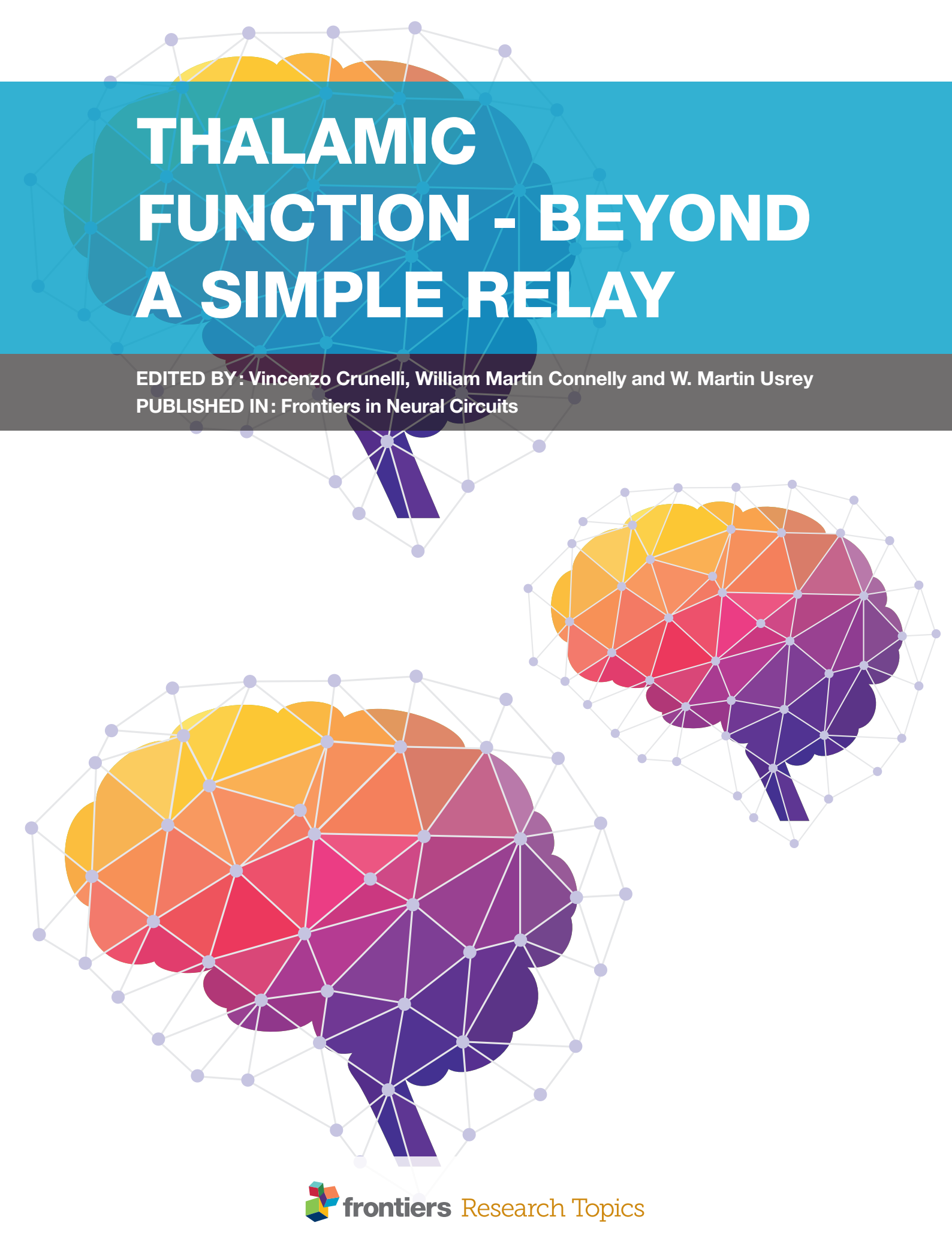


The background of the cover features a stylized brain shape composed of a network of interconnected nodes and lines. The nodes are represented by small circles, and the lines are thin, light gray. The brain is filled with a color gradient that transitions from yellow at the top to orange, then red, and finally to dark purple at the bottom. The title is overlaid on a solid blue horizontal band that spans the width of the image.

THALAMIC FUNCTION - BEYOND A SIMPLE RELAY

EDITED BY: Vincenzo Crunelli, William Martin Connelly and W. Martin Usrey
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THALAMIC FUNCTION - BEYOND A SIMPLE RELAY

Topic Editors:

Vincenzo Crunelli, Cardiff University, UK

William Martin Connelly, Australian National University, Australia

W. Martin Usrey, University of California, Davis, USA

The thalamus is often described as a relay. Typified by sensory pathways, this concept leads to thalamic nuclei being viewed as areas that passively streams information from a single source to the cortex, without affecting the nature of that information. However, diverse intrathalamic connections, the varying synaptic and membrane properties of thalamic neurons and the large number of inputs from non-sensory sources make the idea that the thalamus is just a passive relay unlikely. Furthermore, a large number of thalamic nuclei are not primarily driven by sensory signals nor do they exclusively target the cortex, meaning the thalamus must do more than simply pass sensory signals to the cortex. Finally, there is a wealth of research demonstrating that the thalamus does indeed function in ways that are not captured by the concept of a simple relay. So why, given all of this, is the primary paradigm for describing the thalamus, a relay? This Research Topic covers original research, reviews and hypotheses on thalamic function that explore the concept that the thalamus performs computational tasks other than simply passively relaying information.

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The olfactory thalamus: unanswered questions about the role of the mediodorsal thalamic nucleus in olfaction

Emmanuelle Courtiol^{1,2*} and Donald A. Wilson^{1,2}

¹ Emotional Brain Institute, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY, USA, ² Department of Child and Adolescent Psychiatry, New York University Langone Medical Center, NY, USA

The mediodorsal thalamic nucleus (MDT) is a higher order thalamic nucleus and its role in cognition is increasingly well established. Interestingly, components of the MDT also have a somewhat unique sensory function as they link primary olfactory cortex to orbitofrontal associative cortex. In fact, anatomical evidence firmly demonstrates that the MDT receives direct input from primary olfactory areas including the piriform cortex and has dense reciprocal connections with the orbitofrontal cortex. The functions of this olfactory pathway have been poorly explored but lesion, imaging, and electrophysiological studies suggest that these connections may be involved in olfactory processing including odor perception, discrimination, learning, and attention. However, many important questions regarding the MDT and olfaction remain unanswered. Our goal here is not only to briefly review the existing literature but also to highlight some of the remaining questions that need to be answered to better define the role(s) of the MDT in olfactory processing.

Keywords: olfaction, mediodorsal thalamus, dorsomedial thalamus, piriform cortex, odor response

Introduction

The thalamus is a crucial crossroad structure in the brain that is recognized as a major contributor to the following functions: sensory perception, attention, sleep and arousal, memory, and cognition. Thalamic nuclei can be divided into (at least) two categories: first-order and higher order thalamic relays (Guillery, 1995). The first category, sensory recipient thalamic relays, processes information arriving from the periphery. The second category, higher order thalamic relays, processes information sent from many cortical areas. Higher order thalamic relays are key structures in cortico-thalamo-cortical networks (Sherman and Guillery, 2002; Mitchell et al., 2014; Saalman, 2014).

The mediodorsal thalamic nucleus (MDT) is an example of a higher-order thalamic relay (Mitchell and Chakraborty, 2013). The MDT receives inputs from a wide variety of brain areas including cortical structures (notably the prefrontal cortex), brainstem structures, basal forebrain structures, and other thalamic nuclei (Groenewegen, 1988; Kuroda and Price, 1991a,b; Ray and Price, 1992; Guillery, 1995; Kuroda, 1998). In return, the MDT projects massively to the prefrontal cortex (Leonard, 1969; Krettek and Price, 1977a). The cytoarchitecture and the topographical distribution of the different inputs and outputs have led to the separation of the MDT into three subnuclei in the rat—medial, central, and lateral (Krettek and Price, 1977a; Groenewegen, 1988). The dense reciprocal connections between the MDT and the prefrontal cortex have placed the MDT as a critical structure in the study of cognitive processes.

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Edited by:

William Martin Connelly,
Cardiff University, UK

Reviewed by:

Jane Plailly,
Centre National de la Recherche
Scientifique, France
Burton Slotnick,
American University, USA

*Correspondence:

Emmanuelle Courtiol,
Emotional Brain Institute, Nathan
Kline Institute for Psychiatric
Research, 140 Old Orangeburg
Road, Orangeburg, NY 10962, USA
ecourtio@nki.rfmh.org

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In fact, lesions of the MDT in monkeys and rats are associated with a wide range of cognitive deficits: mnesic deficits, deficits in stimulus-outcome associations, deficits in representation of outcome value, and deficits in action-outcome association (Corbit et al., 2003; Mitchell and Gaffan, 2008; Ostlund and Balleine, 2008; Baxter, 2013; Mitchell and Chakraborty, 2013; Alcaraz et al., 2014; Mair et al., 2015). Electrophysiological recordings of the MDT have also demonstrated the contribution of the MDT in working memory, behavioral flexibility, goal-directed behavior, and stimulus reward-association (Oyoshi et al., 1996; Kawagoe et al., 2007; Yu et al., 2012; Han et al., 2013; Parnaudeau et al., 2013; Mair et al., 2015). The role of the MDT in cognition is thus increasingly well established (reviewed in Baxter, 2013; Funahashi, 2013; Mitchell and Chakraborty, 2013; Mitchell et al., 2014; Mitchell, 2015). In addition to these cognitive functions, the MDT also has a sensory component as the olfactory thalamus. As described below, there are firm anatomical and physiological data demonstrating the relationships among the olfactory cortex, the MDT, and the orbitofrontal associative cortex. These connections are particularly intriguing as they bring together one of the most phylogenetically oldest sensory systems with one of the more recently evolved cortical structures.

Anatomy of the Olfactory Thalamus

The thalamus is the major source of sensory information to the primary sensory cortex for all of the senses except olfaction. In fact, olfactory sensory neurons send their axons directly to the olfactory bulb which in turn projects to the primary olfactory cortex—a region including the piriform cortex, the anterior olfactory nucleus, the olfactory tubercle, the cortical nucleus of the amygdala, and the lateral entorhinal cortex (Price and Powell, 1971; Haberly and Price, 1977; **Figure 1A**). While there is no direct input from the olfactory sensory neurons to the thalamus, the MDT both receives and sends information to primary as well as secondary olfactory areas. An example of a major secondary olfactory area is the orbitofrontal cortex which has strong reciprocal connections with both the MDT and piriform cortex (Illig, 2005). While this review focuses on the MDT, the submedial nucleus of the thalamus also receives olfactory inputs (Price and Slotnick, 1983; Price, 1985).

Olfactory Afferents

Powell et al. (1963) was one of the first to reveal the relationship between the olfactory pathway and the MDT by showing axonal fiber degeneration in the MDT following lesions in the piriform cortex. In addition to the piriform cortex, the MDT also receives direct input from the olfactory tubercle, the basolateral and cortical nuclei of the amygdala, the lateral entorhinal cortex, the anterior olfactory nucleus, the endopiriform nucleus, and the orbitofrontal cortex (**Figure 1A**). The MDT is thus the target of all the primary olfactory areas (e.g., piriform cortex) as well as some secondary olfactory areas (e.g., orbitofrontal cortex). Of note, the olfactory projections are topographical and involve two distinct subregions of the MDT: the medial

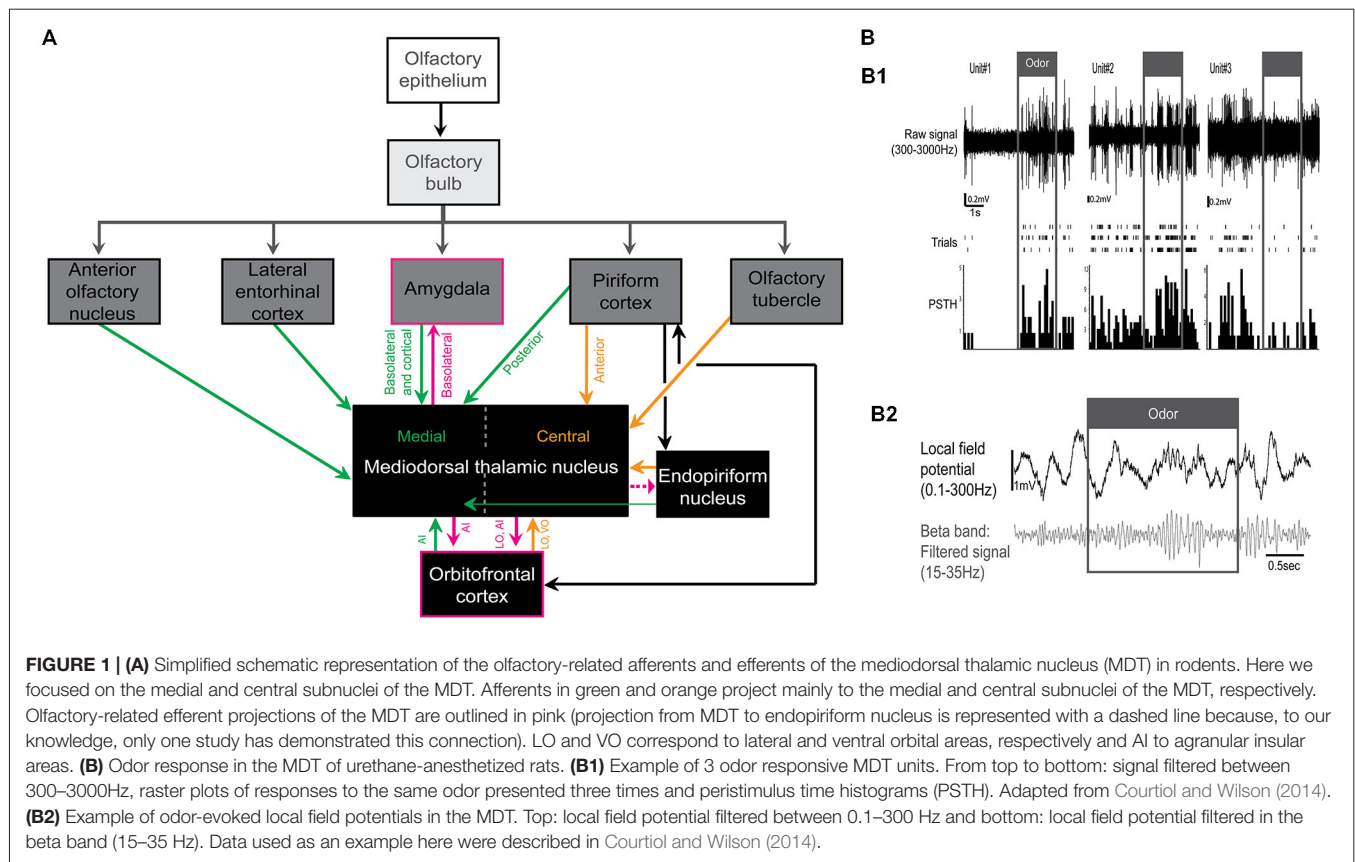
and central subnuclei [in rats: (Powell et al., 1963; Heimer, 1968; Krettek and Price, 1974, 1977b; Inagaki et al., 1983; Price and Slotnick, 1983; Price, 1985; Cornwall and Phillipson, 1988; Kuroda and Price, 1991a,b; Kuroda et al., 1992a,b; Kowianski et al., 1999; Bay and Cavdar, 2013; Wilson et al., 2014); in hamsters: (Ferrer, 1969); **Figure 1A**]. The posterior piriform cortex, the anterior olfactory nucleus, the basolateral and cortical nuclei of the amygdala, the agranular insular areas, and the lateral entorhinal cortex project more medially in the MDT while the rostral piriform cortex (deep layers), the ventral and lateral orbital areas, the olfactory tubercle (polymorphic area), and the ventral endopiriform nucleus project mainly to the central region of the MDT [(Krettek and Price, 1974; Inagaki et al., 1983; Price and Slotnick, 1983; Price, 1985; Ray and Price, 1992; Bay and Cavdar, 2013); a small number of neurons from the endopiriform nucleus also project to the medial MDT (Cornwall and Phillipson, 1988)]. The detailed synaptic organization of the olfactory projections to the MDT is still poorly known. Kuroda et al. (1992a,b) described two types of axon terminals (large and small presynaptic terminals) from the piriform cortex to the central MDT which both appear to be excitatory. However, those two types of axon terminals still need to be characterized physiologically to identify whether or not the cells of origin differ. The targeting of specific cell types with cell-specific viral manipulations (e.g., optogenetics) may help to answer this question.

Olfactory-Related Efferents

The MDT is known to be the origin of dense projections to the frontal cortex in the rat (Groenewegen, 1988). Furthermore, topographical projections from the MDT to olfactory-related structures, including the orbitofrontal cortex and the amygdala, have been established (Krettek and Price, 1977a; Ray and Price, 1992; **Figure 1A**). Interestingly, Kowianski et al. (1999) demonstrated that the endopiriform nucleus receives input from the MDT, though this has not been reported elsewhere.

In essence, the medial subnucleus of the MDT projects to prelimbic and dorsal agranular insular areas, as well as to the basolateral amygdala. The central subnucleus projects to the lateral part of the orbitofrontal cortex and the ventral part of the agranular insular area. Finally, the lateral subnucleus projects to the anterior part of the cingulate area, the medial precentral area, and is reciprocally connected with orbital areas (Krettek and Price, 1977a; Groenewegen, 1988; Ray and Price, 1992). The projections of the MDT to the orbitofrontal cortex and basolateral amygdala are of great interest as those two areas are strongly involved in olfactory perception and odor-guided behavior [in rats: (Schoenbaum et al., 1999; Sevelinges et al., 2004; Feierstein et al., 2006; Roesch et al., 2007; Chapuis et al., 2009); in monkeys: (Tanabe et al., 1975; Rolls et al., 1996); in humans: (Jones-Gotman and Zatorre, 1993; Zald and Pardo, 1997; Gottfried and Zelano, 2011)].

The different anatomical studies provide strong evidence establishing the relationship between the olfactory pathway and the MDT. However questions related to the cells of origin and ultrastructural and synaptic organizations of the olfactory afferents in the MDT, as well as the neurotransmitters involved,



still need to be investigated. Furthermore, given the recent demonstration that piriform cortical neurons projecting to orbitofrontal cortex may be non-randomly spatially organized, more detailed analysis of the olfactory cortex-MDT projection is warranted (Chen et al., 2014).

Electrophysiological Studies of the Olfactory Thalamus

As a first step in understanding the contribution of the MDT in olfactory processing, it is important to characterize how olfactory information is encoded in the MDT. Here, we will describe the physiological responses of the MDT to olfactory stimulation. These data are based on responses recorded in the central and medial portions of the MDT.

First, evoked potentials and extracellular unitary responses in the MDT following the electrical stimulation of the olfactory bulb or lateral olfactory tract of various species have been described [in rats, central subnucleus: (Price and Slotnick, 1983; Price, 1985); in monkeys, medial subnucleus: (Benjamin and Jackson, 1974; Yarita et al., 1980; Takagi, 1986); in rabbits, medial subnucleus: (Jackson and Benjamin, 1974; Imamura et al., 1984)], with percentages of olfactory region-driven MDT units ranging from 16% to approximately 70% [(Imamura et al., 1984): 87/538 units in rabbits and Benjamin and Jackson (1974): approximately 180 units recorded in

monkeys and 127 responsive units were localized in medial MDT].

Second, MDT units can respond to various odorant categories including biological, monomolecular, and mixture odorants [in rats, we observed odor-responsive units in medial, central and lateral MDT, but the boundary delimitation of the different subnuclei was not always clear and may account for the observation of odor-responsive units in the lateral MDT: (Courtiol and Wilson, 2014); in rabbits (Imamura et al., 1984); in monkeys: (Yarita et al., 1980; Takagi, 1986); in cats: (Motokizawa, 1974); **Figure 1B1**]. We observed that 51% of rat MDT units were odor-responsive, Motokizawa (1974) reported 44% of odor-responsive units in cats and Imamura et al. (1984) reported 55% of odor-responsive units in rabbits, although this last percentage was calculated only on MDT neurons responding to lateral olfactory tract shocks (48/87). The tuning of MDT neurons to odorants seems to be dependent on the species, odorant set, and level of anesthesia used. In fact, Courtiol and Wilson (2014) reported that, in anesthetized rats, 63% of units responded to only one odor out of the odor set used. Yet, this tuning has been shown to be more broad in awake monkey and anesthetized rabbits, with 41.5% of MDT neurons responding to four odors and more than 80% responding to two odors or more, respectively (Yarita et al., 1980; Imamura et al., 1984). Nevertheless, two common features of these reports are that MDT

units: (1) primarily display excitatory activities in response to odors although suppressive responses can also be observed (**Figure 1B1**) and (2) are similarly responsive to biological, monomolecular or mixture odorants. In addition, our work and the work of others reported that some MDT units can display respiration-linked activity (Imamura et al., 1984; Courtiol and Wilson, 2014). Interestingly, at least in anesthetized rabbits and unanesthetized monkeys, the majority of odor responsive neurons are sensory specific as they do not respond to visual, auditory nor somatic stimulation (Yarita et al., 1980; Imamura et al., 1984).

Finally, the response of the MDT to odorant stimulation can also be recorded at the network level. In recording the local field potentials in the MDT of urethane-anesthetized rats (Courtiol and Wilson, 2014; **Figure 1B2**), we observed that odorants induce the conjoint emergence of beta frequency oscillations in the MDT and piriform cortex. Interestingly, a subset of MDT units fire in phase with the beta frequency oscillations recorded in the piriform cortex. These beta oscillations may offer an effective mechanism for olfactory information transmission between the piriform cortex and the MDT (Tallon-Baudry et al., 2001).

Taken together, these studies reveal that the MDT can respond to and encode odorant information in a manner similar to other primary and secondary olfactory structures. However, future studies will need to determine: (1) the contribution of each of the olfactory inputs to the MDT response; (2) the impact of the MDT on downstream targets such as the orbitofrontal cortex; and (3) given the rich variety of non-olfactory inputs to MDT, how MDT neurons contribute to multi-sensory associations and contextual effects on odor perception. Performing multi-site unitary recordings and using a large set of odorants in behaving animals may achieve this.

The Still Unclear Role of the MDT in Olfaction

Beyond its basic odor responsiveness, the role of the MDT in olfaction remains unclear. In this last section, we will review the different studies involving the MDT in olfaction, point to some common threads among the available literature, and highlight remaining questions.

Studies of the effect of damage to the MDT in both humans and animal models have provided some useful information about its role in olfaction (Tham et al., 2009). The results of these studies have demonstrated that both humans and animal models with MDT damage are not anosmic and do not present deficits in olfactory detection [in rats and hamsters: (Eichenbaum et al., 1980; Sapolsky and Eichenbaum, 1980); in humans: (Potter and Butters, 1980; Sela et al., 2009)]. Furthermore, humans with Korsakoff's disease presenting MDT damage are not anosmic but odor detection effects vary between studies, probably depending on the extent of the damage (Jones et al., 1975; Potter and Butters, 1980; Pol et al., 2002). While the results of those studies have shown that MDT damage does not affect olfactory detection, they support the fact that MDT lesions do affect other olfactory functions including

olfactory perception, discrimination, learning, and attention. In respect to olfactory perception, Sapolsky and Eichenbaum (1980) demonstrated that MDT-lesioned hamsters show distorted odor preference, i.e., less interest in female and male odors and reduced preference for genital sniffing, leading to maladaptive sexual behaviors. Altered odor preference was also reported in humans. In fact, patients with damage in the MDT present altered olfactory hedonic perception (Rousseaux et al., 1996; Asai et al., 2008; Sela et al., 2009). These results are interesting given the reciprocal connections between the MDT and the amygdala, as the neuronal activity in the amygdala has been shown to be directly influenced by the hedonic valence of olfactory stimuli in humans (Zald and Pardo, 1997). The MDT may thus be part of a network, including the amygdala, involved in the coding of the hedonic valence of the olfactory stimuli.

With respect to discrimination, Eichenbaum et al. (1980) showed that rats with lesions of the MDT exhibit deficits in difficult odor discriminations. For instance, rats with MDT lesions need more trials to reach the discrimination criterion when the task difficulty is increased by using either novel stimuli or perceptually similar stimuli (Eichenbaum et al., 1980; Slotnick and Risser, 1990). However, these deficits can be temporary and alleviated if the animals receive intensive training (Staubli et al., 1987). Deficits in odor discrimination, as well as in odor identification, were also reported in humans with thalamic damage (Sela et al., 2009; Tham et al., 2011a). Notably, Tham et al. (2011b) used both olfactory and visual discrimination tests and demonstrated that patients with MDT damage presented impaired performance selectively for olfactory discrimination compared to visual discrimination. The effects of MDT lesions extend beyond olfactory discrimination given that deficits in olfactory learning have also been observed. For example, rats with central MDT lesions perform as well as controls for preoperatively learned visual discrimination tasks and in the acquisition of a simple go/no-go odor discrimination task (Slotnick and Kaneko, 1981). However MDT-lesioned rats were impaired when performing odor reversal learning. The authors also noticed in a set of preliminary experiments that three MDT-lesioned rats were not impaired in their acquisition of a visual discrimination reversal set suggesting that the deficits were modality specific. Thus, lesions of the MDT seem to induce a severe deficit in reversal learning and the degree of impairment seems to be related to the extent of the lesion (Slotnick and Kaneko, 1981; Staubli et al., 1987; Lu and Slotnick, 1990; Slotnick and Risser, 1990; McBride and Slotnick, 1997). Importantly, one of the reciprocally connected structures of the MDT—the orbitofrontal cortex—has also been involved in reversal learning (Roesch et al., 2007). The MDT-orbitofrontal cortex network may integrate stimulus-outcome associations to flexibly guide goal-directed behavior.

Rats with thalamic lesions which include the MDT also present deficits in an olfactory continuous delayed nonmatching-to-sample task with no effect on an odor discrimination task. Although, when lesions were more restricted to the MDT, the deficits were minimal in this task (Koger and Mair, 1994; Zhang et al., 1998).

The magnitude of the deficits due to MDT lesions is probably related to the extent of the lesions, to the task used, as well as to the difficulty of the task. In fact, Eichenbaum et al. (1980) reported that the major effects of MDT lesions appear when the task complexity is high. Interestingly, the difficulty level of the task may be linked to the attentional demand. An attention deficit may underlie the problems of rodents and humans with damage to the MDT to perform difficult odor discrimination tasks. The role of the MDT in olfactory attention was recently investigated in humans. Plailly et al. (2008) measured attention-dependent network activity using functional magnetic resonance imaging (fMRI). They observed that attending specifically to odor (compared to tone) increases the coupling between piriform cortex to MDT and MDT to orbitofrontal cortex. Corroborating these results, Veldhuizen and Small (2011) observed in humans, using fMRI, activation of the MDT in response to attention to odorants but not tastants. Those two studies support the involvement of the MDT in attention to odors and may be related to the fact that the MDT can be involved in prediction error signaling where the response magnitude of the MDT is significantly higher to unexpected compared to expected odor stimuli (Zelano et al., 2011; Olofsson et al., 2013).

These results obtained with functional imaging are further supported by lesions and neuropsychological studies (Tham et al., 2009, 2011a,b). For example, Tham et al. (2011b) tested whether the MDT was likely involved in top-down directed olfactory attention by using a Target Odor Search Test and showed that patients with damage to the MDT performed poorer verbal-based search than controls. All these studies in humans indicate a possible role of the MDT in olfactory attention processing. However, this idea was debated by Keller (2011) who proposed that the olfactory inputs to the MDT may not be sufficient to support shift of attention toward odors. The question about the contribution of the MDT in olfactory attention still remains open and other studies are required to disentangle it. For example, future studies can assess the impact of lesions of the MDT in rats performing an attention-related task, such as the one described in Ljubojevic et al. (2014) and measure not only their performance relative to controls but also their sampling duration and their latency to reply. Those studies can also assess how and when during the attention-related task the MDT is required by selectively and temporally inhibiting the MDT at different periods of the task using optogenetics.

Lastly, electrophysiological recordings of the MDT in behaving animals may also help to better characterize the temporal contribution of the MDT in olfactory perception and odor-guided behavior. Regarding electrophysiological recordings of the MDT in behaving animals, to the best of our knowledge, there is only one published study recording single-unit activity in the MDT in animals actively engaged in olfactory tasks [in rats: (Kawagoe et al., 2007); *nota bene*: in Yarita et al. (1980), monkeys were awake though odors were presented passively]. Kawagoe et al. (2007) recorded the MDT activity in an olfactory task requiring animals to discriminate odor cues associated with reward or not. They observed that 10% (13/121 units) of MDT neurons recorded in the central and medial subnuclei responded to the odor cue. Most of these cue-responsive neurons

displayed odorant selectivity with a difference of activity between odor cues associated to the same reinforcement category. Combined with this sensory selectivity, the most remarkable effect was that cue-responsive MDT neurons showed strong response preference to cues associated with a reward, and those responses were plastic to extinction and relearning and always related to the reward contingency. The MDT is thus sensitive to stimulus-reward association. Interestingly, the basolateral amygdala and the olfactory tubercle, which project to the medial and central subnuclei, respectively, can also encode the associated outcome of odors, which could contribute to this MDT activity (Schoenbaum et al., 1998; Gadziola et al., 2015). Finally, we recently recorded MDT units in rats performing a two alternative odor discrimination task (Courtiol and Wilson, 2015) and observed that a subset of units were odor selective. Intermingled with this sensory function, we observed that the MDT units displayed activity prior to odor sampling, presumably anticipatory activity, and seemed to encode the choice direction-goal location associated with the odor. This study as well as the one by Kawagoe et al. (2007) emphasize that the MDT may encode both basic sensory as well as complex olfactory functions. This observation is not unique to the MDT as coding both basic sensory and complex olfactory functions has notably been reported in piriform cortex and orbitofrontal cortex (Schoenbaum and Eichenbaum, 1995; Feierstein et al., 2006). With these limited images of MDT function, many questions remain, most notably what are the specific contributions of the MDT in these functions compared to the piriform cortex or the orbitofrontal cortex and what is the MDT adding to olfactory processing?

Conclusion and Perspectives

Despite the unusual anatomy of the olfactory pathway, an olfactory thalamus can be identified—the MDT receives direct input from various olfactory areas (**Figure 1A**). By virtue of this specific pathway, the thalamic contributions to olfaction are woefully unexplored. Lesion studies in the 1980's followed by more recent humans studies have provided the first evidence of the involvement of the MDT in olfactory processing and suggest a role for the MDT in functions ranging from olfactory perception to attention. While this work provides a glimpse of the place of the MDT in olfaction, many questions, as described above, remain unanswered. For example: Is the MDT role in olfaction similar to the role of primary sensory relays such as the lateral geniculate nucleus? If so, can principles of thalamic function be generalized to all sensory systems? We hypothesize that not to be the case. In olfaction, the functions of “primary sensory thalamic relay” including sensory coding, gain control, and state-dependent modulation may be distributed between the olfactory bulb and the piriform cortex (Murakami et al., 2005; Kay and Sherman, 2007). Moreover, given: (1) the anatomical place of the MDT in the olfactory pathway (convergence of many olfactory inputs); (2) the implication of the MDT in higher order functions including olfactory attention; and (3) the MDT being a higher order thalamic relay, the MDT may then be viewed as a higher order olfactory thalamus rather than a

primary sensory thalamic relay. Identifying the precise role of MDT in olfactory perception and odor-guided behavior may be an excellent avenue for exploring broader questions of higher-order thalamic function.

References

- Alcaraz, F., Naneix, F., Desfosses, E., Marchand, A. R., Wolff, M., and Coutureau, E. (2014). Dissociable effects of anterior and mediodorsal thalamic lesions on spatial goal-directed behavior. *Brain Struct. Funct.* doi: 10.1007/s00429-014-0893-7 [Epub ahead of print].
- Asai, H., Uda, F., Hirano, M., and Ueno, S. (2008). Odor abnormalities caused by bilateral thalamic infarction. *Clin. Neurol. Neurosurg.* 110, 500–501. doi: 10.1016/j.clineuro.2008.01.008
- Baxter, M. G. (2013). Mediodorsal thalamus and cognition in non-human primates. *Front. Syst. Neurosci.* 7:38. doi: 10.3389/fnsys.2013.00038
- Bay, H. H., and Cavdar, S. (2013). Regional connections of the mediodorsal thalamic nucleus in the rat. *J. Integr. Neurosci.* 12, 201–219. doi: 10.1142/s021963521350012x
- Benjamin, R. M., and Jackson, J. C. (1974). Unit discharges in the mediodorsal nucleus of the squirrel monkey evoked by electrical stimulation of the olfactory bulb. *Brain Res.* 75, 181–191. doi: 10.1016/0006-8993(74)90740-9
- Chapuis, J., Garcia, S., Messaoudi, B., Thevenet, M., Ferreira, G., Gervais, R., et al. (2009). The way an odor is experienced during aversive conditioning determines the extent of the network recruited during retrieval: a multisite electrophysiological study in rats. *J. Neurosci.* 29, 10287–10298. doi: 10.1523/JNEUROSCI.0505-09.2009
- Chen, C. F., Zou, D. J., Altomare, C. G., Xu, L., Greer, C. A., and Firestein, S. J. (2014). Nonsensory target-dependent organization of piriform cortex. *Proc. Natl. Acad. Sci. U S A* 111, 16931–16936. doi: 10.1073/pnas.1411266111
- Corbit, L. H., Muir, J. L., and Balleine, B. W. (2003). Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *Eur. J. Neurosci.* 18, 1286–1294. doi: 10.1046/j.1460-9568.2003.02833.x
- Cornwall, J., and Phillipson, O. T. (1988). Afferent projections to the dorsal thalamus of the rat as shown by retrograde lectin transport—I. The mediodorsal nucleus. *Neuroscience* 24, 1035–1049. doi: 10.1016/0306-4522(88)90085-1
- Courtio, E., and Wilson, D. A. (2014). Thalamic olfaction: characterizing odor processing in the mediodorsal thalamus of the rat. *J. Neurophysiol.* 111, 1274–1285. doi: 10.1152/jn.00741.2013
- Courtio, E., and Wilson, D. A. (2015). The olfactory thalamus: characterizing single-unit activity of the mediodorsal thalamic nucleus in behaving rats. Association for Chemoreception Sciences Annual Meeting April 22–25, 2015, Bonita Springs, FL.
- Eichenbaum, H., Shedlack, K. J., and Eckmann, K. W. (1980). Thalamocortical mechanisms in odor-guided behavior. I. Effects of lesions of the mediodorsal thalamic nucleus and frontal cortex on olfactory discrimination in the rat. *Brain Behav. Evol.* 17, 255–275. doi: 10.1159/000121803
- Feierstein, C. E., Quirk, M. C., Uchida, N., Sosulski, D. L., and Mainen, Z. F. (2006). Representation of spatial goals in rat orbitofrontal cortex. *Neuron* 51, 495–507. doi: 10.1016/j.neuron.2006.06.032
- Ferrer, N. G. (1969). Efferent projections of the anterior olfactory nucleus. *J. Comp. Neurol.* 137, 309–320. doi: 10.1002/cne.901370305
- Funahashi, S. (2013). Thalamic mediodorsal nucleus and its participation in spatial working memory processes: comparison with the prefrontal cortex. *Front. Syst. Neurosci.* 7:36. doi: 10.3389/fnsys.2013.00036
- Gadziola, M. A., Tylicki, K. A., Christian, D. L., and Wesson, D. W. (2015). The olfactory tubercle encodes odor valence in behaving mice. *J. Neurosci.* 35, 4515–4527. doi: 10.1523/JNEUROSCI.4750-14.2015
- Gottfried, J. A., and Zelano, C. (2011). The value of identity: olfactory notes on orbitofrontal cortex function. *Ann. N. Y. Acad. Sci.* 1239, 138–148. doi: 10.1111/j.1749-6632.2011.06268.x
- Groenewegen, H. J. (1988). Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. *Neuroscience* 24, 379–431. doi: 10.1016/0306-4522(88)90339-9
- Guillery, R. W. (1995). Anatomical evidence concerning the role of the thalamus in corticocortical communication: a brief review. *J. Anat.* 187, 583–592.
- Haberly, L. B., and Price, J. L. (1977). The axonal projection patterns of the mitral and tufted cells of the olfactory bulb in the rat. *Brain Res.* 129, 152–157. doi: 10.1016/0006-8993(77)90978-7
- Han, J., Lee, J. H., Kim, M. J., and Jung, M. W. (2013). Neural activity in mediodorsal nucleus of thalamus in rats performing a working memory task. *Front. Neural Circuits* 7:128. doi: 10.3389/fncir.2013.00128
- Heimer, L. (1968). Synaptic distribution of centripetal and centrifugal nerve fibres in the olfactory system of the rat. An experimental anatomical study. *J. Anat.* 103, 413–432.
- Illig, K. R. (2005). Projections from orbitofrontal cortex to anterior piriform cortex in the rat suggest a role in olfactory information processing. *J. Comp. Neurol.* 488, 224–231. doi: 10.1002/cne.20595
- Imamura, K., Onoda, N., and Takagi, S. F. (1984). Odor response characteristics of thalamic mediodorsal nucleus neurons in the rabbit. *Jpn. J. Physiol.* 34, 55–73. doi: 10.2170/jjphysiol.34.55
- Inagaki, S., Kubota, Y., Shinoda, K., Kawai, Y., and Tohyama, M. (1983). Neurotensin-containing pathway from the endopiriform nucleus and the adjacent prepiriform cortex to the dorsomedial thalamic nucleus in the rat. *Brain Res.* 260, 143–146. doi: 10.1016/0006-8993(83)90776-x
- Jackson, J. C., and Benjamin, R. M. (1974). Unit discharges in the mediodorsal nucleus of the rabbit evoked by electrical stimulation of the olfactory bulb. *Brain Res.* 75, 193–201. doi: 10.1016/0006-8993(74)90741-0
- Jones, B. P., Moskowitz, H. R., and Butters, N. (1975). Olfactory discrimination in alcoholic korsakoff patients. *Neuropsychologia* 13, 173–179. doi: 10.1016/0028-3932(75)90026-3
- Jones-Gotman, M., and Zatorre, R. J. (1993). Odor recognition memory in humans: role of right temporal and orbitofrontal regions. *Brain Cogn.* 22, 182–198. doi: 10.1006/brcg.1993.1033
- Kawagoe, T., Tamura, R., Uwano, T., Asahi, T., Nishijo, H., Eifuku, S., et al. (2007). Neural correlates of stimulus-reward association in the rat mediodorsal thalamus. *Neuroreport* 18, 683–688. doi: 10.1097/wnr.0b013e3280bef9a6
- Kay, L. M., and Sherman, S. M. (2007). An argument for an olfactory thalamus. *Trends Neurosci.* 30, 47–53. doi: 10.1016/j.tins.2006.11.007
- Keller, A. (2011). Attention and olfactory consciousness. *Front. Psychol.* 2:380. doi: 10.3389/fpsyg.2011.00380
- Koger, S. M., and Mair, R. G. (1994). Comparison of the effects of frontal cortical and thalamic lesions on measures of olfactory learning and memory in the rat. *Behav. Neurosci.* 108, 1088–1100. doi: 10.1037/0735-7044.108.6.1088
- Kowianski, P., Lipowska, M., and Moryś, J. (1999). The piriform cortex and the endopiriform nucleus in the rat reveal generally similar pattern of connections. *Folia Morphol. (Warsz)* 58, 9–19.
- Krettek, J. E., and Price, J. L. (1974). A direct input from the amygdala to the thalamus and the cerebral cortex. *Brain Res.* 67, 169–174. doi: 10.1016/0006-8993(74)90309-6
- Krettek, J. E., and Price, J. L. (1977a). The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. *J. Comp. Neurol.* 171, 157–191. doi: 10.1002/cne.901710204
- Krettek, J. E., and Price, J. L. (1977b). Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *J. Comp. Neurol.* 172, 687–722. doi: 10.1002/cne.901720408
- Kuroda, M. (1998). Synaptic connections between the prefrontal cortex and the mediodorsal nucleus of the thalamus. *Kaibogaku Zasshi* 73, 93–106.
- Kuroda, M., and Price, J. L. (1991a). Synaptic organization of projections from basal forebrain structures to the mediodorsal thalamic nucleus of the rat. *J. Comp. Neurol.* 303, 513–533. doi: 10.1002/cne.903030402
- Kuroda, M., and Price, J. L. (1991b). Ultrastructure and synaptic organization of axon terminals from brainstem structures to the mediodorsal thalamic nucleus of the rat. *J. Comp. Neurol.* 313, 539–552. doi: 10.1002/cne.903130313

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- Kuroda, M., López-Mascaraque, L., and Price, J. L. (1992a). Neuronal and synaptic composition of the mediodorsal thalamic nucleus in the rat: a light and electron microscopic Golgi study. *J. Comp. Neurol.* 326, 61–81. doi: 10.1002/cne.903260106
- Kuroda, M., Murakami, K., Kishi, K., and Price, J. L. (1992b). Distribution of the piriform cortical terminals to cells in the central segment of the mediodorsal thalamic nucleus of the rat. *Brain Res.* 595, 159–163. doi: 10.1016/0006-8993(92)91468-t
- Leonard, C. M. (1969). The prefrontal cortex of the rat. I. Cortical projection of the mediodorsal nucleus. II. Efferent connections. *Brain Res.* 12, 321–343. doi: 10.1016/0006-8993(69)90003-1
- Ljubojevic, V., Luu, P., and De Rosa, E. (2014). Cholinergic contributions to supramodal attentional processes in rats. *J. Neurosci.* 34, 2264–2275. doi: 10.1523/JNEUROSCI.1024-13.2014
- Lu, X.-C. M., and Slotnick, B. M. (1990). Acquisition of an olfactory learning-set in rats with lesions of the mediodorsal thalamic nucleus. *Chem. Senses* 15, 713–724. doi: 10.1093/chemse/15.6.713
- Mair, R. G., Miller, R. L., Wormwood, B. A., Francoeur, M. J., Onos, K. D., and Gibson, B. M. (2015). The neurobiology of thalamic amnesia: contributions of medial thalamus and prefrontal cortex to delayed conditional discrimination. *Neurosci. Biobehav. Rev.* 54, 161–174. doi: 10.1016/j.neubiorev.2015.01.011
- McBride, S. A., and Slotnick, B. (1997). The olfactory thalamocortical system and odor reversal learning examined using an asymmetrical lesion paradigm in rats. *Behav. Neurosci.* 111, 1273–1284. doi: 10.1037/0735-7044.111.6.1273
- Mitchell, A. S. (2015). The mediodorsal thalamus as a higher order thalamic relay nucleus important for learning and decision-making. *Neurosci. Biobehav. Rev.* 54, 76–88. doi: 10.1016/j.neubiorev.2015.03.001
- Mitchell, A. S., and Chakraborty, S. (2013). What does the mediodorsal thalamus do? *Front. Syst. Neurosci.* 7:37. doi: 10.3389/fnsys.2013.00037
- Mitchell, A. S., and Gaffan, D. (2008). The magnocellular mediodorsal thalamus is necessary for memory acquisition, but not retrieval. *J. Neurosci.* 28, 258–263. doi: 10.1523/JNEUROSCI.4922-07.2008
- Mitchell, A. S., Sherman, S. M., Sommer, M. A., Mair, R. G., Vertes, R. P., and Chudasama, Y. (2014). Advances in understanding mechanisms of thalamic relays in cognition and behavior. *J. Neurosci.* 34, 15340–15346. doi: 10.1523/JNEUROSCI.3289-14.2014
- Motokizawa, F. (1974). Olfactory input to the thalamus: electrophysiological evidence. *Brain Res.* 67, 334–337. doi: 10.1016/0006-8993(74)90284-4
- Murakami, M., Kashiwadani, H., Kirino, Y., and Mori, K. (2005). State-dependent sensory gating in olfactory cortex. *Neuron* 46, 285–296. doi: 10.1016/j.neuron.2005.02.025
- Olofsson, J. K., Rogalski, E., Harrison, T., Mesulam, M. M., and Gottfried, J. A. (2013). A cortical pathway to olfactory naming: evidence from primary progressive aphasia. *Brain* 136, 1245–1259. doi: 10.1093/brain/awt019
- Ostlund, S. B., and Balleine, B. W. (2008). Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. *J. Neurosci.* 28, 4398–4405. doi: 10.1523/JNEUROSCI.5472-07.2008
- Oyoshi, T., Nishijo, H., Asakura, T., Takamura, Y., and Ono, T. (1996). Emotional and behavioral correlates of mediodorsal thalamic neurons during associative learning in rats. *J. Neurosci.* 16, 5812–5829.
- Parnaudeau, S., O'Neill, P. K., Bolkan, S. S., Ward, R. D., Abbas, A. I., Roth, B. L., et al. (2013). Inhibition of mediodorsal thalamus disrupts thalamofrontal connectivity and cognition. *Neuron* 77, 1151–1162. doi: 10.1016/j.neuron.2013.01.038
- Plailly, J., Howard, J. D., Gitelman, D. R., and Gottfried, J. A. (2008). Attention to odor modulates thalamocortical connectivity in the human brain. *J. Neurosci.* 28, 5257–5267. doi: 10.1523/JNEUROSCI.5607-07.2008
- Pol, H. E. H., Hijman, R., Tulken, C. A. F., Heeren, T. J., Schneider, N., and van Ree, J. M. (2002). Odor discrimination in patients with frontal lobe damage and korsakoff's syndrome. *Neuropsychologia* 40, 888–891. doi: 10.1016/s0028-3932(01)00167-1
- Potter, H., and Butters, N. (1980). An assessment of olfactory deficits in patients with damage to prefrontal cortex. *Neuropsychologia* 18, 621–628. doi: 10.1016/0028-3932(80)90101-3
- Powell, T. P., Cowan, W. M., and Raisman, G. (1963). Olfactory relationships of the diencephalon. *Nature* 199, 710–712. doi: 10.1038/199710b0
- Price, J. L. (1985). Beyond the primary olfactory cortex-olfactory-related areas in the neocortex, thalamus and hypothalamus. *Chem. Senses* 10, 239–258. doi: 10.1093/chemse/10.2.239
- Price, J. L., and Powell, T. P. (1971). Certain observations on the olfactory pathway. *J. Anat.* 110, 105–126.
- Price, J. L., and Slotnick, B. M. (1983). Dual olfactory representation in the rat thalamus: an anatomical and electrophysiological study. *J. Comp. Neurol.* 215, 63–77. doi: 10.1002/cne.902150106
- Ray, J. P., and Price, J. L. (1992). The organization of the thalamocortical connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain-prefrontal cortex topography. *J. Comp. Neurol.* 323, 167–197. doi: 10.1002/cne.903230204
- Roesch, M. R., Stalnaker, T. A., and Schoenbaum, G. (2007). Associative encoding in anterior piriform cortex versus orbitofrontal cortex during odor discrimination and reversal learning. *Cereb. Cortex* 17, 643–652. doi: 10.1093/cercor/bhk009
- Rolls, E. T., Critchley, H. D., and Treves, A. (1996). Representation of olfactory information in the primate orbitofrontal cortex. *J. Neurophysiol.* 75, 1982–1996.
- Rousseaux, M., Muller, P., Gahide, I., Mottin, Y., and Romon, M. (1996). Disorders of smell, taste and food intake in a patient with a dorsomedial thalamic infarct. *Stroke* 27, 2328–2330. doi: 10.1161/01.str.27.12.2328
- Saalmann, Y. B. (2014). Intralaminar and medial thalamic influence on cortical synchrony, information transmission and cognition. *Front. Syst. Neurosci.* 8:83. doi: 10.3389/fnsys.2014.00083
- Sapolsky, R. M., and Eichenbaum, H. (1980). Thalamocortical mechanisms in odor-guided behavior. II. Effects of lesions of the mediodorsal thalamic nucleus and frontal cortex on odor preferences and sexual behavior in the hamster. *Brain Behav. Evol.* 17, 276–290. doi: 10.1159/000121804
- Schoenbaum, G., Chiba, A. A., and Gallagher, M. (1998). Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. *Nat. Neurosci.* 1, 155–159. doi: 10.1038/407
- Schoenbaum, G., Chiba, A. A., and Gallagher, M. (1999). Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. *J. Neurosci.* 19, 1876–1884.
- Schoenbaum, G., and Eichenbaum, H. (1995). Information coding in the rodent prefrontal cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform cortex. *J. Neurophysiol.* 74, 733–750.
- Sela, L., Sacher, Y., Serfaty, C., Yeshurun, Y., Soroker, N., and Sobel, N. (2009). Sparing and impaired olfactory abilities after thalamic lesions. *J. Neurosci.* 29, 12059–12069. doi: 10.1523/JNEUROSCI.2114-09.2009
- Sevelinges, Y., Gervais, R., Messaoudi, B., Granjon, L., and Mouly, A. M. (2004). Olfactory fear conditioning induces field potential potentiation in rat olfactory cortex and amygdala. *Learn. Mem.* 11, 761–769. doi: 10.1101/lm.83604
- Sherman, S. M., and Guillery, R. W. (2002). The role of the thalamus in the flow of information to the cortex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 357, 1695–1708. doi: 10.1098/rstb.2002.1161
- Slotnick, B. M., and Kaneko, N. (1981). Role of mediodorsal thalamic nucleus in olfactory discrimination learning in rats. *Science* 214, 91–92. doi: 10.1126/science.7280684
- Slotnick, B. M., and Risser, J. M. (1990). Odor memory and odor learning in rats with lesions of the lateral olfactory tract and mediodorsal thalamic nucleus. *Brain Res.* 529, 23–29. doi: 10.1016/0006-8993(90)90807-n
- Staubli, U., Schottler, F., and Nejat-Bina, D. (1987). Role of dorsomedial thalamic nucleus and piriform cortex in processing olfactory information. *Behav. Brain Res.* 25, 117–129. doi: 10.1016/0166-4328(87)90005-2
- Takagi, S. F. (1986). Studies on the olfactory nervous system of the old world monkey. *Prog. Neurobiol.* 27, 195–250. doi: 10.1016/0301-0082(86)90022-5
- Tallon-Baudry, C., Bertrand, O., and Fischer, C. (2001). Oscillatory synchrony between human extrastriate areas during visual short-term memory maintenance. *J. Neurosci.* 21:RC177.
- Tanabe, T., Iino, M., and Takagi, S. F. (1975). Discrimination of odors in olfactory bulb, pyriform-amygdaloid areas and orbitofrontal cortex of the monkey. *J. Neurophysiol.* 38, 1284–1296.
- Tham, W. W., Stevenson, R. J., and Miller, L. A. (2009). The functional role of the medio dorsal thalamic nucleus in olfaction. *Brain Res. Rev.* 62, 109–126. doi: 10.1016/j.brainresrev.2009.09.007

- Tham, W. W., Stevenson, R. J., and Miller, L. A. (2011a). The impact of mediodorsal thalamic lesions on olfactory attention and flavor perception. *Brain Cogn.* 77, 71–79. doi: 10.1016/j.bandc.2011.05.008
- Tham, W. W., Stevenson, R. J., and Miller, L. A. (2011b). The role of the mediodorsal thalamic nucleus in human olfaction. *Neurocase* 17, 148–159. doi: 10.1080/13554794.2010.504728
- Veldhuizen, M. G., and Small, D. M. (2011). Modality-specific neural effects of selective attention to taste and odor. *Chem. Senses* 36, 747–760. doi: 10.1093/chemse/bjr043
- Wilson, D. A., Xu, W., Sadrian, B., Courtiol, E., Cohen, Y., and Barnes, D. C. (2014). Cortical odor processing in health and disease. *Prog. Brain Res.* 208, 275–305. doi: 10.1016/b978-0-444-63350-7.00011-5
- Yarita, H., Iino, M., Tanabe, T., Kogure, S., and Takagi, S. F. (1980). A transthalamic olfactory pathway to orbitofrontal cortex in the monkey. *J. Neurophysiol.* 43, 69–85.
- Yu, C., Fan, D., Lopez, A., and Yin, H. H. (2012). Dynamic changes in single unit activity and gamma oscillations in a thalamocortical circuit during rapid instrumental learning. *PLoS One* 7:e50578. doi: 10.1371/journal.pone.0050578
- Zald, D. H., and Pardo, J. V. (1997). Emotion, olfaction and the human amygdala: amygdala activation during aversive olfactory stimulation. *Proc. Natl. Acad. Sci. U S A* 94, 4119–4124. doi: 10.1073/pnas.94.8.4119
- Zelano, C., Mohanty, A., and Gottfried, J. A. (2011). Olfactory predictive codes and stimulus templates in piriform cortex. *Neuron* 72, 178–187. doi: 10.1016/j.neuron.2011.08.010
- Zhang, Y., Burk, J. A., Glode, B. M., and Mair, R. G. (1998). Effects of thalamic and olfactory cortical lesions on continuous olfactory delayed nonmatching-to-sample and olfactory discrimination in rats (*Rattus norvegicus*). *Behav. Neurosci.* 112, 39–53. doi: 10.1037/0735-7044.112.1.39

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Efficient population coding of naturalistic whisker motion in the ventro-posterior medial thalamus based on precise spike timing

Michael R. Bale^{1,2,3*†}, Robin A. A. Ince^{2,4†}, Greta Santagata² and Rasmus S. Petersen^{2*}

¹ School of Life Sciences, University of Sussex, Brighton, UK, ² Faculty of Life Sciences, University of Manchester, Manchester, UK, ³ Instituto de Neurociencias Alicante UMH-CSIC, Sant Joan d'Alacant, Spain, ⁴ Institute of Neuroscience and Psychology, University of Glasgow, Glasgow, UK

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Ileana Hanganu-Opatz,
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Paloma Tamara Gonzalez-Bellido,
University of Cambridge, UK

*Correspondence:

Michael R. Bale,
School of Life Sciences, University
of Sussex, CRPC Building,
Brighton BN1 9QG, UK
m.bale@sussex.ac.uk;
Rasmus S. Petersen,
Faculty of Life Sciences,
University of Manchester,
Stopford Building,
Manchester M13 9PT, UK
r.petersen@manchester.ac.uk

[†] Joint first authors.

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The rodent whisker-associated thalamic nucleus (VPM) contains a somatotopic map where whisker representation is divided into distinct neuronal sub-populations, called “barreloids”. Each barreloid projects to its associated cortical barrel column and so forms a gateway for incoming sensory stimuli to the barrel cortex. We aimed to determine how the population of neurons within one barreloid encodes naturalistic whisker motion. In rats, we recorded the extracellular activity of up to nine single neurons within a single barreloid, by implanting silicon probes parallel to the longitudinal axis of the barreloids. We found that play-back of texture-induced whisker motion evoked sparse responses, timed with millisecond precision. At the population level, there was synchronous activity: however, different subsets of neurons were synchronously active at different times. Mutual information between population responses and whisker motion increased near linearly with population size. When normalized to factor out firing rate differences, we found that texture was encoded with greater informational-efficiency than white noise. These results indicate that, within each VPM barreloid, there is a rich and efficient population code for naturalistic whisker motion based on precisely timed, population spike patterns.

Keywords: vibrissa, VPM, neural coding, information theory, synchrony, somatosensory

Introduction

Thalamus is the gateway to the cerebral cortex and the manner in which sensory signals are encoded by thalamic populations fundamentally constrains cortical computation (Sherman and Guillery, 2009). Although it has long been suspected that neuronal activity within modular, neuronal populations is crucial for thalamo-cortical processing, few studies have simultaneously recorded the activity of multiple neurons within such modules in the thalamus (Desbordes et al., 2008; Temereanca et al., 2008; Wang et al., 2010b). The whisker system is ideal for investigating thalamic population coding (Petersen et al., 2009), since there is a well-defined, modular population of neurons devoted to each whisker at each of the major stations of the sensory pathway (Woolsey and Van der Loos, 1970). In the ventro-posterior medial nucleus (VPM) of the thalamus, each whisker is primarily represented by a cluster of ~250 neurons (in rat) known as a “barreloid” (van der Loos, 1976).

In the whisker system, mechanoreceptors embedded in the whisker follicle-sinus complex project to the cerebral cortex via parallel, topographic pathways through brainstem and thalamus (reviewed by Diamond and Arabzadeh, 2013). The major (lemniscal) pathway, primarily responsible for encoding whisker-object touch, involves VPM (Deschênes et al., 2005). The core of VPM receives ascending input from the principal nucleus of the brainstem and projects primarily (although not exclusively) to layer IV of primary somatosensory cortex (S1) with collaterals to the thalamic reticular nucleus (NRT). Analogously to other sensory systems, VPM conveys ascending sensory signals to cortex and modulates those signals depending on cortical and behavioral state (Temereanca et al., 2008; Sherman and Guillery, 2009). VPM activity is modulated by input from S1 layer 6, NRT and brainstem neuromodulatory centers. VPM is neurochemically more homogeneous than other thalamic nuclei, such as LGN, in being devoid of GABAergic interneurons and consisting of glutamatergic, S1-projecting relay neurons.

By recording the activity of individual neurons, previous studies have determined that VPM neurons can respond to whisker deflection with high (sub-millisecond) spike timing precision and have identified sensory features to which VPM neurons respond (Waite, 1973; Simons and Carvell, 1989; Pinto et al., 2000; Brecht and Sakmann, 2002; Castro-Alamancos, 2002; Yu et al., 2006; Montemurro et al., 2007a; Petersen et al., 2008; Ego-Stengel et al., 2012). At the population level, it is known that sudden whisker contact (e.g., a ramp-and-hold stimulus) evokes a response where different neurons fire within a few milliseconds of each other—commonly termed “synchrony”—and that the degree of synchrony varies with stimulus features (Pinto et al., 2000; Temereanca et al., 2008; Wang et al., 2010b). However, it is not known how VPM neurons, at either single neuron or population level, respond to naturalistic whisker motion. Coding principles for naturalistic stimuli can differ substantially from those for artificial stimuli (Mainen and Sejnowski, 1995; de Ruyter van Steveninck et al., 1997). Our aim here was to investigate how the population of neurons in an individual barreloid of the VPM nucleus encodes texture-induced motion of the corresponding whisker and to test whether its population response is synchronous.

To address these issues, we used multi-microelectrode arrays to record simultaneously the activity of multiple single units from within a single VPM barreloid, in response to play-back of texture-induced whisker motion (Arabzadeh et al., 2005; Wolfe et al., 2008; Lottem and Azouz, 2011; Bale et al., 2013). We found that naturalistic whisker motion evoked precisely timed patterns of population spiking, where different groups of neurons fired synchronously at different times (“dynamic synchrony, DS”). Furthermore, mutual information scaled linearly with population size, suggesting a marked lack of redundancy, and responses exhibited a greater informational efficiency for naturalistic stimuli.

Materials and Methods

Electrophysiology

All experimental procedures were approved both by the UK Home Office and by The University of Manchester Ethical Review Committee. Adult male Wistar rats ($n = 13$, 250–350 g) were anesthetized with urethane (1.5 g/kg body weight) and placed into a stereotaxic apparatus. A craniotomy was made over the hemisphere ipsilateral to whisker stimulation 2.0–4.5 mm posterior and 1.5–4.0 mm lateral to bregma. A single-shank, 32-site silicon probe (single row of recording sites, each area $177 \mu\text{m}^2$, $50 \mu\text{m}$ spacing; recording site impedance lowered by Iridium oxide activation, top and bottom sites used to pass current for marking recording site; Neuronexus, Ann Arbor, MI) was inserted into the brain at 45° to the midline in the coronal plane (dorso-medial to ventro-lateral), to target the contralateral VPM nucleus. During the experiment, position of the probe within VPM was verified by short-latency responses evoked by manual stimulation of the whiskers. The probe was lowered until whisker-evoked activity ceased, and receptive fields characteristic of the ventroposterior lateral nucleus (VPL) emerged on the deepest recording sites (mean 8.3, SD 0.3 mm from pial surface). Extracellular signals were preamplified, digitized at 24.4 kHz, band pass filtered (300–3000 Hz) and continuously stored to hard disk for offline analysis.

Whisker Stimulation

All whiskers were trimmed to 5 mm and receptive fields were mapped for each recording site by manually deflecting single whiskers. The whisker that evoked responses at the greatest number of recording sites was selected for stimulation. This whisker was inserted into a pulled pipette tube attached to a piezoelectric actuator (P/N PL127.10; Physik Instrumente) positioned 1 mm from the face. The actuator was shortened to raise resonant frequency and fixed to an aluminium tube via small plastic screws. The dynamic range of the actuator was 0.8 mm (whisker deflection range $\sim 40^\circ$).

We employed two types of dynamic whisker stimulation: low-pass filtered (300 Hz) white noise (Petersen et al., 2008) and a naturalistic texture stimulus (Bale et al., 2013). The texture stimulus was constructed from optical measurements of whisker motion made from awake rats by Wolfe et al. (2008). Wolfe et al. (2008) trained rats to whisk a textured surface (sandpaper: P150, P400, P800 or P1200) and used a CCD array to measure traces of texture-induced whisker motion in the rostro-caudal plane. In order to construct the stimulus corresponding to a given grade of sandpaper, we considered only periods of the CCD traces during which whiskers were in contact with that sandpaper. Such traces were stitched together so that the final position of one trace equalled the first position of the subsequent one until a sequence of 10 s duration was obtained. To minimise potential contribution of head movement, the resulting sequence was high-pass filtered (FIR at 1 Hz). Finally, to avoid mechanical resonance from the actuator, the sequence was low-pass filtered (FIR 600 Hz). This procedure was repeated for each of the four grades of sandpaper.

The stimulus protocol consisted of 50–100 trials. Each trial consisted of a fixed 10 s sequence of white noise and 1–4 sequences of 5–10 s repeated texture motion. Stimulus sequences were presented in a randomised order and were separated by 1 s intervals without whisker motion. A schematic for an example session (in which a single texture sequence was presented) is shown in **Figure 2A**.

For consistency with the conditions under which the whisker motion data were registered, all whisker motion stimuli were delivered in the rostral-caudal direction. Accurate reproduction of stimulus position, velocity and acceleration was confirmed by optical testing with a phototransistor circuit (Storchi et al., 2012).

Histology

At the end of each experiment, two lesions were made by passing 20 μ A for 6 s through both the deepest and most superficial sites on the silicon probe. As detailed in Montemurro et al. (2007a), animals were perfused transcardially with saline and formalin. Brains were removed, immersed in fixative for at least 1 day and then transferred to a phosphate-buffered 30% (w/v) sucrose solution for a further 48 h. Coronal sections of 50 μ m were stained with cresyl violet. Recovery of the lesion sites confirmed that all single units reported in this study were located in VPM.

Spike Sorting

The first step of our data analysis was to isolate single unit activity from the extracellular recordings. We found that spikes emitted by a given single unit were typically recorded from 1–3 recording sites (three recording sites span 100 μ m). To exploit this information for better single unit isolation, and also to prevent potential double counting of spikes on adjacent channels, we spike sorted 1–3 channels simultaneously (**Figures 1D–F**). We extracted 1 ms segments around voltage-threshold-crossing times, concatenating segments from 1–3 adjacent recording sites and clustered these data in the space of their principal components using a *t*-distribution mixture model (**Figure 1E**, Shoham et al., 2003; Bale and Petersen, 2009). Only clusters exhibiting a clear refractory period were accepted. To test for potential double counting of spikes isolated from adjacent blocks of recording sites, we checked for suspicious peaks (width 0.08 ms, corresponding to two sampling intervals) in the cross-correlation function (bin size = 0.04 ms). Any units contributing to such peaks were excluded. An example recording of four simultaneously recorded adjacent channels is shown in **Figure 1D**, of which two sites were sorted together (channels 5 and 6). The spikes extracted during the sorting routine (from **Figure 1F**) are overlaid.

Localization of Receptive Fields

During each recording, we identified the whisker that evoked the greatest multi-unit activity (MUA) on each recording site (its principal whisker, PW) to manual deflection of individual whiskers. To assess the multi-unit PW quantitatively, we then recorded responses to piezoelectric deflection of the PW in both caudal and rostral directions with a ramp-and-hold protocol. This was repeated for each of the surrounding whiskers. In off-line analysis, we computed the MUA spike count evoked by

caudal and rostral deflection at each recording site (as detailed above, time window 20 ms), for each whisker. For each site, we determined the whisker that evoked the greatest MUA response. Provided that this was significantly greater than the spontaneous spike count (time window 20 ms; Wilcoxon signed ranks), this was deemed the PW. By repeating this analysis for each recording site, the PW at each site with significant whisker response was obtained (**Figure 1C**).

The majority of single units (91%) had a receptive field that matched the multi-unit receptive field of the channel to which it was most closely located. The remaining 9% of single units had a receptive field that corresponded to the MU activity of an adjacent whisker.

Spike Train Sparseness

Sparse codes are metabolically efficient, in that much information is conveyed with few spikes, and are prominent in the brain (Simoncelli and Olshausen, 2001). We measured temporal sparseness (“lifetime sparseness”), using a slight variant of a standard measure (Rolls and Tovee, 1995):

$$S = 1 - \frac{\langle r_t \rangle^2}{\langle r_t^2 \rangle} \quad (1)$$

Here r_t is the value of the peri-stimulus time histogram (PSTH) in time bin t and the angled brackets denote the mean over time bins (bin size 10 ms). If r_t is constant (non-sparse response), $S = 0$. In contrast, if r_t is zero except for a few bins (highly sparse response), S tends to 1 in the limit of many bins. The original Rolls-Tovee measure, a , is related to the one used here through the relation $S = 1 - a$.

Stimulus Sparseness

An important characteristic of sensory stimuli is whether or not they are Gaussian-distributed. This is usefully assessed by measuring “sparseness” through the following, standard index (Hyvärinen et al., 2009). Given samples x of a sensory signal:

$$S_{stim} = -\sqrt{\frac{\pi}{2}} \frac{\langle |x - \mu| \rangle}{\sigma_x} \quad (2)$$

Where μ is the mean, σ_x is the standard deviation of x . $S_{stim} = -1$ for Gaussian distributions; $S > -1$ for distributions with a thinner peak around the mean and fatter tails (“sparse”).

Unit Responsiveness

To determine whether a given unit was responsive to the stimulus (white noise or texture), the unit’s spontaneous firing rate was measured as the spike count in the 0.5 s interval prior to stimulus onset and its evoked firing rate was measured in each successive 0.5 s interval throughout the course of the stimulus. A unit was classified as “responsive” if its firing rate in any of the post-stimulus windows was significantly higher than that in the pre-stimulus window (Wilcoxon signed ranks, $p < 0.001$; Bonferroni corrected for multiple comparisons). Non-responsive units were not considered in later analysis.

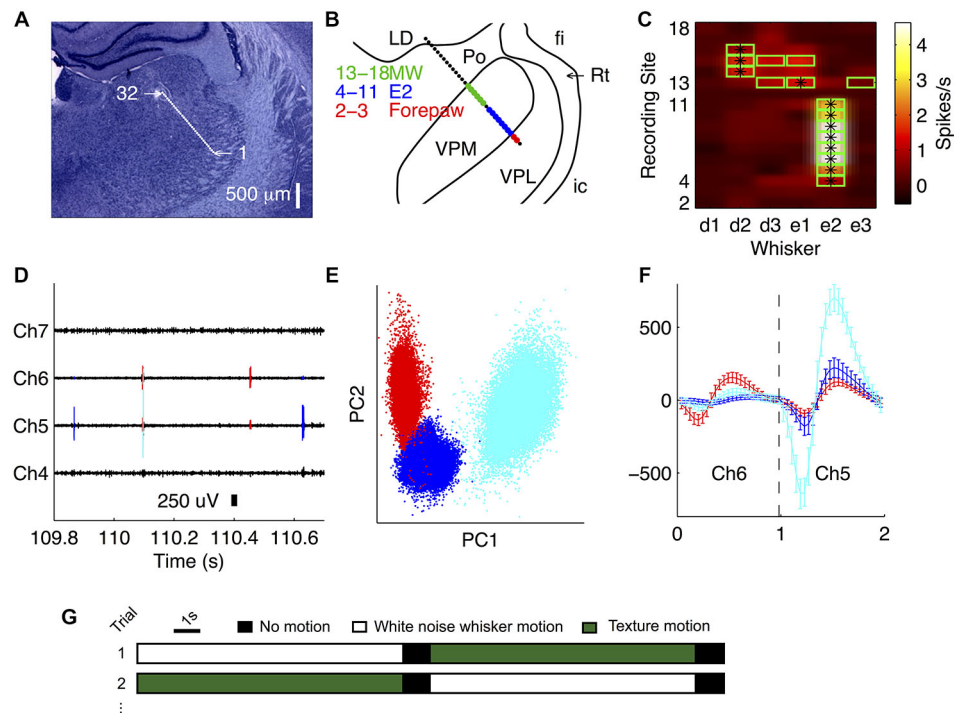


FIGURE 1 | Recording from VPM using multi-site silicon probes. (A) Location of recording sites (32-channel silicon probe, sites spaced by 50 μm) in an example recording. Nissl stained coronal section (3.4 mm posterior to bregma). **(B)** Receptive field map obtained during experiment, superimposed on thalamic nuclei reconstruction. LD, lateral nucleus; fi, fimbria; Rt, thalamic reticular nucleus; ic, internal capsule; VPL, ventroposterior lateral nucleus. MW, multi-whisker. **(C)** Multi-unit activity (MUA) evoked by stimulation of each of six whiskers at each recording site. Green outline signifies units with evoked firing rate significantly greater than spontaneous, consistent with online receptive field mapping multi-whisker (MW) observations in **(B)**. *Denotes the principal whisker (PW) at each recording site. **(D)** Unit identities overlaid on an example trace of the raw recording from the experiment shown in **(B)**. **(E)** Waveforms for all identified spikes projected onto the first two principal components of their multichannel waveforms; each color represents a different unit (matches **D**). **(F)** Average single unit waveforms for three extracted clusters on two different channels from the recording shown in **(B)**. **(G)** Schematic illustrating the experimental protocol.

Jitter Analysis

Since spike timing precision is a limit on a neuron's capacity to transmit information, it is necessary to quantify it (Petersen et al., 2009). To quantify spiking precision, we measured the trial-to-trial variability in spike timing ("jitter") for each unit, as previously described (Montemurro et al., 2007a; Bale et al., 2015). First, we divided the evoked response into 0.8 ms time bins and averaged over trials to form a PSTH, which was then smoothed with a Gaussian filter (SD 1.6 ms). Firing episodes corresponded to local peaks in the PSTH. To focus on reliable episodes we selected peaks which satisfied the following two conditions for the jitter calculation: (1) firing rate was at least half of the maximum for that unit and (2) firing rate exceeded a threshold set according to the null hypothesis that the unit fired randomly at the same average rate ($p = 0.001$). To establish the second condition, we repeatedly simulated random spike trains from a homogeneous Poisson process with the same rate as the time-averaged firing rate of the considered unit. The threshold was set as the 99.9th percentile of these peak heights. For each peak meeting both criteria, we extracted all spikes fired within ± 10 ms of the time of the peak and computed spike times relative to the peak time. These time differences were pooled across both trials and peaks and the resulting distribution fitted to a Gaussian.

Jitter was defined as the SD of this Gaussian. A minority of units were only weakly modulated by the whisker stimulus and did not exhibit well-defined firing episodes, resulting in high and unreliable values of calculated jitter. In the jitter plot (**Figure 2E**), we do not plot the points which lie outside the whiskers (defined as median ± 2.7 SD). There were seven such points for white noise and 10 for texture out of a total of $N = 65$ single units.

Synchrony Analysis

Synchrony is an interesting property of neuronal population activity, since it can enable reliable transmission of information even if individual synapses are weak (Bruno and Sakmann, 2006). As a first test of synchrony, for each pair of simultaneously recorded units, we computed the cross-correlation function for the stimulus-evoked activity on each trial and then averaged the data across trials (binsize 1 ms). This was done separately for the white noise and texture stimuli. For each pair of units, we calculated the peak value of the trial-mean cross-correlation function (the "CCG peak") as well as its standard error. A unit pair was defined as having a significant cross-correlation peak if the CCG peak exceeded at least three standard errors.

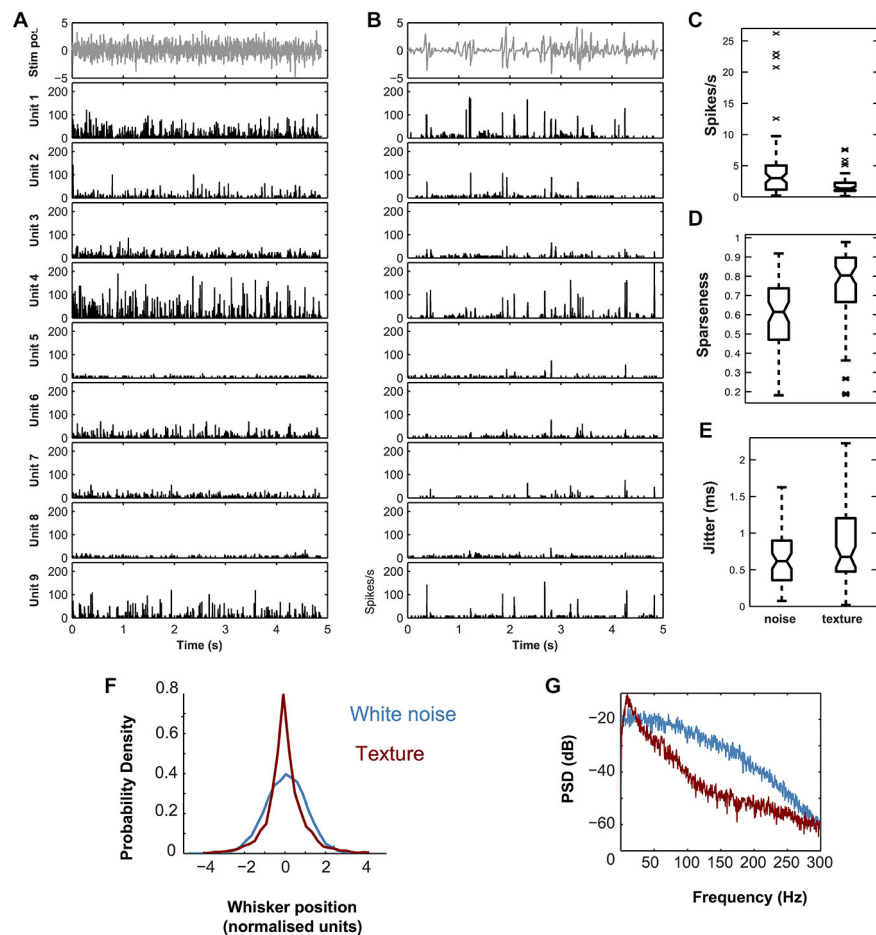


FIGURE 2 | Response properties of simultaneously recorded single VPM units located within a single barreloid to dynamic whisker stimuli. (A) White noise stimulus (top panel) and corresponding evoked PSTHs for nine simultaneously recorded units from the same barreloid (lower panels). **(B)** P150 texture stimulus (top panel) and corresponding evoked PSTHs for the same nine units as in A. PSTHs computed with 0.08 ms bins, and smoothed by convolution with a Gaussian (SD 1 ms). Units ordered by recording location from dorso-medial (top) to ventro-lateral (bottom). **(C)** Distribution of single cell firing rates evoked by white noise compared to that evoked by the sandpaper texture stimulus. **(D)** Corresponding plot for sparseness. **(E)** Corresponding plot for spike timing precision (jitter). In **(C–E)** central mark shows median; box edges show 25th, 75th percentiles; whiskers show range of points not considered outliers (approx. ± 2.7 SD); crosses show outliers. In panel **(C)**, outliers are not shown. **(F)** Probability Density Function (PDF) of piezo position (normalized units) for the white noise (blue) and texture (red) stimuli. **(G)** Power Spectral Density (PSD) of the same.

To test for synchrony in our simultaneously recorded units, we registered the response of each unit in 10 ms time bins, and computed the number of bins in which $n = 2, 3, 4 \dots$ units all fired. As a control, we compared this to the number of bins in which such events would be expected by chance from a statistically independent population of units, with the same single unit spike count distributions. To do this, we shuffled the response of each unit independently across time bins. This preserved the overall firing rate and response diversity, while removing the temporal structure. We then computed the same synchrony measure (number of bins in which different numbers of multiple units fired) for this surrogate data set.

With the simplest (static) type of synchrony, each evoked synchronous population response has the same distribution of population response words (that is, the same set of units fires together). Alternatively, different evoked response events

might have different distributions of response words. We term this dynamic synchrony (DS), since events at different times evoke different patterns of synchrony. To quantify the extent to which synchrony is dynamic we used an information theoretic approach. We first isolated synchronous population firing events by identifying time bins (20 ms) where two or more units fired on at least 5% of trials. We quantified the degree of DS as the mutual information between the population response words and the peri-stimulus time of the response event [Ish information estimator (Montemurro et al., 2007b), Panzeri-Treves bias correction (Panzeri and Treves, 1996)]. To normalize for the potential effect of events of different strengths eliciting the same patterns of synchronous activity but with different probabilities, we consider in the information calculation only trials where at least one unit fired. This is equivalent to removing the response word $[0, \dots, 0]$ from the probability

distribution and renormalizing appropriately. If all synchronous population events elicit the same pattern of synchronous activity the information computed in this way should be zero. If it is not, this shows that different synchronous population events evoke different distributions of response patterns—hence the synchrony is dynamic. This can be interpreted as a model comparison between a model where all events elicit population response words from the same distribution, and one in which each event elicits responses from a different distribution—a different pattern of synchrony. To determine the significance of the observed information values we use a permutation testing approach. The null hypothesis for this test is that the observed synchrony is static; the same distribution of response words is evoked by each event (albeit with possibly different pooled firing rate). We therefore performed 1000 permutation calculations in which the identity of the events that elicited each response were randomly shuffled. Mutual information values are affected by the number of trials as well as the size of the spaces considered. Since different recordings had different numbers of trials, cells and detected events it is therefore difficult to compare information values directly. To address this we standardized (z-scored) the observed information for each recording with respect to the corresponding set of permutation values. This gives a measure of the statistical strength of the result in favor of DS, and provides a measure in standardised (z-score) units that can be compared across different experiments.

Mutual Information Analysis

Mutual information is a powerful, non-linear measure of the correlation between two variables. Applied to neural coding, it quantifies the intuitive notion of the “information” that a neuron (or neurons) conveys about a stimulus. A key advantage is that it makes minimal assumptions on the functional relationship between the encoded parameter (e.g., whisker position) and the neuronal response; a second advantage is that it provides a rigorous yardstick for comparing alternative, candidate neural codes. Mutual information can give useful physiological insight concerning whether, for example, precise spike timing increases the capacity of a neuron to transmit sensory messages or whether different neurons in a population convey the same messages (redundancy) or complementary ones. Application of information theoretic methods to the whisker system has been reviewed elsewhere (Petersen et al., 2009; Ince et al., 2010).

The aim of this analysis was to quantify how much total information a given population response code conveys about the dynamic whisker stimulus. To this end, we used the spike trains evoked by the repeated whisker motion sequences. We employed the well-established method of Strong et al. (1998) and conducted the analysis for simultaneously recorded populations of single units. For a population of units $i = 1, \dots, N$, we computed the response r as the N -element binary word $(r_1, r_2, \dots, r_N)$ within the time bin $(t, t + 10 \text{ ms})$. Here, r_i denotes the (binary) response of unit i where 0 indicates that the unit was inactive (no spikes emitted) in that response bin, and 1 indicates that the unit was active (at least 1 spike emitted); t denotes time with respect to stimulus onset ($t = 0$). In this approach, these time bins are each considered as the response to a particular stimulus; the

stimulus set S is then the set of response bins (implicitly, the small segments of dynamic stimulus preceding each response bin). In this way the explicit consideration of particular stimulus features is replaced by the temporal exploration of a range of inputs from an approximately ergodic process. The method therefore takes into account, without any prior assumptions, all possible stimulus features that might be driving the neural response. The particular number of stimuli available depends on the length of the repeated stimulus segment considered as well as the bin size used. The resulting information value quantifies how discriminable, on average, different response bins (and hence the different preceding stimulus segments) are from each other.

The measurements of r were used to estimate the probability $P(r|t)$ of the neural response r in time bin, t , and the probability $P(r)$, the mean of $P(r|t)$ over all time bins. When a neural response is reliably modulated by a stimulus, it evokes similar responses across repeated trials at a given time, and different responses across different time bins: thus, $P(r|t)$ varies systematically with t . In contrast, when a neuron is insensitive to a stimulus, similar responses are produced for all time bins and $P(r|t)$ is similar to $P(r)$. $I(R; S)$ quantifies how much, on average, $P(r|t)$ differs from $P(r)$:

$$I(R; S) = \left\langle \sum_{r \in R} P(r|t) \log_2 \frac{P(r|t)}{P(r)} \right\rangle_t \quad (3)$$

Here the angled brackets denote an average over all stimulus time bins. $I(R; S)$ quantifies how well, on average, an ideal observer could decide which stimulus time bin was presented from observation of the neural response r on a single trial. $I(R; S)$ has units of bits; one bit of information indicates that, on average, the uncertainty about which stimulus bin was presented is reduced by a factor of two after observation of a single response.

For each experimental session consisting of N units, we computed information values for all possible subpopulations of every size, including single unit information values. For each (sub)-population, the sum of these single unit information values for the population members gives the amount of information that would be conveyed by if the units were transmitting information independently (Averbeck et al., 2006), denoted *independent*. We measured the synergy/redundancy as the difference between the information conveyed by the population and the sum of the single unit information values (Schneidman et al., 2003; Latham and Nirenberg, 2005):

$$\text{Syn}(R_1, R_2; S) = I(R_1, R_2; S) - I(R_1; S) - I(R_2; S) \quad (4)$$

Positive values of $\text{Syn}(R_1, R_2; S)$ indicate synergy; the two response variables carry more information about the stimulus together than they do alone. Negative values of $\text{Syn}(R_1, R_2; S)$ indicate redundancy; the two variables together carry less information than the sum of their individual contributions. To test whether a low synergy/redundancy value for a given population might reflect cancelation between strongly redundant and strongly synergistic unit pairs, we conducted the following analysis. For a given population, we computed the synergy/redundancy of all of its constituent unit pairs.

Positive (synergistic) and negative (redundant) values were summed separately, resulting in a net pairwise synergy value and a net pairwise redundancy value. The maximum absolute value of these two was taken as an indicator of the maximum possible pairwise interaction effect for the population. We then normalized this value with respect to the mutual information conveyed by the population.

To give a more intuitive measure for the mutual information conveyed by a population, we converted bits to bits/s by dividing by the bin width. We also separately normalized for differences in firing rate, by calculating the mutual information per spike (bits/spike), both for single cells and populations.

The probability distributions that appear in equation (3) were computed from a finite number of trials and thus subject to sampling error, which typically inflates estimates of mutual information (Panzeri et al., 2007). Bias correction was performed using the shuffled information estimator, $I_{sh}(R; S)$ together with quadratic extrapolation (Montemurro et al., 2007b; Panzeri et al., 2007). Mutual information values were computed using the PyEntropy package (Ince et al., 2009). The length of the repeated stimulus blocks was either 5 s or 10 s, resulting in 500 or 1000 response bins for each information calculation. The crucial parameter for determining the effectiveness of bias correction is the ratio of the number of trials per stimulus to the number of different response symbols (Ince et al., 2010). This number of trials per stimulus is given by the number of repeated presentations of the continuous stimulus and does not change across different stimulus block lengths or response bin sizes. The number of response symbols (2 to the power of the population size) is also fixed, so the difference in stimulus block lengths does not affect the bias of the information measure. With the available data, the number of trials per stimulus was 50–200 (median 200); the ratio of trials/stimulus to number of response symbols (for the largest population size recorded in each experiment) was 0.2–100 (median 18.75). Simulations have shown (Ince et al., 2009) that with the bias correction employed here, a ratio of ~ 0.25 can be adequately bias corrected: the data here fall within that range. To further test the accuracy of our bias correction, we repeated the analysis with a surrogate data set in which, for each trial, the population responses were shuffled independently across time bins (so that the true information is zero). The resulting mean information values for each population size were expressed as a percentage of the corresponding unshuffled value. In the most demanding case—population size 9—for white noise stimulation, the median was 0.79%; for texture stimulation, the median was 0.85%. This indicates that bias was effectively corrected.

Results

Recording the Population Response of a VPM Barreloid to Whisker Motion

Our primary aim was to investigate how the population of neurons within a single VPM barreloid collectively encodes naturalistic whisker motion. To address this, it was essential to

deliver multiple, identical repeats of controlled, whisker motion sequences. To this end, we implanted 32-channel silicon probes into the VPM of urethanized rats, and recorded simultaneously the responses of multiple single units to deflection of the whiskers. To concentrate recording sites as far as possible within the same barreloid, we implanted probes at a 45° angle (dorso-medial to ventro-lateral), approximately parallel to the long axis of the barreloids (**Figures 1A,B**). To assess microelectrode placement, we identified, for each recording site, the whisker that evoked the greatest multi-unit response to ramp-and-hold deflection (**Figure 1C**; see “Materials and Methods” Section)—the “PW”. In the example of **Figure 1**, the PW was E2 for sites 4–11, E1 for site 13 and D2 for sites 14–16. Overall ($N = 16$ recordings), up to 11 recording sites shared the same principal whisker (mean 7.4, SD 2.4 range 4–11), indicating that they recorded neural activity from the same barreloid. Since the recording sites were spaced at 50 μm intervals along the probe shank, 11 sites spanned 500 μm . This length is consistent with anatomical estimates of barreloid length in adult rats (Haidarliu and Ahissar, 2001).

A piezoelectric actuator was used to “play back” sequences of dynamic whisker motion (Bale et al., 2013; see “Materials and Methods” Section). A naturalistic stimulus was generated from optically registered whisker sweeps of rats actively whisking sandpaper (Wolfe et al., 2008). To benchmark the coding efficiency of this stimulus, we also used low-pass filtered white noise (hereafter abbreviated to “white noise”; **Figure 2A**), which was normalized to have the same standard deviation. Both stimuli were applied to individual whiskers ($N = 16$ recordings), and we recorded neuronal responses from all probe sites simultaneously.

Response of Single VPM Units to Naturalistic Whisker Motion are Temporally Precise and Sparse

Figure 2 shows PSTHs, evoked by white noise (**Figure 2A**) and texture (**Figure 2B**) applied to whisker E2, for a set of 9 responsive single units (see “Materials and Methods” Section) recorded simultaneously from barreloid E2. The single unit responses to the two stimuli were qualitatively similar. Both stimuli evoked responses that consisted of temporally isolated firing episodes. We quantified the spike timing precision of these firing episodes by estimating the trial to trial “jitter” in spike time (**Figure 2E**; see “Materials and Methods” Section). Jitter for both texture and white noise was sub-millisecond (median 0.48 ms, IQR 0.48–1.2 ms and median 0.36 ms, IQR 0.36–0.88 ms respectively). The main differences between the stimuli were that white noise evoked a higher rate of firing events (**Figure 2C**; median 3.0 spikes/s compared to 1.4 spikes/s; signed rank test, $p < 10^{-7}$), while responses to texture exhibited higher temporal sparseness (**Figure 2D**; median 0.80 compared to 0.61; signed rank test, $p < 10^{-9}$). To investigate the source of these differences we compared the stimuli. Compared to white noise, the texture stimulus both had a markedly non-Gaussian “sparse” distribution (**Figure 2F**) and contained less relative power at high frequencies (**Figure 2G**). Using a standard index which

is -1 for a Gaussian and >-1 for sparse distributions (Material and Methods, Equation 2), the white noise had an index value of -1 and the texture -0.85 .

VPM Population Responses Exhibit Dynamic Synchrony

Our finding that texture evokes precisely timed spikes raises the question of the nature of the response at the population level. Motivated by previous studies of the VPM response to periodic whisker stimulation (Bruno and Simons, 2002; Temereanca et al., 2008; Wang et al., 2010b), we investigated whether the population response manifested synchrony, conventionally defined as coincident firing on a time-scale of ~ 10 ms (Wang et al., 2010a). Inspection of the recordings revealed qualitative evidence for synchrony. **Figure 3A** shows a detailed view of the responses of 3 simultaneously recorded single units (a subset of those shown in **Figure 2**) evoked by the texture stimulus. Within this 2.5 s excerpt, there were several times at which the stimulus evoked a marked increase in firing rate in 2 or more units (shaded regions).

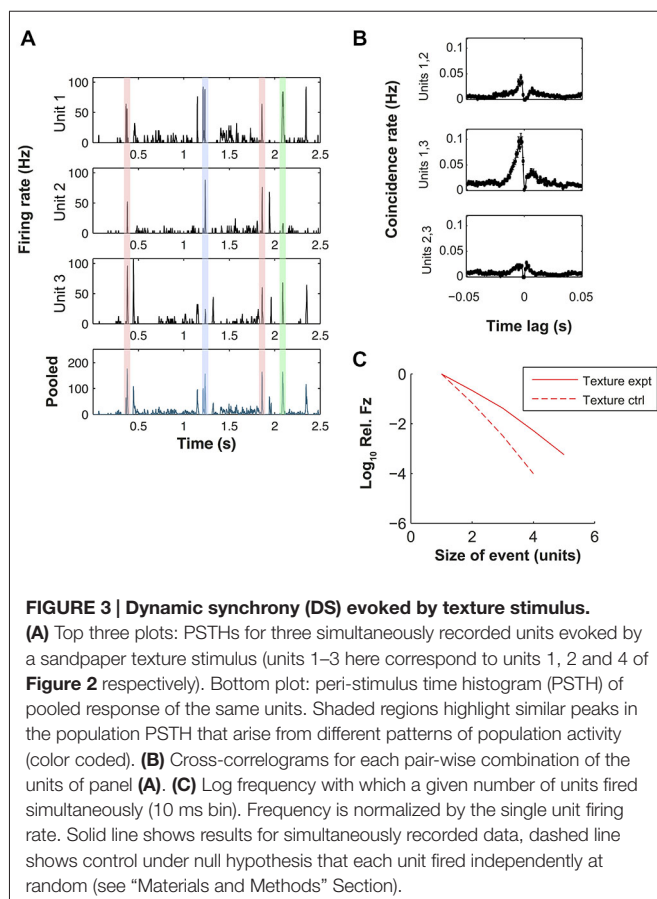
To quantify the synchrony, we first computed cross-correlograms (CCGs) for the texture-evoked activity (**Figure 3B**, for units with PSTHs shown in **Figure 3A**). Eighty four neuron pairs (out of a total of 145) exhibited statistically significant CCG peaks (see “Materials and Methods” Section). For the significant peaks, the CCG peak amplitude was 0.03 ± 0.11

coincidences/spike (median $\pm$ SD), the CCG (absolute) peak lag was 2.5 ± 5.8 ms and the CCG peak width (full width at half maximum, FWHM) was 4.6 ± 3.6 ms. Results for white noise were similar: 71 pairs exhibited significant CCG peaks; for these peaks, CCG peak amplitude was 0.08 ± 0.27 ; lag was 2.0 ± 5.6 ms and CCG peak FWHM was 3.3 ± 5.1 ms.

To extend the analysis of synchrony beyond the pairwise CCGs to neuronal sets of arbitrary size, we used the following procedure. We registered the response of each unit in 10 ms time bins, and computed the number of bins in which $n = 2, 3, 4 \dots$ units all fired on the same trial. As a control, we compared this to the number of such events expected from random firing (see “Materials and Methods” Section). We found, for the texture-evoked response of the nine simultaneously recorded units of **Figure 2A**, that synchronous firing of three and four units occurred more often (14.6 and 66.8 times respectively) than expected from random firing (**Figure 3C**). Synchronous firing of 5 units occurred in the experimental data but never in the control. On average, across all sets of simultaneous recordings, synchrony occurred more frequently than expected by random firing.

These results indicate that both white noise and texture whisker motion induced synchronous firing within the associated VPM barreloid. However, closer inspection of the data revealed that the precise pattern of synchrony changed over time: different constellations of neurons fired at different times during the stimulus. The shaded regions in **Figure 3A** illustrate four response episodes where the pooled firing rate of the population was similar but the mean population spike patterns were, in 3 cases, distinct. For two of the peaks all three units exhibited high firing rate at almost the same time (red shading), but at other times different combinations of units responded: units 1 and 2 but not 3 (blue shading) or units 1 and 3 but not 2 (green shading).

With the simplest (static) type of synchrony, each evoked synchronous population response has the same distribution of population response words (that is, the same set of units fires together). Alternatively, different evoked response events might have different distributions of response words. Since events at different times evoke different patterns of synchrony, we refer to this type of synchrony as dynamic (dynamic synchrony, DS). DS implies that there is a repertoire of different synchronous response patterns and that there is a systematic relationship between a particular stimulus event and the evoked response pattern. DS can be quantified by measuring the strength of this association. Specifically, we quantified DS with a normalized mutual information value (see “Materials and Methods” Section). This value can be interpreted as a z -score: under the null hypothesis of static synchrony, it should take values drawn from a standard normal distribution. For all seven recordings in which synchronous events were reliably identified (3–9 simultaneously recorded units; 3–121 synchronous events) the DS measure was highly significant ($p < 0.001$; permutation test, $N = 1000$). We found that responses to texture stimuli resulted in a DS measure of 35.8 ± 21.1 (standardised units) while for those to white noise, the values were 26.8 ± 23.1 ; but that this difference was not significant (Wilcoxon signed rank test, $p > 0.3$).

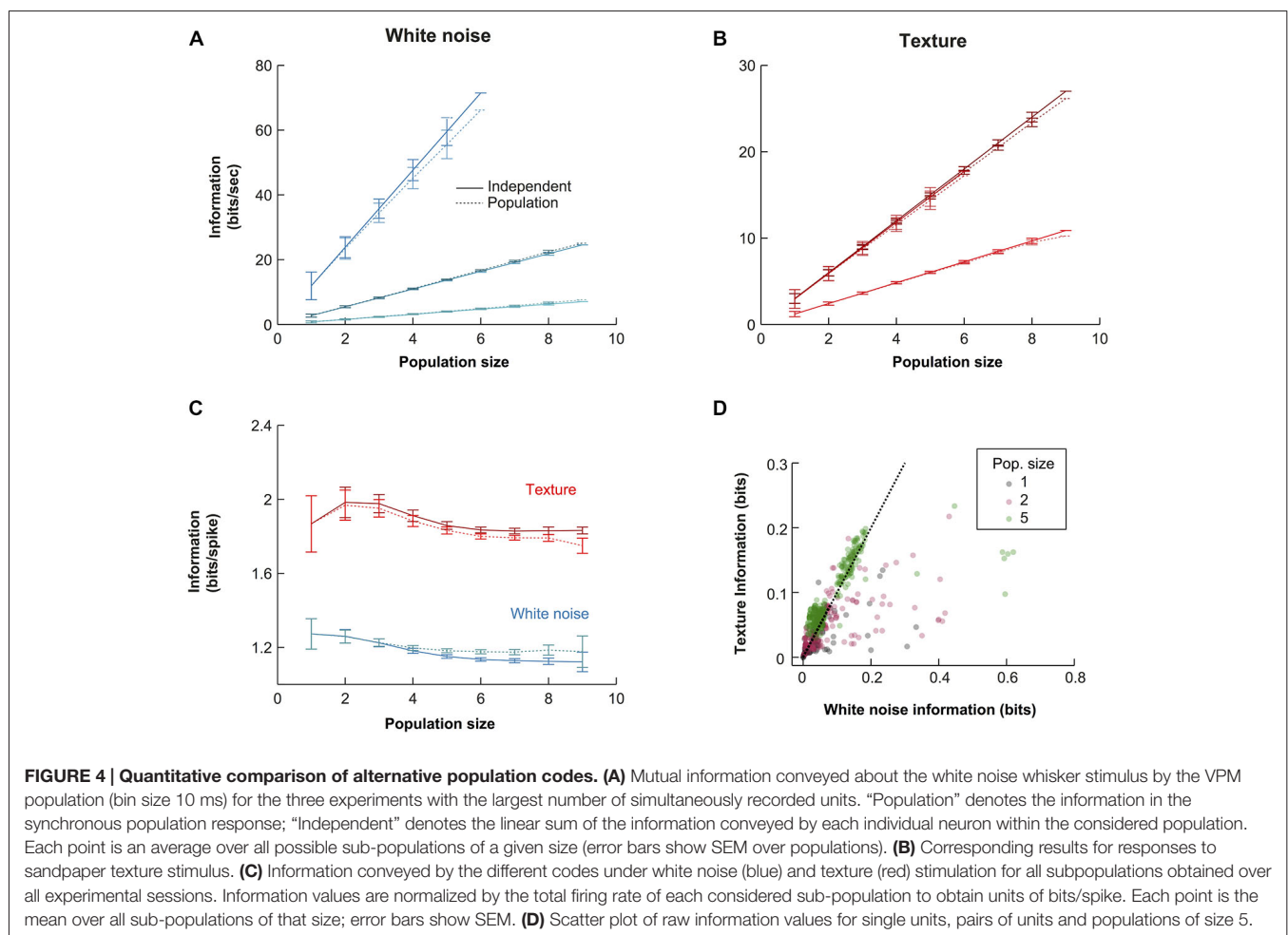


VPM Populations Encode Information Independently

To guide our investigation of the population response, we examined how the information conveyed by the population scaled with population size. Such an analysis can reveal whether the representation is, overall, redundant (sub-linear scaling), independent (linear scaling) or synergistic (super-linear scaling). We estimated the mutual information that the population spike pattern (the simultaneous response of all neurons in a given time bin) conveyed about white noise and texture (see “Materials and Methods” Section). In all cases, we found mutual information to scale approximately linearly with population size. We illustrate this with the three experiments with highest numbers of simultaneously recorded units (**Figure 4**). The similarity between the mutual information available in the population and that in the sum of single unit information values, across a range of population sizes (**Figures 4A,B**, independent solid lines, pattern dotted lines), implies a striking lack of redundancy.

In principle, zero redundancy for a population could mask significant redundancy/synergy in subsets of the population that cancel out. To test this, we first calculated pairwise synergies

and redundancies directly (see “Materials and Methods” Section). For each recording with two or more simultaneous units, we calculated the mean absolute synergy/redundancy value (Equation 4) of each unit pair, as a percentage of the mutual information conveyed by that pair. Across recordings ($N = 11$), the mean was $2.1 \pm 1.7\%$ for white noise and $1.8 \pm 1.0\%$ for textures. This shows that net pairwise interaction effects were small. Second, for recordings in which at least five units were recorded ($N = 3$; those shown in **Figure 4A**), we considered all possible sub-populations of size 5. For each such sub-population, we computed an index which expresses the maximum possible pairwise synergistic/redundant effect, as a percentage of the mutual information conveyed by the sub-population (see “Materials and Methods” Section). This resulted in a maximum pairwise synergy/redundancy cancellation of $2.0 \pm 1.5\%$ ($N = 258$ populations, mean $\pm$ SD). While this analysis does not exclude the possibility of potential higher order effects, it does show that the near-zero redundancy observed at the population level was not a consequence of cancellation between synergy and redundancy in pairs of units.



VPM Populations Encode Naturalistic Texture Motion Efficiently than White Noise Motion

We repeated the information calculations for all recordings ($N = 16$; **Figure 4D**). To factor out the effect of different firing rates, we normalized the mutual information values to units of bits/spike and then averaged the results over all simultaneous recordings of a given size (**Figure 4C**). The normalization to units of bits/spike revealed that the mutual information conveyed per spike was higher for the texture stimulus than for the white noise stimulus. At the single unit level ($N = 65$), white noise stimulation yielded mutual information values of 1.33 ± 0.67 [1.29] bits/spike, while texture stimulation yielded 1.94 ± 1.24 [1.64] bits/spike (mean $\pm$ SD [median]): this difference was significant (signed ranks test; $p < 0.00001$). For populations of size 6 ($N = 169$), the population code conveyed 1.26 ± 0.15 [1.26] bits/spike with white noise and 1.95 ± 0.26 [1.98] bits/spike with texture (signed ranks test; $p < 1e^{-10}$). This difference might mean either that the population transmits more information (in a given period of time) about texture than about white noise or, alternatively, that it conveys the same information but using fewer spikes. The scatter plots in **Figure 4D** show that the mutual information values conveyed by the response in each 10 ms bin were usually similar for the two types of stimuli (although a proportion of population responses were more informative about white noise). Therefore, the lower firing rate evoked by texture (**Figure 2C**) accounts for the finding that texture conveys more bits/spike. The population response conveys about the same amount of information about texture motion as white noise, but does so with fewer spikes. This suggests that the subcortical pathway may be adapted to whisker motion with the statistical characteristics of natural object interactions.

Effect of Noise Correlations on the Population Code

Dynamic synchrony might reflect either: (1) properties of individual units or (2) network properties. To discriminate between these possibilities, we calculated the effect of noise correlations on the population code, using information theory (Schneidman et al., 2003; Latham and Nirenberg, 2005; Chicharro, 2014). Noise correlations are correlations between the responses of simultaneously recorded single units that are not related to, or driven by, the external stimulus; arising instead from intrinsic network activity. The effect of noise correlations can be removed by creating a surrogate data set in which the responses of each unit to a given stimulus are shuffled across trials, with respect to the other members of the population. This surrogate data set is then a model for what the responses would look like if the cells were firing independently. We generated such surrogate data and repeated both the DS analysis and the mutual information based analysis. We found, for both types of stimulus, that shuffling made little difference to the DS measure: the ratio of the shuffled to the unshuffled DS measure was 1.02 [0.82 1.19] (median [min max] across recordings) for white noise and 0.99 [0.90 1.13] for texture. To assess the impact of noise correlations

on mutual information, we computed the difference between the unshuffled and shuffled information, normalized by the unshuffled information. Again, for both types of stimulus, the impact of noise correlations was small: 4.1% [2.4 6.7] for white noise, 2.9% [1.8 4.8] for textures (median [min max] across population sizes). These results indicate that the results reported above reflect the coding properties of individual units rather than network interactions.

Robustness of Results to Differential Parameters of the Mutual Information Estimation

It is necessary to test whether estimates of mutual information are accurate (see “Materials and Methods” Section). To assess the robustness of the main mutual information results to the various parameters of the information theoretic analyses, we performed the following controls. First, we investigated the effect of the length of stimulus. In the full data set, stimulus lengths ranged from 5–40 s, corresponding to 500–4000 10 ms bins. To test whether our results were affected by these differences, we repeated the population mutual information analysis using subsections of the stimulus sequence (1.25–5 s) common to all recordings (**Figure 5A**). In all cases, the texture stimulus evoked more informative responses than white noise (in bits/spike), there was very little redundancy and the values were quantitatively very close to the full results presented in **Figure 4C**. Information values for the shortest texture stimuli (1.25s) were lower, but we expect this is a consequence of the sparse structure of the stimulus. Second, we tested the effect of bin size used for the information analysis (range 10–80 ms; **Figures 5B,C**). Again the results were robust: in all cases, texture evoked higher bits/spike than white noise and redundancy was very low. Information values were lower for larger bin sizes; this indicates the temporal precision of the population response is (as expected from the jitter and CCG results) at least 10 ms.

Discussion

The aim of this study was to investigate how a modular, population of neurons corresponding to one whisker processes complex, naturalistic whisker motion. We focussed on the VPM thalamus, where signals from a given whisker are primarily encoded by a population of ~250 projection neurons within the corresponding barreloid (van der Loos, 1976; Land and Simons, 1985; Sugitani et al., 1990; Haidarliu et al., 2008). We were able to record from up to nine single units within one barreloid simultaneously. In contrast to what has previously been reported with ramp-and-hold stimuli, we found that naturalistic whisker motion evoked a dynamic sequence of population spiking, where different groups of neurons fired synchronously at different times (“dynamic synchrony”).

Previous work on the rat/mouse VPM recorded the activity of 1–2 neurons within one barreloid (Waite, 1973; Simons and Carvell, 1989; Pinto et al., 2000; Brecht and Sakmann, 2002; Yu et al., 2006; Montemurro et al., 2007a; Petersen et al., 2008; Temereanca et al., 2008; Bale and Petersen, 2009; Wang et al., 2010b; Scaglione et al., 2011; Poulet et al., 2012). Here, by using multi-site silicon probes and a novel insertion angle,

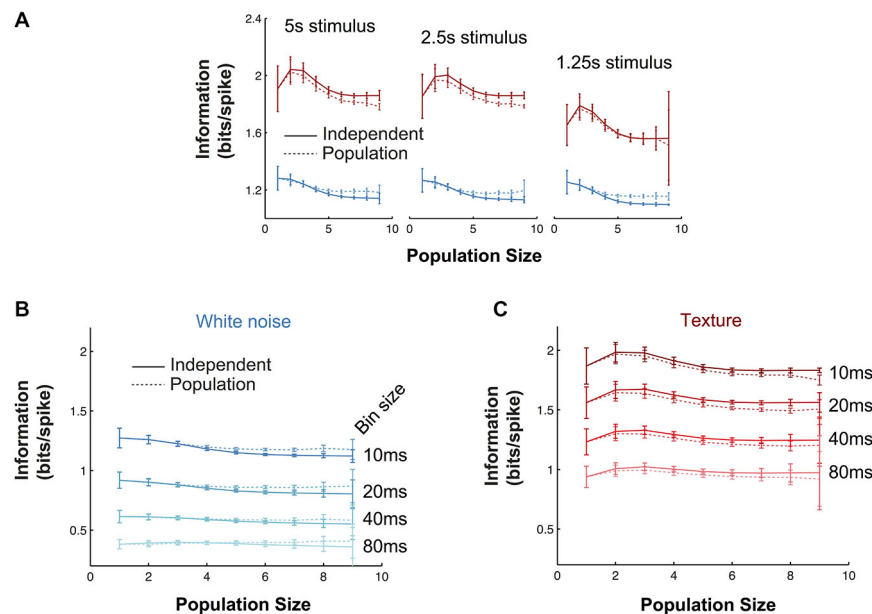


FIGURE 5 | Robustness of information theoretic analyses. (A) Mutual information results over all experiments as a function of population size (details as in **Figure 4C**) when the calculations are performed with stimulus windows of 5, 2.5 and 1.25 s (500, 250 and 125 stimuli respectively). **(B,C)** Mutual information results over all experiments as a function of population size (details as in **Figure 4C**) for a range of response bin sizes (10–80 ms) for the white noise **(B)** and texture **(C)** stimuli.

up to 11 recording sites could be located within a single barreloid. Eleven sites spanned 500 microns, which is consistent with anatomical measurements of barreloid length in adult rats (Haidarliu et al., 2008). This technique enabled us to isolate the activity of up to nine single units within the same barreloid.

“Naturalistic” stimuli, that reflect the complex, dynamic stimuli that animals experience during natural behavior, can evoke responses that differ substantially in reliability and spike timing (Mainen and Sejnowski, 1995; de Ruyter van Steveninck et al., 1997). Although the naturalistic stimulus paradigm is well-established in vision (Simoncelli and Olshausen, 2001), it is only more recently that it has become feasible in the whisker system (Arabzadeh et al., 2005; Lottem and Azouz, 2011; Bale et al., 2013). Our aim here was to study the population response of a VPM barreloid to naturalistic whisker motion. To this end, it was essential to reproduce identical sequences of naturalistic whisker motion on multiple trials. Our approach was to use an anesthetised preparation and to play back sequences of texture-induced whisker motion recorded optically from behaving rats (Wolfe et al., 2008).

Previous work on VPM has shown that rapid whisker motion, such as occurs frequently during a white noise sequence or at the start of a ramp-and-hold stimulus, evokes spikes whose timing is reliable with sub-millisecond precision (Montemurro et al., 2007a). Under some circumstances (periodic whisker deflection), it is known that these spikes occur coincidentally across neurons on a time-scale of ~ 10 ms (“synchrony”; Bruno and Sakmann, 2006; Temereanca et al., 2008; Wang et al., 2010b). We found that naturalistic whisker motion evoked a complex

population response where different subsets of neurons are co-active at different times (**Figure 3A**). In other words, the synchrony was dynamic. We argue that this difference is due to the more complex nature of the stimuli we used. The onset of a ramp-and-hold whisker deflection provokes a correlated increase in position, velocity, acceleration and all higher order temporal derivatives of whisker position (Petersen et al., 2008). Thus, although VPM neurons are tuned to diverse kinetic features (Pinto et al., 2000; Petersen et al., 2008), such a stimulus will tend to evoke a synchronous response from a substantial proportion of the neuron population. Conversely, with a dynamic naturalistic stimulus, the position, velocity and acceleration are decoupled and neurons tuned to different kinetic features tend to fire at different times. Consistent with this interpretation, our quantitative measure of DS was not strongly affected by removing noise correlations from the responses. Moreover, the degree of synchrony exhibited by VPM neuron pairs is known to depend on both deflection velocity and stimulation frequency (Temereanca et al., 2008). Adaptation mechanisms may contribute further dynamism to the response (Wang et al., 2010b). VPM response heterogeneity has also been reported both in the electrical whisking paradigm (Yu et al., 2006) and in the behaving mouse (Gutnisky et al., 2013). These observations are consistent with a model of VPM barreloid function whereby different neurons independently encode different aspects of the on-going whisker motion. Consistent with this, we found that the mutual information conveyed by the population scaled linearly with population size and that this effect was robust to varying the parameters of the

analysis. We found no evidence that this lack of redundancy reflected cancelation between strongly synergistic and strongly redundant pairwise sub-populations.

Individual spikes conveyed more bits/spike for the naturalistic texture stimulus compared to white noise (**Figure 4C**), suggesting that the sensory pathway to thalamus may be optimized towards representing naturalistic stimuli, as has also been suggested in the visual and auditory systems (Dan et al., 1996; Simoncelli and Olshausen, 2001; Lewicki, 2002). Similarly to these other systems, the naturalistic texture stimulus had a notably non-gaussian “sparse” distribution. Compared to white noise, the texture stimulus had relatively greater power at low frequencies. There are likely to be two factors underlying the greater sparseness and informational efficiency of the texture responses: (1) the greater stimulus sparseness and (2) the fact that VPM neurons tend to be velocity-sensitive (Pinto et al., 2000; Petersen et al., 2008) and therefore sensitive to higher stimulus frequency components. Theories of efficient coding (redundancy reduction, predictive coding) predict that, under high signal to noise conditions, there should be little redundancy in the neural representation of a sensory signal (Barlow, 1961; Srinivasan et al., 1982; Atick and Redlich, 1990). There has been extensive investigation of efficient coding in vision (Atick and Redlich, 1990; Simoncelli and Olshausen, 2001; Vinje and Gallant, 2002; Sharpee et al., 2006), a few studies in audition (Smith and Lewicki, 2006; Ming and Holt, 2009) but in somatosensation, the only previous work has been on tactile robots (Hafner et al., 2003; Evans, 2013). As far as we are aware, the current study is the first direct evidence for efficient coding in somatosensation.

Synchrony is potentially important, since it provides a possible explanation for how the thalamocortical pathway might achieve reliable transmission, despite the fact that individual, afferent synapses are typically weak (Bruno and Sakmann, 2006). When a population of thalamic neurons spikes synchronously (on the time-scale of membrane time constant), multiple postsynaptic potentials can summate in a thalamorecipient neuron and trigger postsynaptic spiking. Such an integration mechanism accounts for how stimuli such as ramp-and-hold whisker deflections might trigger a reliable response in thalamorecipient, cortical neurons, and is a potentially effective method of decoding the occurrence and velocity/direction of such stimuli. However, integration is sensitive only to the number of presynaptic spikes and is blind to their origin. It will lose information from a DS code where different patterns of spikes are elicited by different stimulus features. An intriguing possibility is that, through tuning of synaptic strengths (Feldman, 2009) and/or nonlinear dendritic processes (London and Häusser, 2005), cortical neurons are able to perform decoding operations more sophisticated than integration and thereby to discriminate amongst a broad class of sensory signals.

Our findings raise issues for further work. One limitation of the current analysis relates to the information calculations. Mutual information as computed here is a powerful statistic for quantifying, in a general way, the reliability of the population response to a dynamic stimulus. However, it would also be interesting to understand what particular kinematic features of the stimulus drive the population response. Second, although recording from populations of size 9 from a single barreloid is the largest-scale recording yet reported from VPM, some network interaction effects only become appreciable for large population sizes (Roudi et al., 2009), and it would be interesting to achieve still higher population sizes. Finally, it is an important challenge to investigate population coding in the awake, behaving animal. However, since multiple trials of identical whisker motion sequences cannot be delivered under these conditions, the present analysis approaches are inapplicable and new methods will be required.

In sum, our findings suggest that the basic building block of the whisker-related thalamus—the barreloid—encodes naturalistic sensory information in a remarkably efficient manner. Barreloid ensembles exhibit temporally sparse responses that encode rich information about whisker motion through a DS code. The combination of sub-millisecond spike timing precision and diverse tuning leads to a high capacity thalamic population code, which potentially conveys a rich signal about multiple features of whisker motion to cortex. A potentially significant implication of our findings is that it would be advantageous for cortical circuits to act not only as detectors of coincident thalamic activity but also to decode patterns of thalamic population activity.

Author Contributions

MRB, RAAI and RSP designed the experiment. MRB and GS performed neurophysiology. MRB, RAAI and RSP performed data analysis. MRB, RAAI and RSP wrote the manuscript. MRB and RAAI contributed equally and share first authorship of this manuscript.

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References

- Arabzadeh, E., Zorzin, E., and Diamond, M. E. (2005). Neuronal encoding of texture in the whisker sensory pathway. *PLoS Biol.* 3:e17. doi: 10.1371/journal.pbio.0030017
- Atick, J. J., and Redlich, A. N. (1990). Towards a theory of early visual processing. *Neural Comput.* 2, 308–320. doi: 10.1162/neco.1990.2.3.308
- Averbeck, B. B., Latham, P. E., and Pouget, A. (2006). Neural correlations, population coding and computation. *Nat. Rev. Neurosci.* 7, 358–367. doi: 10.1038/nrn1888

- Bale, M. R., Campagner, D., Erskine, A., and Petersen, R. S. (2015). Microsecond-scale timing precision in rodent trigeminal primary afferents. *J. Neurosci.* 35, 5935–5940. doi: 10.1523/jneurosci.3876-14.2015
- Bale, M. R., Davies, K., Freeman, O. J., Ince, R. A. A., and Petersen, R. S. (2013). Low-dimensional sensory feature representation by trigeminal primary afferents. *J. Neurosci.* 33, 12003–12012. doi: 10.1523/jneurosci.0925-13.2013
- Bale, M. R., and Petersen, R. S. (2009). Transformation in the neural code for whisker deflection direction along the lemniscal pathway. *J. Neurophysiol.* 102, 2771–2780. doi: 10.1152/jn.00636.2009
- Barlow, H. (1961). “Possible principles underlying the transformations of sensory messages,” in *Sensory Communication*, ed. W. Rosenblith (Cambridge, MA: MIT Press), 217–234.
- Brecht, M., and Sakmann, B. (2002). Whisker maps of neuronal subclasses of the rat ventral posterior medial thalamus, identified by whole-cell voltage recording and morphological reconstruction. *J. Physiol.* 538, 495–515. doi: 10.1113/jphysiol.2001.012334
- Bruno, R. M., and Sakmann, B. (2006). Cortex is driven by weak but synchronously active thalamocortical synapses. *Science* 312, 1622–1627. doi: 10.1126/science.1124593
- Bruno, R. M., and Simons, D. J. (2002). Feedforward mechanisms of excitatory and inhibitory cortical receptive fields. *J. Neurosci.* 22, 10966–10975.
- Castro-Alamancos, M. A. (2002). Properties of Primary Sensory (Lemniscal) Synapses in the Ventrobasal Thalamus and the Relay of High-Frequency Sensory Inputs. *J. Neurophysiol.* 87, 946–953. doi: 10.1152/jn.00426.2001
- Chicharro, D. (2014). A causal perspective on the analysis of signal and noise correlations and their role in population coding. *Neural Comput.* 26, 999–1054. doi: 10.1162/neco_a_00588
- Dan, Y., Atick, J. J., and Reid, R. C. (1996). Efficient coding of natural scenes in the lateral geniculate nucleus: experimental test of a computational theory. *J. Neurosci.* 16, 3351–3362.
- Desbordes, G., Jin, J., Weng, C., Lesica, N. A., Stanley, G. B., and Alonso, J. M. (2008). Timing precision in population coding of natural scenes in the early visual system. *PLoS Biol.* 6:e324. doi: 10.1371/journal.pbio.0060324
- Deschênes, M., Timofeeva, E., Lavallée, P., and Dufresne, C. (2005). The vibrissal system as a model of thalamic operations. *Prog. Brain Res.* 149, 31–40. doi: 10.1016/s0079-6123(05)49003-2
- Diamond, M. E., and Arabzadeh, E. (2013). Whisker sensory system - From receptor to decision. *Prog. Neurobiol.* 103, 28–40. doi: 10.1016/j.pneurobio.2012.05.013
- Ego-Stengel, V., Cam, J. L., and Shulz, D. E. (2012). Coding of apparent motion in the thalamic nucleus of the rat vibrissal somatosensory system. *J. Neurosci.* 32, 3339–3351. doi: 10.1523/jneurosci.3890-11.2012
- Evans, M. H. (2013). “Efficient coding in the whisker system: biomimetic pre-processing for robots?” in *Biomimetic and Biohybrid Systems Lecture Notes in Computer Science*, eds N. F. Lepora, A. Mura, H. G. Krapp, P. F. M. J. Verschure, and T. J. Prescott (Berlin Heidelberg: Springer), 364–367.
- Feldman, D. E. (2009). Synaptic mechanisms for plasticity in neocortex. *Annu. Rev. Neurosci.* 32, 33–55. doi: 10.1146/annurev.neuro.051508.135516
- Gutnisky, D. A., To, M.-S., Bale, M. R., Petersen, R. S., and Svoboda, K. (2013). Transformation of neural codes between the ventral posteromedial nucleus of the thalamus and layer 4 of barrel cortex during active sensation. *Soc. Neurosci. Abstr.* 39, 743.17.
- Hafner, V. V., Fend, M., Lungarella, M., Pfeifer, R., König, P., and Paul, K. (2003). “Optimal coding for naturally occurring whisker deflections,” in *Artificial Neural Networks and Neural Information Processing—ICANN/ICONIP 2003 Lecture Notes in Computer Science*, eds O. Kaynak, E. Alpaydin, E. Oja, and L. Xu (Berlin, Heidelberg: Springer), 805–812.
- Haidarliu, S., and Ahissar, E. (2001). Size gradients of barreloids in the rat thalamus. *J. Comp. Neurol.* 429, 372–387. doi: 10.1002/1096-9861(20010115)429:3<372::aid-cne2>3.0.co;2-3
- Haidarliu, S., Yu, C., Rubin, N., and Ahissar, E. (2008). Lemniscal and extralemniscal compartments in the VPM of the rat. *Front. Neuroanat.* 2:4. doi: 10.3389/neuro.05.004.2008
- Hyvärinen, A., Hurri, J., and Hoyer, P. O. (2009). *Natural Image Statistics: A Probabilistic Approach to Early Computational Vision*. London: Springer.
- Ince, R. A. A., Petersen, R. S., Swan, D. C., and Panzeri, S. (2009). Python for information theoretic analysis of neural data. *Front. Neuroinformatics* 3:4. doi: 10.3389/neuro.11.004.2009
- Ince, R. A. A., Senatore, R., Arabzadeh, E., Montani, F., Diamond, M. E., and Panzeri, S. (2010). Information-theoretic methods for studying population codes. *Neural Netw.* 23, 713–727. doi: 10.1016/j.neunet.2010.05.008
- Land, P. W., and Simons, D. J. (1985). Metabolic and structural correlates of the vibrissae representation in the thalamus of the adult rat. *Neurosci. Lett.* 60, 319–324. doi: 10.1016/0304-3940(85)90597-x
- Latham, P. E., and Nirenberg, S. (2005). Synergy, redundancy and independence in population codes, revisited. *J. Neurosci.* 25, 5195–5206. doi: 10.1523/jneurosci.5319-04.2005
- Lewicki, M. S. (2002). Efficient coding of natural sounds. *Nat. Neurosci.* 5, 356–363. doi: 10.1038/nn831
- London, M., and Häusser, M. (2005). Dendritic computation. *Annu. Rev. Neurosci.* 28, 503–532. doi: 10.1146/annurev.neuro.28.061604.135703
- van der Loos, H. (1976). Barreloids in mouse somatosensory thalamus. *Neurosci. Lett.* 2, 1–6. doi: 10.1016/0304-3940(76)90036-7
- Lottem, E., and Azouz, R. (2011). A unifying framework underlying mechanotransduction in the somatosensory system. *J. Neurosci.* 31, 8520–8532. doi: 10.1523/jneurosci.6695-10.2011
- Mainen, Z. F., and Sejnowski, T. J. (1995). Reliability of spike timing in neocortical neurons. *Science* 268, 1503–1506. doi: 10.1126/science.7770778
- Ming, V. L., and Holt, L. L. (2009). Efficient coding in human auditory perception. *J. Acoust. Soc. Am.* 126, 1312–1320. doi: 10.1121/1.3158939
- Montemurro, M. A., Panzeri, S., Maravall, M., Alenda, A., Bale, M. R., Brambilla, M., et al. (2007a). Role of precise spike timing in coding of dynamic vibrissa stimuli in somatosensory thalamus. *J. Neurophysiol.* 98, 1871–1882. doi: 10.1152/jn.00593.2007
- Montemurro, M. A., Senatore, R., and Panzeri, S. (2007b). Tight data-robust bounds to mutual information combining shuffling and model selection techniques. *Neural Comput.* 19, 2913–2957. doi: 10.1162/neco.2007.19.11.2913
- Panzeri, S., Senatore, R., Montemurro, M. A., and Petersen, R. S. (2007). Correcting for the Sampling Bias Problem in Spike Train Information Measures. *J. Neurophysiol.* 98, 1064–1072. doi: 10.1152/jn.00559.2007
- Panzeri, S., and Treves, A. (1996). Analytical estimates of limited sampling biases in different information measures. *Netw. Comput. Neural Syst.* 7, 87–107.
- Petersen, R. S., Brambilla, M., Bale, M. R., Alenda, A., Panzeri, S., Montemurro, M. A., et al. (2008). Diverse and temporally precise kinetic feature selectivity in the vpm thalamic nucleus. *Neuron*. 60, 890–903. doi: 10.1016/j.neuron.2008.09.041
- Petersen, R. S., Panzeri, S., and Maravall, M. (2009). Neural coding and contextual influences in the whisker system. *Biol. Cybern.* 100, 427–446. doi: 10.1007/s00422-008-0290-5
- Pinto, D. J., Brumberg, J. C., and Simons, D. J. (2000). Circuit dynamics and coding strategies in rodent somatosensory cortex. *J. Neurophysiol.* 83, 1158–1166.
- Poulet, J. F. A., Fernandez, L. M. J., Crochet, S., and Petersen, C. C. H. (2012). Thalamic control of cortical states. *Nat. Neurosci.* 15, 370–372. doi: 10.1038/nn.3035
- Rolls, E. T., and Tovee, M. J. (1995). Sparseness of the neuronal representation of stimuli in the primate temporal visual cortex. *J. Neurophysiol.* 73, 713–726.
- Roudi, Y., Nirenberg, S., and Latham, P. E. (2009). Pairwise maximum entropy models for studying large biological systems: when they can work and when they can't. *PLoS Comput. Biol.* 5:e1000380. doi: 10.1371/journal.pcbi.1000380
- de Ruyter van Steveninck, R. R., Lewen, G. D., Strong, S. P., Koberle, R., and Bialek, W. (1997). Reproducibility and variability in neural spike trains. *Science* 275, 1805–1808. doi: 10.1126/science.275.5307.1805
- Scaglione, A., Moxon, K. A., Aguilar, J., and Foffani, G. (2011). Trial-to-trial variability in the responses of neurons carries information about stimulus location in the rat whisker thalamus. *Proc. Natl. Acad. Sci.* 108, 14956–14961. doi: 10.1073/pnas.1103168108
- Schneidman, E., Bialek, W., and Berry, M. J. (2003). Synergy, redundancy and independence in population codes. *J. Neurosci.* 23, 11539–11553.
- Sharpee, T. O., Sugihara, H., Kurgansky, A. V., Rebrik, S. P., Stryker, M. P., and Miller, K. D. (2006). Adaptive filtering enhances information transmission in visual cortex. *Nature*. 439, 936–942. doi: 10.1038/nature045541
- Sherman, S. M., and Guillery, R. (2009). *Exploring the Thalamus and Its Role in Cortical Function*. 2nd Edn. Cambridge, MA: MIT Press.
- Shoham, S., Fellows, M. R., and Normann, R. A. (2003). Robust, automatic spike sorting using mixtures of multivariate t-distributions. *J. Neurosci. Methods* 127, 111–122. doi: 10.1016/s0165-0270(03)00120-1

- Simoncelli, E. P., and Olshausen, B. A. (2001). Natural image statistics and neural representation. *Annu. Rev. Neurosci.* 24, 1193–1216. doi: 10.1146/annurev.neuro.24.1.1193
- Simons, D., and Carvell, G. (1989). Thalamocortical response transformation in the rat vibrissa/barrel system. *J. Neurophysiol.* 61, 311–330.
- Smith, E. C., and Lewicki, M. S. (2006). Efficient auditory coding. *Nature* 439, 978–982. doi: 10.3410/f.1031209.368716
- Srinivasan, M. V., Laughlin, S. B., and Dubs, A. (1982). Predictive coding: a fresh view of inhibition in the retina. *Proc. R. Soc. Lond. B. Biol. Sci.* 216, 427–459. doi: 10.1098/rspb.1982.0085
- Storchi, R., Bale, M. R., Biella, G. E. M., and Petersen, R. S. (2012). Comparison of latency and rate coding for the direction of whisker deflection in the subcortical somatosensory pathway. *J. Neurophysiol.* 108, 1810–1821. doi: 10.1152/jn.00921.2011
- Strong, S. P., Koberle, R., de Ruyter van Steveninck, R. R., and Bialek, W. (1998). Entropy and information in neural spike trains. *Phys. Rev. Lett.* 80, 197–200. doi: 10.1103/PhysRevLett.80.197
- Sugitani, M., Yano, J., Sugai, T., and Ooyama, H. (1990). Somatotopic organization and columnar structure of vibrissae representation in the rat ventrobasal complex. *Exp. Brain Res.* 81, 346–352. doi: 10.1007/bf00228125
- Temereanca, S., Brown, E. N., and Simons, D. J. (2008). Rapid changes in thalamic firing synchrony during repetitive whisker stimulation. *J. Neurosci.* 28, 11153–11164. doi: 10.1523/jneurosci.1586-08.2008
- Vinje, W. E., and Gallant, J. L. (2002). Natural stimulation of the nonclassical receptive field increases information transmission efficiency in V1. *J. Neurosci.* 22, 2904–2915. doi: 10.3410/f.1006578.82409
- Waite, P. M. E. (1973). Somatotopic organization of vibrissal responses in the ventro-basal complex of the rat thalamus. *J. Physiol.* 228, 527–540. doi: 10.1113/jphysiol.1973.sp010098
- Wang, H. P., Spencer, D., Fellous, J. M., and Sejnowski, T. J. (2010a). Synchrony of thalamocortical inputs maximizes cortical reliability. *Science* 328, 106–109. doi: 10.1126/science.1183108
- Wang, Q., Webber, R. M., and Stanley, G. B. (2010b). Thalamic synchrony and the adaptive gating of information flow to cortex. *Nat. Neurosci.* 13, 1534–1541. doi: 10.1038/nn.2670
- Wolfe, J., Hill, D. N., Pahlavan, S., Drew, P. J., Kleinfeld, D., and Feldman, D. E. (2008). Texture coding in the rat whisker system: slip-stick versus differential resonance. *PLoS Biol.* 6:e215. doi: 10.1371/journal.pbio.0060215
- Woolsey, T. A., and Van der Loos, H. (1970). The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res.* 17, 205–242. doi: 10.1016/0006-8993(70)90079-x
- Yu, C., Derdikman, D., Haidarliu, S., and Ahissar, E. (2006). Parallel thalamic pathways for whisking and touch signals in the rat. *PLoS Biol.* 4:e124. doi: 10.1371/journal.pbio.0040124

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A computational relationship between thalamic sensory neural responses and contrast perception

Yaoguang Jiang¹, Gopathy Purushothaman² and Vivien A. Casagrande^{1,2,3*}

¹ Department of Psychology, Vanderbilt University, Nashville, TN, USA, ² Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA, ³ Department of Ophthalmology and Visual Sciences, Vanderbilt University, Nashville, TN, USA

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*Correspondence:

Vivien A. Casagrande,
Department of Cell and
Developmental Biology, Vanderbilt
University, PMB407935 RM U-3218
MRB 3, 465 21st Avenue South,
Nashville, TN 37240-7935, USA
vivien.casagrande@vanderbilt.edu

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Uncovering the relationship between sensory neural responses and perceptual decisions remains a fundamental problem in neuroscience. Decades of experimental and modeling work in the sensory cortex have demonstrated that a perceptual decision pool is usually composed of tens to hundreds of neurons, the responses of which are significantly correlated not only with each other, but also with the behavioral choices of an animal. Few studies, however, have measured neural activity in the sensory thalamus of awake, behaving animals. Therefore, it remains unclear how many thalamic neurons are recruited and how the information from these neurons is pooled at subsequent cortical stages to form a perceptual decision. In a previous study we measured neural activity in the macaque lateral geniculate nucleus (LGN) during a two alternative forced choice (2AFC) contrast detection task, and found that single LGN neurons were significantly correlated with the monkeys' behavioral choices, despite their relatively poor contrast sensitivity and a lack of overall interneuronal correlations. We have now computationally tested a number of specific hypotheses relating these measured LGN neural responses to the contrast detection behavior of the animals. We modeled the perceptual decisions with different numbers of neurons and using a variety of pooling/readout strategies, and found that the most successful model consisted of about 50–200 LGN neurons, with individual neurons weighted differentially according to their signal-to-noise ratios (quantified as d-primes). These results supported the hypothesis that in contrast detection the perceptual decision pool consists of multiple thalamic neurons, and that the response fluctuations in these neurons can influence contrast perception, with the more sensitive thalamic neurons likely to exert a greater influence.

Keywords: lateral geniculate nucleus (LGN), perception, contrast, neural model, choice probability

Introduction

From smelling a flower to recognizing the face of a loved one, every perceptual task we face, simple or complex, involves a number of neurons in a wide range of brain areas. Of essential interest to neuroscientists is the number of sensory neurons needed to sustain a perception, and the way these neurons are decoded at a later stage to form various decisions. Theoretically, every perceptual task can be accomplished by engaging only the few sensory neurons that are the most sensitive for that task (i.e., the lower envelope principle; Barlow, 1995; Parker and Newsome, 1998).

In reality, however, a variety of factors such as the response variances of single neurons and the positive noise correlations between pairs of neurons constrain the pool size, requiring at least 10–1000 sensory neurons in an average sized decision pool (Shadlen et al., 1996; Cook and Maunsell, 2002; Purushothaman and Bradley, 2005; Cohen and Newsome, 2009; Liu et al., 2013). In sensory cortex, such perceptual decision pools have two prominent features. First, stimulus-independent, random fluctuations of sensory neural responses are known to covary with the perceptual decisions of the animal. The strength of this covariation is quantified as “choice probability” (Britten et al., 1996). Weak but significantly above chance choice probabilities have been observed in a number of sensory cortical areas (Britten et al., 1996; Dodd et al., 2001; Cook and Maunsell, 2002; Grunewald et al., 2002; Uka and Deangelis, 2004; Liu and Newsome, 2005; Purushothaman and Bradley, 2005; Uka et al., 2005; Nienborg and Cumming, 2006; Palmer et al., 2007). Second, cortical sensory neurons are also correlated with each other in their random response fluctuations (Averbeck et al., 2006; Cohen and Kohn, 2011). This correlation, known as the interneuronal noise correlation, is likely to reflect the shared feedforward, feedback, or lateral connections between neurons (Zohary et al., 1994; Shadlen and Newsome, 1998; Bair et al., 2001; Reich et al., 2001; Cohen and Maunsell, 2009). Previous modeling work has revealed that interneuronal correlations can have a profound influence on the choice probability structure of the decision pool (Shadlen et al., 1996; Cohen and Newsome, 2009; Nienborg and Cumming, 2010; Haefner et al., 2013).

Such interneuronal correlation or choice probability measurements, however, are rarely made in subcortical structures (but see Liu et al., 2013). In the mammalian visual system, the retina sends direct input to the lateral geniculate nucleus (LGN) of the thalamus which, in turn, relays this information to the visual cortex. Recently, we reported the first study in which LGN neural responses were examined in detail while the animals were required to make perceptual decisions using the information available within the receptive fields of those LGN neurons (Jiang et al., 2015). In a two alternative forced choice (2AFC) contrast detection task, we found that the majority of single LGN parvocellular (P) and magnocellular (M) neurons were not as sensitive as the monkeys. Importantly, the covariation between neural responses and perceptual decisions, measured as choice probability, was significant for both P and M neurons, even though the average interneuronal correlation between LGN neuron pairs was not different from zero. Additionally, both neural sensitivity and choice probability evolved throughout the stimulus presentation time, with M neurons exhibiting faster and more transient response profiles than P neurons (Jiang et al., 2015).

Taking advantage of this previously characterized dataset and using a computational approach, we investigated in this study how single LGN neurons contribute to our perception of contrast. We built a series of models to explore the interaction between the size of the decision pool, the duration of integration time, and the pooling/readout strategy of the neural system. Because previous experimental and computational work has

suggested a positive relationship between neural sensitivity, choice probability, and readout weight (for example see Britten et al., 1996; Shadlen et al., 1996; Purushothaman and Bradley, 2005; Haefner et al., 2013; Liu et al., 2013), we examined not only the standard uniform readout model but also several alternative weighted readout schemes in which individual neurons were assigned different weights based on their sensitivities. We accepted or rejected these models based on their ability to account for the behavioral performance of the monkeys as well as the measured choice probability values for LGN neurons (see Jiang et al., 2015). Aspects of the modeling data presented here have been published in abstract form (Jiang et al., 2012, 2013).

Materials and Methods

All the experimental procedures regarding surgical preparation, animal training, stimulus presentation, and physiological recordings have been described in detail in previous publications (Jiang et al., 2013, 2015), and are therefore only briefly repeated here when relevant.

Subjects

Two macaque monkeys (monkey 1: *Macaca radiata*, male, 7 kg, 10 years old; monkey 2: *Macaca mulatta*, male, 8 kg, 12 years old) served as subjects. The monkeys were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the guidelines of Vanderbilt University Animal Care and Use Committee under an approved protocol. The monkeys underwent sterile procedures for the implantation of head posts and recording chambers. The chambers were centered over the right LGN of monkey 1 (AP = 7, ML = 12.5) and the left LGN of monkey 2 (AP = 7, ML = 12).

Visual Stimulus Presentations and Behavioral Tasks

The monkeys were first trained to fixate on a central fixation spot for an extended period of time. Next, the monkeys were trained to perform a two-alternative forced choice (2AFC) contrast detection task, in which a contrast stimulus was presented either at the receptive field location of the cell being recorded, or at a symmetrical location in the opposite visual hemi-field, for a fixed duration (200 ms). The monkeys saccaded to one of the two target locations to indicate the side on which the stimulus was presented. The stimulus diameter was always the sum of the classical receptive field diameter (center and surround) plus the fixation window diameter. During each recording session, stimuli of 5 or 9 different contrast levels (including 0% contrast, or blank trials, where no physical stimulus was presented) were presented at each location. Different contrast levels and presentation locations (i.e., left or right) were randomly mixed, with equal probabilities of left or right appearance and higher proportions of low to medium contrast trials to ensure accurate estimations of the psychophysical threshold.

Psychometric Functions

The proportion of correct responses from the monkeys was plotted for each contrast, and a Weibull function was fitted to the data:

$$P(c) = 1 - 0.5 * e^{-\left(\frac{c}{\alpha}\right)^\beta} \quad (1)$$

Where $P(c)$ is the probability of correct responses at contrast level c , α is the contrast level that supports threshold performance (82% correct), and β is the slope of the function.

Cell Mapping and Classification

LGN cells were hand mapped using first a flashlight and then an elongated bar with a sharp contrast profile. Cells were classified as ON-center or OFF-center cells, and Parvocellular (P) or Magnocellular (M) cells, based on their visually driven responses (Norton and Casagrande, 1982; Norton et al., 1988; Xu et al., 2001; Royal et al., 2006; Jiang et al., 2015).

Neurometric Functions

Basic procedures in computing neurometric functions were similar to those described in previous studies (Barlow et al., 1971; Britten et al., 1992; Purushothaman and Bradley, 2005). For every contrast level, an ROC (Receiver Operating Characteristic) curve was computed (Green and Swets, 1966). Each ROC curve plotted, for all possible signal detection criteria (spikes), the proportion of stimulus-inside-receptive-field trials where the spike count exceeded a certain criterion, against the proportion of stimulus-outside-receptive-field trials that exceeded the same criterion. Next each area-under-ROC curve value was calculated and plotted against its corresponding contrast, a Weibull function ([1], as described above) was fitted, and the neurometric threshold and slope were obtained from the fitted curve. All the LGN cells that could be clearly mapped and maintained long enough to characterize both the psychophysical and neural responses (>150 trials, overall psychophysical performance >65% correct) in the detection task were included in this analysis (overall: $n = 89$ neurons; monkey 1: $n = 61$; monkey 2: $n = 28$). We identified in this dataset 41 ON-center P neurons, 27 OFF-center P neurons, 19 ON-center M neurons, and 2 OFF-center M neurons. We found that the average neurometric threshold ($54.4 \pm 4.78\%$ contrast, $n = 89$ neurons, in 0–150 ms integration time windows) was significantly different from the simultaneously measured psychometric threshold ($5.76 \pm 0.72\%$ contrast, $P = 0.000$, Wilcoxon signed rank test). The average ratio of neurometric to psychometric threshold was 40.74 ± 10.75 , indicating that the average LGN neuron was much less sensitive than the monkey in contrast detection (Jiang et al., 2015).

Choice Probability

Basic procedures in computing choice probabilities also were similar to those described in previous studies (Britten et al., 1996; Purushothaman and Bradley, 2005). The choice probability for a certain contrast was measured by

plotting, as an ROC curve, the proportion of choice-inside-receptive-field trials (i.e., trials in which the monkey saccaded towards the receptive field location) against the proportion of choice-outside-receptive-field trials that exceeded the spike count criteria, and computing the area under that curve. The significance of individual or population choice probabilities was assessed using permutation tests (Britten et al., 1996; Jiang et al., 2015). To accurately estimate choice probability, only neural recordings that met the following criteria were included in this analysis: (1) Behavior *ratio* (choice-inside/choice-outside-receptive-field trials) > 0.25 and <4; and (2) For every contrast level that was included in the choice probability computation, at least 10 choice-inside and 10 choice-outside-receptive-field trials were recorded. Out of the 89 neurons in the above dataset, 75 (54 P neurons, 21 M neurons) were included in the choice probability analysis according to these criteria. We found in this dataset that, in the absence of any physical stimulus (i.e., 0% contrast, blank trials only), the average choice probability was 0.54 ± 0.01 for LGN P neurons and 0.54 ± 0.01 for LGN M neurons, both above chance according to permutation tests (P neuron: $P = 0.015$, M neuron: $P = 0.033$; Jiang et al., 2015).

Pooling Model

The basic structure of our pooling model was similar to other bottom up pooling models previously proposed to account for the psychophysical threshold and choice probabilities measured during behavioral tasks (Shadlen et al., 1996; Purushothaman and Bradley, 2005; Cohen and Newsome, 2009; Haefner et al., 2013; Liu et al., 2013). Briefly, to simulate a perceptual decision pool of n units, n single neurons were randomly chosen, with replacement, from our entire dataset. To construct a single trial at a given contrast, we simulated each neuron's response by randomly drawing a number from a Gaussian distribution; the mean and variance of this distribution were determined by that neuron's measured response at that contrast level. In each trial, the model made a "choice" by comparing the summed activity of the neural pool at the test contrast level to the summed activity of the same neurons at the reference contrast (i.e., blank, 0% contrast trials). This procedure was repeated 50 times (i.e., to simulate 50 trials) for each of the 5 contrast levels, and the simulated "psychophysical" performance was recorded as the percentage of correct "choices" at each contrast. This performance was fitted with a cumulative Weibull function [1], and threshold and slope parameters were extracted as described above. The choice probability for each simulated neuron was quantified as the covariation between the simulated neural response and the simulated "psychophysical choice" at 0% contrast. The parameters for the model included the number of neurons in the pool ($n = 1$ –512 neurons), the integration time window ($t = 25$ –200 ms), the Fano factor ($f = 0.25$ –3.0), the interneuronal noise correlation ($r = 0$ –0.3), and the downstream pooling noise ($p = 0$ –4.0). For each parameter combination, the set of simulations described above (50 trials * 5 contrast levels) was repeated 200 times, each time with a new random

sample of n neurons (with replacement), thus giving reliable estimations of the model performance. The overall fitness of the model was evaluated by computing a Goodness-of-Fit (GoF) index:

$$\text{GoF} = (1 - 1/3 * (|\text{simulated threshold} - \text{measured threshold}| / \text{measured threshold} + |\text{simulated P choice probability} - \text{measured P choice probability}| / \text{measured P choice probability} + |\text{simulated M choice probability} - \text{measured M choice probability}| / \text{measured M choice probability})) * 100\% \quad (2)$$

Model Parameters

In our simulations we typically used fixed or experimentally measured values (Jiang et al., 2015) for variables such as the Fano factor, the interneural correlation, and the pooling noise (with the exception of **Figure 1**, where these parameters were systematically varied to probe the basic properties of the pooling model). We consider our choices for these parameter values to be neurobiologically realistic and meaningful for the following reasons: (1) The Fano factor: Recordings in anesthetized as well as alert animals have reported significant variabilities in the responses of single cortical neurons, with the Fano factor (response variance/mean) averaging 1.0–3.0 (Tolhurst et al., 1983; McAdams and Maunsell, 1999; Oram et al., 1999; Gu et al., 2007). The Fano factor of subcortical visual neurons, however, is relatively low (i.e., <1.0). This is true for retinal ganglion cells (Levine et al., 1992; Berry et al., 1997; Reich et al., 1997) as well as LGN cells (Kara et al., 2000). The Fano factors measured in our detection task (Jiang et al., 2015) and used in our models (0.8–1.4, depending on integration time) were in agreement these previously reported measurements. (2) Interneural correlation: In sensory cortex, interneural correlations between pairs of nearby neurons are typically weak but positive (~ 0.1 – 0.2 ; Averbeck et al., 2006). For the LGN P-P and M-M neuron pairs, because convergent feedforward, divergent feedforward, and lateral connections are sparser than those in the cortex (Casagrande and Xu, 2004; Nassi and Callaway, 2009), it is not surprising that we found an average interneural correlation (0.028) that was not significantly different from 0.0. The interneural correlation for a P-M neuron pair is very likely to be even smaller, as the P and M pathways receive different retinal inputs, remain segregated in different layers of the LGN (Casagrande and Norton, 1991; Nassi and Callaway, 2009), and retain separate feedback loops with V1 (Ichida and Casagrande, 2002; Briggs and Usrey, 2009, 2011; Ichida et al., 2014). In our model the P-M correlation was fixed at 0.01, but our simulations could always approach >99% GoF at some parameter combinations, given any P-M correlation values between 0.0–0.05 (data not shown). (3) Pooling noise: The downstream pooling noise can be thought of as the average Fano factor of the cortical neurons onto which LGN neurons converge (Shadlen et al., 1996). In our simulations this number was fixed at 2.0, which is the average estimation of the Fano factor in cortex (see above). The success of our simulations (i.e., approaching >99% GoF at some parameter combinations), however, did not

depend on this assumption. Similar model results could be obtained by simply assuming a true Poisson distribution for all downstream neurons (i.e., pooling noise = 1.0).

Uniform Pooling and Alternative Pooling Strategies

In this paper, we examined uniform pooling models as well as several alternative weighted pooling models. All of these models shared the same overall structure:

$$X_{\text{pooled}} = \sum_{i=1}^n w_i x_i \quad (3)$$

Where X_{pooled} is the summed activity of the perceptual decision pool, x_i is the response of a single neuron, and w_i is the readout weight assigned to this neuron. Within this structure, the uniform pooling model simply assigned equal weights (i.e., $w_i = 1.0$) to all neurons in the decision pool, regardless of their sensitivities (Shadlen et al., 1996). An alternative weighted pooling strategy, in contrast, calculated the sensitivity of each individual neuron and assigned weights accordingly. Depending on how this neural sensitivity was quantified, there were three main categories of weighted pooling schemes: (1) Amplitude-per-trial (amp/trial) weighted scheme, where every neuron was weighted according to its response amplitude in every trial ($w_i \propto x_i$), with the neuron with the highest spike rate carrying a weight of 1.0; (2) Mean amplitude (mean amp) weighted scheme, where every neuron was weighted according to its average response amplitude ($w_i \propto \bar{x}_i$) at high contrast (80–99%), with the neuron with the highest average spike rate carrying a weight of 1.0; and (3) D-prime weighted scheme, where every neuron was weighted by its d-prime value ($w_i \propto d'_i$) at high contrast (80–99%), with the neuron with the greatest d-prime carrying a weight of 1.0. Here d-prime was defined as:

$$d'_i = (\bar{x}_i - \bar{x}_{0i}) / \sqrt{\frac{s_i^2 + s_{0i}^2}{2}} \quad (4)$$

Where $\bar{x}_i$ is the neuron's mean response amplitude at high contrast (80–99%), $\bar{x}_{0i}$ is its mean response amplitude at reference contrast (i.e., blank, 0% contrast), and s_i and s_{0i} represent the corresponding standard deviations. Because of their potential deviations from normality, for both weight and d-prime distributions we reported medians as well as means. Additionally, to characterize the spread of a distribution, we reported the interquartile range:

$$\text{Interquartile Range (IQR)} = Q_3 - Q_1 \quad (5)$$

Where Q_1 is the 1st quartile (i.e., 25% percentile), and Q_3 is the 3rd quartile (i.e., 75% percentile) in the range. To characterize the skewness of a distribution, we reported the skewness index:

$$\text{Skewness index (SI)} = \frac{\frac{1}{n} \sum_{i=1}^n (x_i - \bar{x})^3}{\left(\sqrt{\frac{1}{n} \sum_{i=1}^n (x_i - \bar{x})^2} \right)^3} \quad (6)$$

Where n is the sample size, and $\bar{x}$ is the mean of the sample distribution.

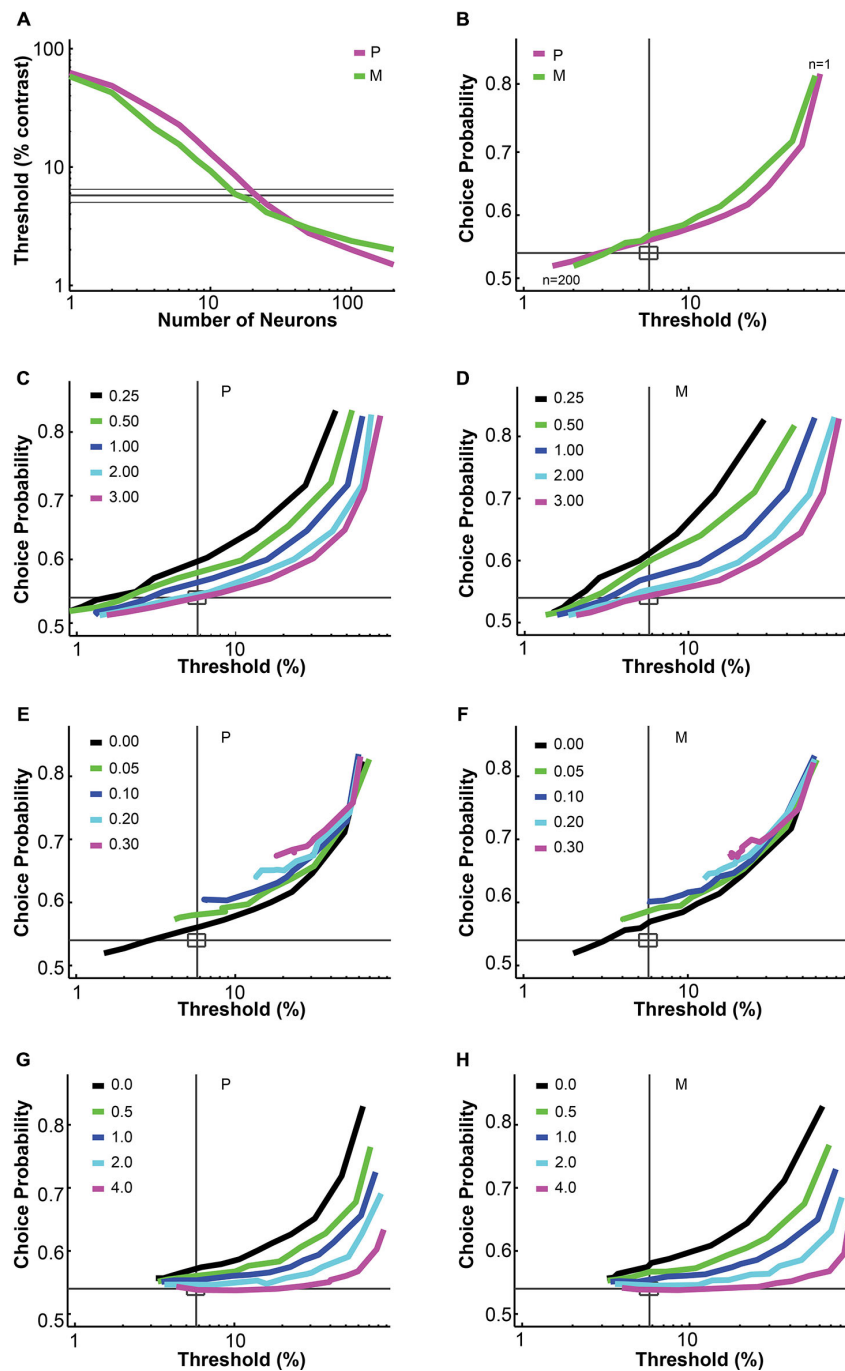


FIGURE 1 | Parametric analysis of the pooling model. (A,B) Increasing the number of neurons in the pool decreased the simulated threshold and choice probability. Interneuronal correlation and pooling noise were assumed to be 0; integration time was 0–150 ms. Magenta: simulated P neuron; green: simulated M neuron; thick gray line: measured mean psychophysical threshold/choice probability; thin gray line/rectangle: mean $\pm$ SEM. **(C,D)** Increasing the Fano factor increased simulated threshold but maintained choice probability, for both P neurons **(C)** and M neurons **(D)**. Interneuronal correlation and pooling noise were assumed to be 0; integration time was 0–150 ms. As in **(B)**, each simulation line moved from the upper right corner to the lower left corner as more neurons were added in the pool. Gray line: measured mean psychophysical threshold/choice probability; rectangle: mean $\pm$ SEM. **(E,F)** Increasing interneuronal correlation increased simulated threshold and choice probability, for both P neurons **(E)** and M neurons **(F)**. The Fano factor was assumed to be 1.03 (measured value); pooling noise was assumed to be 0; integration time was 0–150 ms. As in **(B)**, each simulation line moved from the upper right corner to the lower left corner as more neurons were added in the pool. Legends as in **(C,D)**. **(G,H)** Increasing pooling noise increased simulated threshold and decreased choice probability, for both P neurons **(G)** and M neurons **(H)**. The Fano factor was assumed to be 1.03 (measured value); interneuronal correlation was assumed to be 0.028 (measured value); integration time was 0–150 ms. As in **(B)**, each simulation line moved from the upper right corner to the lower left corner as more neurons were added in the pool. Legends as in **(C,D)**. **(A,B)** and **(E–H)** were adapted from Jiang et al. (2015).

Results

As previously reported (Jiang et al., 2015), we found that single LGN P and M neurons, although not as sensitive as the monkeys in detecting contrast, were significantly correlated with the behavioral choices of the monkeys during a 2AFC contrast detection task. Based on these experimental data, we report in this paper a series of modeling results in the following order: first we describe the basic parameters of the uniform pooling model and its performance in different time frames, then we compare several alternative weighted pooling schemes to the uniform pooling model, and finally we zoom in on one of the best performing weighted schemes and examine its structure in detail.

Uniform Pooling Model: Parameters

The uniform pooling model we built was similar to a number of previous models used to account for psychophysical performance and choice probability measurements based on sensory neural responses (Shadlen et al., 1996; Purushothaman and Bradley, 2005; Cohen and Newsome, 2009; Haefner et al., 2013; Liu et al., 2013). Inputs to the model were single LGN P and M neural responses at different contrasts. Outputs were the simulated “psychophysical” threshold and the simulated choice probabilities for individual neurons. In agreement with Shadlen et al. (1996), this uniform pooling model behaved predictably when certain model parameters were changed. Specifically, increasing the number of neurons in the pool (n) decreased the psychophysical threshold and choice probability values (Figures 1A,B). Increasing the Fano factor increased the simulated threshold but maintained the same choice probability values (Figures 1C,D). Increasing the interneuronal correlation increased the threshold as well as choice probability values (Figures 1E,F). Increasing the downstream pooling noise increased the threshold and decreased choice probability values (Figures 1G,H). For the simulation results reported in the following sections, the Fano factor and interneuronal noise correlation were fixed at experimentally measured values and the pooling noise, which could be considered as the Fano factor of downstream neurons onto which LGN neurons converge, was assumed to be 2.0 (see “Material and Methods” Section).

Uniform Pooling Model: Performance

The first pooling scheme we investigated was the uniform pooling model, in which the responses of all neurons were weighted equally and summed up to form perceptual decisions. In this model, the simulated psychometric threshold consistently decreased as: (1) more neurons (n) were added into the pool; and (2) the integration time window (t) was extended. Specifically, we found that: (1) at extremely short intervals (25 ms), incorporating a large number of neurons from both the P and M populations ($n = 512$ P neurons, 512 M neurons) still failed to achieve great psychophysical sensitivities (i.e., threshold $< 10\%$ contrast), but incorporating a large number of M neurons ($n = 256$ –512) rather than P neurons was more beneficial to model performance (Figure 2A); (2) at relatively brief intervals (50 ms), preferably

incorporating a large number of M neurons ($n = 256$ –512) rather than P neurons produced good psychophysical performance (i.e., threshold $< 10\%$ contrast; Figure 2B); (3) at medium to long intervals (75–200 ms), a wider range of M/P neuron combinations ($n = 64$ –512) yielded good model performance (Figures 2C–F).

Next we analyzed the simulated choice probabilities for the P and M populations and compared them to the measured choice probability distributions (Jiang et al., 2015). Briefly, in the 2AFC contrast detection task, we found that in the absence of any physical stimulus (i.e., 0% contrast, blank trials only), the average choice probability was 0.54 ± 0.01 for LGN P neurons and 0.54 ± 0.01 for LGN M neurons, both above chance according to permutation tests (P neuron: $P = 0.015$, M neuron: $P = 0.033$). In the uniform pooling model, the simulated choice probability distributions for P and M neurons ($n = 512$ neurons, $t = 0$ –150 ms, P choice probability = 0.54 ± 0.00 , M choice probability = 0.54 ± 0.00) resembled their experimentally measured counterparts ($P > 0.05$, permutation tests; Figures 3A,B). These choice probability patterns remained unchanged throughout the 200 ms stimulus presentation time ($n = 512$ neurons, $P > 0.05$, 1-way ANOVAs; Figures 3C,D).

To evaluate the overall performance of this model, a Goodness-of-Fit (GoF) index (see equation [2]) was reported for each (n , t) parameter combination. A GoF (ranging from 0–100%) reflected three factors equally: (1) how close the simulated “psychophysical” threshold approached the measured psychophysical threshold; (2) how close the simulated P population choice probability approached the measured average choice probability for P neurons; and (3) how close the simulated M population choice probability approached the measured average choice probability for M neurons. A GoF of 100% indicated that our simulation perfectly matched the observed psychometric threshold and the choice probabilities for both types of neurons. By changing the duration of the integration window (25–200 ms), we found that: (1) at extremely short intervals (25 ms), even incorporating a large number of neurons from both groups ($n = 512$ P neurons, 512 M neurons) still failed to reproduce the observed threshold and choice probabilities (Figure 4A); (2) in 50 ms, preferably incorporating a large number of M neurons ($n = 256$ –512) could explain the observed threshold and choice probabilities (Figure 4B); (3) in 75 ms, incorporating a large number of either P or M neurons ($n = 128$ –512) could achieve good overall model performance (Figure 4C); and (4) at medium to long intervals (100–200 ms), a smaller number of P and M neurons were needed ($n = 32$ –128) to achieve good model performance, but further increasing the number of neurons resulted in a decrease in model performance (Figures 4D–F).

Alternative Pooling Schemes

In this section we examine several alternative pooling schemes where, instead of assigning the same weight to all neurons, each individual neuron was weighted differentially based on its response rate or sensitivity. We investigated three main categories of weighted pooling schemes, namely the

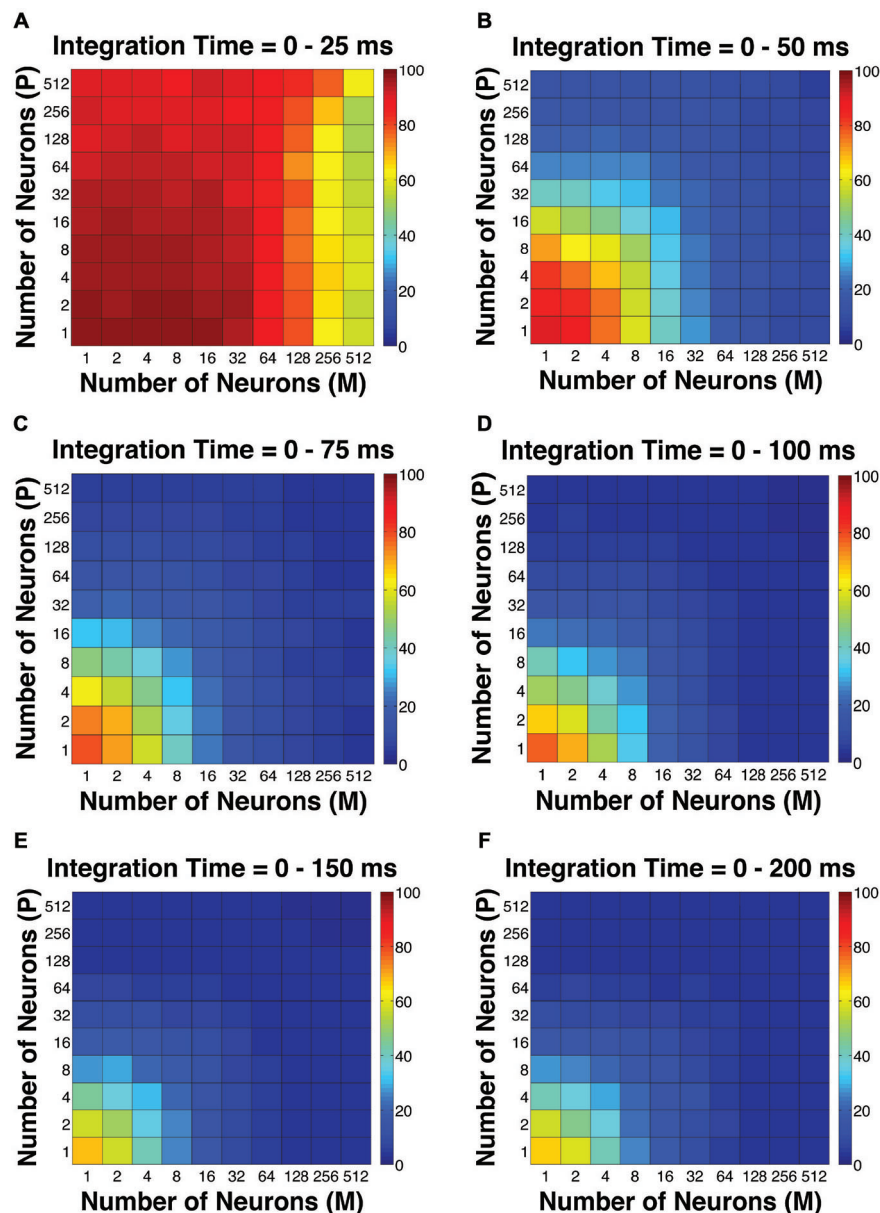


FIGURE 2 | The simulated “psychophysical” thresholds for different pool sizes (n) and integration time windows (t). Red indicates high threshold (100% contrast), whereas blue indicates low threshold (0% contrast). **(A)** $t = 0-25$ ms. **(B)** $t = 0-50$ ms. **(C)** $t = 0-75$ ms. **(D)** $t = 0-100$ ms. **(E)** $t = 0-150$ ms. **(F)** $t = 0-200$ ms.

amplitude-per-trial (amp/trial) weighted, the mean amplitude (mean amp) weighted, and the d-prime weighted schemes (see “Material and Methods” Section). First we compared the simulated psychometric thresholds and found that for all pooling schemes the average psychometric threshold decreased with time ($n = 1-512$ neurons, $F = 920.22$, $P = 0.00$, 2-way ANOVA main effect for time). Furthermore, there was a significant difference in psychometric thresholds among different pooling schemes ($F = 54.82$, $P = 0.00$, 2-way ANOVA main effect for pooling strategy), and this difference changed across time ($F = 8.43$, $P = 0.00$, 2-way ANOVA

interaction effect; **Figure 5A**). Next we examined whether these alternative pooling strategies improved the sensitivity of the model when compared to the uniform pooling strategy. Here the mean amplitude weighted and the d-prime weighted models could be further divided into two subcategories, respectively, depending on whether P and M neurons were weighted separately or together in the model. Among all of these alternative pooling schemes, we found that only the d-prime weighted schemes consistently improved the psychophysical performance when compared with the uniform pooling scheme (mean difference, d-prime 1 = $-4.88 \pm 0.42\%$ contrast;

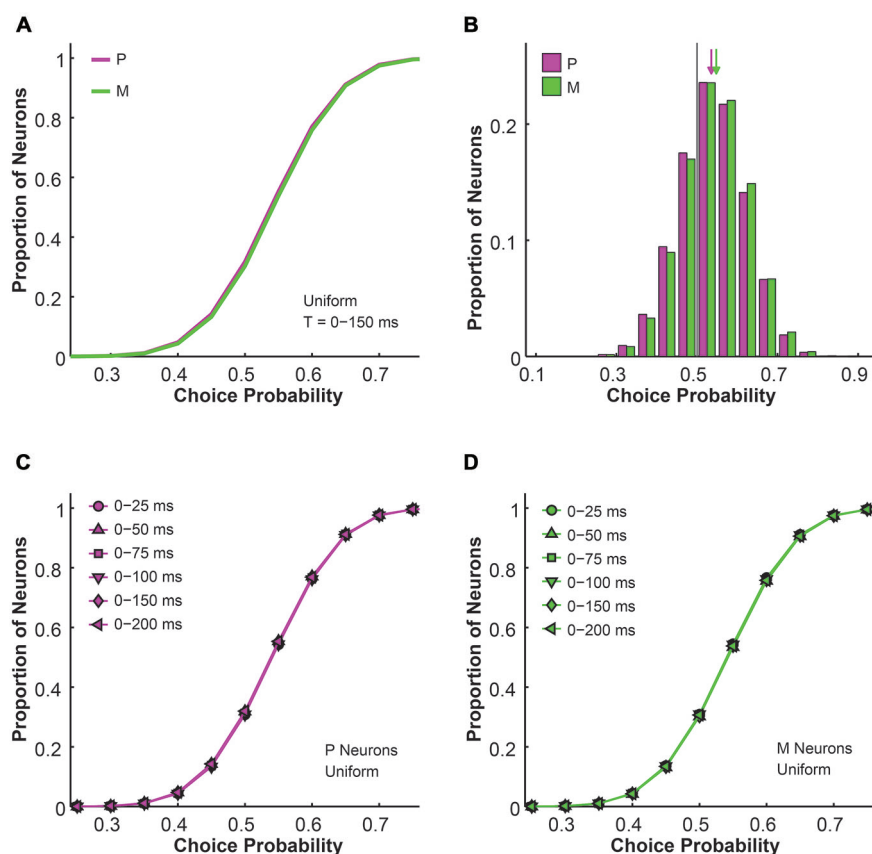


FIGURE 3 | The simulated choice probabilities of P and M neurons for different pool sizes (n) and integration time windows (t). (A) Cumulative choice probability distributions for P (magenta) and M (green) neurons in 200 simulations ($n = 512$ neurons, $t = 0$ – 150 ms). **(B)** Choice probability distributions for P (magenta) and M (green) neurons in 200 simulations ($n = 512$ neurons, $t = 0$ – 150 ms). Arrow: mean choice probability; solid line: choice probability = 0.5. **(C)** Cumulative choice probability distributions for P (magenta) neurons in different integration time windows ($n = 512$ neurons, 200 simulations for each integration time). **(D)** Cumulative choice probability distributions for M (green) neurons in different integration time windows ($n = 512$ neurons, 200 simulations for each integration time).

mean difference, d -prime 2 = $-4.67 \pm 0.43\%$ contrast; $P < 0.05$, Tukey's HSD tests for multiple comparisons). The mean amplitude weighted schemes and the amplitude per trial weighted scheme all failed to perform as well as the uniform pooling scheme in terms of the threshold (mean difference, mean amp 1 = $2.59 \pm 0.21\%$ contrast; mean difference, mean amp 2 = $3.31 \pm 0.23\%$ contrast; mean difference, amp/trial = $7.51 \pm 0.28\%$ contrast; $P < 0.05$, Tukey's HSD tests for multiple comparisons). Additionally, the two subtypes of mean amplitude weighted models did not differ from each other in terms of their simulated thresholds, and the two subtypes of d -prime weighted models did not differ from each other either ($P > 0.05$, Tukey's HSD tests for multiple comparisons; **Figure 5B**). Finally, the minimal psychophysical threshold achieved by the model also decreased with time in all pooling schemes and plateaued at around 50–75 ms after stimulus onset ($n = 1$ – 512 neurons, minimal threshold = 2–3% contrast; **Figure 5C**). Taken together, **Figures 5A–C** demonstrated that the d -prime weighted pooling strategies were the most optimal in terms of the simulated psychophysical performance, and this advantage over other

pooling strategies was the most apparent in short integration time windows (25–50 ms).

Next, we compared the simulated choice probabilities in these different pooling schemes. First, in a fixed time window ($t = 0$ – 150 ms), the overall choice probability distributions for simulated P or M neurons did not differ significantly among the pooling schemes ($n = 512$ neurons, $P > 0.05$, 1-way ANOVAs; **Figures 5D,E**). Second, these choice probability distributions did not change with time in the case of the amplitude per trial and mean amplitude weighted models ($n = 512$ neurons, $P > 0.05$, 2-way ANOVAs main effect for time; **Figures 5F,G**). In the d -prime weighted pooling model, however, the choice probability distributions did shift significantly as the integration time window was extended ($n = 512$ neurons, $F = 4.86$, $P = 0.00$, 2-way ANOVA main effect for time; **Figure 5H**), corresponding well to the temporal dynamics of choice probability that were experimentally measured in LGN P and M neurons (see Figure 7 of Jiang et al., 2015).

Finally, we investigated the overall performance of different pooling strategies by comparing their GoF indices in different time windows. We found that for all pooling schemes the overall

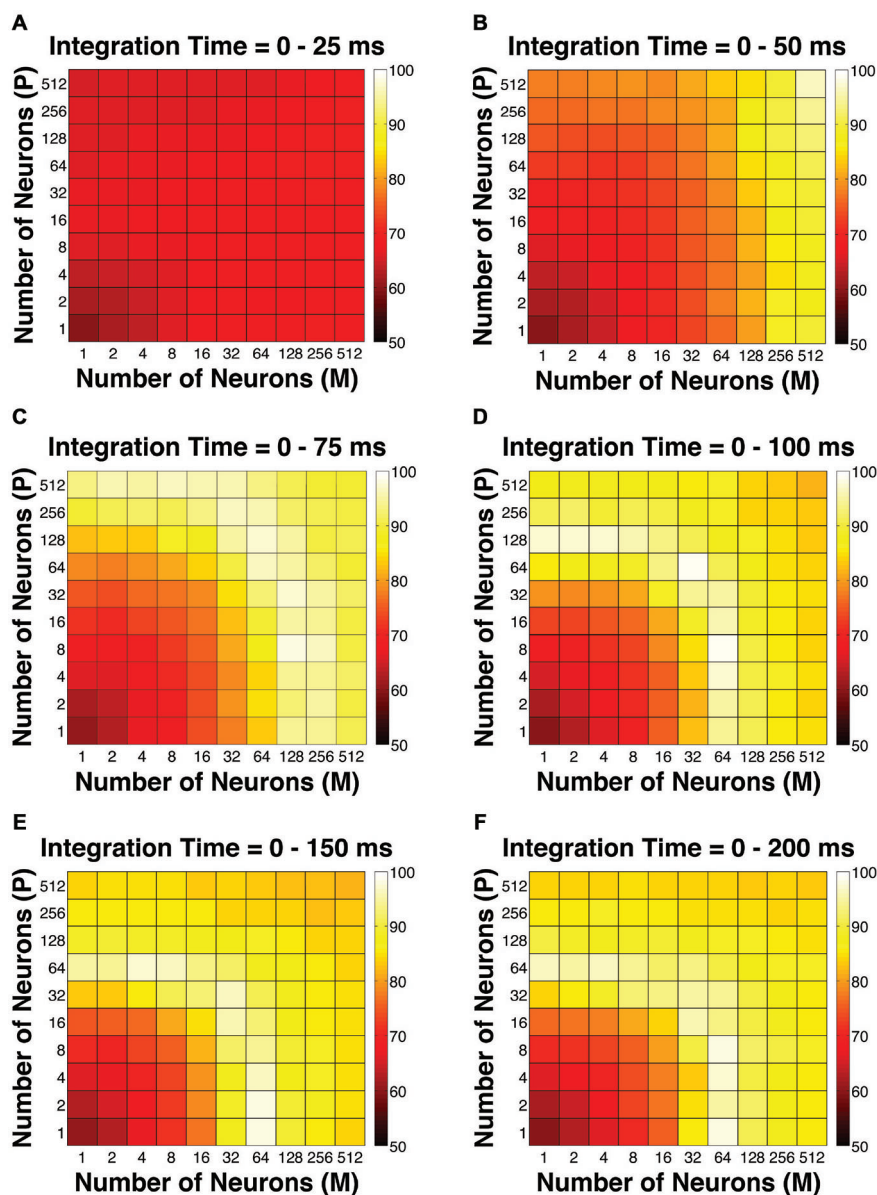


FIGURE 4 | The Goodness-of-Fit (GoF) indices for different pool sizes (n) and integration time windows (t). A GoF of 100% (white) indicates that the model perfectly matches the observed psychometric threshold as well as choice probabilities for both P and M neurons. (A) $t = 0-25$ ms. (B) $t = 0-50$ ms. (C) $t = 0-75$ ms. (D) $t = 0-100$ ms. (E) $t = 0-150$ ms. (F) $t = 0-200$ ms. (A,B) and (D-F) were adapted from Jiang et al. (2015).

model performance improved with time ($n = 1-512$ neurons, $F = 223.78$, $P = 0.00$, 2-way ANOVA main effect for time). Furthermore, there was a significant difference in overall fitness among different pooling schemes ($F = 19.06$, $P = 0.00$, 2-way ANOVA main effect for pooling strategy), and this difference changed across time ($F = 2.75$, $P = 0.00$, 2-way ANOVA interaction effect; **Figure 6A**). Next, we examined whether the alternative pooling strategies improved upon the performance of the uniform pooling model. We found that, again, only the d-prime weighted schemes consistently improved the overall fitness of the model (mean difference, d-prime $1 = 1.10 \pm 0.15\%$

GoF; mean difference, d-prime $2 = 0.95 \pm 0.15\%$ GoF; $P < 0.05$, Tukey's HSD tests for multiple comparisons). The mean amplitude weighted schemes did not differ significantly from the uniform pooling scheme (mean difference, mean/amp $1 = -0.64 \pm 0.25\%$ GoF; mean difference, mean/amp $2 = -0.79 \pm 0.26\%$ GoF; $P > 0.05$, Tukey's HSD tests for multiple comparisons), whereas the amplitude per trial weighted scheme failed to perform as well as the uniform pooling scheme (mean difference, amp/trial $1 = -2.49 \pm 0.29\%$ GoF; $P < 0.05$, Tukey's HSD test for multiple comparisons). Additionally, the two subtypes of mean amplitude weighted models did not differ from each other in

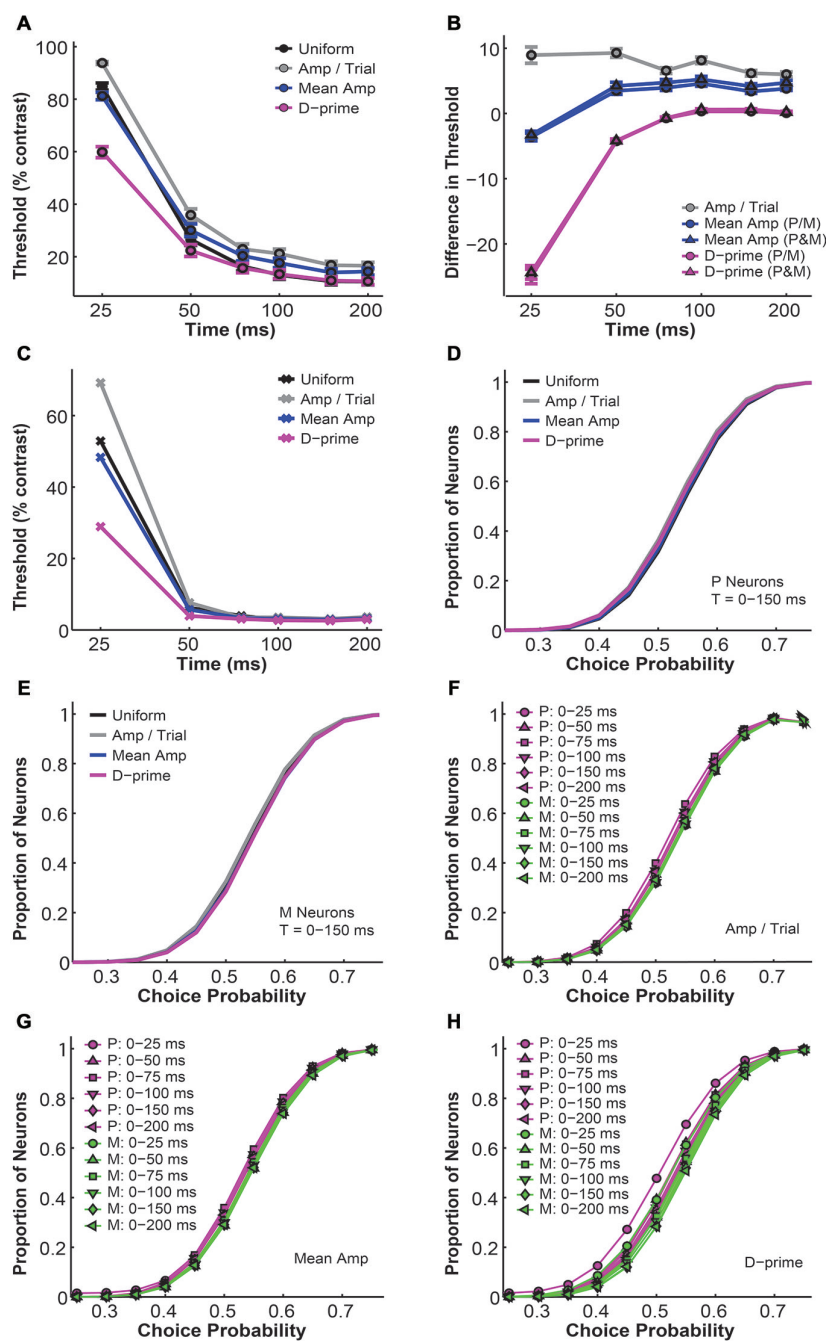


FIGURE 5 | A comparison of threshold and choice probability values derived from different pooling schemes. (A) The average psychometric threshold decreased with time in all pooling schemes ($n = 1-512$ neurons). Black: uniform pooling (as above); gray: every neuron weighted by its response amplitude in every trial; blue: every neuron weighted by its average response amplitude at high contrast (80–99%); error bar: mean $\pm$ SEM. **(B)** Different pooling schemes yielded significantly different psychometric thresholds when compared with the uniform pooling model ($n = 1-512$ neurons). Gray: every neuron weighted by its response amplitude in every trial; blue with circle: every neuron weighted by its average response amplitude at high contrast (80–99%), with P and M neurons weighted separately in reference to their respective maximal responses; blue with triangle: every neuron weighted by its average response amplitude at high contrast (80–99%), with P and M neurons weighted together in reference to one maximal response; magenta with circle: every neuron weighted by its d-prime value at high contrast (80–99%), with P and M neurons weighted separately in reference to their respective maximal d-primes; magenta with triangle: every neuron weighted by its d-prime value at high contrast (80–99%), with P and M neurons weighted together in reference to one maximal d-prime; y axis: the difference in psychometric threshold (alternative pooling scheme—uniform, % contrast); error bar: mean $\pm$ SEM. **(C)** The minimal psychometric threshold achieved by the model decreased with time in all pooling schemes. **(D,E)** Cumulative choice probability distributions for P **(D)** and M **(E)** neurons ($n = 512$ neurons, $t = 0-150$ ms) in different pooling schemes. **(F–H)** Cumulative choice probability distributions for P (magenta) and M (green) neurons in different integration time windows ($n = 512$ neurons), with every neuron weighted by its response amplitude in every trial **(F)**, by its average response amplitude **(G)**, or by its d-prime **(H)**.

terms of their GoF indices, and neither did the two subtypes of d-prime weighted models ($P > 0.05$, Tukey's HSD tests for multiple comparisons; **Figure 6B**). Additionally, the maximal GoF achieved by the model also increased with time in all pooling schemes and plateaued at around 50–75 ms after stimulus onset ($n = 1$ –512 neurons, 98–99% GoF; **Figure 6C**). Finally, the number of neurons needed to achieve the maximal GoF decreased with time in all pooling schemes, but only the uniform and the d-prime weighted pooling schemes were able to achieve maximal fitness (98–99% GoF) with fewer than 100 neurons (**Figure 6D**). Taken together, **Figures 6A–D** demonstrated that the d-prime weighted pooling strategies were the most optimal in terms of the overall performance, and this advantage over other pooling strategies was the most apparent in short integration time windows (25–50 ms).

d-prime Weighted Pooling Scheme: Performance

Of all the alternative pooling strategies described above, we were the most interested in the d-prime weighted strategy because of its superior performance. In the next few sections we discuss in detail the performance, structure and properties of the d-prime model. First, in terms of the simulated psychophysical threshold, at extremely short integration time windows (25 ms), the d-prime model failed to achieve good psychophysical performance (i.e., threshold $< 10\%$ contrast) even when it incorporated a large number of neurons from both the P and M populations ($n = 512$ P neurons, 512 M neurons), but incorporating a large number of M neurons ($n = 128$ –512) rather than P neurons was more beneficial to model performance (**Figure 7A**). At relatively brief intervals (50 ms), incorporating a large number of either P or M neurons ($n = 256$ –512) could achieve good psychophysical performance (i.e., threshold $< 10\%$ contrast; **Figure 7B**). At medium to long intervals (75–200 ms), a wider range of M/P neuron combinations ($n = 64$ –512) yielded good model performance (**Figures 7C–F**). Comparing **Figure 7** (d-prime pooling) to **Figure 2** (uniform pooling), it is clear that the d-prime model behaved rather similarly to the uniform pooling model in terms of its psychophysical performance, but there were apparent differences between the two models in the 25 ms and 50 ms time windows. Specifically, the d-prime model achieved much lower psychophysical thresholds than the uniform model in both time windows (25 ms: mean difference = $-25.00 \pm 1.13\%$ contrast, $P = 0.00$, Wilcoxon signed rank test; 50 ms: mean difference = $-4.25 \pm 0.28\%$ contrast, $P = 0.00$, Wilcoxon signed rank test).

In terms of the overall fitness quantified as GoF, at extremely short integration time windows (25 ms), the d-prime model failed to reproduce the observed threshold and choice probabilities even when it incorporated a large number of neurons from both the P and M populations ($n = 512$ P neurons, 512 M neurons; **Figure 8A**). In 50 ms windows, incorporating a large number of either P or M neurons ($n = 256$ –512) could explain the observed threshold and choice probabilities (**Figure 8B**). Finally, at medium to long intervals (75–200 ms), a wider range of M/P neuron combinations ($n = 32$ –256) yielded good model performance, but further increasing the

number of neurons would result in a decrease in model performance (**Figures 8C–F**). Comparing **Figure 8** (d-prime pooling) with **Figure 4** (uniform pooling), it is clear that the temporal evolution of the GoF index for the d-prime model resembled that for the uniform model, but there were apparent differences between the two models in the 50 ms and 75 ms time windows. To be more precise, in the 50 ms window, the d-prime model demonstrated better overall performance than the uniform model (mean difference = $4.85 \pm 0.51\%$ GoF, $P = 0.00$, Wilcoxon signed rank test). In the 75 ms window, in contrast, the overall performance did not differ between the two types of pooling models (mean difference = $0.00 \pm 0.48\%$ GoF, $P = 0.30$, Wilcoxon signed rank test), but the number of neurons needed to achieve good model performance ($>90\%$ GoF) was significantly reduced in the d-prime model (d-prime model: $n = 64$ –256 neurons, uniform model: $n = 128$ –512 neurons).

d-prime Weighted Pooling Scheme: Structure

Next, we examined the relationship between d-primes, weights, and choice probabilities for different cell types within the d-prime pooling model. First, intuitively, as the model was allowed to integrate firing rate information for longer durations, the overall d-prime distributions extended accordingly, for both P and M populations ($F = 87.74$, $P = 0.00$, 2-way ANOVA main effect for time; **Figures 9A,B**). Next, in a fixed time window of medium duration ($n = 512$ neurons, $t = 0$ –150 ms), we compared the d-prime distributions for P and M neurons. In P neurons, the average d-prime was 1.70 ± 0.00 and the median was 1.40. In M neurons, the average d-prime was 1.69 ± 0.00 and the median was 1.69 as well. Even though the average d-primes were similar, the shapes of the distributions differed dramatically between the two cell types, with the P d-prime distribution much more widely spread (P interquartile range = 2.05, M interquartile range = 1.25) and positively skewed (P skewness index = 0.73, M skewness index = 0.12; **Figures 9C,D**). In the same time window ($t = 0$ –150 ms), the pooling weight of each individual neuron was directly determined by its d-prime value, and the weight distributions for P and M neurons were therefore very reminiscent of the their corresponding d-prime distributions in terms of shape. As the pooling weight of a neuron could not exceed 1.0, however, the weight distributions were scaled-down versions of the corresponding d-prime distributions (P weight: mean = 0.29 ± 0.00 , median = 0.23; M weight: mean = 0.44 ± 0.00 , median = 0.43). As a result, the weight distributions for P and M neurons still differed from each other in terms of skewness (P skewness index = 0.74, M skewness index = 0.15), but they were no longer distinguishable in terms of spread (P interquartile range = 0.35, M interquartile range = 0.32; **Figures 9E,F**).

We also analyzed the simulated choice probabilities for P and M neurons in the same time window ($t = 0$ –150 ms), and found that the P and M choice probabilities in the d-prime model (P choice probability = 0.53 ± 0.00 , M choice probability = 0.54 ± 0.00) resembled their experimentally measured counterparts (Jiang et al., 2015, also see above; **Figure 9G**). Furthermore, individual choice probability values were positively correlated

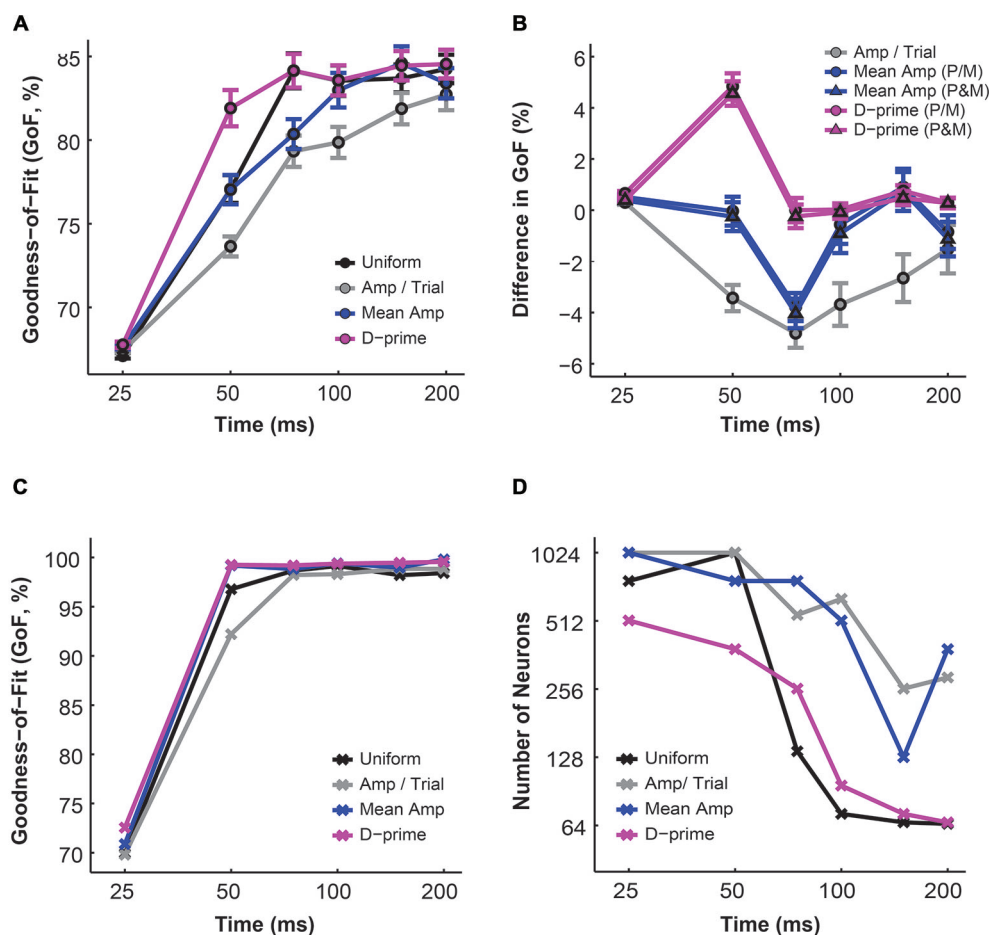


FIGURE 6 | A comparison of the GoF indices derived from different pooling schemes. (A) The average GoF increased with time in all pooling schemes ($n = 1-512$ neurons). Black: uniform pooling (as above); gray: every neuron weighted by its response amplitude in every trial; blue: every neuron weighted by its average response amplitude at high contrast (80–99%); magenta: every neuron weighted by its d-prime value at high contrast (80–99%); error bar: mean $\pm$ SEM. **(B)** Different pooling schemes yielded significantly different GoFs when compared with the uniform pooling model ($n = 1-512$ neurons). Gray: every neuron weighted by its response amplitude in every trial; blue with circle: every neuron weighted by its average response amplitude at high contrast (80–99%), with P and M neurons weighted separately in reference to their respective maximal responses; blue with triangle: every neuron weighted by its average response amplitude at high contrast (80–99%), with P and M neurons weighted together in reference to one maximal response; magenta with circle: every neuron weighted by its d-prime value at high contrast (80–99%), with P and M neurons weighted separately in reference to their respective maximal d-primes; magenta with triangle: every neuron weighted by its d-prime value at high contrast (80–99%), with P and M neurons weighted together in reference to one maximal d-prime; y axis: the difference in GoF (alternative pooling scheme—uniform, % GoF); error bar: mean $\pm$ SEM. **(C)** The maximal GoF achieved by the model increased with time in all pooling schemes. **(D)** The number of neurons needed to achieve the maximal GoF decreased with time in all pooling schemes.

with d-prime values for both P neurons ($r = 0.08$, $P = 0.00$) and M neurons ($r = 0.04$, $P = 0.00$; **Figure 9H**), indicating that the more sensitive LGN neurons were also more correlated with the behavioral choices of the monkeys.

d-Prime Weighted Pooling Scheme: Which One to Choose?

As mentioned above, the d-prime weighted pooling scheme could be further divided into two subtypes depending on whether the P and M populations were weighted separately or together. These two types of d-prime models were indistinguishable from each other in terms of overall fitness, but we were interested in comparing their structures and detailed properties as well as

making inferences as to which model was neurobiologically more meaningful. In the previous section we described the relationship between d-prime, weight, and choice probability in the scenario where P and M neurons were weighted separately according to their respective maximal d-primes, and in this section we perform similar analyses on the alternative d-prime model where P and M neurons were weighted together.

First, as the d-prime value is a direct reflection of the signal-to-noise ratio of single neural responses, it is not surprising that the d-prime distributions remained the same regardless of the pooling strategy (compare **Figures 9C,D** to **10A,B**). Specifically, for the P population, the average d-prime here was 1.70 ± 0.00 and the median was 1.40. For the M population, the average

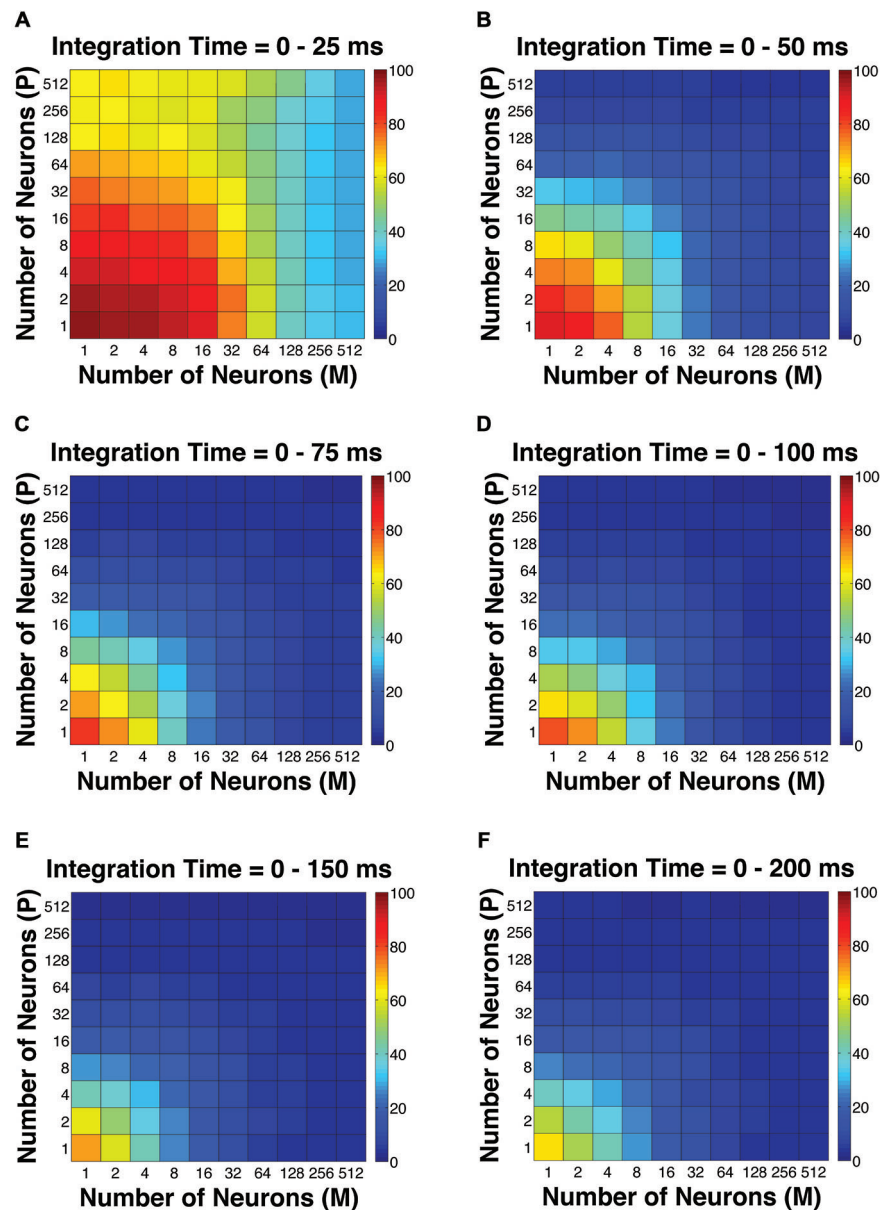


FIGURE 7 | The simulated “psychophysical” thresholds in the d-prime weighted scheme for different pool sizes (n) and integration time windows (t). Red indicates high threshold (100% contrast), whereas blue indicates low threshold (0% contrast). **(A)** $t = 0-25$ ms. **(B)** $t = 0-50$ ms. **(C)** $t = 0-75$ ms. **(D)** $t = 0-100$ ms. **(E)** $t = 0-150$ ms. **(F)** $t = 0-200$ ms.

d-prime here was 1.70 ± 0.00 and the median was 1.70 as well. Additionally, the P and M d-prime distributions differed significantly in their shapes, with the P d-prime distribution much more widely spread (P interquartile range = 2.04, M interquartile range = 1.25) and positively skewed (P skewness index = 0.73, M skewness index = 0.11; **Figures 10A,B**). The weight distributions for P and M neurons in the same time window ($t = 0-150$ ms), however, were very different between the two types of d-prime models (compare **Figures 9E,F** to **10C,D**). Specifically, when P and M neurons were pooled together, as

was the case here, the weight distributions were still scaled-down versions of their corresponding d-prime distributions (P weight: mean = 0.29 ± 0.00 , median = 0.24; M weight: mean = 0.29 ± 0.00 , median = 0.29), but both distributions retained their shape and spread. In other words, the weight distributions for P and M neurons still differed from each other in terms of both spread (P interquartile range = 0.36, M interquartile range = 0.21) and skewness (P skewness index = 0.74, M skewness index = 0.14; **Figures 10C,D**). Two-way ANOVAs confirmed that while the d-prime distributions did not differ

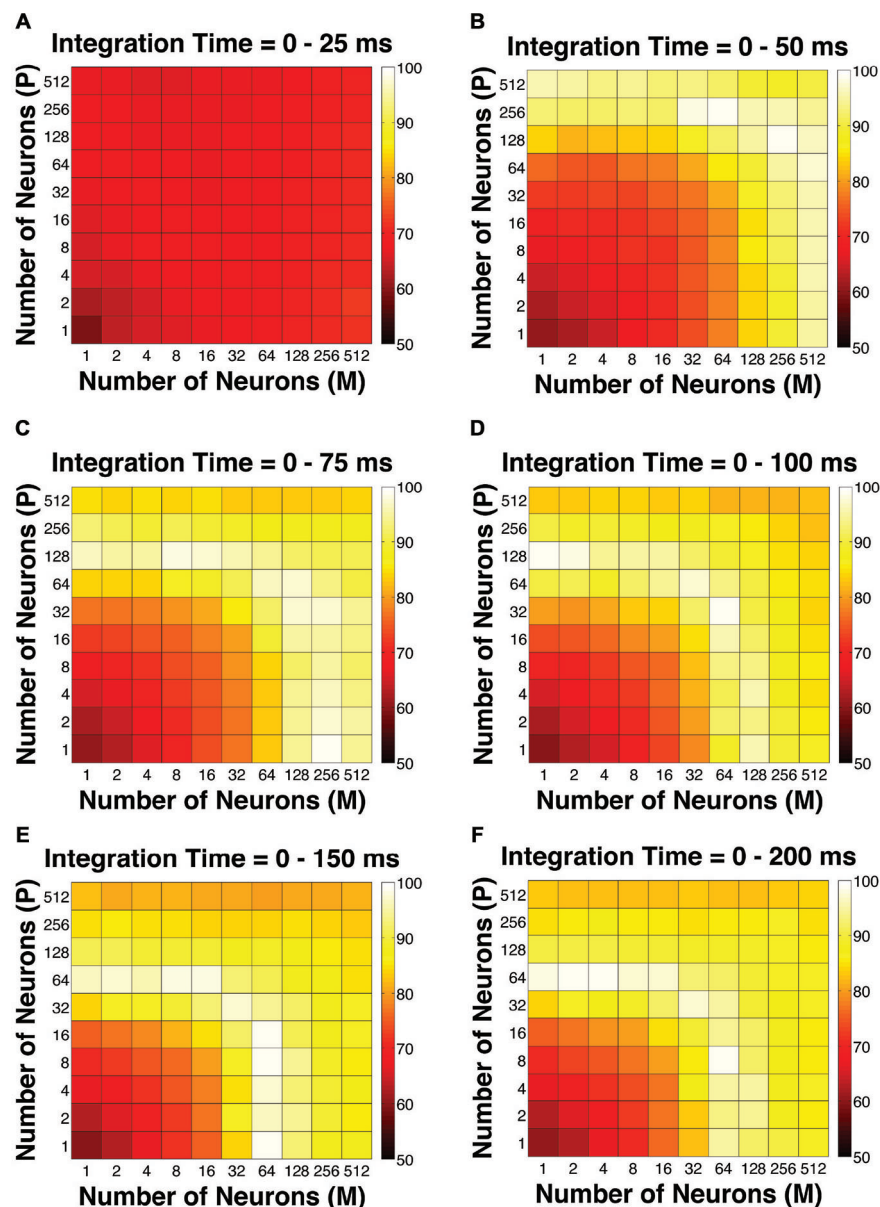


FIGURE 8 | The GoF indices in the d-prime weighted scheme for different pool sizes (n) and integration time windows (t). A GoF of 100% (white) indicates that the model perfectly matches the observed psychometric threshold as well as choice probabilities for both P and M neurons. (A) $t = 0-25$ ms. (B) $t = 0-50$ ms. (C) $t = 0-75$ ms. (D) $t = 0-100$ ms. (E) $t = 0-150$ ms. (F) $t = 0-200$ ms.

($F = 0.01$, $P = 0.91$, 2-way ANOVA main effect for pooling strategy), the weight distributions differed dramatically between the two pooling schemes ($F = 12073.18$, $P = 0.00$, 2-way ANOVA main effect for pooling strategy). This difference in the weight distributions was presumably due to the fact that, compared with M d-prime distributions, P d-prime distributions were more widely spread with greater maximal values. Thus, when P and M populations were scaled together, as was the case here, both were most likely scaled in reference to the d-primes of a subset of P neurons, thus preserving the shapes as well as spreads of these distributions. When P and M populations were scaled separately,

as was the case above, M neurons were scaled to a lesser degree when compared with P neurons, rendering the spreads of the two distributions indistinguishable.

We also analyzed the simulated choice probabilities for P and M neurons in the same time window ($t = 0-150$ ms), and found that in this d-prime model the P and M choice probabilities (P choice probability = 0.53 ± 0.00 , M choice probability = 0.54 ± 0.00) also resembled their experimentally measured counterparts (Jiang et al., 2015, also see above; **Figure 10E**). Furthermore, these P and M choice probability distributions developed throughout the 200 ms stimulus presentation time ($n = 512$ neurons, $F = 3.78$,

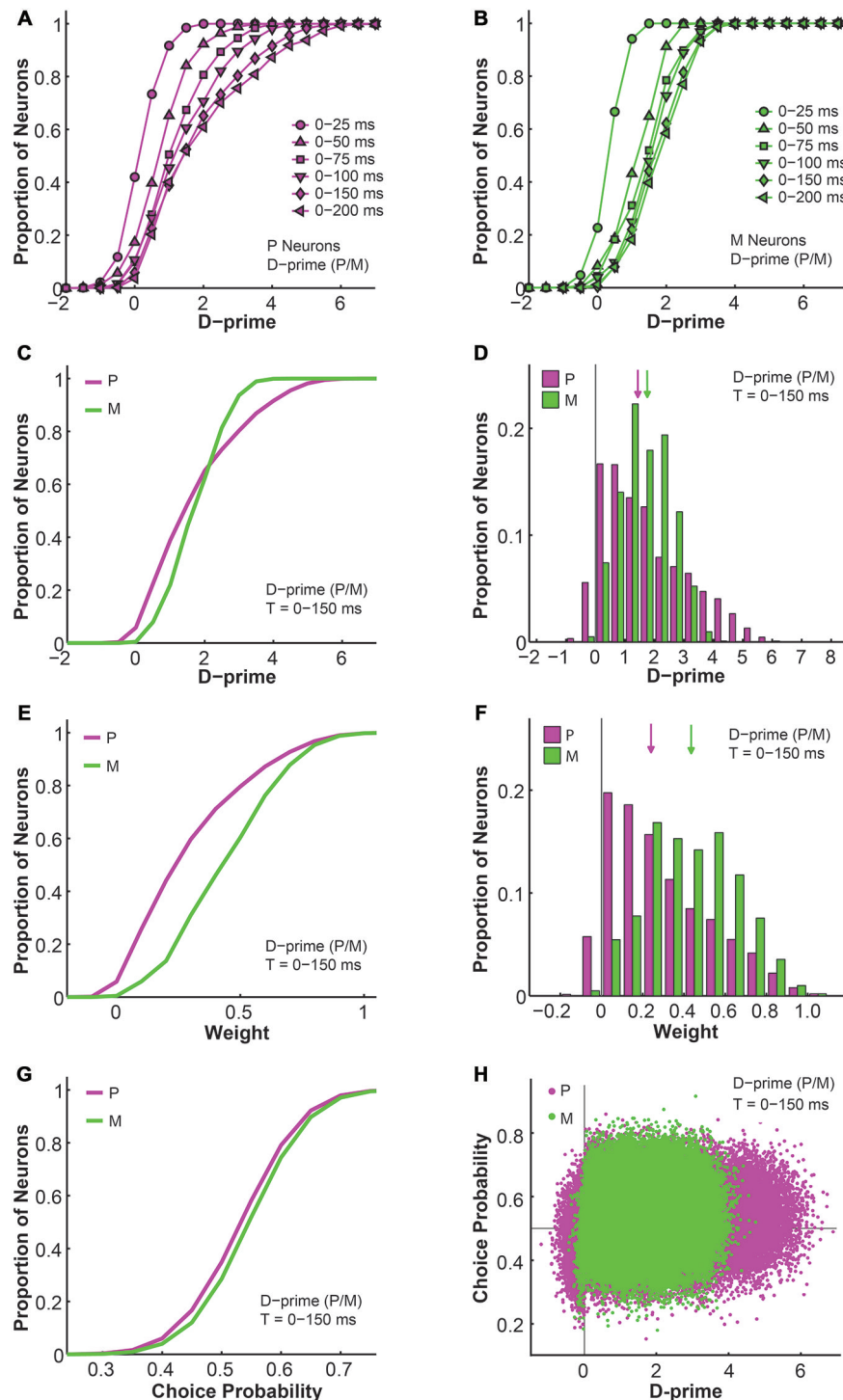


FIGURE 9 | Further analysis of the d-prime weighted pooling scheme: the relationship between d-prime, weight, and choice probability.

(A,B) Cumulative d-prime distributions for P (magenta, A) or M (green, B) neurons in different integration time windows ($n = 512$ neurons). (C) Cumulative d-prime distributions for P (magenta) and M (green) neurons in a 0–150 ms window ($n = 512$ neurons, 200 simulations). (D) D-prime distributions for P (magenta) and M (green) neurons in a 0–150 ms window ($n = 512$ neurons, 200 simulations). Arrow: median d-prime; solid line: d-prime = 0. (E) Cumulative weight distributions for P (magenta) and M (green) neurons in the same 0–150 ms window ($n = 512$ neurons, 200 simulations). (F) Weight distributions for P (magenta) and M (green) neurons in the same 0–150 ms window ($n = 512$ neurons, 200 simulations). Arrow: median weight; solid line: weight = 0. (G) Cumulative choice probability distributions for P (magenta) and M (green) neurons in the same 0–150 ms window ($n = 512$ neurons, 200 simulations). (H) Choice probability values were positively correlated with d-primes ($n = 512$ neurons, $t = 0$ –150 ms, 200 simulations). Horizontal line: choice probability = 0.5; vertical line: d-prime = 0.

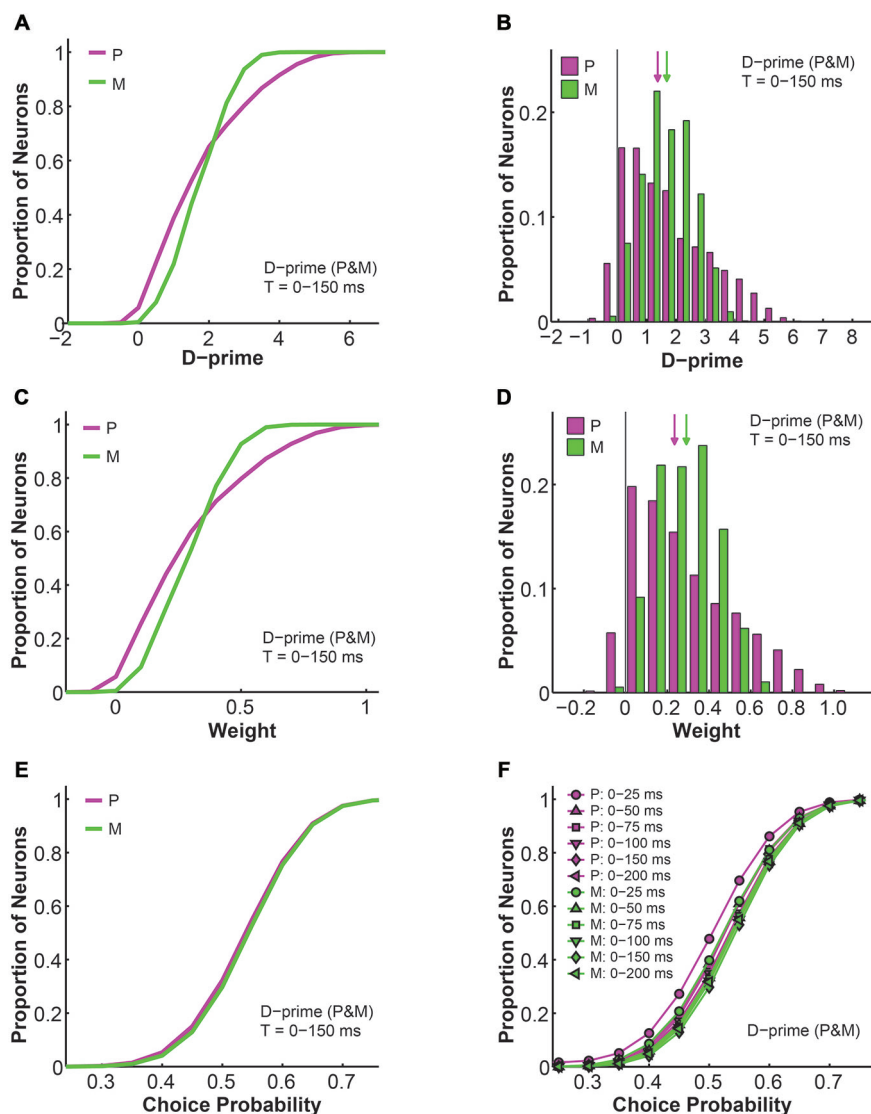


FIGURE 10 | Further analysis of the d-prime weighted pooling scheme: P and M neurons weighted together in reference to one maximal d-prime.

(A) Cumulative d-prime distributions for P (magenta) and M (green) neurons in a 0–150 ms window ($n = 512$ neurons, 200 simulations). (B) D-prime distributions for P (magenta) and M (green) neurons in a 0–150 ms window ($n = 512$ neurons, 200 simulations). Arrow: median d-prime; solid line: d-prime = 0. (C) Cumulative weight distributions for P (magenta) and M (green) neurons in the same 0–150 ms window ($n = 512$ neurons, 200 simulations). (D) Weight distributions for P (magenta) and M (green) neurons in the same 0–150 ms window ($n = 512$ neurons, 200 simulations). Arrow: median weight; solid line: weight = 0. (E) Cumulative choice probability distributions for P (magenta) and M (green) neurons in the same 0–150 ms window ($n = 512$ neurons, 200 simulations). (F) Cumulative choice probability distributions for P (magenta) and M (green) neurons in different integration time windows ($n = 512$ neurons).

$P = 0.00$, 2-way ANOVA main effect for time), much like in the other d-prime model (compare **Figures 5H** to **10F**), and confirming the temporal dynamics that we had observed in the LGN of awake monkeys (Jiang et al., 2015).

Discussion

We previously reported that, in a 2AFC contrast detection task, single LGN P and M neurons demonstrated significant choice probabilities despite their relatively poor neural sensitivities (Jiang et al., 2015). In this study, we examined quantitatively the

effects of the neural pool size, the Fano factor, the interneuronal correlation and the downstream pooling noise on the simulated psychophysical performance and choice probability values. We investigated different pooling/readout schemes that ranged from basic, uniform pools to more optimal pools that preferably weighted the more sensitive single neurons. We compared these pooling strategies in integration time windows of different durations, and found that the most successful model consisted of a medium number of LGN neurons ($n = \sim 30$ –250) in medium to long integration time windows (75–200 ms), with individual neurons weighted differentially according to their d-prime

values. These results indicated that both the psychophysical threshold and the LGN choice probabilities during contrast detection could be fully explained using simple, bottom-up pooling models without assuming significant interneuronal correlations, and that such modeling efforts helped elucidate the complicated relationship between neural sensitivity, readout weight, and choice probability. We now consider the significance of these results in light of previous experimental and theoretical findings.

Pooling/Readout Strategies

The primate LGN provides major feedforward input to the visual cortex, and is an essential thalamic gateway to conscious vision (Sherman and Guillery, 2001; Jones, 2007; Schmid et al., 2010; Casagrande and Ichida, 2011; Saalmann and Kastner, 2011). The effort to understand how the information carried by LGN cells is utilized at later stages is therefore of great importance. Very generally, pooling/readout rules can be divided into two categories. In the first, perceptual decisions are based on signals provided by one or several of the most sensitive sensory neurons (i.e., lower envelope principle; Barlow, 1995). In the second category, perceptual decisions are based on some form of pooled responses from many sensory neurons. The uniform pooling as well as alternative weighted pooling schemes used in this paper all fall into the second category.

The lower envelope principle, however, always remains a theoretical possibility. This is because even in a detection task such as ours, where the sensitivities of most single neurons failed to match the psychophysical sensitivity of the subject, there were still a small but significant proportion (13.5%; Jiang et al., 2015) of single cells that matched or even outperformed the subject. That being said, if the lower envelope principle were true, we would expect a choice probability distribution that is qualitatively different from what was observed in physiological recordings. Briefly, if only a few neurons contribute to a perceptual decision, all of them should demonstrate very significant choice probabilities, with the rest of the entire neural population exhibiting chance choice probabilities (Nienborg et al., 2012; Haefner et al., 2013; but see below for the influence of interneuronal correlation on choice probability). In reality, most cortical recordings have reported a broad distribution of weakly significant choice probabilities (for example, see Britten et al., 1996; Uka and Deangelis, 2004; Liu and Newsome, 2005; Purushothaman and Bradley, 2005; Nienborg and Cumming, 2006; Palmer et al., 2007; Price and Born, 2010; Liu et al., 2013), a result that was confirmed in the LGN (Jiang et al., 2015).

Consequently, our current study as well as a number of other computational studies (Shadlen et al., 1996; Purushothaman and Bradley, 2005; Cohen and Newsome, 2009; Haefner et al., 2013) arrived at the conclusion that an ideal perceptual decision pool consists of not just a few, but rather tens to hundreds of single sensory neurons. In this type of broad decision pool, the readout weight profile, or pooling strategy, of the neural system can be inferred from experimentally measurable quantities such as the behavioral threshold and the choice probability distributions (Haefner et al., 2013; Liu et al., 2013), as demonstrated in the current study.

The d-Prime Weighted Pooling Model

According to signal detection theory (Green and Swets, 1966), d-prime is one of the most useful and widely used descriptors of signal-to-noise ratio. The d-prime model was one of several selective weighted pooling models that we examined in this paper. In this model the readout weight of each neuron was determined by its d-prime value at high contrast, with the neuron with the greatest d-prime value carrying a weight of 1.0. Our simulations showed that the d-prime weighted model provided a parsimonious and complete account of all of our experimental data including the monkeys' psychophysical performance and the population distributions of LGN choice probabilities.

Compared with the simple uniform pooling scheme, the d-prime model was superior in several major ways: (1) The d-prime model achieved lower average and minimal psychophysical thresholds (**Figures 5A–C**), especially in shorter integration time windows; (2) The d-prime model more faithfully reflected the temporal developments of choice probabilities in LGN P and M neurons (**Figure 5H**); and (3) The d-prime model achieved greater average and maximal model fitness (**Figures 6A–C**) with fewer neurons (**Figure 6D**), especially in shorter time windows. Additionally, the d-prime weighted model also demonstrated a clear, direct relationship between choice probability and neural sensitivity (**Figure 9H**), indicating that neurons with higher signal-to-noise ratios were also more correlated with perceptual choices. This correlation was even more pronounced in shorter integration time windows, where fewer neurons demonstrated high signal-to-noise ratios (e.g., d-prime vs. choice probability, $t = 0\text{--}25$ ms: r for P neurons = 0.22, $P = 0.00$; r for M neurons = 0.28, $P = 0.00$. $t = 0\text{--}50$ ms: r for P neurons = 0.18, $P = 0.00$; r for M neurons = 0.18, $P = 0.00$). According to previous theoretical work (Haefner et al., 2013; Moreno-Bote et al., 2014), when choice probabilities and neural sensitivities (i.e., d-primes) exhibit such direct correlations, it is an indication that the pooling/readout strategy is optimal for the task. Last but not least, the uniform pooling scheme assumes that even after extensive practice of a perceptual task, the initial pattern of widespread and diffuse synaptic connections will remain unrefined. In reality, however, perceptual learning is known to dramatically alter the properties of single sensory neurons (Sasaki et al., 2010; Kumano and Uka, 2013; Watanabe and Sasaki, 2015). Therefore, neurobiologically speaking, the d-prime weighted model is also the more plausible solution *in vivo*.

If the d-prime weighted pooling strategy is indeed utilized in the neural system, our simulations make several specific predictions that can be tested in future psychophysical and physiological recordings: (1) A single LGN neuron's d-prime value should be directly correlated with its choice probability (**Figure 9H**), and this correlation should be stronger in shorter integration time windows (see above); (2) A single LGN neuron's d-prime and choice probability values should both develop throughout the stimulus presentation time (see **Figures 5H, 9A,B, 10F**). This prediction was already confirmed in our previous publication (**Figures 7C,D** in Jiang et al., 2015); and

(3) More importantly, if LGN responses are optimally pooled in subsequent stages, humans and monkeys should be able to maintain the same contrast detection performance with stimulus durations as short as 50–75 ms (see **Figures 5A–C**), even though LGN choice probabilities may decrease in such short integration time windows (**Figures 5H, 10F**).

As mentioned above, the d-prime weighted pooling model could be further divided into two types, with one weighing P and M neurons separately according to their respective maximal d-primes, and one weighing P and M neurons together according to one maximal d-prime. These two models were indistinguishable in terms of their overall performance, but they did differ from each other in their readout weight and choice probability distributions. When considered separately, M neurons were significantly more heavily weighted than P neurons (**Figure 9F**). In contrast, when weighted together, M neurons only had a very slight advantage over P neurons (**Figure 10D**). Computationally, we could not rule out one model in favor of the other. Neurobiologically, the former scenario is more likely to occur only in layer 4 of V1, where LGN P and M inputs remain segregated (Casagrande and Xu, 2004; Nassi and Callaway, 2009). The latter readout scheme, in contrast, is more likely to occur everywhere else in the cortex, where LGN P and M inputs are mixed and integrated.

The Limitations of the Pooling Models

First, to appropriately interpret our modeling results, it is important to understand the role of interneuronal correlation in perceptual decision making. In medium-sized decision pools such as ours, interneuronal noise correlations can strongly influence not only the choice probability structure, but also the readout weight distribution (Chen et al., 2006; Haefner et al., 2013). In cortex, interneuronal correlations are considered to be mostly unavoidable (Averbeck et al., 2006; Cohen and Kohn, 2011) because of the extensively shared connections between neurons (Zohary et al., 1994; Shadlen and Newsome, 1998; Bair et al., 2001; Reich et al., 2001; Averbeck et al., 2006; Cohen and Maunsell, 2009). Recent studies, however, reported overall interneuronal correlations not different from chance in chronic recordings from large populations of V1 neurons in awake monkeys (Ecker et al., 2010, 2014). Compared to the visual cortex, neural circuitry in the LGN is simpler (Casagrande and Norton, 1991; Nassi and Callaway, 2009) and highly specific to cell types (Casagrande and Xu, 2004; Briggs and Usrey, 2011; Ichida et al., 2014). Furthermore, cognitive factors such as attention (Cohen and Maunsell, 2009; Mitchell et al., 2009) and perceptual learning (Gu et al., 2011) are known to decrease the existing interneuronal correlations in a perceptual decision task. It is therefore not entirely surprising that we found LGN interneuronal correlations to be not significantly different from 0.0 during a contrast detection task (Jiang et al., 2015).

Even though we were able to successfully model experimentally measured psychophysical performance and choice probabilities without assuming any significant

interneuronal correlations, we could not rule out the possibility that, in reality, there exist some fine patterns within the LGN interneuronal correlation structure. In fact, recent modeling work has revealed that it is not the average interneuronal correlation level, but the structure of a specific type of differential correlation, that determines choice probability values in a perceptual decision pool (Haefner et al., 2013; Moreno-Bote et al., 2014). Briefly, in cortex, interneuronal correlations are known to be stronger for similarly tuned neurons rather than dissimilarly tuned ones (Zohary et al., 1994; Maynard et al., 1999; Averbeck and Lee, 2003; Gu et al., 2011; Adibi et al., 2013). In this scenario, the neurons at the center of the decision pool could have the largest choice probabilities simply because they are most correlated with all the other neurons in the same pool. In other words, choice probabilities could decrease in the direction of the pool boundaries solely because of the correlation structure, but not the readout weight structure, of the decision pool (Chen et al., 2006; Haefner et al., 2013). This is a possibility that we did not model, and therefore could not rule out for the LGN perceptual decision pool. Furthermore, thalamic interneuronal correlations may be qualitatively different from those measured in the cortex, as LGN neurons sharing the same retinal inputs are known to exhibit very strong temporal correlations in their firing patterns (Alonso et al., 1996; Dan et al., 1998). This is also a possible correlation structure that we did not explore in our models, and as a result we could not rule out its potential influence on the psychophysical sensitivity and choice probabilities of LGN neurons.

Additionally, our pooling models were abstract representations of the minimal computations required to account for our experimental data. These models did not specify and were not critically dependent on, for example, exactly when and where a perceptual “choice” is made *in vivo*. Furthermore, even though we were able to simulate neural pools of infinitely large sizes for a large number of trials, the accuracies of these simulations were constrained by the sample sizes in our original experimental data (Jiang et al., 2015). Finally, although we took into consideration the temporal evolution of a variety of critical factors such as the mean and variance of neural response, the d-prime, and the choice probability, we did not characterize how temporal changes in other parameters such as the interneuronal correlation and the downstream pooling noise might influence model performance. Despite these limitations, we believe that our modeling results clearly and unarguably support the hypothesis that the neural pool consists of not just a few very sensitive neurons but many neurons, likely 100 or more, at the level of the visual thalamus, and that the response fluctuations in these thalamic neurons can influence perception, with the more sensitive neurons exerting a bigger influence on perception.

Author Contributions

YJ, GP and VC conceptualized and designed the study, YJ collected and analyzed the data, YJ and VC interpreted the results, YJ drafted the manuscript, YJ and VC revised the manuscript, YJ, GP and VC approved the final version of the

manuscript and agreed to be accountable for all aspects of the work.

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References

- Adibi, M., McDonald, J. S., Clifford, C. W., and Arabzadeh, E. (2013). Adaptation improves neural coding efficiency despite increasing correlations in variability. *J. Neurosci.* 33, 2108–2120. doi: 10.1523/jneurosci.3449-12.2013
- Alonso, J. M., Usrey, W. M., and Reid, R. C. (1996). Precisely correlated firing in cells of the lateral geniculate nucleus. *Nature*. 383, 815–819. doi: 10.1038/383815a0
- Averbeck, B. B., Latham, P. E., and Pouget, A. (2006). Neural correlations, population coding and computation. *Nat. Rev. Neurosci.* 7, 358–366. doi: 10.1038/nrn1888
- Averbeck, B. B., and Lee, D. (2003). Neural noise and movement-related codes in the macaque supplementary motor area. *J. Neurosci.* 23, 7630–7641.
- Bair, W., Zohary, E., and Newsome, W. T. (2001). Correlated firing in macaque visual area MT: time scales and relationship to behavior. *J. Neurosci.* 21, 1676–1697.
- Barlow, H. B. (1995). “The neuron doctrine in perception,” in *The Cognitive Neurosciences*, ed. M. S. Gazzaniga (Cambridge, MA: The MIT Press), 415–435.
- Barlow, H. B., Levick, W. R., and Yoon, M. (1971). Responses to single quanta of light in retinal ganglion cells of the cat. *Vision Res. Suppl* 3, 87–101. doi: 10.1016/0042-6989(71)90033-2
- Berry, M. J., Warland, D. K., and Meister, M. (1997). The structure and precision of retinal spike trains. *Proc. Natl. Acad. Sci. U. S. A.* 94, 5411–5416. doi: 10.1073/pnas.94.10.5411
- Briggs, F., and Usrey, W. M. (2009). Parallel processing in the corticogeniculate pathway of the macaque monkey. *Neuron*. 62, 135–146. doi: 10.1016/j.neuron.2009.02.024
- Briggs, F., and Usrey, W. M. (2011). Corticogeniculate feedback and visual processing in the primate. *J. Physiol.* 589, 33–40. doi: 10.1113/jphysiol.2010.193599
- Britten, K. H., Newsome, W. T., Shadlen, M. N., Celebrini, S., and Movshon, J. A. (1996). A relationship between behavioral choice and the visual responses of neurons in macaque MT. *Vis. Neurosci.* 13, 87–100. doi: 10.1017/s095252380000715x
- Britten, K. H., Shadlen, M. N., Newsome, W. T., and Movshon, J. A. (1992). The analysis of visual motion: a comparison of neuronal and psychophysical performance. *J. Neurosci.* 12, 4745–4765.
- Casagrande, V. A., and Ichida, J. M. (2011). “Processing in the Lateral Geniculate Nucleus (LGN),” in *Adler’s Physiology of the Eye* 11th edition, eds L. A. Levin, S. F. E. Nilsson, J. Ver Hoeve, S. Wu, P. L. Kaufman and A. Alm (Amsterdam: Elsevier), 574–585.
- Casagrande, V. A., and Norton, T. T. (1991). “The lateral geniculate nucleus: A review of its physiology and function,” in *The Neural Basis of Visual Function*, ed. A. G. Leventhal (London, UK: MacMillan Press), 41–84. doi: 10.1007/springerreference.184100
- Casagrande, V. A., and Xu, X. (2004). “Parallel visual pathways: a comparative perspective,” in *The Visual Neurosciences*, eds L. Chalupa and J. S. Werner (Cambridge, MA: The MIT Press), 494–506.
- Chen, Y., Geisler, W. S., and Seidemann, E. (2006). Optimal decoding of correlated neural population responses in the primate visual cortex. *Nat. Neurosci.* 9, 1412–1420. doi: 10.1038/nn1792
- Cohen, M. R., and Kohn, A. (2011). Measuring and interpreting neuronal correlations. *Nat. Neurosci.* 14, 811–819. doi: 10.1038/nn.2842
- Cohen, M. R., and Maunsell, J. H. (2009). Attention improves performance primarily by reducing interneuronal correlations. *Nat. Neurosci.* 12, 1594–1600. doi: 10.1038/nn.2439
- Cohen, M. R., and Newsome, W. T. (2009). Estimates of the contribution of single neurons to perception depend on timescale and noise correlation. *J. Neurosci.* 29, 6635–6648. doi: 10.1523/jneurosci.5179-08.2009
- Cook, E. P., and Maunsell, J. H. (2002). Dynamics of neuronal responses in macaque MT and VIP during motion detection. *Nat. Neurosci.* 5, 985–994. doi: 10.1038/nn924
- Dan, Y., Alonso, J. M., Usrey, W. M., and Reid, R. C. (1998). Coding of visual information by precisely correlated spikes in the lateral geniculate nucleus. *Nat. Neurosci.* 1, 501–507. doi: 10.1038/2217
- Dodd, J. V., Krug, K., Cumming, B. G., and Parker, A. J. (2001). Perceptually bistable three-dimensional figures evoke high choice probabilities in cortical area MT. *J. Neurosci.* 21, 4809–4821.
- Ecker, A. S., Berens, P., Cotton, R. J., Subramaniam, M., Denfield, G. H., Cadwell, C. R., et al. (2014). State dependence of noise correlations in macaque primary visual cortex. *Neuron*. 82, 235–248. doi: 10.1016/j.neuron.2014.02.006
- Ecker, A. S., Berens, P., Keliris, G. A., Bethge, M., Logothetis, N. K., and Tolias, A. S. (2010). Decorrelated neuronal firing in cortical microcircuits. *Science*. 327, 584–587. doi: 10.1126/science.1179867
- Green, D. M., and Swets, J. A. (1966). *Signal Detection Theory and Psychophysics*. New York, NY: Wiley.
- Grunewald, A., Bradley, D. C., and Andersen, R. A. (2002). Neural correlates of structure-from-motion perception in macaque V1 and MT. *J. Neurosci.* 22, 6195–6207.
- Gu, Y., Deangelis, G. C., and Angelaki, D. E. (2007). A functional link between area MSTd and heading perception based on vestibular signals. *Nat. Neurosci.* 10, 1038–1047. doi: 10.1038/nn1935
- Gu, Y., Liu, S., Fetsch, C. R., Yang, Y., Fok, S., Sunkara, A., et al. (2011). Perceptual learning reduces interneuronal correlations in macaque visual cortex. *Neuron*. 71, 750–761. doi: 10.1016/j.neuron.2011.06.015
- Haefner, R. M., Gerwin, S., Macke, J. H., and Bethge, M. (2013). Inferring decoding strategies from choice probabilities in the presence of correlated variability. *Nat. Neurosci.* 16, 235–242. doi: 10.1038/nn.3309
- Ichida, J. M., and Casagrande, V. A. (2002). Organization of the feedback pathway from striate cortex (V1) to the lateral geniculate nucleus (LGN) in the owl monkey (*Aotus trivirgatus*). *J. Comp. Neurol.* 454, 272–283. doi: 10.1002/cne.10441
- Ichida, J. M., Mavity-Hudson, J. A., and Casagrande, V. A. (2014). Distinct patterns of corticogeniculate feedback to different layers of the lateral geniculate nucleus. *Eye Brain* 6(Suppl. 1), 57–73. doi: 10.2147/EB.S64281
- Jiang, Y., Yampolsky, D., Purushothaman, G., and Casagrande, V. A. (2012). Neural response in the lateral geniculate nucleus (LGN) of awake, behaving monkeys during a contrast detection task: comparison of psychometric and neurometric functions. Abstract retrieved from Society for Neuroscience database (Accession No. 175.115/CC112).
- Jiang, Y., Yampolsky, D., Purushothaman, G., and Casagrande, V. A. (2013). Neural sensitivity in the lateral geniculate nucleus (LGN) of awake, behaving monkeys during a contrast detection task: comparison of neurometric and psychometric functions. *J. Vis.* 13, 1026. Abstract retrieved from Vision Sciences Society. doi: 10.1167/13.9.1026

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- Jiang, Y., Yampolsky, D., Purushothaman, G., and Casagrande, V. A. (2015). Perceptual Decision Related Activity in the Lateral Geniculate Nucleus (LGN). *J. Neurophysiol.* 114, 717–735. doi: 10.1152/jn.00068.2015
- Jones, E. G. (2007). *The Thalamus*. Cambridge, UK: Cambridge University Press.
- Kara, P., Reinagel, P., and Reid, R. C. (2000). Low response variability in simultaneously recorded retinal, thalamic and cortical neurons. *Neuron*. 27, 635–646. doi: 10.1016/S0896-6273(00)00072-6
- Kumano, H., and Uka, T. (2013). Neuronal mechanisms of visual perceptual learning. *Behav. Brain Res.* 249, 75–80. doi: 10.1016/j.bbr.2013.04.034
- Levine, M. W., Cleland, B. G., and Zimmerman, R. P. (1992). Variability of responses of cat retinal ganglion cells. *Vis. Neurosci.* 8, 277–279. doi: 10.1017/S0952523800002923
- Liu, J., and Newsome, W. T. (2005). Correlation between speed perception and neural activity in the middle temporal visual area. *J. Neurosci.* 25, 711–722. doi: 10.1523/JNEUROSCI.4034-04.2005
- Liu, S., Gu, Y., Deangelis, G. C., and Angelaki, D. E. (2013). Choice-related activity and correlated noise in subcortical vestibular neurons. *Nat. Neurosci.* 16, 89–97. doi: 10.1038/nn.3267
- Maynard, E., Hatsopoulos, N., Ojakangas, C., Acuna, B., Sanes, J., Normann, R., et al. (1999). Neuronal interactions improve cortical population coding of movement direction. *J. Neurosci.* 19, 8083–8093.
- McAdams, C. J., and Maunsell, J. H. (1999). Effects of attention on the reliability of individual neurons in monkey visual cortex. *Neuron*. 23, 765–773. doi: 10.1016/S0896-6273(01)80034-9
- Mitchell, J. F., Sundberg, K. A., and Reynolds, J. H. (2009). Spatial attention decorrelates intrinsic activity fluctuations in macaque area V4. *Neuron*. 63, 879–888. doi: 10.1016/j.neuron.2009.09.013
- Moreno-Bote, R., Beck, J., Kanitscheider, I., Pitkow, X., Latham, P., and Pouget, A. (2014). Information-limiting correlations. *Nat. Neurosci.* 17, 1410–1417. doi: 10.1038/nn.3807
- Nassi, J. J., and Callaway, E. M. (2009). Parallel processing strategies of the primate visual system. *Nat. Rev. Neurosci.* 10, 360–372. doi: 10.1038/nrn2619
- Nienborg, H., Cohen, M. R., and Cumming, B. G. (2012). Decision-related activity in sensory neurons: correlations among neurons and with behavior. *Annu. Rev. Neurosci.* 35, 463–483. doi: 10.1146/annurev-neuro-062111-150403
- Nienborg, H., and Cumming, B. (2010). Correlations between the activity of sensory neurons and behavior: how much do they tell us about a neuron's causality? *Curr. Opin. Neurobiol.* 20, 376–381. doi: 10.1016/j.conb.2010.05.002
- Nienborg, H., and Cumming, B. G. (2006). Macaque V2 neurons, but not V1 neurons, show choice-related activity. *J. Neurosci.* 26, 9567–9578. doi: 10.1523/JNEUROSCI.2256-06.2006
- Norton, T. T., and Casagrande, V. A. (1982). Laminar organization of receptive-field properties in lateral geniculate nucleus of bush baby (*Galago crassicaudatus*). *J. Neurophysiol.* 47, 715–741.
- Norton, T. T., Casagrande, V. A., Irvin, G. E., Sesma, M. A., and Petry, H. M. (1988). Contrast-sensitivity functions of W-, X- and Y-like relay cells in the lateral geniculate nucleus of bush baby, *Galago crassicaudatus*. *J. Neurophysiol.* 59, 1639–1656.
- Oram, M., Wiener, M., Lestienne, R., and Richmond, B. (1999). Stochastic nature of precisely timed spike patterns in visual system neuronal responses. *J. Neurophysiol.* 81, 3021–3033.
- Palmer, C., Cheng, S. Y., and Seidemann, E. (2007). Linking neuronal and behavioral performance in a reaction-time visual detection task. *J. Neurosci.* 27, 8122–8137. doi: 10.1523/JNEUROSCI.1940-07.2007
- Parker, A. J., and Newsome, W. T. (1998). Sense and the single neuron: probing the physiology of perception. *Annu. Rev. Neurosci.* 21, 227–277. doi: 10.1146/annurev-neuro.21.1.227
- Price, N. S., and Born, R. T. (2010). Timescales of sensory-and decision-related activity in the middle temporal and medial superior temporal areas. *J. Neurosci.* 30, 14036–14045. doi: 10.1523/JNEUROSCI.2336-10.2010
- Purushothaman, G., and Bradley, D. C. (2005). Neural population code for fine perceptual decisions in area MT. *Nat. Neurosci.* 8, 99–106. doi: 10.1038/nn1373
- Reich, D. S., Mechler, F., and Victor, J. D. (2001). Independent and redundant information in nearby cortical neurons. *Science*. 294, 2566–2568. doi: 10.1126/science.1065839
- Reich, D. S., Victor, J. D., Knight, B. W., Ozaki, T., and Kaplan, E. (1997). Response variability and timing precision of neuronal spike trains *in vivo*. *J. Neurophysiol.* 77, 2836–2841.
- Royal, D. W., Sary, G., Schall, J. D., and Casagrande, V. A. (2006). Correlates of motor planning and postsaccadic fixation in the macaque monkey lateral geniculate nucleus. *Exp. Brain Res.* 168, 62–75. doi: 10.1007/s00221-005-0093-z
- Saalmann, Y. B., and Kastner, S. (2011). Cognitive and perceptual functions of the visual thalamus. *Neuron*. 71, 209–223. doi: 10.1016/j.neuron.2011.06.027
- Sasaki, Y., Nanez, J. E., and Watanabe, T. (2010). Advances in visual perceptual learning and plasticity. *Nat. Rev. Neurosci.* 11, 53–60. doi: 10.1038/nrn2737
- Schmid, M. C., Mrowka, S. W., Turchi, J., Saunders, R. C., Wilke, M., Peters, A. J., et al. (2010). Blindsight depends on the lateral geniculate nucleus. *Nature*. 466, 373–377. doi: 10.1038/nature08956
- Shadlen, M. N., Britten, K. H., Newsome, W. T., and Movshon, J. A. (1996). A computational analysis of the relationship between neuronal and behavioral responses to visual motion. *J. Neurosci.* 16, 1486–1510.
- Shadlen, M. N., and Newsome, W. T. (1998). The variable discharge of cortical neurons: implications for connectivity, computation and information coding. *J. Neurosci.* 18, 3870–3896.
- Sherman, S. M., and Guillery, R. W. (2001). *Exploring the thalamus*. San Diego, CA: Academic.
- Tolhurst, D. J., Movshon, J., and Dean, A. (1983). The statistical reliability of signals in single neurons in cat and monkey visual cortex. *Vision Res.* 23, 775–785. doi: 10.1016/0042-6989(83)90200-6
- Uka, T., and Deangelis, G. C. (2004). Contribution of area MT to stereoscopic depth perception: choice-related response modulations reflect task strategy. *Neuron*. 42, 297–310. doi: 10.1016/S0896-6273(04)00186-2
- Uka, T., Tanabe, S., Watanabe, M., and Fujita, I. (2005). Neural correlates of fine depth discrimination in monkey inferior temporal cortex. *J. Neurosci.* 25, 10796–10802. doi: 10.1523/JNEUROSCI.1637-05.2005
- Watanabe, T., and Sasaki, Y. (2015). Perceptual learning: toward a comprehensive theory. *Annu. Rev. Psychol.* 66, 197–221. doi: 10.1146/annurev-psych-010814-015214
- Xu, X., Ichida, J. M., Allison, J. D., Boyd, J. D., Bonds, A. B., and Casagrande, V. A. (2001). A comparison of koniocellular, magnocellular and parvocellular receptive field properties in the lateral geniculate nucleus of the owl monkey (*Aotus trivirgatus*). *J. Physiol.* 531, 203–218. doi: 10.1111/j.1469-7793.2001.0203j.x
- Zohary, E., Shadlen, M. N., and Newsome, W. T. (1994). Correlated neuronal discharge rate and its implications for psychophysical performance. *Nature*. 370, 140–143. doi: 10.1038/371358c0

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Gating of tactile information through gamma band during passive arm movement in awake primates

Weiguo Song^{*†} and Joseph T. Francis[†]

Department of Physiology and Pharmacology, SUNY Downstate Medical Center, Brooklyn, NY, USA

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W. Martin Usrey,
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Reviewed by:

Heather Read,
University of Connecticut, USA
Ya-tang Li,
California Institute of Technology, USA

*Correspondence:

Weiguo Song
weiguo.song@yahoo.com

†Present Address:

Weiguo Song,
Department of Physiology,
Pharmacology and Neuroscience, The
City College of New York,
New York, NY, USA
Joseph T. Francis,
Department of Biomedical
Engineering, Cullen College of
Engineering, University of Houston,
Houston, TX, USA

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To make precise and prompt action in a dynamic environment, the sensorimotor system needs to integrate all related information. The inflow of somatosensory information to the cerebral cortex is regulated and mostly suppressed by movement, which is commonly referred to as sensory gating or gating. Sensory gating plays an important role in preventing redundant information from reaching the cortex, which should be considered when designing somatosensory neuroprosthetics. Gating can occur at several levels within the sensorimotor pathway, while the underlying mechanism is not yet fully understood. The average sensory evoked potential is commonly used to assess sensory information processing, however the assumption of a stereotyped response to each stimulus is still an open question. Event related spectral perturbation (ERSP), which is the power spectrum after time-frequency decomposition on single trial evoked potentials (total power), could overcome this limitation of averaging and provide additional information for understanding the underlying mechanism. To this aim, neural activities in primary somatosensory cortex (S1), primary motor cortex (M1), and ventral posterolateral (VPL) nucleus of thalamus were recorded simultaneously in two areas (S1 and M1 or S1 and VPL) during passive arm movement and rest in awake monkeys. Our results showed that neural activity at different recording areas demonstrated specific and unique response frequency characteristics. Tactile input induced early high frequency responses followed by low frequency oscillations within sensorimotor circuits, and passive movement suppressed these oscillations either in a phase-locked or non-phase-locked manner. Sensory gating by movement was non-phase-locked in M1, and complex in sensory areas. VPL showed gating of non-phase-locked at gamma band and mix of phase-locked and non-phase-locked at low frequency, while S1 showed gating of phase-locked and non-phase-locked at gamma band and an early phase-locked elevation followed by non-phase-locked gating at low frequency. Granger causality (GC) analysis showed bidirectional coupling between VPL and S1, while GC between M1 and S1 was not responsive to tactile input. Thus, these results suggest that tactile input is dominantly transmitted along the ascending direction from VPL to S1, and the sensory input is suppressed during movement through a bottom-up strategy within the gamma-band during passive movement.

Keywords: somatosensory cortex, thalamus, sensory gating, time-frequency representation, Granger causality

INTRODUCTION

Understanding how sensory information is processed in dynamic environments will provide important basic information on neural encoding and for designing realistic sensory prosthetics, such as when, where and how to provide effective sensory feedback without affecting the ongoing action. In recent years the study of, and production of brain machine interfaces (BMIs), has become popular in biomedical engineering. The study of BMIs has also lead to some interesting basic neuroscience research (Jackson et al., 2006; Ganguly and Carmena, 2009; Marsh et al., 2015). Initially most BMIs decoded intention, however increasing efforts are being put into neuroprosthetics that stimulate the brain directly, such as toward repair of damaged neural systems (Kerr et al., 2012; Li et al., 2015), or as sensory inputs (Brockmeier et al., 2011; Li et al., 2013, 2014; Tabot et al., 2014). Our overall strategy on this last front has been to recreate cortical neural responses to touch by directly stimulating the VPL thalamus or somatosensory cortex. Thus, knowing how passive and active movements change the neural representation will become key for our system to produce the appropriate cortical neural response under these different types of movement. Toward this goal we present here work from passive movement.

Sensory inputs are initiated from peripheral receptor and transmitted through the spinal cord via thalamus to cortex, and sensory information could be regulated at each of these different levels during behavior. Movement could activate peripheral sensory receptors that activate neurons along the somatosensory pathway, however sensory information is commonly suppressed during movement (Chapin and Woodward, 1982; Chapman et al., 1988; Jiang et al., 1991; Urbain and Deschênes, 2007; Song and Francis, 2013), during the preparatory period before movement onset (Nelson et al., 1991; Ogata et al., 2009; Seki and Fetz, 2012), and even during observation of movement (Voisin et al., 2011). Sensory gating can occur at spinal (Ghez and Pisa, 1972; Seki and Fetz, 2012), brainstem (Furuta et al., 2008), and thalamic levels (Aguilar and Castro-Alamancos, 2005; Lavallée et al., 2005; Urbain and Deschênes, 2007), and is stronger during active movement than passive movement (London and Miller, 2012; Seki and Fetz, 2012).

Sensory evoked potentials (SEPs), which are thought to represent postsynaptic potentials from cells in the vicinity of the recording electrodes, are commonly used for evaluation of sensory information processing (Starr and Cohen, 1985; Seki and Fetz, 2012). Traditional SEP calculations are based on the assumption that a stereotyped pattern of phase-locked electrical activity is superimposed onto an independent stationary stochastic process, which is canceled out during averaging. However, the amplitude and latency of the evoked potential are not constant across trials and may depend on the ongoing activity and carry information (Scaglione et al., 2011). To overcome this limitation, event related spectral perturbation (ERSP) analysis was designed by applying time-frequency decomposition to single trials before averaging (Delorme and Makeig, 2004). By calculating the spectral power from either single trial evoked potentials or trial averaged SEP, effects from phase-locked and non-phase-locked responses may be teased out

(Tallon-Baudry et al., 1996). It has been indicated that phase-locked and non-phase-locked responses arise from different sources (Kalcher and Pfurtscheller, 1995; David et al., 2006), thus the dissection of each contribution may help to better understand the mechanism of sensory gating.

Most findings about sensory gating have been based on individual neural structures or from stand-alone observations (Starr and Cohen, 1985; Marlinski et al., 2012; Seki and Fetz, 2012). As there are extensive anatomical and functional interconnections between and within somatosensory areas (Deschênes et al., 1998; Hunnicutt et al., 2014; Kinnischtzke et al., 2014), some of the changes triggered in one region may influence changes in other regions. However, it is still unclear how movement modulates the interactions between regions distributed across sensory-motor circuits and how sensory information is processed by the intrinsic circuit without a behavioral task.

Granger causality (GC) provides an efficient way to probe the causal/directional coupling between two signals, and has been used to test the interactions between two brain structures (Brovelli et al., 2004). To this aim, local field potentials (LFPs) were recorded simultaneously from microelectrode arrays implanted in cortical areas (S1, M1) and the VPL. Neuronal oscillatory activity in each area was assessed after time-frequency decomposition on single trial evoked responses and trial averaged SEP, and the functional connections between areas were assessed by using GC.

MATERIALS AND METHODS

Four rhesus monkeys (A, male 4.1 Kg; J, male 3.9 Kg; K, female 3.7 Kg; N, male 5.3 Kg) were used in these experiments. Care and treatment of the animals during all stages of the experiments were approved by the Division of Laboratory Animal Resources and Institutional Animal Care and Use Committee of SUNY Downstate Medical Center.

Surgical Procedure

Following our detailed procedure for head-post and electrode array implantation (Chhatbar et al., 2010), 96-Platinum-Iridium microelectrode arrays (10 by 10; electrode pitch 400 μ m and electrode length 1.0 or 1.5 mm; Blackrock Microsystems) were pneumatically inserted in left S1/M1 hand representation area, which demonstrated clear response from a sharp probing electrode during touching the right fingers. The M1 and S1 arrays were placed on the bank of pre and post-central sulcus, respectively (**Figure 1B**). To implant the deep VPL array, MRI images were acquired with 3T Siemens scanner before surgery, and were registered onto the atlas of a standard rhesus brain (Frey et al., 2011). With the guidance of the image and the stereotaxic coordination, a 24-channel linear array (LMA, Microprobes Inc.) was inserted in the VPL nucleus of thalamus on the left hemisphere (monkey N; **Figure 1B**). Similar to S1 implantation, the implantation location of VPL array was further confirmed during electrode insertion by using the receptive field mapping technique.

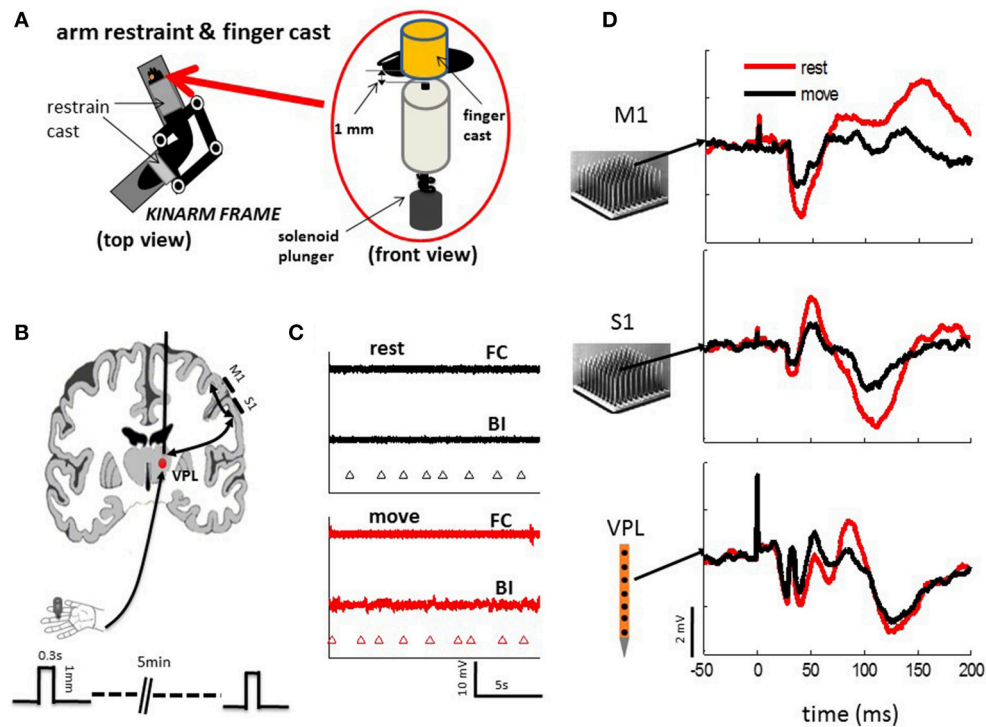


FIGURE 1 | Experimental setups. (A) Monkeys were seated comfortably in a non-human primate chair with their right arm and hand restrained on a KINARM (BKIN Technologies), which allows free movement of the arm in the horizontal plane. The fingers are held in place by cast that allows tactile stimuli to be delivered reliably from a solenoid plunger under both the resting condition and passive movement of the arm. (B) Neuronal activities were recorded from electrode arrays in M1 and S1 (10 by 10, Blackrock Microsystems) or S1 and VPL (24 sites linear array, Microprobes Corn) during tactile stimulation to the finger pad. Three-hundred to six-hundred stimuli were randomly delivered via the tactile stimulator (A) at a mean frequency of 0.5 Hz under resting and passive arm movement. Each tactile stimulus (one stroke of solenoid) was applied for 0.3 s on the skin, which indents the finger pad of either index, middle, or ring finger by 1 mm. (C) EMG activities from the major muscle were (BI, biceps; FC, flexor carpi) recorded during rest and movement. There were no obvious active movements observed during either rest or passive movement. Triangle marker indicate the time of tactile stimulation. (D) Example evoked responses from a single channel of each array in M1, S1, and VPL show from typical session.

Neural Recording

Recordings began 3 weeks after implantation surgery. Neuronal activities were acquired through unity gain head-stages (Plexon Inc.) with a multichannel acquisition processor (MAP, Plexon Inc.). LFPs from different recording areas were acquired simultaneously (M1 and S1 or S1 and VPL) (see example in **Figure 1D**). LFPs were amplified (gain 500–1000), filtered (0.3–200 Hz) and digitized at a sampling frequency of 1 or 2 kHz. Up to 32 channels (every three channel on the 96 array) of LFP in M1 and S1, and up to 23 channels in VPL were acquired. Power line noise (60 Hz) was removed with offline notch filter (iirnotch, Matlab). Previously we reported unit activities in S1 from monkeys A and N (Song and Francis, 2013), while this paper uses different datasets with LFP recordings.

Testing Protocols

The testing procedure was reported previously (Song et al., 2013). In short, monkeys were seated quietly in a primate chair with their right arms restrained to the KINARM exoskeletal robotic system (BKIN Technologies), and the fingers to be tactile stimulated were put in a finger cast, which was modified by drilling a hole through a cylinder plastic tube. A plunger was attached to the bottom side. Then identical tactile stimuli

were randomly delivered by indenting a finger pad (around 1 mm in depth; 0.2–0.3 s duration; 0.5 Hz mean frequency) with a solenoid actuator (plunger diameter: 1 mm; STA-195201, Ladex Inc.; see **Figure 1A**), which was controlled by a PC via a digital card (PCI-6229, National Instruments Inc.). During tactile stimulation the KINARM was either locked in place (rest) or moved slowly and smoothly by an experimenter within the horizontal plane (30 by 40 cm). Sessions of active arm movement were excluded, as they could be felt by the experimenter and validated with electromyography recordings from a few major forearm muscles (**Figure 1C**). Each daily training session consisted of several 5-min tactile stimulation epochs (300–600 epochs each session) of either resting or passively moving. In this paper, only the recordings from the left sides of M1, S1 and VPL, which are contralateral to the tested right hand, are present.

Data Analysis

Time-frequency Representation

Neural response epochs (trials), which were defined as from 100 ms before and 200 ms after each tactile stimulus onset, were recorded during tactile stimulation on the finger pads. After rejection of epochs contaminated with artifacts by visually

checking the baseline neural activity (100 ms before stimulation onset). SEPs were calculated as the average response to tactile stimuli across all epochs. ERSP was calculated from time-frequency decomposition of single trial response (total power) or trial averaged SEP (phase-locked power; Delorme and Makeig, 2004). We further calculated time-frequency decomposition of the single trial response after SEP was removed, which was termed non-phase-locked power (Cohen and Donner, 2013). The difference between total power, non-phase-locked and phase-locked power could help tease out the underlying contributions from phase-locked and non-phase-locked components (Tallon-Baudry et al., 1996). To obtain optimal time-frequency resolution, we applied wavelet decomposition methods. To further reduce the sensitivity of noisy trials, the ERSP was normalized (subtract mean and divided by standard deviation in each whole trial) before averaging across trials (Grandchamp and Delorme, 2011) and then baseline corrected (subtract mean power of baseline and divided by standard deviation of baseline power). To compare the gating effect across areas, each gating map (rest-move) was normalized to the maximum power in the map. The oscillatory activity was assessed at low frequency (beta-band: 14–30 Hz) and gamma-band (30–80 Hz). Then the power spectrum was averaged across sessions to give a grand average (see Figures 2–4).

Granger Causality

GC has been used to research the temporal interactions between brain areas (Brovelli et al., 2004). To study the causal relation between two signals, the time series of these signals are first modeled with bivariate autoregressive process as

$$x(t) = \sum_{i=1}^N a_{11,i}x(t-i) + \sum_{i=1}^N a_{12,i}y(t-i) + E_1(t), \quad (1)$$

$$y(t) = \sum_{i=1}^N a_{21,i}x(t-i) + \sum_{i=1}^N a_{22,i}y(t-i) + E_2(t), \quad (2)$$

where N is the order of the autoregressive model, $a_{11,i}$, $a_{12,i}$, $a_{21,i}$ and $a_{22,i}$ are the regression coefficients, and $E_1(t)$ and $E_2(t)$ are the prediction error with covariance matrix $\Sigma = \begin{pmatrix} \Sigma_{11} & \Sigma_{12} \\ \Sigma_{21} & \Sigma_{22} \end{pmatrix}$. Then Equations (1) and (2) are transformed into frequency domain

$$\begin{pmatrix} X(f) \\ Y(f) \end{pmatrix} = H \begin{pmatrix} E_1(f) \\ E_2(f) \end{pmatrix}, \quad (3)$$

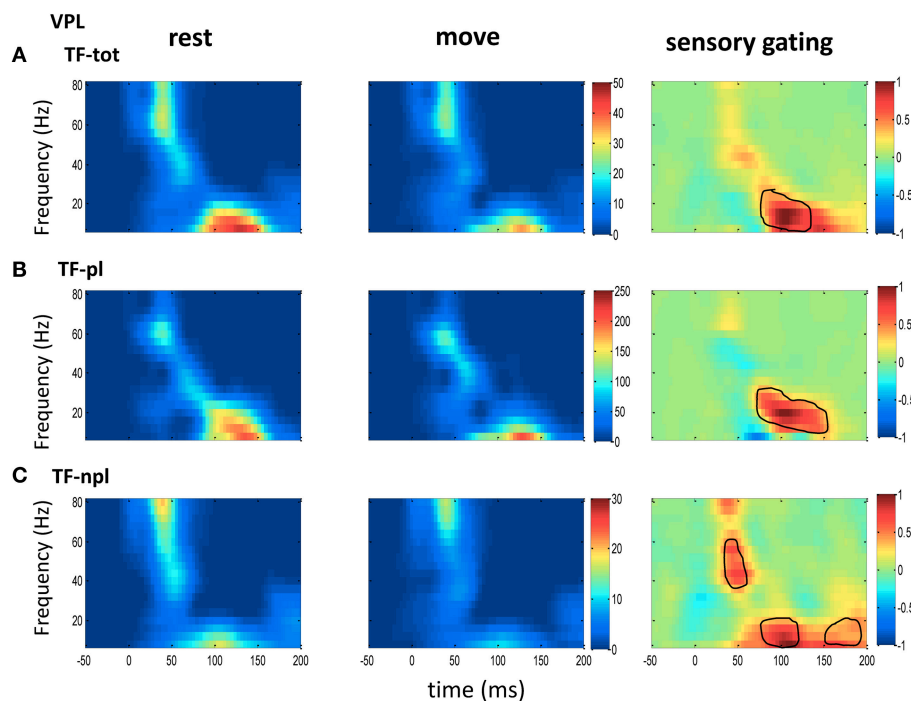


FIGURE 2 | The grand average powers in VPL. (A) Total powers during rest (left) and move (right) show early high gamma oscillations followed by low frequency oscillation. Movement induced sensory gating (right: rest-move) occurs at low frequency bands (right). **(B)** The phase-locked power, which is time-frequency representation on trial-averaged SEPs, shows similar pattern to that of total power but shifted toward low frequency band. **(C)** The non-phase-locked power shows stronger oscillation at gamma band than at low frequency band, and sensory gating presents at both gamma band and low frequency band. Colorbar in each map of rest and move condition represents normalized power (std of baseline); Colorbar of the sensory gating map was normalized to maximum value of the map, and elements within circle represent significantly changed ($p < 0.05$ for signrank test, $n = 10$).

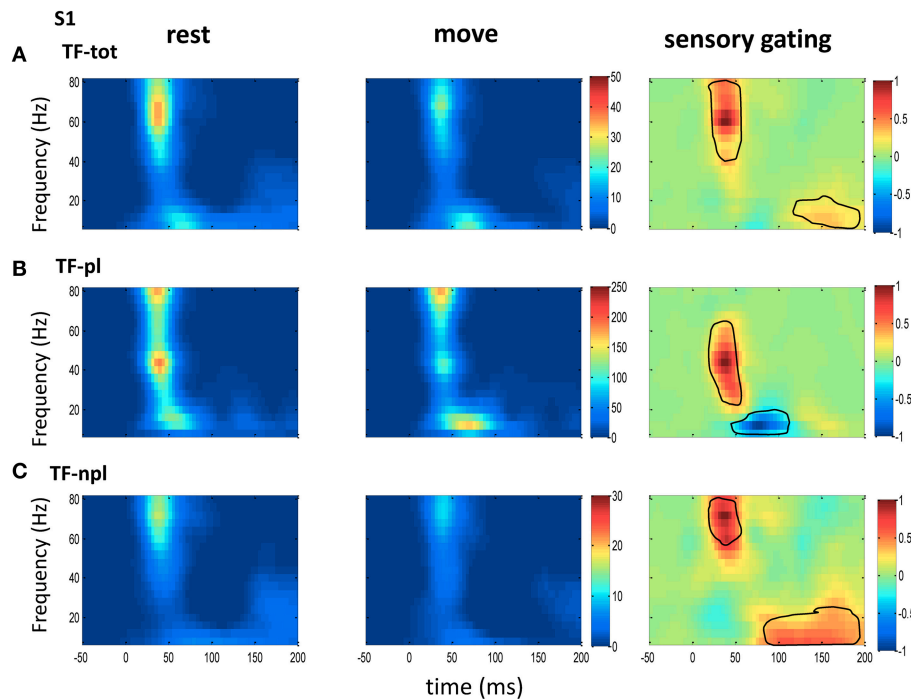


FIGURE 3 | The grand average powers in S1. (A) Total powers during rest (left) and move (right) show early high gamma oscillations followed by weak low frequency oscillations, which starts earlier than that in VPL (**Figure 2**). Movement induced sensory gating (right) is stronger at high gamma band than later low frequency band. **(B)** Compared to that of total power, the phase-locked power shows low frequency shifted pattern, and the sensory gating only at low gamma band while an elevation at low frequency band. **(C)** Non-phase-locked power shows dominant high frequency oscillation, and sensory gating presents strong early high gamma band and weak late low frequency band. The same convention of the colorbar and normalization is used in each map as in **Figure 2** ($p < 0.05$ for signrank test, $n = 26$).

where H is the transfer matrix with $H = \begin{pmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{pmatrix}^{-1}$. Then the spectral matrix of the system can be calculated as

$$S(f) = \langle H(f) \Sigma H^*(f) \rangle \quad (4)$$

where $*$ corresponds to transposition and complex conjugation of $H(f)$. Finally, the GC from y to x is expressed as

$$GC_{y \rightarrow x}(f) = -\ln \left| \left(1 - \left(\Sigma_{22} - \frac{\Sigma_{12}^2}{\Sigma_{11}} \right) |H_{12}(f)|^2 / S_{11}(f) \right) \right|, \quad (5)$$

and x to y as

$$GC_{x \rightarrow y}(f) = -\ln \left| \left(1 - \left(\Sigma_{11} - \frac{\Sigma_{21}^2}{\Sigma_{22}} \right) |H_{21}(f)|^2 / S_{22}(f) \right) \right|. \quad (6)$$

The LFPs from different channels of each recording array were averaged together before use to yield a low-noise representation to build the model. Following the procedure to calculate GC (Brovelli et al., 2004), the ensemble mean of SEP from each recording area was subtracted point-wise from each epoch time series, and then the amplitude was divided by the temporal standard deviation to give equal weight for different recording areas and epochs. GC between two areas was calculated with

the toolbox developed by Seth (Seth, 2010) with spectral autoregressive modeling from BSMART (Cui et al., 2008). The order of the model was chosen based on Akaike information criterion (AIC), which drops monotonically with increased model order. When considering the small decrease in AIC for order higher than 10, a maximum order of 10 was used. A 100 ms window with 4 ms moving step was used in this model. Various lengths of windows and steps were also tested, and the overall results were consistent. The GC at each frequency was normalized to its baseline (100 ms before tactile onset), and represented as ratio change over baseline. To confirm the GC in individual sessions was not from random connections, we cross-validated the GC by using a bootstrap strategy (resampling with replacement from original data while preserving both serial order and causal relations: $n = 500$). The pattern (peaks and latencies at difference frequency bands) of the grand mean map agrees with the pattern in the bootstrapped map over 95% confidence level.

Statistical Analysis

Similar patterns were found across animals, thus all the data from different monkeys was pooled to have a statistical test. Parametric paired or unpaired two sample test (ttest or ttest2, Matlab) was used between different conditions for a normal distribution, and non-parametric rank test (ranksum or signrank, Matlab) was used otherwise. The normality was tested with Jarque-Bera test

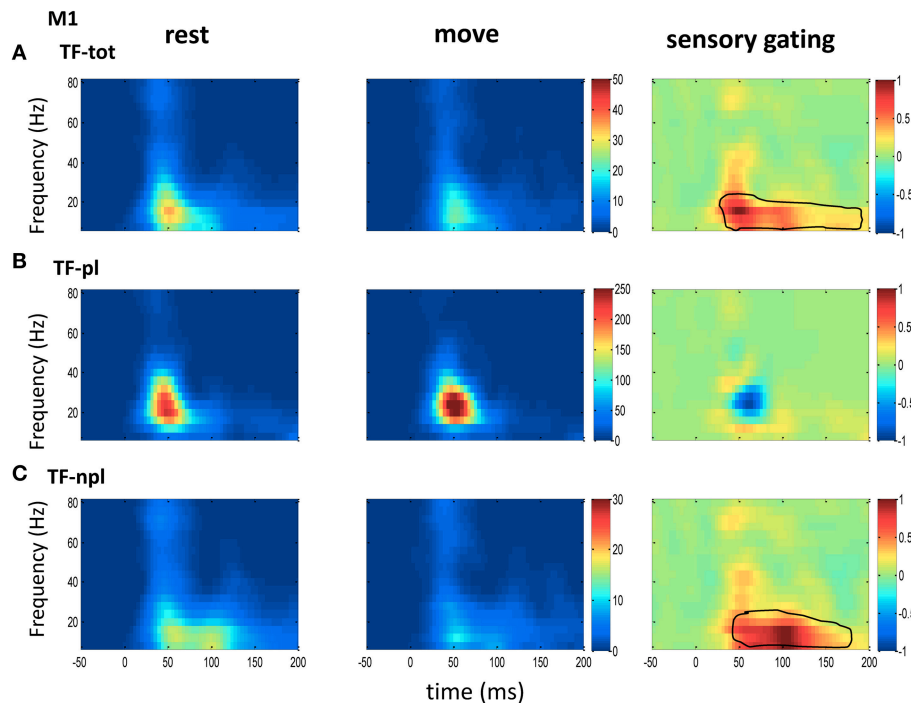


FIGURE 4 | The grand average powers in M1. (A) Different from the powers in sensory areas (VPL of **Figure 2** and S1 of **Figure 3**), total powers during rest (left) and move (middle) in M1 show oscillatory activities dominantly at low frequency band. Movement induced sensory gating is also at low frequency band, which starts earlier than those in sensory areas (VPL and S1). (B) The phase-locked power shows similar pattern to that of total power, but different from the total power, instead of sensory gating, there is a small sensory elevation at low gamma band. (C) Compared the non-phase-locked power with sensory areas, the sensory gating in M1 only presents at low frequency band. The same convention of the colorbar and normalization is used in each map as in **Figure 2** ($p < 0.05$ for signrank test, $n = 16$).

(jbstest, Matlab). The significance level of each test was set at 0.05, unless stated otherwise. In each sensory gating map, area within a circle represents elements significantly different from zero. Only meaningful area, which was defined as more than 200 elements (equivalent to 20 ms by 10 Hz) connected, was drawn. All analyses were performed using Matlab (MathWorks Inc.).

RESULTS

Power spectrum in each area and GC between two simultaneously recorded areas (VPL vs. S1 and M1 vs. S1) were analyzed for each session. A total of 26 sessions (10 from VPL and S1; 16 from M1 and S1) of LFP responses to tactile stimulation were recorded in four quiet awake monkeys. There were around 300–600 epochs under both rest and passive arm movement conditions in each session.

Power Spectrum and Sensory Gating by Movement

To understand the mechanism underlying sensory gating, the grand average of total power, non-phase-locked power and phase-locked power were calculated at each recording area (**Figures 2–4**). The total power showed distinct pattern for each

area and frequency band, and it was modulated by movement in each area. The total power in sensory areas (VPL and S1) showed short bursts of high frequency oscillations (at 45 ms after tactile input), which were followed by low frequency oscillations. M1 was dominated by low frequency oscillations (at 50 ms after tactile input), which encode movement related information (Rickert et al., 2005). The total power was significantly suppressed by movement at gamma band in S1 and at low frequencies across all areas (sensory gating of **Figures 2A, 3A, 4A**). While low frequency oscillations were stronger in M1 than in sensory areas (VPL and S1) during both rest and movement. When comparing the gating of phase-locked and non-phase-locked power, the non-phase-locked power was suppressed in high frequency band following tactile input in VPL (**Figures 2B,C**). In S1, there were immediate suppressions for both phase-locked and non-phase-locked power, while the suppression was in higher frequency band from phase-locked than from non-phase-locked component. Surprisingly, there was a low frequency enhancing in the phase-locked power while suppressing in non-phase-locked power (**Figures 3B,C**). In M1, the gating was from the non-phase-locked power at low frequency band and no significant change was found in the phase-locked power during movement and rest (**Figures 4B,C**). In summary, sensory gating was initiated from gamma band in both non-phase-locked oscillations of VPL and S1 and phase-locked oscillation of S1, and

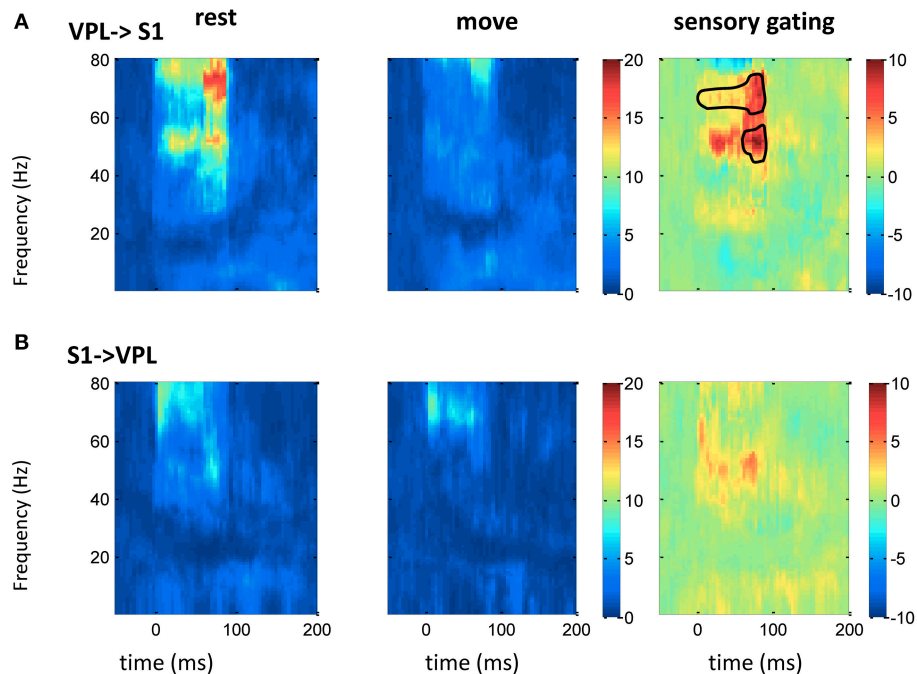


FIGURE 5 | GC between VPL and S1 in frequency domain. After normalizing the GC at each frequency to its baseline (100 ms before tactile onset), the time-frequency GC map shows strong directional coupling from VPL to S1 (**A**) than that from S1 to VPL (**B**) during rest (left) and movement condition (middle). The movement induced sensory gating (right), which is the difference of rest and move, is dominantly at gamma frequency band and is stronger and faster from VPL to S1 than that from S1 to VPL. Each heat-map is the mean time-frequency representation across sessions ($n = 10$). Area within circle represents elements significantly different from zero ($p < 0.05$ for signrank test, $n = 10$).

then followed by non-phase-locked oscillation in M1 and further a low frequency suppression in VPL and S1.

Granger-causality and the Effect of Movement

The above spectrum analyses showed that neural oscillatory activities were regulated differently by movement at different areas. As there exist anatomical and functional connections between these areas, response at these areas might interact each other. Thus, GC between two areas ($n = 16$ between S1 and M1; $n = 10$ between S1 and VPL) was analyzed. Compared with traditional correlation analysis, GC provides directional information, which helps to resolve the temporal relations between the regions. There was bidirectional GC between VPL and S1, which was modulated by sensory input dominantly at gamma band. As expected, the GC from VPL to S1 was stronger than that from S1 to VPL, and movement suppressed the GC (**Figure 5**). The suppression of GC was stronger along the ascending direction than descending direction (right of **Figure 5**). Although both M1 and S1 showed sensory modulated oscillations (**Figures 2–4**), the GC between S1 and M1 was not strongly modulated by the tactile input. Movement significantly (while weakly compared with GC between VPL and S1) increased the GC at low frequency band from S1 to M1 (**Figure 6A**), which might originate from the non-phase-locked low frequency oscillation in M1 and S1 (**Figures 3, 4**). The GC from M1 to S1 was not significantly changed (**Figure 6B**).

DISCUSSION

Phase-locked and Non-phase-locked Sensory Gating Show Frequency Dependency and Area Specificity

Sensory information is regulated during sensorimotor integration in dynamic environments (Ghez and Pisa, 1972; Chapin and Woodward, 1982; London and Miller, 2013), as well as by attention and cognition (Bollimunta et al., 2011), while the underlying mechanisms are not fully understood. The difference between the total power, the phase-locked power and non-phase-locked power at each area suggests that sensory gating may not only come from the phase-locked stereotyped responses, but is also present in the non-phase-locked ongoing activities. The phase-locked and non-phase-locked responses were thought to reflect different neural processes and represent different underlying neuronal mechanisms (David et al., 2006). Sensory gating in motor cortex was only found in the low frequency of the total power not in the phase-locked power, which shows that the non-phase-locked low frequency oscillation plays an important role during even passive movement. This low frequency oscillation (alpha/beta) is commonly observed in sensorimotor cortex and regulated by movement or attention (Bollimunta et al., 2011; Davis et al., 2012), and it is thought to be an indication of an idling state of the brain, but it may also play a role in sensory-motor integration (Başar et al., 1997; Brovelli et al., 2004). On the other hand, high frequency

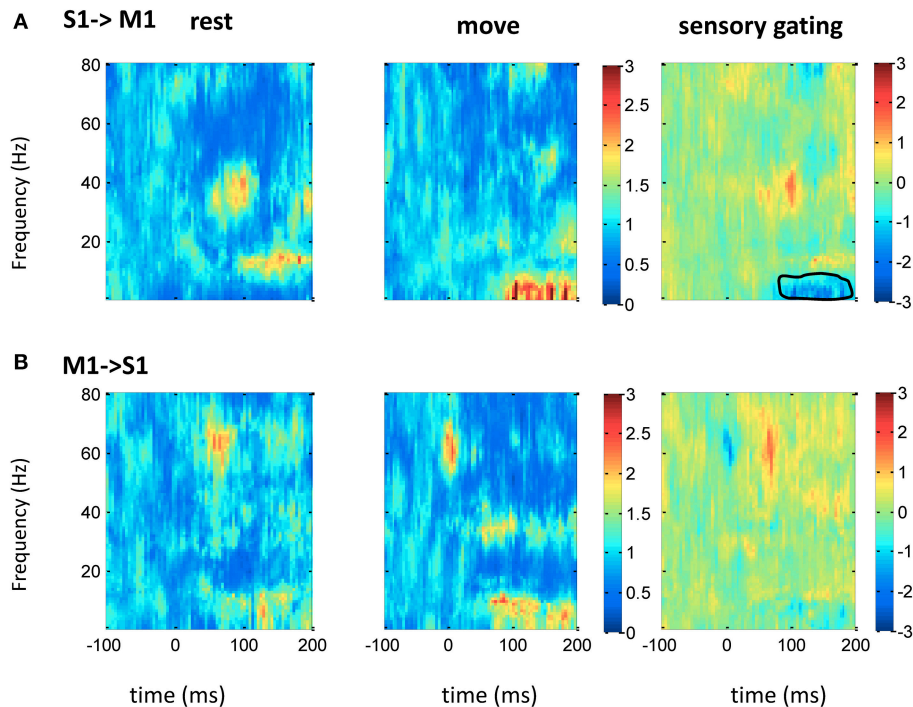


FIGURE 6 | GC between M1 and S1 in frequency domain. After normalizing the GC at each frequency to its baseline (100 ms before tactile onset), the time-frequency GC map does not show clear directional coupling pattern from M1 to S1 (A) or from S1 to VPL (B) during rest (left) and movement condition (middle). Instead of sensory gating, movement significantly increases the GC from S1 to M1 at low frequency band (right). Each heatmap is the mean time-frequency representation across sessions ($n = 16$). The same convention of legend is used as in Figure 5.

gamma oscillations have been linked to perception, stimulus specificity and higher-level cognition (Tallon-Baudry et al., 1996; Schaefer et al., 2006; Haegens et al., 2011). S1 showed strong gamma band gating during movement in both the total power and phase-locked power, while the phase-locked power shifted toward low gamma. This is not surprising, as phase-locked power was calculated from trial averaged SEP, which may have canceled out some high frequency “noise.” In line with what has been found in visual and auditory systems (Bertrand and Tallon-Baudry, 2000; Trautner et al., 2006), the gamma oscillation in sensory areas could also be related with tactile representation or stimulus onset through bottom-up mechanisms of feature binding and to enhance sensory transmission (Paik et al., 2009). This putative feature binding ability in S1 was interrupted or suppressed during movement, and it was mostly through non-phase-locked ongoing oscillations, thus further caused the suppression of GC at gamma band (Figure 5). The presence of both high and low frequency gating in S1 might imply multiple neural populations oscillating at different frequencies, which would allow parallel computation of information within the same region (Crone et al., 2001), or the same population from different feedback loops. The gating of the total power in VPL was dominantly from the phase-locked effect of low frequency band, while there exists non-phase-locked effect at gamma band. This suggests that temporal coding or synchrony could be important during regulation of sensory input in VPL.

Sensory Gating Through Gamma Band Along the Ascending Direction

Sensory gating by movement was observed at different individual areas/levels and under different tasks (Jiang et al., 1991; McCormick and Bal, 1994; Aguilar and Castro-Alamancos, 2005; Urbain and Deschênes, 2007; Furuta et al., 2008; Ogata et al., 2009; Seki and Fetz, 2012), but by using GC we showed that there were only strong directional coupling between sensory areas (S1 vs. VPL). GC along the ascending direction was larger than that along the descending direction. This agrees with the direction of sensory transmission, and corticothalamic projections also demonstrated GC from S1 to VPL. Interestingly, although gamma and low frequency oscillations presented in both VPL and S1, GC was only found in gamma, which indicates that gamma oscillations could bind sensory input across areas. Gamma rhythms are thought to be involved in interregional communication and selection of salient stimuli (Fries, 2009). This frequency dependent sensory information processing was also found recently in the primate visual system, and it was suggested that rhythms of different frequencies act as distinct channels that differentially route top-down and bottom-up signals (Bastos et al., 2015). Movement strongly suppressed directional coupling from VPL to S1, which suggests that sensory gating was from a bottom-up strategy. While the suppression of gamma band power only in S1 not in VPL suggests lateral inhibition might be strongly involved within S1, which also affects sensory processing at VPL (Figure 2). Surprisingly the

directional coupling between S1 to M1 did not show significant modulation to tactile stimulation, and there is a weak while significant enhancement at low frequency band. This might come from the non-phase-locked oscillations at S1 and M1 (Figures 3, 4). The stronger sensory information transmission from S1 to M1 during movement than during rest might arise from the activation of the sensory neurons by movement (Fetz et al., 1980; Soso and Fetz, 1980; Cohen et al., 1994). This suggests that the low frequency oscillation did not bind across the network due to sensory input specifically. The non-tactile modulated directional coupling between M1 and S1 could arise from reciprocal connections between S1 and M1 or from common drive to M1 and S1 from thalamus (Asanuma and Fernandez, 1974). But as there was no simultaneous recording between VPL and M1, the direct interaction between them was not testable in this study. It is worth mentioning that compared with the previous movement related active sensing tasks (London and Miller, 2012; Seki and Fetz, 2012), the passive arm movement task had neither an explicitly motivated motor planning phase or active sensing phase nor any reward or cognition involvement,

thus it represents intrinsic information processing within the circuit, and the modulation most likely comes from low level sensory information processing. GC between M1 and S1 did not modulate to tactile stimulation further suggesting no high level network involvement in our task, thus our sensory gating was dominantly arising from lower level via a bottom-up mechanism.

In conclusion, tactile stimulation evoked oscillatory activities across the sensorimotor loop, while movement suppressed the oscillation either in a phase-locked or non-phase-locked manner dependent on frequency band and area. Tactile information is dominantly transmitted along the ascending direction from VPL to S1, which is regulated during movement through a bottom-up mechanism within the gamma-band.

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REFERENCES

- Aguilar, J. R., and Castro-Alamancos, M. A. (2005). Spatiotemporal gating of sensory inputs in thalamus during quiescent and activated states. *J. Neurosci.* 25, 10990–11002. doi: 10.1523/JNEUROSCI.3229-05.2005
- Asanuma, H., and Fernandez, J. J. (1974). Organization of projection from the thalamic relay nuclei to the motor cortex in the cat. *Brain Res.* 71, 515–522. doi: 10.1016/0006-8993(74)90995-0
- Başar, E., Schürmann, M., Başar-Eroglu, C., and Karakaş, S. (1997). Alpha oscillations in brain functioning: an integrative theory. *Int. J. Psychophysiol.* 26, 5–29. doi: 10.1016/S0167-8760(97)00753-8
- Bastos, A. M., Vezoli, J., Bosman, C. A., Schoffelen, J. M., Oostenveld, R., Dowdall, J. R., et al. (2015). Visual areas exert feedforward and feedback influences through distinct frequency channels. *Neuron* 85, 390–401. doi: 10.1016/j.neuron.2014.12.018
- Bertrand, O., and Tallon-Baudry, C. (2000). Oscillatory gamma activity in humans: a possible role for object representation. *Int. J. Psychophysiol.* 38, 211–223. doi: 10.1016/S0167-8760(00)00166-5
- Bollimunta, A., Mo, J., Schroeder, C. E., and Ding, M. (2011). Neuronal mechanisms and attentional modulation of corticothalamic alpha oscillations. *J. Neurosci.* 31, 4935–4943. doi: 10.1523/JNEUROSCI.5580-10.2011
- Brockmeier, A. J., Choi, J. S., Distasio, M. M., Francis, J. T., and Principe, J. C. (2011). Optimizing microstimulation using a reinforcement learning framework. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2011, 1069–1072. doi: 10.1109/iembs.2011.6090249
- Brovelli, A., Ding, M., Ledberg, A., Chen, Y., Nakamura, R., and Bressler, S. L. (2004). Beta oscillations in a large-scale sensorimotor cortical network: directional influences revealed by Granger causality. *Proc. Natl. Acad. Sci. U.S.A.* 101, 9849–9854. doi: 10.1073/pnas.0308538101
- Chapin, J. K., and Woodward, D. J. (1982). Somatic sensory transmission to the cortex during movement: gating of single cell responses to touch. *Exp. Neurol.* 78, 654–669. doi: 10.1016/0014-4886(82)90082-6
- Chapman, C. E., Jiang, W., and Lamarre, Y. (1988). Modulation of lemniscal input during conditioned arm movements in the monkey. *Exp. Brain Res.* 72, 316–334. doi: 10.1007/BF00250254
- Chhatbar, P. Y., von Kraus, L. M., Semework, M., and Francis, J. T. (2010). A bio-friendly and economical technique for chronic implantation of multiple microelectrode arrays. *J. Neurosci. Methods* 188, 187–194. doi: 10.1016/j.jneumeth.2010.02.006
- Cohen, D. A., Prud'homme, M. J., and Kalaska, J. F. (1994). Tactile activity in primate primary somatosensory cortex during active arm movements: correlation with receptive field properties. *J. Neurophysiol.* 71, 161–172.
- Cohen, M. X., and Donner, T. H. (2013). Midfrontal conflict-related theta-band power reflects neural oscillations that predict behavior. *J. Neurophysiol.* 110, 2752–2763. doi: 10.1152/jn.00479.2013
- Crone, N. E., Boatman, D., Gordon, B., and Hao, L. (2001). Induced electrocorticographic gamma activity during auditory perception. *Brazier Award-winning article, 2001. Clin. Neurophysiol.* 112, 565–582. doi: 10.1016/S1388-2457(00)00545-9
- Cui, J., Xu, L., Bressler, S. L., Ding, M., and Liang, H. (2008). BSMAR: a Matlab/C toolbox for analysis of multichannel neural time series. *Neural Netw.* 21, 1094–1104. doi: 10.1016/j.neunet.2008.05.007
- David, O., Kilner, J. M., and Friston, K. J. (2006). Mechanisms of evoked and induced responses in MEG/EEG. *Neuroimage* 31, 1580–1591. doi: 10.1016/j.neuroimage.2006.02.034
- Davis, N. J., Tomlinson, S. P., and Morgan, H. M. (2012). The role of beta-frequency neural oscillations in motor control. *J. Neurosci.* 32, 403–404. doi: 10.1523/JNEUROSCI.5106-11.2012
- Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134, 9–21. doi: 10.1016/j.jneumeth.2003.10.009
- Deschênes, M., Veinante, P., and Zhang, Z. W. (1998). The organization of corticothalamic projections: reciprocity versus parity. *Brain Res. Rev.* 28, 286–308. doi: 10.1016/S0165-0173(98)00017-4
- Fetz, E. E., Finocchio, D. V., Baker, M. A., and Soso, M. J. (1980). Sensory and motor responses of precentral cortex cells during comparable passive and active joint movements. *J. Neurophysiol.* 43, 1070–1089.
- Frey, S., Pandya, D. N., Chakravarty, M. M., Bailey, L., Petrides, M., and Collins, D. L. (2011). An MRI based average macaque monkey stereotaxic atlas and space (MNI monkey space). *Neuroimage* 55, 1435–1442. doi: 10.1016/j.neuroimage.2011.01.040
- Fries, P. (2009). Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annu. Rev. Neurosci.* 32, 209–224. doi: 10.1146/annurev.neuro.051508.135603
- Furuta, T., Timofeeva, E., Nakamura, K., Okamoto-Furuta, K., Togo, M., Kaneko, T., et al. (2008). Inhibitory gating of vibrissa inputs in the brainstem. *J. Neurosci.* 28, 1789–1797. doi: 10.1523/JNEUROSCI.4627-07.2008
- Ganguly, K., and Carmena, J. M. (2009). Emergence of a stable cortical map for neuroprosthetic control. *PLoS Biol.* 7:e1000153. doi: 10.1371/journal.pbio.1000153
- Ghez, C., and Pisa, M. (1972). Inhibition of afferent transmission in cuneate nucleus during voluntary movement in the cat. *Brain Res.* 40, 145–155. doi: 10.1016/0006-8993(72)90120-5

- Grandchamp, R., and Delorme, A. (2011). Single-trial normalization for event-related spectral decomposition reduces sensitivity to noisy trials. *Front. Psychol.* 2:236. doi: 10.3389/fpsyg.2011.00236
- Haegens, S., Nacher, V., Luna, R., Romo, R., and Jensen, O. (2011). alpha-Oscillations in the monkey sensorimotor network influence discrimination performance by rhythmical inhibition of neuronal spiking. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19377–19382. doi: 10.1073/pnas.1117190108
- Hunnicutt, B. J., Long, B. R., Kusefoglul, D., Gertz, K. J., Zhong, H., and Mao, T. Y. (2014). A comprehensive thalamocortical projection map at the mesoscopic level. *Nat. Neurosci.* 17, 1276–1285. doi: 10.1038/nn.3780
- Jackson, A., Mavoori, J., and Fetzi, E. E. (2006). Long-term motor cortex plasticity induced by an electronic neural implant. *Nature* 444, 56–60. doi: 10.1038/nature05226
- Jiang, W., Chapman, C. E., and Lamarre, Y. (1991). Modulation of the cutaneous responsiveness of neurons in the primary somatosensory cortex during conditioned arm movements in the monkey. *Exp. Brain Res.* 84, 342–354.
- Kalcher, J., and Pfurtscheller, G. (1995). Discrimination between phase-locked and non-phase-locked event-related EEG activity. *Electroencephalogr. Clin. Neurophysiol.* 94, 381–384. doi: 10.1016/0013-4694(95)00040-6
- Kerr, C. C., Neymotin, S. A., Chadderdon, G. L., Fietkiewicz, C. T., Francis, J. T., and Lytton, W. W. (2012). Electrostimulation as a prosthesis for repair of information flow in a computer model of neocortex. *IEEE Trans. Neural Syst. Rehabil. Eng.* 20, 153–160. doi: 10.1109/TNSRE.2011.2178614
- Kinnischtzke, A. K., Simons, D. J., and Fanselow, E. E. (2014). Motor cortex broadly engages excitatory and inhibitory neurons in somatosensory barrel cortex. *Cereb. Cortex* 24, 2237–2248. doi: 10.1093/cercor/bht085
- Lavallée, P., Urbain, N., Dufresne, C., Bokor, H., Acsády, L., and Deschênes, M. (2005). Feedforward inhibitory control of sensory information in higher-order thalamic nuclei. *J. Neurosci.* 25, 7489–7498. doi: 10.1523/JNEUROSCI.2301-05.2005
- Li, K., Dura-Bernal, S., Francis, J. T., Lytton, W., and Principe, J. C. (2015). “Repairing lesions via kernel adaptive inverse control in a biomimetic model of sensorimotor cortex,” in *IEEE 7th International EMBS Conference*. (Montpellier). doi: 10.1109/ner.2015.7146663
- Li, L., Brockmeier, A. J., Choi, J. S., Francis, J. T., Sanchez, J. C., and Principe, J. C. (2014). A tensor-product-kernel framework for multiscale neural activity decoding and control. *Comput. Intell. Neurosci.* 2014:870160. doi: 10.1155/2014/870160
- Li, L., Park, I. M., Brockmeier, A., Chen, B., Seth, S., Francis, J. T., et al. (2013). Adaptive inverse control of neural spatiotemporal spike patterns with a reproducing kernel Hilbert space (RKHS) framework. *IEEE Trans. Neural Syst. Rehabil. Eng.* 21, 532–543. doi: 10.1109/TNSRE.2012.2200300
- London, B. M., and Miller, L. E. (2012). Responses of somatosensory area 2 neurons to actively and passively generated limb movements. *J. Neurophysiol.* 109, 1505–1513. doi: 10.1152/jn.00372.2012
- London, B. M., and Miller, L. E. (2013). Responses of somatosensory area 2 neurons to actively and passively generated limb movements. *J. Neurophysiol.* 109, 1505–1513. doi: 10.1152/jn.00372.2012
- Marlinski, V., Sirota, M. G., and Beloozerova, I. N. (2012). Differential gating of thalamocortical signals by reticular nucleus of thalamus during locomotion. *J. Neurosci.* 32, 15823–15836. doi: 10.1523/JNEUROSCI.0782-12.2012
- Marsh, B. T., Tarigoppula, V. S., Chen, C., and Francis, J. T. (2015). Toward an autonomous brain machine interface: integrating sensorimotor reward modulation and reinforcement learning. *J. Neurosci.* 35, 7374–7387. doi: 10.1523/JNEUROSCI.1802-14.2015
- McCormick, D. A., and Bal, T. (1994). Sensory gating mechanisms of the thalamus. *Curr. Opin. Neurobiol.* 4, 550–556. doi: 10.1016/0959-4388(94)90056-6
- Nelson, R. J., Smith, B. N., and Douglas, V. D. (1991). Relationships between sensory responsiveness and premovement activity of quickly adapting neurons in areas 3b and 1 of monkey primary somatosensory cortex. *Exp. Brain Res.* 84, 75–90.
- Ogata, K., Okamoto, T., Yamasaki, T., Shigeto, H., and Tobimatsu, S. (2009). Pre-movement gating of somatosensory-evoked potentials by self-initiated movements: the effects of ageing and its implication. *Clin. Neurophysiol.* 120, 1143–1148. doi: 10.1016/j.clinph.2009.01.020
- Paik, S. B., Kumar, T., and Glaser, D. A. (2009). Spontaneous local gamma oscillation selectively enhances neural network responsiveness. *PLoS Comput. Biol.* 5:e1000342. doi: 10.1371/journal.pcbi.1000342
- Rickert, J., Oliveira, S. C., Vaadia, E., Aertsen, A., Rotter, S., and Mehring, C. (2005). Encoding of movement direction in different frequency ranges of motor cortical local field potentials. *J. Neurosci.* 25, 8815–8824. doi: 10.1523/JNEUROSCI.0816-05.2005
- Scaglione, A., Moxona, K. A., Aguilar, J., and Foffani, G. (2011). Trial-to-trial variability in the responses of neurons carries information about stimulus location in the rat whisker thalamus. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14956–14961. doi: 10.1073/pnas.1103168108
- Schaefer, A. T., Angelo, K., Spors, H., and Margrie, T. W. (2006). Neuronal oscillations enhance stimulus discrimination by ensuring action potential precision. *PLoS Biol.* 4:e163. doi: 10.1371/journal.pbio.0040163
- Seki, K., and Fetzi, E. E. (2012). Gating of sensory input at spinal and cortical levels during preparation and execution of voluntary movement. *J. Neurosci.* 32, 890–902. doi: 10.1523/JNEUROSCI.4958-11.2012
- Seth, A. K. (2010). A MATLAB toolbox for Granger causal connectivity analysis. *J. Neurosci. Methods* 186, 262–273. doi: 10.1016/j.jneumeth.2009.11.020
- Song, W., and Francis, J. T. (2013). Tactile information processing in primate hand somatosensory cortex (S1) during passive arm movement. *J. Neurophysiol.* 110, 2061–2070. doi: 10.1152/jn.00893.2012
- Song, W., Kerr, C. C., Lytton, W. W., and Francis, J. T. (2013). Cortical plasticity induced by spike-triggered microstimulation in primate somatosensory cortex. *PLoS ONE* 8:e57453. doi: 10.1371/journal.pone.0057453
- Soso, M. J., and Fetzi, E. E. (1980). Responses of identified cells in postcentral cortex of awake monkeys during comparable active and passive joint movements. *J. Neurophysiol.* 43, 1090–1110.
- Starr, A., and Cohen, L. G. (1985). ‘Gating’ of somatosensory evoked potentials begins before the onset of voluntary movement in man. *Brain Res.* 348, 183–186. doi: 10.1016/0006-8993(85)90377-4
- Tabot, G. A., Kim, S. S., Winberry, J. E., and Bensmaia, S. J. (2014). Restoring tactile and proprioceptive sensation through a brain interface. *Neurobiol. Dis.* doi: 10.1016/j.nbd.2014.08.029. [Epub ahead of print].
- Tallon-Baudry, C., Bertrand, O., Delpuech, C., and Pernier, J. (1996). Stimulus specificity of phase-locked and non-phase-locked 40 Hz visual responses in human. *J. Neurosci.* 16, 4240–4249.
- Trautner, P., Rosburg, T., Dietl, T., Fell, J., Korzyukov, O. A., Kurthen, M., et al. (2006). Sensory gating of auditory evoked and induced gamma band activity in intracranial recordings. *Neuroimage* 32, 790–798. doi: 10.1016/j.neuroimage.2006.04.203
- Urbain, N., and Deschênes, M. (2007). Motor cortex gates vibrissal responses in a thalamocortical projection pathway. *Neuron* 56, 714–725. doi: 10.1016/j.neuron.2007.10.023
- Voisin, J. I., Rodrigues, E. C., Hetu, S., Jackson, P. L., Vargas, C. D., Malouin, F., et al. (2011). Modulation of the response to a somatosensory stimulation of the hand during the observation of manual actions. *Exp. Brain Res.* 208, 11–19. doi: 10.1007/s00221-010-2448-3

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Exploring functions for the non-lemniscal auditory thalamus

Charles C. Lee*

Department of Comparative Biomedical Sciences, Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA, USA

The functions of the medial geniculate body (MGB) in normal hearing still remain somewhat enigmatic, in part due to the relatively unexplored properties of the non-lemniscal MGB nuclei. Indeed, the canonical view of the thalamus as a simple relay for transmitting ascending information to the cortex belies a role in higher-order forebrain processes. However, recent anatomical and physiological findings now suggest important information and affective processing roles for the non-primary auditory thalamic nuclei. The non-lemniscal nuclei send and receive feedforward and feedback projections among a wide constellation of midbrain, cortical, and limbic-related sites, which support potential conduits for auditory information flow to higher auditory cortical areas, mediators for transitioning among arousal states, and synchronizers of activity across expansive cortical territories. Considered here is a perspective on the putative and unresolved functional roles of the non-lemniscal nuclei of the MGB.

Keywords: auditory, thalamus, cortex, corticothalamic, thalamocortical, amygdala

INTRODUCTION

The medial geniculate body (MGB) is the main thalamic nucleus associated with audition, receiving direct synaptic inputs from the inferior colliculus (IC; Calford and Aitkin, 1983; Peruzzi et al., 1997; Crabtree, 1998; Wenstrup, 2005), thalamic reticular nucleus (TRN; Crabtree, 1998), and cerebral cortex (Winer et al., 1999, 2001), among other sources (Winer, 1992). Its projections target the cerebral cortex primarily, but also extend to subcortical sites, such as the amygdala (LeDoux et al., 1991; Bordi and LeDoux, 1994) and TRN (Crabtree, 1998; Lee and Imaizumi, 2013). Classically, the MGB can be divided into three main divisions based on cytoarchitectural, connectional, and physiological criteria (Calford and Aitkin, 1983; Calford, 1983; Imig and Morel, 1985; Clerci and Coleman, 1990; Hashikawa et al., 1991; Smith et al., 2012; Imaizumi and Lee, 2015): the ventral (MGBv), dorsal (MGBd), and medial (MGBm) divisions (Winer, 1984; Rouiller et al., 1989); although, further subdivisions are proposed in some species, particularly within the dorsal division (Jones, 2007; Lee and Winer, 2011b).

Among these MGB divisions, the principal, or lemniscal nucleus, the ventral division (MGBv) receives topographically organized projections from the central nucleus of the IC and projects to tonotopically-organized areas of the auditory cortex (AI; McMullen and de Venecia, 1993; Lee et al., 2004a; de la Mothe et al., 2006b; Lee and Winer, 2008a; Llano and Sherman, 2008; Razak and Fuzessery, 2010; Hackett et al., 2011; Smith et al., 2012). In contrast, the dorsal division nuclei are non-tonotopically organized and are connectionally affiliated with corresponding non-tonotopically organized regions of the midbrain (dorsal division of the IC; ICd) and auditory cortex (e.g., secondary auditory cortex (AII); Huang and Winer, 2000; Smith et al., 2012). Finally, and perhaps most enigmatic, the medial division of the MGB receives polymodal

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William Martin Connelly,
Australian National University,
Australia

Reviewed by:

Martha E. Bickford,
University of Louisville, USA
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*Correspondence:

Charles C. Lee
cclee@lsu.edu

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inputs from the IC and projects broadly across many tonotopic, non-tonotopic, multimodal and limbic cortical areas (Lee and Winer, 2008a; Imaizumi and Lee, 2015), terminating notably in cortical layers 1 and 6 (Huang and Winer, 2000) and also in the amygdala (LeDoux et al., 1991).

While the physiological properties of the tonotopic ventral division of the MGB have been intensively investigated (Aitkin and Webster, 1972; Calford and Webster, 1981; Imig and Morel, 1985; Morel and Imig, 1987; Miller et al., 2001, 2002), similar studies of the non-lemniscal MGB nuclei in relation to the ventral division are ongoing (Aitkin, 1973; Calford and Aitkin, 1983; Calford, 1983; Rouiller et al., 1989; Bordi and LeDoux, 1994; Bartlett and Smith, 1999; Edeline et al., 1999; Wenstrup, 1999; He and Hu, 2002; He, 2002; Anderson et al., 2007; Anderson and Linden, 2011; Bartlett and Wang, 2011). Indeed, this is not unique to the auditory system, as the roles of non-primary thalamic nuclei in other systems have generally not been well defined (Sherman and Guillery, 2006; Jones, 2007; Cruikshank et al., 2012). However, we have suggested that some of these non-primary nuclei likely have important roles in the transfer of information to higher auditory cortical centers (Lee and Sherman, 2010a, 2011), while others likely are involved in emotive and affective processing of auditory information (Iwata et al., 1986; Weinberger, 2011). Modern experimental approaches will likely shed light on those thalamic nuclei whose functions have yet to be defined (Cruikshank et al., 2012).

NON-LEMNISCAL AUDITORY THALAMIC NUCLEI AS INFORMATION-BEARING CONDUITS

Many thalamic and cortical projections converge in each auditory cortical area, with the most numerous extrinsic inputs arising from other ipsilateral cortical areas (~80% of the total extrinsic input to each auditory area in the cat, **Figure 1**; Lee and Winer, 2011a). Similar connectional patterns organize auditory regions in many mammalian species, including the monkey (Hackett et al., 1998; de la Mothe et al., 2006a,b), cat (Lee and Winer, 2008a,b,c), bat (Fitzpatrick et al., 1998), rat (Roger and Arnault, 1990; Shi and Cassell, 1997), mouse (Llano and Sherman, 2008; Oh et al., 2014; Takemoto et al., 2014), ferret (Bizley et al., 2005), and gerbil (Budinger et al., 2000; Takesian et al., 2012). Due to the preponderance of such corticocortical convergence, hierarchical cortical models form the basis for many connectional frameworks linking these auditory areas (**Figure 1**; Rouiller et al., 1991; Kaas and Hackett, 2000; Hackett, 2011; Lee and Winer, 2011a,b), similar to those proposed for the visual and somatosensory systems (Felleman and Van Essen, 1991).

The role of the thalamus has generally been disregarded in these hierarchical cortical models beyond that of the primary thalamic nuclei and instead the non-primary nuclei are often assigned a modulatory role (Olshausen et al., 1993). Canonically then, auditory information is often viewed as ascending through the central auditory lemniscal pathway from the cochlea through the brainstem, midbrain (IC), and

thalamus (MGBv) until it reaches the primary auditory area and is subsequently processed through the copious corticocortical network (**Figure 1**). However, as we have noted above, every auditory cortical area receives some fraction of its convergent input from the thalamus (~10% of the total extrinsic input, **Figure 1**; Lee and Winer, 2008a, 2011a,b). Why then should these non-lemniscal thalamic inputs to higher auditory cortical areas have no role in auditory information processing?

Indeed, we have previously argued that, despite their relative minority, these higher-order auditory thalamocortical connections provide an important alternate conduit for conveying information between cortical areas via a *corticothalamocortical* route (**Figure 2**: red pathway; Lee and Sherman, 2010a, 2011). This route originates from layer 5 of a lower-order auditory cortical area (e.g., AI) and terminates non-reciprocally in a higher-order thalamic nucleus (e.g., MGBd; Bartlett et al., 2000; Huang and Winer, 2000; Llano and Sherman, 2008). These layer 5 neurons potentially may branch to innervate motor centers, serving as an efference copy of motor signals to higher auditory centers, as has been similarly suggested for the visual and somatosensory systems (Guillery, 2003; Sherman and Guillery, 2011). The superior colliculus may be the most likely target for such an auditory efference copy (Harting et al., 1992; Chabot et al., 2013), as similar branching of layer 5 CT neurons appears absent to the IC (Wong and Kelly, 1981; Lee et al., 2011).

Completing this circuit, the higher-order thalamic nucleus then projects to a higher order auditory cortical area (e.g., AII; **Figure 2**: red pathway; Lee and Sherman, 2008; Llano and Sherman, 2008). Neuronal projections along this alternate corticothalamocortical route have anatomical and physiological properties suited for high-fidelity neuronal conduits for information processing in the nervous system, which we have previously termed “driver” or “class-1” pathways (Lee and Sherman, 2010a, 2011). The driver-like projections typically exhibit thick axons with giant terminal endings and depressing synapses that activate only ionotropic glutamate receptors (iGluRs; Lee and Sherman, 2010a, 2011). Thus, for example, the layer 5 corticothalamic pathway from AI exhibits thick axons that end in giant terminals in the dorsal division of the MGB (Ojima, 1994; Winer et al., 1999; Llano and Sherman, 2008). Such synapses then, despite their numerical minority, can exert a potent influence on their postsynaptic targets, much like the numerically sparse retinogeniculate projection (Sherman and Guillery, 1998; Winer et al., 1999; Bartlett et al., 2000; Llano and Sherman, 2008). In addition, the thalamocortical pathways from MGBv and MGBd to AI and AII, respectively, both exhibit driver-like, high-probability of release synapses, characterized by a depressing response to paired stimulation that activates only iGluRs, that while weak individually, are highly-reliable and can synchronize to drive receptive field formation in the cortex (Rose and Metherate, 2005; Bruno and Sakmann, 2006; Lee and Sherman, 2008). As such, in this framework, higher-order auditory thalamic nuclei, like the MGBd, are proposed as driver-like conduits for information flowing from lower auditory

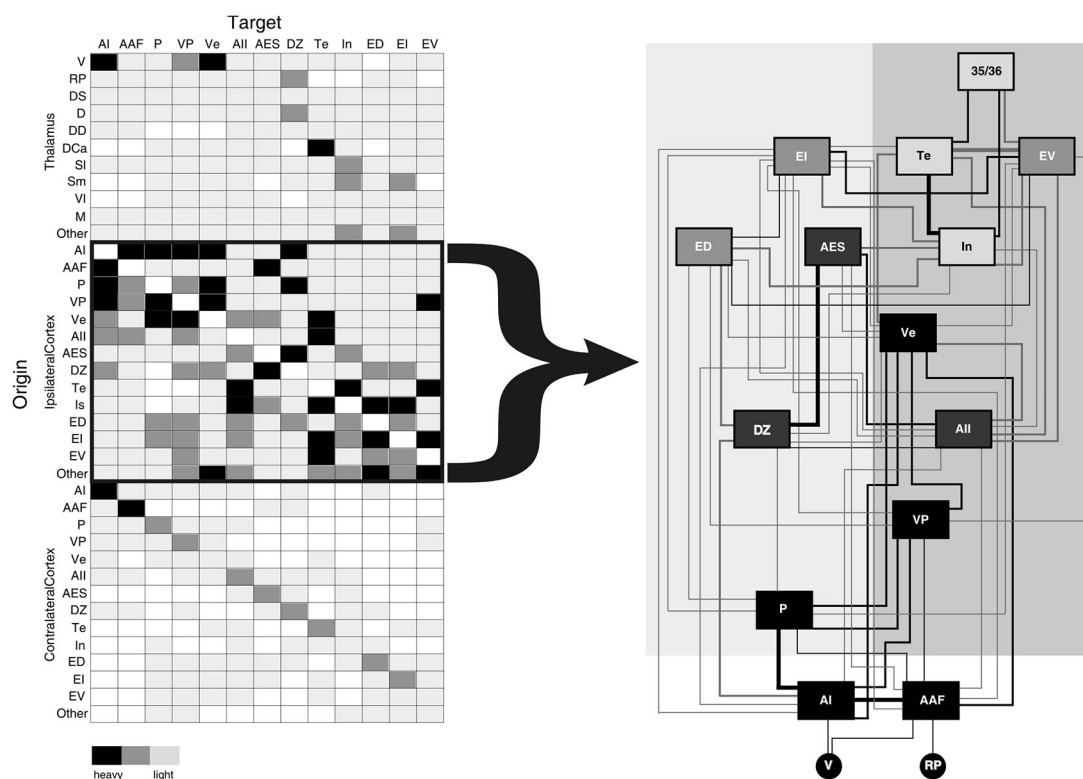
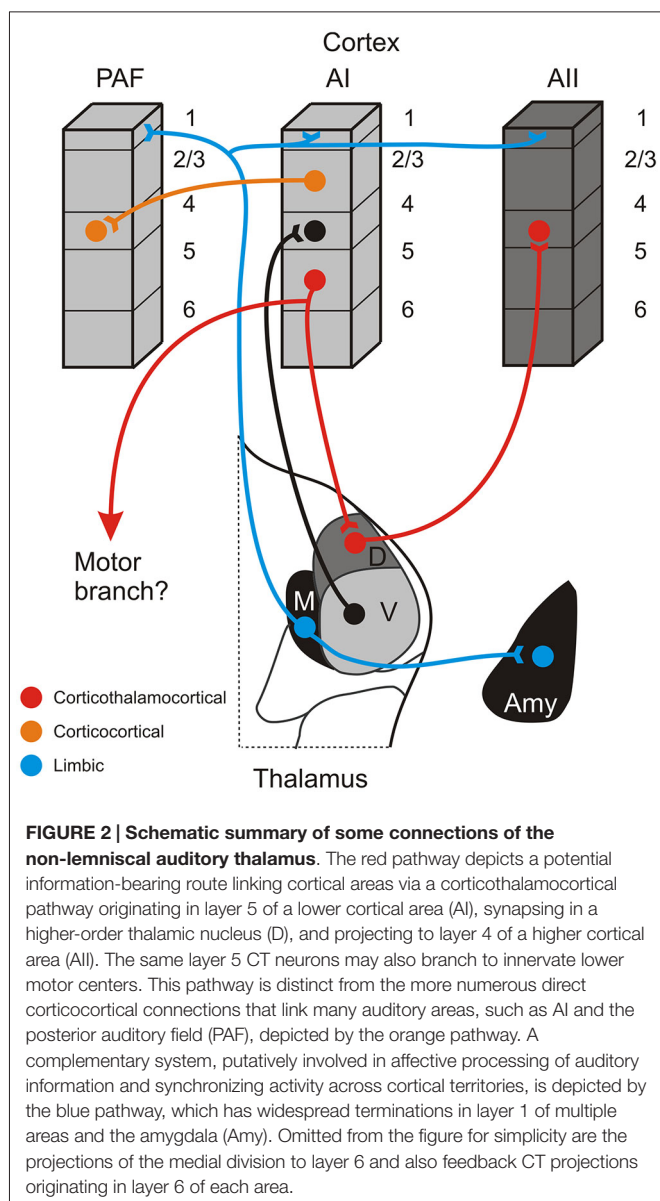


FIGURE 1 | Schematic summary of convergent projections from thalamic and cortical sources to the thirteen areas of the cat auditory cortex (AI). Left panel depicts a simplified schematic of the relative input arising from each thalamic and cortical source based on the percent input to each area. Right panel depicts a serial hierarchical ordering of auditory cortical areas in the cat based on the laminar origins of cortical projections. Such a cortical hierarchy (right panel) reflects the notion that the numerous corticocortical projections (left panel) are the main determinants of information processing in higher cortical areas. Neglected in this view are roles for the several non-primary thalamocortical (as well as the commissural cortical) inputs to each auditory area, which also contribute fewer, yet potentially salient convergent information to each area. Shading intensity on left panel depicts relative strength of inputs (heavy, medium, weak). Area box shading in right panel indicates type of auditory area (black = tonotopic, dark gray = non-tonotopic, medium gray = polymodal association, light gray = limbic) and line weights reflect average connectional strength indicated in left panel. Figure adapted from Lee and Winer (2011a). Abbreviations: AAF, anterior auditory field; AES, anterior ectosylvian sulcal area; AI, primary auditory area; AII, second auditory cortex; D, dorsal division of the medial geniculate body (MGB); DCa, dorsal caudal nucleus of the MGB; DD, deep dorsal nucleus of the MGB; DS, dorsal superficial division of the MGB; DZ, dorsal auditory zone; ED, dorsal posterior ectosylvian area; EI, intermediate posterior ectosylvian area; EV, ventral posterior ectosylvian area; In, insular cortical area; M, medial division of the MGB; MGB, medial geniculate body; P, posterior auditory area; RP, rostral pole of the MGB; SI, lateral supragenicular nucleus; Sm, medial supragenicular nucleus; Te, temporal auditory area; V, ventral division of the MGB; Ve, ventral auditory area; VI, ventrolateral nucleus of the MGB; VP, ventroposterior auditory area.

cortical areas to higher auditory cortical areas (**Figure 2: red pathway**).

We have demonstrated the plausibility of such a corticothalamocortical conduit, both anatomically and physiologically, for very early stages of the auditory cortical pathway in mice (i.e., AI-MGBd-AII; Lee and Sherman, 2008, 2009, 2010b); however, it is still unknown the extent to which these corticothalamocortical pathways are linked beyond these areas (Lee and Sherman, 2011). Still, it appears likely that the anatomical substrates exist for corticothalamocortical pathways to link all auditory cortical areas (Winer et al., 1999, 2001; Huang and Winer, 2000; Smith et al., 2012). In particular in the cat, the giant, driver-like corticothalamocortical terminals originate from all auditory cortical areas and target various nuclei in the dorsal MGB (e.g., dorsal nucleus, dorsal superficial nucleus, deep dorsal, etc.; Winer et al., 1999), which in turn project to layer 4 of

several auditory cortical areas (Huang and Winer, 2000; Lee and Winer, 2008a). Interestingly, giant corticothalamocortical projections originating from different areas may target the same thalamic nucleus, such as the projections to MGBd from areas AI, AAF, In, and AII in the cat (Winer et al., 1999) or areas AI and AAF in the mouse (Llano and Sherman, 2008), establishing potential hubs for convergent information processing in the thalamus. Indeed, such convergent corticothalamocortical geometries are perhaps more parsimonious with the notion of each thalamic nucleus and cortical area forming units of degenerate, web-like, processing ensembles (Lee and Winer, 2011a,b), rather than strictly limited by serial hierarchical processing networks (Felleman and Van Essen, 1991). However, defining the precise nature of these corticothalamocortical routes through each thalamic nucleus and auditory cortical area will require further neuroanatomical and physiological studies.



CORTICOCORTICAL vs. CORTICOTHALAMOCORTICAL

An open question here is the manner in which different auditory areas interact, whether via the direct corticocortical route, the indirect corticothalamocortical route, or a combination of both routes (Figure 2: red vs. orange pathways; Felleman and Van Essen, 1991; Rouiller et al., 1991; Lee and Winer, 2011b). Although we have previously posited that this alternate route exists between AI and AII (Lee and Sherman, 2008, 2010b, 2011), it remains unclear the extent to which these alternate corticothalamocortical pathways prevail throughout the auditory forebrain. That is, are certain cortical areas preferentially linked via corticocortical or corticothalamocortical connections? What benefits accrue to information processing via these types of pathways? How are these routes organized globally across all

auditory areas? Of course, these issues are unresolved, but some connectional observations may be pertinent to deciphering them.

In general, groups of physiologically similar areas are related by their forebrain connections. This principle is particularly evident in the monkey (Hackett et al., 1998; Kaas and Hackett, 2000), where auditory areas are grouped into core, belt and parabelt regions based on connectivity and physiology (de la Mothe et al., 2006a,b). In the cat, tonotopic areas are preferentially linked by their cortical and thalamic inputs, while the non-tonotopic, association and limbic areas likewise each have distinct connectional affiliations (Lee et al., 2004a,b, 2011; Lee and Winer, 2005, 2008a,b,c, 2011a,b; Lee, 2013).

However, physiologically different areas generally have much sparser direct corticocortical connections (Fitzpatrick et al., 1998; Budinger et al., 2000; Bizley et al., 2005; de la Mothe et al., 2006a; Lee and Winer, 2008c). For example, in the cat, similar areas, such as AI and posterior auditory field (PAF), are linked via numerous corticocortical and thalamocortical connections (Figure 1; Lee et al., 2004a; Lee and Winer, 2008c). Both of these areas receive direct inputs from the ventral division of the MGB (MGBv) to which they send feedback reciprocal corticothalamic projections that originate in layer 6 (Winer et al., 2001; Lee and Winer, 2008a). But, in comparison, physiologically dissimilar areas, the primary (AI) and secondary auditory cortices (AII), are weakly interconnected by corticocortical and thalamocortical connections (Figure 1; Lee and Winer, 2008c, 2011a).

How then might information be transferred between these auditory cortical areas in the cat: AI, P, and AII? Conjecturing based on the connectivity in the cat, we would suggest that the alternate *corticothalamocortical* route may preferentially transfer information between the physiologically dissimilar areas (tonotopic and non-tonotopic), AI and AII, via layer 5 of AI to MGBd and then to layer 4 of AII (Figure 2: red pathway; Lee and Sherman, 2008, 2010a, 2011). On the other hand, the *corticocortical* route might be utilized preferentially for transferring information between physiologically similar (tonotopic) areas, such as AI and PAF (Figure 2: orange pathway; Morel and Imig, 1987; Lee and Winer, 2008a).

By comparison, in primates, connections among areas with similar physiological properties (e.g., core area connections) also tend to be greater than inter-group connections (e.g., core to belt area connections), although the magnitude of these inter-group connections seems greater in primates compared with cats (Hackett et al., 1998; Kaas and Hackett, 1998; de la Mothe et al., 2006a, 2012). It is plausible, therefore, that species-specific constraints govern the degree to which corticocortical and corticothalamocortical pathways are utilized, perhaps akin to the species-specific evolutionary trade-offs in the MGB that differ in their utilization of interneuronal or reticulothalamic inhibitory inputs (Winer and Larue, 1996).

However, rather than forming the basis of a strict prediction, one might better approach these conjectures as a framework for deciphering future physiological investigations to consider both

the corticocortical and corticothalamocortical routes as potential neural substrates in auditory forebrain operations. The question then of utility of these two pathways in auditory forebrain operations might be better construed as one of degree, rather than that of hegemony.

THE MEDIAL DIVISION OF THE MGB

A caveat to this notion of the non-lemniscal MGB nuclei as conduits for information flow to higher auditory cortical areas is the medial division of the MGB. Unlike the nuclei of the dorsal division, the medial division does not appear to be a major nuclear target of the giant, driver-like corticothalamic projections that establish the first leg of the corticothalamocortical pathway (**Figure 2**; Winer et al., 1999; Llano and Sherman, 2008). Furthermore, unlike both the ventral and dorsal divisions, the medial division does not project specifically to one or a few cortical areas, but rather projects broadly across nearly all auditory cortical regions, terminating prominently in cortical layer 1, rather than the classical thalamic input layer 4 (Huang and Winer, 2000; Jones, 2003; Lee and Winer, 2008a; Llano and Sherman, 2008; Smith et al., 2012). As such, the neuroanatomical substrates supporting the corticothalamocortical pathway, as initially formulated, appear to be lacking for the medial division of the MGB, but see Cruikshank et al. (2012) for a consideration of similar thalamic projections in the prefrontal cortex.

Instead then, the prevailing notion for the medial division of the MGB considers it to be part of the matrix system of thalamic nuclei, proposed by Jones (2001) in his core-matrix model of thalamic organization. In this framework, thalamic nuclei are distinguished on the basis of the expression of different calcium binding proteins, i.e., parvalbumin is highly expressed in the core thalamic cells, as in the ventral division of the MGB, while calbindin is expressed strongly in the matrix cells, as in the medial division of the MGB (Hashikawa et al., 1991; Molinari et al., 1995; Jones, 2001, 2003; Lu et al., 2009). The thalamocortical projection patterns of these cell types are similarly distinct, with the core thalamic neurons projecting to layer 4 in specific areas, while the matrix neurons project diffusely across the cortex, targeting layer 1 and potentially different classes of excitatory projection neurons (**Figure 2**; blue lines; Hashikawa et al., 1991; Molinari et al., 1995; Jones, 2001; Harris and Shepherd, 2015). Likewise, the functions for these two systems are proposed to be distinct, with the core thalamocortical system analogous to the first and higher-order pathways discussed above, while the

matrix thalamocortical system, of which the medial division is a part, likely exerts control over broad cortical territories, possibly regulating excitability and synchronizing activity in response to different behavioral arousal states (Mitani and Shimokouchi, 1985; Hipp et al., 2011). Yet, this parcellation alone does not capture the full complexity of the auditory thalamus, since the dorsal division, in part, may also be considered part of the matrix system (Hashikawa et al., 1991; Molinari et al., 1995; Jones, 2001, 2003; Lu et al., 2009). Thus, additional neuroanatomical and physiological features must further distinguish the unique roles of the medial division from those of the dorsal and ventral division (LeDoux et al., 1985, 1991; Iwata et al., 1986; Cruikshank et al., 1992).

In this regard, the connections of the medial division of the MGB with the limbic-related nuclei in the amygdala position it uniquely to alter auditory forebrain networks in affective and emotional responses to aversive stimuli (**Figure 2**; blue line; LeDoux et al., 1985, 1991; Iwata et al., 1986; Cruikshank et al., 1992). The same regions of the amygdala also receive descending convergent inputs from the AI (Romanski et al., 1993), which may in turn affect other auditory cortical areas (McDonald and Jackson, 1987; Miyashita et al., 2007), perhaps establishing essential circuits for synchronizing and coalescing auditory forebrain ensembles in response to salient affective stimuli (Winer, 2006; Weinberger, 2011). Moreover, due to its central position in the distribution of afferent information to both the amygdala and cortex, the medial division of the MGB may act as the central hub for auditory fear conditioning (Weinberger et al., 1995; Weinberger, 2011).

Overall though, it is clear that the operations of the non-lemniscal medial and dorsal division nuclei of the MGB extend and enhance the operations of the auditory thalamus beyond that of a simple relay for acoustic information entering the auditory cortical network. The ultimate challenge for future investigations will be to specifically parse their interrelated roles in global auditory forebrain processes and the emergent construction of holistic auditory percepts.

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REFERENCES

- Aitkin, L. M. (1973). Medial geniculate body of the cat: responses to tonal stimuli of neurons in medial division. *J. Neurophysiol.* 36, 275–283.
- Aitkin, L. M., and Webster, W. R. (1972). Medial geniculate body of the cat: organization and responses to tonal stimuli of neurons in ventral division. *J. Neurophysiol.* 35, 365–380.
- Anderson, L. A., and Linden, J. F. (2011). Physiological differences between histologically defined subdivisions in the mouse auditory thalamus. *Hear. Res.* 274, 48–60. doi: 10.1016/j.heares.2010.12.016
- Anderson, L. A., Wallace, M. N., and Palmer, A. R. (2007). Identification of subdivisions in the medial geniculate body of the guinea pig. *Hear. Res.* 228, 156–167. doi: 10.1016/j.heares.2007.02.005
- Bartlett, E. L., and Smith, P. H. (1999). Anatomic, intrinsic and synaptic properties of dorsal and ventral division neurons in rat medial geniculate body. *J. Neurophysiol.* 81, 1999–2016.
- Bartlett, E. L., and Wang, X. (2011). Correlation of neural response properties with auditory thalamus subdivisions in the awake marmoset. *J. Neurophysiol.* 105, 2647–2667. doi: 10.1152/jn.00238.2010

- Bartlett, E. L., Stark, J. M., Guillery, R. W., and Smith, P. H. (2000). Comparison of the fine structure of cortical and collicular terminals in the rat medial geniculate body. *Neurosci.* 100, 811–828. doi: 10.1016/s0306-4522(00)00340-7
- Bizley, J. K., Nodal, F. R., Nelken, I., and King, A. J. (2005). Functional organization of ferret auditory cortex. *Cereb. Cortex* 15, 1637–1653. doi: 10.1093/cercor/bhi042
- Bordi, F., and LeDoux, J. E. (1994). Response properties of single units in areas of rat auditory thalamus that project to the amygdala. II. Cells receiving convergent auditory and somatosensory inputs and cells antidromically activated by amygdala stimulation. *Exp. Brain Res.* 98, 275–286. doi: 10.1007/bf00228415
- Bruno, R. M., and Sakmann, B. (2006). Cortex is driven by weak but synchronously active thalamocortical synapses. *Science* 312, 1622–1627. doi: 10.1126/science.1124593
- Budinger, E., Heil, P., and Scheich, H. (2000). Functional organization of auditory cortex in the Mongolian gerbil (*Meriones unguiculatus*). IV. Connections with anatomically characterized subcortical structures. *Eur. J. Neurosci.* 12, 2452–2474. doi: 10.1046/j.1460-9568.2000.00143.x
- Calford, M. B. (1983). The parcellation of the medial geniculate body of the cat defined by the auditory response properties of single units. *J. Neurosci.* 3, 2350–2364.
- Calford, M. B., and Aitkin, L. M. (1983). Ascending projections to the medial geniculate body of the cat: evidence for multiple, parallel auditory pathways through thalamus. *J. Neurosci.* 3, 2365–2380.
- Calford, M. B., and Webster, W. R. (1981). Auditory representation within principal division of cat medial geniculate body: an electrophysiology study. *J. Neurophysiol.* 45, 1013–1028. doi: 10.1007/978-1-4684-3908-3_17
- Chabot, N., Mellott, J. G., Hall, A. J., Tichenoff, E. L., and Lomber, S. G. (2013). Cerebral origins of the auditory projection to the superior colliculus of the cat. *Hear. Res.* 300, 33–45. doi: 10.1016/j.heares.2013.02.008
- Clerc, W. J., and Coleman, J. R. (1990). Anatomy of the rat medial geniculate body. I. cytoarchitecture, myeloarchitecture and neocortical connectivity. *J. Comp. Neurol.* 297, 14–31. doi: 10.1002/cne.902970103
- Crabtree, J. W. (1998). Organization in the auditory sector of the cat's thalamic reticular nucleus. *J. Comp. Neurol.* 390, 167–182. doi: 10.1002/(sici)1096-9861(19980112)390:2167::aid-cne13.0.co;2-#
- Cruikshank, S. J., Ahmed, O. J., Stevens, T. R., Patrick, S. L., Gonzalez, A. N., Elmaleh, M., et al. (2012). Thalamic control of layer 1 circuits in prefrontal cortex. *J. Neurosci.* 32, 17813–17823. doi: 10.1523/jneurosci.3231-12.2012
- Cruikshank, S. J., Edeline, J. M., and Weinberger, N. M. (1992). Stimulation at a site of auditory-somatosensory convergence in the medial geniculate nucleus is an effective unconditioned stimulus for fear conditioning. *Behav. Neurosci.* 106, 471–483. doi: 10.1037/0735-7044.106.3.471
- de la Mothe, L. A., Blumell, S., Kajikawa, Y., and Hackett, T. A. (2006a). Cortical connections of the auditory cortex in marmoset monkeys: core and medial belt regions. *J. Comp. Neurol.* 496, 27–71. doi: 10.1002/cne.20923
- de la Mothe, L. A., Blumell, S., Kajikawa, Y., and Hackett, T. A. (2006b). Thalamic connections of the auditory cortex in marmoset monkeys: core and medial belt regions. *J. Comp. Neurol.* 496, 72–96. doi: 10.1002/cne.20924
- de la Mothe, L. A., Blumell, S., Kajikawa, Y., and Hackett, T. A. (2012). Cortical connections of auditory cortex in marmoset monkeys: lateral belt and parabelt regions. *Anat. Rec. (Hoboken)* 295, 800–821. doi: 10.1002/ar.22451
- Edeline, J. M., Manunta, Y., Nodal, F. R., and Bajo, V. M. (1999). Do auditory responses recorded from awake animals reflect the anatomical parcellation of the auditory thalamus? *Hear. Res.* 131, 135–152. doi: 10.1016/s0378-5955(99)00026-x
- Felleman, D. J., and Van Essen, D. C. (1991). Distributed hierarchical processing in the primate cerebral cortex. *Cereb. Cortex* 1, 1–47. doi: 10.1093/cercor/1.1.1
- Fitzpatrick, D. C., Olsen, J. F., and Suga, N. (1998). Connections among functional areas in the mustached bat auditory cortex. *J. Comp. Neurol.* 391, 366–396. doi: 10.1002/(sici)1096-9861(19980216)391:3<366::aid-cne6>3.0.co;2-#
- Guillery, R. W. (2003). Branching thalamic afferents link action and perception. *J. Neurophysiol.* 90, 539–548. doi: 10.1152/jn.00337.2003
- Hackett, T. A. (2011). Information flow in the auditory cortical network. *Hear. Res.* 271, 133–146. doi: 10.1016/j.heares.2010.01.011
- Hackett, T. A., Barkat, T. R., O'Brien, B. M., Hensch, T. K., and Polley, D. B. (2011). Linking topography to tonotopy in the mouse auditory thalamocortical circuit. *J. Neurosci.* 31, 2983–2995. doi: 10.1523/jneurosci.5333-10.2011
- Hackett, T. A., Stepniewska, I., and Kaas, J. H. (1998). Subdivisions of auditory cortex and ipsilateral cortical connections of the parabelt auditory cortex in macaque monkeys. *J. Comp. Neurol.* 394, 475–495. doi: 10.1002/(sici)1096-9861(19980518)394:4475::aid-cne63.0.co;2-z
- Harris, K. D., and Shepherd, G. M. (2015). The neocortical circuit: themes and variations. *Nat. Neurosci.* 18, 170–181. doi: 10.1038/nn.3917
- Harting, J. K., Updyke, B. V., and Van Lieshout, D. P. (1992). Corticotectal projections in the cat: anterograde transport studies of twenty-five cortical areas. *J. Comp. Neurol.* 324, 379–414. doi: 10.1002/cne.903240308
- Hashikawa, T., Rausell, E., Molinari, M., and Jones, E. G. (1991). Parvalbumin- and calbindin-containing neurons in the monkey medial geniculate complex: differential distribution and cortical layer specific projections. *Brain Res.* 544, 335–341. doi: 10.1016/0006-8993(91)90076-8
- He, J. (2002). OFF responses in the auditory thalamus of the guinea pig. *J. Neurophysiol.* 88, 2377–2386. doi: 10.1152/jn.00083.2002
- He, J., and Hu, B. (2002). Differential distribution of burst and single-spike responses in auditory thalamus. *J. Neurophysiol.* 88, 2152–2156.
- Hipp, J. F., Engel, A. K., and Siegel, M. (2011). Oscillatory synchronization in large-scale cortical networks predicts perception. *Neuron* 69, 387–396. doi: 10.1016/j.neuron.2011.07.003
- Huang, C. L., and Winer, J. A. (2000). Auditory thalamocortical projections in the cat: laminar and areal patterns of input. *J. Comp. Neurol.* 427, 302–331. doi: 10.1002/1096-9861(20001113)427:2302::aid-cne103.0.co;2-j
- Imaizumi, K., and Lee, C. C. (2015). “Auditory thalamocortical transformations,” in *Encyclopedia of Computational Neuroscience*, eds D. Jaeger and R. Jung (New York: Springer), 278–292.
- Imig, T. J., and Morel, A. (1985). Tonotopic organization in ventral nucleus of medial geniculate body in the cat. *J. Neurophysiol.* 53, 309–340.
- Iwata, J., Ledoux, J. E., Meeley, M. P., Arneric, S., and Reis, D. J. (1986). Intrinsic neurons in the amygdaloid field projected to by the medial geniculate body mediate emotional responses conditioned to acoustic stimuli. *Brain Res.* 383, 195–214. doi: 10.1016/0006-8993(86)90020-x
- Jones, E. G. (2001). The thalamic matrix and thalamocortical synchrony. *Trends Neurosci.* 24, 595–601. doi: 10.1016/s0166-2236(00)01922-6
- Jones, E. G. (2003). Chemically defined parallel pathways in the monkey auditory system. *Ann. N. Y. Acad. Sci.* 999, 218–233. doi: 10.1196/annals.1284.033
- Jones, E. G. (2007). *The Thalamus*. Cambridge: Cambridge University Press.
- Kaas, J. H., and Hackett, T. A. (1998). Subdivisions of auditory cortex and levels of processing in primates. *Audiol. Neurotol.* 3, 73–85.
- Kaas, J. H., and Hackett, T. A. (2000). Subdivisions of auditory cortex and processing streams in primates. *Proc. Natl. Acad. Sci. U S A* 97, 11793–11799. doi: 10.1073/pnas.97.22.11793
- LeDoux, J. E., Farb, C. R., and Romanski, L. M. (1991). Overlapping projections to the amygdala and striatum from auditory processing areas of the thalamus and cortex. *Neurosci. Lett.* 134, 139–144. doi: 10.1016/0304-3940(91)90526-y
- LeDoux, J. E., Ruggiero, D. A., and Reis, D. J. (1985). Projections to the subcortical forebrain from anatomically defined regions of the medial geniculate body in the rat. *J. Comp. Neurol.* 242, 182–213. doi: 10.1002/cne.902420204
- Lee, C. C. (2013). Thalamic and cortical pathways supporting auditory processing. *Brain Lang.* 126, 22–28. doi: 10.1016/j.bandl.2012.05.004
- Lee, C. C., and Imaizumi, K. (2013). Functional convergence of thalamic and intrinsic inputs in cortical layers 4 and 6. *Neurophysiol.* 45, 396–406. doi: 10.1007/s11062-013-9385-2
- Lee, C. C., and Sherman, S. M. (2008). Synaptic properties of thalamic and intracortical inputs to layer 4 of the first- and higher-order cortical areas in the auditory and somatosensory systems. *J. Neurophysiol.* 100, 317–326. doi: 10.1152/jn.90391.2008
- Lee, C. C., and Sherman, S. M. (2009). Modulator property of the intrinsic cortical projections from layer 6 to layer 4. *Front. Syst. Neurosci.* 3:3. doi: 10.3389/neuro.06.003.2009
- Lee, C. C., and Sherman, S. M. (2010a). Drivers and modulators in the central auditory pathways. *Front. Neurosci.* 4, 79–86. doi: 10.3389/neuro.01.014.2010

- Lee, C. C., and Sherman, S. M. (2010b). Topography and physiology of ascending streams in the auditory tectothalamic pathway. *Proc. Natl. Acad. Sci. U S A* 107, 372–377. doi: 10.1073/pnas.0907873107
- Lee, C. C., and Sherman, S. M. (2011). On the classification of pathways in the auditory midbrain, thalamus and cortex. *Hear. Res.* 276, 79–87. doi: 10.1016/j.heares.2010.12.012
- Lee, C. C., and Winer, J. A. (2005). Principles governing auditory cortex connections. *Cereb. Cortex* 15, 1804–1814. doi: 10.1093/cercor/bhi057
- Lee, C. C., and Winer, J. A. (2008a). Connections of cat auditory cortex: I. Thalamocortical system. *J. Comp. Neurol.* 507, 1879–1900. doi: 10.1002/cne.21611
- Lee, C. C., and Winer, J. A. (2008b). Connections of cat auditory cortex: II. Commissural system. *J. Comp. Neurol.* 507, 1901–1919. doi: 10.1002/cne.21614
- Lee, C. C., and Winer, J. A. (2008c). Connections of cat auditory cortex: III. Corticocortical system. *J. Comp. Neurol.* 507, 1920–1943. doi: 10.1002/cne.21613
- Lee, C. C., and Winer, J. A. (2011a). Convergence of thalamic and cortical pathways in cat auditory cortex. *Hear. Res.* 274, 85–94. doi: 10.1016/j.heares.2010.05.008
- Lee, C. C., and Winer, J. A. (2011b). “A synthesis of auditory cortical connections: thalamocortical, commissural and corticocortical systems,” in *The auditory cortex* eds J. A. and Winer C. E. Schreiner (New York: Springer), 147–170.
- Lee, C. C., Imaizumi, K., Schreiner, C. E., and Winer, J. A. (2004a). Concurrent tonotopic processing streams in auditory cortex. *Cereb. Cortex* 14, 441–451. doi: 10.1093/cercor/bhh006
- Lee, C. C., Schreiner, C. E., Imaizumi, K., and Winer, J. A. (2004b). Tonotopic and heterotopic projection systems in physiologically defined auditory cortex. *Neurosci.* 128, 871–887. doi: 10.1016/j.neuroscience.2004.06.062
- Lee, C. C., Kishan, A. U., and Winer, J. A. (2011). Wiring of divergent networks in the central auditory system. *Front. Neuroanat.* 5:46. doi: 10.3389/fnana.2011.00046
- Llano, D. A., and Sherman, S. M. (2008). Evidence for non-reciprocal organization of the mouse auditory thalamocortical-corticocortical projections systems. *J. Comp. Neurol.* 507, 1209–1227. doi: 10.1002/cne.21602
- Lu, E., Llano, D. A., and Sherman, S. M. (2009). Different distributions of calbindin and calretinin immunostaining across the medial and dorsal divisions of the mouse medial geniculate body. *Hear. Res.* 257, 16–23. doi: 10.1016/j.heares.2009.07.009
- McDonald, A. J., and Jackson, T. R. (1987). Amygdaloid connections with posterior insular and temporal cortical areas in the rat. *J. Comp. Neurol.* 262, 59–77. doi: 10.1002/cne.902620106
- McMullen, N. T., and de Venecia, R. K. (1993). Thalamocortical patches in auditory neocortex. *Brain Res.* 620, 317–322. doi: 10.1016/0006-8993(93)90173-k
- Miller, L. M., Escabi, M. A., Read, H. L., and Schreiner, C. E. (2001). Functional convergence of response properties in the auditory thalamocortical system. *Neuron* 32, 151–160. doi: 10.1016/s0896-6273(01)00445-7
- Miller, L. M., Escabi, M. A., Read, H. L., and Schreiner, C. E. (2002). Spectrotemporal receptive fields in the lemniscal auditory thalamus and cortex. *J. Neurophysiol.* 87, 516–527.
- Mitani, A., and Shimokouchi, M. (1985). Neuronal connections in the primary auditory cortex: an electrophysiological study in the cat. *J. Comp. Neurol.* 235, 417–429. doi: 10.1002/cne.902350402
- Miyashita, T., Ichinohe, N., and Rockland, K. S. (2007). Differential modes of termination of amygdalothalamic and amygdalocortical projections in the monkey. *J. Comp. Neurol.* 502, 309–324. doi: 10.1002/cne.21304
- Molinari, M., Dell’anna, M. E., Rausell, E., Leggio, M. G., Hashikawa, T., and Jones, E. G. (1995). Auditory thalamocortical pathways defined in monkeys by calcium-binding protein immunoreactivity. *J. Comp. Neurol.* 362, 171–194.
- Morel, A., and Imig, T. J. (1987). Thalamic projections to fields A, AI, P and VP in the cat auditory cortex. *J. Comp. Neurol.* 265, 119–144. doi: 10.1002/cne.902650109
- Oh, S. W., Harris, J. A., Ng, L., Winslow, B., Cain, N., Mihalas, S., et al. (2014). A mesoscale connectome of the mouse brain. *Nature* 508, 207–214. doi: 10.1038/nature13186
- Ojima, H. (1994). Terminal morphology and distribution of corticocortical fibers originating from layers 5 and 6 of cat primary auditory cortex. *Cereb. Cortex* 4, 646–663. doi: 10.1093/cercor/4.6.646
- Olshausen, B. A., Anderson, C. H., and Van Essen, D. C. (1993). A neurobiological model of visual attention and invariant pattern recognition based on dynamic routing of information. *J. Neurosci.* 13, 4700–4719.
- Peruzzi, D., Bartlett, E., Smith, P. H., and Oliver, D. L. (1997). A monosynaptic GABAergic input from the inferior colliculus to the medial geniculate body in rat. *J. Neurosci.* 17, 3766–3777.
- Razak, K. A., and Fuzessery, Z. M. (2010). Development of parallel auditory thalamocortical pathways for two different behaviors. *Front. Neuroanat.* 4:134. doi: 10.3389/fnana.2010.00134
- Roger, M., and Arnault, P. (1990). Ventral temporal cortex in the rat: connections of secondary auditory areas Te2 and Te3. *J. Comp. Neurol.* 302, 110–123. doi: 10.1002/cne.903020109
- Romanski, L. M., Clugnet, M. C., Bordin, F., and Ledoux, J. E. (1993). Somatosensory and auditory convergence in the lateral nucleus of the amygdala. *Behav. Neurosci.* 107, 444–450. doi: 10.1037/0735-7044.107.3.444
- Rose, H. J., and Metherate, R. (2005). Auditory thalamocortical transmission is reliable and temporally precise. *J. Neurophysiol.* 94, 2019–2030. doi: 10.1152/jn.00860.2004
- Rouiller, E. M., Rodrigues-Dageaff, C., Simm, G., De Ribaupierre, Y., Villa, A., and De Ribaupierre, F. (1989). Functional organization of the medial division of the medial geniculate body of the cat: tonotopic organization, spatial distribution of response properties and cortical connections. *Hear. Res.* 39, 127–142. doi: 10.1016/0378-5955(89)90086-5
- Rouiller, E. M., Simm, G. M., Villa, A. E., De Ribaupierre, Y., and De Ribaupierre, F. (1991). Auditory corticocortical interconnections in the cat: evidence for parallel and hierarchical arrangement of the auditory cortical areas. *Exp. Brain Res.* 86, 483–505. doi: 10.1007/bf00230523
- Sherman, S. M., and Guillery, R. W. (1998). On the actions that one nerve cell can have on another: distinguishing “drivers” from “modulators”. *Proc. Natl. Acad. Sci. U S A* 95, 7121–7126. doi: 10.1073/pnas.95.12.7121
- Sherman, S. M., and Guillery, R. W. (2006). *Exploring the Thalamus and its Role in Cortical Function*. London: MIT Press.
- Sherman, S. M., and Guillery, R. W. (2011). Distinct functions for direct and transthalamic corticocortical connections. *J. Neurophysiol.* 106, 1068–1077. doi: 10.1152/jn.00429.2011
- Shi, C. J., and Cassell, M. D. (1997). Cortical, thalamic and amygdaloid projections of the rat temporal cortex. *J. Comp. Neurol.* 382, 153–175. doi: 10.1002/(sici)1096-9861(19970602)382:2153::aid-cne23.0.co;2-2
- Smith, P. H., Uhlrich, D. J., Manning, K. A., and Banks, M. I. (2012). Thalamocortical projections to rat auditory cortex from the ventral and dorsal divisions of the medial geniculate nucleus. *J. Comp. Neurol.* 520, 34–51. doi: 10.1002/cne.22682
- Takemoto, M., Hasegawa, K., Nishimura, M., and Song, W. J. (2014). The insular auditory field receives input from the lemniscal subdivision of the auditory thalamus in mice. *J. Comp. Neurol.* 522, 1373–1389. doi: 10.1002/cne.23491
- Takesian, A. E., Kotak, V. C., and Sanes, D. H. (2012). Age-dependent effect of hearing loss on cortical inhibitory synapse function. *J. Neurophysiol.* 107, 937–947. doi: 10.1152/jn.00515.2011
- Weinberger, N. M. (2011). The medial geniculate, not the amygdala, as the root of auditory fear conditioning. *Hear. Res.* 274, 61–74. doi: 10.1016/j.heares.2010.03.093
- Weinberger, N. M., Javid, R., and Lapan, B. (1995). Heterosynaptic long-term facilitation of sensory-evoked responses in the auditory cortex by stimulation of the magnocellular medial geniculate body in guinea pigs. *Behav. Neurosci.* 109, 10–17. doi: 10.1037/0735-7044.109.1.10
- Wenstrup, J. J. (1999). Frequency organization and responses to complex sounds in the medial geniculate body of the mustached bat. *J. Neurophysiol.* 82, 2528–2544.
- Wenstrup, J. J. (2005). “The tectothalamic system,” in *The Inferior Colliculus* eds J. A. Winer and C. E. Schreiner (New York: Springer), 200–230.
- Winer, J. A. (1984). The human medial geniculate body. *Hear Res.* 15, 225–247.
- Winer, J. A. (1992). “The functional architecture of the medial geniculate body and the primary auditory cortex,” in *Springer Handbook of Auditory Research, Vol. 1, The Mammalian Auditory Pathways: Neuroanatomy,*

- eds D. B. Webster, A. N. Popper, and R. R. Fay (New York: Springer-Verlag), 222–409.
- Winer, J. A. (2006). Decoding the auditory corticofugal systems. *Hear. Res.* 212, 1–8. doi: 10.1016/j.heares.2005.06.014
- Winer, J. A., and Larue, D. T. (1996). Evolution of GABAergic circuitry in the mammalian medial geniculate body. *Proc. Natl. Acad. Sci. U S A* 93, 3083–3087. doi: 10.1073/pnas.93.7.3083
- Winer, J. A., Diehl, J. J., and Larue, D. T. (2001). Projections of auditory cortex to the medial geniculate body of the cat. *J. Comp. Neurol.* 430, 27–55. doi: 10.1002/1096-9861(20010129)430:127::AID-CNE10133.0.CO;2-8
- Winer, J. A., Larue, D. T., and Huang, C. L. (1999). Two systems of giant axons terminals in the cat medial geniculate body: convergence of cortical and GABAergic inputs. *J. Comp. Neurol.* 413, 181–197.
- Wong, D., and Kelly, J. P. (1981). Differentially projecting cells in individual layers of the auditory cortex: a double-labeling study. *Brain Res.* 230, 362–366. doi: 10.1016/0006-8993(81)90416-9
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Basal ganglia—thalamus and the “crowning enigma”

Marianela Garcia-Munoz and Gordon W. Arbuthnott*

Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

When Hubel (1982) referred to layer 1 of primary visual cortex as “... a ‘crowning mystery’ to keep area-17 physiologists busy for years to come ...” he could have been talking about any cortical area. In the 80’s and 90’s there were no methods to examine this neuropile on the surface of the cortex: a tangled web of axons and dendrites from a variety of different places with unknown specificities and doubtful connections to the cortical output neurons some hundreds of microns below. Recently, three changes have made the crowning enigma less of an impossible mission: the clear presence of neurons in layer 1 (L1), the active conduction of voltage along apical dendrites and optogenetic methods that might allow us to look at one source of input at a time. For all of those reasons alone, it seems it is time to take seriously the function of L1. The functional properties of this layer will need to wait for more experiments but already L1 cells are GAD67 positive, i.e., inhibitory! They could reverse the sign of the thalamic glutamate (GLU) input for the entire cortex. It is at least possible that in the near future normal activity of individual sources of L1 could be detected using genetic tools. We are at the outset of important times in the exploration of thalamic functions and perhaps the solution to the crowning enigma is within sight. Our review looks forward to that solution from the solid basis of the anatomy of the basal ganglia output to motor thalamus. We will focus on L1, its afferents, intrinsic neurons and its influence on responses of pyramidal neurons in layers 2/3 and 5. Since L1 is present in the whole cortex we will provide a general overview considering evidence mainly from the somatosensory (S1) cortex before focusing on motor cortex.

Keywords: ventromedial, ventrolateral, ventral anterior, midline-intralaminar, basal ganglia, motor cortex

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Giuseppe Di Giovanni,
University of Malta, Malta
Masahiko Takada,
Kyoto University, Japan

*Correspondence:

Gordon W. Arbuthnott
gordon@oist.jp

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LAYER 1: THE CROWNING ENIGMA

Our interest in layer 1 (L1) of cortex sprang from the knowledge that it was the final destination of basal ganglia output. In spite of the fact that the whole cortex is represented in striatum and movement is certainly not its only function, here we review thalamic output to motor cortex because of our long-standing interest in movement and the basal ganglia. Similarly L1 is not only present in motor cortex but invests the complete cortex. In theorizing about basal ganglia and their influence on the “crowning enigma” it is as important to remember that movement is only the most obvious output and the easiest outcome to measure from both the dark basements of the brain and its crowing glory. With its plastic spines and input from so many parts of the environment L1 might be the best place to provide the necessary context for movements, or for perceptions, both of which, like all decisions of the cortex, need information from many sources including historical experience that can be coded in the pattern of spines.

NEURONS INTRINSIC TO LAYER 1

L1 is recognized throughout the entire cerebral cortex. Cajal (1890) described for the first time horizontal cells in L1 later called Cajal-Retzius and Lorente De Nó (1922) using Golgi staining in mouse auditory cortex provided the first evidence of “non-specific” thalamic afferents to L1. For a review see Marin-Padilla and Marin-Padilla (1982).

From work performed in mice and rat neocortex (rostral, central and caudal areas) three types of neurons in L1 are described as non-pyramidal GABAergic: Cajal-Retzius, elongated neurogliaform and single bouquet. Cajal-Retzius are the earliest born (embryonic 10–11 days; Soda et al., 2003; Anstötz et al., 2014) and they have an oval shape with a prominent long spiny dendrite (two occasionally) that runs horizontally along L1 (Imamoto et al., 1994). Their horizontal axon extends about 1.7 mm and serves as anchor of dendritic tufts of pyramidal neurons of layers 2/3 and 5 (Anstötz et al., 2014). These neurons participate in layering and connectivity during development (Zecevic and Rakic, 2001; Soda et al., 2003). During the second postnatal week most Cajal-Retzius cells suffer apoptotic death (del Río et al., 1995; Chowdhury et al., 2010) and have nearly disappeared at P14 (Anstötz et al., 2014).

The elongated neurogliaform type comprises 30–40% of neurons in L1, they have a characteristic dense axonal arbor confined to L1 and are coupled electrically. These cells express GABA_A receptors and mostly display non-adapting late spiking action potentials. In the monkey sensory-motor cortex intense GABA_A immunostaining outlines somas of pyramidal and non-pyramidal cells in layers 1–3 (Huntley et al., 1990). Single bouquet cells express vasoactive intestinal peptide (VIP) and mostly display adapting early spiking to depolarizing current injections (Jiang et al., 2013; Ma et al., 2014).

Following the Petilla terminology for interneuron firing patterns (Ascoli et al., 2008) there are four different types of interneurons in L1: neurogliaform, classical-accommodating, fast-spiking and burst-spiking (Wozny and Williams, 2011). The most common type is the fast spiking, with no frequency adaptation and pronounced fast afterhyperpolarizations (Zhou and Hablitz, 1996; Wozny and Williams, 2011; Li et al., 2012; Muralidhar et al., 2013). Correlation of function and morphology with colocalization of neuronal markers and specific neuronal proteins has produced four different subtypes of agranular neocortical GABAergic neurons. Two are found in L1: the calretinin/alpha-actin-2 and somatostatin subtypes (Kubota et al., 2011).

AFFERENTS TO LAYER 1

It is estimated that 4000–5000 glutamate (GLU) containing axons reach any given square millimeter of rat L1 (Rubio-Garrido et al., 2009) to selectively target apical dendritic tufts (Herkenham, 1986; Arbutnott et al., 1990; Lu and Lin, 1993). **Figure 1** illustrates the extension of neocortical L1 projections from ventromedial (VM) that include forelimb and hindlimb areas. Dendritic tufts of layer 5 corticospinal, corticostriatal and corticothalamic neurons are all subject to modulation from L1

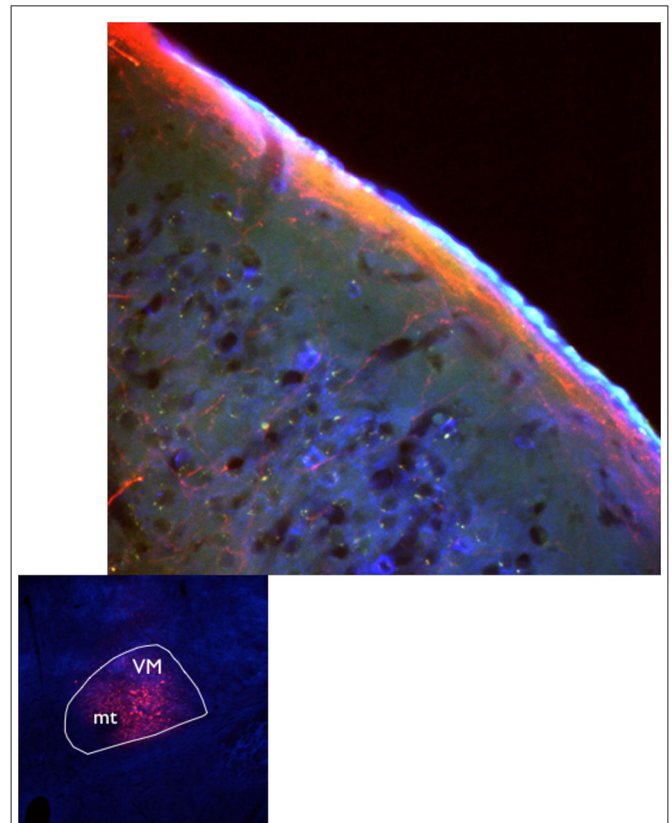


FIGURE 1 | Axons from the ventromedial nucleus of the motor thalamus delineate layer 1 in motor cortex. Axon terminal fields in cortical L1 seen after VM injection of AAV10 containing turbo red and GCaMP6 in C57Bl6J mice (Jáidar Benavides and Arbutnott, 2013; Jáidar et al., 2015).

(Gao and Zheng, 2004). Moreover, important modulation of dendritic tufts of layer 2/3 pyramidal neurons takes place in L1 (see “Responses Mediated by Activation of Layer 1” Section).

In motor cortex afferents reaching L1 are exclusively from motor thalamus (MT) and midline rhomboid nucleus (Ohtake and Yamada, 1989; Van Der Werf et al., 2002; Vertes et al., 2015). Other midline/intralaminar nuclei (i.e., centrolateral, centromedian, paracentral, posterior and parafascicular) terminate although not exclusively, in L1 of motor related areas (Royce and Mourey, 1985; Royce et al., 1989; Jones, 2007; Mohammed and Jain, 2014).

Neuronal Processes that Mingle in Layer 1

Axons that run along L1 originate in higher cortical areas, thalamic specific and non-specific nuclei (Mitchell and Cauller, 2001) and brainstem specific neurotransmitter producing nuclei. Norepinephrine (NE) originates in the pontine locus coeruleus, serotonin (5HT) in the midbrain raphe nuclei, dopamine (DA) in the ventral mesencephalon and acetylcholine (ACh) in the basal forebrain. In general these neurotransmitter-containing fibers enter below layer 6 and ascend sending collateral branches at all levels. L1 is particularly filled with dense axonal terminals and long branching collaterals (Levitt and Moore, 1978, 1979). Cortical NE innervates the marginal zone at embryonic 18–21

days and its participation in pyramidal cell development and layering was highlighted following locus coeruleus lesions in newborn rats (Felten et al., 1982). Similarly, 5HT is related to neuronal development, differentiation and migration (Rubenstein, 1998). The participation of DA and ACh will be indicated below (see “Modulatory Role of Neurotransmitters Released in L1” Section) associated to responses mediated by activation of L1.

Other neuronal processes in L1 come from cortical interneurons, mainly from axons of somatostatin-positive Martinotti cells contained in layers 2–6 (Thomson and Lamy, 2007; Muralidhar et al., 2013), vertical dendrites from bipolar interneurons (layers 1–3) that run horizontally once in L1, and apical dendrites of layer 5 pyramidal cells (Larsen and Callaway, 2006) and layer 2/3 (Walcott and Langdon, 2001). Intrinsic axonal arborizations from L1 run either in the horizontal plane along the layer or descend in the vertical plane to frequently synapse with interneurons of deeper layers (Zhu and Zhu, 2004; Jiang et al., 2013).

Motor Thalamus

Motor thalamus (MT) is considered the area where afferents from globus pallidus (GPi or entopeduncular nucleus, EP), substantia nigra reticulata (SNR) and deep cerebellar nuclei form terminal fields in separate nuclei of the ventral thalamus: ventrolateral (VL), ventral anterior (VA) and ventromedial (VM). According to Scheibel and Scheibel (1967) the best way to conceptualize MT is to look at a horizontal section of the brain through the rostral half of thalamus.

MT Inputs

Terminal sites of afferent axons to MT are conserved across species (Antal et al., 2014) and establish multiple synapses with neurons in VM (Kultas-Ilinsky and Ilinsky, 1990; Kuroda and Price, 1991; Sakai et al., 1998; Tsumori et al., 2002; Bodor et al., 2008).

The use of a new anatomical technique with a resolution like “the old Golgi staining” (Furuta et al., 2001) has refined previous findings of inputs to MT (Deniau et al., 1978; Uno et al., 1978; Bava et al., 1979; MacLeod et al., 1980; Chevalier and Deniau, 1982; Matsuda and Nakamura, 1982; Ueki, 1983). As a result the VA/VL complex is divided in two sections: the rostromedial area immunoreactive to calbindin and GAD67 and the caudolateral area immunoreactive to VGluT2. These results sparked the idea of associating the neurotransmitter markers with the sites of origin calling the GABAergic GAD67-immunoreactive neurons the “inhibitory zone” and the glutamatergic VGluT2-immunoreactive neurons the “excitatory zone”. It is important to note that although immunoreactivities to GAD67 and VGluT2 vary in intensity, they can be found at variable levels throughout MT. VM and VA/VL contain axon terminals of both GABA and GLU in different proportions. GABAergic terminals from SNR and GPi(EP) terminate in VM and the rostroventral VA/VL and cerebellar GLU terminals in the caudodorsal portion of VA/VL (Kuramoto et al., 2011).

In rats and monkeys, the GAD67-immunoreactive axon terminals are large (Bodor et al., 2008; Kuramoto et al., 2011)

with a synaptic arrangement of the typical thalamic “detonator or driver”-type input that favors neurotransmitter spillover and volume transmission (Destexhe and Sejnowski, 1995; Agnati and Fuxe, 2000; Diamond, 2002; Agnati et al., 2008) and provides an ideal form of communication between neighboring neurons as has been observed in other thalamic areas (Bright and Brickley, 2008; Errington et al., 2011; Bright and Smart, 2013; Herd et al., 2013; Ye et al., 2013).

MT Outputs

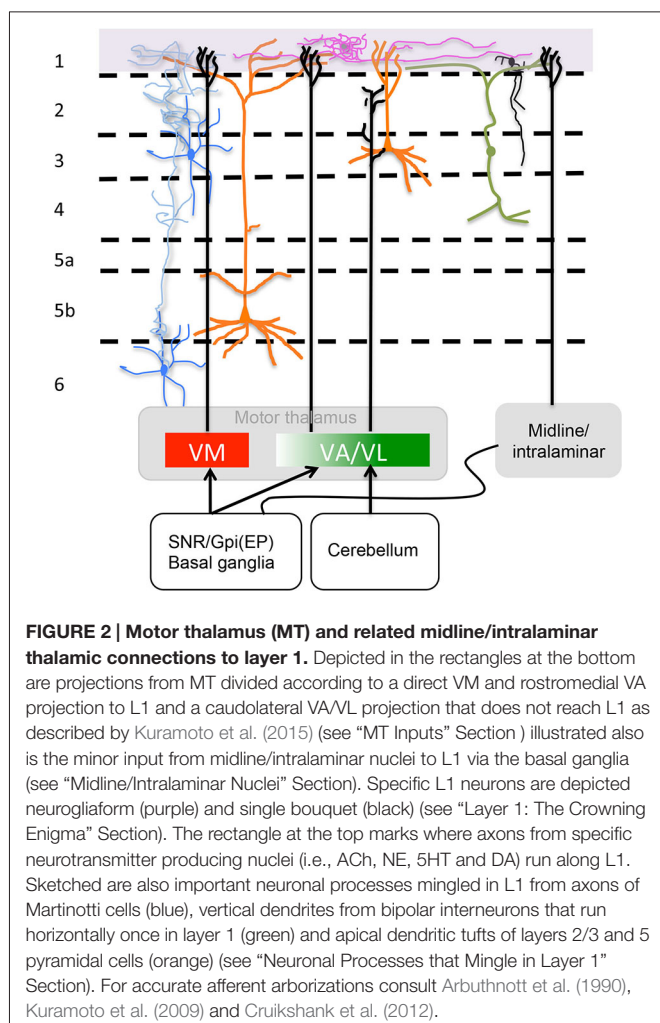
MT projects to prefrontal cortex (Middleton and Strick, 1994), motor cortex (Hoover and Strick, 1999), supplementary motor area (SMA) and pre-SMA (Akkal et al., 2007). Axonal processes from ventromedial (VM) and ventral anterior (VA) thalamic nuclei terminate in L1 (Donoghue and Ebner, 1981; Arbuthnott et al., 1990; Desbois and Villanueva, 2001; Mitchell and Cauller, 2001; Kuramoto et al., 2009, 2015; Rubio-Garrido et al., 2009).

VM neurons project to extensive motor associated cortical areas including the forelimb and hindlimb regions (Tennant et al., 2011; Deffeyes et al., 2015). These results are consistent with the findings of Arbuthnott et al. (1990) following antidromically driven VM neurons over a similarly extensive cortical area. The other areas that receive fibers from VM according to Kuramoto et al. (2009) are primary somatosensory (S1) and associated sensory orbital and cingulate areas. **Figure 2** presents a sketch of afferents to L1, its neuronal types some of neuronal processes found at this level.

MT Functional Output

The motor function of MT reflects the function of its afferent nuclei: optimization of motor sequences, sensory motor control, switching of attention and decision-making of cerebellum e.g., D’Angelo et al. (2011) and attention, implicit learning, habit formation and selection of appropriate motor activity of basal ganglia e.g., Lanciego and Vázquez (2012). How this information is consolidated and expressed depends not only on integrative processes in MT but also on the reentrant cortical input from thalamus (Magill et al., 2004; Bosch-Bouju et al., 2013; Nakamura et al., 2014) and the anatomical reality of a reentrant thalamic pathway to L1.

Initial functional evidence of MT (VA, VM) in relation to basal ganglia output indicated that increases in GPi(EP) and SNR activity resulted in decreased VM activity (Deniau et al., 1978; Patino and Garcia-Munoz, 1985) and increased cortical activity (Tanibuchi et al., 2009). Monosynaptic inhibitory postsynaptic potentials were recorded in VM to stimulation of SNR or GPi(EP) (MacLeod et al., 1980; Ueki, 1983). Recently it has been reported that the similar electrophysiological characteristics of VA and VM suggest they could form a single nucleus recipient of inputs from basal ganglia (Nakamura et al., 2014). Coherence between cortical oscillatory activity (electrocorticograms, ECoG) and action potentials has been reported for cortex-basal ganglia (Magill et al., 2004) or cortex-thalamus (Nakamura et al., 2014). Nakamura et al. (2014) observed that MT neuronal spike discharges are phase-locked to ongoing cortical slow oscillations, and that the two neuronal populations of MT (defined by their



GLU or GABA inputs) preferentially discharge at the ascending phase of the cortical oscillation.

VM and VA are assumed to carry information about movement from basal ganglia, though it will be strongly modified information. Deep brain stimulation in the subthalamic nucleus changes dramatically MT responses, decreases beta oscillations and improves Parkinson's disease symptoms (Anderson et al., 2015) possibly via cortex. Moreover, thalamic deep brain stimulation is not only an effective treatment for movement disorders but also for pain and epilepsy. Nociceptive neurons are located in the lateral parts of VM (lateral to MT) they respond to painful stimulation of the whole body in rats and project to the entire layer 1 of the dorsolateral neocortex (Monconduit and Villanueva, 2005) and cortical epileptic activity during absence seizures is accompanied by rhythmic burst activity in VM (Paz et al., 2007), although the terminals seem widely spread in cortex L1 inhibitory neurons could target specific pyramidal cells.

Midline/Intralaminar Nuclei

The lateral, ventral and posterior groups of midline and intralaminar nuclei interact with MT and its afferent sites according to Van Der Werf et al. (2002). The ascribed

functions of these three groups are cognitive for the lateral (centrolateral, CL; centromedian anterior, CM; and paracentral, PC nuclei), sensory for the ventral (rhomboid, Rh; reuniens, Re; centromedian, CM and posterior, Po) and multisensory for the posterior group (parafascicular, Pf). Among the midline/intralaminar thalamic nuclei Rh, Re and Pf are consistently reported to project to striatum and also to cortical L1 (Berendse and Groenewegen, 1990; Mohammed and Jain, 2014). Optical stimulation of midline thalamic neurons (e.g., Re, PC, CM and Rh) preferentially drives L1 interneurons that often trigger feedforward inhibition of other L1 interneurons and L2/3 pyramidal neurons in medial prefrontal and secondary motor cortex (Cruikshank et al., 2012).

Projections from midline/intralaminar thalamus on their way to cortex on occasions bifurcate to reenter basal ganglia (Killackey and Ebner, 1973; Cesaro et al., 1979; Jinnai and Matsuda, 1981; Royce, 1983; Macchi et al., 1984) and in other cases separate groups of neurons send axons to striatal and cortical targets (Sadikot et al., 1992). **Figure 2** illustrates ascending inputs to L1 (motor areas) from MT and midline/intralaminar nuclei. The topic of dual projections is discussed by Jones and Leavitt (1974). Afferents from these nuclei to striatum have been reported in *monkeys* (Macchi et al., 1984; Nakano et al., 1990; Fenelon et al., 1991; Sadikot et al., 1992; Giménez-Amaya et al., 1995) *cats* (Sato et al., 1979; Beckstead, 1984; Takada et al., 1986) and *rodents* (Dube et al., 1988).

Giménez-Amaya et al. (1995) raised important questions regarding the double thalamic projection to striatum and cortex: Is their function modulatory at both ends? Are the contacted neurons a subset of neurons with common outputs or functions? Axonal branches from thalamic afferents that synapse in striatum on their way to cortex may contribute to striatal multisensory responses (Reig and Silberberg, 2014). Since the early work it was suggested that some afferents on L1 originated from the midline/intralaminar nuclei (Jones and Powell, 1969, 1970a,b; Strick, 1973; Strick and Sterling, 1974). Medium to large caliber axons from VA afferents converged with fine caliber afferents from intralaminar nuclei in L1 (Killackey and Ebner, 1973). Concurrent afferent information from MT and intralaminar nuclei to L1 and striatum may contribute to the animal's awareness of position in space necessary for posture and head orientation (Barter et al., 2015) and also contribute to improvement of symptoms of Parkinson's disease induced by deep brain stimulation (Jouve et al., 2010).

RESPONSES MEDIATED BY ACTIVATION OF LAYER 1

The evidence presented in this section is a summary of the neuronal circuitry activated by L1 or compilation of experimental observations from different cortical areas i.e., S1, medial prefrontal, neocortical and of course specific motor cortex responses relevant to this review. Although the summary underlies general features, important anatomical distinctions between motor/frontal cortex and other cortical areas must be considered (Weiler et al., 2008).

Activation of the Distal Tuft of Pyramidal Cells in L1

Electrophysiological responses of L1 interneurons are mediated by excitatory GLU (i.e., AMPA, kainite and NMDA) and inhibitory GABA_A receptors (Li et al., 2012). Thalamic afferents are in intimate contact with the distal part of the apical dendrite or dendritic tuft that reach L1 and provide a substantial AMPA and NMDA-mediated excitatory synaptic drive that generates subthreshold and suprathreshold voltage responses through the tuft. AMPA receptors mediate unitary depolarizing potentials and NMDA receptors mediate an extensive depolarizing input that leads to the generation in the dendritic tuft of calcium-dependent regenerative action potentials. In summary, inputs to L1 produce regenerative calcium spikes that can induce sodium axosomatic action potentials (Larkum and Zhu, 2002; Larkum et al., 2009). A general organization of cortical synaptic interactions emphasizes a descending influence on cortical output from layer 2/3 to layer 5 for rat S1 (Jiang et al., 2013) as well as motor cortex (Weiler et al., 2008) previously believed to have a predominantly horizontal synaptic interaction.

Influence of the Distal Dendritic Tuft in L1 on Cortical Output

The presence of a cooperative integration between the distal dendritic tuft and the axosomatic compartment of pyramidal cells has been studied following co-activation of both compartments. It has been observed for example, that axosomatic action potentials can be generated by the coincidence of back propagation of action potentials and the effect of distal dendritic excitatory potentials (Larkum et al., 2004) and that volleys of excitatory postsynaptic potentials generated from distal apical dendritic sites facilitate action potential discharges (Williams, 2005).

Expression of specific channels in dendritic tufts that mediate different conductances such as the voltage-activated potassium outward conductance (K_v channels) or the hyperpolarization-activated current (I_h) plus the effects of specific activation of neurotransmitter receptors are important mechanisms for microcircuit control. K_v channels in the distal apical dendrite of layer 5 pyramidal neurons control the interaction between subthreshold tuft excitatory input and trunk spike generation (Harnett et al., 2013). These channels are co-localized with hyperpolarization activated cyclic nucleotide-gated nonselective cation (HCN) channels that are expressed at a high density in the tuft (≈ 85 channels/ μm^2) and at low density in the somatic region with two different effects: an inhibitory action in the tuft that controls initiation and propagation of dendritic spikes, and an excitatory action in the soma that decreases the threshold for action potentials (Harnett et al., 2015). Also I_h -mediated currents make corticospinal neurons susceptible to attenuation of GLU responses but not corticostriatal neurons. Similarly, the blockade of I_h results in increased layer 2/3-driven spiking in corticospinal, but not in corticostriatal neurons (Sheets et al., 2011). This emphasizes differential influences of L1 on cortical output.

Modulatory Role of Neurotransmitters Released in L1

The neuromodulatory role of axons from several neurotransmitter systems (e.g., DA, NE, 5HT, ACh) on L1 is also important in the control of tuft currents and local interneurons. For example, α -adrenergic agents decrease I_h in corticospinal neurons thereby amplifying the impact of excitatory postsynaptic potentials with an increase in action potentials (Sheets et al., 2011). Likewise, the synergistic action of DA D1 and D2 receptors induces a depolarizing shift in the I_h activation curve that results in depolarization of L1 interneurons that can alter tonic cortical inhibition (Wu and Hablitz, 2005; Zhou et al., 2013).

Other neurotransmitters acting on L1 are also relevant for cortical function. For example, ascending ACh axons extend preferential terminations in L1 (Kristt et al., 1985) where neurons express high concentrations of nicotinic receptors (Komal et al., 2015), and an inhibitory interaction of DA D1/5 receptor on $\alpha 7$ nicotinic receptors has been observed in prefrontal L1 (Komal et al., 2015). This DA-ACh interaction can have important consequences in learning and memory. Optical stimulation of cholinergic axons induces excitatory potentials in L1 neurons and generates di-synaptic nicotine receptor-induced prolonged postsynaptic inhibition in layer 2/3 that could be associated with the effects of nicotine on arousal (Arroyo et al., 2012).

Activation of Layer 1 in Motor Cortex

L1 at the level of motor cortex contains afferents from MT specifically VM and VA (Herkenham, 1979; Arbutnott et al., 1990; Kuramoto et al., 2009, 2015; Rubio-Garrido et al., 2009) and from midline/intralaminar nuclei (e.g., Re, PC, CM, Pf and Rh; Berendse and Groenewegen, 1990; Cruikshank et al., 2012). It seems that stimulation of these afferents in L1 can produce changes in synaptic efficacy in motor cortex, for a review see Yu and Zuo (2011). Reorganization of motor representations is a common phenomenon seen after peripheral transection of nerves, repetitive limb movement or motor learning results (Elbert et al., 1995; Sanes and Donoghue, 2000; Harms et al., 2008; Plowman and Kleim, 2010).

Long-term Changes in Dendritic Spines of Pyramidal Neurons are Facilitated by L1 Stimulation

Direct and indirect activation of tufts of apical dendrites by brief stimulation of L1 *in vitro* induces long-term depression of layer 2/3 neurons (Walcott and Langdon, 2001). These changes in postsynaptic response are associated to structural changes likely related to activation of immediate early genes for instance, the activity-regulated cytoskeleton-associated proteins (Arc) and cFos proteins are increased in layer 2/3 and 180 μm from pia in motor cortex as a consequence of learning complex motor tasks (Kleim et al., 1996; Cao et al., 2015). Structural changes include increases in dendritic spine density and cortical map expansion of limb or paw representation. Increases in synapse/neuron ratio are reported for motor cortex layers 5 and 2/3 contralateral to the trained forelimb (Kleim et al., 1996) likely related to consolidation of learning (Kleim et al., 2004). Enlargement of dendritic spines in L1 (Harms et al., 2008) are also a good indicator of modifications of synaptic connectivity

and the influence of this layer on the apical tuft of pyramidal neurons.

Recent *in vivo* visualization of dendritic spines has revealed specific neuronal changes related to motor activity and learned tasks in layer 2/3 of motor cortex in mice under head fixation. Ensembles of neurons display calcium transients in layer 2/3 during performance of a learned task (Komiya et al., 2010; Huber et al., 2012; Hira et al., 2013; Masamizu et al., 2014) and a parallel pruning and growth of dendritic spines likely important for task performance occurs in the apical tuft of neurons active during acquisition of a task in layer 5 (Xu et al., 2009; Yang et al., 2009) and layer 2/3 (Peters et al., 2014; Ma et al., 2015). The presence of DA is crucial for dendritic spine turnover of layer 5 pyramidal tuft (Guo et al., 2015).

Neurotransmitters Released in L1 Modulate Plasticity in Motor Cortex

Learning changes information processing in cortical microcircuits; interest on how neuronal interactions between cortical layers ultimately result in behavior is of outmost relevance. Letzkus et al. (2011) have studied how Ach released in L1 following aversive foot shocks modifies cortical microcircuits involved in learning. Ach produces a nicotinic-dependent excitation of L1 neurons that results in inhibition of layer 2/3 parvalbumin interneurons. This inhibition in turn disinhibits pyramidal neurons. In order for mice to associate a sound with foot shock and express fear, the disinhibition of pyramidal neurons associated with foot shock must take place; pharmacological or optical blockade of pyramidal neuron disinhibition prevents learning. Most likely, similar effects induced by other neurotransmitters released in L1 (e.g., DA and 5HT) are involved in microcircuits involved in learning.

Horizontal L1 Connections between Motor and Sensorimotor Cortex

L1 forms cortico-cortical connections that provide feedback during behavioral tasks. Whisker touch activates L1 of vibrissal motor cortex that in turn sends excitatory input to L1 of S1 barrel cortex to excite dendritic tufts of layer 5 pyramidal neurons (Xu et al., 2012). The large calcium signals recorded in L1 in the S1 cortex during active facial whisker-object contact recorded by these authors in another set of experiments reinforce the concept of a top-down processing of behavioral relevant outputs as well as the horizontal cortico-cortical influence (Harnett et al., 2013).

SUMMARY AND CONCLUSIONS

Highlights on Advances in the Field

- Motor-related information from basal ganglia and cerebellum enters motor cortex via MT. This thalamic region has recently received a different demarcation thanks to new anatomical methods with very good resolution. As a result VA/VL has been divided in rostromedial and caudolateral areas immunoreactive to GAD67 and VGluT2, respectively. VM and rostromedial VA/VL receive GABAergic input from basal ganglia (SNR and GPi(EP)) and caudolateral VA/VL receives

GLU terminals from cerebellum. VM and rostromedial VA/VL project to L1 and caudolateral VA/VL to layer 2/3.

- Apart from cerebellar and basal ganglia influences relayed to motor cortex via MT, indirect cerebellar inputs reach basal ganglia through midline/intralaminar outputs.
- Plastic related changes in spines of pyramidal dendritic tufts make learning-related structural changes a likely substrate for learning in L1.

Voids in the Field that will be Interesting to Fill

- What is the role of the large synaptic terminals from SNR to VM described as “detonator drives” that typically favor neurotransmitter spillover and volume transmission?
- What is the function of the synaptic contacts made in basal ganglia from MT and midline/intralaminar axons as they travel to motor cortex? Which neurons are being contacted?
- What is the function of the electrically coupled elongated neurogliaform interneurons?
- Is 5HT released in L1 involved in learning-induced dendritic changes as are other neurotransmitters released in the area?

Speculations on the Function of Layer 1

The excitatory input of L1 as studied in many cortical areas regulates the active regenerative electrical activity of dendrites of pyramidal cells of layer 2/3 and 5. This excitatory top down control on the dendritic tuft is crucial for integration and further generation of axosomatic discharge.

Recent combined electrophysiological and behavioral observations indicate that L1 can be considered as a driver of pyramidal neurons with important behavioral consequences such as attention, expectation, anticipation, and action command.

We would like to consider projections from MT and midline/intralaminar nuclei to L1 as a regulatory entity of pyramidal cell excitability in motor cortex. These projections can provide the necessary binding input to trigger or suppress final patterns of activity that would ultimately generate appropriate behavioral responses. Motor thalamus has already been labeled a “super integrator” (Bosch-Bouju et al., 2013) and the MT and midline/intralaminar nuclei projections to L1 provide a step further in the integration of motor, cognitive and motivational aspects to produce, in collaboration with thalamic inputs that ultimately reach layer 5, an appropriate response according to the surrounding environment. Hence, projections from MT and midline/intralaminar nuclei could resolve conflicting alternative response patterns and give continuity to cortical function as proposed by Edelman and Gally (2013) and Damasio (1989).

A source of concern when dealing with MT and midline/intralaminar afferents to L1 is the extent of their projection as observed in other neocortical areas. It is hard to conceive the point-to-point modulation suggested by some electrophysiological results e.g., Jiang et al. (2013). Perhaps there are two kinds of inputs, one that involves a restricted command and another that provides a wide-range informative action as suggested by anatomical studies e.g., Arbuthnott et al. (1990).

A mundane example could be a “specific” reverse 911-emergency phone call ordering individual immediate evacuation vs. a “general” regional sound alarm of an approaching hurricane. Does L1 command both types of system? There is still plenty of mystery in the superficial cortical layer but at least forefront tools are shedding new light and making the future an exciting time to be studying this mysterious crown of the cortex.

Changes in dendritic spines induced by motor activity and learning observed in dendritic tufts in L1 underline the functional

rewiring observed as changes in spine morphology and neuronal dynamics (Yuste and Bonhoeffer, 2001; Holtmaat and Svoboda, 2009).

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REFERENCES

- Agnati, L. F., and Fuxe, K. (2000). Volume transmission as a key feature of information handling in the central nervous system possible new interpretative value of the Turing's B-type machine. *Prog. Brain Res.* 125, 3–19. doi: 10.1016/s0079-6123(00)25003-6
- Agnati, L. F., Guidolin, D., Carone, C., Dam, M., Genedani, S., and Fuxe, K. (2008). Understanding neuronal molecular networks builds on neuronal cellular network architecture. *Brain Res. Rev.* 58, 379–399. doi: 10.1016/j.brainresrev.2007.11.002
- Akkal, D., Dum, R. P., and Strick, P. L. (2007). Supplementary motor area and presupplementary motor area: targets of basal ganglia and cerebellar output. *J. Neurosci.* 27, 10659–10673. doi: 10.1523/jneurosci.3134-07.2007
- Anderson, C. J., Sheppard, D. T., Huynh, R., Anderson, D. N., Polar, C. A., and Dorval, A. D. (2015). Subthalamic deep brain stimulation reduces pathological information transmission to the thalamus in a rat model of parkinsonism. *Front. Neural Circuits* 9:31. doi: 10.3389/fncir.2015.00031
- Anstötz, M., Cosgrove, K. E., Hack, I., Mugnaini, E., Maccaferri, G., and Lübke, J. H. (2014). Morphology, input-output relations and synaptic connectivity of Cajal-Retzius cells in layer 1 of the developing neocortex of CXCR4-EGFP mice. *Brain Struct. Funct.* 219, 2119–2139. doi: 10.1007/s00429-013-0627-2
- Antal, M., Beneduce, B. M., and Regehr, W. G. (2014). The substantia nigra conveys target-dependent excitatory and inhibitory outputs from the basal ganglia to the thalamus. *J. Neurosci.* 34, 8032–8042. doi: 10.1523/JNEUROSCI.0236-14.2014
- Arbutnot, G. W., MacLeod, N. K., Maxwell, D. J., and Wright, A. K. (1990). Distribution and synaptic contacts of the cortical terminals arising from neurons in the rat ventromedial thalamic nucleus. *Neuroscience* 38, 47–60. doi: 10.1016/0306-4522(90)90373-c
- Arroyo, S., Bennett, C., Aziz, D., Brown, S. P., and Hestrin, S. (2012). Prolonged disinaptic inhibition in the cortex mediated by slow, non- α 7 nicotinic excitation of a specific subset of cortical interneurons. *J. Neurosci.* 32, 3859–3864. doi: 10.1523/JNEUROSCI.0115-12.2012
- Ascoli, G. A., Alonso-Nanclares, L., Anderson, S. A., Barrionuevo, G., Benavides-Piccion, R., Burkhalter, A., et al. (2008). Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nat. Rev. Neurosci.* 9, 557–568. doi: 10.1038/nrn2402
- Barter, J. W., Li, S., Sukharnikova, T., Rossi, M. A., Bartholomew, R. A., and Yin, H. H. (2015). Basal ganglia outputs map instantaneous position coordinates during behavior. *J. Neurosci.* 35, 2703–2716. doi: 10.1523/JNEUROSCI.3245-14.2015
- Bava, A., Cicirata, F., Licciardello, S., Li Volsi, G., and Panto, M. R. (1979). Fastigial nuclei projections on the ventralis lateralis (VL) thalamic nucleus neurons. *Brain Res.* 168, 169–175. doi: 10.1016/0006-8993(79)90134-3
- Beckstead, R. M. (1984). The thalamostriatal projection in the cat. *J. Comp. Neurol.* 223, 313–346. doi: 10.1002/cne.902230302
- Berendse, H. W., and Groenewegen, H. J. (1990). Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. *J. Comp. Neurol.* 299, 187–228. doi: 10.1002/cne.902990206
- Bodor, A. L., Giber, K., Rovó, Z., Ulbert, I., and Acsády, L. (2008). Structural correlates of efficient GABAergic transmission in the basal ganglia-thalamus pathway. *J. Neurosci.* 28, 3090–3102. doi: 10.1523/JNEUROSCI.5266-07.2008
- Bosch-Bouju, C., Hyland, B. I., and Parr-Brownlie, L. C. (2013). Motor thalamus integration of cortical, cerebellar and basal ganglia information: implications for normal and parkinsonian conditions. *Front. Comput. Neurosci.* 7:163. doi: 10.3389/fncom.2013.00163
- Bright, D. P., and Brickley, S. G. (2008). Acting locally but sensing globally: impact of GABAergic synaptic plasticity on phasic and tonic inhibition in the thalamus. *J. Physiol.* 586, 5091–5099. doi: 10.1111/jphysiol.2008.158576
- Bright, D. P., and Smart, T. G. (2013). Protein kinase C regulates tonic GABA(A) receptor-mediated inhibition in the hippocampus and thalamus. *Eur. J. Neurosci.* 38, 3408–3423. doi: 10.1111/ejn.12352
- Cajal, S. R. (1890). Sobre la existencia de células nerviosas especiales de la primera capa de las circonvoluciones cerebrales. *Gac. Med. Catalana* 15, 225–228.
- Cao, V. Y., Ye, Y., Mastwal, S., Ren, M., Coon, M., Liu, Q., et al. (2015). Motor learning consolidates arc-expressing neuronal ensembles in secondary motor cortex. *Neuron* 86, 1385–1392. doi: 10.1016/j.neuron.2015.05.022
- Cesaro, P., Nguyen-Legros, J., Berger, B., Alvarez, C., and Albe-Fessard, D. (1979). Double labelling of blanchard neurons in the central nervous system of the rat by retrograde axonal transport of horseradish peroxidase and iron dextran complex. *Neurosci. Lett.* 15, 1–7. doi: 10.1016/0304-3940(79)91519-2
- Chevalier, G., and Deniau, J. M. (1982). Inhibitory nigral influence on cerebellar evoked responses in the rat ventromedial thalamic nucleus. *Exp. Brain Res.* 48, 369–376. doi: 10.1007/bf00238613
- Chowdhury, T. G., Jimenez, J. C., Bomar, J. M., Cruz-Martin, A., Cantle, J. P., and Portera-Cailliau, C. (2010). Fate of cajal-retzius neurons in the postnatal mouse neocortex. *Front. Neuroanat.* 4:10. doi: 10.3389/neuro.05.010.2010
- Cruikshank, S. J., Ahmed, O. J., Stevens, T. R., Patrick, S. L., Gonzalez, A. N., Elmaleh, M., et al. (2012). Thalamic control of layer 1 circuits in prefrontal cortex. *J. Neurosci.* 32, 17813–17823. doi: 10.1523/JNEUROSCI.3231-12.2012
- Damasio, A. R. (1989). The brain binds entities and events by multiregional activation from convergence zones. *Neural Comput.* 1, 123–132. doi: 10.1162/neco.1989.1.1.123
- D'Angelo, E., Mazzarello, P., Prestori, F., Mapelli, J., Solinas, S., Lombardo, P., et al. (2011). The cerebellar network: from structure to function and dynamics. *Brain Res. Rev.* 66, 5–15. doi: 10.1016/j.brainresrev.2010.10.002
- Deffeyes, J. E., Touvykine, B., Quessy, S., and Dancuse, N. (2015). Interactions between rostral and caudal cortical motor areas in the rat. *J. Neurophysiol.* 113, 3893–3904. doi: 10.1152/jn.00760.2014
- del Río, J. A., Martínez, A., Fonseca, M., Auladell, C., and Soriano, E. (1995). Glutamate-like immunoreactivity and fate of Cajal-Retzius cells in the murine cortex as identified with calretinin antibody. *Cereb. Cortex* 5, 13–21. doi: 10.1093/cercor/5.1.13
- Deniau, J. M., Lackner, D., and Feger, J. (1978). Effect of substantia nigra stimulation on identified neurons in the VL-VA thalamic complex: comparison between intact and chronically decorticated cats. *Brain Res.* 145, 27–35. doi: 10.1016/0006-8993(78)90793-x
- Desbois, C., and Villanueva, L. (2001). The organization of lateral ventromedial thalamic connections in the rat: a link for the distribution of nociceptive signals to widespread cortical regions. *Neuroscience* 102, 885–898. doi: 10.1016/s0306-4522(00)00537-6
- Destexhe, A., and Sejnowski, T. J. (1995). G protein activation kinetics and spillover of gamma-aminobutyric acid may account for differences between inhibitory responses in the hippocampus and thalamus. *Proc. Natl. Acad. Sci. U S A* 92, 9515–9519. doi: 10.1073/pnas.92.21.9515

- Diamond, J. S. (2002). A broad view of glutamate spillover. *Nat. Neurosci.* 5, 291–292. doi: 10.1038/nn0402-291
- Donoghue, J. P., and Ebner, F. F. (1981). The laminar distribution and ultrastructure of fibers projecting from three thalamic nuclei to the somatic sensory-motor cortex of the opossum. *J. Comp. Neurol.* 198, 389–420. doi: 10.1002/cne.901980303
- Dube, L., Smith, A. D., and Bolam, J. P. (1988). Identification of synaptic terminals of thalamic or cortical origin in contact with distinct medium-size spiny neurons in the rat neostriatum. *J. Comp. Neurol.* 267, 455–471. doi: 10.1002/cne.902670402
- Edelman, G. M., and Gally, J. A. (2013). Reentry: a key mechanism for integration of brain function. *Front. Integr. Neurosci.* 7:63. doi: 10.3389/fnint.2013.00063
- Elbert, T., Pantev, C., Wienbruch, C., Rockstroh, B., and Taub, E. (1995). Increased cortical representation of the fingers of the left hand in string players. *Science* 270, 305–307. doi: 10.1126/science.270.5234.305
- Errington, A. C., Di Giovanni, G., Crunelli, V., and Cope, D. W. (2011). mGluR control of interneuron output regulates feedforward tonic GABA_A inhibition in the visual thalamus. *J. Neurosci.* 31, 8669–8680. doi: 10.1523/JNEUROSCI.0317-11.2011
- Felten, D. L., Hallman, H., and Jonsson, G. (1982). Evidence for a neurotropic role of noradrenergic neurons in the postnatal development of rat cerebral cortex. *J. Neurocytol.* 11, 119–135. doi: 10.1007/bf01258008
- Fenelon, G., Francois, C., Percheron, G., and Yelnik, J. (1991). Topographic distribution of the neurons of the central complex (centre median-parafascicular complex) and of other thalamic neurons projecting to the striatum in macaques. *Neuroscience* 45, 495–510. doi: 10.1016/0306-4522(91)90244-i
- Furuta, T., Tomioka, R., Taki, K., Nakamura, K., Tamamaki, N., and Kaneko, T. (2001). *In vivo* transduction of central neurons using recombinant sindbis virus: golgi-like labeling of dendrites and axons with membrane-targeted fluorescent proteins. *J. Histochem. Cytochem.* 49, 1497–1508. doi: 10.1177/002215540104901203
- Gao, W. J., and Zheng, Z. H. (2004). Target-specific differences in somatodendritic morphology of layer V pyramidal neurons in rat motor cortex. *J. Comp. Neurol.* 476, 174–185. doi: 10.1002/cne.20224
- Giménez-Amaya, J. M., McFarland, N. R., de las Heras, S., and Haber, S. N. (1995). Organization of thalamic projections to the ventral striatum in the primate. *J. Comp. Neurol.* 354, 127–149. doi: 10.1002/cne.903540109
- Guo, L., Xiong, H., Kim, J. I., Wu, Y. W., Lalchandani, R. R., Cui, Y., et al. (2015). Dynamic rewiring of neural circuits in the motor cortex in mouse models of parkinson's disease. *Nat. Neurosci.* 18, 1299–1309. doi: 10.1038/nn.4082
- Harms, K. J., Rioult-Pedotti, M. S., Carter, D. R., and Dunaevsky, A. (2008). Transient spine expansion and learning-induced plasticity in layer 1 primary motor cortex. *J. Neurosci.* 28, 5686–5690. doi: 10.1523/JNEUROSCI.0584-08.2008
- Harnett, M. T., Magee, J. C., and Williams, S. R. (2015). Distribution and function of HCN channels in the apical dendritic tuft of neocortical pyramidal neurons. *J. Neurosci.* 35, 1024–1037. doi: 10.1523/JNEUROSCI.2813-14.2015
- Harnett, M. T., Xu, N. L., Magee, J. C., and Williams, S. R. (2013). Potassium channels control the interaction between active dendritic integration compartments in layer 5 cortical pyramidal neurons. *Neuron* 79, 516–529. doi: 10.1016/j.neuron.2013.06.005
- Herd, M. B., Brown, A. R., Lambert, J. J., and Belelli, D. (2013). Extrasynaptic GABA(A) receptors couple presynaptic activity to postsynaptic inhibition in the somatosensory thalamus. *J. Neurosci.* 33, 14850–14868. doi: 10.1523/JNEUROSCI.1174-13.2013
- Herkenham, M. (1979). The afferent and efferent connections of the ventromedial thalamic nucleus in the rat. *J. Comp. Neurol.* 183, 487–517. doi: 10.1002/cne.901830304
- Herkenham, M. (1986). "New perspectives on the organization and evolution of nonspecific thalamocortical projections," in *Cerebral Cortex*, ed. E. G. Jones (New York: Plenum), 403–445.
- Hira, R., Ohkubo, F., Ozawa, K., Isomura, Y., Kitamura, K., Kano, M., et al. (2013). Spatiotemporal dynamics of functional clusters of neurons in the mouse motor cortex during a voluntary movement. *J. Neurosci.* 33, 1377–1390. doi: 10.1523/JNEUROSCI.2550-12.2013
- Holtmaat, A., and Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat. Rev. Neurosci.* 10, 647–658. doi: 10.1038/nrn2699
- Hoover, J. E., and Strick, P. L. (1999). The organization of cerebellar and basal ganglia outputs to primary motor cortex as revealed by retrograde transneuronal transport of herpes simplex virus type 1. *J. Neurosci.* 19, 1446–1463.
- Hubel, D. H. (1982). Cortical neurobiology: a slanted historical perspective. *Annu. Rev. Neurosci.* 5, 363–370. doi: 10.1146/annurev.ne.05.030182.002051
- Huber, D., Gutnisky, D. A., Peron, S., O'Connor, D. H., Wiegert, J. S., Tian, L., et al. (2012). Multiple dynamic representations in the motor cortex during sensorimotor learning. *Nature* 484, 473–478. doi: 10.1038/nature11039
- Huntley, G. W., de Blas, A. L., and Jones, E. G. (1990). GABA_A receptor immunoreactivity in adult and developing monkey sensory-motor cortex. *Exp. Brain Res.* 82, 519–535. doi: 10.1007/bf00228794
- Imamoto, K., Karasawa, N., Isomura, G., and Nagatsu, I. (1994). Cajal-Retzius neurons identified by GABA immunohistochemistry in layer I of the rat cerebral cortex. *Neurosci. Res.* 20, 101–105. doi: 10.1016/0168-0102(94)90027-2
- Jáidar Benavides, O., and Arbutnott, G. W. (2013). *Section 3.2: The Thalamocortical Output from Basal Ganglia, Figure 2*. Available online at: <https://groups.oist.jp/bmbu>
- Jáidar, O., Roome, C. J., Nakano, Y., Garcia-Munoz, M., Bernd, K., and Arbutnott, G. W. (2015). "Thalamocortical axons and deep cortical layer dendritic arbor in motor cortex layer I display a dynamic array of calcium transients during locomotion and systemic blockade of dopamine receptors," in *British Neuroscience Association Festival of Neuroscience*, Vol. 23, Poster Ref. P1-C-008, Theme: C: Sensory and Motor Systems, Edinburgh.
- Jiang, X., Wang, G., Lee, A. J., Stornetta, R. L., and Zhu, J. J. (2013). The organization of two new cortical interneuronal circuits. *Nat. Neurosci.* 16, 210–218. doi: 10.1038/nn.3305
- Jinnai, K., and Matsuda, Y. (1981). Thalamocaudate projection neurons with a branching axon to the cerebral motor cortex. *Neurosci. Lett.* 26, 95–99. doi: 10.1016/0304-3940(81)90332-3
- Jones, E. G. (2007). *The Thalamus*. New York: Cambridge University Press, 808–874.
- Jones, E. G., and Leavitt, R. Y. (1974). Retrograde axonal-transport and demonstration of nonspecific projections to cerebral-cortex and striatum from thalamic intralaminar nuclei in rat, cat and monkey. *J. Comp. Neurol.* 154, 349–377. doi: 10.1002/cne.901540402
- Jones, E. G., and Powell, T. P. (1969). The cortical projection of the ventroposterior nucleus of the thalamus in the cat. *Brain Res.* 13, 298–318. doi: 10.1016/0006-8993(69)90289-3
- Jones, E. G., and Powell, T. P. (1970a). Connexions of the somatic sensory cortex of the rhesus monkey. 3. Thalamic connexions. *Brain* 93, 37–56. doi: 10.1093/brain/93.1.37
- Jones, E. G., and Powell, T. P. (1970b). An electron microscopic study of the laminar pattern and mode of termination of afferent fibre pathways in the somatic sensory cortex of the cat. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 257, 45–62. doi: 10.1098/rstb.1970.0007
- Jouve, L., Salin, P., Melon, C., and Kerkerian-Le Goff, L. (2010). Deep brain stimulation of the center median-parafascicular complex of the thalamus has efficient anti-parkinsonian action associated with widespread cellular responses in the basal ganglia network in a rat model of parkinson's disease. *J. Neurosci.* 30, 9919–9928. doi: 10.1523/JNEUROSCI.1404-10.2010
- Killackey, H., and Ebner, F. (1973). Convergent projection of three separate thalamic nuclei on to a single cortical area. *Science* 179, 283–285. doi: 10.1126/science.179.4070.283
- Kleim, J. A., Hogg, T. M., Vandenberg, P. M., Cooper, N. R., Bruneau, R., and Rempel, M. (2004). Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *J. Neurosci.* 24, 628–633. doi: 10.1523/jneurosci.3440-03.2004
- Kleim, J. A., Lussnig, E., Schwarz, E. R., Comery, T. A., and Greenough, W. T. (1996). Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. *J. Neurosci.* 16, 4529–4535.
- Komal, P., Estakhr, J., Kamran, M., Renda, A., and Nashmi, R. (2015). cAMP-dependent protein kinase inhibits alpha7 nicotinic receptor activity in layer

- 1 cortical interneurons through activation of D1/D5 dopamine receptors. *J. Physiol.* 593, 3513–3532. doi: 10.1113/jp270469
- Komiyama, T., Sato, T. R., O'Connor, D. H., Zhang, Y. X., Huber, D., Hooks, B. M., et al. (2010). Learning-related fine-scale specificity imaged in motor cortex circuits of behaving mice. *Nature* 464, 1182–1186. doi: 10.1038/nature08897
- Kristt, D. A., McGowan, R. A., Jr., Martin-Mackinnon, N., and Solomon, J. (1985). Basal forebrain innervation of rodent neocortex: studies using acetylcholinesterase histochemistry, Golgi and lesion strategies. *Brain Res.* 337, 19–39. doi: 10.1016/0006-8993(85)91606-3
- Kubota, Y., Shigematsu, N., Karube, F., Sekigawa, A., Kato, S., Yamaguchi, N., et al. (2011). Selective coexpression of multiple chemical markers defines discrete populations of neocortical GABAergic neurons. *Cereb. Cortex* 21, 1803–1817. doi: 10.1093/cercor/bhq252
- Kultas-Ilinsky, K., and Ilinsky, I. A. (1990). Fine structure of the magnocellular subdivision of the ventral anterior thalamic nucleus (VAmc) of Macaca mulatta: II. Organization of nigrothalamic afferents as revealed with EM autoradiography. *J. Comp. Neurol.* 294, 479–489. doi: 10.1002/cne.902940314
- Kuramoto, E., Fujiyama, F., Nakamura, K. C., Tanaka, Y., Hioki, H., and Kaneko, T. (2011). Complementary distribution of glutamatergic cerebellar and GABAergic basal ganglia afferents to the rat motor thalamic nuclei. *Eur. J. Neurosci.* 33, 95–109. doi: 10.1111/j.1460-9568.2010.07481.x
- Kuramoto, E., Furuta, T., Nakamura, K. C., Unzai, T., Hioki, H., and Kaneko, T. (2009). Two types of thalamocortical projections from the motor thalamic nuclei of the rat: a single neuron-tracing study using viral vectors. *Cereb. Cortex* 19, 2065–2077. doi: 10.1093/cercor/bhn231
- Kuramoto, E., Ohno, S., Furuta, T., Unzai, T., Tanaka, Y. R., Hioki, H., et al. (2015). Ventral medial nucleus neurons send thalamocortical afferents more widely and more preferentially to layer 1 than neurons of the ventral anterior-ventral lateral nuclear complex in the rat. *Cereb. Cortex* 25, 221–235. doi: 10.1093/cercor/bht216
- Kuroda, M., and Price, J. L. (1991). Ultrastructure and synaptic organization of axon terminals from brainstem structures to the mediadorsal thalamic nucleus of the rat. *J. Comp. Neurol.* 313, 539–552. doi: 10.1002/cne.903130313
- Lanciego, J. L., and Vázquez, A. (2012). The basal ganglia and thalamus of the long-tailed macaque in stereotaxic coordinates. A template atlas based on coronal, sagittal and horizontal brain sections. *Brain Struct. Funct.* 217, 613–666. doi: 10.1007/s00429-011-0370-5
- Larkum, M. E., and Zhu, J. J. (2002). Signaling of layer 1 and whisker-evoked Ca²⁺ and Na⁺ action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons *in vitro* and *in vivo*. *J. Neurosci.* 22, 6991–7005.
- Larkum, M. E., Nevian, T., Sandler, M., Polsky, A., and Schiller, J. (2009). Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* 325, 756–760. doi: 10.1126/science.1171958
- Larkum, M. E., Senn, W., and Lüscher, H. R. (2004). Top-down dendritic input increases the gain of layer 5 pyramidal neurons. *Cereb. Cortex* 14, 1059–1070. doi: 10.1093/cercor/bhh065
- Larsen, D. D., and Callaway, E. M. (2006). Development of layer-specific axonal arborizations in mouse primary somatosensory cortex. *J. Comp. Neurol.* 494, 398–414. doi: 10.1002/cne.20754
- Letzkus, J. J., Wolff, S. B., Meyer, E. M., Tovote, P., Courtin, J., Herry, C., et al. (2011). A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature* 480, 331–335. doi: 10.1038/nature10674
- Levitt, P., and Moore, R. Y. (1978). Noradrenaline neuron innervation of the neocortex in the rat. *Brain Res.* 139, 219–231. doi: 10.1016/0006-8993(78)90925-3
- Levitt, P., and Moore, R. Y. (1979). Development of the noradrenergic innervation of neocortex. *Brain Res.* 162, 243–259. doi: 10.1016/0006-8993(79)90287-7
- Li, X. Y., Chen, T., Descalzi, G., Koga, K., Qiu, S., and Zhuo, M. (2012). Characterization of neuronal intrinsic properties and synaptic transmission in layer I of anterior cingulate cortex from adult mice. *Mol. Pain* 8:53. doi: 10.1186/1744-8069-8-53
- Lorente De Nó, R. (1922). La corteza cerebral del ratón. *Trabajo de Investigación Biológica Madrid* 20, 41–78.
- Lu, S. M., and Lin, R. C. (1993). Thalamic afferents of the rat barrel cortex: a light- and electron-microscopic study using Phaseolus vulgaris leucoagglutinin as an anterograde tracer. *Somatosens. Mot. Res.* 10, 1–16. doi: 10.1010/08990229309028819
- Ma, J., Yao, X. H., Fu, Y., and Yu, Y. C. (2014). Development of layer 1 neurons in the mouse neocortex. *Cereb. Cortex* 24, 2604–2618. doi: 10.1093/cercor/bht114
- Ma, L., Qiao, Q., Tsai, J. W., Yang, G., Li, W., and Gan, W. B. (2015). Experience-dependent plasticity of dendritic spines of layer 2/3 pyramidal neurons in the mouse cortex. *Dev. Neurobiol.* doi: 10.1002/dneu.22313 [Epub ahead of print].
- Macchi, G., Bentivoglio, M., Molinari, M., and Minciacchi, D. (1984). The thalamo-caudate versus thalamo-cortical projections as studied in the cat with fluorescent retrograde double labeling. *Exp. Brain Res.* 54, 225–239. doi: 10.1007/bf00236222
- MacLeod, N. K., James, T. A., Kilpatrick, I. C., and Starr, M. S. (1980). Evidence for a GABAergic nigrothalamic pathway in the rat. II. Electrophysiological studies. *Exp. Brain Res.* 40, 55–61. doi: 10.1007/bf00236662
- Magill, P. J., Sharott, A., Bolam, J. P., and Brown, P. (2004). Brain state-dependency of coherent oscillatory activity in the cerebral cortex and basal ganglia of the rat. *J. Neurophysiol.* 92, 2122–2136. doi: 10.1152/jn.00333.2004
- Marin-Padilla, M., and Marin-Padilla, T. M. (1982). Origin, prenatal development and structural organization of layer I of the human cerebral (motor) cortex. A Golgi study. *Anat. Embryol. (Berl)* 164, 161–206. doi: 10.1007/bf00318504
- Masamizu, Y., Tanaka, Y. R., Tanaka, Y. H., Hira, R., Ohkubo, F., Kitamura, K., et al. (2014). Two distinct layer-specific dynamics of cortical ensembles during learning of a motor task. *Nat. Neurosci.* 17, 987–994. doi: 10.1038/nn.3739
- Matsuda, Y., and Nakamura, M. (1982). Mode of activation of thalamic neurons by stimulation of the cerebellar fastigial nuclei in the cat. *Brain Res.* 241, 370–373. doi: 10.1016/0006-8993(82)91081-2
- Middleton, F. A., and Strick, P. L. (1994). Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. *Science* 266, 458–461. doi: 10.1126/science.7939688
- Mitchell, B. D., and Cauller, L. J. (2001). Corticocortical and thalamocortical projections to layer I of the frontal neocortex in rats. *Brain Res.* 921, 68–77. doi: 10.1016/s0006-8993(01)03084-0
- Mohammed, H., and Jain, N. (2014). Two whisker motor areas in the rat cortex: evidence from thalamocortical connections. *J. Comp. Neurol.* 522, 528–545. doi: 10.1002/cne.23424
- Monconduit, L., and Villanueva, L. (2005). The lateral ventromedial thalamic nucleus spreads nociceptive signals from the whole body surface to layer I of the frontal cortex. *Eur. J. Neurosci.* 21, 3395–3402. doi: 10.1111/j.1460-9568.2005.04160.x
- Muralidhar, S., Wang, Y., and Markram, H. (2013). Synaptic and cellular organization of layer 1 of the developing rat somatosensory cortex. *Front. Neuroanat.* 7:52. doi: 10.3389/fnana.2013.00052
- Nakamura, K. C., Sharott, A., and Magill, P. J. (2014). Temporal coupling with cortex distinguishes spontaneous neuronal activities in identified basal ganglia-recipient and cerebellar-recipient zones of the motor thalamus. *Cereb. Cortex* 24, 81–97. doi: 10.1093/cercor/bhs287
- Nakano, K., Hasegawa, Y., Tokushige, A., Nakagawa, S., Kayahara, T., and Mizuno, N. (1990). Topographical projections from the thalamus, subthalamic nucleus and pedunculopontine tegmental nucleus to the striatum in the Japanese monkey, macaca fuscata. *Brain Res.* 537, 54–68. doi: 10.1016/0006-8993(90)90339-d
- Ohtake, T., and Yamada, H. (1989). Efferent connections of the nucleus reuniens and the rhomboid nucleus in the rat: an anterograde PHA-L tracing study. *Neurosci. Res.* 6, 556–568. doi: 10.1016/0168-0102(89)90044-8
- Patino, P., and Garcia-Munoz, M. (1985). Electrophysiological thalamic responses evoked by dopamine-receptor stimulation into the striatum. *Brain Res.* 361, 1–9. doi: 10.1016/0006-8993(85)91268-5
- Paz, J. T., Chavez, M., Sallet, S., Deniau, J. M., and Chazpiere, S. (2007). Activity of ventral medial thalamic neurons during absence seizures and modulation of cortical paroxysms by the nigrothalamic pathway. *J. Neurosci.* 27, 929–941. doi: 10.1523/jneurosci.4677-06.2007
- Peters, A. J., Chen, S. X., and Komiyama, T. (2014). Emergence of reproducible spatiotemporal activity during motor learning. *Nature* 510, 263–267. doi: 10.1038/nature13235
- Plowman, E. K., and Kleim, J. A. (2010). Motor cortex reorganization across the lifespan. *J. Commun. Disord.* 43, 286–294. doi: 10.1016/j.jcomdis.2010.04.005
- Reig, R., and Silberberg, G. (2014). Multisensory integration in the mouse striatum. *Neuron* 83, 1200–1212. doi: 10.1016/j.neuron.2014.07.033

- Royce, G. J. (1983). Single thalamic neurons which project to both the rostral cortex and caudate nucleus studied with the fluorescent double labeling method. *Exp. Neurol.* 79, 773–784. doi: 10.1016/0014-4886(83)90041-9
- Royce, G. J., and Mourey, R. J. (1985). Efferent connections of the centromedian and parafascicular thalamic nuclei: an autoradiographic investigation in the cat. *J. Comp. Neurol.* 235, 277–300. doi: 10.1002/cne.902350302
- Royce, G. J., Bromley, S., Gracco, C., and Beckstead, R. M. (1989). Thalamocortical connections of the rostral intralaminar nuclei: an autoradiographic analysis in the cat. *J. Comp. Neurol.* 288, 555–582. doi: 10.1002/cne.902880404
- Rubenstein, J. L. (1998). Development of serotonergic neurons and their projections. *Biol. Psychiatry* 44, 145–150. doi: 10.1016/s0006-3223(98)00133-4
- Rubio-Garrido, P., Pérez-de-Manzo, F., Porrero, C., Galazo, M. J., and Clascá, F. (2009). Thalamic input to distal apical dendrites in neocortical layer 1 is massive and highly convergent. *Cereb. Cortex* 19, 2380–2395. doi: 10.1093/cercor/bhn259
- Sadikot, A. F., Parent, A., and François, C. (1992). Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: a PHA-L study of subcortical projections. *J. Comp. Neurol.* 315, 137–159. doi: 10.1002/cne.903150203
- Sakai, S. T., Grofova, I., and Bruce, K. (1998). Nigrothalamic projections and nigrothalamocortical pathway to the medial agranular cortex in the rat: single- and double-labeling light and electron microscopic studies. *J. Comp. Neurol.* 391, 506–525. doi: 10.1002/(sici)1096-9861(19980222)391:4<506::aid-cne7>3.0.co;2-4
- Sanes, J. N., and Donoghue, J. P. (2000). Plasticity and primary motor cortex. *Annu. Rev. Neurosci.* 23, 393–415. doi: 10.1146/annurev.neuro.23.1.393
- Sato, M., Itoh, K., and Mizuno, N. (1979). Distribution of thalamo-caudate neurons in the cat as demonstrated by horseradish peroxidase. *Exp. Brain Res.* 34, 143–153. doi: 10.1007/bf00238347
- Scheibel, M. E., and Scheibel, A. B. (1967). Structural organization of nonspecific thalamic nuclei and their projection toward cortex. *Brain Res.* 6, 60–94. doi: 10.1016/0006-8993(67)90183-7
- Sheets, P. L., Suter, B. A., Kiritani, T., Chan, C. S., Surmeier, D. J., and Shepherd, G. M. (2011). Corticospinal-specific HCN expression in mouse motor cortex: I(h)-dependent synaptic integration as a candidate microcircuit mechanism involved in motor control. *J. Neurophysiol.* 106, 2216–2231. doi: 10.1152/jn.00232.2011
- Soda, T., Nakashima, R., Watanabe, D., Nakajima, K., Pastan, I., and Nakanishi, S. (2003). Segregation and coactivation of developing neocortical layer 1 neurons. *J. Neurosci.* 23, 6272–6279.
- Strick, P. L. (1973). Light-microscopic-analysis-of-the-cortical-projection-of-the-thalamic-ventrolateral-nucleus-in-the-cat. *Brain Res.* 55, 1–24. doi: 10.1016/0006-8993(73)90485-x
- Strick, P. L., and Sterling, P. (1974). Synaptic termination of afferents from the ventrolateral nucleus of the thalamus in the cat motor cortex. A light and electron microscopy study. *J. Comp. Neurol.* 153, 77–106. doi: 10.1002/cne.901530107
- Takada, M., Ng, G., and Hattori, T. (1986). Single pallidal neurons project both to the striatum and thalamus in the rat. *Neurosci. Lett.* 69, 217–220. doi: 10.1016/0304-3940(86)90482-9
- Tanibuchi, I., Kitano, H., and Jinnai, K. (2009). Substantia nigra output to prefrontal cortex via thalamus in monkeys. I. Electrophysiological identification of thalamic relay neurons. *J. Neurophysiol.* 102, 2933–2945. doi: 10.1152/jn.91287.2008
- Tennant, K. A., Adkins, D. L., Donlan, N. A., Asay, A. L., Thomas, N., Kleim, J. A., et al. (2011). The organization of the forelimb representation of the C57BL/6 mouse motor cortex as defined by intracortical microstimulation and cytoarchitecture. *Cereb. Cortex* 21, 865–876. doi: 10.1093/cercor/bhq159
- Thomson, A. M., and Lamy, C. (2007). Functional maps of neocortical local circuitry. *Front. Neurosci.* 1, 19–42. doi: 10.3389/neuro.01.1.1.002.2007
- Tsumori, T., Yokota, S., Ono, K., and Yasui, Y. (2002). Synaptic organization of GABAergic projections from the substantia nigra pars reticulata and the reticular thalamic nucleus to the parafascicular thalamic nucleus in the rat. *Brain Res.* 957, 231–241. doi: 10.1016/s0006-8993(02)03554-0
- Ueki, A. (1983). The mode of nigro-thalamic transmission investigated with intracellular recording in the cat. *Exp. Brain Res.* 49, 116–124. doi: 10.1007/bf00235546
- Uno, M., Ozawa, N., and Yoshida, M. (1978). The mode of pallido-thalamic transmission investigated with intracellular recording from cat thalamus. *Exp. Brain Res.* 33, 493–507. doi: 10.1007/bf00235570
- Van Der Werf, Y. D., Witter, M. P., and Groenewegen, H. J. (2002). The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness. *Brain Res. Brain Res. Rev.* 39, 107–140. doi: 10.1016/s0165-0173(02)00181-9
- Vertes, R. P., Linley, S. B., and Hoover, W. B. (2015). Limbic circuitry of the midline thalamus. *Neurosci. Biobehav. Rev.* 54, 89–107. doi: 10.1016/j.neubiorev.2015.01.014
- Walcott, E. C., and Langdon, R. B. (2001). Short-term plasticity of extrinsic excitatory inputs to neocortical layer 1. *Exp. Brain Res.* 136, 143–151. doi: 10.1007/s002210000582
- Weiler, N., Wood, L., Yu, J., Solla, S. A., and Shepherd, G. M. (2008). Top-down laminar organization of the excitatory network in motor cortex. *Nat. Neurosci.* 11, 360–366. doi: 10.1038/nn2049
- Williams, S. R. (2005). Encoding and decoding of dendritic excitation during active states in pyramidal neurons. *J. Neurosci.* 25, 5894–5902. doi: 10.1523/jneurosci.0502-05.2005
- Wozny, C., and Williams, S. R. (2011). Specificity of synaptic connectivity between layer 1 inhibitory interneurons and layer 2/3 pyramidal neurons in the rat neocortex. *Cereb. Cortex* 21, 1818–1826. doi: 10.1093/cercor/bhq257
- Wu, J., and Hablitz, J. J. (2005). Cooperative activation of D1 and D2 dopamine receptors enhances a hyperpolarization-activated inward current in layer I interneurons. *J. Neurosci.* 25, 6322–6328. doi: 10.1523/jneurosci.1405-05.2005
- Xu, N. L., Harnett, M. T., Williams, S. R., Huber, D., O'connor, D. H., Svoboda, K., et al. (2012). Nonlinear dendritic integration of sensory and motor input during an active sensing task. *Nature* 492, 247–251. doi: 10.1038/nature11601
- Xu, T., Yu, X., Perlik, A. J., Tobin, W. F., Zweig, J. A., Tennant, K., et al. (2009). Rapid formation and selective stabilization of synapses for enduring motor memories. *Nature* 462, 915–919. doi: 10.1038/nature08389
- Yang, G., Pan, F., and Gan, W. B. (2009). Stably maintained dendritic spines are associated with lifelong memories. *Nature* 462, 920–924. doi: 10.1038/nature08577
- Ye, Z., McGee, T. P., Houston, C. M., and Brickley, S. G. (2013). The contribution of delta subunit-containing GABA_A receptors to phasic and tonic conductance changes in cerebellum, thalamus and neocortex. *Front. Neural Circuits* 7:203. doi: 10.3389/fncir.2013.00203
- Yu, X., and Zuo, Y. (2011). Spine plasticity in the motor cortex. *Curr. Opin. Neurobiol.* 21, 169–174. doi: 10.1016/j.conb.2010.07.010
- Yuste, R., and Bonhoeffer, T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu. Rev. Neurosci.* 24, 1071–1089. doi: 10.1146/annurev.neuro.24.1.1071
- Zecevic, N., and Rakic, P. (2001). Development of layer I neurons in the primate cerebral cortex. *J. Neurosci.* 21, 5607–5619.
- Zhou, F. M., and Hablitz, J. J. (1996). Layer I neurons of rat neocortex. I. Action potential and repetitive firing properties. *J. Neurophysiol.* 76, 651–667.
- Zhou, W. L., Oikonomou, K. D., Short, S. M., and Antic, S. D. (2013). Dopaminergic regulation of dendritic calcium: fast multisite calcium imaging. *Methods Mol. Biol.* 1064, 123–138. doi: 10.1007/978-1-62703-251-3_9
- Zhu, Y., and Zhu, J. J. (2004). Rapid arrival and integration of ascending sensory information in layer 1 nonpyramidal neurons and tuft dendrites of layer 5 pyramidal neurons of the neocortex. *J. Neurosci.* 24, 1272–1279. doi: 10.1523/jneurosci.4805-03.2004

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Effects of the Concomitant Activation of ON and OFF Retinal Ganglion Cells on the Visual Thalamus: Evidence for an Enhanced Recruitment of GABAergic Cells

Giovanni Montesano^{1,2,3†}, Marcello Belfiore^{1,2†}, Maddalena Ripamonti^{1,2}, Alessandro Arena^{1,2}, Jacopo Lamanna^{1,2}, Mattia Ferro^{1,2}, Vincenzo Zimarino¹, Alessandro Ambrosi^{1,2} and Antonio Malgaroli^{1,2*}

¹ Neurobiology of Learning Unit, Division of Neuroscience, Scientific Institute San Raffaele, Milan, Italy, ² Università Vita-Salute San Raffaele, Milan, Italy, ³ Ophthalmology, Azienda Ospedaliera San Paolo, Milan, Italy

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Edited by:

Vincenzo Crunelli,
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Ya-tang Li,

California Institute of Technology, USA

*Correspondence:

Antonio Malgaroli
malgaroli.antonio@univr.it

[†] These authors have contributed
equally to this work.

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A fundamental question in vision neuroscience is how parallel processing of Retinal Ganglion Cell (RGC) signals is integrated at the level of the visual thalamus. It is well-known that parallel ON-OFF pathways generate output signals from the retina that are conveyed to the dorsal lateral geniculate nucleus (dLGN). However, it is unclear how these signals distribute onto thalamic cells and how these two pathways interact. Here, by electrophysiological recordings and c-Fos expression analysis, we characterized the effects of pharmacological manipulations of the retinal circuit aimed at inducing either a selective activation of a single pathway, OFF RGCs [intravitreal L-(+)-2-Amino-4-phosphonobutyric, L-AP4] or an unregulated activity of all classes of RGCs (intravitreal 4-Aminopyridine, 4-AP). In *in vivo* experiments, the analysis of c-Fos expression in the dLGN showed that these two manipulations recruited active cells from the same area, the lateral edge of the dLGN. Despite this similarity, the unregulated co-activation of both ON and OFF pathways by 4-AP yielded a much stronger recruitment of GABAergic interneurons in the dLGN when compared to L-AP4 pure OFF activation. The increased activation of an inhibitory thalamic network by a high level of unregulated discharge of ON and OFF RGCs might suggest that cross-inhibitory pathways between opposing visual channels are presumably replicated at multiple levels in the visual pathway, thus increasing the filtering ability for non-informative or noisy visual signals.

Keywords: dLGN, retina, 4-AP, 4-aminopyridine, inhibitory neurons, visual thalamus, c-Fos, NeuN

INTRODUCTION

The lateral geniculate nucleus (LGN), which allows information transfer from the retina to the visual cortex, has a complex role in vision that is still not fully understood and clearly must go beyond the simple regulation of transfer efficiency of visual signals (Hubel and Wiesel, 1961; Guillery and Sherman, 2002). This structure is composed of a variety of intrinsic neuronal cells that can be categorized into long range relay neurons connecting to the visual cortex, the

thalamocortical neurons, and GABAergic interneurons which are randomly scattered within the LGN (Gabbott and Bacon, 1994). Both cell types are targeted by the input from retinal ganglion cells (RGCs), known to invade the LGN from its lateral border (Kim et al., 2010; Dhande et al., 2011), forming large rosette-like presynaptic boutons on thalamic cells (Li et al., 2003). The activation of interneurons by the retino-thalamic axons provides feedforward inhibition of thalamocortical neurons. This inhibitory input controls the number and the precision of visually evoked spikes and refines the receptive fields of thalamocortical neurons (Berardi and Morrone, 1984; Blitz and Regehr, 2003, 2005). Thalamic neurons are also reached by a very large number of non-retinal inputs which originate from the thalamic reticular nucleus, the superior colliculus, layer 6 cortical neurons [i.e., the cortico-thalamic feedback, which enters the dLGN from its medial border (Jacobs et al., 2007; Su et al., 2011)], the pedunculopontine tegmentum, the parabigeminal nucleus, and the pretectum (Bickford et al., 2000). In other mammals, evidence exists that this nucleus might also be reached by afferents from the substantia reticularis (Francesconi et al., 1988). The synaptic contacts made by non-retinal inputs, together with those of *in situ* GABAergic interneurons, account for the large majority of LGN synaptic connections (Van Horn et al., 2000). They participate in visual perception and its modulation, for example during the different sleep-wake states.

In rodents, the LGN complex is usually subdivided in its dorsal portion (dLGN), the intergeniculate leaflet (IGL; the pregeniculate nucleus of primates), and in the ventral lateral geniculate nucleus (vLGN). While the IGL and the vLGN belong to the circadian rhythm system (Morin and Allen, 2006), the dLGN is the image forming region of the LGN. Based on conventional histological stainings, the LGN lamination seen in high mammals (Polyak, 1957; Hubel and Wiesel, 1961) is not visible in the rodent dLGN. In fact, most of RGC axons cross at the optic chiasm to reach the dLGN of the opposite brain hemisphere. Ipsilateral retinal axons which represent just the ~3–5% of the total RGCs fibers (Polyak, 1957; Jeffery, 1984) segregate in a specific dLGN subregion (Godement et al., 1980, 1984; Reese, 1988), a rostro-ventral central structure named the “hidden lamina,” suggestive of a primordial lamination plan (Reese, 1984, 1988). This finding was confirmed and extended by more refined labeling methods based on transgenic lines expressing fluorescent proteins in specific subsets of RGCs (Huberman et al., 2008, 2009; Kim et al., 2010; Rivlin-Etzion et al., 2011). These anatomical findings need to be substantiated by functional recordings to evaluate the relevance of these neuronal-synaptic laminae. Interestingly, electrophysiological recordings from rat LGN have demonstrated a comparable response to ipsilateral and contralateral stimuli on a large fraction of dLGN neurons, suggesting a much larger superimposition of crossed and uncrossed axon collaterals (Grieve, 2005). Based on these results binocular integration in rodents might already occur inside the visual thalamus (Grieve, 2005). The functional organization of the dLGN activation pattern can be established by the expression of the c-Fos gene product, a member of the Immediate Early Gene family, whose expression

is calcium regulated through CREB (cAMP response element-binding protein) phosphorylation (Sheng et al., 1990; Flavell and Greenberg, 2008). In fact, cellular levels of the c-Fos protein have been shown to be able to report neuronal firing and synaptic regulation (Murphy et al., 1991). Based on immunocytochemical detection or using specific mouse lines where the c-Fos promoter drives the expression of reporter molecules (Greferath et al., 2004; Murphy et al., 2004), staining for c-Fos can be effectively used to trace the spatial distribution of active cells such as those of the visual thalamus (Montero and Jian, 1995; Correa-Lacarcel et al., 2000; Greferath et al., 2004; Lu et al., 2004; Dai et al., 2009). In most of these studies, the light stimulation induced the appearance of some c-Fos positive cells in the dLGN, although no functional segregation or lamination of active cells was reported (Montero and Jian, 1995; Correa-Lacarcel et al., 2000; Greferath et al., 2004).

The aim of our study was to characterize the effects of two different (chaotic or highly coherent) retinal inputs to the dLGN in terms of different patterns of c-Fos expression in this nucleus. We used monocular ON-OFF light pattern stimulation in association with exposure of RGCs to either 4-Aminopyridine (4-AP) or L-(+)-2-Amino-4-phosphonobutyric (L-AP4). 4-AP was used to produce random increase in the excitability of both ON and OFF ganglion cells, hence in their unregulated firing. On the contrary, L-AP4, an ON-pathway inhibitor (Slaughter and Miller, 1981), was applied to promote a much more coherent RGC firing. Our results provide evidence that these two modalities of retina activation, which are expected to convey different amounts of visual information, based on their low or high level of coherence in RGC activities, both activate the intrinsic inhibitory circuit of the dLGN but to a different extent. In the presence of noisy or non-coherent input, as during 4-AP administration, this inhibitory network of dLGN GABAergic interneurons appeared to be more strongly recruited, possibly in a physiological attempt aimed at filtering out non-coherent or noisy signals. Interestingly, the vast majority of active neurons appeared not randomly distributed across the dLGN, with a higher density of active cells near the lateral border. Our findings provide insights into the functional architecture of the rat dLGN and suggest that the activity pattern of dLGN cells follows to some extent but do not superimpose with the well-known distribution of crossed retino-geniculate fibers (Huberman et al., 2008, 2009; Kim et al., 2010; Rivlin-Etzion et al., 2011).

MATERIALS AND METHODS

Animal Care Procedures

Research and animal care procedures were approved by our Institutional Animal Care and Use Committee for Good Animal Experimentation in accordance with the Italian Ministero della Salute code of practice for the care and use of animals for scientific purposes (IACUC number: 54o). Experiments were carried out on adult male Sprague Dawley rats (weight 175–200 g). Before experiments, animals were individually caged with free access to food and water *ad libitum* and were exposed to 12 h light/dark cycles at 23°C constant room temperature. For

the c-Fos expression experiments, to obtain a pure monocular light response, the functionality of right eye was abolished. This procedure was applied because, although the input of ipsilateral retinal axons is just ~3–5% of the total fiber input (Polyak, 1957; Jeffery, 1984), functional recordings have demonstrated a large dLGN responses to ipsilateral stimuli suggestive of a large divergence of uncrossed axons (Grieve, 2005). The alternative procedure, i.e., suturing of the eyelid above the eyeball with or without the application of dark glue material on its external surface, was not selected because of potential tonic and non-coherent activation of the retina by light filtering through the eyelid and/or by increased external pressure on the eyeball. To this aim, animals were deeply anesthetized with sevoflurane (Sevorane, Abbott) and their retina surgically removed. After surgery, we applied a local anesthetic (Lidocaine) to reduce animal discomfort. An antibiotic powder (Cicatrene, Johnson and Johnson) was then added followed by the right eyelid suturing to prevent infections. Rats were then housed in darkness for 48 h before experimenting to reduce background levels of c-Fos expression arising from ambient light and from the right eye surgical procedure. In these conditions no change in the activity of the dLGN contralateral to the removed eye could be detected when comparing the number of c-Fos positive cells between the two dLGNs (ipsilateral and contralateral to the enucleated eye) of rats kept in darkness for 2 h when one eye was enucleated ($p > 0.10$). Surgery was always performed under sevoflurane anesthesia and animals were sacrificed with an overdose of sodium pentobarbital.

Ganglion Cell Recordings from Isolated Retinas

Retinal preparations were obtained as described in a published protocol (Schmidt and Kofuji, 2011). Briefly, rats were dark adapted for several hours and then euthanized in a dark ambient via CO₂ asphyxiation. Always minimizing light exposure, both eyes were enucleated and opened by puncturing the eyeball at the limbus. Then the cornea was removed with a circular incision and the lens removed with forceps. Finally, forceps were used to tear up the sclera freeing the retina completely. The retina was then cut in four pieces. To avoid long-term changes by pharmacological manipulations, only one recording was obtained from each piece of the retina, which was then discarded. Every step after the removal of the sclera were performed in CO₂/O₂ saturated Ames' medium (Sigma) with 0.19% Sodium Bicarbonate. The same solution was used to maintain the tissue after dissection. Retina preparations were allowed to dark adapted for 1 h prior to use. Loose patch recording from single RGCs were obtained from these acute retina preparations of rat retinas using 5–8 MOhm electrodes filled with Ames' medium. During electrophysiological recordings, the quartered pieces of the retina were continuously superfused with CO₂/O₂ saturated Ames' medium kept at room temperature (24°C). Electrophysiological Signals were acquired using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA), filtered at 2–5 kHz and digitally acquired at 20 kHz using a 16-bit analog-to-digital interface (Digidata, Axon) controlled by the pClamp acquisition software. During recordings, drugs

(4-Aminopyridine, 4-AP, 0.02 mM) were dissolved in Ames' medium and applied through the bath perfusion system. If not otherwise indicated, salts and chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). Light stimuli consisted of full field illumination of the retinal preparation using a LED placed under the microscope condenser. The light pulses lasted 1 s with 19 s interval between pulses. Thirty sweeps were recorded before during the application of 4-AP for every analyzed cell. In the described conditions retinas were available for recording for up to 8 h after dissection. In preliminary experiments we could obtain stable recordings from single cells for 1.5 h.

As opposed to 4-AP, L-AP4 is a widely known tool for investigating the retinal circuitry and its effects on such circuit have been extensively studied since 1981 (Slaughter and Miller, 1981). We were able to reproduce such known effects in our experimental setting but no formal analysis was performed (see **Supplementary Figure 1** for an exemplar recording).

Visual Stimulation

For light stimulation, we placed animals inside a Perspex tube leaving out their neck and head. The head was clamped by a custom device, which lowered the head to an angle of 30° with respect to the animal horizontal plane. Animals were then positioned in front of a cathode flat monitor (100 Hz refresh frequency). To induce the activation of ON-OFF retinal fields, rats were visually stimulated with black and white vertical bars [height = 30 cm, width = 2 cm, irradiance: white bars 37 mW/m²; black bars 0.11 mW/m² at a wavelength of 509 nm, the peak of rat M-cones absorbance (Jacobs et al., 2001)]. By angling the screen to 45° (with respect to the animal longitudinal rostral-caudal axis) the monitor was placed in front of the animal left eye. The distance from the screen was set to obtain a spatial frequency of 0.126 cycles/degree. In the experiments presented here, we used a pattern reversal at 2 Hz and the stimulation protocol lasted for 2 h. Based on a set of preliminary experiments, this length of the experiment was set to allow optimal c-Fos protein expression levels. The day of the experiment, we kept the no-light group in the dark for 2 h in the same experimental setting as in the light stimulation experiments except for the fact that the monitor was turned off. To determine if light activation protocols and other manipulations (intravitreal drug administrations) were indeed effective, the c-Fos activation pattern of RGCs was first analyzed. When light induced c-Fos enhancement could not be detected in the retina, the brains of the corresponding animals were discarded and not further processed. Only two rats were excluded based on this criterion and in both cases the retinal stimulation was not effective due the presence of blood filling the vitreal chamber after drug injection.

Intravitreal Drug Administration

For drug treated subgroups, animals were injected intravitreally either with the selected drug or with physiological saline and then kept in the darkness for 30 min before experimenting to allow drug equilibration. For the injections, rats were deeply anesthetized with sevoflurane and injected intravitreally

with the selected drug using a 10 μ l Hamilton syringe coupled to the 30G eye injection needle through a Teflon tubing (TE50) filled with mineral oil. The injection site was in between the cornea and the sclera (limbus), and the appropriate intravitreal needle position was determined by visual inspection through the iris. The injection volume was set to 5 μ l. L-(+)-2-Amino-4-phosphonobutyric (L-AP4, Tocris Bioscience) and 4-aminopyridine (4-AP, Sigma Aldrich) were diluted in a Tyrode saline solution containing: NaCl (119 mM), KCl (5 mM), Hepes (25 mM) CaCl₂ (2 mM), MgCl₂ (2 mM), Glucose (6 g/L), which was buffered to pH 7.4. The final concentration for both drugs in the vitreous chamber was approximately 2 mM. Right eyes were injected for Visual Evoked Potential (VEP) experiments and left eyes for c-Fos experiments.

Immunolabelings

At the end of the stimulation protocols, rats were sacrificed with an overdose of sodium pentobarbital (Thiopental, intraperitoneal injection 30 mg/Kg; Inresa-LD) within a time span from 02:00 p.m. to 06:00 p.m. Via the ascending aorta, we quickly perfused the circulation by gravity, first with a cold buffered solution containing heparin (0.12 M phosphate buffer, 4°C; Heparin 5000 UI/ml) followed by 4% paraformaldehyde dissolved in 0.12 M sodium phosphate buffer. At the end of the perfusion, brains were removed and post-fixed overnight in the same fixative solution (4°C). Brains were included in 4% agar and coronal sections (35 μ m thick) were obtained at regular space intervals to reconstruct the lateral geniculate nucleus (LGN) from both hemispheres slices using a Vibratome (Leica VT1000 S Vibrating Blade Microtome). To extract the retina, after intra-cardiac perfusion with fixative, the eye was removed and the cornea gently sectioned. The eye-cup was then post-fixed in 4% paraformaldehyde solution overnight (4°C). The whole retina was gently removed and left free floating in a multi-well dish. For immunolabeling, free-floating sections and retinas were initially washed with a solution containing: 0.12 M phosphate buffer, 0.3% Triton X-100, 1% BSA, pH 7.4. Slices and retinas were incubated overnight at 4°C with a mixture of primary antibodies dissolved in the same solution (rabbit anti c-Fos, 1:300 dilution, Santa Cruz Biotechnology K-25; IgG1 mouse anti NeuN, 1:300 dilution, Millipore, MAB377; IgG2A mouse anti GAD65 and anti GAD67, 1:300 dilution; CHEMICON MAB351 and MAB5406). After incubation with primary antibodies, retinas and slices were extensively washed and incubated for 2 h at room temperature with a mixture of species-specific fluorescent secondary antibodies (anti rabbit-CY5 or anti rabbit-Alexa647 conjugated donkey anti mouse IgG, 1:200 dilution; anti mouse-FITC conjugated donkey IgG, 1:200 dilution; Jackson ImmunoResearch; Southern Biotech, A-21126, anti mouse IgG2A, FITC conjugated donkey, 1:200 dilution; Molecular Probes, 1080-02, anti mouse IgG1, Alexa647 conjugated donkey, 1:200 dilution). After extensive washing, brain sections and retinas were mounted on glass slides and covered with coverslips using an aqueous mounting medium (Fluorsave, MERK). In a set of preliminary experiments, we tested for the specificity of primary and secondary antibodies

and we excluded cross-reactivity. In addition, we evaluated the level and the excitation-emission spectrum of background fluorescence of retinas and of the LGN structure. For the detection of c-Fos expression, we used secondary antibodies emitting in the far-red (CY5 or Alexa647 fluorescent probes) since in this portion of the spectrum the auto-fluorescence of the LGN tissue is very faint.

Image Acquisition and Analysis

Fluorescent images were acquired for every slice (400 μ m) by one photon confocal microscopy (10 $\times$ –63 $\times$ magnification; Axioscope LSM 510, Zeiss) and fused in composite images or collages to reconstruct every LGN section. Based on a set of preliminary experiments we determined the best acquisition parameters (excitation intensity, gain, and pinhole settings; no background subtraction) used throughout this study. For each animal, the LGN analysis was restricted to $n = 9$ sequential and equally spaced coronal brain slices (including both hemispheres). Composite images of ipsilateral and contralateral hemispheres were band-pass filtered with a Fast Fourier Transform algorithm, fluorescent cells detected by a particle analyser tool and segmented using the Watershed algorithm (ImageJ, NIH). The best threshold for detection was established for every filtered image according to the distributions of the fluorescent signal and background noise (Max Entropy algorithm, ImageJ, NIH). In addition, all detected NeuN and c-Fos positive cells were visually scrutinized before final counting. Although the analogic level of c-Fos cell activation provides important information, we preferred to concentrate on a more elementary digital classification (number of active vs. inactive cells) because: (i) the time frame to combine a thorough spatial analysis with accurate estimates of cell-fluorescence (requiring high magnification and specific acquisition settings) was disproportionate large; (ii) c-Fos intensities showed unwanted slice to slice variability and also more subtle local changes because of heterogeneity in background fluorescence and/or in the efficacy of the staining procedure; (iii) depending on the timeframe and degree of activation the c-Fos protein doesn't remain concentrated in the nuclear and perinuclear areas but diffuses away in dendritic branches, often out of focus. To attribute cells to one of the three LGN subdivisions (dLGN, vLGN, and IGL), it was critical to define the borders of these structures more precisely. The boundaries derived from the detectable anatomical features and from the characteristic spatial distribution of NeuN positive cells in these nuclei. For counting of c-Fos/NeuN single and double positive cells, neurons were detected either in the NeuN channel or in the c-Fos channels. Based on their morphology and fluorescence values (see above), neurons were considered single or double positive NeuN/c-Fos cells. This analysis and the characterization of their spatial distribution was obtained by custom routines written in Matlab (Mathworks). A Matlab routine was developed to subdivide every dLGN coronal section in a series of stripes of equal thickness, running parallel to the lateral edge of the dLGN. C-Fos and NeuN positive cells were counted in every stripe, values refer to crude counts or counts normalized for stripe area. The thickness of these stripes was selected to avoid disproportionate fluctuations of the cell-count

index essentially when the number of cells/stripe drops to a very low value. For every dLGN, after searching for the section with highest count of cells labeled by NeuN, the stripe thickness was then set in all sections according to the square root of this value.

Visual Evoked Potentials (VEPs) Recordings

For VEP recordings, we used Sprague-Dawley rats matched for strain, age, and sex with those used in c-Fos experiments (weight 175–200 g). Very small stainless steel screws (diameter ~ 1 mm) were positioned above the left and right binocular visual cortex and used as recording electrodes. For electrode positioning, rats were deeply anesthetized with the volatile anesthetic sevoflurane (Sevorane, Abbott), the skull was exposed and using a stereotaxic apparatus to find the correct stereotaxic coordinates of the binocular visual cortex, small holes were drilled in the skull without exceeding the bone thickness. The electrodes were screwed and connected to gold pin terminals, a liquid methacrylate resin (REPORT/N Salmoiraghi Produzione Dentaria Srl, Italy) was polymerized above them leaving just the electrode endings exposed. After surgery, gentamicin-sulfate (1.5 mg/Kg; i.p. injection twice a day) and dexamethasone-phosphate (0.2 mg/Kg; one subcutaneous injection) were administered. Three days after surgery, animals were prepared for experiments. Rats were deeply anesthetized and a sterile polyethylene catheter was positioned inside the trachea for mechanical ventilation (SERVO ventilator 900C—Siemens, 3% Sevoflurane). A volume-controlled ventilator delivered a tidal volume of 6 ml with a respiratory rate of 80–85 breath cycles/min and volume of 0.5 L/min. A gas analyser (Vamos, Dräger) monitored the inspiratory and the expiratory concentrations of the anesthetic and CO₂ level. Rats were placed on an anti-vibration breadboard over a heating pad to maintain their body temperature in the physiological range (36–37°C). In order to obtain muscle relaxation, rats were curarized (Atracurium, 5 mg/kg, caudal vein injection every 20 min). During experiments, rats were kept in a dark environment, the left eye was covered by a black patch and the right eye stimulated with a white LED [trains of 300 ms light pulses, 0.1 Hz, $n = 30$ per train; white LED irradiance was set to 111 $\mu\text{W}/\text{cm}^2$ measured at the 509 nm wavelength, the peak of rat M-cones absorbance Jacobs et al., 2001]. Gold-pin electrodes were connected to a custom-made DC-decoupled amplifier with a fixed gain ($G_{\text{IN}} = 100$). The differential signals coming from the amplifier were low-pass filtered ($\text{GPRE} = 50$, $f_p = 1$ KHz) and further amplified (210 A amplifier, Brown Lee Precision). Data were digitized at 20 KHz sampling frequency using a ITC18 16-bit data acquisition interface (HEKA), connected to a computer running a custom data acquisition software developed in LabVIEW environment (National Instruments). For VEP measurements, a differential recording was obtained between the left and the right V1 skull electrodes. The ground potential was obtained by an additional hook electrode positioned on the external ear. The animals together with the first amplification stage, was kept inside a Faraday cage. VEPs were recorded before and after intravitreal drug administration using the same recording and light stimulation protocols.

DATA ANALYSIS AND STATISTICAL METHODS

Spike Train Analysis

Autocorrelograms were calculated on recordings of 2 min of spontaneous activity with a lag window of 2 s (from -2 s to $+2$ s) with 100 ms bin-size and normalized setting the lag-zero counts equal to one. The power spectra were obtained as the Fourier transform of the autocorrelograms. Periodic and non-periodic behaviors were selected based on an Periodicity Index calculated as the height of the highest peak in the normalized power spectrum divided by the length of its base. The height of the peak was calculated as the difference from the lowest surrounding minima and only peaks above a defined threshold were considered (0.008). If no peaks could be detected or the Periodicity Index was too low (below 0.003), the cell was classified as non-periodic. The selectivity of the cell responses for the light stimuli was calculated as a Discrimination Index $= (A1 - A2)/(A1 + A2)$, where A1 is the number of spikes during the light pulse and A2 is the number of spikes right after the stimulus calculated on a time window of the same duration as the light pulse. It is positive for ON cells and negative for OFF cells and tends to zero if there is an equal probability of firing during and after the stimulus. The entropy of the Interspike Interval (ISIs) was calculated as $H = -\sum_i (ISI_i / \sum_j ISI_j) \times \log_2 (ISI_i / \sum_j ISI_j)$ on a peristimulus time window of 4 s (1 s before and 1 s after the light pulse). The Standardized Entropy (H_s) was then obtained dividing by the maximum entropy value of each ISI series [calculated as $\log_2(n)$ where n is the number of ISIs in the series] to account for differences in the number of spikes. All calculations were made for each cell in each experimental condition on a single trace obtained as the superimposition of 30 sweeps. Finally, the significance of the effect of 4-AP on the discrimination ability of the cell was tested using a logistic regression model with a Logit link function where $\text{logit}(p) = \log(p/(1-p))$. The model included random effects to account for clustered observations coming from retinal preparations of the same rat (Rat random effect) and from the same cell (i.e., before and after 4-AP, Cell random effect). In this model, the spikes were counted using the same time window as in the Discrimination Index, but were classified as successes and failures: spikes were considered as successes if they occurred during the light pulse for ON cells or after the light pulse for OFF cells. Otherwise they were counted as failures. Successes and failures were used as the response variable to match the assumption of binomial distribution of the data.

Monte Carlo Methods and Spatial Analysis

Monte Carlo methods were used to generate random samples of activated cells in the dLGN using the experimental NeuN data sets. To compare the simulated random activated cell patterns to the experimental observations, we measured the entropy of the cell distribution for each analyzed brain LGN section. To calculate the entropy values we used the Voronoi tessellation generated by each cell-point pattern, for both random and experimental data sets. For the Voronoi tessellation all locations were associated in space with their closest member(s) using the Euclidean distance (Okabe et al., 2000). The Voronoi diagram

composed by ordinary Voronoi polygons was used to compute the entropy of each point pattern on a finite surface with a slightly modified version of the Shannon's equation for entropy (Chapman, 1970):

$$H_{c-Fos} = - \sum_{i=1}^{n_{c-Fos}} \frac{A_i}{\sum_{j=1}^{n_{c-Fos}} A_j} \cdot \ln \frac{A_i}{\sum_{j=1}^{n_{c-Fos}} A_j}$$

where n_{c-Fos} is the total number of the points (cells) generating the observed pattern, A_i is the area of the Voronoi polygon associated to point i . For each dLGN section, we computed the entropy H_{sim} for $N_{sim} = 50,000$ random samples drawn from the NeuN set, with sample size n_{c-Fos} . For each slice, the p -value of the observed entropy value H_{c-Fos} was calculated as $p = n/N_m$ where n is the number of simulated entropy values H_{sim} less than to the observed entropy H_{c-Fos} .

To compare neuronal density in brain sections, we used frequency counts in k dLGN stripes (double positive NeuN and c-Fos neurons). To verify the null hypothesis of the uniform distribution across stripes, a G -test was performed on the observed frequencies of NeuN cells. To take into account the reduction in the area of stripes due to the dLGN curvature, the expected frequency in each stripe was normalized to the ratio of the stripe area over the whole dLGN section area. Hence the test statistic becomes:

$$G = 2 \cdot \sum_{i=1}^k O_i \cdot \ln \frac{O_i}{E_i}$$

Where k is the number of stripes the dLGN section was subdivided into, O_i is the observed frequency in each stripe and E_i is the expected frequency in each stripe calculated as:

$$E_i = \frac{S_i}{\sum_{i=1}^k S_i} \cdot n_{NeuN}$$

where S_i is the area of each stripe and n_{NeuN} is the total number of NeuN cells in the dLGN section. Under the null hypothesis, the test statistic has χ^2 distribution with $k-1$ degrees of freedom. To verify if the median value of double positive NeuN/c-Fos neurons was smaller (closer to the lateral edge) than the median value of the NeuN cell population, for each dLGN section N_{sim} random samples were extracted from the NeuN set (sample size n_{c-Fos}). Then, for each sample the position of each cell along the latero-medial axis was calculated as before and the median value for each sample calculated. The p -value was calculated as above.

VEP Analysis

To better visualize coherence of VEPs pattern across successive trials, we plotted the recorded voltage values over time for each trial or sweep in a heat-map graph. In order to evaluate possible changes of the ON and OFF responses induced by L-AP4 and 4-AP treatments, we analyzed individual VEP trials in the 0–150 ms time window (ON response) and in the 300–450 ms time window (OFF response). For each trial we computed the height of the first positive peak in the OFF and ON responses,

and the integral of the root mean square or quadratic mean (RMS) for the ON and OFF responses in the two time windows listed above. The presence of significant differences between treatments was evaluated by means of permutation statistical methods based on t -type statistics, to avoid any assumption on parental distribution (Pesarin and Salmaso, 2010). $P < 0.05$ were considered significant. All VEP statistical analyses were performed in R environment.

Statistical Test to Analyze Significant Differences in Cell Counts

To assess possible differences in cell counts between different treatments, we fitted a Generalized Linear Mixed Model (GLMM) to the data (McCulloch et al., 2008). In more details, the observed number of c-Fos positive cells in each portion of the dLGN was considered as a random variable with Poisson distribution. The parameter λ of the Poisson distribution was allowed to depend both on the area of the considered dLGN structure and the applied treatment with a logarithmic link function.

$$\ln \lambda = (\alpha_0 + \alpha_1 I_t) + (\alpha_2 + \alpha_3 I_t) \cdot \text{area}$$

where I_t is the dummy variable that identifies the treatments to be compared, area is the area of each dLGN area section. In such parameterization, α_0 identifies the intercept relative to the first treatment, α_1 is the difference of the intercepts of the two treatments, α_2 identifies the slope of the first treatment and α_3 is the difference between the slopes. We added a random effect to take into account possible dependencies among brain slices belonging to the same rat. The null hypothesis, $H_0: \alpha_1 = \alpha_3 = 0$ was verified by means of the Likelihood Ratio test statistic with p -values computed by means of bootstrap (Faraway, 2006; McCulloch et al., 2008). Statistical analyses were performed in Matlab and R environment.

Statistical Test to Analyze Significant Differences in c-Fos Expression of GAD-positive Cells

GAD-positive cells were identified on $63\times$ confocal raw images. Then, a circular area of 20 pixel radius centered on each GAD positive cell was used to measure c-Fos expression. For every image, a distribution of the c-Fos background was generated sampling the image with 20 pixel radius circular areas (avoiding previously selected putative c-Fos positive cells) several times (1000) and this was used as the null hypothesis distribution to test the value of c-Fos expression of each GAD positive cell. Fluorescence values greater than the mean of the background plus twice the standard deviation of the background were used to identify c-Fos positive cells among GAD positive interneurons (see **Supplementary Figure 2**). A logistic regression was then used to test the effect of drug treatment over c-Fos expression in these neurons. A similar method was employed to test the even distribution of GAD positive cells, where a generalized linear model for counting processes was used to assess the homogeneity of cells counts across all locations, and the probability of high c-Fos expression at a given lateral-medial location, again using a logistic regression (see Results).

RESULTS

Effects of 4-AP on Isolated Rat Retinas

In this study, loose-patch recordings from single RGCs in isolated rat retinas were used to investigate the effects of 4-Aminopyridine (4-AP) on the RGC firing rate. Recordings were obtained from 20 RGCs from 8 animals. The firing rate before and after the application of 4-AP (0.02 mM) was obtained in each RGC recording. In both conditions (before and after 4-AP) spontaneous activity and the evoked response to light

stimulation were recorded. In these experiments spontaneous activity was evaluated over a period of 2 min before and after 4-AP, always preceded by a brief period of dark adaptation (5 min). The results showed that after the application of 4-AP the spontaneous activity of ganglion cells was generally increased (mean fold increase = 6.2, IQR = 5.17, see **Figure 1**). Since in some cells a shift to a periodic firing pattern was detected following the application of 4-AP, autocorrelograms of spontaneous spike series were calculated to evaluate such periodicities. Periodic behavior was assessed via the analysis of

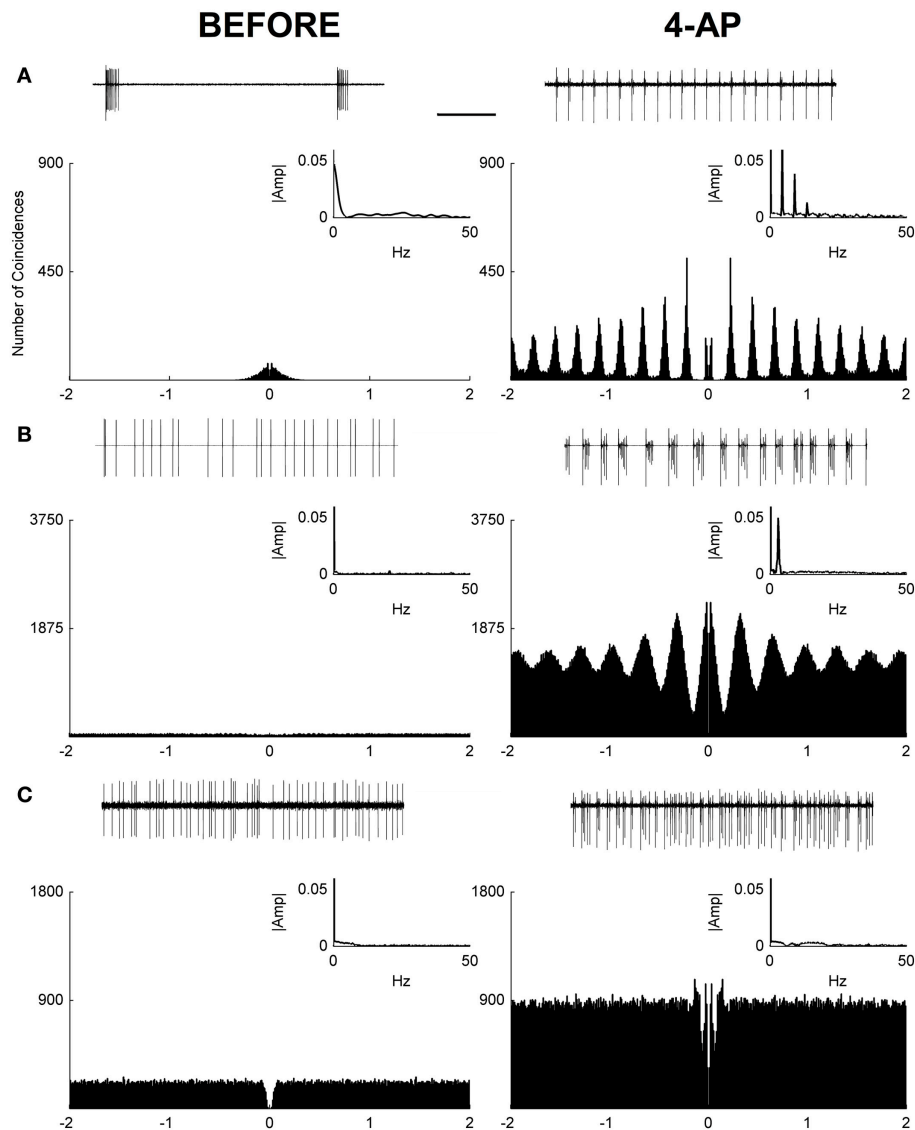
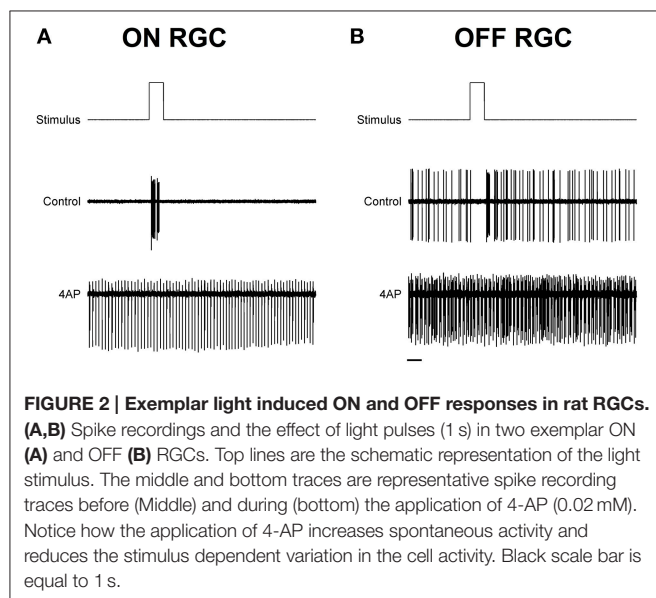


FIGURE 1 | Autocorrelograms and power spectra of RGC spontaneous activity recorded from rat isolated retinas. (A–C) Spike extracellular recordings (top traces), autocorrelograms (main plot), and power spectra (small insets) in three exemplar cells before (left) and during (right) the application of 4-AP (0.02 mM). In some cases (12 out of 20 RGCs) a periodic behavior was observed in the presence of 4-AP with frequencies in the range of 2.2–5 Hz (mean $\pm$ STD = 3.52 ± 0.86). Three different behaviors could be detected in the 4-AP conditions, periodic non-bursting (**A**), periodic bursting (**B**), non-periodic (**C**). The periodic non-bursting behavior was characterized by groups of one or two spikes repeated at regular intervals with sharp peaks in the autocorrelogram and harmonic components in the power spectrum. For periodic bursting, highly regular bursting activity, with dilated peaks in the autocorrelogram and no harmonic components in the power spectrum was observed. The non-periodic behavior consisted of a flat autocorrelogram and no clear dominant frequencies in the power spectrum. The black scale bar (1 s, top of panel **A**) applies to all spike recording traces (**A–C**).

Power Spectra and the calculation of a Periodicity Index (see Materials and Methods). Based on this analysis 12 over 20 cells showed periodic firing behavior at low frequencies after the application of 4-AP in the range of 2.2–5 Hz (mean predominant frequency = 3.52 Hz, STD = ± 0.86). Three major classes of cells could be identified: periodic non-bursting, periodic bursting, and non-periodic (**Figure 1**). The periodic non-bursting behavior was characterized by groups of one or two spikes repeated at regular intervals with sharp peaks in the autocorrelogram and harmonic components in the power spectrum. For periodic bursting, highly regular bursting activity, with dilated peaks in the autocorrelogram and no harmonic components in the power spectrum was observed. The non-periodic behavior consisted of a flat autocorrelogram and no clear dominant frequencies in the power spectrum.

To test the light evoked response, the isolated retinas were stimulated with full field light pulses (1 s pulses) repeated every 20 s (30 pulses for each experimental condition). Based on these data, we could classify cells based on a Discrimination Index (see Materials and Methods). Cells with a positive discrimination index (meaning they were more likely to respond to sudden increase in luminance) were classified as ON ganglion cells ($n = 9$), while cells with a negative discrimination index (meaning they were more likely to respond to sudden decrease in luminance) were classified as OFF ganglion cells ($n = 11$). No biphasic ON-OFF cells were encountered during this study. Although the application of 4-AP increased spontaneous activity, it reduced the stimulus dependent variation in cell activity, i.e., cells were less able to distinguish between dark and light (**Figure 2**). Indeed the discrimination index was generally closer to 0 both in ON and OFF cells after the application of 4-AP, meaning that cells were equally likely to fire during their appropriate stimulus and without any stimulation (Discrimination Index Mean Absolute Deviation from 0: 0.68 in Controls, 0.13 with 4-AP, $p < 0.01$ calculated using a logistic model as described in the Materials and Methods Section, **Figure 3**). As the discrimination was reduced,

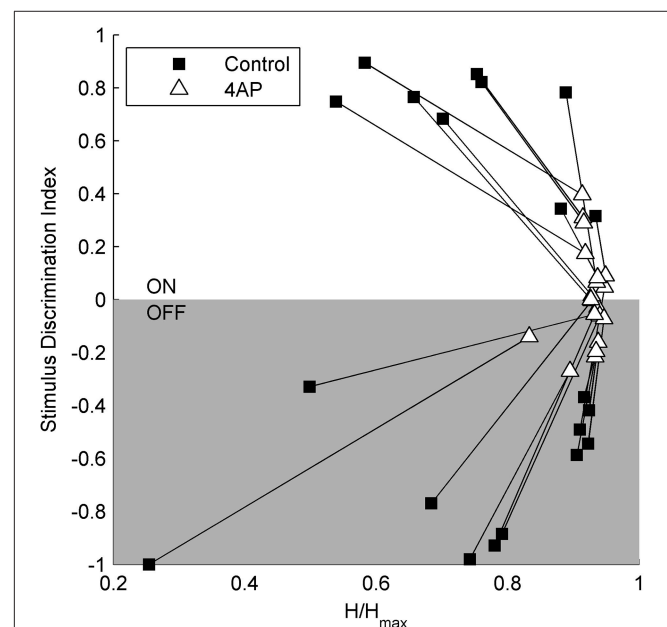


the standardized entropy H_s (see Materials and Methods) of the Interspike Intervals (ISIs) was closer to its maximum value, suggesting a reduction in the information transfer (mean H_s before 4-AP = 0.75, STD = 0.18; mean H_s after 4-AP = 0.93, STD = 0.03; **Figure 3**).

Selective Suppression of ON-OFF Thalamo-cortical Responses by 4-AP and L-AP4

The second step was to assess the efficacy of the two drugs used throughout the study when administered to live animals with intravitreal injections.

The first drug we tested was 4-AP; specifically we wanted to examine the *in vivo* effects of the circuit alterations measured on the isolated retina. The second drug tested was L-AP4, a mGluR-6 agonist known to block the activity of ON bipolar cells (Slaughter and Miller, 1981). In the latter case, the rationale was



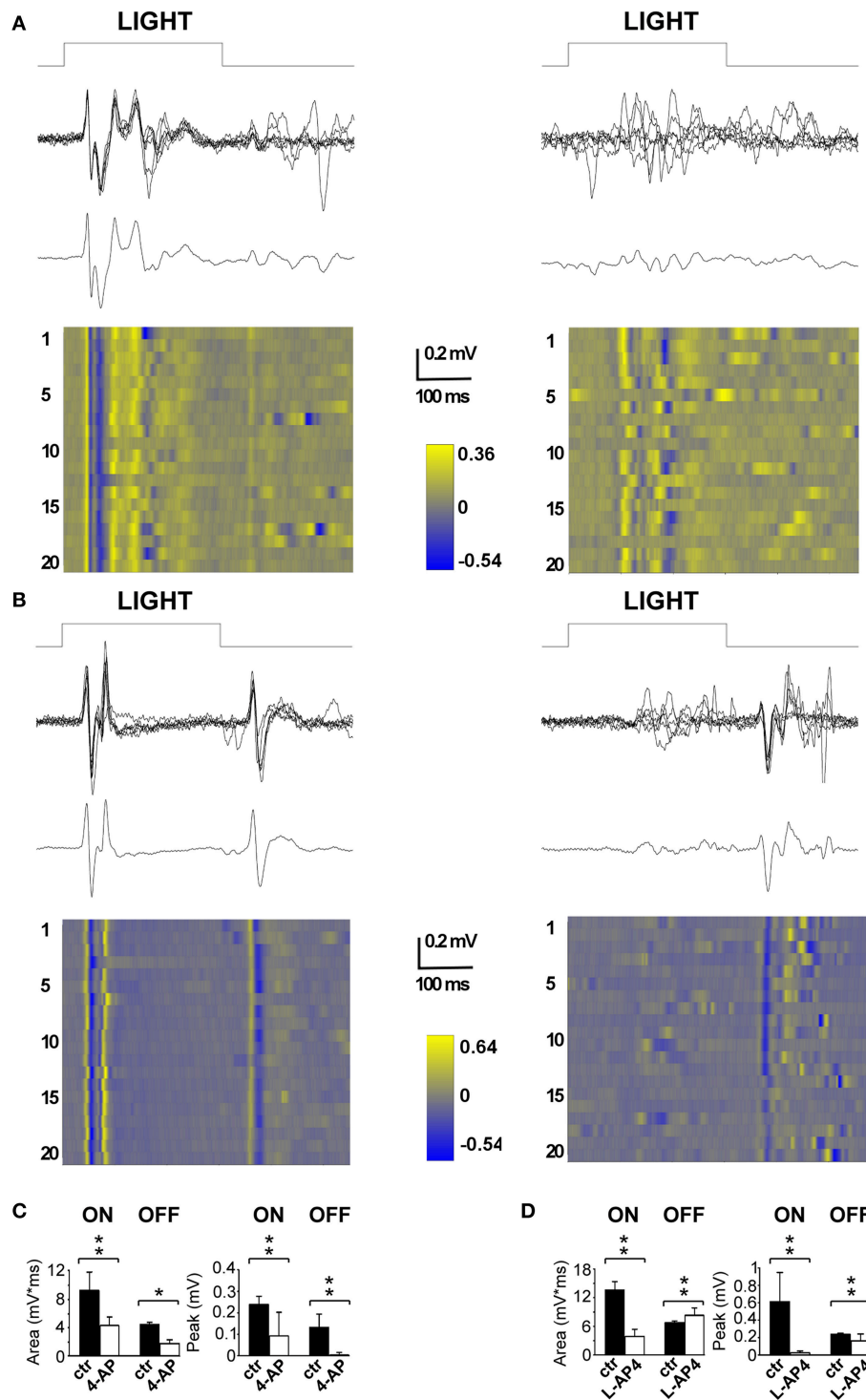


FIGURE 4 | Effects of 4-AP and L-AP4 on visual evoked potentials (VEPs) recorded from the V1 area. (A,B) Representative VEPs recorded before (left) and after (right) the application of 4-AP (2 mM) **(A)** and L-AP4 (2 mM) **(B)**. Rats were stimulated with light pulses lasting 300 ms ($111 \mu\text{W}/\text{cm}^2$) before (traces on the left) and after (traces on the right) the intravitreal injection of either 4-AP **(A)** or L-AP4 **(B)**. In both panels, above it is plotted the superimposition of $n = 6$ individuals sweeps, below the corresponding ensemble average. Notice how in control conditions (traces on the left) after the change in luminance there is a clear change in voltage (ON cortical response) and that the interruption of the stimulation also produces an effect which is smaller and less reproducible in shape across different experiments (OFF cortical response). **(C,D)** Quantitative analysis for the ON and OFF responses before (black bars) and after (white bars) intravitreal injections of 4-AP **(C)** and L-AP4 **(D)**. Bars plot the integral of root mean square or quadratic mean (RMS) (right bars in **C,D**) or the peak amplitude of the first positive response (left bars in **C,D**). The RMS of the ON and OFF responses were integrated for 150 ms from the start (ON) or from the end (OFF) of light pulses. All pair comparisons yielded significant differences ($n = 3$ rats for each condition; 60 sweeps before and after drug treatment; stars indicate significance: *indicates p -values < 0.05, **indicates p -values < 0.01; permutation tests based on t -type statistics).

to obtain a single pathway output from the retina, in contrast with the uncoordinated ON-OFF RGC activity produced by 4-AP. To assess the efficacy of intravitreal injection of the selected drugs, we recorded visual evoked potentials (VEPs) induced by flash stimulation before and after the intravitreal injection to obtain internal comparisons and to minimize the effects of inter animal variability among recordings. In these experiments, we sampled V1 activity on anesthetized and ventilated animals (see Materials and Methods for details). As depicted in **Figure 4**, a repeated stimulation with 300 ms light flashes in control conditions produced a very clear and reliable response across successive trials, whose amplitude and waveform was preserved over time (stimulation rate 0.1 Hz; flash intensity $111 \mu\text{W}/\text{cm}^2$) (left traces in **Figures 4A,B**). The early biphasic ON-response peaked on average at 40 ms (P wave) and 50 ms (N wave) from the beginning of the light stimulus. Overall the ON response lasted from 30 to 200 ms. In most cases a clear OFF-response could also be detected. The initial positive P wave of the OFF response peaked at 70 ms from the end of the light stimulus. When 4-AP was administered via intravitreal injection (2 mM; eye contralateral to the V1 recording site) it clearly reduced the detectable visual cortex responses seen in control conditions. The early phase of the ON and OFF responses almost completely disappeared leaving just some highly variable asynchronous activity ($p < 0.05$; **Figure 4A**, right). On the contrary, when the selective group III metabotropic glutamate receptor agonist L-AP4 was tested, we observed, as expected, a selective inhibition of the ON response, contralateral to the injected eye, ($p < 0.05$; **Figure 4B**) while the OFF response was still clearly present. **Figures 4C,D** shows the quantitative results across all experiments to illustrate the important reduction of the entire ON and OFF waveform by 4-AP, while L-AP4 essentially spared the OFF response, with a small, although statistically significant, change in the shape and duration of its waveform.

Effects of ON-OFF Visual Patterns on dLGN Activity

For this experiment, rats were subdivided in two experimental groups, the no-light and the light-stimulated groups. Light stimulated animals received monocular light stimulation with 2 Hz reversal, at constant overall luminance, of black and white reversing vertical stripes (0.5 cycle/degree; left eye stimulation) (see Materials and Methods). To study the distribution of active

neurons in the visual thalamus, we used the activity-dependent labeling method based on the expression of the c-Fos gene protein product (Sheng et al., 1990; Flavell and Greenberg, 2008) on $10\times$ magnification images of the dLGN. Active neurons, independently of their level of c-Fos activation, were grouped together in a single class (c-Fos positive), which was distinct from the inactive cell group (c-Fos negative) (see Materials and Methods for distinction). Animals were dark reared for 48 h before the experiment to reduce the basal level of c-Fos expression in thalamic neurons. In the no-light condition, very spare active cells could be identified inside the dLGN, the image-forming subregion of the LGN structure (**Table 1**). Following monocular ON-OFF light stimulation, the number of active cells in the dLGN did not change significantly with respect to the no-light condition (**Table 1**, $p > 0.05$; see **Supplementary Figure 3**). No significant differences were also found between contralateral and ipsilateral LGNs ($p > 0.05$). Since ON-OFF light stimulations induced a very clear activation in the matching retinas (**Table 2**), these results might suggest the presence of some kind of inhibitory mechanisms counteracting a strong activation of postsynaptic dLGN cells, preventing a detectable expression of c-Fos.

Effects of Concomitant ON and OFF RGCs Activation by 4-AP on dLGN Activity

We showed that 4-AP can produce a random chaotic activation of RGCs shunting the upstream retinal circuit regulation. To test the effect of concomitant random activation of the ON

TABLE 2 | NeuN+/c-Fos+ cell counts (cells/mm²) in the retina for the conditions tested.

	Darkness	Grid	Rats
Control	31 (± 6)	138 (± 41)	8
4-AP	120 (± 69)	160 (± 56)	8
L-AP4	161 (± 107)	96 (± 58)	6

Table reports cells counts (cells/mm²) of c-Fos positive cells among NeuN positive cells in the retina for various conditions. Notice how, even in the control group, a clear increase in c-Fos positive cell density could be found after visual stimulation, as opposed to the dLGN behavior in the same conditions. The last column reports the number of animals tested for each pharmacological manipulation, equally subdivided between darkness and visual stimulation.

TABLE 1 | NeuN+/c-Fos+ cell counts (cells/mm²) in the dLGN for the conditions tested.

	Darkness	Grid	Darkness	Grid	Rats	Slice/Rat
	Contralateral		Ipsilateral			
Control	45 (± 34)	47 (± 17)	45 (± 34)	38 (± 20)	8	9
4-AP	151 (± 28)	228 (± 45)	35 (± 30)	40 (± 20)	8	9
L-AP4	63 (± 44)	442 (± 60)	41 (± 25)	35 (± 21)	6	9

Table reports cells counts (cells/mm²) of c-Fos positive cells among NeuN positive cells in the dLGN (contralateral and ipsilateral to the tested eye) for various conditions. Notice the different behavior of the 4-AP group compared to the L-AP4 group: in the first no differential activation could be detected between visual stimulation and darkness condition, while in the latter a clear increase in c-Fos expression could be detected after visual stimulation. The fifth column reports the number of animals tested for each pharmacological manipulation, equally subdivided between darkness and visual stimulation. The last column reports the number of slices analyzed per each animal.

and OFF pathways on thalamic c-Fos expression, we injected 4-AP in one eye (2 mM). Following the application of 4-AP, in darkness conditions, we observed a ~ 4 fold increase in the number of active RGCs in the retina (Table 2). As presented in Figure 5A, the injection 4-AP increased the number of active neurons in the contralateral dLGN with respect to animals kept in darkness in control conditions (Table 1, $p < 0.05$). The ON-OFF light-stimulation after the injection of 4-AP produced a clear activation of the contralateral dLGN as compared to the

visual stimulation alone ($p < 0.05$) but most of this effect was not visually driven. In fact, in the 4-AP experimental group, the ON-OFF light stimulation produced a small and not statistically significant increment in the number of activated cells in the retina (33%) and dLGN (51%) with respect to the no-light condition (Figures 5A,B) ($p = 0.32$). No significant activation of the ipsilateral dLGN could be detected. In these 4-AP experiments, the distribution of active neurons in the contralateral dLGN was not homogeneous, with an increased density of c-Fos positive

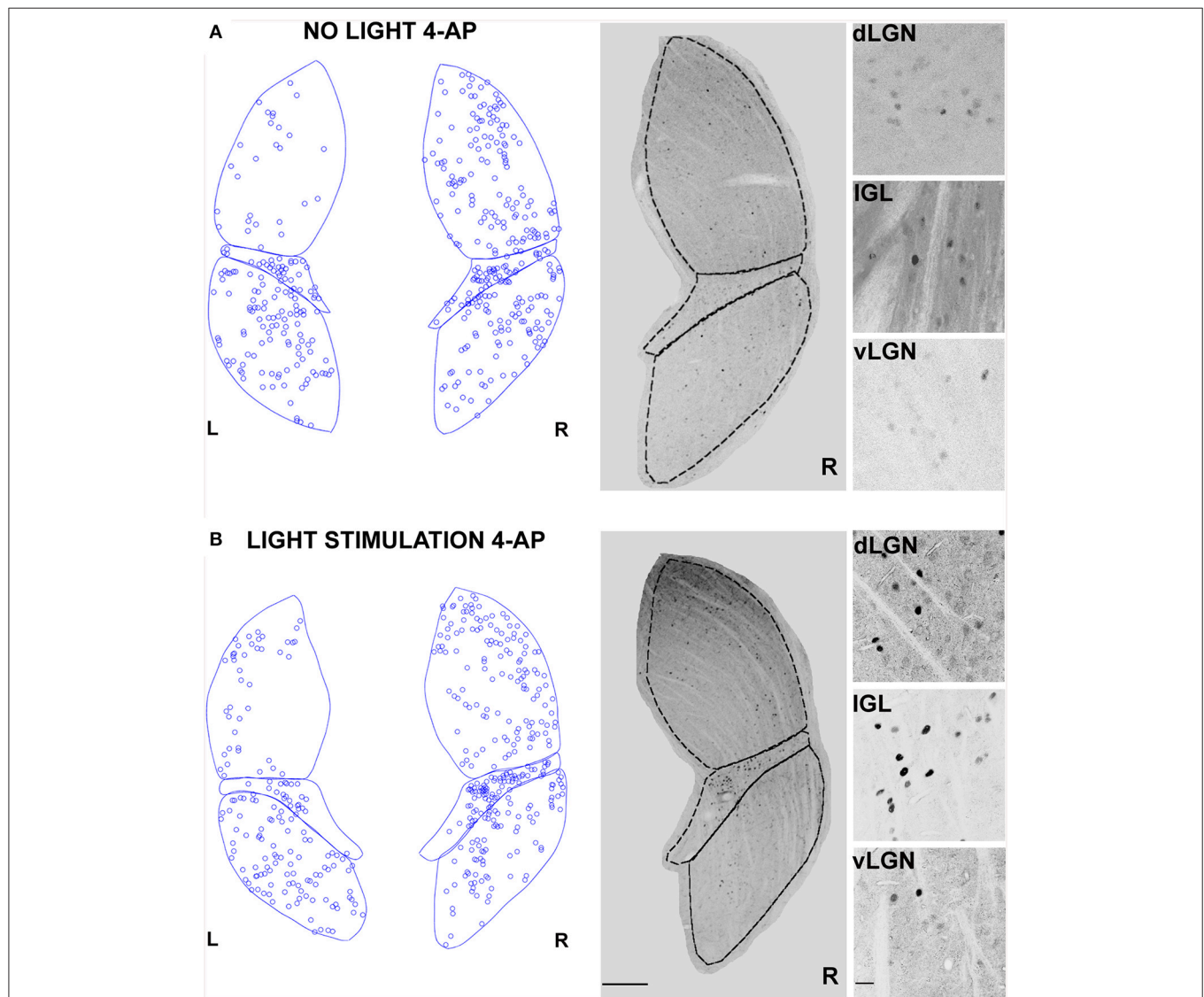


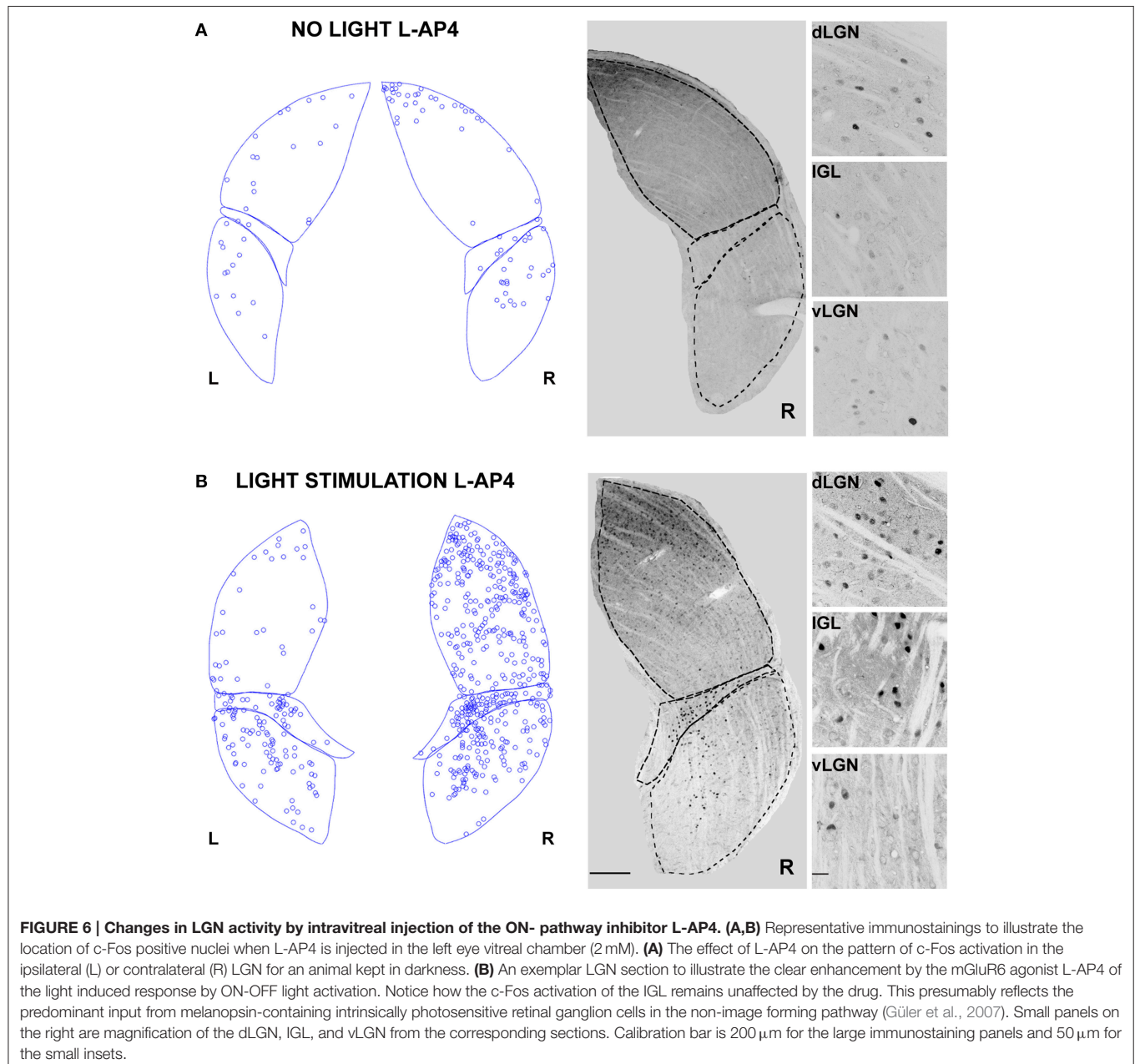
FIGURE 5 | Changes in LGN activity by intravitreal injection of 4-aminopyridine (4-AP). (A,B) Representative immunostainings (right) and digital reconstructions (left) of the right (R) and left (L) LGNs (same coronal sections). Circles report the location of identified c-Fos positive cells (for segmentation algorithm, see Materials and Methods). In these experiments the A-type potassium channel blocker 4-aminopyridine was injected in the left eye vitreal chamber (2 mM). (A) An exemplar result to illustrate the effect of 4-AP on c-Fos activation pattern in the no-light condition (animal kept in darkness). (B) The effect of ON-OFF light activation in the presence of 4-AP. Light-stimulation was obtained with alternating black and white vertical bars at constant overall luminance (white bars 37 mW/m²; black bars 0.11 mW/m²; 2 h; 2 Hz refresh rate; 0.5 cycle/degree; left eye stimulation). The three small panels on the right are magnification of the dLGN, IGL, and vLGN from the corresponding sections. Continuous and dashed lines indicate edges of dLGN, IGL, and vLGN. Notice how while 4-AP increases the basal activation of the dLGN (see data in the text, also compare L and R), the effect of light stimulation on this structure is not noticeable (Compare R in A with B). Calibration bar is 200 μ m for the large immunostaining panels and 50 μ m for the small insets.

cells near the lateral edge (see below). This contrasted with a homogeneous distribution of neurons across the dLGN structure (staining for the neuronal marker NeuN Mullen et al., 1992; Kim et al., 2009; see below and Figure 9).

Effects of ON Retinal Pathway Blockade by L-AP4 On dLGN Activity

In contrast with the previous experiment, we wanted to test the effect of a pure single channel (OFF) activation on the c-Fos expression in dLGN. To obtain such selective activation, we intravitreally injected L-AP4, known to inhibit the activation of ON-bipolar cells (Slaughter and Miller, 1981), sparing the OFF retinal pathway. This treatment produced little neuronal

activation in both the retina and the LGN in the absence of a visual stimulus if compared to the darkness in control conditions (Table 2; $p > 0.05$) (Figure 6A) but a clear recruitment could be driven by visual stimulation both in the retina ($\sim 600\%$, Table 2) and in the dLGN (Figure 6B and Table 1, $p < 0.01$). The presence of a visually driven response and the degree of dLGN recruitment seen with L-AP4 injection followed by visual stimulation clearly differed from the results obtained with 4-AP injection. The strong light induced response in a condition where the activity of the ON channel is suppressed confirms previous results on tonic inhibition of the OFF bipolar cells by the ON pathway (Zaghloul et al., 2003; Margolis and Detwiler, 2007). No significant activation of the ipsilateral dLGN could be detected.



As after the injection of 4-AP, active thalamic neurons appeared to cluster near the lateral edge of the dLGN (see later). This result suggests that the lateral border of the dLGN, irrespectively of the ON and OFF modality, concentrates neurons with either the highest firing activity or with the lowest inhibitory input.

Selective Recruitment of dLGN GABAergic Interneurons

In all experiments presented up to now, dLGN cells were identified by counterstaining for the neuronal marker NeuN (1704 ± 505 NeuN positive cells/mm², mean $\pm$ SD; $n = 38$ dLGN sections) (Mullen et al., 1992). From preliminary inspection, we realized that many active cells in the 4-AP experimental group, most likely neurons because of their morphological shape (based on interference contrast imaging), were either unstained or very faintly stained with antibodies against the NeuN antigen (**Figure 7A**). When datasets of c-Fos positive cells were subdivided into NeuN positive and negative cells (threshold for inclusion = background fluorescence noise + 3 times its SD), 4-aminopyridine with or without monocular light stimulation was found to activate mainly NeuN negative cells ($73 \pm 25\%$ of active cells were NeuN negative; mean $\pm$ SD; $n = 30$ dLGN sections). These active cells were usually surrounded by a large number of inactive NeuN positive cells (**Figure 7A**). On the contrary, in the L-AP4 treated group, light recruited mainly neurons expressing high levels of NeuN protein ($65 \pm 11\%$ of active cells were NeuN positive; mean $\pm$ SD; $n = 32$ dLGN sections) (**Figure 7B**). Although these results are in contrast with the general idea that the nuclear antigen NeuN, corresponding

to the protein product of the Fox-3 gene (Kim et al., 2009), is a reliable marker for neurons, it has been reported that, in some brain areas, *bona fide* neurons do not stain with antibodies against NeuN (Mullen et al., 1992; Weyer and Schilling, 2003); in the cerebellum these cells coincide with well-known GABAergic neurons (Weyer and Schilling, 2003).

Therefore, to clarify this issue and determine if NeuN negative cells could belong to a population of GABAergic interneurons, we double-immunolabeled dLGN cells with a mixture of antibodies against the GABA synthesizing enzymes (GAD65 and GAD67) together with antibodies against the NeuN antigen. As depicted in **Figure 8A**, the GAD positive cells were fewer than the NeuN positive cells (278 ± 140 GAD positive cells/mm², mean $\pm$ SD; $n = 49$ fields) and most of these cells were either devoid or very weakly counterstained for the neuronal antigen NeuN (this observation was not a general finding in all brain areas, see reticular nucleus staining in **Supplementary Figure 4**). GAD staining does not allow single cell identification at $10\times$ magnification. Therefore, we used $63\times$ magnification to locate GAD positive cells and determine the percentage of inhibitory neurons stained with NeuN antibody. We found that 53.3% of the GAD positive cells showed very faint or no staining for NeuN ($n = 272$ cells, 107 fields, 15 slices from 5 animals).

Then, we run a second set of experiments to test the main hypothesis that 4-AP injection was more effective in recruiting inhibitory interneurons than L-AP4 injection. A total of six animals were injected (three with 4-AP and three with L-AP4) and then exposed visual stimulation. dLGN sections

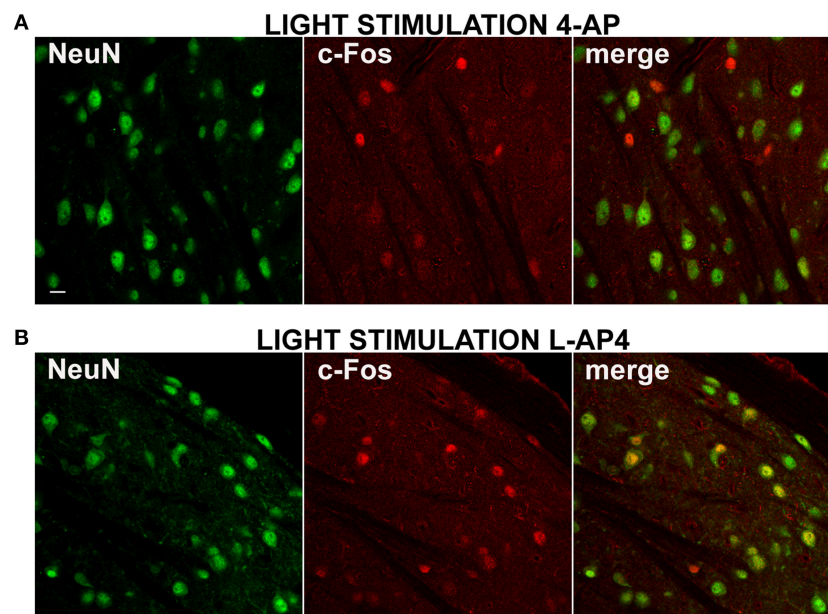
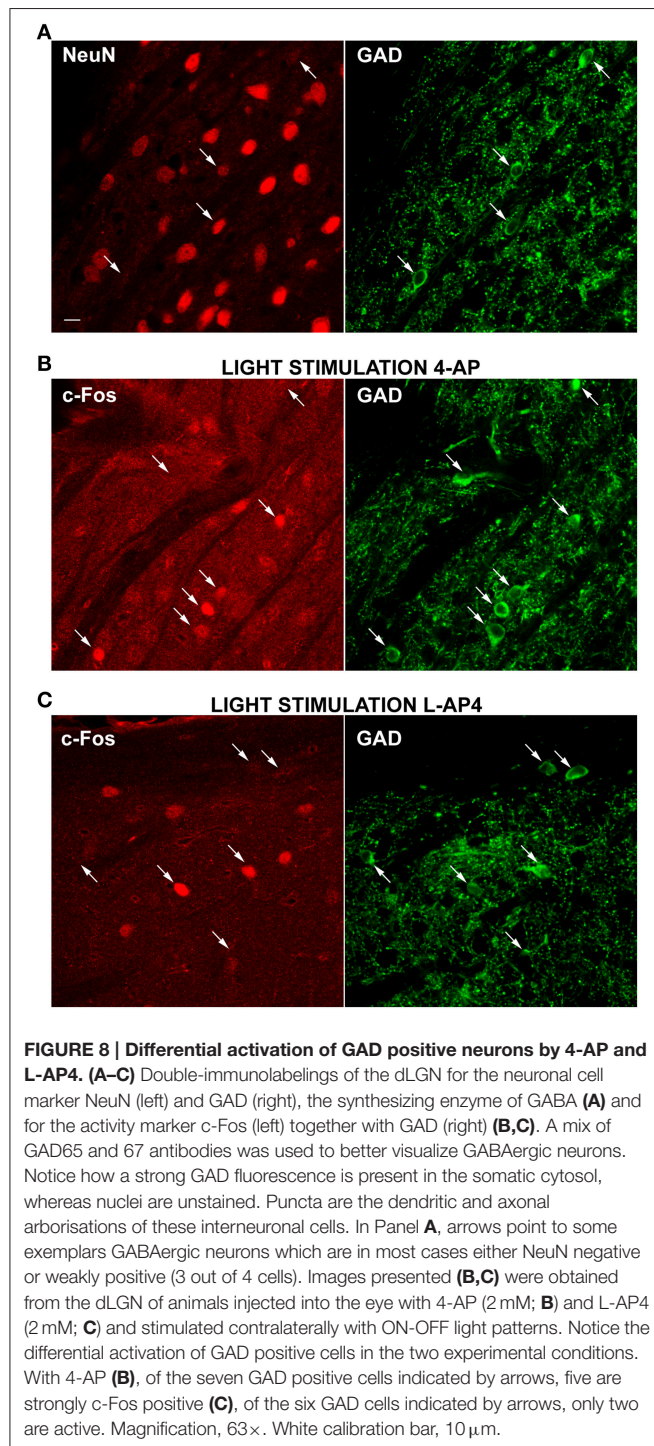


FIGURE 7 | Differential activation of NeuN positive neurons by 4-AP and L-AP4. (A,B) Double immunostaining for the neuronal marker NeuN (left) and the activity marker c-Fos (middle) and the merge image (right) in the dorsal portion of the lateral geniculate nucleus. Rats were injected into the eye with 4-AP (2 mM; **A**) and L-AP4 (2 mM; **B**). In **(A,B)** dLGN NeuN and c-Fos images were obtained from the same optical section of contralateral hemisphere. Notice how with 4-AP, almost all c-Fos positive cells are NeuN negative or weakly positive. The opposite is seen with L-AP4, where most active cells are NeuN positive. Magnification, $63\times$. White calibration bar, $10\mu\text{m}$.



were then double stained for c-Fos and GAD and the activation of inhibitory neurons analyzed again at 63 $\times$ magnification (see Table 3). We found that 64 and 33% of GAD positive cells expressed high c-Fos levels, in the 4-AP and L-AP4 experimental groups respectively (see Table 3, $p < 0.001$; see Supplementary Figure 2 and Materials and Methods for threshold selection and statistics) (Figures 8B,C). These results suggest that the preferred phenotype of activated neurons

differed significantly between these two activation modalities. L-AP4 elicits a weaker response of inhibitory interneurons, thus yielding a larger c-Fos expression in the dLGN, mostly by NeuN positive neurons. On the other side, 4-AP is able to achieve a stronger recruitment of inhibitory interneurons, and this reflects in the smaller number of overall active neurons between 4-AP and L-AP4 treatments with light stimulation (although presumably because of the high variability in cell counts and sample size this reduction did not reach the significance level, $p = 0.09$).

We also analyzed the spatial distribution of GAD positive and GAD/c-Fos positive cells. To this aim we acquired high magnification (63 $\times$) images in nine separate fields per each slice. These fields were distributed along both the lateral-medial axis and the dorso-ventral axis (lateral, central, medial positions combined with superior, central and inferior positions). Each field position was then recorded and GAD positive cells, c-Fos positive cells and double positive cells counted. Regarding the distribution of GAD cells, when number and density of these cells were counted in each one of these locations, repeating this for 83 different areas, by using a generalized linear model with a Poisson error distribution, we could test the null hypothesis of homogeneous cell counts across all different locations. With this analysis no evidence for divergence from the null hypothesis could be found (p always above 0.48) confirming both our visual impression and previous findings (Gabbott and Bacon, 1994) that the density of GABAergic cells does not change along the superior-inferior and lateral-medial axes of the dLGN. Then we estimated the probability that a GAD positive cell at a given lateral-medial location was also expressing c-Fos at high level (same threshold used above). We found, by using a logistic regression, that highly active GABAergic interneurons were localized closer to the lateral edge ($p = 0.033$).

Statistical Analysis of the Spatial Distribution of Active Neurons

The results presented up to now suggest two different modalities of dLGN activation in the presence of either 4-AP or L-AP4. We therefore asked the question if these differences correlated with a different distribution of active neurons over this thalamic sub-region. Upon visual inspection, the c-Fos activation pattern showed a non-homogenous spatial distribution of activated neurons with more active cells located on the lateral edge of this structure. To study this feature in deeper detail, we analyzed the spatial distribution of activated (c-Fos positive) cells identified as neurons based on their staining for the neuronal marker NeuN (10 $\times$ magnification images). Based on the comparative observation of LGN sections by fluorescence and interference contrast imaging, NeuN positive cells represent the large majority of neuronal cells that could be bona fide identified in the dLGN structure. To estimate the spatial randomness of activated neurons (NeuN positive), we analyzed the distribution of c-Fos positive cells in those experiments where 4-AP or L-AP4 treatments were associated with ON-OFF light stimulation. dLGN sections were reconstructed and subdivided using the Voronoi tessellation method based on the point pattern of double

TABLE 3 | GAD+, c-Fos+, and GAD+/c-Fos+ cell counts (cells/field) at 63× magnification in the dLGN after visual stimulation and intravitreal drug injection.

Treatment	Rat	Slices	cFos+ Cells	GAD+ Cells	GAD+/cFos+ Cells	cFos+ per Field	GAD+ per Field	GAD+/cFos+ per Field
4-AP	3	8	351	123	79	7.313	2.563	1.646
L-AP4	3	10	642	162	54	8.447	2.132	0.711

Table reports cells counts (cells/field) of GAD positive (column 3), c-Fos positive (column 4), and double GAD/c-Fos positive cells (column 5) in the dLGN following visual stimulation and intravitreal drug injection. All cells were identified at 63× magnification to allow precise detection of GAD positive cells. Highly c-Fos expressing cells (refer to text for selection threshold) were identified as c-Fos positive cells. The last three columns represent the same counts normalized by the number of fields analyzed in each condition. The first column report the number of animal tested in each condition, the second column reports the total number of slices analyzed per condition (multiple fields were analyzed for each slice).

NeuN/c-Fos positive cells (data not shown). Individual areas of the Voronoi polygons, each one associated to an identified neuron belonging to a single dLGN slice, were used to calculate the entropy value of the experimental activation pattern. In this analysis the null hypothesis was that the observed pattern of activated cells was not different from a random activation of the NeuN data set (the *p*-value was estimated by Monte Carlo methods, see Materials and Methods Section). In L-AP4 injected animals, the spatial pattern of activated cells was found significantly different from a random extraction from the corresponding NeuN data set in every dLGN section analyzed ($n = 6$; p always < 0.001). For 4-AP experiments the same null hypothesis could not be rejected in two out of six cases (significance level at 95%). The latter result could be explained either by a more homogeneous activation of the NeuN set when the A-type potassium channels blocker 4-AP is used or by some intrinsic limitation of the test procedure due to a reduced sample size of activated neurons under this experimental condition. To further evaluate this issue, and clarify if the observed inhomogeneity could be partially attributed to a subtle increase in cell density in the lateral portion of the dLGN, we subdivided this structure into lateral-medial stripes with a constant thickness, drawn parallel to the dLGN lateral edge (See Materials and Methods). For every stripe, we computed the frequency of NeuN positive neurons and activated cells. The pictures and histograms presented in **Figure 9** depict the frequency of NeuN positive and NeuN/c-Fos double positive neurons for individual stripes. In agreement with the results presented above, NeuN positive neurons showed a uniform spatial distribution when their frequency was corrected by the individual stripe area (the null hypothesis of uniformity could not be rejected with *p*-values always > 0.4). In order to test whether c-Fos positive neurons could simply be a random activation of the uniform NeuN neuronal population, from each available slice, by Monte Carlo Sampling we extracted $N = 10,000$ random samples from the NeuN data set, with a sample size corresponding to the experimental number of activated cells in each slice. The distribution of the median values of these random samples was used as an estimate of the test distribution under the null hypothesis. The results lead to a clear evidence of lateralization of activated neurons in the dLGN structure for both L-AP4 and 4-AP groups (*p*-values always < 0.05 , Bonferroni-Holm correction). This suggest that despite the crossed retino-thalamic input reaching both medial and lateral LGN districts, the lateral border concentrate most of the activity behaving as a sort of functional stripe.

DISCUSSION

In this study we characterized the effects of two different (chaotic or highly coherent) retinal inputs to the dLGN in terms of different patterns of c-Fos expression in this nucleus. As a reporter of activity, we used the expression of the early gene c-Fos paired with specific labeling of neuronal cells (Sheng et al., 1990; Murphy et al., 1991, 2004; Montero and Jian, 1995; Correa-Lacarcnel et al., 2000; Greferath et al., 2004; Lu et al., 2004; Flavell and Greenberg, 2008; Dai et al., 2009). Despite the fact that some anatomical features of lamination have been previously described in the rat visual thalamus, with segregation of ipsilateral and contralateral RGC axon terminals (Godement et al., 1980, 1984; Reese, 1988; Huberman et al., 2008, 2009; Kim et al., 2010; Rivlin-Etzion et al., 2011), little information is available on the spatial and functional organization of cells and synapses across the dLGN structure. In the past, the analysis of visually driven c-Fos activation in the rat thalamus has provided evidence for some recruitment of neuronal cells in the dLGN (Montero and Jian, 1995; Correa-Lacarcnel et al., 2000; Lu et al., 2004). In none of these studies a functional lamination of the dLGN response was reported, presumably because the integrated firing activity in the thalamus is never very extensive and in standard conditions few c-Fos positive cells are detected. Indeed in our experimental conditions, no significant recruitment could be induced by monocular ON-OFF light stimulation, suggesting that either the intrinsic properties of dLGN cells or a mixture of excitation and inhibition at the level of the retina and/or dLGN could prevent a sufficient firing in thalamocortical cells or in neighboring interneurons. On the other hand, this might be simply due to the low activity resolution of c-Fos expression measurement, which can only highlight intense activity of neural cells. However, to our knowledge, besides the very recently developed Campari method (Fosque et al., 2015), which would have required rat transgenesis, c-Fos is still an extremely useful method when it comes to characterizing the spatial position and the phenotype of such large networks of active/inactive cells.

To highlight this regulatory properties of dLGN we wanted to eliminate the upstream filtering of the retinal circuitry. From *in vitro* experiments on isolated retinas we found that 4-AP, an A-type potassium channel blocker, is able to increase the activity of RGCs in a non-regulated manner, leading to a parallel independent activation of the two ON-OFF retinal channels and effectively shunting retinal cross regulation. When 4-AP was administered *in vivo* via intravitreal injection the number of active cells found in the dLGN was strongly increased. As a confirmation of the chaotic and non-stimulus dependent

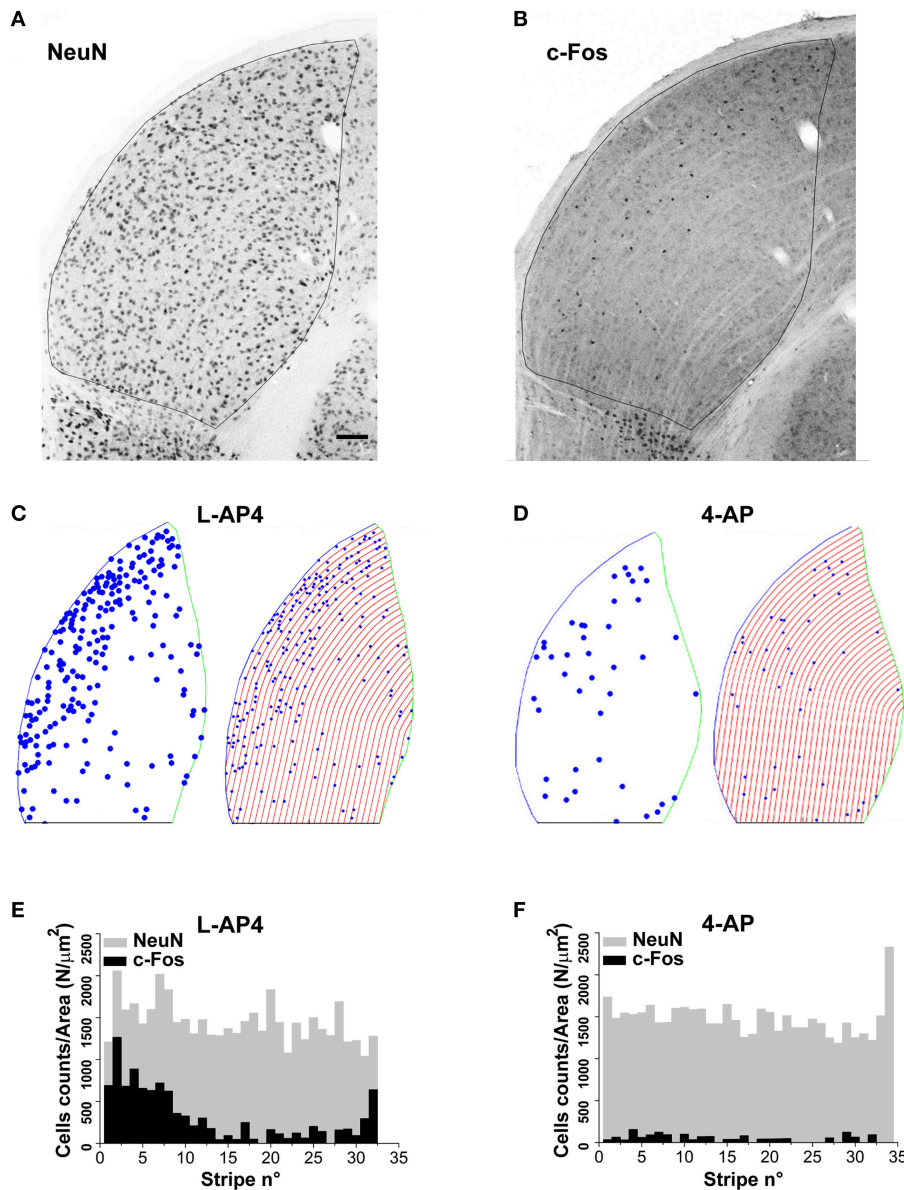


FIGURE 9 | Lateralization of active NeuN positive neurons in the dLGN structure. (A,B) Double immunostaining for the neuronal marker NeuN (A) and the c-Fos protein (B) in the dorsal portion of the right lateral geniculate nucleus. These two panels refer to an animal whose left eye was injected with 4-AP (2 mM) and then light stimulated. **(C,D)** Digital reconstructions to illustrate the location of double positive NeuN-c-Fos neurons and the procedure used to subdivide the dLGN structure in parallel stripes of constant thickness (see Materials and Methods Section for the procedure used to subdivide the LGN in stripes). The panels illustrate two exemplar experiments where the left eye was injected with either L-AP4 (2 mM; C) or 4-AP (2 mM; D) and then light stimulated. **(E,F)** Histograms plot the number of NeuN positive cells (gray bars) and double positive NeuN-c-Fos neurons (black bars) in each stripe. The histograms plot raw cell counts for sequential stripes from a single section normalized by the corresponding stripe area to compensate for the reduction of stripe area due to the LGN curvature. Histograms of normalized counts always displayed a fairly uniform distribution of NeuN cells along the latero-medial axis ($p > 0.4$) while double positive NeuN-c-Fos neurons always displayed a skewed distribution with higher density for stripes near the lateral edge of the dLGN. To test if the median value of double positive NeuN-c-Fos neurons was significantly smaller (closer to the lateral edge) than the median value of the NeuN cell population, for each dLGN section 10.000 random samples were extracted from the NeuN set (sample size equal to the observed NeuN-c-Fos sample size). Then, for each sample ($n = 2$ randomly selected sections from each animal; $n = 3$ animals for each experimental condition) the position of each cell along the lateral-medial axis was calculated as before and the median value for each sample calculated. The p -value was calculated as described in the Materials and Methods Section with Monte Carlo methods. P -values were then adjusted for multiplicity by means of the Bonferroni-Holm procedure. Results indicate that in every case the null hypothesis (double positive neurons median value not smaller than NeuN set median value) could be rejected (p always < 0.05 using Bonferroni-Holm correction).

activation of RGCs exposed to 4-AP, no significant differences could be found between the no-light and light stimulated animals. Also, no stimulus dependent activity could be detected via VEP recordings. One interesting aspect emerged from the analysis of 4-AP experiments. With or without light stimulation roughly 70% of c-Fos positive cells were found to be NeuN negative. The use of antibodies against the GABA synthesizing enzymes (GAD65 and GAD 67), a reliable markers of GABAergic cells (Meyer et al., 2011), provided clear evidence that many active cells were GABAergic interneurons, and that most of these GABAergic cells (53%) were NeuN negative. Previous reports have provided clear evidence that GABAergic interneurons are functionally heterogeneous (Williams et al., 1996). An opposite behavior emerged when RGCs were exposed to L-AP4, an mGluR6 receptor agonist which is known to block the ON retinal pathway (Slaughter and Miller, 1981). In this latter case the ON component of VEP recordings was abolished while leaving the OFF component almost unaltered. Also, a clear difference between the no light and light condition could be detected, with a much weaker recruitment of GABAergic interneurons than with 4-AP and a diffused stimulus driven activation of NeuN positive neurons within the dLGN when compared to the no-light condition. These results suggest the existence of a population of GABAergic interneurons belonging to a strong inhibitory circuitry that is set to filter out the visual signal in the presence of non-coherent tonic RGC firing, as in the case of 4-AP exposure. On the contrary, a single channel (OFF) input to dLGN, as in the case of L-AP4 administration upon light stimulation, might be less effective in recruiting such inhibitory circuitry, leading to a stimulus induced activation of dLGN neurons that is even stronger than in the no drug condition. However, it has to be noticed that an additional explanation to such stronger recruitment of dLGN cell could also arise from the suppression of channel cross inhibition within the retina itself when exposed to L-AP4 (Margolis and Detwiler, 2007; Liang and Freed, 2010). In the future it would be important to clarify the contribution of all these components to this functional behavior, possibly attributing specific roles to one or more of the previously described categories of GABAergic interneurons of the dLGN (Williams et al., 1996). Also it would be important to ascertain if thalamocortical cells participate in the filtration process of tonic and non-coherent visual inputs, by their transition from bursting firing mode to a less effective tonic discharge, because of tonic depolarization with inactivation of T-type voltage activated calcium channels and/or deactivation of Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (Jahnsen and Llinás, 1984; McCormick and Feese, 1990; McCormick and Pape, 1990). At this stage, considering the complexity of the thalamic circuitry, the different variety of GABAergic cells (Williams et al., 1996), the many modulatory pathways and the short- and long-feedback loops impinging upon thalamocortical neurons (Burke and Jervie Sefton, 1966; Berardi and Morrone, 1984; Murphy et al., 1999; Blitz and Regehr, 2003, 2005; Chen and Regehr, 2003; Cudeiro and Sillito, 2006; Hammer et al., 2014), it is difficult to provide a conclusive explanation without further experimental evidence. Our results apparently contrast with previous findings (Montero and Jian,

1995) that showed no significant difference in the soma areas of c-Fos positive and c-Fos negative cells in the dLGN after light stimulation. In that case it was concluded that small interneurons and larger relay cells were equally activated. It has to be noted, however, that these results are not directly comparable: Montero and Jian stimulated using a campimeter and were able to detect a large number of c-Fos positive cells in the pigmented rat and did not use any drug. Preferential activation of cell subtypes, as in our case, might be highlighted with c-Fos expression only after pharmacological manipulation aimed at pushing this neural system to its physiological limits, eventually recruiting its regulatory circuitry to a large extent.

Regarding dLGN neurons, which were identified as NeuN positive, presumably thalamocortical neurons, our anatomical staining suggests that no spatial clustering and/or segregation of these cells are detectable. When this analysis was extended to functional segregation, we found evidence for non-homogeneous distribution of active cells inside the dLGN structure in the 4-AP and L-AP4 experiments (c-Fos/NeuN double positive cells). In most cases a significant lateralization of active neurons, with a higher density of active cells near the lateral border of the dLGN, was detected. It is not easy to explain how such functional segregation could be generated and what its role might be in the processing of the visual information, although some functional segregation of visual features processing has been reported in the dLGN (Dhande and Huberman, 2014; Bickford et al., 2015). This external functional lamina seems to correlate with the entry point of retino-thalamic axons known to access the dLGN from the lateral edge. Despite these considerations, our results do not correlate with the presence of contralateral axons also on the medial border of the dLGN (Reese, 1988; Kim et al., 2010). This functional segregation of active neurons in the dLGN might therefore reflect a possible gradient of inhibition which gets stronger in the lateral-medial direction, possibly due to either the intrinsic inhibitory circuitry or to the cortical-thalamic inhibitory feedback, whose fibers are known to enter the dLGN from its medial border (Su et al., 2011).

AUTHOR CONTRIBUTIONS

AAr, MB, GM, MR, MF performed and design the experiments; AAm, JL, GM performed data analysis; AM designed research; AM, GM, and VZ wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fncir.2015.00077>

Supplementary Figure 1 | Exemplar light induced ON and OFF responses in a rat ON-OFF RGC. Spike recordings and the effect of light pulses (1 s) in two exemplar ON-OFF RGCs. Top lines are the schematic representation of the light stimulus. The middle and bottom track are representative spike recording before (middle) and during (bottom) the application of L-AP4 (20 μ M). Notice how the application of L-AP4 abolishes the ON response leaving the OFF response unaltered. Black scale bar is equal to 1 s.

Supplementary Figure 2 | Distribution of c-Fos Fluorescence-Threshold ratio for GAD positive cells. Histograms representing the distribution of c-Fos fluorescence intensity of GAD positive cells divided by the field specific threshold used for selection of highly active cells [Threshold = Mean (Background) + 2 x std(Background)]. The red vertical lines divide the highly active from the lowly active GAD positive cells. Highly active cells are represented with red bars. On the y axis normalized frequencies are reported. The top histogram refers to cells from L-AP4 injected animals, while bottom histogram refers to cells from 4-AP injected animals. The number of GAD positive cells is reported in each histogram. Notice how the percentage of highly active cells is greatly increased in the 4-AP group.

Supplementary Figure 3 | LGN neuronal activity pattern in control conditions (A,B). Representative c-Fos immunostainings of the right LGN (R) and digital reconstructions from the same coronal sections to visualize active neurons

in the right (R) and left (L) LGNs from rats kept in darkness (A) or light-stimulated (B) with alternating black and white vertical bars at constant overall luminance (white bars 37 mW/m²; black bars 0.11 mW/m²; 2 h; 2 Hz refresh rate; 0.5 cycle/degree; left eye stimulation). The three small panels on the right are magnification of the dLGN, IGL, and vLGN from the corresponding sections. Continuous and dashed lines indicate edges of dLGN, IGL, and vLGN. In the digital reconstruction, circles report the location of identified c-Fos positive cells (for segmentation algorithm, see Materials and Methods). Few spare cells are active in the dLGN in the no-light and following ON-OFF light-stimulation, while clear activity is detected in the IGL and vLGN. The calibration bar is 200 μ m for the large immunostaining panels and 50 μ m for the small insets.

Supplementary Figure 4 | NeuN, GAD, and c-Fos staining for the reticular nucleus. Since the reticular nucleus is one of the main inhibitory input to the dLGN, this nucleus was inspected to assess if the lack of c-Fos expression following visual stimulation in the dLGN could be due to its strong activation. This is an exemplar image from a rat after monocular visual stimulation (see Materials and Methods). (A) Double staining for GAD (on the left) and c-Fos (on the right), clearly showing the lack of c-Fos expression by GAD positive cells. (B) Double staining for GAD (on the left) and NeuN (on the right), clearly showing how, in the reticular nucleus, differently from the dLGN, all GAD+ cells are intensely stained by NeuN.

REFERENCES

- Berardi, N., and Morrone, M. C. (1984). The role of gamma-aminobutyric acid mediated inhibition in the response properties of cat lateral geniculate nucleus neurones. *J. Physiol.* 357, 505–523. doi: 10.1113/jphysiol.1984.sp015514
- Bickford, M. E., Ramcharan, E., Godwin, D. W., Erisir, A., Gnadt, J., and Sherman, S. M. (2000). Neurotransmitters contained in the subcortical extraretinal inputs to the monkey lateral geniculate nucleus. *J. Comp. Neurol.* 424, 701–717. doi: 10.1002/1096-9861(20000904)424:4<701::AID-CNE11>3.0.CO;2-B
- Bickford, M. E., Zhou, N., Krahe, T. E., Govindaiah, G., and Guido, W. (2015). Retinal and tectal “driver-like” inputs converge in the shell of the mouse dorsal lateral geniculate nucleus. *J. Neurosci.* 35, 10523–10534. doi: 10.1523/JNEUROSCI.3375-14.2015
- Blitz, D. M., and Regehr, W. G. (2003). Retinogeniculate synaptic properties controlling spike number and timing in relay neurons. *J. Neurophysiol.* 90, 2438–2450. doi: 10.1152/jn.00562.2003
- Blitz, D. M., and Regehr, W. G. (2005). Timing and specificity of feed-forward inhibition within the LGN. *Neuron* 45, 917–928. doi: 10.1016/j.neuron.2005.01.033
- Burke, W., and Jervie Sefton, A. (1966). Discharge patterns of principal cells and interneurons in lateral geniculate nucleus of rat. *J. Physiol.* 187, 201–212. doi: 10.1113/jphysiol.1966.sp008083
- Chapman, G. P. (1970). The application of information theory to the analysis of population distributions in space. *Econ. Geogr.* 46, 317–331. doi: 10.2307/143147
- Chen, C., and Regehr, W. G. (2003). Presynaptic modulation of the retinogeniculate synapse. *J. Neurosci.* 23, 3130–3135. doi: 10.3410/f.1013375.190301
- Correa-Lacárcel, J., Pujante, M. J., Terol, F. F., Almenar-García, V., Puchades-Orts, A., Ballesta, J. J., et al. (2000). Stimulus frequency affects c-fos expression in the rat visual system. *J. Chem. Neuroanat.* 18, 135–146. doi: 10.1016/S0891-0618(00)00038-7
- Cudeiro, J., and Sillito, A. M. (2006). Looking back: corticothalamic feedback and early visual processing. *Trends Neurosci.* 29, 298–306. doi: 10.1016/j.tins.2006.05.002
- Dai, Y., Sun, X., and Chen, Q. (2009). Differential induction of c-Fos and c-Jun in the lateral geniculate nucleus of rats following unilateral optic nerve injury with contralateral retinal blockade. *Exp. Brain Res.* 193, 9–18. doi: 10.1007/s00221-008-1591-6
- Dhande, O. S., Hua, E. W., Guh, E., Yeh, J., Bhatt, S., Zhang, Y., et al. (2011). Development of single retinofugal axon arbors in normal and beta2 knock-out mice. *J. Neurosci.* 31, 3384–3399. doi: 10.1523/JNEUROSCI.4899-10.2011
- Dhande, O. S., and Huberman, A. D. (2014). Retinal ganglion cell maps in the brain: implications for visual processing. *Curr. Opin. Neurobiol.* 24, 133–142. doi: 10.1016/j.conb.2013.08.006
- Faraway, J. (ed.). (2006). *Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models*. Boca Raton, FL: Chapman and Hall/CRC.
- Flavell, S. W., and Greenberg, M. E. (2008). Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. *Annu. Rev. Neurosci.* 31, 563–590. doi: 10.1146/annurev.neuro.31.060407.125631
- Fosque, B. F., Sun, Y., Dana, H., Yang, C. T., Ohyama, T., Tadross, M. R., et al. (2015). Neural circuits. Labeling of active neural circuits *in vivo* with designed calcium integrators. *Science* 347, 755–760. doi: 10.1126/science.1260922
- Francesconi, W., Muller, C. M., and Singer, W. (1988). Cholinergic mechanisms in the reticular control of transmission in the cat lateral geniculate nucleus. *J. Neurophysiol.* 59, 1690–1718.
- Gabbott, P. L., and Bacon, S. J. (1994). Two types of interneuron in the dorsal lateral geniculate nucleus of the rat: a combined NADPH diaphorase histochemical and GABA immunocytochemical study. *J. Comp. Neurol.* 350, 281–301. doi: 10.1002/cne.903500211
- Godement, P., Saillour, P., and Imbert, M. (1980). The ipsilateral optic pathway to the dorsal lateral geniculate nucleus and superior colliculus in mice with prenatal or postnatal loss of one eye. *J. Comp. Neurol.* 190, 611–626. doi: 10.1002/cne.901900402
- Godement, P., Salaün, J., and Imbert, M. (1984). Prenatal and postnatal development of retinogeniculate and retinocollicular projections in the mouse. *J. Comp. Neurol.* 230, 552–575. doi: 10.1002/cne.902300406
- Greferath, U., Nag, N., Zele, A. J., Bui, B. V., Wilson, Y., Vingrys, A. J., et al. (2004). Fos-tau-LacZ mice expose light-activated pathways in the visual system. *Neuroimage* 23, 1027–1038. doi: 10.1016/j.neuroimage.2004.06.044
- Grieve, K. L. (2005). Binocular visual responses in cells of the rat dLGN. *J. Physiol. (Lond.)* 566, 119–124. doi: 10.1113/jphysiol.2005.090878
- Guillery, R. W., and Sherman, S. M. (2002). Thalamic relay functions and their role in corticocortical communication: generalizations from the visual system. *Neuron* 33, 163–175. doi: 10.1016/S0896-6273(01)00582-7
- Güler, A. D., Altimus, C. M., Ecker, J. L., and Hattar, S. (2007). Multiple photoreceptors contribute to nonimage-forming visual functions predominantly through melanopsin-containing retinal ganglion cells. *Cold Spring Harb. Symp. Quant. Biol.* 72, 509–515. doi: 10.1101/sqb.2007.72.074
- Hammer, S., Carrillo, G. L., Govindaiah, G., Monavarfeshani, A., Bircher, J. S., Su, J., et al. (2014). Nuclei-specific differences in nerve terminal distribution, morphology, and development in mouse visual thalamus. *Neural Dev.* 9:16. doi: 10.1186/1749-8104-9-16

- Hubel, D. H., and Wiesel, T. N. (1961). Integrative action in the cat's lateral geniculate body. *J. Physiol.* 155, 385–398. doi: 10.1113/jphysiol.1961.sp006635
- Huberman, A. D., Manu, M., Koch, S. M., Susman, M. W., Lutz, A. B., Ullian, E. M., et al. (2008). Architecture and activity-mediated refinement of axonal projections from a mosaic of genetically identified retinal ganglion cells. *Neuron* 59, 425–438. doi: 10.1016/j.neuron.2008.07.018
- Huberman, A. D., Wei, W., Elstrott, J., Stafford, B. K., Feller, M. B., and Barres, B. A. (2009). Genetic identification of an On-Off direction-selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. *Neuron* 62, 327–334. doi: 10.1016/j.neuron.2009.04.014
- Jacobs, E. C., Campagnoni, C., Kampf, K., Reyes, S. D., Kalra, V., Handley, V., et al. (2007). Visualization of corticofugal projections during early cortical development in a tau-GFP-transgenic mouse. *Eur. J. Neurosci.* 25, 17–30. doi: 10.1111/j.1460-9568.2006.05258.x
- Jacobs, G. H., Fenwick, J. A., and Williams, G. A. (2001). Cone-based vision of rats for ultraviolet and visible lights. *J. Exp. Biol.* 204, 2439–2446.
- Jahnsen, H., and Llinás, R. (1984). Electrophysiological properties of guinea-pig thalamic neurones: an *in vitro* study. *J. Physiol.* 349, 205–226. doi: 10.1113/jphysiol.1984.sp015153
- Jeffery, G. (1984). Retinal ganglion cell death and terminal field retraction in the developing rodent visual system. *Brain Res.* 315, 81–96. doi: 10.1016/0165-3806(84)90079-8
- Kim, I. J., Zhang, Y., Meister, M., and Sanes, J. R. (2010). Laminar restriction of retinal ganglion cell dendrites and axons: subtype-specific developmental patterns revealed with transgenic markers. *J. Neurosci.* 30, 1452–1462. doi: 10.1523/JNEUROSCI.4779-09.2010
- Kim, K. K., Adelstein, R. S., and Kawamoto, S. (2009). Identification of neuronal nuclei (NeuN) as Fox-3, a new member of the Fox-1 gene family of splicing factors. *J. Biol. Chem.* 284, 31052–31061. doi: 10.1074/jbc.M109.052969
- Li, J., Wang, S., and Bickford, M. E. (2003). Comparison of the ultrastructure of cortical and retinal terminals in the rat dorsal lateral geniculate and lateral posterior nuclei. *J. Comp. Neurol.* 460, 394–409. doi: 10.1002/cne.10646
- Liang, Z., and Freed, M. A. (2010). The ON pathway rectifies the OFF pathway of the mammalian retina. *J. Neurosci.* 30, 5533–5543. doi: 10.1523/JNEUROSCI.4733-09.2010
- Lu, B., Coffey, P., and Lund, R. (2004). Increased c-fos-like immunoreactivity in the superior colliculus and lateral geniculate nucleus of the rd mouse. *Brain Res.* 1025, 220–225. doi: 10.1016/j.brainres.2004.06.081
- Margolis, D. J., and Detwiler, P. B. (2007). Different mechanisms generate maintained activity in ON and OFF retinal ganglion cells. *J. Neurosci.* 27, 5994–6005. doi: 10.1523/JNEUROSCI.0130-07.2007
- McCormick, D. A., and Feeser, H. R. (1990). Functional implications of burst firing and single spike activity in lateral geniculate relay neurons. *Neuroscience* 39, 103–113. doi: 10.1016/0306-4522(90)90225-S
- McCormick, D. A., and Pape, H. C. (1990). Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *J. Physiol.* 431, 291–318. doi: 10.1113/jphysiol.1990.sp018331
- McCulloch, C. E., Searle, S. R., and Neuhaus, J. M. (eds.). (2008). *Generalized, Linear, and Mixed Models*. Hoboken, NJ: Wiley & Sons, Inc.
- Meyer, H. S., Schwarz, D., Wimmer, V. C., Schmitt, A. C., Kerr, J. N., Sakmann, B., et al. (2011). Inhibitory interneurons in a cortical column form hot zones of inhibition in layers 2 and 5A. *Proc. Natl. Acad. Sci. U.S.A.* 108, 16807–16812. doi: 10.1073/pnas.1113648108
- Montero, V. M., and Jian, S. (1995). Induction of c-fos protein by patterned visual stimulation in central visual pathways of the rat. *Brain Res.* 690, 189–199. doi: 10.1016/0006-8993(95)00620-6
- Morin, L. P., and Allen, C. N. (2006). The circadian visual system, 2005. *Brain Res. Rev.* 51, 1–60. doi: 10.1016/j.brainresrev.2005.08.003
- Mullen, R. J., Buck, C. R., and Smith, A. M. (1992). NeuN, a neuronal specific nuclear protein in vertebrates. *Development* 116, 201–211.
- Murphy, M., Greferath, U., Nag, N., Nithianantharajah, J., and Wilson, Y. M. (2004). Tracing functional circuits using c-Fos regulated expression of marker genes targeted to neuronal projections. *Front. Biosci.* 9, 40–47. doi: 10.2741/1203
- Murphy, P. C., Duckett, S. G., and Sillito, A. M. (1999). Feedback connections to the lateral geniculate nucleus and cortical response properties. *Science* 286, 1552–1554. doi: 10.1126/science.286.5444.1552
- Murphy, T. H., Worley, P. F., and Baraban, J. M. (1991). L-type voltage-sensitive calcium channels mediate synaptic activation of immediate early genes. *Neuron* 7, 625–635. doi: 10.1016/0896-6273(91)90375-A
- Okabe, A., Boots, B., and Sugihara, K. (eds.). (2000). *Spatial Tessellations: Concepts and Applications of Voronoi Diagrams, 2nd Edn*. Chichester: Wiley & Sons, Inc.
- Pesarin, F., and Salmaso, L. (eds.). (2010). *Permutation Tests for Complex Data*. Chichester: Wiley & Son, Ltd.
- Polyak, S. (ed.). (1957). *The Vertebrate Visual System*. Chicago, IL: University of Chicago Press.
- Reese, B. E. (1984). The projection from the superior colliculus to the dorsal lateral geniculate nucleus in the rat. *Brain Res.* 305, 162–168. doi: 10.1016/0006-8993(84)91133-8
- Reese, B. E. (1988). “Hidden lamination” in the dorsal lateral geniculate nucleus: the functional organization of this thalamic region in the rat. *Brain Res.* 472, 119–137. doi: 10.1016/0165-0173(88)90017-3
- Rivlin-Etzion, M., Zhou, K., Wei, W., Elstrott, J., Nguyen, P. L., Barres, B. A., et al. (2011). Transgenic mice reveal unexpected diversity of on-off direction-selective retinal ganglion cell subtypes and brain structures involved in motion processing. *J. Neurosci.* 31, 8760–8769. doi: 10.1523/JNEUROSCI.0564-11.2011
- Schmidt, T. M., and Kofuji, P. (2011). An isolated retinal preparation to record light response from genetically labeled retinal ganglion cells. *J. Vis. Exp.* 47:2367. doi: 10.3791/2367
- Sheng, M., McFadden, G., and Greenberg, M. E. (1990). Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. *Neuron* 4, 571–582. doi: 10.1016/0896-6273(90)90115-V
- Slaughter, M. M., and Miller, R. F. (1981). 2-amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. *Science* 211, 182–185. doi: 10.1126/science.6255566
- Su, J., Haner, C. V., Imbery, T. E., Brooks, J. M., Morhardt, D. R., Gorse, K., et al. (2011). Reelin is required for class-specific retinogeniculate targeting. *J. Neurosci.* 31, 575–586. doi: 10.1523/JNEUROSCI.4227-10.2011
- Van Horn, S. C., Erisir, A., and Sherman, S. M. (2000). Relative distribution of synapses in the A-laminae of the lateral geniculate nucleus of the cat. *J. Comp. Neurol.* 416, 509–520. doi: 10.1002/(SICI)1096-9861(20000124)416:4<509::AID-CNE7>3.0.CO;2-H
- Weyer, A., and Schilling, K. (2003). Developmental and cell type-specific expression of the neuronal marker NeuN in the murine cerebellum. *J. Neurosci. Res.* 73, 400–409. doi: 10.1002/jnr.10655
- Williams, S. R., Turner, J. P., Anderson, C. M., and Crunelli, V. (1996). Electrophysiological and morphological properties of interneurons in the rat dorsal lateral geniculate nucleus *in vitro*. *J. Physiol.* 490(Pt 1), 129–147. doi: 10.1113/jphysiol.1996.sp021131
- Zaghloul, K. A., Boahen, K., and Demb, J. B. (2003). Different circuits for ON and OFF retinal ganglion cells cause different contrast sensitivities. *J. Neurosci.* 23, 2645–2654.

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Vestibular Interactions in the Thalamus

Rajiv Wijesinghe¹, Dario A. Protti² and Aaron J. Camp^{1*}

¹ Sensory Systems and Integration Laboratory, Sydney Medical School, Discipline of Biomedical Science, University of Sydney, Sydney, NSW, Australia, ² Vision Laboratory, Sydney Medical School, Discipline of Physiology, University of Sydney, Sydney, NSW, Australia

It has long been known that the vast majority of all information en route to the cerebral cortex must first pass through the thalamus. The long held view that the thalamus serves as a simple high fidelity relay station for sensory information to the cortex, however, has over recent years been dispelled. Indeed, multiple projections from the vestibular nuclei to thalamic nuclei (including the ventrobasal nuclei, and the geniculate bodies)-regions typically associated with other modalities- have been described. Further, some thalamic neurons have been shown to respond to stimuli presented from across sensory modalities. For example, neurons in the rat anterodorsal and laterodorsal nuclei of the thalamus respond to visual, vestibular, proprioceptive and somatosensory stimuli and integrate this information to compute heading within the environment. Together, these findings imply that the thalamus serves crucial integrative functions, at least in regard to vestibular processing, beyond that imparted by a “simple” relay. In this mini review we outline the vestibular inputs to the thalamus and provide some clinical context for vestibular interactions in the thalamus. We then focus on how vestibular inputs interact with other sensory systems and discuss the multisensory integration properties of the thalamus.

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Elizabeth Quinlan,
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USA
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Ludwig-Maximilians-University,
Germany

*Correspondence:

Aaron J. Camp
aaron.camp@sydney.edu.au

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INTRODUCTION

The vestibular system differs from the other primary sensory systems in a number of fundamental ways. Most sensory systems are organized in a linear fashion, where peripheral organ fibers project primarily through a modality-specific thalamic nucleus (for example LGN for the visual system, MGN for the auditory system) and only then onto their respective cortical or subcortical targets. These ordered projections through the thalamus create a sensory map that closely matches that created in the periphery, and this tends to be maintained by downstream thalamocortical projections [for review, see Jones (1985)].

Abbreviations: CL, Centrolateral Nucleus; CM, Centromedian Nucleus; IL, Interlaminar Nuclei; IVN, Inferior Vestibular Nucleus; LGN, Lateral Geniculate Nucleus; LVN, Lateral Vestibular Nucleus; LP, Lateral Posterior Nucleus; LD, Lateral Dorsal Nucleus; MGN, Medial Geniculate Nucleus; MVN, Medial Vestibular Nucleus; PNF, Parafascicular Nucleus; SpVN, spinal (descending) vestibular Nucleus; SuVN, Superior Vestibular Nucleus; VA, Ventroanterior Nucleus; VI, Ventral Intermediate Nucleus; VL, Ventrolateral Nucleus; VP, Ventral Posterior Nucleus; VPL, Ventral Posterior Lateral Nucleus; VPM, Ventral Posterior Medial Nucleus.

This organization, along with ordered corticothalamic feedback mechanisms, allows the thalamus to play filtering and modulatory roles in sensory processing (see Sherman, 2007 and references within). Hair cells within the vestibular epithelia are oriented such that they are direction-selective (Lindeman, 1969), however when afferent fibers that relay information from these cells in the semicircular canals, utricle and saccule all converge onto the brainstem vestibular nuclei this signal then becomes more complex. For example, some thalamic neurons receiving vestibular information appear to selectively fire when the organism's head is orientated in a specific direction within the environment, much like a compass (for comprehensive review, see Wiener and Taube, 2005). The brainstem vestibular nuclei project directly to the thalamus, however there is no dedicated vestibular thalamic nucleus that contains a direction or orientation selective map. Further, vestibular thalamic projections are widely distributed to subcortical (Lai et al., 2000), cerebellar (Kotchabhakdi and Walberg, 1978) and cortical (de Waele et al., 2001; Miyamoto et al., 2005) regions that have been shown to be involved in vestibular processing.

Cortical processing of vestibular information is poorly understood compared to other sense modalities such as vision, audition and touch. The perceptual correlate of vestibular activation does not usually correspond to a unimodal sensation, as it occurs with vision and audition. Natural activation of the vestibular system due to head motion and locomotion typically involves activation of visual, vestibular and somatosensory systems and consequently the resulting perceptual correlate are sensations that involve visual, vestibular and somatosensory characteristics. Vestibular-responsive neurons were initially described in somatosensory cortex in areas 3a (Grusser et al., 1990) and area 2v (Buttner and Buettner, 1978). More recently, head direction neurons responsive to vestibular stimulation have also been identified in regions within the classical Papez circuit. Whether signals from different modalities reach these regions independently or they converge at earlier stages is not completely understood yet. There is, however, evidence consistent with integration at the level of the thalamus.

These fundamental observations highlight that the vestibular system has a unique structure amongst the sensory systems, and raises some important questions. In particular, what role does the thalamus play in vestibular processing? In this mini-review we will explore the current understanding of vestibular thalamic function. In the first section, we summarize anatomical studies of vestibulothalamic projections, and suggest that they may be organized into modality-specific processing streams that permit multisensory integration. In the second section, we analyze physiological studies exploring how vestibular thalamic neurons respond to vestibular information, and clinical observations that give us insights into vestibular processing within the thalamus. In the final section, we make hypotheses about the mechanisms of multisensory integration using the vestibular system as an example, and suggest that the thalamus is well placed to represent a unique subcortical locus of multisensory integration.

VESTIBULOTHALAMIC PROJECTIONS

Peripheral vestibular stimulation has been shown to cause strong activation within the thalamus (Deecke et al., 1974; Buttner and Henn, 1976; Blum et al., 1979). Tracing studies performed in rat (Shiroyama et al., 1999), cat (Kotchabhakdi et al., 1980), and monkey (Meng et al., 2007), as well as radiological investigations in humans (Kirsch et al., 2015) have demonstrated multiple projections from vestibular nuclei to the thalamus. The primary thalamic targets are the ventrobasal, ventrolateral and intralaminar nuclei and the geniculate bodies (for a recent review, see Lopez and Blanke, 2011). Many of these nuclei are not specific to the vestibular system, and therefore receive inputs from a number of different peripheral sensory and cortical regions. There is some evidence to suggest that these vestibulothalamic circuits may form discrete, functionally specialized pathways that integrate vestibular with other modality-specific signals within the thalamus (Lopez and Blanke, 2011).

Inter alia, the ventrobasal nuclei (ventral posterolateral, VPL; ventral posteromedial, VPM; and ventral intermediate, VI) receive dominant vestibular inputs from bilateral superior vestibular nucleus (SuVN), and contralateral medial vestibular nucleus (MVN) via the medial longitudinal fasciculus (MLF; Nagata, 1986; Matesz et al., 2002). VPM neurons have been shown to respond directly to vestibular nerve stimulation, as well as simulated translational and rotational movements (Marlinski and Mccrea, 2008). These nuclei in turn project to well known vestibular cortical areas such as the anterior suprasylvian cortex (Deecke et al., 1979), posterior cruciate region and the intraparietal sulcus (for review, see Brandt and Dieterich, 1999). The ventral posterior nuclei (VP or VPP) in particular is the source of the major projection to a well studied vestibular cortical region in the macaque monkey, the PIVC (Akbarian et al., 1992). However, it has been shown that some posterior thalamic nuclei such as the VP are also activated during deep somatic and cutaneous stimulation (Deecke et al., 1977). In addition, the VPI projects directly to primary somatosensory cortex (Deecke et al., 1974), as well as vestibular regions of the secondary association somatosensory cortex (3aV) and posterior parietal cortex (Matsuzaki et al., 2004). These observations show that the thalamus contains the neural circuits necessary to mediate the integration of vestibular and somatosensory signals.

The ventrolateral nuclei (ventral anterior, VA; ventral lateral, VL) receive inputs primarily from LVN, MVN and SuVN via the medial longitudinal fasciculus (MLF; Maciewicz et al., 1982; Nakano et al., 1985; Nagata, 1986). Interestingly, the VL also receives inputs from vestibular cerebellar nuclei such as the fastigial and dentate nuclei via the superior cerebellar peduncle (Sawyer et al., 1994a,b), while the VA receives strong inputs from the basal ganglia (Percheron et al., 1996). The VA-VL complex projects to primary motor and premotor cortices (Brodmann 4 and 6), suggesting that this circuit may represent a major vestibulomotor pathway. The intralaminar nuclei (CL or PFN in rat, CM) receive inputs primarily from ipsilateral MVN, SuVN and spinal vestibular nucleus (SpVN);

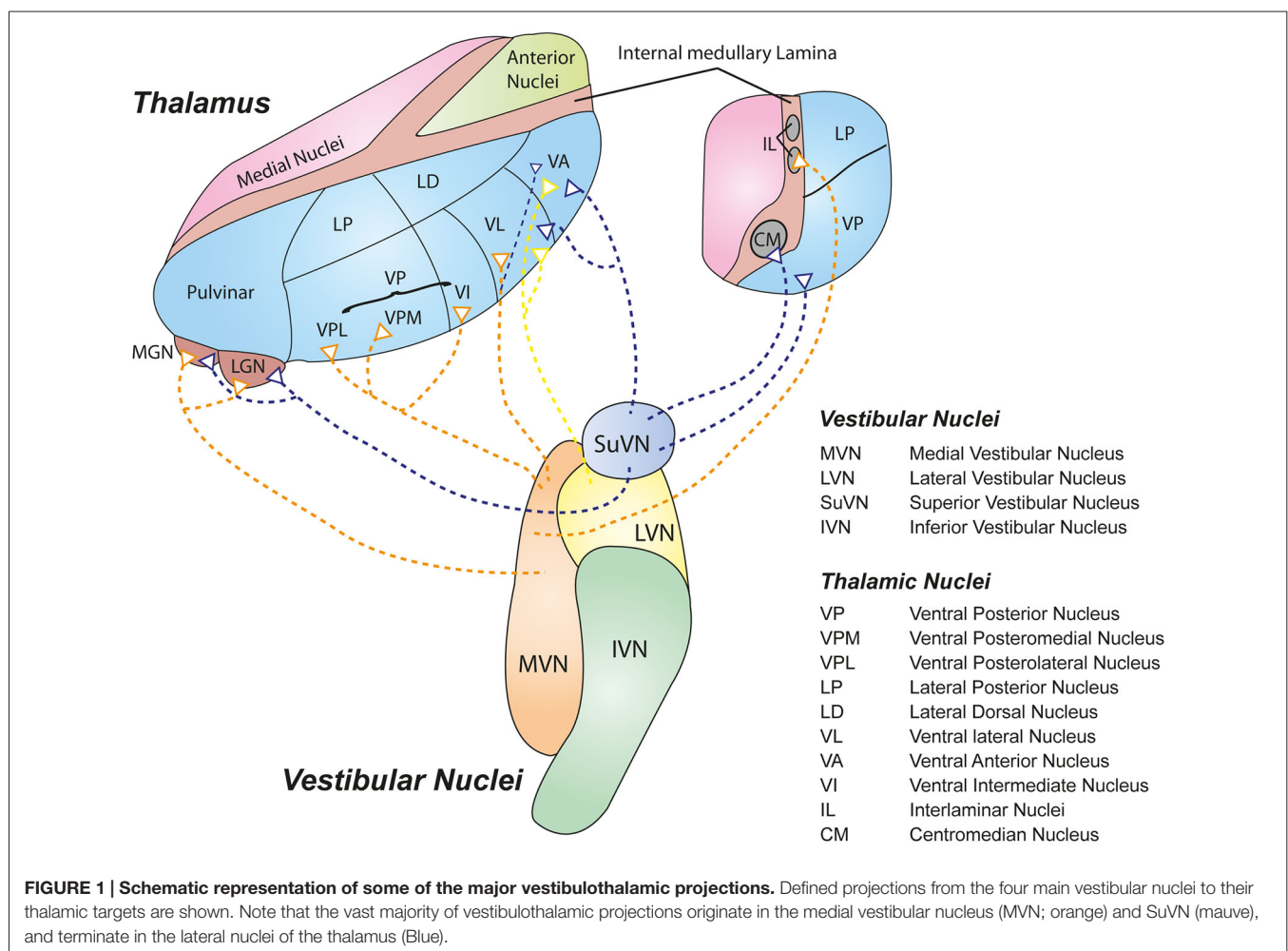
Magnin and Kennedy, 1979; Matsuo et al., 1994). Specifically, the PFN has been shown to be a crucial synapse for a vestibulo-thalamo-striatal pathway proposed to be involved in controlling body and limb movements (for review, see Stiles and Smith, 2015). Further, the geniculate bodies (MGN, LGN, SGN) receive inputs from DVN, SuVN and MVN (Kotchabhakdi et al., 1980; Mergner et al., 1981). Despite their primary role in auditory and visual signalling (MGN and LGN respectively), these centers have also been shown to respond to vestibular signals (Liedgren and Schwarz, 1976; Roucoux-Hanus and Boisacq-Schepens, 1977), suggesting that these thalamic nuclei may participate in subcortical multi-sensory integration. **Figure 1** illustrates some of the major pathways connecting the central vestibular nuclei and the thalamus. Note that most of the inputs terminate in the lateral nuclei of the thalamus.

THALAMIC VESTIBULAR PROCESSING

The vestibular nuclei project heavily to the spinal cord and cerebellum, and these pathways mediate important reflexes that maintain postural control and motor learning (Granit

and Pompeiano, 1979; Pompeiano and Allum, 1988). As is evident from the discussion above, there are multiple, divergent vestibular projections that course through the thalamus. However there is limited information about what role the thalamus plays (in concert with the vestibular system) in postural control, and only recently has work begun to delineate its fundamental vestibular processing characteristics.

Clinical observations provide some useful insights into the thalamic contribution to vestibular processing. Postural disorders are increasingly well recognized in patients recovering from strokes, some of which cannot solely be attributed to the unilateral loss of muscular control. For example, Pusher syndrome is an active righting movement observed following strokes where the patient “push[es] actively with non-paretic extremities to the side contralateral to the brain lesion” (Karnath et al., 2005). The lesion induces an abnormality in the perception of the body’s alignment in relation to gravity, and has been described in patients with circumscribed posterior thalamic lesions as well as other adjacent brain structures (Karnath et al., 2005). Specifically, patients with pusher syndrome perceive their body to be positioned vertically despite approximately 18 degrees



of tilt of the subjective postural vertical in the roll plane (for review see Karnath, 2007). Interestingly the subjective visual vertical appears remarkably undisturbed in pusher syndrome (Karnath et al., 2000). The observation that cortical infarcts are also capable of inducing this syndrome (Johannsen et al., 2006; Karnath et al., 2008) or other perturbations of subjective visual vertical (Baier et al., 2012) suggests that the posterior thalamus is important but not unique in generating this perception of verticality. Another interesting insight comes from studies of patients suffering from vestibular migraine, a central vestibular disorder characterized by an abnormal vestibular percept without peripheral vestibulopathy (for recent review, see Stolte et al., 2015). Functional magnetic resonance imaging (fMRI) studies in these patients during vestibular stimulation demonstrated increased BOLD signal within the thalamus compared to both healthy controls and migraineurs with aura (Russo et al., 2014). These observations demonstrate that the thalamus plays an integral role in the generation of complex vestibular percepts, and that abnormalities in thalamic processing may account for the distortion of these percepts observed in certain clinical situations.

A number of *in vivo* electrophysiology studies have demonstrated the vestibular evoked responses of thalamic neurons. Meng et al. (2007) recorded the responses of thalamic neurons in the ventral posterior and ventral lateral thalamic nuclei of macaque monkeys. In general, they found that vestibular-responsive thalamic neurons show mixed selectivity for rotations and translations within different planes, however they tend to be more responsive to translations within the yaw plane. Subsequently, a small subset of thalamic neurons was found to be responsive to both vestibular and visual inputs (Meng and Angelaki, 2010). **Figure 2** shows the translation direction tuning to both visual and vestibular stimulation for one example multisensory neuron. Similar responses were also observed for rotational stimuli. In addition, experiments by Marlinski and colleagues demonstrated that neurons tended to respond either to ipsi- or contralateral rotation, with no neurons displaying bidirectional sensitivity (Marlinski and Mccrea, 2008). Experiments performed in both light and dark environments demonstrate that the vestibular signal in the thalamus is independent of visual signals, however the addition of visual information does

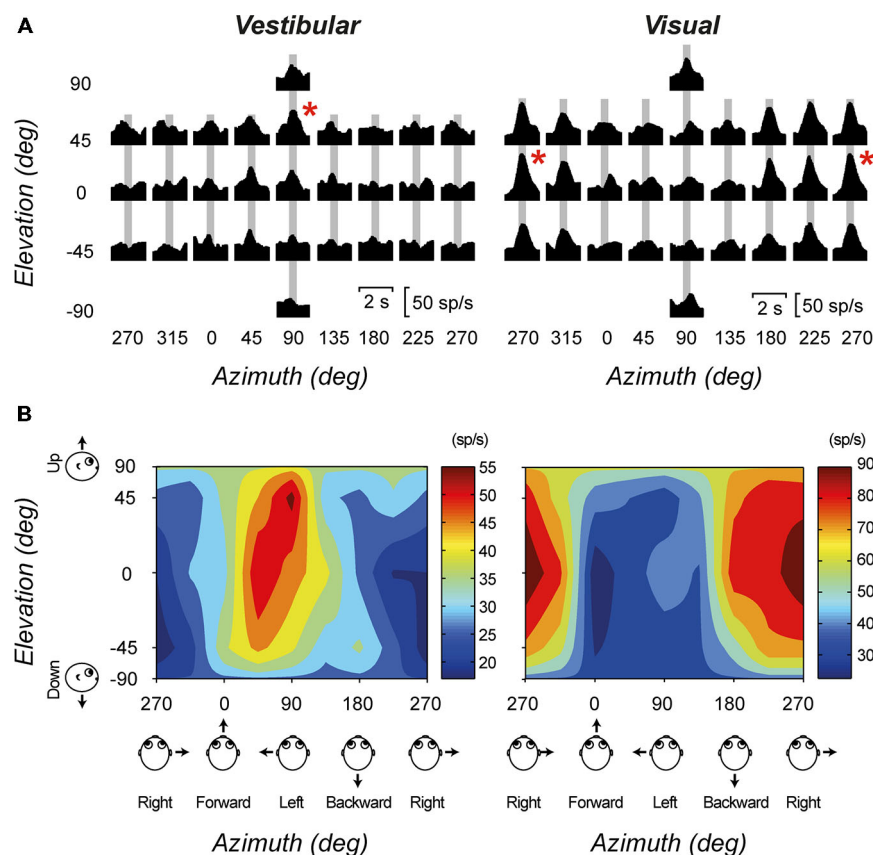


FIGURE 2 | An example cell with significant tuning during both the visual (optic flow) and vestibular stimulus conditions. (A) Response PSTHs for each of 26 directions during translation. Red asterisks mark the maximum response directions. The corresponding peak-times are marked by gray bars. **(B)** The corresponding color contour tuning (at peak time) for each stimulus condition. Figure modified from Meng and Angelaki (2010) with permission from the Journal of Neurophysiology.

increase the sensitivity of these neurons to incoming inputs. Both groups showed a large range of responsiveness to rotational stimulation, in both amplitude and phase. Their experiments demonstrated a large number of vestibular responsive areas within the thalamus, outside of the classically described vestibulothalamic projection zones. Interestingly, Marlinski and colleagues demonstrated small clusters of vestibular-responsive neurons within the VP nucleus, and postulate that these may be analog to the discrete regions seen within the macaque thalamus that project to specific vestibular-specific cortical regions (Marlinski and Mccrea, 2008).

Few investigators have studied the intrinsic properties of neurons within the thalamus that receive vestibular inputs. One recent study by Kulkarni and colleagues examined the properties of rat anterodorsal thalamic neurons in the presence of synaptic blockade (Kulkarni et al., 2011). They found that single neurons were able to sustain long periods of regular firing over many minutes with minimal adaptation. This response was particularly evident when induced by hyperpolarizing as opposed to depolarizing pulses. Work studying the synaptic profile of anterodorsal thalamic neurons suggests that they are characteristic of those within a first order relay nucleus, with a driver input from the mammillary bodies and modulatory input from cortical feedback (Petrof and Sherman, 2009). It would be interesting to see whether these neurons also display similar output profiles to other first-order relay thalamocortical neurons such as those seen in the lateral geniculate nucleus (Gutierrez et al., 2001; Wijesinghe et al., 2013).

MULTISENSORY INTEGRATION IN THE THALAMUS

Perceptually, the vestibular system is integral in generating higher order sensory phenomena that require input from other sensory systems. For example, creating the sense of the visual vertical requires input from both visual and vestibular neurons (Vingerhoets et al., 2009); postural stability requires input from proprioceptive neurons and vestibular neurons (Bronstein, 1999). Studies have also unexpectedly suggested a role for the vestibular system in cognitive domains such as memory and learning (Smith and Zheng, 2013) and body representation (Mast et al., 2014). As outlined above, thalamic nuclei receiving vestibular afferents are also heavily involved in the processing of information from other sensory systems. This raises the possibility that the thalamus may serve as an important locus for multisensory integration. Indeed, a recent study shows tantalizing evidence that driver (primary and secondary vestibular) and modulatory (visual) inputs onto cerebellar granule cells have different strength and temporal course. In addition, when these inputs are co-activated they produce an enhanced response with a characteristic first spike latency that allows temporal coding of multisensory inputs (Chabrol et al., 2015). It is tempting to speculate, as the authors do, that similar mechanisms may also be involved in the integration of synaptic inputs of different sensory modalities

in other thalamic neurons and HD cells that receive vestibular information.

Studies comparing stroke patients with a degree of somatosensory loss to normal controls have suggested that interactions between vestibular and somatosensory information is dependent on the function of the posterolateral thalamus (Barra et al., 2010). Further, psychophysical studies have demonstrated that multisensory integration is impaired in patients with Parkinson's disease, possibly be due to thalamic dysfunction induced by lack of facilitation by ascending cholinergic systems (Muller et al., 2013). Interestingly, one study reporting on two patients with Parkinson's following deep brain stimulation of the subthalamic nucleus showed changes in the subjective visual vertical (Mike et al., 2009)- a feature typically associated with peripheral and/or central vestibular dysfunction- again suggestive of vestibular thalamic interactions.

Experimentally, a model system for multisensory integration is the head-direction (HD) system, which contains neurons that encode head orientation in the horizontal (yaw) plane independently of location within an environment (for review, see Taube, 2007). HD neurons have been found in a number of regions within the classical Papez circuit, such as the anterodorsal thalamus, lateral mammillary nuclei, and the retrosplenial and entorhinal cortices (see Wiener and Taube, 2005 and references within for details). The vestibular system plays an integral role in the generation of the HD signal, as demonstrated by behavioral studies assessing spatial navigation while restricting sensory cues or with specific brain regions deactivated (Yoder and Taube, 2014). A recent *in vivo* study analyzing the head-direction cell response to both active and passive movements has shown that vestibular input is necessary and sufficient to generate the HD signal (Shinder and Taube, 2011), highlighting the central role of the vestibular system in this localization pathway.

Thalamic HD neurons have been the focus of recent investigations aiming to elucidate the specific mechanisms behind the HD signal. Using rats on a moving treadmill, Enkhjargal et al. (2014) studied the effect of more complex movements involving multiple frames of reference with conflicting sensory inputs. They found that thalamic HD neurons displayed complex spatial firing patterns that depended on the combinations of facing direction and movement direction; that is, their activity depends on perceived directional heading, optic flow, vestibular and proprioceptive information (Enkhjargal et al., 2014). Interestingly, the directionality seen with the head-direction signal may be dependent on the intrinsic properties of anterodorsal thalamic neurons, specifically their propensity for irregular firing (Tsanov et al., 2014). The finding that the HD signal is also present when motionless (Shinder and Taube, 2014) may suggest that intrinsic mechanisms are capable of maintaining the HD signal once it has been generated. Recent investigations analyzing ensembles of HD-cells within the anterodorsal nucleus and post-subiculum found coherent activations during awake and sleep network states, suggesting that the HD representation may be generated internally within

thalamic circuits and modulated by sensory inputs (Peyrache et al., 2015). Another exciting new discovery has shown that parahippocampal grid cell activity may be dependent upon the thalamic head direction signal (Winter et al., 2015), highlighting a central role of the thalamus as a major locus for multisensory integration in the generation of complex perceptual phenomena, particularly in regard to the vestibular contributions to navigation.

SUMMARY

Our understanding of the vestibular interactions within the thalamus remains nascent, however is growing quite quickly. Clinical and experimental observations are piecing together a clearer picture of how vestibular signals are processed and combined with other sensory signals to form complex representations of the environment and our actions within it. Despite this, the underlying cellular mechanisms that mediate these processes remain unclear. What are the characteristics of individual thalamic neurons in these multisensory pathways? What are the intrinsic properties of these neurons that allow

this filtering and integration to take place? What synaptic and intrinsic properties mediate the processing of disparate and conflicting signals from different sensory systems? What is clear, however, is that the thalamus is a point of convergence for signals from essentially all sensory modalities, and that groups of, and even individual neurons within the Thalamus, have the capacity to compute a variety of output functions depending on the signals received. This feature alone is sufficient to clearly define the Thalamus as “beyond a simple relay”.

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REFERENCES

- Akbadian, S., Grusser, O. J., and Guldin, W. O. (1992). Thalamic connections of the vestibular cortical fields in the squirrel monkey (*Saimiri sciureus*). *J. Comp. Neurol.* 326, 423–441.
- Baier, B., Suchan, J., Karnath, H. O., and Dieterich, M. (2012). Neural correlates of disturbed perception of verticality. *Neurology* 78, 728–735. doi: 10.1212/wnl.0b013e318248e544
- Barra, J., Marquer, A., Joassin, R., Reymond, C., Metge, L., Chauvineau, V., et al. (2010). Humans use internal models to construct and update a sense of verticality. *Brain* 133, 3552–3563. doi: 10.1093/brain/awq311
- Blum, P. S., Day, M. J., Carpenter, M. B., and Gilman, S. (1979). Thalamic components of the ascending vestibular system. *Exp. Neurol.* 64, 587–603. doi: 10.1016/0014-4886(79)90234-6
- Brandt, T., and Dieterich, M. (1999). The vestibular cortex. Its locations, functions and disorders. *Ann. N Y Acad. Sci.* 871, 293–312. doi: 10.1007/978-1-4471-0527-5_13
- Bronstein, A. M. (1999). The interaction of otolith and proprioceptive information in the perception of verticality. The effects of labyrinthine and CNS disease. *Ann. N Y Acad. Sci.* 871, 324–333. doi: 10.1111/j.1749-6632.1999.tb09195.x
- Buttner, U., and Buettner, U. W. (1978). Parietal cortex (2v) neuronal activity in the alert monkey during natural vestibular and optokinetic stimulation. *Brain Res.* 153, 392–397. doi: 10.1016/0006-8993(78)90421-3
- Buttner, U., and Henn, V. (1976). Thalamic unit activity in the alert monkey during natural vestibular stimulation. *Brain Res.* 103, 127–132. doi: 10.1016/0006-8993(76)90692-2
- Chabrol, F. P., Arenz, A., Wiechert, M. T., Margrie, T. W., and Digregorio, D. A. (2015). Synaptic diversity enables temporal coding of coincident multisensory inputs in single neurons. *Nat. Neurosci.* 18, 718–727. doi: 10.1038/nn.3974
- de Waele, C., Baudonniere, P. M., Lepecq, J. C., Tran Ba Huy, P., and Vidal, P. P. (2001). Vestibular projections in the human cortex. *Exp. Brain Res.* 141, 541–551. doi: 10.1007/s00221-001-0894-7
- Deecke, L., Mergner, T., and Becker, W. (1979). Neuronal responses to natural vestibular stimuli in the cat's anterior suprasylvian gyrus. *Adv. Otorhinolaryngol.* 25, 74–81. doi: 10.1016/0006-8993(79)90052-0
- Deecke, L., Schwarz, D. W., and Fredrickson, J. M. (1974). Nucleus ventroposterior inferior (VPI) as the vestibular thalamic relay in the rhesus monkey. I. Field potential investigation. *Exp. Brain Res.* 20, 88–100. doi: 10.1007/bf00239019
- Deecke, L., Schwarz, D. W., and Fredrickson, J. M. (1977). Vestibular responses in the rhesus monkey ventroposterior thalamus. II. Vestibulo-proprioceptive convergence at thalamic neurons. *Exp. Brain Res.* 30, 219–232. doi: 10.1007/bf00237252
- Enkhjargal, N., Matsumoto, J., Chinzorig, C., Berthoz, A., Ono, T., and Nishijo, H. (2014). Rat thalamic neurons encode complex combinations of heading and movement directions and the trajectory route during translocation with sensory conflict. *Front. Behav. Neurosci.* 8:242. doi: 10.3389/fnbeh.2014.00242
- Granit, R., and Pompeiano, O. (1979). *Reflex Control of Posture and Movement*. Holland: Elsevier.
- Grusser, O. J., Pause, M., and Schreier, U. (1990). Localization and responses of neurons in the parieto-insular vestibular cortex of awake monkeys (*macaca-fascicularis*). *J. Physiol.* 430, 537–557.
- Gutierrez, C., Cox, C. L., Rinzel, J., and Sherman, S. M. (2001). Dynamics of low-threshold spike activation in relay neurons of the cat lateral geniculate nucleus. *J. Neurosci.* 21, 1022–1032.
- Johannsen, L., Broetz, D., Naegel, T., and Karnath, H. O. (2006). “Pusher syndrome” following cortical lesions that spare the thalamus. *J. Neurol.* 253, 455–463. doi: 10.1007/s00415-005-0025-7
- Jones, E. G. (1985). *The Thalamus*. New York and London: Plenum Press.
- Karnath, H. O. (2007). Pusher syndrome—a frequent but little-known disturbance of body orientation perception. *J. Neurol.* 254, 415–424. doi: 10.1007/s00415-006-0341-6
- Karnath, H. O., Ferber, S., and Dichgans, J. (2000). The origin of contraversive pushing: evidence for a second graviceptive system in humans. *Neurology* 55, 1298–1304. doi: 10.1212/wnl.55.9.1298
- Karnath, H. O., Johannsen, L., Broetz, D., and Kuker, W. (2005). Posterior thalamic hemorrhage induces “pusher syndrome”. *Neurology* 64, 1014–1019. doi: 10.1212/wnl.65.10.1682
- Karnath, H. O., Suchan, J., and Johannsen, L. (2008). Pusher syndrome after ACA territory infarction. *Eur. J. Neurol.* 15, e84–e85. doi: 10.1111/j.1468-1331.2008.02187.x
- Kirsch, V., Keeser, D., Hergenroeder, T., Erat, O., Ertl-Wagner, B., Brandt, T., et al. (2015). Structural and functional connectivity mapping of the vestibular circuitry from human brainstem to cortex. *Brain Struct. Funct.* doi: 10.1007/s00429-014-0971-x [Epub ahead of print].
- Kotchabhakdi, N., Rinvik, E., Walberg, F., and Yingchareon, K. (1980). The vestibulothalamic projections in the cat studied by retrograde axonal transport of horseradish peroxidase. *Exp. Brain Res.* 40, 405–418. doi: 10.1007/bf00236149

- Kotchabhakdi, N., and Walberg, F. (1978). Cerebellar afferent projections from the vestibular nuclei in the cat: an experimental study with the method of retrograde axonal transport of horseradish peroxidase. *Exp. Brain Res.* 31, 591–604. doi: 10.1007/bf00239814
- Kulkarni, M., Zhang, K., and Kirkwood, A. (2011). Single-cell persistent activity in anterodorsal thalamus. *Neurosci. Lett.* 498, 179–184. doi: 10.1016/j.neulet.2011.02.051
- Lai, H., Tsumori, T., Shiroyama, T., Yokota, S., Nakano, K., and Yasui, Y. (2000). Morphological evidence for a vestibulo-thalamo-striatal pathway via the parafascicular nucleus in the rat. *Brain Res.* 872, 208–214. doi: 10.1016/S0006-8993(00)02457-4
- Liedgren, S. R., and Schwarz, D. W. (1976). Vestibular evoked potentials in thalamus and basal ganglia of the squirrel monkey (*Saimiri sciureus*). *Acta. Otolaryngol.* 81, 73–82.
- Lindeman, H. H. (1969). Studies on the morphology of the sensory regions of the vestibular apparatus with 45 figures. *Ergeb. Anat. Entwicklungsgesch.* 42, 1–113. doi: 10.1007/978-3-662-24992-5
- Lopez, C., and Blanke, O. (2011). The thalamocortical vestibular system in animals and humans. *Brain Res. Rev.* 67, 119–146. doi: 10.1016/j.brainresrev.2010.12.002
- Maciewicz, R., Phipps, B. S., Bry, J., and Highstein, S. M. (1982). The vestibulothalamic pathway: contribution of the ascending tract of Deiters. *Brain Res.* 252, 1–11. doi: 10.1016/0006-8993(82)90973-8
- Magnin, M., and Kennedy, H. (1979). Anatomical evidence of a third ascending vestibular pathway involving the ventral lateral geniculate nucleus and the intralaminar nuclei of the cat. *Brain Res.* 171, 523–529. doi: 10.1016/0006-8993(79)91056-4
- Marlinski, V., and Mccrea, R. A. (2008). Activity of ventroposterior thalamus neurons during rotation and translation in the horizontal plane in the alert squirrel monkey. *J. Neurophysiol.* 99, 2533–2545. doi: 10.1152/jn.00761.2007
- Mast, F. W., Preuss, N., Hartmann, M., and Grabherr, L. (2014). Spatial cognition, body representation and affective processes: the role of vestibular information beyond ocular reflexes and control of posture. *Front. Integr. Neurosci.* 8:44. doi: 10.3389/fnint.2014.00044
- Matesz, C., Bacska, T., Nagy, E., Halasi, G., and Kulik, A. (2002). Efferent connections of the vestibular nuclei in the rat: a neuromorphological study using PHA-L. *Brain Res. Bull.* 57, 313–315. doi: 10.1016/S0361-9230(01)00726-2
- Matsuo, S., Hosogai, M., and Nakao, S. (1994). Ascending projections of posterior canal-activated excitatory and inhibitory secondary vestibular neurons to the mesodiencephalon in cats. *Exp. Brain Res.* 100, 7–17. doi: 10.1007/bf00227274
- Matsuzaki, R., Kyuhou, S., Matsuura-Nakao, K., and Gemba, H. (2004). Thalamo-cortical projections to the posterior parietal cortex in the monkey. *Neurosci. Lett.* 355, 113–116. doi: 10.1016/j.neulet.2003.10.066
- Meng, H., and Angelaki, D. E. (2010). Responses of ventral posterior thalamus neurons to three-dimensional vestibular and optic flow stimulation. *J. Neurophysiol.* 103, 817–826. doi: 10.1152/jn.00729.2009
- Meng, H., May, P. J., Dickman, J. D., and Angelaki, D. E. (2007). Vestibular signals in primate thalamus: properties and origins. *J. Neurosci.* 27, 13590–13602. doi: 10.1523/JNEUROSCI.3931-07.2007
- Mergner, T., Deecke, L., and Wagner, H. J. (1981). Vestibulo-thalamic projection to the anterior suprasylvian cortex of the cat. *Exp. Brain Res.* 44, 455–458. doi: 10.1007/bf00238841
- Mike, A., Balas, I., Varga, D., Janszky, J., Nagy, F., and Kovacs, N. (2009). Subjective visual vertical may be altered by bilateral subthalamic deep brain stimulation. *Mov. Disord.* 24, 1556–1557. doi: 10.1002/mds.22605
- Miyamoto, T., Fukushima, K., Takada, T., de Waele, C., and Vidal, P. P. (2005). Saccular projections in the human cerebral cortex. *Ann. N Y Acad. Sci.* 1039, 124–131. doi: 10.1196/annals.1325.012
- Muller, M. L., Albin, R. L., Kotagal, V., Koeppe, R. A., Scott, P. J., Frey, K. A., et al. (2013). Thalamic cholinergic innervation and postural sensory integration function in Parkinson's disease. *Brain* 136, 3282–3289. doi: 10.1093/brain/awt247
- Nagata, S. (1986). The vestibulothalamic connections in the rat: a morphological analysis using wheat germ agglutinin-horseradish peroxidase. *Brain Res.* 376, 57–70.
- Nakano, K., Kohno, M., Hasegawa, Y., and Tokushige, A. (1985). Cortical and brain stem afferents to the ventral thalamic nuclei of the cat demonstrated by retrograde axonal transport of horseradish peroxidase. *J. Comp. Neurol.* 231, 102–120. doi: 10.1002/cne.902310109
- Percheron, G., Francois, C., Talbi, B., Yelnik, J., and Fenelon, G. (1996). The primate motor thalamus. *Brain Res. Brain Res. Rev.* 22, 93–181. doi: 10.1016/S0165-0173(96)00003-3
- Petrof, I., and Sherman, S. M. (2009). Synaptic properties of the mammillary and cortical afferents to the anterodorsal thalamic nucleus in the mouse. *J. Neurosci.* 29, 7815–7819. doi: 10.1523/JNEUROSCI.1564-09.2009
- Peyrache, A., Lacroix, M. M., Petersen, P. C., and Buzsaki, G. (2015). Internally organized mechanisms of the head direction sense. *Nat. Neurosci.* 18, 569–575. doi: 10.1038/nn.3968
- Pompeiano, O., and Allum, J. H. J. (1988). *Vestibulospinal Control of Posture and Locomotion* Holland: Elsevier.
- Roucoux-Hanus, M., and Boisacq-Schepens, N. (1977). Ascending vestibular projections: further results at cortical and thalamic levels in the cat. *Exp. Brain Res.* 29, 283–292. doi: 10.1007/bf00237048
- Russo, A., Marcelli, V., Esposito, F., Corvino, V., Marcuccio, L., Giannone, A., et al. (2014). Abnormal thalamic function in patients with vestibular migraine. *Neurology* 82, 2120–2126. doi: 10.1212/wnl.0000000000000496
- Sawyer, S. F., Tepper, J. M., and Groves, P. M. (1994a). Cerebellar-responsive neurons in the thalamic ventroanterior-ventrolateral complex of rats: light and electron microscopy. *Neuroscience* 63, 725–745. doi: 10.1016/0306-4522(94)90518-5
- Sawyer, S. F., Young, S. J., Groves, P. M., and Tepper, J. M. (1994b). Cerebellar-responsive neurons in the thalamic ventroanterior-ventrolateral complex of rats: *in vivo* electrophysiology. *Neuroscience* 63, 711–724. doi: 10.1016/0306-4522(94)90517-7
- Sherman, S. M. (2007). The thalamus is more than just a relay. *Curr. Opin. Neurobiol.* 17, 417–422. doi: 10.1016/j.conb.2007.07.003
- Shinder, M. E., and Taube, J. S. (2011). Active and passive movement are encoded equally by head direction cells in the anterodorsal thalamus. *J. Neurophysiol.* 106, 788–800. doi: 10.1152/jn.01098.2010
- Shinder, M. E., and Taube, J. S. (2014). Self-motion improves head direction cell tuning. *J. Neurophysiol.* 111, 2479–2492. doi: 10.1152/jn.00512.2013
- Shiroyama, T., Kayahara, T., Yasui, Y., Nomura, J., and Nakano, K. (1999). Projections of the vestibular nuclei to the thalamus in the rat: a Phaseolus vulgaris leucoagglutinin study. *J. Comp. Neurol.* 407, 318–332. doi: 10.1002/(sici)1096-9861(19990510)407:3318::aid-cne23.3.co;2-8
- Smith, P. F., and Zheng, Y. (2013). From ear to uncertainty: vestibular contributions to cognitive function. *Front. Integr. Neurosci.* 7:84. doi: 10.3389/fnint.2013.00084
- Stiles, L., and Smith, P. F. (2015). The vestibular-basal ganglia connection: balancing motor control. *Brain Res.* 1597, 180–188. doi: 10.1016/j.brainres.2014.11.063
- Stolte, B., Holle, D., Naegel, S., Diener, H. C., and Obermann, M. (2015). Vestibular migraine. *Cephalalgia* 35, 262–270. doi: 10.1177/0333102414535113
- Taube, J. S. (2007). The head direction signal: origins and sensory-motor integration. *Annu. Rev. Neurosci.* 30, 181–207. doi: 10.1146/annurev.neuro.29.051605.112854
- Tsanov, M., Chah, E., Noor, M. S., Egan, C., Reilly, R. B., Aggleton, J. P., et al. (2014). The irregular firing properties of thalamic head direction cells mediate turn-specific modulation of the directional tuning curve. *J. Neurophysiol.* 112, 2316–2331. doi: 10.1152/jn.00583.2013
- Vingerhoets, R. A., de Vrijer, M., Van Gisbergen, J. A., and Medendorp, W. P. (2009). Fusion of visual and vestibular tilt cues in the perception of visual vertical. *J. Neurophysiol.* 101, 1321–1333. doi: 10.1152/jn.90725.2008
- Wiener, S. I., and Taube, J. S. (2005). *Head Direction Cells and the Neural Mechanisms of Spatial Orientation*. Cambridge, MA: MIT Press.

- Wijesinghe, R., Solomon, S. G., and Camp, A. J. (2013). Noise normalizes firing output of mouse lateral geniculate nucleus neurons. *PLoS One*. 8:e57961. doi: 10.1371/journal.pone.0057961
- Winter, S. S., Clark, B. J., and Taube, J. S. (2015). Spatial navigation. Disruption of the head direction cell network impairs the parahippocampal grid cell signal. *Science* 347, 870–874. doi: 10.1126/science.1259591
- Yoder, R. M., and Taube, J. S. (2014). The vestibular contribution to the head direction signal and navigation. *Front. Integr. Neurosci.* 8:32. doi: 10.3389/fnint.2014.00032

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Corticothalamic Synaptic Noise as a Mechanism for Selective Attention in Thalamic Neurons

Sébastien Béhuret^{1*}, Charlotte Deleuze^{1,2} and Thierry Bal^{1*}

¹ Unité de Neurosciences, Information et Complexité, Centre National de la Recherche Scientifique FRE-3693, Gif-sur-Yvette, France, ² Institut National de la Santé et de la Recherche Médicale U 1127, Centre National de la Recherche Scientifique UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle Épinrière, Paris, France

A reason why the thalamus is more than a passive gateway for sensory signals is that two-third of the synapses of thalamocortical neurons are directly or indirectly related to the activity of corticothalamic axons. While the responses of thalamocortical neurons evoked by sensory stimuli are well characterized, with ON- and OFF-center receptive field structures, the prevalence of synaptic noise resulting from neocortical feedback in intracellularly recorded thalamocortical neurons *in vivo* has attracted little attention. However, *in vitro* and modeling experiments point to its critical role for the integration of sensory signals. Here we combine our recent findings in a unified framework suggesting the hypothesis that corticothalamic synaptic activity is adapted to modulate the transfer efficiency of thalamocortical neurons during selective attention at three different levels: First, on ionic channels by interacting with intrinsic membrane properties, second at the neuron level by impacting on the input-output gain, and third even more effectively at the cell assembly level by boosting the information transfer of sensory features encoded in thalamic subnetworks. This top-down population control is achieved by tuning the correlations in subthreshold membrane potential fluctuations and is adapted to modulate the transfer of sensory features encoded by assemblies of thalamocortical relay neurons. We thus propose that cortically-controlled (de-)correlation of subthreshold noise is an efficient and swift dynamic mechanism for selective attention in the thalamus.

Keywords: thalamic gateway, thalamocortical system, sensory transfer, selective attention, corticothalamic feedback, synaptic noise, gain control, activity decorrelation

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*Correspondence:

Sébastien Béhuret
behuret@unic.cnrs-gif.fr;
Thierry Bal
bal@unic.cnrs-gif.fr

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INTRODUCTION

Importance of the Corticothalamic Feedback

Nearly all sensory information transmitted to the neocortex, and thus critical to perception and attention, is relayed by the thalamus. Thalamocortical (TC) neurons in the dorsolateral geniculate nucleus (dLGN) relay visual input from retinal ganglion cells to the cortex, from which they receive massive feedback (Erişir et al., 1997; Van Horn et al., 2000), as well as synaptic and non-synaptic influences from other sources (Casagrande et al., 2005). A third of the synapses received by a thalamocortical cell have a direct cortical origin. Disynaptic inhibitory inputs from GABAergic local interneurons and neurons of the reticular thalamic nucleus (NRT), which both receive monosynaptic cortical inputs, account for another third of synapses onto TC cells (Erişir et al., 1997; Van Horn et al., 2000; Sherman and Guillery, 2002). Thus, two-third of synapses contacting a single

TC neuron are related directly or indirectly to the activity of layer 6 corticothalamic (CT) axons. The CT feedback has long been considered as having a strong influence on the control of sensory information transfer by thalamocortical cells (Sherman and Koch, 1986; Koch, 1987; Ahissar, 1997; Sherman, 2001; Sillito and Jones, 2002) and could be involved in selective attention (O'Connor et al., 2002; Casagrande et al., 2005; Saalmann and Kastner, 2009), with recent evidences pointing out that corticothalamic feedback alters orientation-selectivity in human LGN during attention (Ling et al., 2015) and is involved in goal-directed attention in the mouse, via the disynaptic pathway involving the NRT (Ahrens et al., 2014). However, the mechanisms of cortically-driven attention in the thalamus remain an intriguing open question.

A well-known hypothesis endows the corticothalamic feedback and the NRT with a searchlight function (Crick, 1984) or focal attention (Montero, 1999) by enhancing selectively the receptivity of targeted TC neuron populations to attended sensory features. Others consider the thalamus as an “active blackboard” onto which the cortex could write down the results of its computation (Mumford, 1991). Some of these exciting hypotheses only began to be demonstrated recently (Wimmer et al., 2015) in awake and attentive animals due to technical limitations using conventional *in vivo* approaches.

Studies of the functional impact of the CT feedback *in vivo* are mainly restricted to extracellular recordings. They reveal an increased contrast gain in the anesthetized macaque (Przybylski et al., 2000), a spatial sharpening of thalamic receptive field and its ON-OFF antagonism (Temereanca and Simons, 2004), the facilitation of lateral geniculate nucleus activity in the awake cat (Waleszczyk et al., 2005), or attentive monkey (McAlonan et al., 2006), the synchronizing action on thalamic neurons involved in the detection of co-aligned elements in the visual field (Murphy et al., 1999; Wang et al., 2006) as well as the enhancement of the surround antagonism during motion processing (Sillito et al., 2006).

Synaptic activity originating from projections of cortical layer 6 is not well known, with a substantial proportion of corticothalamic cells that remain weakly active or silent in anesthetized or awake animals. Nevertheless, there are behavioral circumstances in which the corticothalamic feedback could be engaged (see Discussion), thus providing TC neurons with synaptic activity of cortical origin.

The Corticothalamic Feedback Provides Neurons with Synaptic Noise

In vivo, neurons are constantly exposed to background barrages of synaptic inputs, called “synaptic noise,” which likely interact with their membrane properties (Steriade, 2001; Destexhe et al., 2003) and impact on their response to “meaningful” sensory synaptic input (Destexhe and Paré, 1999; Anderson et al., 2000). The sensitivity of individual TC cells to sensory input can be assessed by their input-output transfer function, which evaluates the probability of firing in response to synaptic inputs of given amplitudes. In cortical cells the fluctuation of membrane conductances is able to change the gain, i.e., the slope of the input-output spike transfer function (Hô and Destexhe,

2000; Chance et al., 2002; Shu et al., 2003), and to increase neuronal responsiveness (Hô and Destexhe, 2000). Likewise, thalamocortical cells recorded *in vivo* are also in a high-conductance state, in particular during corticothalamic barrages (Contreras et al., 1996; Steriade, 2001), enabling interactions with the synaptic input during sensory integration.

The increase in detection sensitivity to sensory input by TC neurons likely results from a change in the tuning of cortically-driven synaptic inputs during attention. In the higher level visual cortical area V4, individual neurons respond to attended stimuli that are not salient enough to elicit a response when unattended. The lower threshold of response and increase in sensitivity is reflected in a leftward shift in the contrast-response function without a substantial increase in the firing response to high-contrast stimuli (Reynolds et al., 2000). During a similar attentive task, the firing of thalamic relay neurons increases slightly (Casagrande et al., 2005; McAlonan et al., 2006, 2008). However, individual TC neurons were recorded extracellularly, providing no information on the intracellular mechanisms responsible for the attention-dependent effects.

The precise structure of thalamic activity resulting from cortical modulation and the nature and origin of the CT feedback are still elusive. To date it remains unclear how the cortical input affects the transfer of sensory signals to the neocortex, and the dynamical brain processes that are involved in the control of thalamic activity. A detailed modeling of activity of layer 6 seems presently an unreachable target, since it would require taking into account network interactions with all other cortical layers and other related cortical areas. Instead we model the synaptic noise as a configurable activity pattern transmitted by fluctuating excitatory and inhibitory conductances for which we control the statistical structure. We investigate how the cortical input affect the relay of sensory signals by injecting these patterns into biological TC cells in visual and somatosensory thalamic nuclei.

Synaptic Noise Acts at Three Different Levels within the Thalamocortical Circuit

In a series of studies using dynamic clamp in slices *in vitro*, we show that synaptic noise acting at the ionic channel, neuron and network levels, tunes the relay function of the thalamus, thus promoting the idea that the thalamus is more than a passive gateway for the relay of sensory inputs to the neocortex.

The ionic channel level: In TC cells, when membrane potential is depolarized, the T-type calcium channels were previously thought to be inactivated. We found that the rapid membrane potential fluctuations that result from background synaptic noise influence the dynamics of T-type channels, making a fraction of these channels available for activation, thus boosting the spike responsiveness of individual thalamocortical neurons during wake-like states (Deleuze et al., 2012). We demonstrate that the activation of T-channels during wake-like states is a major determinant for single-spike and burst occurrence during tonic firing, forming a multi-spike code that improves coding capabilities, and provides robustness to the thalamocortical transfer of sensory inputs.

The neuron level: By adjusting synaptic conductance parameters, such as the amplitude of fluctuations or the ratio of

excitation and inhibition, we demonstrate that synaptic noise tunes the gain and sensitivity of individual neuron spiking probability function (Wolfart et al., 2005), which results from the stochastic resonance between sensory synaptic inputs and membrane potential fluctuations. By regulating the intensity of background activity, the cortex could thus exert a fast and efficient control of the thalamic relay through instantaneous adjustment of gain and of bursting probability, which may be related to selective attention mechanisms.

The network level: A novel property, which could participate to the cellular mechanisms of selective attention, emerges at the level of the neuronal population, where recipient cortical cells receive input from a number of thalamocortical cells. The stochastic resonance distributed in the afferent network of thalamic neurons provides an emerging signal filtering property critically controlled by synaptic noise (de-)correlation across the thalamic assembly. We found that synaptic noise, presumably under the fast control of neocortical feedback, facilitates the synchronization of spikes propagated to the neocortex by decorrelating the activity of thalamocortical cells (Béhuiret et al., 2013). The resulting population-based stochastic facilitation can selectively boost the information transfer of sensory signals to neocortex.

We therefore propose that corticothalamic feedback exerts its function not only by exciting or inhibiting thalamocortical cells, but also by using a separate “channel” of modulatory information: The statistical structure of the cortical background synaptic input, including the mean and variance of synaptic conductances, the ratio of excitation and inhibition, and the correlation of synaptic noise across TC cells. From these works emerges the hypothesis that cortically-induced synaptic noise endows TC neurons with a function of selective attention, where rapid modulations of the statistical structure of synaptic noise determine the selection and deselection of sensory signals during attention.

The Hypothesis: Synaptic Noise Provides a Mechanism for Selective Attention in Thalamic Neurons

Attentional modulation originating in higher-level visual areas and focusing its action on low-level visual areas is central to the “Reverse Hierarchy Theory” (Hochstein and Ahissar, 2002). It posits that the “pop-out” phenomenon, which allows one to perceive a visual stimulus without being aware of the smaller details that it is made of, emerges initially from activity within high-level areas using their large receptive fields. Filling-in perceptual details demands focused attention, and it is proposed that later “reentrant” feedback to lower levels progressively adds details available in the small receptive fields found in primary areas. The nature of this feedback is unknown and we speculate that synaptic bombardment directed to neurons encoding specific features in the thalamocortical system could play a role in the selection of relevant sensory signals.

A number of experimental observations indicate that correlations present in thalamic and cortical activities change

during focused attention. On the one hand, some studies report increased correlations in the alpha-beta (8–30 Hz) (Bekisz and Wróbel, 1993, 2003) and gamma (30–80 Hz) (Bouyer et al., 1981; Fries et al., 2001; Fries, 2009) bands. On the other hand, studies report decreased correlations in LFP signals during attentive tasks (Cohen and Maunsell, 2009; Mitchell et al., 2009), including in the alpha-beta bands (Fries et al., 2001). It is possible that changes in activity correlation depend on the attentional goals, as it is suggested by functional magnetic resonance imaging data recorded from human visual cortex (Al-Aidroos et al., 2012).

In this paper we propose a mechanism to reconcile these opposing findings: It was stated in Sillito et al. (1994) that “Selective attention may involve the cortical feedback to focus the appropriate circuitry onto the attended stimulus feature.” We suggest that this focus might be achieved—by decreasing correlations—, improving sensory coding of selected stimulus features, while the surrounding subnetworks coding for other features irrelevant to the task, would relax in a state of correlations associated with lower sensitivity. Therefore, we propose that (de-)correlation of the synaptic bombardment across TC neurons at the network level provides a plausible mechanism for selective attention in the thalamocortical system.

SYNAPTIC BOMBARDMENT TUNES SPIKE TRANSFER TO CORTEX IN INDIVIDUAL THALAMIC NEURONS

Synaptic Noise Controls the Cell's Sensitivity to Synaptic Inputs

In this section we illustrate how background synaptic noise affects individual thalamic neurons. We used the dynamic-clamp technique to reproduce the electrical impacts of the opening of ion channels in the membrane of intracellularly recorded biological TC neurons by injecting artificial conductances at the recording site through the glass pipette (Figure 1A). Dynamic-clamp relies on establishing a real-time loop between the computer-controlled injected current and the constantly updated and recorded membrane potential (Figure 1B, reviewed in Piwkowska et al., 2009). We simulated the noisy synaptic environment of the activated “wake-like” state in LGN and somatosensory thalamic neurons recorded *in vitro*. We injected sequences of sensory-like AMPA conductances (Figure 1C, quiescent) together with thousands of static (Figure 1C, static) or fluctuating (Figure 1C, noise) cortical synaptic inputs, mimicked by excitatory and inhibitory synaptic background conductances. In those protocols the sensory AMPA conductance amplitudes are randomly generated to reproduce a realistic spectrum of input synchrony degree. We describe below that background conductance noise significantly changes the burstiness and the input-output transfer function of thalamic relay neurons (Wolfart et al., 2005).

The probabilistic input-output curve in Figures 1D,E defines the neuronal responsiveness over a range of inputs of different amplitude and are characterized by their slope and position, forming multiplicative and additive gains, respectively (Rothman

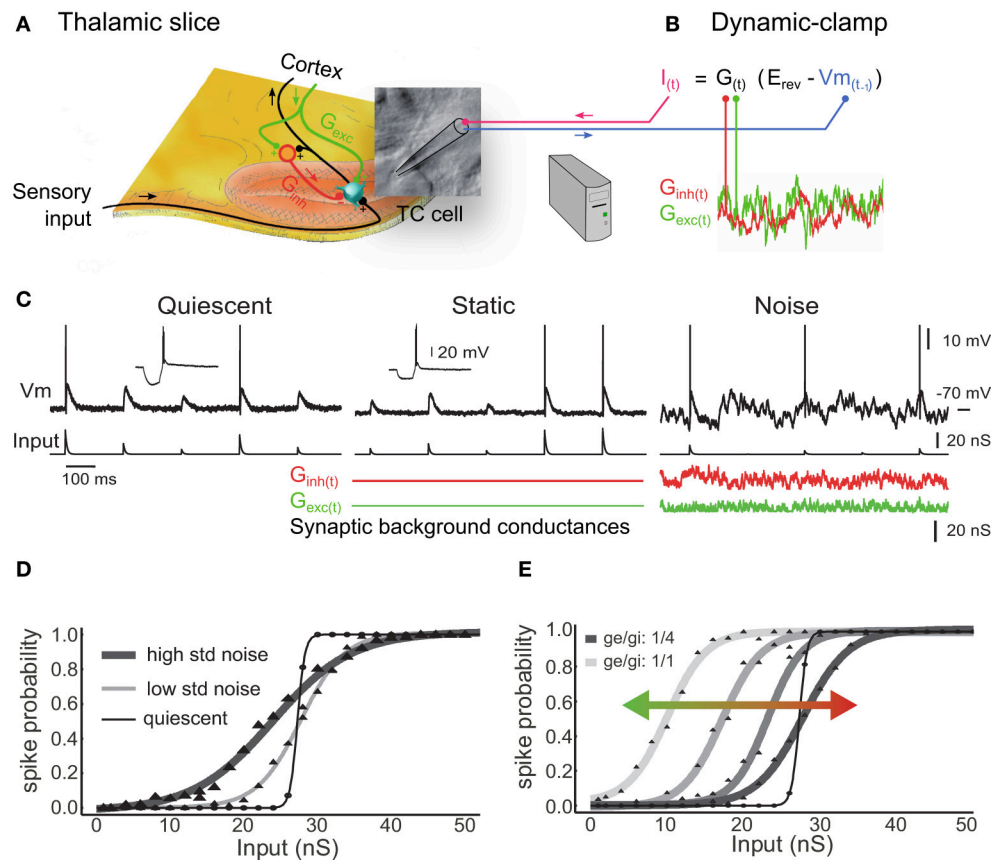


FIGURE 1 | Synaptic noise tunes the transfer function of thalamocortical cells recorded *in vitro*. (A) Illustration of a thalamic slice where a TC cell is recorded using a patch pipette. (B) The dynamic-clamp technique is used to stimulate TC cells with artificial sensory inputs and synaptic conductances, thus mimicking the impact of the corticothalamic feedback during sensory integration. (C) Voltage during injection of discrete retinal-like input conductance (quiescent) and with additional inhibitory plus excitatory background conductance that were either non-fluctuating (static) or stochastically fluctuating (noise). Combined inhibitory and excitatory conductances reduced the input resistance to ~50% (insets in quiescent and static). (D) Probabilities of input conductance strengths to evoke at least one spike within a 20 ms delay, fitted to sigmoid functions. In the noise condition (but not static; not shown, see in Wolfart et al., 2005), a multiplicative gain is induced, corresponding to a slope change of the response curve, and characterized by an increased sensitivity to small inputs and a decreased sensitivity to large inputs. Decreasing the variance of noise conductance values from high voltage variance noise (high std noise; 3.65 mV; $n = 24$) to low noise (low std noise; 2.6 mV; $n = 5$) changes the input-output slope and the sensitivity to small inputs. (E) Changing the ratio of excitatory/inhibitory conductances (1/1, 1/2, 1/3, and 1/4) induces an additive gain that shifts the dynamic input sensitivity range of the transfer function toward smaller inputs (leftward green shift) for higher ratios and toward larger inputs for lower ratios (rightward red shift). Modified from Wolfart et al. (2005).

et al., 2009; Silver, 2010) (this is similar to the psychometric curves of contrast-response functions when probing the correlates of attention in monkey neurons).

We found that a step-like transfer function characterized the response of TC neurons in absence of subthreshold membrane fluctuations (quiescent condition in **Figures 1C,D**), providing a steep probabilistic input-output curve that presents poor encoding capabilities. Conversely, under the influence of noise the response probability was linearized, adopting intermediate values between 0 and 1 over a larger dynamic input range (high and low std noise in **Figure 1D**). This feature provides great flexibility for a possible top-down control of the thalamocortical transfer function through at least two mechanisms detailed in the following.

First, changing the variance of background conductance, which is reflected by a change in amplitude of voltage

fluctuations, tunes the input-output gain of TC cells and their sensitivity to sensory inputs (**Figure 1D**). For instance, an EPSP generated by a 20 nS conductance is not detected in the quiescent state, but becomes progressively detectable when increasing the amplitude of stochastic membrane potential fluctuations. This multiplicative scaling by noise, which corresponds to a change in the slope of the transfer function, can be explained by the fact that the probability for small-amplitude inputs to evoke a spike can only be enhanced by noise (floor effect), whereas for larger-amplitude inputs, the probability can only be reduced by noise (ceiling effect) (Shu et al., 2003). This effect is linked to stochastic resonance, where synaptic noise-induced fluctuations randomly adding up to sensory-evoked EPSPs produce a linearized input-output transfer function. Note that the stochastic resonance effect is also of importance for information transfer at the population level and will be described later.

Second, changing the ratio of conductance excitation to inhibition shifts the response curve along the input axis. **Figure 1E** shows that decreasing the strength of the inhibitory conductance results in a leftward shift of the curve and an increase in the cell's sensitivity to smaller sensory EPSPs (green arrow). Conversely, increasing the strength of the inhibition results in a rightward shift of the curve and an increase in the cell's sensitivity to larger sensory EPSPs (red arrow). We tested several ratios of excitatory to inhibitory conductance, ranging from 1/1 to 1/4, that produced only little changes in membrane potential (**Figure 1E**, V_m mean $\pm$ SD; 1/1 ratio: -64.8 ± 1.0 ; 1/2 ratio: -66.2 ± 1.1 ; 1/3 ratio: -66.9 ± 1.2 ; 1/4 ratio: -66.2 ± 1.3 ; $n = 4$), but were associated with significant shifts of the response curve (1/4 ratio: 53.5 ± 10.8 nS versus 1/1 ratio: 37.6 ± 10.3 nS; $p = 0.034$; one-sided test; $n = 4$).

In conclusion we show here two separate mechanisms by which background synaptic activity is able to control the input-output curve of TC neurons in a flexible manner, by changing either the gain (slope change = multiplicative change) or the sensitivity (position on the x-axis = additive change) of the neuronal transfer function or both. Consistently with the effects of synaptic noise that were observed in cortical neurons (Shu et al., 2003), we propose that the variance of synaptic noise amplitude and the ratio of conductance excitation to inhibition in thalamic neurons is a potent dual mechanism for sensory transfer modulation, which is separate from the classical V_m modulation that occurs through discrete postsynaptic excitation and inhibition. However, it remains unclear whether or not the cortex is capable of adjusting these parameters precisely and rapidly. These mechanisms remain a possibility that should be tested experimentally, for example by recording thalamic neurons intracellularly to assess individual conductance fluctuations and conductance ratios during increasingly-demanding attentive tasks.

Interactions of Subthreshold Synaptic Fluctuations with the T-Type Calcium Current

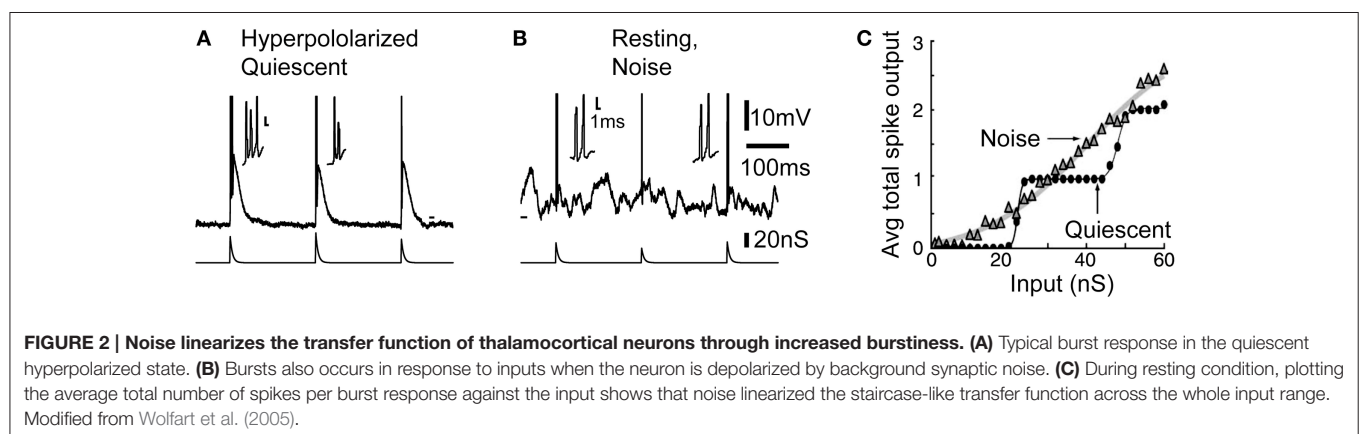
A striking difference with cortical pyramidal neurons is that in quiescent thalamocortical cells, the gain is highly dependent

on membrane potential level and input frequencies. In TC cells low-threshold calcium current boosts the response to synaptic inputs at hyperpolarized levels, for low frequencies limited to approximately <10 Hz (McCormick and Feese, 1990) (**Figure 2A**). This effect is explained by a cumulative inactivation of the T-type channels at higher frequencies. The inability of T-type channels to follow high frequencies provides thalamocortical cells with low-pass filter properties when they are hyperpolarized.

However, in presence of noise, this voltage-dependent response behavior is largely reduced, and the gain of the input-output curve characterizing the response probability remains similar at the potentials and frequencies tested (for details see Figures 3, 4 in Wolfart et al., 2005). Therefore, the presence of subthreshold voltage fluctuations masks the intrinsic, non-linear response behavior of thalamocortical cells, and equips them with a robust, quasi voltage-independent transfer function (Wolfart et al., 2005; Deleuze et al., 2012).

We found that part of this property of linearization results from increased burstiness. In the noise condition, even at resting and depolarized potentials where T-type calcium channels are thought to be fully inactivated, high-frequency multi-spike responses, made of two or three and sometime four spikes, often occurred, therefore mixing single-spikes and short duration bursts in response to inputs (**Figure 2B**). Without synaptic background, the cell behaves as a high-pass filter, detecting only strong inputs with no discrimination of strength above a certain threshold (see the staircase-like response in **Figure 2C**, quiescent). Conversely, in presence of noise the number of spikes grows proportionally to input strength on average, resulting in a linear transfer function that is sensitive to a wide-range of input amplitude (**Figure 2C**, noise).

Thus, in the presence of synaptic background activity, probabilistic “mixing” of single-spike and burst responses forms a multi-spike code, presumably controlled by the cortical input, that provides better encoding capabilities. It could enable TC cells to reliably detect and gradually respond to different degrees of input synchrony.



Single-Spike and Burst Responses at Depolarized Membrane Potential Rely on T-Current

What could be the origin of the burst firing occurring in presence of synaptic bombardment in the depolarized state? Is it solely determined by synaptic inputs, or is the low-threshold T-current underlying the bursting mode also involved? We describe below that the activation of T-channels plays a major role in mixing single-spikes with bursts, and promotes responses with multiple spikes at depolarized membrane potentials where T-current was previously thought to be inactive (Deleuze et al., 2012).

In vivo cortical activity depolarizes TC neurons within a 10 mV range of potential, from approximately -70 mV up to -60 mV (Dossi et al., 1992). T-type calcium channels are classically thought to be fully inactivated in the -60 mV voltage range associated with the wake state (Coulter et al., 1989; Crunelli et al., 1989). Indeed, the role of T-channels has been restricted to rhythmic bursting during sleep or to occasional isolated bursts during sensory processing (Guido and Weyand, 1995; Ramcharan et al., 2000; Fanselow et al., 2001; Swadlow and Gusev, 2001; Martinez-Conde et al., 2002). Bursts were identified by a preceding period of silence, thought to be associated with hyperpolarization that deinactivate T-channels (Llinás and Steriade, 2006; Wang et al., 2007).

However, there is a high density of T-channels in TC cells (Bessaïh et al., 2008; Dreyfus et al., 2010) that far exceeds the number of channels required to generate a typical calcium spike following hyperpolarization. This excess of available channels results in a window T-current (Dreyfus et al., 2010), demonstrating the presence of a number of deinactivated T-channels in the -60 mV voltage range. We characterized the role of T-current in the transfer of sensory inputs using TTA-P2, a highly specific blocker of T-type channels (Dreyfus et al., 2010), and KO mice lacking the T-channel subunit expressed in thalamus. We show that available T-channels are involved in the boost of synaptic inputs and single-spike responses in the presence of background noise and explain the presence of burst responses to synaptic inputs seen in this depolarized state (Figures 2, 3).

We submitted somatosensory TC neurons to dynamic-clamp protocols similar to those described in Figure 1 under normal (control), blocked, and artificial T-current conditions. Neurons were maintained at strictly the same depolarized membrane potential (-58 mV), and dynamic-clamp sequences were replayed identically across all conditions (Figure 3A). In these highly controlled conditions that would be very challenging to obtain *in vivo*, we found that only the largest AMPA conductances are able to evoke firing when T-current is antagonized (Figure 3A, I_T block), as shown by the rightward shift of the corresponding input-output transfer function (Figure 3B, I_T block). This decrease in sensitivity when T-current is blocked is accompanied by a reduced burstiness. Not only the threshold to trigger single-spike responses becomes higher but the probability of generating burst responses drastically decreases (Figure 3C). In summary, this analysis shows that T-current promotes burstiness during wake-like depolarized potentials,

enabling TC cells to integrate a range of sensory amplitudes in an efficient multi-spike code. Note that burst responses still occurs in the absence of T-current, and are very similar to the ones evoked in the presence of the T-current. Therefore, the involvement of T-current during bursts cannot be determined solely on the basis of the inter-spike intervals (see Deleuze et al., 2012 for details).

We demonstrated that the synaptic noisy input, presumably controlled by the cortex during focused attention, can tune the input-output transfer function of individual TC cells while promoting an efficient representation of sensory input amplitudes. In the brain, neurons are all different, and while the CT input must be topographically precise to enable directed activity modulations under attention, it is very unlikely that it is tuned in such a way as to accommodate the membrane properties of each TC cell. Therefore, there must be mechanisms responsible for the normalization of the input-output transfer functions. In the next section, we show that T-current has a determinant role in stabilizing the transfer function of TC cells across a range of membrane potentials, thus helping the cortex to exert its modulating influence across a diversity of neuronal properties.

T-Channel Recruitment During Synaptic Noise Stabilizes the Transfer Function Across Voltage Changes

T-current not only boosts the tonic firing and burst occurrence at depolarized potentials but, due to its graded deinactivation with increasing hyperpolarization, also stabilizes the excitability of the neuronal population across a range of membrane voltages spanned by TC neurons during the waking state. We show here that the interaction of the T-current with background synaptic noise confers robustness to the response of TC neurons.

The magnitude of a single retinogeniculate EPSP may vary little, but the effective retinogeniculate EPSPs depend on variable degrees of temporal summation, such that the effective input has a larger magnitude range (Turner et al., 1994; Usrey et al., 1998). Thus, Poisson-rate stimulation protocol allows varying the effective input EPSP magnitudes in a physiological way, as a result of summation (Turner et al., 1994). In Figure 4, TC neurons are successively maintained at different membrane potentials, ranging from -55 to -72 mV, while being submitted to the same temporal sequence of Poisson-distributed AMPA conductances and synaptic noise, in the presence of T-current or when T-current is blocked using TTA-P2.

When T-current is present, the evoked firing remains quite stable with only few spikes disappearing upon hyperpolarization (Figure 4A). In contrast, when T-current is blocked the firing responses of TC neurons are consistently and strongly decreased upon hyperpolarization (Figure 4B). Quantification of the neuronal firing with respect to two mean membrane potentials, i.e., $-55/-60$ mV and $-67/-72$ mV, shows a consistent decrease upon hyperpolarization when T-current is blocked (Figure 4C, right), whereas in presence of T-current, the neuronal firing increases in five neurons but decreases in the remaining eight neurons (Figure 4C, left). Overall, when considering the population of TC neurons, the mean firing rate remains almost stable throughout the 10 mV hyperpolarization in presence of

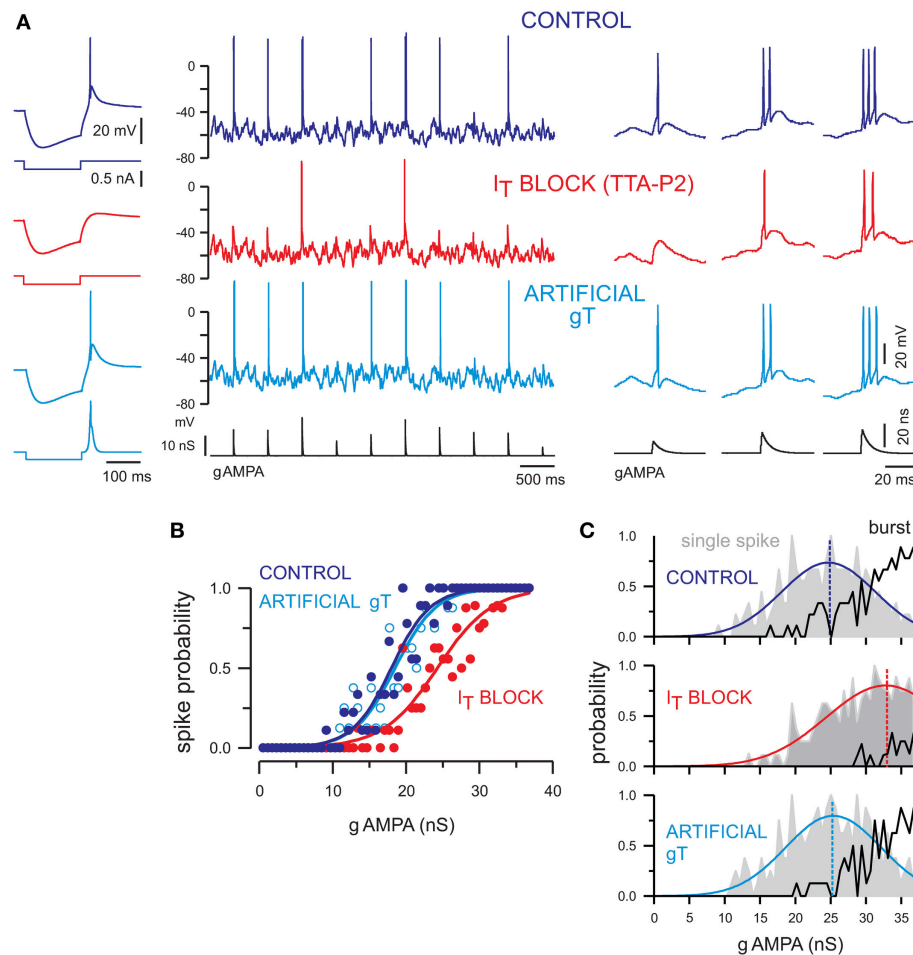


FIGURE 3 | T-current tunes the transfer function of TC neurons and contributes to single-spike and burst firing at depolarized membrane potentials.

(A) Left panel. The rebound low-threshold spike evoked in a TC neuron following a hyperpolarizing current step (control condition) is blocked by TTA-P2 and restored by dynamic clamp injection of gT. Middle panel. Voltage traces of a TC neuron injected with a sequence of AMPA conductances (gAMPA) of different amplitudes in control condition and in the presence of TTA-P2. The neuron received the same fluctuating excitatory and inhibitory conductance noise in each condition and displayed a mean membrane potential of -58 mV. The smallest AMPA conductances failed to evoke a spike when the T-current was blocked (red) and the spike probability was restored upon artificial gT injection (light blue). Right panel. Zoom on the firing activity in response to gAMPA of increasing amplitudes from the recording shown on the middle panel. Both single-spike and burst responses were conditioned by the presence of the T-current. **(B)** Transfer functions of the neuron presented in **(A)** show that the T-current block shifted the input-output curve toward larger AMPA conductances [same color code as in **(A)**]. Recovery was obtained with injection of gT. **(C)** Histograms present the probability of single-spike (gray area) and burst (black curve) generation as a function of the gAMPA amplitude in each condition. In the absence of T-current, the single-spike probability curve was shifted toward larger gAMPA and the burst probability was drastically reduced. The single-spike probability was fitted to a Gaussian function (colored line) to estimate the gAMPA conductance leading to the maximal probability (dashed line). Modified from Deleuze et al. (2012).

T-current (**Figure 4D**; $96 \pm 25\%$ of the firing rate measured at depolarized potential; $n = 13$).

Therefore, our results suggest that T-current enables TC neurons to operate in a dynamic range of membrane potentials optimally. Another way to describe this property is that it maintains the neuronal sensitivity to sensory inputs stable across voltage changes, by stabilizing the transfer function of TC neurons when T-current is present (**Figure 4E**, control). In contrast, the transfer function of TC neurons is shifted toward a sensitivity to larger inputs upon hyperpolarization when T-current is blocked (**Figure 4E**, I_T block) or absent in mice that lack endogenous T-channels (**Figure 4F**).

In conclusion of this first part devoted to the integration properties of individual thalamocortical neurons, we show that synaptic noise tunes the integration of sensory input. Instead of a staircase-like input-output curve with limited coding capabilities for different input amplitudes, a linear response curve across the whole input range is generated, suggesting that, during synaptic noise, sensory signals can be relayed to the cortex with different efficiencies. Furthermore, the combination of synaptic noise with thalamic membrane properties generated by the T-current, gives a global responsiveness that is more stable at all membrane potentials, thus providing robustness to the thalamocortical transfer of sensory inputs. In the following we will consider how

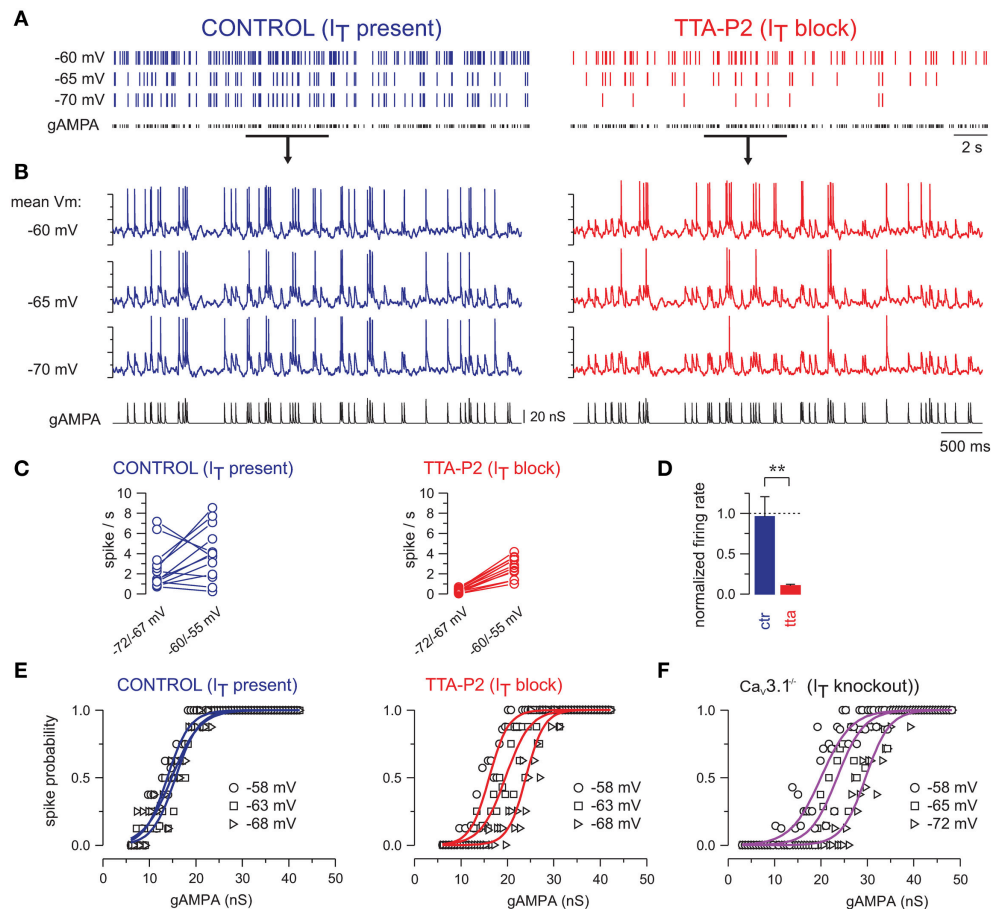


FIGURE 4 | T-current provides robustness to the response of TC neurons across a large range of membrane potentials. (A) Spike raster plots of a TC neuron injected with gAMPA of fixed amplitude following a Poisson-distribution (mean frequency: 10 Hz), while the mean membrane potential was successively maintained at -60 , -65 , and -70 mV. **(B)** Intracellular activities recorded during the time windows indicated by the black line in **(A)**. In control condition the firing of the neuron remained almost invariant across the entire voltage range, but strongly decreased upon hyperpolarization when T-current was blocked. **(C)** Firing frequencies calculated in each neuron successively maintained at a membrane potential between $-60/-55$ mV and between $-72/-67$ mV while being submitted to the same gAMPA/noise sequences. Hyperpolarization induced either a decrease or an increase in firing frequency in control condition (CTR; $n = 13$) but a systematic decrease in the presence of TTA-P2 (TTA; $n = 14$). **(D)** In each neuron, the firing frequency at hyperpolarized potentials was normalized to the one at $-60/-55$ mV. Comparison of the mean values obtained with (CTR) and without T-current (TTA; $**p < 0.01$; independent t -test) suggests that at the level of the TC neuronal population, T-channels rescued the voltage dependent decrease in firing induced by hyperpolarization. **(E)** Transfer functions were quasi-invariant in the presence of the T-current but drastically shifted toward larger gAMPA values upon hyperpolarization when the T-current was blocked. **(F)** Similar voltage dependence of the transfer functions was observed in TC neurons recorded in Cav3.1 $^{-/-}$ knock-out mice devoid of T-current. **(E,F)** are from Deleuze et al. (2012).

cortically-induced synaptic noise can control responsiveness at the higher level of integration of the thalamic cell assembly.

A MECHANISM OF TOP-DOWN CONTROL OF SIGNAL TRANSMISSION EMERGES AT THE THALAMIC POPULATION LEVEL

Convergence of Thalamocortical Neurons Onto Recipient Cortical Cells

Is it realistic to address the question of the efficiency of cortically-induced modulations of the thalamic sensory transfer solely from the interactions observed at the single-cell level, or does it emerge from higher order interactions within the network?

There are important functional distinctions when considering either the isolated cell or the mesoscopic organization of a cell assembly. Our data obtained from experiments in individual cells, show that the background synaptic noise controls the cell responsiveness in a probabilistic manner, and the repetition of trials of similar inputs is necessary to average the response over time and build up the full description of the input-output transfer function. In the whole brain, the need for an immediate response makes trial averaging in individual cells impossible. Therefore, there must be mechanisms responsible for the rapid extraction of the probability function underlying neuronal responsiveness.

A large number of TC neurons, ranging from 15 to 125 in the cat (Alonso et al., 2001), form cell assemblies that converge onto individual recipient cortical neurons in primary visual cortex

(Peters, 2002). We propose that this anatomical convergence of thalamocortical axons toward a recipient layer 4 cortical neuron is a critical circuit feature that allows a higher level of integration, where the targeted cortical neuron can decode the probabilistic signal integration distributed within its afferent thalamic circuit (Béhuret et al., 2013). In addition, the synaptic activity resulting from top-down cortical inputs can modulate this distributed integration process, thus providing a network-level mechanism for selective attention in the thalamus.

We tested this population-level control in networks of biological thalamic neurons forming a population of TC cells presynaptic to a recipient cortical cell (illustrated in **Figure 5**). We quantified the functional impact of the corticothalamic feedback on sensory information transfer in both computational model and iteratively constructed biological networks (Béhuret et al., 2013). In accordance with the dynamic-clamp paradigms used in our previous studies, biological TC cells were stimulated with retinal-like sensory inputs together with background synaptic noise mimicking the cortical input. But here, the method allowed not only to control critical parameters such as the mean and variance of the background conductances, but also the level of independence of the synaptic noise across thalamic cells.

Background Synaptic Noise Tunes the Information Transfer of Sensory Signals

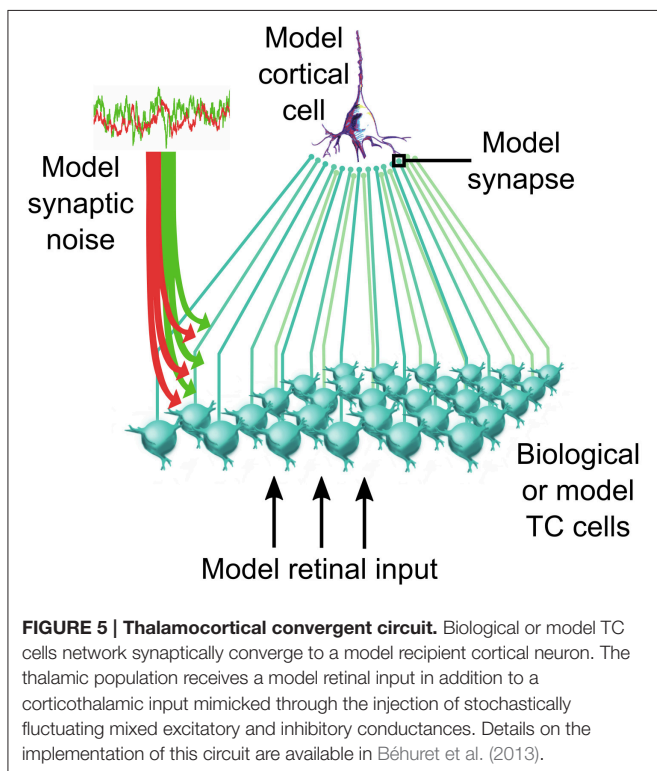
As previously shown in individual TC cells, the input-output transfer function of retinal-like signals is measured as a spiking probability. In our model and biological networks of thalamic

neurons, the transfer of sensory inputs to the receiver cortical cell is best captured with mutual information (Béhuret et al., 2013). We define the “transfer efficiency” as the transmitted information between the artificial retinal input and the cortical output spike-train. Application of the mutual information method to our network of one layer of neurons interposed between the input and the output is straightforward, and reflects faithfully the transfer properties of the circuit, when compared to other classical methods such as spike-transfer probability and spike-train cross-correlation analysis (see **Figure S1** in Béhuret et al., 2013).

We varied the mean and variance of both excitatory and inhibitory components of synaptic noise, and found that the information transfer of sensory inputs is finely tuned by these two parameters at the level of the thalamic population. This effect results from an adjustment of the gain at the cellular level, where the spike response probability of each TC cell is shaped by the characteristics of the noise bombardment (Temereanca and Simons, 2004; Wolfart et al., 2005; Silver, 2010). The cumulation of gain adjustments at the population level further enabled the recipient cortical cell to integrate the converging thalamocortical lines and decode in a single trial the probabilistic function of presynaptic TC cells lumped together.

A systematic exploration of the parametric space allowed us to determine optimal synaptic noise parameters, corresponding to the maximization of the sensory transfer efficiency by means of mutual information. Optimal noises generated by cortical synaptic inputs are revealed by the elongated hot spot of efficient transfers in **Figure 6A**, and are characterized by quasi-balanced levels of excitation and inhibition over a wide range of conductance states. Small conductance fluctuation amplitudes, generating small voltage fluctuations ranging from 1.0 to 1.4 mV in thalamic neurons (standard deviation after removal of spikes), is optimal for high transfer efficiency (**Figure 6B**). Therefore, small noisy fluctuations in membrane potentials observed *in vivo*, rather than being insignificant, could in fact reflect such mechanism of population gain control.

The anatomical convergence of thalamocortical synapses to a receiver cortical cell is adapted to detect thalamic synchrony. This can be seen in **Figure 6C** where typical activity regimes and their corresponding cortical spike-triggered averages (STA) are shown. In the optimal regimes (arrow 1 and 2 in **Figures 6A,B**), the STA show an increase of the thalamic input synchrony a few milliseconds before cortical spikes. Low and high conductance states, which represent different levels of conductance input strength, lead to similar activity regimes and are both as effective for the relay of sensory information (see Discussion). In the silent regime (arrow 3), no spikes are evoked due to the concomitant action of strong inhibition and weak excitation. In the saturated regime (arrow 4), thalamic and cortical neurons are firing in a tonic mode due to a saturating level of excitation, resulting in a non-specific cortical STA. The above results suggest that optimally tuned background synaptic noise, as reflected by the hot spots in **Figures 6A,B**, facilitates the synchronization of sensory-evoked thalamic spikes, and hence their detection by the receiver cortical cell. In other regimes, the synchronization of



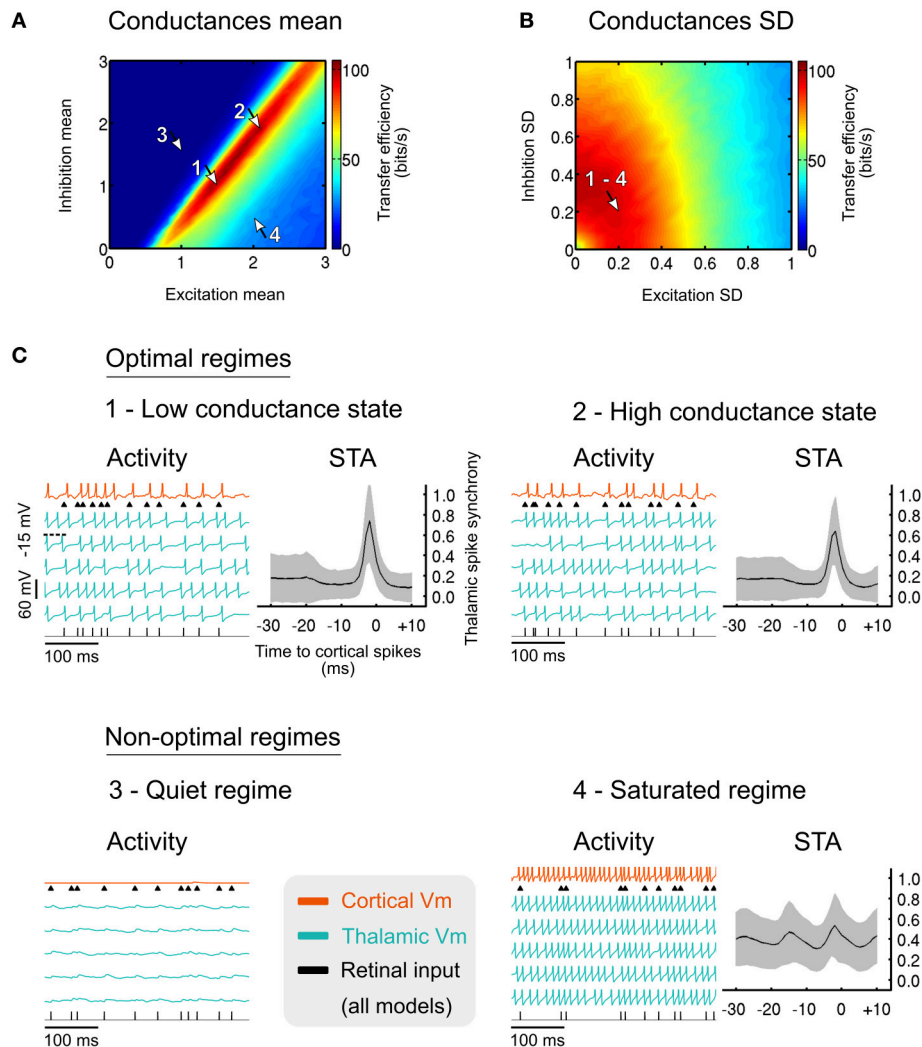


FIGURE 6 | (A) Effect of the cortical excitatory and inhibitory mean input conductances on the sensory transfer efficiency. Mean conductances were normalized relative to the resting conductance of the thalamic cells. Arrows indicate specific operating regimes illustrated in **(C)**. The normalized conductance standard deviations were normalized relative to their respective means, and set to 0.2 for both excitation and inhibition. **(B)** Similar to **(A)** for the standard deviation of the excitatory and inhibitory conductances. The normalized conductance means were set to 1.5 for the excitation and 1.0 for the inhibition. **(C)** Membrane voltage traces for the four specific regimes denoted by the arrows in **(A,B)**. The thalamic spike synchrony was measured with cortical spike-triggered average. The number of thalamic spikes evoked in the corresponding regimes was averaged using a bin size of 1 ms and was then normalized to the total number of TC cells. Grayed areas represent the standard deviation of the counts across all cortical spikes ($n > 10^3$ in every bin). Modified from Béhuret et al. (2013).

thalamic spikes is prevented, which further decouples the cortical spikes from the retinal input.

Given these results, the relay of sensory features encoded by presynaptic thalamic populations could depend on the precise timing of thalamic spikes. During successful transfer of retinal spikes, the synchronization of thalamic spikes falls within a ~ 10 ms time window (**Figure 6C**, optimal regimes STA), which is consistent with the spiking opportunity window for thalamic spikes (Pouille and Scanziani, 2001), the thalamic synchronization tuning resulting from adaptation (Wang et al., 2010), and retinogeniculate paired-spike enhancements (Usrey et al., 1998; Kara and Reid, 2003). This view is also confirmed by a recent study that demonstrates the importance of thalamic

synchrony for cortical feature selectivity, with the most efficient transmission at a level of thalamic synchrony in the range of 10–20 ms (Kelly et al., 2014).

Therefore, our results indicate that the cortical feedback tunes the sensory information transfer by switching thalamocortical activity regimes, which has an impact on the firing rate and synchrony of thalamic spikes. This modulatory effect observed at the population level may account for the modulation of sensory transfer in the thalamus during attention. However, the mechanisms implementing selective attention at the circuit level may not be as straightforward as a firing rate modulation, as it is suggested by studies showing changes in activity correlations during focused attention (see Introduction). In the following

simulations and experiments we investigated the functional impact of synaptic noise (de-)correlation across TC cells, a critical feature in the control of information transfer.

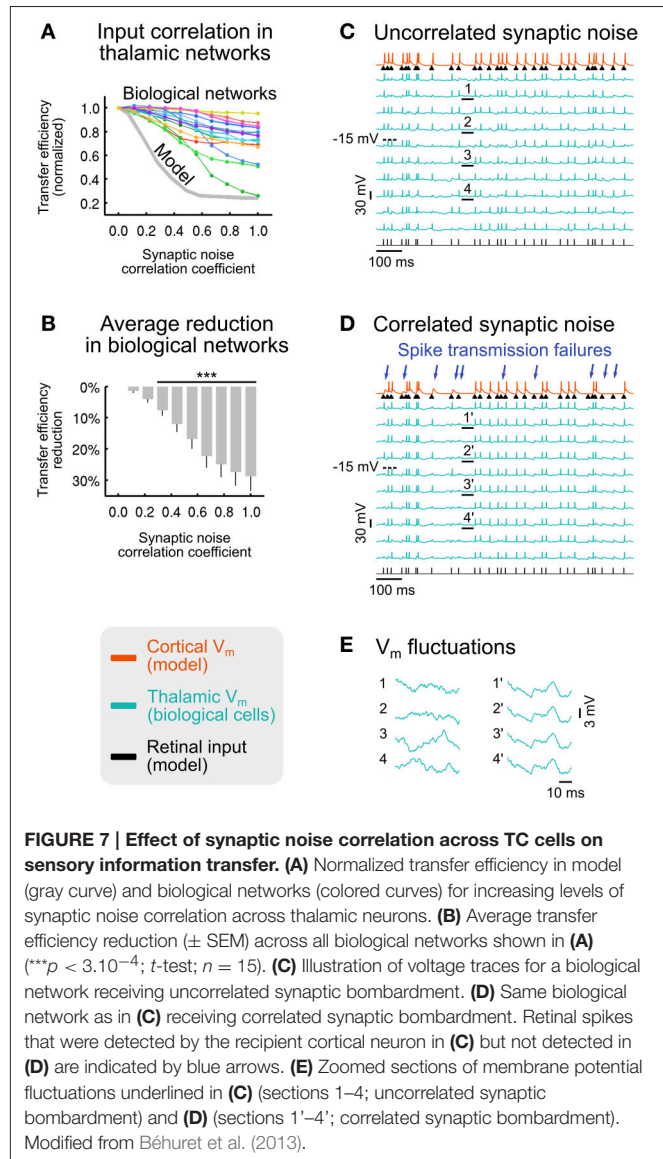
Synaptic Noise Decorrelation Boosts Sensory Information Transfer

To explore the effects of synaptic noise (de-)correlation, we imposed a range of correlation levels in the synaptic bombardment across TC cells, ranging from complete desynchronization, as in the previous parts of this study, to full synchronization, in the model (Figure 7A, gray curve) and in networks of biological thalamic neurons (Figure 7A, colored curves). We found that the sensory transfer efficiency decreases with increasing levels of correlation in the synaptic noise. Desynchronized (uncorrelated) top-down input was thus highly efficient in promoting retinal signals transfer to the recipient cortical neuron, while correlated input has the opposite effect of strongly reducing the relay by up to 76%. As seen throughout our analysis, background noise (de-)correlation is not an all-or-none “permissive” mechanism, suggesting that the brain may be able to gradually adjust the information transfer of selected sensory signals. Furthermore, the retinocortical transfer is not entirely switched off even with a fully correlated corticothalamic synaptic bombardment.

The average transfer efficiency reduction in the tested biological networks becomes highly significant for correlation coefficients larger than 0.33 (Figure 7B), suggesting that low correlation levels have a strong impact during sensory processing. We also explored in model circuits how the correlations imposed in the top-down input affected the correlations between thalamic spikes. In model circuits, a synaptic noise correlation of 0.28 had the minor effect of increasing the pairwise correlations of thalamic spiking activity from ~ 3 to $\sim 11\%$, while imposing a major reduction of the transfer efficiency by more than 50%. This analysis shows that differences in thalamic pairwise spike correlations, so small that they may not be detected using dual recordings *in vivo*, can nonetheless strongly impact thalamocortical processing. Our results are consistent with data showing that neurons with similar orientation tuning in the primary visual cortex of awake macaques virtually share no correlation (Ecker et al., 2010), and a study stressing the high impact of the low correlations in neural populations (Schneidman et al., 2006).

The deleterious effect of synaptic noise correlation can also be seen on activity traces. We found that, when compared to the uncorrelated condition (Figure 7C), $\sim 30\%$ of retinal spikes were not detected by the recipient cortical neuron in the correlated synaptic bombardment condition (Figure 7D), although the thalamic firing rate was nearly identical in both conditions (uncorrelated: 20.3 Hz; correlated: 21.0 Hz). This is reflected by a significant reduction of the retino-cortical spike transfer probability, from 0.97 to 0.75 (average across non-overlapping activity windows; $p < 10^{-7}$; paired-sample *t*-test; $n = 10$).

These analyses in model and biological networks point out the determinant role of synaptic noise decorrelation in the transfer of sensory signals. However, we have seen in the



previous section that optimal background noise facilitates the synchrony of thalamic spikes, but how can this aspect be reconciled with the positive effects of noise decorrelation? When synaptic bombardment is highly correlated across the thalamic population, sensory-evoked thalamic spikes are either amplified or attenuated simultaneously in every TC cell (Figure 7D) as a result of membrane voltage fluctuations being nearly identical on the basis of similar intrinsic membrane properties (Figure 7E, right traces). Although this configuration leads to highly synchronous thalamic spikes, this uniformization of the thalamic population response is detrimental for the transfer of sensory information. We explain this counter-intuitive effect in the following way. Strong depolarizations randomly triggered by the correlated top-down noise sometimes lead to non-sensory spikes in every TC cell simultaneously, and further transmit retina-unrelated spikes to the receiver cortical cell. Similarly, randomly induced top-down hyperpolarizations in register with

retinal spikes sometimes prevent TC cells from responding. Therefore, correlated background inputs, which result in an error-prone spiking behavior across the thalamic population, have deleterious effects on the transfer of sensory signals, and substantially decouple the causal link between the retinal input and the cortical output. Conversely, weakly correlated or uncorrelated inputs promotes a diversity of subthreshold thalamic responses (**Figure 7E**, left traces), with each thalamic spike being independently amplified or attenuated by synaptic noise (**Figure 7C**). This stochastic resonance property of synaptic noise (Rudolph and Destexhe, 2001; McDonnell and Abbott, 2009) allows each presynaptic TC cell to independently detect sensory signals for which the recipient cortical cell is selective, and results collectively in a stochastic facilitation process (McDonnell and Ward, 2011) across the thalamic population.

In summary, the exploration of information transfer properties in model and biological networks reveals that decorrelation of the synaptic bombardment facilitates the transfer of sensory signals to the cortex, an effect which only emerges from the collective action of TC cells. In light of these data, the decorrelation of the CT input appears to be a potent mechanism by which the neocortex could exert its action on sensory inputs. This view is also supported by a recent modeling study suggesting that the corticogeniculate feedback enables efficient representations of input information by decorrelating LGN responses (Zabbah et al., 2014). Combined with a dynamic modulation of the mean and variance of the cortical feedback input, these mechanisms could be used by the brain to actively filter the sensory information that is conveyed by retinal ganglion cells, reflecting both attentional processes and active stimulus filtering under the supervision of cortical areas.

We finally considered an extreme mode of correlation, largely present in the brain in the form of widespread synchronized oscillations of various but specific frequencies, that are known to impair signal transfer during sleep (Le Masson et al., 2002; Dang-Vu et al., 2010) and absence epilepsy (Hughes, 2009), promote loss of consciousness (Ching et al., 2010), and show reduced magnitude during focal attention (Bollimunta et al., 2011). We investigated to which extent such oscillation-induced correlations imposed in the convergent structure of the thalamic network would affect signal transmission.

Synchronized Oscillations in the Thalamus Block Sensory Information

Thalamocortical oscillations are stereotyped in frequency and amplitude, lack the broadband variability of the cortical noise and are widely present in the thalamocortical system during wakefulness and sleep. During relaxed wakefulness, the electroencephalogram (EEG) exhibits robust rhythms in the alpha band (8–13 Hz), which decelerate to theta (2–7 Hz) frequencies during early sleep (Hughes et al., 2004; Hughes and Crunelli, 2007), followed by the 10–14 Hz spindles waves and the slow (<1 Hz) rhythms during non-REM sleep (Steriade et al., 1993; for a review see Crunelli and Hughes, 2010). We proposed earlier that spindles, which are perhaps among the best-understood synchronized oscillations generated endogenously in the thalamocortical system during slow wave sleep (von Krosigk

et al., 1993; for a review see McCormick and Bal, 1997), have the property of imposing a temporal decorrelation of retinal input and thalamic relay output, resulting in the functional disconnection of the cortex from the sensory drive (Le Masson et al., 2002). Conversely, during wakefulness, the waning of synchronized oscillations, and in particular the decrease of power in the alpha (Bollimunta et al., 2011) and mu (Jones et al., 2010) bands seem to be associated with attention. Altogether these data suggest that thalamocortical oscillations are involved in sensory filtering during attention.

We induced oscillations in model thalamic populations by injecting sine-wave currents of varying amplitude and frequency in relay cells, in addition to retinal-like sensory inputs and uncorrelated synaptic bombardment. We found that imposing synchronized oscillations across the thalamic population results in a large decrease of the sensory transfer efficiency (**Figure 8A**). In contrast, desynchronized oscillations, where the phase is homogeneously distributed across the thalamic population, have only a minimal effect on the transfer efficiency (**Figure 8B**).

Small oscillation amplitudes that did not produce any visible rhythmic activity in the membrane potentials of relay cells (**Figure 8C**, top trace for 0.1 nA) largely degraded the transfer efficiency in the synchronized condition (arrow 1 in **Figure 8A**). By contrast, larger oscillation amplitudes that substantially entrained thalamic membrane potentials (**Figure 8C**, bottom trace for 0.4 nA), had only a minor impact on the sensory transfer property in the desynchronized condition (arrow 2 in **Figure 8B**). This comparison between small synchronized and large desynchronized oscillations emphasizes the detrimental effect of modulatory input correlation on sensory transfer, even for oscillation amplitudes so small that they could barely be detected in membrane potential traces.

Our results provide new insight into the role of coherent oscillations in the thalamocortical system. Oscillations may be used by the brain as an effective way to regulate the information transfer of sensory signals. This can be seen with sleep spindles, which are associated to sleep robustness. Spindles are spatially correlated in the thalamocortical system (Contreras et al., 1996), and EEG data show that people having more spindles during sleep are more likely to stay asleep in noisy situations (Dang-Vu et al., 2010), suggesting that spindle oscillations block auditory signals. Signal decoupling by means of synchronized oscillations is most likely to reach its maximal impact in situations of anesthesia or epilepsy. A theoretical study based on human EEG recordings proposed that the thalamocortical coherence induced by Propofol, a short-acting hypnotic agent, is a generative mechanism for the loss of conscious sensory experience (Ching et al., 2010). It is possible that synchronized oscillations in the alpha band are part of an active attentional suppression mechanism aimed at ignoring irrelevant or distracting information (Foxe and Snyder, 2011). Interestingly, it is proposed that this suppression mechanism plays a key role in filtering inputs to primary sensory neocortex, and can be improved in cognitive therapies based on mindfulness meditation (Kerr et al., 2013). Standardized mindfulness alleviates chronic pain and depression relapses,

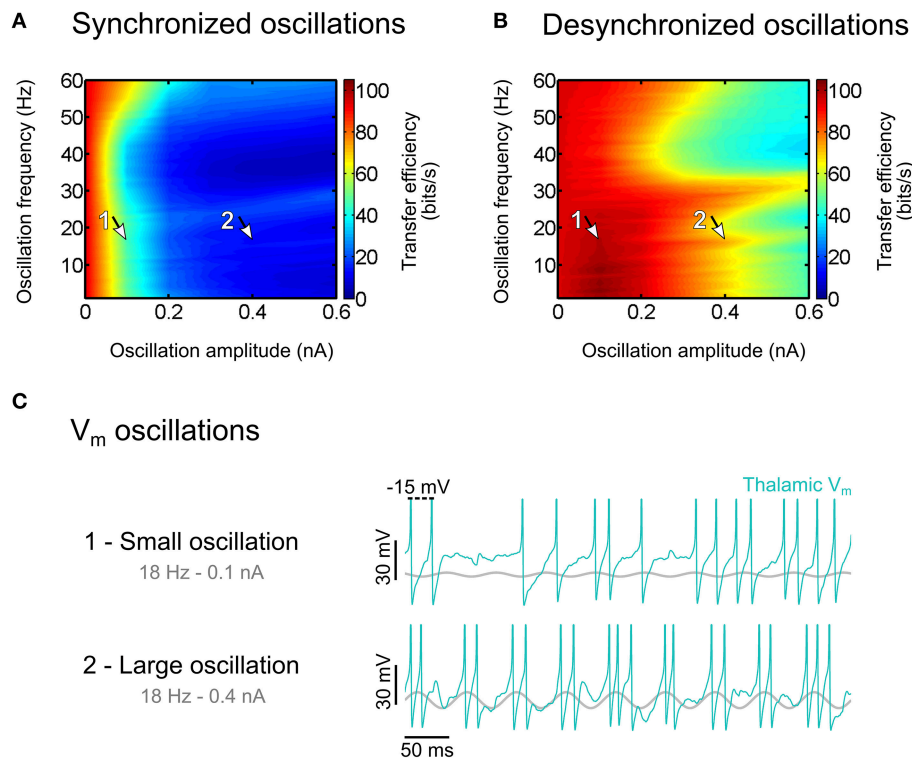


FIGURE 8 | Effect of thalamic oscillations on sensory information transfer. (A) Transfer efficiency for synchronized oscillations. (B) Same as (A) for desynchronized oscillations. (C) Thalamic membrane potential traces for oscillation amplitudes of 0.1 nA [arrow 1 in (A), degraded transfer] and 0.4 nA [arrow 2 in (B), permissive transfer]. Modified from Béhuret et al. (2013).

and this could result from optimized attentional modulation of 7–14 Hz alpha rhythms.

The overall evidence suggests that synchronized oscillations may be used by the brain to set the thalamic gateway into a default, non-permissive oscillatory regime, limiting the transfer of sensory signals during sleep, and perhaps during wakefulness, in thalamic regions not attended by the cortical feedback. In the next section, we propose a phenomenological model of selective attention explaining how the cortical feedback may become decorrelated, modulate the spatial coherence of thalamic oscillations, and boost the signal transfer of attended sensory features.

Selective Attention: A Feedback Decorrelation Model

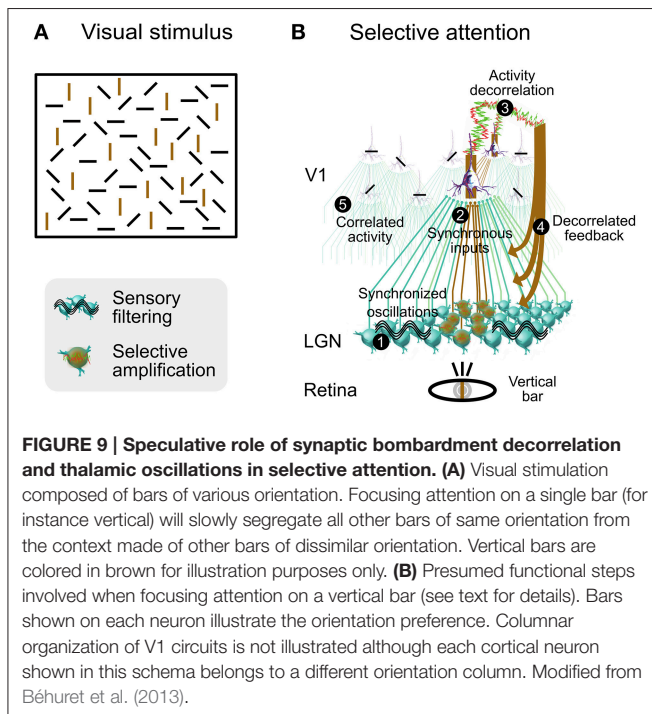
We illustrate how selective attention may involve synaptic noise decorrelation in a functional scenario consisting of a visual stimulus composed of bars of four different orientations (Figure 9A). When our attention is focused on a single bar (for instance vertical), then all other bars of same orientation are segregated from the context made of other bars of dissimilar orientation and become selectively perceived. This effect is rather slow and demands an attentional effort, unlike the classical pop-out that is automatic and fast (Hochstein and Ahissar, 2002). This slower perception process could depend on a feedback cascade from higher-level cortical areas, and on horizontal synaptic

connections in V1. Note that the color of the bar is not an issue here, the effect is the same when bars are all of the same color.

We propose that a decorrelated cortical feedback modulates the spatial coherence of thalamic oscillations in regions that are retinotopically aligned with bars similar to the one being focused, thus boosting their sensory transfer to the cortex. Based upon this decorrelation mechanism, we describe a phenomenological model in several steps (Figure 9B) for this visual effect, produced by contrasting levels of activity correlation between V1 columns selective to the orientation of the attended bar and other columns:

1 - Initial State of the Thalamic Gateway: Oscillations in the thalamus are generated by various known mechanisms such as intrinsic properties, gap junctions and synaptic interactions with NRT (McCormick and Bal, 1997; Hughes et al., 2004; Lorincz et al., 2009). They are synchronized in the alpha-beta range between V1 and dLGN during attention (Bekisz and Wróbel, 2003), an effect which may rely on corticothalamic projections (Contreras et al., 1996). In this state the thalamic gateway is marginally permissive to sensory signals, and highly synchronized sensory input and the activation of T-type channels may be necessary to reliably relay sensory information to the neocortex.

2 - Synchronous Sensory Inputs to Cortical Area V1: The contour of the focused bar is detected by retinal ganglion cells, which further discharge synchronously. In the retinotopic



stream activated by the attended vertical bar, thalamocortical relay cells are activated by the synchronous retinal inputs, and activate cortical cells that are selective to the orientation of the focused bar.

3 - Decorrelation of Activity in Cortical Area V1 in Same-Orientation Columns: Sensory input to V1 area leads to activity decorrelation (Renart et al., 2010; Middleton et al., 2012), switching visual cortex from synchronous to asynchronous states (Tan et al., 2014), and feedback from high-level visual areas may contribute to or reinforce this decorrelation (Hochstein and Ahissar, 2002; Greenberg et al., 2012). We predict that an LFP electrode precisely located within the target region of the focused attention in V1 would reveal decreased levels of correlation in the alpha-beta or gamma frequency ranges or both. Decorrelated activity propagates to other columns via connections between columns of same orientation (Bosking et al., 1997; Angelucci et al., 2002; Lund et al., 2003). At this stage, the emerging activity in V1 is characterized by decorrelated activity in columns that are selective to an orientation matching that of the attended bar, and by correlated activity in columns selective to other orientations. Correlated activity could be further amplified by the lack of thalamocortical input resulting from the underlying filtering of sensory information (this notion of sensory filtering is explained in step 5).

4 - Selective Amplification of Sensory Signals by Foci of Corticothalamic Decorrelation: Regions of decorrelated activity in V1 send a decorrelated corticothalamic synaptic bombardment to their target neurons in the dLGN and in the NRT, disrupting the synchronization of thalamocortical

oscillations, and boosting the sensory transfer at these specific locations. We infer from the retinotopic organization of the top-down projections that the target neurons in the dLGN are specifically tuned to detect features matching bars with an orientation similar to the one being focused.

5 - Filtering of Irrelevant Information by Synchronized Activity: In regions aside from decorrelated corticothalamic foci, the descending cortical input remains correlated. At these locations, thalamic oscillations and correlated feedback impose a reduction in the transfer efficiency of sensory signals that are unrelated to the focused bar and to other bars of similar orientation. High correlations in V1 are essentially sustained by horizontal activity, intracortical recurrent activity, and thalamocortical oscillations. For instance, increased synchronization in the gamma frequency range may stem from elevated oscillatory retinal frequencies (Troy and Robson, 2009), intracortical mechanisms including high-frequency bursting (Gray and McCormick, 1996) and interneurons activity (Traub et al., 1998). Likewise, synchronization in the alpha-beta frequency range may be driven by intrinsic thalamic oscillations described in step 1.

The originality of our proposal is that, for a given attentional task, correlated and decorrelated activities coexist, which is consistent with studies reporting either increased or decreased correlations in the alert animals and specific correlation patterns in humans performing attentive tasks (see Introduction). Thalamocortical regions being the focus of selective attention would present low correlations, while other regions would present higher correlations, possibly sustained by a default, non-permissive oscillatory regime of the thalamic gateway. An experimental consequence would be that detection of increased or decreased correlations in neuronal activity should entirely depend upon the location of an LFP electrode, which picks up signals from restricted cell populations, and primarily reflects synaptic activity local coherence. We therefore predict the existence of dynamic functional maps of correlation and decorrelation in V1 and in the thalamus that reflect the deployment of attention in the early visual system. These maps differ from classical cortical maps, such as those of orientation and ocular dominance in V1, in that they should vary according to the stimuli and the attentional context, and their distribution should change from moment to moment.

In summary, we envision a mechanism of selective sensory attention by which top-down cortical inputs could create an ever and rapidly changing landscape of islands of highly efficient sensory spike transfer in a network otherwise functionally decoupled from its inputs.

DISCUSSION

Synaptic Noise Determines the Transfer of Sensory Signals in the Thalamus

In this paper we summarize our recent findings supporting the hypothesis that corticothalamic synaptic activity is adapted to modulate the sensory transfer of thalamocortical neurons at

different levels. At the ionic channel and neuronal levels we show that the noisy high-conductance state, mixing inhibition and excitation, that neurons experience *in vivo* (Steriade, 2001; Destexhe et al., 2003) interacts with neuronal built-in integrative properties and influences the transfer function of individual TC neurons (Wolfart et al., 2005; Deleuze et al., 2012). At the network level, the axonal projections of many thalamocortical neurons converge onto individual cortical neurons, enabling receiver cortical neurons to collect many thalamocortical inputs, each of them firing one or several spikes in response to sensory input. We show *in vitro* and *in computo* (Béhuret et al., 2013) that this circuit feature endows the receiver cortical cell with the capability to extract the response probability function of the thalamic cell assembly. We found that this integrative property is under the tuning control of synaptic noise. Importantly, at the thalamic population level, the stochastic facilitation of sensory transfer emerges via a cortical mechanism of decorrelation of subthreshold synaptic noise and oscillations.

Activity desynchronization may reflect an active computing principle for the selection of sensory information during attention, which is suggested by studies showing reduced interneuronal correlations in visual area V4 of monkeys performing an attentive task (Reynolds et al., 2000; Cohen and Maunsell, 2009; Mitchell et al., 2009) (for a review, see Reynolds and Chelazzi, 2004). For instance, multiple units recording in this area revealed that fluctuations in firing rate, which are correlated across relatively large populations of neurons, are reduced by spatially selective attention (Cohen and Maunsell, 2009; Mitchell et al., 2009). These attention-dependent reductions in correlated firing could produce a far greater improvement in signal-to-noise ratio than increases in firing rate associated with attention would do (Mitchell et al., 2009). This view is also supported by a number of experimental and theoretical studies pointing to the importance of activity desynchronization for an improved sensory processing (Cohen and Maunsell, 2009; Mitchell et al., 2009; Ching et al., 2010; Dang-Vu et al., 2010; Jones et al., 2010; Bollimunta et al., 2011; Béhuret et al., 2013).

Several non-exclusive mechanisms may also contribute to selective attention via permissive or suppressive action on sensory transfer in the thalamus (Casagrande et al., 2005). The neuromodulation of membrane properties of relay cells is part of them and it has the potential of strongly changing the input resistance of the cells via tonic synaptic activities. The activity of afferents coming from brainstem triggers neurotransmitters release in the thalamus (acetylcholine, noradrenaline, etc.), which contributes to synaptic noise and modulation of membrane properties. These synaptic influences depolarize thalamic relay neurons in a voltage range at which rhythmic oscillations are not prevalent, and promotes a single spike activity as well as enhanced sensory responses (McCormick, 1992). We had previously shown in hybrid circuits that application of noradrenaline increased both efficiency and reliability of retinal spike transfer to cortex (Le Masson et al., 2002). The neuromodulation course of action however does not seem to match the spatial and temporal precision needed for visual attention (Casagrande et al., 2005). In contrast, the cortical control of thalamic signal transfer by a tunable mixed excitatory

and inhibitory synaptic background activity, as proposed in this paper, presents several advantages over the modulation by neuromodulators: It is dynamic, fast, and topographically precise.

Impact of Synaptic Noise on Membrane Properties

By injecting stochastic conductances into thalamocortical neurons, we show that the transfer function of TC neurons is strongly influenced by conductance noise. We propose that the duality of spiking modes in thalamic relay neurons, with bursting during sleep and tonic single-spikes firing during wake, no longer holds if synaptic background activity is taken into account. During sleep, thalamocortical cell bursting is part of a large-scale synchronized activity, and this “proper” burst mode transmits state-dependent information to the cortex, different from the information transferred by single spikes (McCormick and Bal, 1997; Sherman, 2001; Steriade, 2001). However, during activated states when TC neurons receive background synaptic inputs, single-spike and burst responses may both contribute to an efficient encoding of visual information (Reinagel et al., 1999). Thus, the co-occurrence of single-spikes and bursts in response to excitatory stimuli, as well as the more graded aspect of bursts in a multi-spike code, suggest that with background synaptic activity, there is indeed no clear distinction between single spikes and bursts.

The combined effect at the network level of synaptic noise acting on individual TC neurons promotes an efficient information transfer of sensory inputs to the cortex. Peak transfer efficiencies are obtained for nearly balanced regimes of excitation and inhibition, with a total synaptic noise conductance ranging from a low to a high conductance state. The conductance state commonly refers to the strength of the synaptic input. Although the functional distinction between the low and high conductance states is not apparent from the presented results, it is indeed an important one. In high conductance states, synaptic noise is characterized by larger conductances and has a stronger modulating influence on voltage fluctuations. In our protocols there was no extra-cortical competitive input to the thalamic layer, therefore both low and high conductances states led to similar voltage fluctuations in TC neurons. However, in presence of competitive inputs, synaptic noise in a high conductance state would drive the voltage dynamics of TC cells more effectively. Thus, in the intact brain where TC neurons receive additional inputs from regions of the brainstem and basal forebrain (Sherman and Guillery, 1996), it is possible that the conductance state due to synaptic noise forms yet another channel by which the cortex exerts its influence on sensory transfer, with a maximal modulating strength reached during the high conductance state.

We show that the T-current strengthens the input-output relationship of sensory-like input of every TC neuron studied, in full agreement with a recent study showing that T-current underlies the homeostasis of the input-output relation (Hong et al., 2014). Furthermore, we found a cell-to-cell variability in the stability of the neuronal response over membrane voltages. This heterogeneity may originate from the different

T-current densities that are expressed in each neuron; this possibility remains to be tested experimentally. Large differences in neuronal properties can interfere with information processing in thalamic networks, yet some degree of variability is beneficial for sensory signal transmission to the cortex, an effect which relies on the decorrelation of sensory responses across neurons receiving shared inputs (Béhuret et al., 2013). This view is also supported by a study in the olfactory bulb glomerulus showing that variability in intrinsic properties of mitral cells increases the information content of the cell population (Padmanabhan and Urban, 2010; Wilson, 2010).

Thus, cell-to-cell differences in T-current density among the thalamocortical population appear to be valuable to information processing at a population level, and may contribute to the optimization of sensory transfer to the cortex. However, alteration of cellular biophysical properties is a slow adaptive process (Nelson and Turrigiano, 2008) which is presumably not suited to achieve fast and reversible dynamical regulation of sensory transfer. In contrast, input synaptic variability is a dynamical process governed by the presynaptic activity of thousands of neurons and is capable of adapting rapidly, perhaps instantaneously, to the needs of sensory transfer. Because in thalamic neurons, background synaptic input originates mainly from the cortex, these results support a determinant role of cortical layer 6 for the control of sensory information transfer across the thalamic gateway.

The Elusive Activity of Layer 6 Corticothalamic Neurons

In regard to the high degree of receptive field specificity of layer 6 neurons (Swadlow and Weyand, 1987) and of their massive projections to the thalamus (and to other cortical layers), it has been a long-standing enigma that these neurons were often found largely unresponsive or firing at low rate in the anesthetized (Kelly et al., 2001) and especially in the awake animal (Swadlow and Weyand, 1987; Swadlow, 1989; O'Connor et al., 2010).

A few studies suggest that the corticothalamic feedback is engaged in the behaving animals. Inputs to the primary sensory cortex from another functionally related cortical area are critical to alter the excitability of corticothalamic neurons in lightly sedated rats (Lee et al., 2008), where the enhancement of firing in deep layers of motor cortex facilitates whisker-evoked responses in both topographically aligned S1 corticothalamic neurons and thalamic VPM neurons. In mouse V1, layer 6 neurons projecting to thalamus are spontaneously active and their activity increases during unspecific full-field visual stimulation (Figure 1D in Olsen et al., 2012). Surprisingly, when layer 6 activity is artificially increased in a highly correlated manner by broad optogenetic photostimulations or by full-field visual stimulation, it has a suppressive effect on the dLGN and on other cortical layers (Olsen et al., 2012). By contrast, silencing layer 6 via photostimulation of V1 inhibitory neurons strongly facilitates dLGN activity (Olsen et al., 2012). However, similar experiments in A1 and V1 had no visible effects on the average firing of auditory thalamic neurons (Li et al., 2013a), and of dLGN neurons (Li et al., 2013b).

Broad photostimulation and full-field stimulation may lack the specificity of physiological activity during natural perception and attention. In the optogenetic silencing experiments when the light is shone through the cortex, the spatially uniform activation and the temporal patterns generated in layer 6 GABAergic neurons and in inhibitory neurons in other layers may differ from that evoked by visual and auditory stimuli. The fact that the firing rate of thalamic neurons is often unchanged after silencing the cortex is likely a result of a concurrent decrease of excitatory drive from layer 6 and inhibitory drive from the TRN which also receives direct excitation from layer 6 of the cortex (Li et al., 2013b). When optogenetic photostimulation was more specific, targeting a layer 6 neuron sub-population, it was capable of driving both increases and decreases in visually evoked spike count, even in simultaneously recorded cells, without affecting burst frequency (Denman and Contreras, 2015). The observed effects result from a balance of monosynaptic excitation and disinaptic inhibition that can be tipped toward either inhibition or excitation.

These results point to the great complexity of the layer 6 circuits and are not contradictory with the proposed selective attention hypothesis. We show in particular that the decorrelation of top-down synaptic bombardment do not act on the average activity of individual thalamic relay cells. Therefore, it could be difficult to capture this effect with random multi-unit recording in the thalamus.

Corticothalamic Feedback and Selective Attention

The convergent synaptic organization of the thalamocortical circuit forms the structural kernel upon which selective attention may act. This topology of relay neurons in the visual system is essential because it provides a locus of control over the transfer of sensory information. First, it allows recipient cortical cells to average in a single trial the spiking probability function of each afferent relay cell, which is presumably controlled by the cortical input. This averaging distributed over the presynaptic thalamic population enables a reliable and rapid decoding of feedforward sensory signals. Second, thalamic output spike synchrony provides a means for feature selectivity in cortical area V1, turning cortical cells into synchrony detectors, and enabling the cortex to extract sensory features encoded by the coactivation of afferent relay cells. Modulations of the mean and variance of background synaptic noise may tune the transfer of sensory information by adjusting thalamic synchrony. Third, the concomitant synaptic bombardment exerted by the descending corticothalamic feedback results in a tunable stochastic facilitation of the feedforward inputs. This effect—controlled by synaptic noise decorrelation at the subthreshold level—and possibly supervised by cortical areas, enables fine adjustments in transmitted sensory information, and therefore appears to be a potent mechanism by which the cortex may regulate the transfer of selected sensory signals during attention.

We suggested that oscillations, which are the hallmark of sleep and absence epilepsy, and are associated with a drop of sensory perception, could be another possible mechanism contributing to thalamic selective attention. The filtering of unattended sensory

signals is possibly accounted by a default thalamic regime, characterized by weak thalamocortical ensemble oscillations, that leads to the synchronous entrainment of TC cells and results in a decoupling of sensory inputs from the cortex. By contrast, dynamic modulations exerted by the cortical feedback could provide a switch mechanism to augment the sensory throughput of the thalamus (Crandall et al., 2015), thus enabling a controlled transfer of sensory information to the cortex. We therefore propose that one important role of the cortical feedback is to decorrelate subthreshold synaptic noise and oscillations in order to adjust the transfer efficiency of selected sensory signals, informed by context and prior knowledge.

Our prediction is that background synaptic activity in thalamocortical regions focused by attention presents low correlations, while activity in neglected regions outside of the focus presents high correlations. Therefore, in agreement with the view of Foxe and Snyder (2011), we think that alpha/mu oscillations are not so much carrier waves for information propagation but rather the signature of information transfer suppression by providing uniformity to cellular responses. A prediction that remains to be tested is that activity correlations should be lower in thalamic and V1 regions that are selective to attended features. Experimental validation of this prediction remains a major issue because of the yet unsolved technical challenge of identifying and recording simultaneously all neurons belonging to a thalamic network that converges to the same recipient cortical cell. A challenging alternative would be to record simultaneously many TC cells intracellularly in order to unravel the correlation level of the synaptic bombardment during attentive tasks.

METHODS SUMMARY

Ethics Statement

All *in vitro* research procedures concerning the experimental animals and their care were performed in accordance with the local animal welfare committees and adhered to the American Physiological Society's Guiding Principles in the Care and Use of Animals, to Center for Interdisciplinary Research in Biology and European Guidelines Directive 2010/63/EU, to European Council Directive 86/609/EEC, to European Treaties Series 123, and were also approved by the regional ethics committee "Ile-de-France Sud" (Certificate 05-003). *In vitro* slice experiments were conducted in rodents bred in the Central CNRS Animal Care at Gif-sur-Yvette (French Agriculture Ministry Authorization: B91-272-105) under the required veterinary and National Ethical Committee supervision. Every precaution was taken to minimize stress and the number of animals used in each series of experiments. Animals were housed in standard 12 h light/dark cycles and food and water were available *ad libitum*.

Slice Preparation

In vitro experiments were performed on 300–350 μm -thick slices from the dLGN and VPM of Wistar rats 4–6 weeks old for sharp recordings, 14–25 days old for patch recordings, C57BL/6J wild-type or Cav3.1^{-/-} (Kim et al., 2001) mice 14–23 days old, or adult guinea pigs. Animals were anesthetized with

sodium pentobarbital (30 mg/kg) or inhaled isoflurane before decapitation, craniectomy and brain removal. The brain was rapidly removed and immersed in a cold "cutting" solution, and slices were prepared with a vibratome in which the NaCl was replaced with sucrose while maintaining an equivalent osmotic pressure. Slices were then incubated in a "recovery" solution for 30 min to 2 h before recording, in either interface style or submerged recording chambers.

Electrophysiology

During recording, the slices were incubated in an oxygenated artificial aCSF. Whole-cell patch-clamp recordings and intracellular sharp recordings of TC neurons were performed using a Multiclamp 700B (Molecular Devices) or an AxoClamp 2B (Axon Instruments) amplifier. Dynamic-clamp was used to insert sensory-like inputs and cortically-induced synaptic noise in TC neurons.

Dynamic-Clamp

The dynamic-clamp technique (Robinson and Kawai, 1993; Sharp et al., 1993; Piwkowska et al., 2009) was used to inject computer-generated conductances in real neurons. When using sharp electrodes, dynamic-clamp was coupled with an Active Electrode Compensation (Brette, 2009) that allows the removal of electrode noise from intracellular voltage recordings in real time. The dynamic-clamp software is based on a custom ADC/DAC program used for data acquisition and analysis (Elphy2, developed at UNIC by Gérard Sadoc) and is interfaced with a real-time NEURON environment (Sadoc et al., 2009), in which the NEURON simulator v6.0 (Hines and Carnevale, 1997) was modified and recompiled to run under the INtime stack (TenAsys), a kernel driver enabling real-time operation under Microsoft Windows OS. Stimulation protocols were run in real time with the acquisition card at 10–20 kHz.

Sensory Input

Sensory glutamatergic AMPA inputs were mediated by conductance-based synaptic currents described by:

$$I_{\text{AMPA}} = g_{\text{AMPA}} (E_{\text{AMPA}} - V_m)$$

where g_{AMPA} is the evoked conductance in the post-synaptic compartment and $E_{\text{AMPA}} = 0 \text{ mV}$ the reversal potential.

Synaptic Noise

Background synaptic noise was simulated by fluctuating conductances generated as independent stochastic processes and mimicking the effect of thousands of stochastically glutamate- and GABA-releasing synapses (Destexhe et al., 2001). The total injected synaptic current I_{noise} was composed of two conductances, excitatory G_{exc} and inhibitory G_{inh} :

$$I_{\text{noise}} = G_{\text{exc}} (E_{\text{exc}} - V_m) + G_{\text{inh}} (E_{\text{inh}} - V_m)$$

where $E_{\text{exc}} = 0 \text{ mV}$ and $E_{\text{inh}} = -75 \text{ mV}$ are the reversal potentials for the excitatory and inhibitory conductances, respectively.

Data Analysis

A spike was considered as a response to the input when it occurred within 20 ms after the stimulus onset. Responses were considered as multi-spike if the interspike interval was <10 ms. The transfer efficiency of the retinocortical sensory signal transfer was calculated by means of mutual information theoretical analysis:

$$MI(S; R) = \sum_s P(s) \sum_r P(r/s) \log_2 \frac{P(r/s)}{P(r)}$$

where S denotes the stimulation, R the response, $P(s)$ the probability of presentation of the stimulus pattern s , $P(r)$ the probability of presentation of the response pattern r and $P(r/s)$ the probability to obtain the response pattern r in response to the stimulus pattern s .

Modeling and Simulations

Model neurons were described with the equation:

$$C_m \frac{dV_m}{dt} = I_{leak} + I_{Na} + I_h + I_K + I_T + I_{noise}$$

where V_m is membrane potential, C_m is the capacitance of the cell, I_{leak} is the leak current, I_{Na} is the voltage-dependent Na^+ current, I_h is the hyperpolarization-activated non-specific cationic current, I_K is the delayed rectifier K^+ current, I_T is the T-type Ca^{2+} current, and I_{noise} is the synaptic noise.

The T-current was simulated by a Hodgkin–Huxley-like model:

$$I_T = \bar{g}_{Ca} m^2 h (E_{Ca} - V_m)$$

where $\bar{g}_{Ca}$ is the maximal conductance, and m and h the activation and inactivation variables, respectively.

Activation and inactivation gates follow the simple two-state kinetic scheme introduced by Hodgkin and Huxley (1952).

Iteratively constructed biological networks were built using a two-step procedure. First, biological TC neurons were sequentially recorded under various stimulus conditions. Second, recorded membrane potentials were replayed off-line in a model circuit to simulate the synaptic convergence of the thalamic layer onto a model cortical cell.

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SUPPLEMENTARY INFORMATION

All recordings and analyses performed in this study are described elsewhere. For the ionic channel level, knock-out mice, TTA, and T-current protocols are described in Deleuze et al. (2012). For the neuron level, input-output transfer functions are described in Wolfart et al. (2005) and Deleuze et al. (2012). For the population level, biologically iteratively constructed networks, model circuits and mutual information transfer analysis are described in Béhuret et al. (2013).

REFERENCES

- Ahissar, E. (1997). Decoding temporally encoded sensory input by cortical oscillations and thalamic phase comparators. *Proc. Natl. Acad. Sci. U.S.A.* 94, 11633–11638. doi: 10.1073/pnas.94.21.11633
- Ahrens, S., Jaramillo, S., Yu, K., Ghosh, S., Hwang, G.-R., Paik, R., et al. (2014). ErbB4 regulation of a thalamic reticular nucleus circuit for sensory selection. *Nat. Neurosci.* 18, 104–111. doi: 10.1038/nn.3897
- Al-Aidroos, N., Said, C. P., and Turk-Browne, N. B. (2012). Top-down attention switches coupling between low-level and high-level areas of human visual cortex. *Proc. Natl. Acad. Sci. U.S.A.* 109, 14675–14680. doi: 10.1073/pnas.1202095109
- Alonso, J. M., Usrey, W. M., and Reid, R. C. (2001). Rules of connectivity between geniculate cells and simple cells in cat primary visual cortex. *J. Neurosci.* 21, 4002–4015.
- Anderson, J. S., Lampl, I., Gillespie, D. C., and Ferster, D. (2000). The contribution of noise to contrast invariance of orientation tuning in cat visual cortex. *Science* 290, 1968–1972. doi: 10.1126/science.290.5498.1968
- Angelucci, A., Levitt, J. B., Walton, E. J. S., Hupe, J.-M., Bullier, J., and Lund, J. S. (2002). Circuits for local and global signal integration in primary visual cortex. *J. Neurosci.* 22, 8633–8646.
- Béhuret, S., Deleuze, C., Gomez, L., Frégnac, Y., and Bal, T. (2013). Cortically-controlled population stochastic facilitation as a plausible substrate for guiding sensory transfer across the thalamic gateway. *PLoS Comput. Biol.* 9:e1003401. doi: 10.1371/journal.pcbi.1003401
- Bekisz, M., and Wróbel, A. (1993). 20 Hz rhythm of activity in visual system of perceiving cat. *Acta Neurobiol. Exp. (Wars)* 53, 175–182.
- Bekisz, M., and Wróbel, A. (2003). Attention-dependent coupling between beta activities recorded in the cat's thalamic and cortical representations of the central visual field. *Eur. J. Neurosci.* 17, 421–426. doi: 10.1046/j.1460-9568.2003.02454.x
- Bessaïh, T., Leresche, N., and Lambert, R. C. (2008). T current potentiation increases the occurrence and temporal fidelity of synaptically evoked burst firing in sensory thalamic neurons. *Proc. Natl. Acad. Sci. U.S.A.* 105, 11376–11381. doi: 10.1073/pnas.0801484105
- Bollimunta, A., Mo, J., Schroeder, C. E., and Ding, M. (2011). Neuronal mechanisms and attentional modulation of corticothalamic alpha oscillations. *J. Neurosci.* 31, 4935–4943. doi: 10.1523/JNEUROSCI.5580-10.2011
- Bosking, W. H., Zhang, Y., Schofield, B., and Fitzpatrick, D. (1997). Orientation selectivity and the arrangement of horizontal connections in tree shrew striate cortex. *J. Neurosci.* 17, 2112–2127.
- Bouyer, J. J., Montaron, M. F., and Rougeul, A. (1981). Fast fronto-parietal rhythms during combined focused attentive behaviour and immobility in cat: cortical and thalamic localizations. *Electroencephalogr. Clin. Neurophysiol.* 51, 244–252. doi: 10.1016/0013-4694(81)90138-3

- Brette, R. (2009). Generation of correlated spike trains. *Neural Comput.* 21, 188–215. doi: 10.1162/neco.2009.12.07-657
- Casagrande, V. A., Sáry, G., Royal, D., and Ruiz, O. (2005). On the impact of attention and motor planning on the lateral geniculate nucleus. *Prog. Brain Res.* 149, 11–29. doi: 10.1016/S0079-6123(05)49002-0
- Chance, F. S., Abbott, L. F., and Reyes, A. D. (2002). Gain modulation from background synaptic input. *Neuron* 35, 773–782. doi: 10.1016/S0896-6273(02)00820-6
- Ching, S., Cimenser, A., Purdon, P. L., Brown, E. N., and Kopell, N. J. (2010). Thalamocortical model for a propofol-induced alpha-rhythm associated with loss of consciousness. *Proc. Natl. Acad. Sci. U.S.A.* 107, 22665–22670. doi: 10.1073/pnas.1017069108
- Cohen, M. R., and Maunsell, J. H. R. (2009). Attention improves performance primarily by reducing interneuronal correlations. *Nat. Neurosci.* 12, 1594–1600. doi: 10.1038/nn.2439
- Contreras, D., Destexhe, A., Sejnowski, T. J., and Steriade, M. (1996). Control of spatiotemporal coherence of a thalamic oscillation by corticothalamic feedback. *Science* 274, 771–774. doi: 10.1126/science.274.5288.771
- Coulter, D. A., Huguenard, J. R., and Prince, D. A. (1989). Calcium currents in rat thalamocortical relay neurones: kinetic properties of the transient, low-threshold current. *J. Physiol.* 414, 587–604. doi: 10.1113/jphysiol.1989.sp017705
- Crandall, S. R., Cruikshank, S. J., and Connors, B. W. (2015). A corticothalamic switch: controlling the thalamus with dynamic synapses. *Neuron* 86, 768–782. doi: 10.1016/j.neuron.2015.03.040
- Crick, F. (1984). Function of the thalamic reticular complex: the searchlight hypothesis. *Proc. Natl. Acad. Sci. U.S.A.* 81, 4586–4590. doi: 10.1073/pnas.81.14.4586
- Crunelli, V., and Hughes, S. W. (2010). The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat. Neurosci.* 13, 9–17. doi: 10.1038/nn.2445
- Crunelli, V., Lightowler, S., and Pollard, C. E. (1989). A T-type Ca²⁺ current underlies low-threshold Ca²⁺ potentials in cells of the cat and rat lateral geniculate nucleus. *J. Physiol.* 413, 543. doi: 10.1113/jphysiol.1989.sp017668
- Dang-Vu, T. T., McKinney, S. M., Buxton, O. M., Solet, J. M., and Ellenbogen, J. M. (2010). Spontaneous brain rhythms predict sleep stability in the face of noise. *Curr. Biol.* 20, R626–R627. doi: 10.1016/j.cub.2010.06.032
- Deleuze, C., David, F., Béhuret, S., Sadoc, G., Shin, H.-S., Uebele, V. N., et al. (2012). T-type calcium channels consolidate tonic action potential output of thalamic neurons to neocortex. *J. Neurosci.* 32, 12228–12236. doi: 10.1523/JNEUROSCI.1362-12.2012
- Denman, D. J., and Contreras, D. (2015). Complex effects on *in vivo* visual responses by specific projections from mouse cortical layer 6 to dorsal lateral geniculate nucleus. *J. Neurosci.* 35, 9265–9280. doi: 10.1523/JNEUROSCI.0027-15.2015
- Destexhe, A., and Paré, D. (1999). Impact of network activity on the integrative properties of neocortical pyramidal neurons *in vivo*. *J. Neurophysiol.* 81, 1531–1547.
- Destexhe, A., Rudolph, M., Fellous, J. M., and Sejnowski, T. J. (2001). Fluctuating synaptic conductances recreate *in vivo*-like activity in neocortical neurons. *Neuroscience* 107, 13–24. doi: 10.1016/S0306-4522(01)00344-X
- Destexhe, A., Rudolph, M., and Paré, D. (2003). The high-conductance state of neocortical neurons *in vivo*. *Nat. Rev. Neurosci.* 4, 739–751. doi: 10.1038/nrn1198
- Dossi, R. C., Nuñez, A., and Steriade, M. (1992). Electrophysiology of a slow (0.5–4 Hz) intrinsic oscillation of cat thalamocortical neurones *in vivo*. *J. Physiol.* 447, 215–234.
- Dreyfus, F. M., Tschertner, A., Errington, A. C., Renger, J. J., Shin, H.-S., Uebele, V. N., et al. (2010). Selective T-type calcium channel block in thalamic neurons reveals channel redundancy and physiological impact of IT window. *J. Neurosci.* 30, 99–109. doi: 10.1523/JNEUROSCI.4305-09.2010
- Ecker, A. S., Berens, P., Keliris, G. A., Bethge, M., Logothetis, N. K., and Tolias, A. S. (2010). Decorrelated neuronal firing in cortical microcircuits. *Science* 327, 584–587. doi: 10.1126/science.1179867
- Erişir, A., Van Horn, S. C., and Sherman, S. M. (1997). Relative numbers of cortical and brainstem inputs to the lateral geniculate nucleus. *Proc. Natl. Acad. Sci. U.S.A.* 94, 1517–1520. doi: 10.1073/pnas.94.4.1517
- Fanselow, E. E., Sameshima, K., Baccala, L. A., and Nicolelis, M. A. (2001). Thalamic bursting in rats during different awake behavioral states. *Proc. Natl. Acad. Sci. U.S.A.* 98, 15330–15335. doi: 10.1073/pnas.261273898
- Foxe, J. J., and Snyder, A. C. (2011). The role of alpha-band brain oscillations as a sensory suppression mechanism during selective attention. *Front. Psychol.* 2:154. doi: 10.3389/fpsyg.2011.00154
- Fries, P. (2009). Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annu. Rev. Neurosci.* 32, 209–224. doi: 10.1146/annurev.neuro.051508.135603
- Fries, P., Reynolds, J. H., Rorie, A. E., and Desimone, R. (2001). Modulation of oscillatory neuronal synchronization by selective visual attention. *Science* 291, 1560–1563. doi: 10.1126/science.1055465
- Gray, C. M., and McCormick, D. A. (1996). Chattering cells: superficial pyramidal neurons contributing to the generation of synchronous oscillations in the visual cortex. *Science* 274, 109–113. doi: 10.1126/science.274.5284.109
- Greenberg, A. S., Verstynen, T., Chiu, Y.-C., Yantis, S., Schneider, W., and Behrmann, M. (2012). Visuotopic cortical connectivity underlying attention revealed with white-matter tractography. *J. Neurosci.* 32, 2773–2782. doi: 10.1523/JNEUROSCI.5419-11.2012
- Guido, W., and Weyand, T. (1995). Burst responses in thalamic relay cells of the awake behaving cat. *J. Neurophysiol.* 74, 1782–1786.
- Hines, M. L., and Carnevale, N. T. (1997). The NEURON simulation environment. *Neural Comput.* 9, 1179–209. doi: 10.1162/neco.1997.9.6.1179
- Hochstein, S., and Ahissar, M. (2002). View from the top: hierarchies and reverse hierarchies in the visual system. *Neuron* 36, 791–804. doi: 10.1016/S0896-6273(02)01091-7
- Hodgkin, A. L., and Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117, 500–544. doi: 10.1016/S0092-8240(05)80004-7
- Hô, N., and Destexhe, A. (2000). Synaptic background activity enhances the responsiveness of neocortical pyramidal neurons. *J. Neurophysiol.* 84, 1488–1496.
- Hong, S. Z., Kim, H. R., and Fiorillo, C. D. (2014). T-type calcium channels promote predictive homeostasis of input-output relations in thalamocortical neurons of lateral geniculate nucleus. *Front. Comput. Neurosci.* 8:98. doi: 10.3389/fncom.2014.00098
- Hughes, J. R. (2009). Absence seizures: a review of recent reports with new concepts. *Epilepsy Behav.* 15, 404–412. doi: 10.1016/j.yebeh.2009.06.007
- Hughes, S. W., and Crunelli, V. (2007). Just a phase they're going through: the complex interaction of intrinsic high-threshold bursting and gap junctions in the generation of thalamic alpha and theta rhythms. *Int. J. Psychophysiol.* 64, 3–17. doi: 10.1016/j.ijpsycho.2006.08.004
- Hughes, S. W., Lörincz, M., Cope, D. W., Blethyn, K. L., Kékesi, K. A., Parri, H. R. R., et al. (2004). Synchronized oscillations at alpha and theta frequencies in the lateral geniculate nucleus. *Neuron* 42, 253–268. doi: 10.1016/S0896-6273(04)00191-6
- Jones, S. R., Kerr, C. E., Wan, Q., Pritchett, D. L., Härmäläinen, M., and Moore, C. I. (2010). Cued spatial attention drives functionally relevant modulation of the mu rhythm in primary somatosensory cortex. *J. Neurosci.* 30, 13760–13765. doi: 10.1523/JNEUROSCI.2969-10.2010
- Kara, P., and Reid, R. C. (2003). Efficacy of retinal spikes in driving cortical responses. *J. Neurosci.* 23, 8547–8557.
- Kelly, M. K., Carvell, G. E., Hartings, J. A., and Simons, D. J. (2001). Axonal conduction properties of antidromically identified neurons in rat barrel cortex. *Somatosens. Mot. Res.* 18, 202–210. doi: 10.1080/01421590120072196
- Kelly, S. T., Kremkow, J., Jin, J., Wang, Y., Wang, Q., Alonso, J.-M., et al. (2014). The role of thalamic population synchrony in the emergence of cortical feature selectivity. *PLoS Comput. Biol.* 10:e1003418. doi: 10.1371/journal.pcbi.1003418
- Kerr, C. E., Sacchet, M. D., Lazar, S. W., Moore, C. I., and Jones, S. R. (2013). Mindfulness starts with the body: somatosensory attention and top-down modulation of cortical alpha rhythms in mindfulness meditation. *Front. Hum. Neurosci.* 7:12. doi: 10.3389/fnhum.2013.00012
- Kim, D., Song, I., Keum, S., Lee, T., Jeong, M. J., Kim, S. S., et al. (2001). Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type Ca(2+) channels. *Neuron* 31, 35–45. doi: 10.1016/S0896-6273(01)00343-9
- Koch, C. (1987). The action of the corticofugal pathway on sensory thalamic nuclei: a hypothesis. *Neuroscience* 23, 399–406. doi: 10.1016/0306-4522(87)90064-9
- Lee, S., Carvell, G. E., and Simons, D. J. (2008). Motor modulation of afferent somatosensory circuits. *Nat. Neurosci.* 11, 1430–1438. doi: 10.1038/nn.2227

- Le Masson, G., Renaud-Le Masson, S., Debay, D., and Bal, T. (2002). Feedback inhibition controls spike transfer in hybrid thalamic circuits. *Nature* 417, 854–858. doi: 10.1038/nature00825
- Li, L. Y., Li, Y. T., Zhou, M., Tao, H. W., and Zhang, L. I. (2013a). Intracortical multiplication of thalamocortical signals in mouse auditory cortex. *Nat. Neurosci.* 16, 1179–1181. doi: 10.1038/nn.3493
- Li, Y. T., Ibrahim, L. A., Liu, B. H., Zhang, L. I., and Tao, H. W. (2013b). Linear transformation of thalamocortical input by intracortical excitation. *Nat. Neurosci.* 16, 1324–1330. doi: 10.1038/nn.3494
- Ling, S., Pratte, M. S., and Tong, F. (2015). Attention alters orientation processing in the human lateral geniculate nucleus. *Nat. Neurosci.* 18, 496–498. doi: 10.1038/nn.3967
- Llinás, R. R., and Steriade, M. (2006). Bursting of thalamic neurons and states of vigilance. *J. Neurophysiol.* 95, 3297–3308. doi: 10.1152/jn.00166.2006
- Lorincz, M. L., Kékesi, K. A., Juhász, G., Crunelli, V., and Hughes, S. W. (2009). Temporal framing of thalamic relay-mode firing by phasic inhibition during the alpha rhythm. *Neuron* 63, 683–696. doi: 10.1016/j.neuron.2009.08.012
- Lund, J. S., Angelucci, A., and Bressloff, P. C. (2003). Anatomical substrates for functional columns in macaque monkey primary visual cortex. *Cereb. Cortex* 13, 15–24. doi: 10.1093/cercor/13.1.15
- Martinez-Conde, S., Macknik, S. L., and Hubel, D. H. (2002). The function of bursts of spikes during visual fixation in the awake primate lateral geniculate nucleus and primary visual cortex. *Proc. Natl. Acad. Sci. U.S.A.* 99, 13920–13925. doi: 10.1073/pnas.212500599
- McAlonan, K., Cavanaugh, J., and Wurtz, R. H. (2006). Attentional modulation of thalamic reticular neurons. *J. Neurosci.* 26, 4444–4450. doi: 10.1523/JNEUROSCI.5602-05.2006
- McAlonan, K., Cavanaugh, J., and Wurtz, R. H. (2008). Guarding the gateway to cortex with attention in visual thalamus. *Nature* 456, 391–394. doi: 10.1038/nature07382
- McCormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog. Neurobiol.* 39, 337–388.
- McCormick, D. A., and Bal, T. (1997). Sleep and arousal: thalamocortical mechanisms. *Annu. Rev. Neurosci.* 20, 185–215. doi: 10.1146/annurev.neuro.20.1.185
- McCormick, D. A., and Feese, H. R. (1990). Functional implications of burst firing and single spike activity in lateral geniculate relay neurons. *Neuroscience* 39, 103–113. doi: 10.1016/0306-4522(90)90225-S
- McDonnell, M. D., and Abbott, D. (2009). What is stochastic resonance? Definitions, misconceptions, debates, and its relevance to biology. *PLoS Comput. Biol.* 5:e1000348. doi: 10.1371/journal.pcbi.1000348
- McDonnell, M. D., and Ward, L. M. (2011). The benefits of noise in neural systems: bridging theory and experiment. *Nat. Rev. Neurosci.* 12, 415–426. doi: 10.1038/nrn3061
- Middleton, J. W., Omar, C., Doiron, B., and Simons, D. J. (2012). Neural correlation is stimulus modulated by feedforward inhibitory circuitry. *J. Neurosci.* 32, 506–518. doi: 10.1523/JNEUROSCI.3474-11.2012
- Mitchell, J. F., Sundberg, K. A., and Reynolds, J. H. (2009). Spatial attention decorrelates intrinsic activity fluctuations in macaque area V4. *Neuron* 63, 879–888. doi: 10.1016/j.neuron.2009.09.013
- Montero, V. M. (1999). Amblyopia decreases activation of the corticogeniculate pathway and visual thalamic reticularis in attentive rats: a “focal attention” hypothesis. *Neuroscience* 91, 805–817. doi: 10.1016/S0306-4522(98)00632-0
- Mumford, D. (1991). On the computational architecture of the neocortex. *Biol. Cybern.* 65, 135–145. doi: 10.1007/BF00202389
- Murphy, P. C., Duckett, S. G., and Sillito, A. M. (1999). Feedback connections to the lateral geniculate nucleus and cortical response properties. *Science* 286, 1998–2000. doi: 10.1126/science.286.5444.1552
- Nelson, S. B., and Turrigiano, G. G. (2008). Strength through diversity. *Neuron* 60, 477–482. doi: 10.1016/j.neuron.2008.10.020
- O'Connor, D. H., Fukui, M. M., Pinsk, M. A., and Kastner, S. (2002). Attention modulates responses in the human lateral geniculate nucleus. *Nat. Neurosci.* 5, 1203–1209.
- O'Connor, D. H., Peron, S. P., Huber, D., and Svoboda, K. (2010). Neural activity in barrel cortex underlying vibrissa-based object localization in mice. *Neuron* 67, 1048–1061. doi: 10.1016/j.neuron.2010.08.026
- Olsen, S. R., Bortone, D. S., Adesnik, H., and Scanziani, M. (2012). Gain control by layer six in cortical circuits of vision. *Nature* 483, 47–52. doi: 10.1038/nature10835
- Padmanabhan, K., and Urban, N. N. (2010). Intrinsic biophysical diversity decorrelates neuronal firing while increasing information content. *Nat. Neurosci.* 13, 1276–1282. doi: 10.1038/nn.2630
- Peters, A. (2002). Examining neocortical circuits: some background and facts. *J. Neurocytol.* 31, 183–193. doi: 10.1023/A:1024157522651
- Piwkowska, Z., Destexhe, A., and Bal, T. (2009). “Associating living cells and computational models: an introduction to dynamic clamp principles and its applications,” in *Dynamic-Clamp*, eds T. Bal and A. Destexhe (New York, NY: Springer), 1–30. doi: 10.1007/978-0-387-89279-5_1
- Pouille, F., and Scanziani, M. (2001). Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. *Science* 293, 1159–1163. doi: 10.1126/science.1060342
- Przybylski, A. W., Gaska, J. P., Foote, W., and Pollen, D. A. (2000). Striate cortex increases contrast gain of macaque LGN neurons. *Vis. Neurosci.* 17, 485–494. doi: 10.1017/S0952523800174012
- Ramcharan, E. J., Gnadt, J. W., and Sherman, S. M. (2000). Burst and tonic firing in thalamic cells of unanesthetized, behaving monkeys. *Vis. Neurosci.* 17, 55–62. doi: 10.1017/S0952523800171056
- Reinagel, P., Godwin, D., Sherman, S. M., and Koch, C. (1999). Encoding of visual information by LGN bursts. *J. Neurophysiol.* 81, 2558–2569.
- Renart, A., de la Rocha, J., Bartho, P., Hollender, L., Parga, N., Reyes, A., et al. (2010). The asynchronous state in cortical circuits. *Science* 327, 587–590. doi: 10.1126/science.1179850
- Reynolds, J. H., and Chelazzi, L. (2004). Attentional modulation of visual processing. *Annu. Rev. Neurosci.* 27, 611–647. doi: 10.1146/annurev.neuro.26.041002.131039
- Reynolds, J. H., Pasternak, T., and Desimone, R. (2000). Attention increases sensitivity of V4 neurons. *Neuron* 26, 703–714. doi: 10.1016/S0896-6273(00)81206-4
- Robinson, H. P., and Kawai, N. (1993). Injection of digitally synthesized synaptic conductance transients to measure the integrative properties of neurons. *J. Neurosci. Methods* 49, 157–165. doi: 10.1016/0165-0270(93)90119-C
- Rothman, J. S., Cathala, L., Steuber, V., and Silver, R. A. (2009). Synaptic depression enables neuronal gain control. *Nature* 457, 1015–1018. doi: 10.1038/nature07604
- Rudolph, M., and Destexhe, A. (2001). Do neocortical pyramidal neurons display stochastic resonance? *J. Comput. Neurosci.* 11, 19–42. doi: 10.1023/A:1011200713411
- Saalmann, Y. B., and Kastner, S. (2009). Gain control in the visual thalamus during perception and cognition. *Curr. Opin. Neurobiol.* 19, 408–414. doi: 10.1016/j.conb.2009.05.007
- Sadoc, G., Le Masson, G., Foutry, B., Le Franc, Y., Piwkowska, Z., Destexhe, A., et al. (2009). “Re-creating *in vivo*-like activity and investigating the signal transfer capabilities of neurons: dynamic-clamp applications using real-time neuron,” in *Dynamic-Clamp*, eds T. Bal and A. Destexhe (New York, NY: Springer), 287–320. doi: 10.1007/978-0-387-89279-5_14
- Schneidman, E., Berry, M. J. II, Segev, R., and Bialek, W. (2006). Weak pairwise correlations imply strongly correlated network states in a neural population. *Nature* 440, 1007–1012. doi: 10.1038/nature04701
- Sharp, A. A., O'Neil, M. B., Abbott, L. F., and Marder, E. (1993). Dynamic clamp: computer-generated conductances in real neurons. *J. Neurophysiol.* 69, 992–995.
- Sherman, S. M. (2001). Tonic and burst firing: dual modes of thalamocortical relay. *Trends Neurosci.* 24, 122–126. doi: 10.1016/S0166-2236(00)01714-8
- Sherman, S. M., and Guillery, R. W. (1996). Functional organization of thalamocortical relays. *J. Neurophysiol.* 76, 1367–1395.
- Sherman, S. M., and Guillery, R. W. (2002). The role of the thalamus in the flow of information to the cortex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 357, 1695–1708. doi: 10.1098/rstb.2002.1161
- Sherman, S. M., and Koch, C. (1986). The control of retinogeniculate transmission in the mammalian lateral geniculate nucleus. *Exp. Brain Res.* 63, 1–20. doi: 10.1007/BF00235642
- Shu, Y., Hasenstaub, A., Badoual, M., Bal, T., and McCormick, D. A. (2003). Barrages of synaptic activity control the gain and sensitivity of cortical neurons. *J. Neurosci.* 23, 10388–10401.

- Sillito, A. M., Cudeiro, J., and Jones, H. E. (2006). Always returning: feedback and sensory processing in visual cortex and thalamus. *Trends Neurosci.* 29, 307–316. doi: 10.1016/j.tins.2006.05.001
- Sillito, A. M., and Jones, H. E. (2002). Corticothalamic interactions in the transfer of visual information. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 357, 1739–1752. doi: 10.1098/rstb.2002.1170
- Sillito, A. M., Jones, H. E., Gerstein, G. L., and West, D. C. (1994). Feature-linked synchronization of thalamic relay cell firing induced by feedback from the visual cortex. *Nature* 369, 479–482. doi: 10.1038/369479a0
- Silver, R. A. (2010). Neuronal arithmetic. *Nat. Rev. Neurosci.* 11, 474–489. doi: 10.1038/nrn2864
- Steriade, M. (2001). Impact of network activities on neuronal properties in corticothalamic systems. *J. Neurophysiol.* 86, 1–39.
- Steriade, M., Nuñez, A., and Amzica, F. (1993). A novel slow (< 1 Hz) oscillation of neocortical neurons *in vivo*: depolarizing and hyperpolarizing components. *J. Neurosci.* 13, 3252–3265.
- Swadlow, H. A. (1989). Efferent neurons and suspected interneurons in S-1 vibrissa cortex of the awake rabbit: receptive fields and axonal properties. *J. Neurophysiol.* 62, 288–308.
- Swadlow, H. A., and Gusev, A. G. (2001). The impact of “bursting” thalamic impulses at a neocortical synapse. *Nat. Neurosci.* 4, 402–408. doi: 10.1038/86054
- Swadlow, H. A., and Weyand, T. G. (1987). Corticogeniculate neurons, corticotectal neurons, and suspected interneurons in visual cortex of awake rabbits: receptive-field properties, axonal properties, and effects of EEG arousal. *J. Neurophysiol.* 57, 977–1001.
- Tan, A. Y. Y., Chen, Y., Scholl, B., Seidemann, E., and Priebe, N. J. (2014). Sensory stimulation shifts visual cortex from synchronous to asynchronous states. *Nature* 509, 226–229. doi: 10.1038/nature13159
- Temereanca, S., and Simons, D. J. (2004). Functional topography of corticothalamic feedback enhances thalamic spatial response tuning in the somatosensory Whisker/Barrel system. *Neuron* 41, 639–651. doi: 10.1016/S0896-6273(04)00046-7
- Traub, R. D., Spruston, N., Soltesz, I., Konnerth, A., Whittington, M. A., and Jefferys, G. R. (1998). Gamma-frequency oscillations: a neuronal population phenomenon, regulated by synaptic and intrinsic cellular processes, and inducing synaptic plasticity. *Prog. Neurobiol.* 55, 563–575. doi: 10.1016/S0301-0082(98)00020-3
- Troy, J. B., and Robson, J. G. (2009). Steady discharges of X and Y retinal ganglion cells of cat under photopic illuminance. *Vis. Neurosci.* 9, 535. doi: 10.1017/S0952523800001784
- Turner, J. P., Leresche, N., Guyon, A., Soltesz, I., and Crunelli, V. (1994). Sensory input and burst firing output of rat and cat thalamocortical cells: the role of NMDA and non-NMDA receptors. *J. Physiol.* 480, 281–295. doi: 10.1113/jphysiol.1994.sp020359
- Usrey, W. M., Reppas, J. B., and Reid, R. C. (1998). Paired-spike interactions and synaptic efficacy of retinal inputs to the thalamus. *Nature* 395, 384–387. doi: 10.1038/26487
- Van Horn, S. C., Erişir, A., and Sherman, S. M. (2000). Relative distribution of synapses in the A-laminae of the lateral geniculate nucleus of the cat. *J. Comp. Neurol.* 416, 509–520.
- von Krosigk, M., Bal, T., and McCormick, D. A. (1993). Cellular mechanisms of a synchronized oscillation in the thalamus. *Science* 261, 361–364. doi: 10.1126/science.8392750
- Waleszczyk, W. J., Bekisz, M., and Wróbel, A. (2005). Cortical modulation of neuronal activity in the cat's lateral geniculate and perigeniculate nuclei. *Exp. Neurol.* 196, 54–72. doi: 10.1016/j.expneurol.2005.07.006
- Wang, Q., Webber, R. M., and Stanley, G. B. (2010). Thalamic synchrony and the adaptive gating of information flow to cortex. *Nat. Neurosci.* 13, 1534–1541. doi: 10.1038/nn.2670
- Wang, W., Jones, H. E., Andolina, I. M., Salt, T. E., and Sillito, A. M. (2006). Functional alignment of feedback effects from visual cortex to thalamus. *Nat. Neurosci.* 9, 1330–1336. doi: 10.1038/nn1768
- Wang, X., Wei, Y., Vaingankar, V., Wang, Q., Koepsell, K., Sommer, F. T., et al. (2007). Feedforward excitation and inhibition evoke dual modes of firing in the cat's visual thalamus during naturalistic viewing. *Neuron* 55, 465–478. doi: 10.1016/j.neuron.2007.06.039
- Wilson, R. I. (2010). It takes all kinds to make a brain. *Nat. Neurosci.* 13, 1158–1160. doi: 10.1038/nn1010-1158
- Wimmer, R. D., Schmitt, L. I., Davidson, T. J., Nakajima, M., Deisseroth, K., and Halassa, M. M. (2015). Thalamic control of sensory selection in divided attention. *Nature* 526, 705–709. doi: 10.1038/nature15398
- Wolfart, J., Debay, D., Le Masson, G., Destexhe, A., and Bal, T. (2005). Synaptic background activity controls spike transfer from thalamus to cortex. *Nat. Neurosci.* 8, 1760–1767. doi: 10.1038/nn1591
- Zabbah, S., Rajaei, K., Mirzaei, A., Ebrahimpour, R., and Khaligh-Razavi, S.-M. (2014). The impact of the lateral geniculate nucleus and corticogeniculate interactions on efficient coding and higher-order visual object processing. *Vision Res.* 101C, 82–93. doi: 10.1016/j.visres.2014.05.006

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Thalamic Circuit Mechanisms Link Sensory Processing in Sleep and Attention

Zhe Chen^{1,2†}, Ralf D. Wimmer^{2,3†}, Matthew A. Wilson⁴ and Michael M. Halassa^{1,2,3,5*}

¹ Department of Psychiatry, NYU Langone Medical Center, New York, NY, USA, ² Department of Neuroscience and Physiology, NYU School of Medicine, New York, NY, USA, ³ The Neuroscience Institute, NYU School of Medicine, New York, NY, USA, ⁴ Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA, ⁵ Center for Neural Science, New York University, New York, NY, USA

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*Correspondence:

Michael M. Halassa
michael.halassa@nyumc.org

[†]These authors have contributed
equally to this work.

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The correlation between sleep integrity and attentional performance is normally interpreted as poor sleep causing impaired attention. Here, we provide an alternative explanation for this correlation: common thalamic circuits regulate sensory processing across sleep and attention, and their disruption may lead to correlated dysfunction. Using multi-electrode recordings in mice, we find that rate and rhythmicity of thalamic reticular nucleus (TRN) neurons are predictive of their functional organization in sleep and suggestive of their participation in sensory processing across states. Surprisingly, TRN neurons associated with spindles in sleep are also associated with alpha oscillations during attention. As such, we propose that common thalamic circuit principles regulate sensory processing in a state-invariant manner and that in certain disorders, targeting these circuits may be a more viable therapeutic strategy than considering individual states in isolation.

Keywords: thalamic reticular nucleus, thalamic inhibition, attention, sleep spindles

INTRODUCTION

Impaired sleep is a complaint that is widely encountered in clinical practice, encompassing disorders that are primarily brain-based and others that are not (Zisapel, 2007). Because sleep is restorative of brain function (Siegel, 2009; Xie et al., 2013), it is often targeted by hypnotics (Walsh et al., 1995) and behavioral therapy (Edinger et al., 2001; Martínez et al., 2014), with the idea that rescuing sleep will lead to general improvement in cognition regardless of disease etiology. Targeting sleep, while undoubtedly helpful in neurodevelopmental disorders like schizophrenia and autism (Cortese et al., 2013; Wamsley et al., 2013), may be insufficient for cognitive enhancement. Sleep disruption in such disorders is seen in the context of impaired attention (Neumann et al., 2006), executive function (Xu et al., 2014) and working memory (Collins et al., 2014; Kaller et al., 2014), but this comorbidity may be the result of dysfunctional circuits normally required for sleep and cognition, rather than impaired sleep causing cognitive deficits. In support of this notion, studies have shown that both schizophrenia and autism exhibit reduction in fast electrical rhythms known as sleep spindles; phasic 7–15 Hz oscillations seen in surface electroencephalographic (EEG) recordings during sleep (Limoges et al., 2005; Ferrarelli et al., 2010). Spindles are generated by interactions between thalamic reticular nucleus (TRN) neurons and thalamo-cortical relay neurons. The TRN is a group of GABAergic neurons that surround thalamic relay neurons and

provide them with a major source of inhibition (Steriade et al., 1985, 1987; Pinault, 2004; Halassa et al., 2011). The TRN is also known to be involved in attentional processing (McAlonan et al., 2006; Wimmer et al., 2015), and manipulating the TRN causes changes in both attention (Halassa et al., 2014; Ahrens et al., 2015; Wimmer et al., 2015) and sleep (Kim et al., 2012). As such, it would be reasonable to speculate that perturbed TRN circuits lead to correlated disruptions in sleep and attention. Turning this speculation into a testable model requires knowing the degree of overlap between TRN circuits that are engaged in sleep and those that are engaged in attention. That is, to what extent does spindle-generating circuitry engage in attentional processing? In this study, we attempted to answer this question by examining electrophysiological activity of identified TRN neurons across these two behavioral states. Surprisingly, not only did we find that spindle-generating TRN circuits engage in attentional processing, but we also discover that this engagement involves alpha oscillations, a waking rhythm with computational properties similar to spindles. These findings support a model in which common thalamic circuits regulate sensory processing across sleep and attention, and suggest that targeting these circuits or their computational principles may be an effective therapeutic strategy in neurodevelopmental disorders.

METHODS

VGAT-ChR2 mice were obtained from the Jackson Labs and maintained on a C57Bl6/J background. VGAT-Cre mice were backcrossed to C57Bl6/J mice for at least six generations. All experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Massachusetts Institute of Technology (MIT), the New York University Langone Medical Center and the US National Institutes of Health.

Virus Injections and Drive Implantations

For all surgical procedures, VGAT-cre mice were anesthetized with 1% isoflurane and mounted on a stereotactic frame. To transfect visually connected TRN neurons with channelrhodopsin 2 (ChR2), 0.5–0.8 μ l of pseudotyped retrograde lenti-virus (lenti-EF1 α -DIO-ChR2-EGFP, Halassa et al., 2014) were injected into the lateral geniculate nucleus (LGN; from bregma: AP, -2.1 mm, ML, 2 mm, DV, -2.5 mm). For electrophysiological experiments, drive implants with 12 independently adjustable microdrives carrying 1–2 stereotrodes [12.5 micron nichrome or 25 micron tungsten (California Fine Wire Company, Grover Beach, CA)] and a fixed optical fiber (Doric Lenses, Quebec, Canada) targeting caudal TRN were used (Halassa et al., 2014). After drilling a 2–3 mm craniotomy (center coordinates: AP, -2 mm, ML, 2.5 mm) followed by a durotomy, the implant was attached to a stereotaxic arm and lowered in a 15° angle (relative to midline) until electrodes penetrated the cortex (<500 microns). Three stainless steel screws were implanted as EEG (prefrontal location and cerebellar reference) and ground (cerebellar). Two wires (A-M systems, Carlsborg, WA) were inserted into the neck muscle to serve as EMG. Dental cement was used to fix the implant to the skull. Mice

were allowed to recover for at least one week before recordings began.

Electrophysiology and Recording

Upon recovery of implant surgery, each animal was connected to a custom made 32-channel preamplifier headstage (Neuralynx). A recording session consisted of 1–2 h behavioral testing followed by 1–2 h of post-behavioral sleep recording, allowing for neuronal activity analysis both during sleep and wakefulness. All data were recorded using a Neuralynx Digilynx recording system. Signals from each stereotrode were amplified and filtered between 0.1 Hz and 9 kHz and digitized at ~ 30 kHz. LFPs were collected from a single channel on each stereotrode and chosen based on recording quality (absence of low-frequency noise and movement artifacts). Stereotrodes were slowly lowered (over several days) in 125–250 micron steps to collect spike activity. Spike sorting was performed offline using the MClust toolbox (<http://redishlab.neuroscience.umn.edu/mclust/MClust.html>), based on spike amplitudes and energies on the two electrodes of each stereotrode. Units were separated by hand, and cross-correlation and autocorrelation analyses were used to confirm unit separation. The stability of the neuronal activity over time was visually verified (see examples in Supplementary Figure 1). Identification of TRN units was described previously (Wimmer et al., 2015). For identification of optogenetically-tagged visual TRN neurons, a fiber optic patch cord (Doric Lenses) delivered light from a 473 nm laser (Opto Engine) to the fiber optic connector on the animal's implant. Prior to connecting to the animal, laser power was measured and titrated to ~ 10 mW using a neutral density filter (Thorlabs). Power at the tip of the implanted fiber was $\sim 50\%$ of this value, based on measurements prior to surgery. Thus, there was 4–5 mW of power at the fiber tip, or 140–180 mW mm $^{-2}$ for a 200-micron fiber. An analog stimulus generator was used to control laser pulses of 10 ms duration and 0.01 Hz frequency.

Visual Detection Task

We trained mice on a visual detection task that required attentional engagement. Animals were food restricted to and maintained at 85–90% of their *ad libitum* body weight. Body weight was monitored daily and the amount of regular mouse chow (LabDiet, St. Louis, MO) was adjusted based on the number of rewards the mice received during behavioral training and testing. Experiments were conducted in a standard modular test chamber (Med Associates, St. Albans, VT). The chamber was modified to form an isosceles triangle: 23 $\times$ 24 cm (base $\times$ height). The front wall contained two white light emitting diodes, 6.5 cm apart, mounted below two nose-pokes. A third nose-poke with response detector was centrally located on the grid floor, 6 cm away from the base wall and two small Plexiglas walls (3 $\times$ 5 cm), opening at an angle of 20°, served as a guide to the poke. All nose-pokes contained an infrared LED/infrared phototransistor pair for response detection. At the level of the floor-mounted poke, two headphone speakers were introduced into each sidewall of the box, allowing for sound delivery. Trial logic was controlled by custom software running on a microcontroller. Liquid reward consisting of 10 μ l of evaporated

milk was delivered directly to the lateral nose-pokes via a single-syringe pump.

A white noise auditory stimulus signaled the opportunity to initiate a trial. Mice were required to hold their snouts for 500–700 ms into the floor mounted nose-poke unit for successful initiation (stimulus anticipation period). In a few sessions, holding time was increased up to 900 ms to investigate the impact of a prolonged anticipatory window. Following initiation, a stimulus light (500 ms) was presented either to the left or to the right. Responding at the corresponding nose-poke resulted in a liquid reward (10 μ l evaporated milk) dispensed directly at the nose-poke.

Optogenetic Inactivation Experiments

Following successful training on the visual detection task, three VGAT-ChR2 mice were anesthetized with 1% isoflurane and implanted with 3–5 mm long optic fibers (Doric Lenses, Quebec, Canada) targeting bilateral LGN (AP, -2.1 , ML, ± 2 , DV, -2.1) and primary visual cortex (V1: AP, -3.5 , ML, ± 2.5 , DV, -0.5). For determining visual detection psychometric function, visual stimulus duration was 0.1 s and the light was randomly displayed at one of five different intensities (0.15, 0.3, 0.6, 0.9, 1.2 lumens). For LGN or V1 inactivation, laser trains of blue light (50 Hz, 18 ms pulses, 90% duty cycle) at an intensity of 8 mW were delivered bilaterally on every other trial during stimulus presentation. Optogenetic drive of inhibitory neurons in the ChR2 mouse suppresses activity in the excitatory neurons (Zhao et al., 2011) and has been previously used to silence cortical and thalamic regions (Halassa et al., 2011; Guo et al., 2014; Wimmer et al., 2015). Performance was assessed based on the fraction of correct responses relative to chance level (50%, γ). Visual detection threshold (α) and maximum performance (λ) were estimated by fitting performance across stimulation intensities with a logistic function:

$$F(x; \alpha, \beta, \lambda, \gamma) = \gamma + \frac{(1 - \gamma - \lambda)}{1 + \exp(-\beta(x - \alpha))}$$

where x corresponds to the five stimulus intensity levels expressed as a percentage of maximum stimulus intensity. The fraction of correct trials was summed across sessions and the overall performance as a function of stimulus intensity was fit using maximum likelihood estimation implemented in the Palamedes psychophysical toolbox (<http://www.palamedestoolbox.org/>). Estimation of α was made via non-parametric bootstrap analysis of curve fits.

Analysis of Vigilance State Dependent Firing

Unit activity during the different vigilance states was determined as previously described (Halassa et al., 2014). Based on simultaneous EEG and EMG recordings, behavioral epochs were classified into three states: wake, slow-wave sleep (SWS), and rapid eye movement (REM) sleep. Wake epochs were identified by high EMG activity, and the REM epochs were determined by a low EMG activity and high EEG theta/delta power ratio. The remaining epochs were treated as SWS epochs and scoring

was visually verified. Minimum criteria for wake and SWS were >16 s and REM was >5 s. Activity of individual TRN neurons was determined for each vigilance state by calculating the firing rate with 1 s bin size and computing the mean of all instantaneous binned firing rates of the same state. Cells with firing rate ratio wake/SWS >1 were considered to be wake-active, and cells with ratio wake/SWS <1 was considered to be sleep-active.

Spectral Analysis

Multitapered spectral analyses were performed using the Chronux toolbox (www.chronux.org; Mitra and Bokil, 2008), which included the LFP spectrogram and spike-field coherence (SFC). The SFC measures phase synchronization between the LFP and spike times as a function of frequency. In all analyses, to reduce the estimation bias we used the multi-taper method instead of an arbitrary windowing method. Specifically, we chose a half-bandwidth parameter W such that the windowing functions are maximally concentrated within $[-W, W]$. We chose $W > 1/T$ (where T denotes temporal duration) such that the Slepian taper functions are well concentrated in frequency and have bias reducing characteristic (Bokil et al., 2010). In terms of Chronux function setup, we used the tapers setup $[TW, K]$, where $TW = 3$ is the time-bandwidth product, and $K = 2TW - 1 = 5$ is the number of tapers. In addition, since the taper functions are mutually orthogonal, they give independent spectral estimates. In turn, by reducing one taper one can compute robust, Jackknife-based confidence intervals on all estimates, even for a single trial. In the visual detection task, we computed SFC at both in-attention window (0 to the first 1 s after trial initiation, $[0, 1]$ s) and outside-attention window (10–11 s after trial initiation, $[10, 11]$ s). In all time-frequency analyses, we used a window size of 500 ms (which covers at least 4 alpha cycles) and a moving window step size of 10 ms.

Spike-Phase Synchrony Analysis

We applied a Hilbert transform to compute an analytic signal and its instantaneous phase value for the LFP. We filtered the LFP within a specific frequency band of interest, e.g., the alpha band (9–15 Hz). For each TRN unit, we constructed a circular spike-phase histogram (24 bins within 360°) and measured the spike-LFP phase synchrony in two steps. First, a neuron was considered phase locked only if the distribution of the spike phase angles departed from a uniform circular distribution (Rayleigh's test for circular uniformity, $P < 0.05$). We used a standard and well-established circular statistics MATLAB toolbox (MathWorks Inc., Natick, MA) for statistical analyses (Berens, 2009). TRN cells with firing rate below 1 Hz were excluded in this analysis. Next, to further quantify the degree of spike phase locking, we applied an inverse cosine transformation to the spike count vector derived from the spike phase histogram (Halassa et al., 2014), and computed the Pearson correlation statistic from the sample scatter plot. A high degree of correlation indicates a well fit of the cosine phase tuning of spike data or high spike-phase synchrony. $P < 0.05$ was considered to be statistically significant.

Pairwise Neuronal Spike-Time Synchrony Analysis

We computed the spike-time synchrony between paired TRN cells during sleep and awake states (time lag $[-500, 500]$ ms, bin size 10 ms). We then computed the mean and SD of the cross-correlation profile (which is non-negative and non-symmetric). The correlation value above or below 2 SD was considered significant. We integrated the significant correlation value in a small window $[-50, 50]$ ms, shaded area of **Figure 1H** and computed the averaged Z-score as a measure of synchrony. Positive Z-score indicates an excitatory effect from the trigger cell to the target cell, whereas negative Z-score indicates an inhibitory

effect. All comparisons in **Figure 1I** failed the test for normality (Anderson-Darling test, V-V $n = 46$ pairs, $P < 1e-5$, PC-PC $n = 144$ $P < 1e-5$, NC-NC $n = 164$, $P < 1e-5$, V-PC $n = 108$, $P < 1e-5$, V-NC $n = 108$, $P < 1e-5$, PC-NC $n = 131$, $P < 1e-5$) and were compared using Wilcoxon rank-sum test.

Group Average Spike Phase Modulation

We applied a Hilbert transform to compute an analytic signal and its instantaneous phase value (MATLAB function “hilbert”) for the local LFP. During sleep or attention, we band-passed the LFP within the spindle or alpha frequency band (9–15 Hz). For each TRN unit, we constructed a spike-phase histogram (within

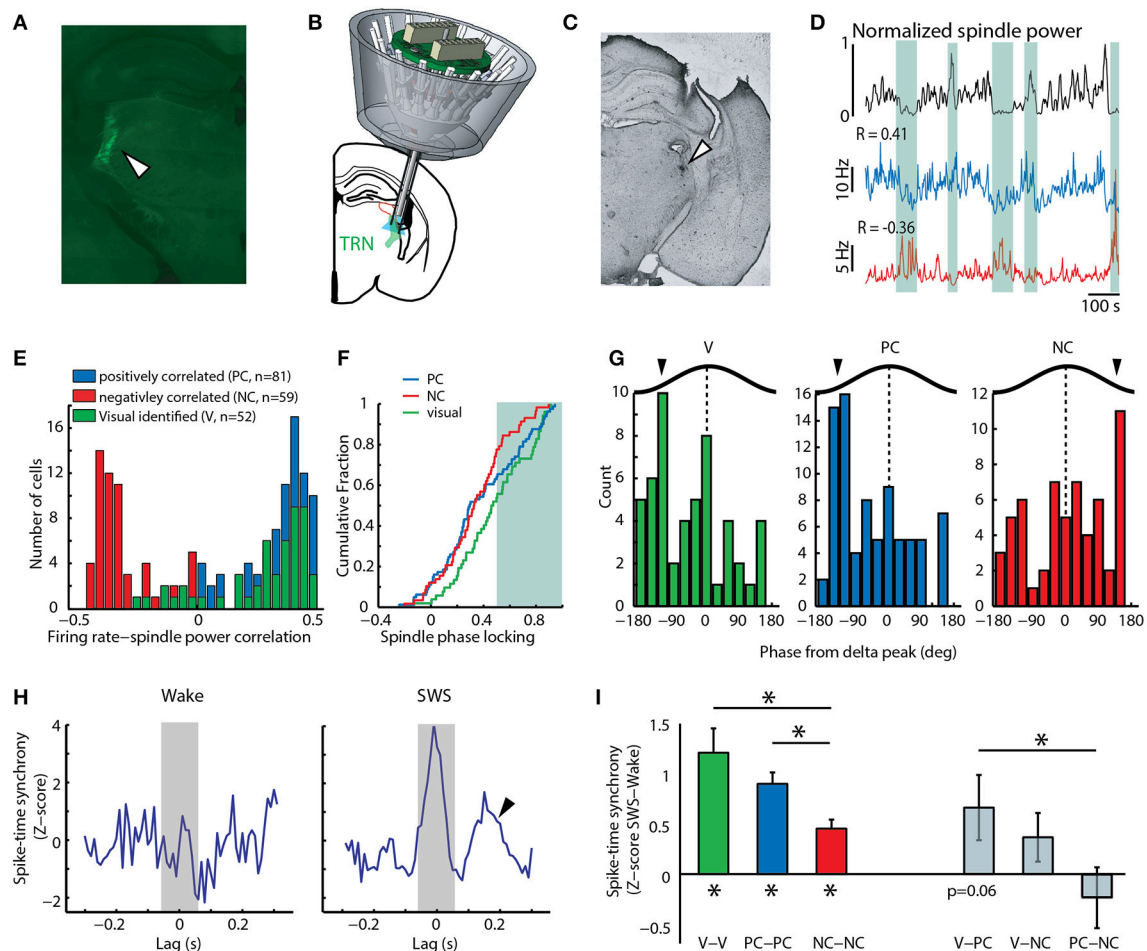


FIGURE 1 | Positive correlation to spindles among TRN neurons predicts involvement in sensory processing. (A) Histological section showing Cre-dependent retrograde tagging of visTRN. **(B)** Cartoon depiction of multi-electrode implant with optical fiber targeting caudal TRN, where visTRN neurons are located. **(C)** Histological verification of TRN targeting in one mouse. **(D)** Examples of two untagged TRN neurons displaying positive (blue) and negative (red) correlation to spindle power (black). Shaded areas highlight portions of high or low spindle power and the opposing changes in firing rate between the positive and negative correlated example neurons. **(E)** Population data reveals a bimodal distribution of rate-spindle power correlation among TRN populations (PC, $n = 81$; NC, $n = 59$), with visTRN neurons ($n = 52$) exhibiting a predominantly positive correlation. **(F)** visTRN neurons show higher spindle phase locking values compared to NC neurons. PC neurons are more visTRN-like at higher phase-locking values (shaded area). **(G)** visTRN and PC neurons show similar phase preferences to delta waves in sleep, which is opposite to NC neurons. **(H)** Example spike-time synchrony (Z-score) plot from a PC neuronal pair, showing elevated values in SWS compared to wake. Only the integral of the correlation value $[-50, 50]$ ms (shaded area) was taken into account for quantification. Arrow head denotes second peak at 200 ms, suggestive of delta entrainment. **(I)** Group data of quantifying phenomenon in panel h; note that visTRN and PC populations exhibit the highest synchrony values, and both are significantly different than NC neurons ($*P < 0.05$, Wilcoxon rank-sum test). When comparing across group, visTRN and PC heterologous pairs are the only ones that show a value of marginal significance ($P = 0.06$, Wilcoxon signed-rank test).

0–360°), which measures the spike phase modulation (SPM). For each TRN cell group (either visTRN, PC or NC), we computed the weighted mean and weighted standard deviation (SD) of the group SPM according to the number of spikes of each cell (**Figure 4B**). For instance, let N_i denote the number of spike from the i -th cell, and let SPM_i denote the SPM curve computed from the spike-phase histogram spike from the i -th cell, the group mean and SD are computed as (Halassa et al., 2014)

$$SPM_{\text{mean}} = \frac{\sum_i N_i SPM_i}{\sum_i N_i},$$

$$SPM_{\text{SD}} = \sqrt{\frac{\sum_i N_i (SPM_i - SPM_{\text{mean}})^2}{\sum_i N_i}}$$

Statistical Analysis

All data with $n \geq 50$ was tested for normality using Anderson-Darling test. For data that were non-normal, unipolar (e.g., data are all positive) or of small sample size, non-parametric statistics were used. When comparing two odds ratios from two independent sample groups, we first computed the sample proportions p_1 and p_2 based on sample sizes n_1 and n_2 . The null hypothesis H_0 is assumed to be $p_1 = p_2$. We then computed the z -score using the formula

$$z = \frac{p_1 - p_2}{\sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}}}$$

where the denominator denotes the standard error (SE). The confidence intervals (CIs) for the difference of two odds are $(p_1 - p_2) \pm z$ SE. Then the one-sided or two-sided P -valued associated with the z -value can be computed ($z = 1.96$ for a 95% CI and $z = 2.58$ for a 99% CI). We reject the null hypothesis H_0 if $P < 0.05$.

To determine neurons with significant firing rate modulation, we computed the mean firing rate during the task period (FR_task) and baseline (FR_baseline) at each trial, where the task period is defined within [0, 0.5] s after trial initiation and baseline is defined as 2.5 s before trial initiation. Then the relative firing rate change was calculated:

$$\text{Relative rate change} = \frac{\text{mean of FR_task} - \text{mean of FR_baseline}}{\text{mean of FR_baseline}}$$

A neuron was considered to be significantly modulated if two conditions were satisfied: First, the absolute value of the Relative rate change was >0.2 . Second, the difference between the matched samples in the vectors FR_task and FR_baseline came from a distribution whose median was non-zero (two-sided Wilcoxon signed-rank test).

RESULTS

To begin investigating the overlap between sleep and attention microcircuits in the TRN, we analyzed a previously collected electrophysiological recordings dataset containing activity of identified TRN neurons in freely behaving mice (Halassa et al.,

2014). In each recording session, mice were run on an attention-requiring detection task, after which they were allowed to sleep. Based on previous analysis, we concluded that the TRN is composed of functional subnetworks defined by anatomical connectivity, and that this architecture allows the TRN to independently control its thalamic targets in a behaviorally relevant manner (Halassa et al., 2014). We focused our analysis here on TRN neurons that are connected to visual thalamus (visTRN), which we had anatomically and physiologically identified by retrograde, Cre-dependent optogenetic tagging (**Figures 1A–C**, Supplementary Figure 2). Similar to results previously obtained from rostral TRN, we found that during slow wave sleep (SWS), this caudal region contained untagged neurons whose firing rates were positively correlated (PC) and others that were negatively correlated (NC) to spindle power (**Figures 1D,E**). The majority of visTRN neurons (44/52) tended to be PC to this measure, and overall, these neurons showed higher phase-locking to locally-recorded spindles than NC neurons (**Figure 1F**). Interestingly, PC neurons showed a mixed distribution, approaching visTRN neurons at higher phase locking values (**Figure 1F**). These findings confirm earlier results that had proposed a sensory origin for spindles (Sato et al., 2007; Timofeev and Chauvette, 2013; Halassa et al., 2014), and raise the possibility that at least a proportion of PC neurons are sensory in nature. The PC group might include a small fraction of unlabeled visTRN neurons. However, in contrast to the 90% of optogenetically tagged visTRN neurons that responded to full field visual stimulation, only 18% of PC neurons were visually responsive. This small potential misclassification cannot account for the fact that 40% of PC neurons exhibited spindle phase locking values equivalent to visTRN.

If PC neurons contained a sensory population, we would expect them to share physiological features with visTRN, and that these features would be indicative of engagement in sensory processing. During SWS, PC neurons showed three features that were “sensory-like.” First, PC and visTRN neurons showed a preference to the same delta (0.5–4 Hz) phase (**Figure 1G**, $P < 0.001$, binomial test), suggesting that they are entrained by similar cortical connectivity mechanisms (Slézia et al., 2011; Sheroziya and Timofeev, 2014). Second, analysis of vigilance state dependent firing revealed that visTRN and PC neurons had a substantially larger proportion that was “sleep-active” than NC neurons (vis: 23/52, PC: 35/81, NC: 7/59; $P < 0.01$, two-sample proportion test), suggesting similar mechanistic regulation by arousal states (Halassa et al., 2014). Third, while all caudal TRN neurons showed enhanced spike-time synchrony during SWS compared to wake (**Figure 1H** and left side of **Figure 1I**), PC and visTRN showed higher values compared to NC neurons ($P < 0.01$, Wilcoxon rank-sum test). SWS synchrony among pairs containing one visTRN and one PC neuron reached marginal significance ($P = 0.06$, Wilcoxon signed-rank test), compared to values obtained from other heterologous pairs, which were all non-significant (right side of **Figure 1I**). The higher proportion of sleep-active cells and the generally more robust sleep synchrony within and across visTRN and PC neurons suggest that these populations provide more inhibition to their thalamic targets compared to NC neurons. This is

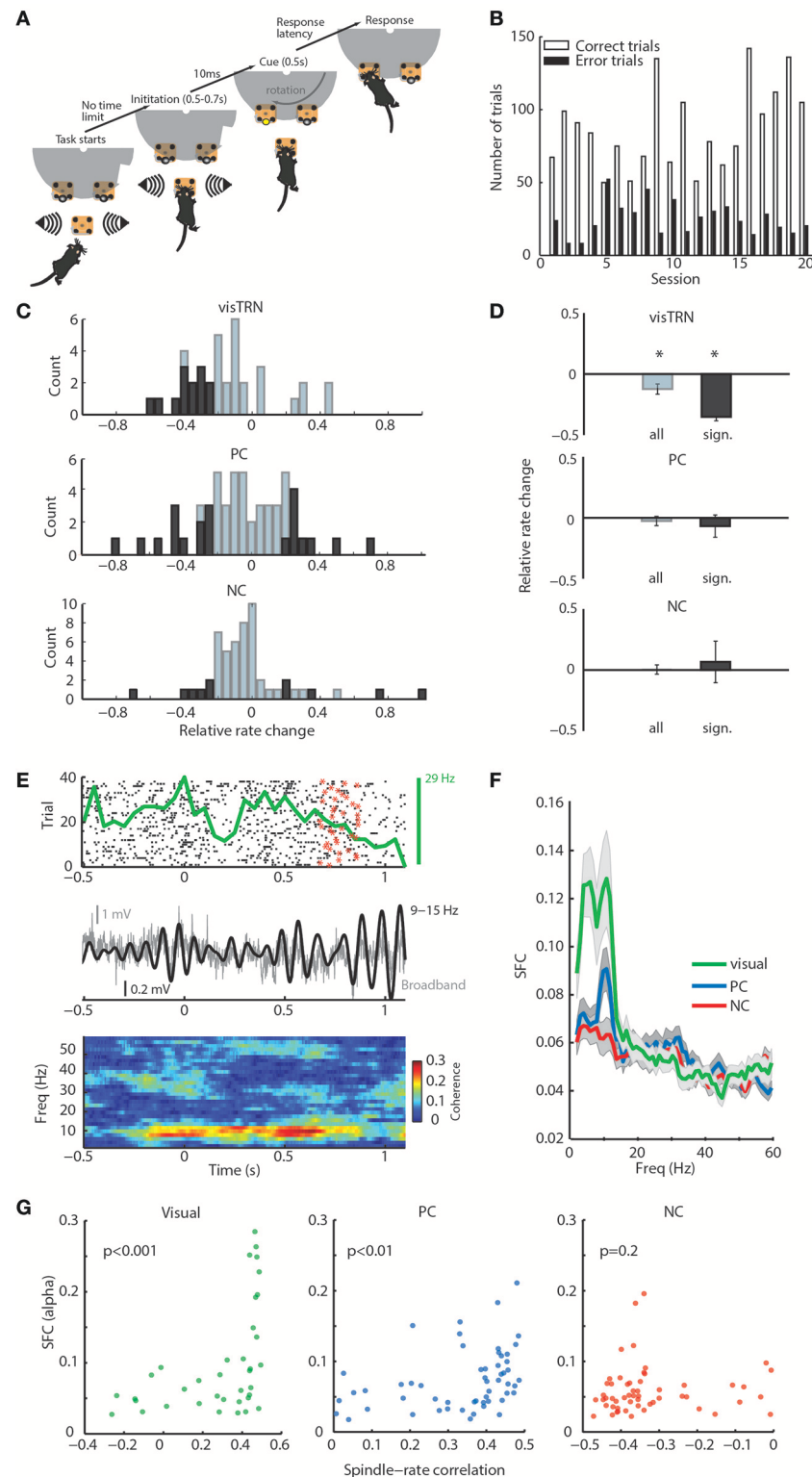


FIGURE 2 | General and modality-specific sensory engagement of TRN neuronal populations. (A) Schematic diagram of visual detection task. **(B)** Task performance showing the proportion of correct and incorrect trials for each of the 20 recorded sessions. **(C)** Distribution of rate modulation values of TRN neurons in the stimulus anticipatory period of the task shown separately for the three groups (visTRN, $n = 37$; PC, $n = 55$; NC, $n = 55$). Dark colors denote significance per cell (see statistical criterion in Methods). Note that all significant visTRN neurons show negative modulation; PC neurons, while showing a comparable number of neurons, are split and NC neurons show very few significantly modulated neurons. **(D)** Grouped data for panel (C). * $P < 0.05$ (one-sided Wilcoxon signed rank test). Only (Continued)

FIGURE 2 | Continued

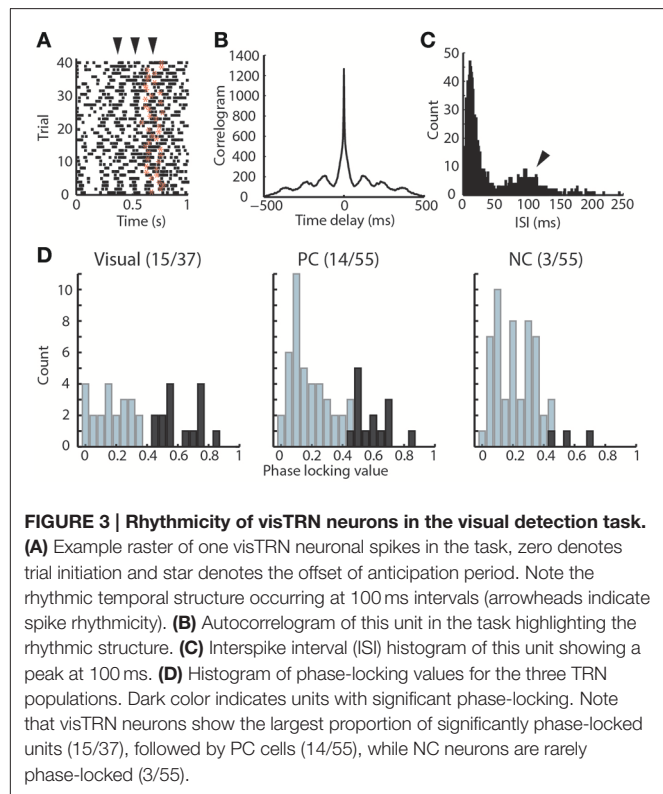
visTRN neurons show a significant decrease in firing rate, consistent with increased gain in visual thalamic sensory processing. **(E)** Example showing a positive correlation between a visTRN neuronal rate (spike raster: black dots; star denotes the offset of anticipation period at each trial; PSTH: top green curve), and alpha amplitude (middle black curve) during the task (0 denotes trial initiation). This positive correlation is reflected in alpha spike-field coherence (SFC) observed during stimulus anticipation and presentation (bottom). **(F)** alpha SFC specific to visTRN neurons within the anticipatory window ([0, 1] s). **(G)** alpha SFC during awake attention covaries significantly with spindle-rate correlation in visTRN ($P < 0.001$, $\rho = 0.6$) and PC ($P < 0.01$, $\rho = 0.42$), but not NC neurons ($P = 0.2$, $\rho = 0.17$; P -values denote significance for Spearman's rank correlation).

supportive of the notion that, just like visTRN, PC neurons are involved in sensory processing, perhaps by rendering their targets less responsive to sensory stimuli during sleep.

To further examine sensory-related features across the three TRN populations, we analyzed their activities in a visual detection task, as had been previously described (Halassa et al., 2014). The task consisted of self-initiated trials, where a food deprived mouse had to appropriately position its head for 500–700 ms, after which a bright (1.2 lumens), 500 ms white light LED stimulus was presented. The location of the visual stimulus indicated the side at which the reward (20 μ L of evaporated milk) was available (**Figure 2A**), and the mouse had 15 s to collect it. Under these conditions, the visual detection task performance is dependent on sensory processing at the level of LGN but not primary visual cortex V1, as determined by optogenetic inactivation experiments (Supplementary Figure 3). Mice performed this task with high accuracy (in 40% of the sessions, mice performed at $\geq 84\%$ accuracy; 20 sessions, $n = 2$ animals, **Figure 2B**).

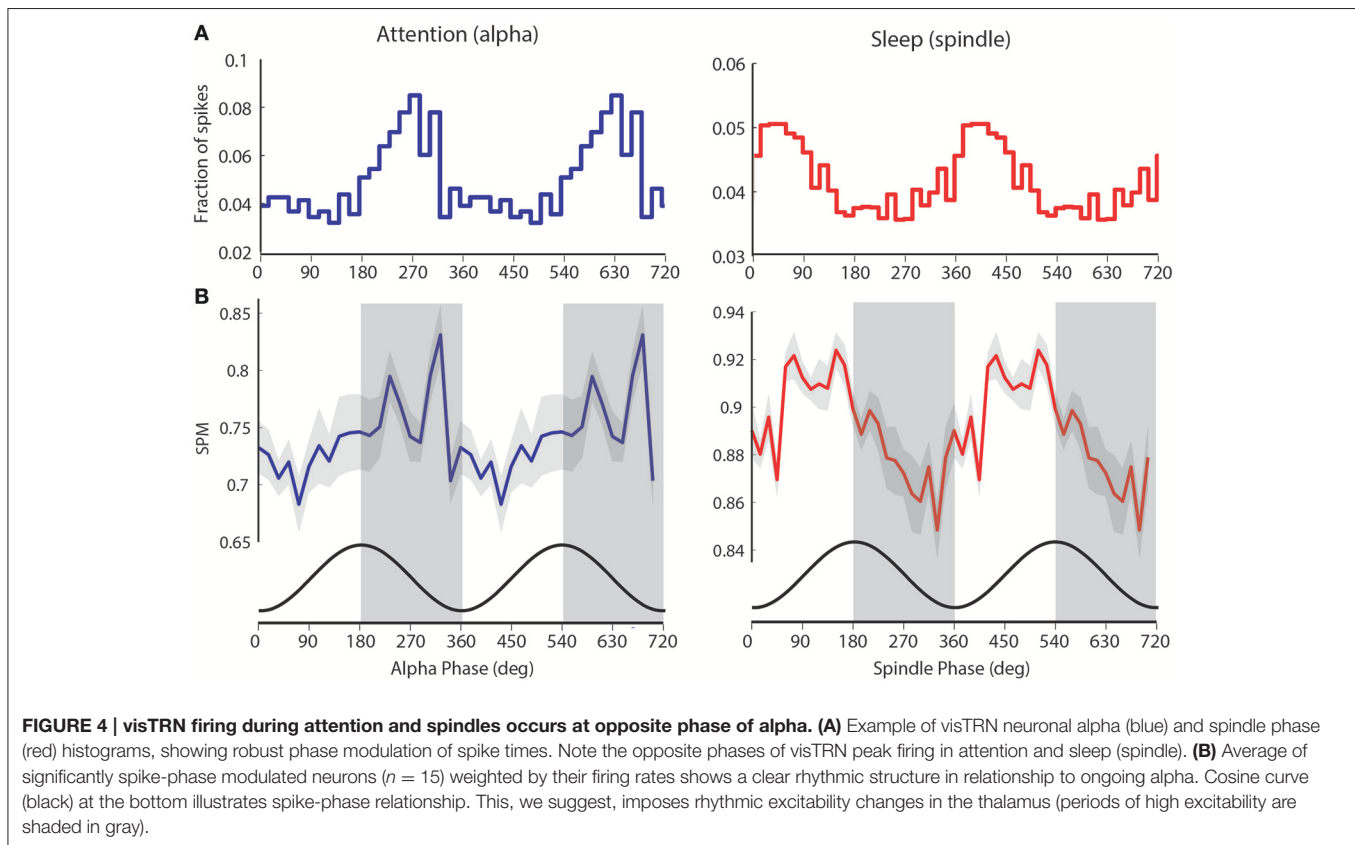
Our previous study showed that, as a population, visTRN neurons exhibited a decrease in firing rate during the stimulus anticipation period (Halassa et al., 2014). Here, we evaluated firing rate changes on a cell-by-cell basis, where the magnitude, direction and significance of change were all considered. We found that the relative rate change distribution for visTRN neurons exhibited a leftward shift (**Figure 2C**, top; and Supplementary Figure 4 for a representative cell). All visTRN cells showing a significant change exhibited a decrease in firing rate (**Figure 2C**, top). PC neurons had a comparable proportion of significantly modulated neurons as visTRN, but only 12/21 showed a decrease (**Figure 2C**, middle). In comparison to visTRN and PC, NC neurons showed a very small number of significantly modulated cells (**Figure 2C**, bottom). As a population, only visTRN neurons showed a reduction in firing rate during anticipation of a visual stimulus (**Figure 2D**), supporting previous findings that TRN contains modality-specific subnetworks that provide independent control over thalamic targets (Halassa et al., 2014).

We noted that the rate changes observed across visTRN neurons were correlated with changes in local field potential (LFP) power, particularly in the alpha band (9–15 Hz; **Figure 2E**). To quantify this observation, we examined the SFC between visTRN neuronal rates and locally recorded LFP. There was a robust increase in visTRN-alpha band SFC around stimulus anticipation and presentation (**Figure 2F**), yet there was no associated change in alpha power (Supplementary Figure 5). While PC neurons showed a smaller alpha SFC peak in that same period, its value did not reach significance when compared against baseline (Supplementary Figure 6). This suggests that



enhanced alpha SFC is specific to visTRN during visual stimulus anticipation, and given the spectral overlap between alpha and spindles, it also suggests that TRN neurons show sensory-related dynamics that are qualitatively similar across states. In support of this conclusion, we observed a strong positive correlation between alpha-SFC in attention and firing rate-spindle correlation in sleep (**Figure 2G**).

Previous data in awake cats suggested a role for thalamic inhibition related to alpha oscillation in visual perception (Lorincz et al., 2009). We reasoned that if alpha oscillation was relevant to performance, then it would have a relationship to TRN neuronal spike times, and would therefore support a role for temporally-structured thalamic inhibition in attention. We found that TRN neurons themselves exhibit rhythmicity at alpha frequency in the task (**Figures 3A–C**). To evaluate the relationship between TRN rhythmicity and alpha in the LFP, we examined alpha phase-locking of TRN neurons. We found that visTRN neurons showed the largest proportion of significantly phase-locked cells, followed by PC neurons, while the NC group only contained very few phase-locked neurons



(Figure 3D). This finding suggested that alpha rhythmicity may reflect a temporally-matched visTRN structure, providing rhythmic inhibition to visual thalamus (LGN). Consistent with this notion, we found that visTRN neurons fired preferentially within half of the alpha cycle, with relatively fewer cells firing at 0–180° (left side of Figure 4A).

To further explore the computational significance of this temporal structure, we weighted the preferred visTRN alpha phase-distribution with the number of spikes generated by each visTRN neuron. This revealed a more robust rhythmic structure of visTRN activity in the anticipatory window (left side of Figure 4B). This particular result is consistent with the predictive role of alpha phase in visual perception suggested by human studies (Busch et al., 2009), as it proposes a model in which the TRN imposes ~50 ms windows of alternating excitability in thalamus that can explain this perceptual dependence (Figure 4B). A corresponding rhythmic process was observed with spindles during sleep, but interestingly, visTRN firing occurred at the opposite phase of spindles compared to alpha (right side of Figures 4A,B).

DISCUSSION

For several years, the TRN has been considered a monolithic structure where its neurons provide uniform inhibition to thalamic targets (Llinas and Steriade, 2006). This notion had been largely based on the biophysical homogeneity encountered when

TRN neurons were examined in acute (Huguenard and Prince, 1992; Bal and McCormick, 1993) or anesthetized preparations (Contreras et al., 1993; but see Lee et al., 2007). By performing electrophysiological recordings of TRN neurons in a behavioral context, we discovered that these cells are physiologically heterogeneous. Our previous findings gave rise to the notion that the TRN is composed of multiple subnetworks, each defined by its projections to a distinct thalamic target (Halassa et al., 2014). As such, thalamic inhibition may be regionally controlled, in a manner that is dependent on behavioral demand. This study confirms and extends this notion along several dimensions.

First, the finding that untaged spindle-correlated TRN neurons show activity modulation consistent with them being sensory in nature confirms the notion that spindles are sensory-related dynamics (Figures 1G–I). Importantly, this finding provides a mechanistic explanation for the well-known correlation between spindle density and gating of sensory inputs during sleep (Dang-Vu et al., 2010; Wimmer et al., 2012). Sensory TRN neuronal firing rates (a correlate of spindles) will likely determine the degree of thalamic sensory throughput during sleep.

Second, the preferential and uniform modulation of visTRN neurons in the visual detection task (Figures 2C,D) provides additional and independent evidence that thalamic inhibition can be controlled in a modality-specific manner. This also confirms the notion that inhibitory control of thalamic sensory processing does not simply co-vary with general arousal, but can rather

be more precisely matched to behavioral demand. The finding that the PC population included significant firing rate increases supports the idea that modalities which are not relevant to solve the task might be suppressed. In another study, we fully evaluated this idea by developing a behavior that requires modality-specific suppression of sensory inputs (Wimmer et al., 2015).

Third, while rhythmic TRN firing in relation to spindles has been observed in sleep, to our knowledge this study is the first to show a similar rhythmic engagement during an attentional state. Just as sensory TRN neuronal firing rates show broad correlation to spindle power in sleep (Figure 1E), they do so to alpha oscillations in attention (Figures 2E–F). In fact, the degree to which a neuronal firing rate is correlated to spindle in sleep predicts its correlation to alpha in attention (Figure 2G). This finding is highly significant, suggesting a common biophysical substrate for rhythmic TRN neuronal engagement across states. Such notion would be consistent with computational studies showing that TRN-thalamic microcircuits generate rhythmic oscillations at ~10 Hz (Destexhe et al., 1993). The inverse phase relationship between visTRN firing during awake alpha and sleep spindle activity (Figure 4) might indicate overlapping but non-identical mechanisms. The stronger rhythmicity of visTRN neurons observed in attention further supports the notion of modality-specific TRN engagement in sensory processing, and extends it beyond changes in firing rate.

Does TRN rhythmicity during sensory processing result in functional consequences? One can hypothesize that TRN spiking in relationship to alpha phase (Figure 4) would result in waxing and waning thalamic inhibition at the alpha frequency. This would imply that alpha oscillations would be associated with shifts in sensitivity to incoming stimuli. Meaning, at near-threshold conditions inputs that arrive at the phase associated with least inhibition would be most likely detected, while those arriving at the opposite phase would not. Interestingly, recent human psychophysics/EEG experiments have provided empirical support to this notion; alpha phase predicts the detection of near-threshold visual stimuli (Busch et al., 2009). Furthermore, the speed of alpha oscillations across individuals appears to set the limits for temporal vision (Samaha and Postle, 2015). Our data suggest that the circuits underlying temporal attention may be

thalamic, and that their boundaries are set by the biophysical properties of thalamic-TRN interactions. It is important to note that our study does not show any change in alpha power during the attention task (Supplementary Figure 5), and that our findings are consistent with the role of alpha phase in attentional processing as recently observed in primates (Saalmann et al., 2012).

The question of how the brain processes sensory information across states is a long standing one in neuroscience (Lee and Dan, 2012). Here, we have shown an unexpected overlap in thalamic mechanisms of sensory processing during sleep and attention. The finding that spindles in sleep may be an alpha in attention counterpart is intriguing, and calls for more mechanistic studies of both oscillations. More generally, our data set the stage for guided investigation of how the TRN controls thalamic gain and timing, impacting sensory processing across states.

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AUTHOR CONTRIBUTIONS

MH conceived and supervised all aspects of the study. RW collected all data and analyzed behavioral aspects of the data. ZC analyzed electrophysiological aspects of the data. MW supported the initial phase of the project. MH wrote the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fncir.2015.00083>

REFERENCES

- Ahrens, S., Jaramillo, S., Yu, K., Ghosh, S., Hwang, G. R., Paik, R., et al. (2015). ErbB4 regulation of a thalamic reticular nucleus circuit for sensory selection. *Nat. Neurosci.* 18, 104–111. doi: 10.1038/nn.3897
- Bal, T., and McCormick, D. A. (1993). Mechanisms of oscillatory activity in guinea-pig nucleus reticularis thalami *in vitro*: a mammalian pacemaker. *J. Physiol.* 468, 669–691. doi: 10.1113/jphysiol.1993.sp019794
- Berens, P. (2009). CircStat: a MATLAB toolbox for circular statistics. *J. Stat. Softw.* 31, 1–21. doi: 10.18637/jss.v031.i10
- Bokil, H., Andrews, P., Kulkarni, J. E., Mehta, S., and Mitra, P. P. (2010). Chronux: a platform for analyzing neural signals. *J. Neurosci. Methods* 192, 146–151. doi: 10.1016/j.jneumeth.2010.06.020
- Busch, N. A., Dubois, J., and VanRullen, R. (2009). The phase of ongoing EEG oscillations predicts visual perception. *J. Neurosci.* 29, 7869–7876. doi: 10.1523/JNEUROSCI.0113-09.2009
- Collins, A. G., Brown, J. K., Gold, J. M., Waltz, J. A., and Frank, M. J. (2014). Working memory contributions to reinforcement learning impairments in schizophrenia. *J. Neurosci.* 34, 13747–13756. doi: 10.1523/JNEUROSCI.0989-14.2014
- Contreras, D., Curró Dossi, R., and Steriade, M. (1993). Electrophysiological properties of cat reticular thalamic neurones *in vivo*. *J. Physiol.* 470, 273–294. doi: 10.1113/jphysiol.1993.sp019858
- Cortese, S., Brown, T. E., Corkum, P., Gruber, R., O'Brien, L. M., Stein, M., et al. (2013). Assessment and management of sleep problems in youths with attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 52, 784–796. doi: 10.1016/j.jaac.2013.06.001
- Dang-Vu, T. T., McKinney, S. M., Buxton, O. M., Solet, J. M., and Ellenbogen, J. M. (2010). Spontaneous brain rhythms predict sleep stability in the face of noise. *Curr. Biol.* 20, R626–R627. doi: 10.1016/j.cub.2010.06.032

- Destexhe, A., McCormick, D. A., and Sejnowski, T. J. (1993). A model for 8–10 Hz spindling in interconnected thalamic relay and reticularis neurons. *Biophys. J.* 65, 2473–2477. doi: 10.1016/S0006-3495(93)81297-9
- Edinger, J. D., Wohlgemuth, W. K., Radtke, R. A., Marsh, G. R., and Quillian, R. E. (2001). Does cognitive-behavioral insomnia therapy alter dysfunctional beliefs about sleep? *Sleep* 24, 591–599. Available online at: <http://www.journalsleep.org/ViewAbstract.aspx?pid=25596>
- Ferrarelli, F., Peterson, M. J., Sarasso, S., Riedner, B. A., Murphy, M. J., Benca, R. M., et al. (2010). Thalamic dysfunction in schizophrenia suggested by whole-night deficits in slow and fast spindles. *Am. J. Psychiatry* 167, 1339–1348. doi: 10.1176/appi.ajp.2010.09121731
- Guo, Z. V., Li, N., Huber, D., Ophir, E., Gutnisky, D., Ting, J. T., et al. (2014). Flow of cortical activity underlying a tactile decision in mice. *Neuron* 81, 179–194. doi: 10.1016/j.neuron.2013.10.020
- Halassa, M. M., Chen, Z., Wimmer, R. D., Brunetti, P. M., Zhao, S., Zikopoulos, B., et al. (2014). State-dependent architecture of thalamic reticular subnetworks. *Cell* 158, 808–821. doi: 10.1016/j.cell.2014.06.025
- Halassa, M. M., Siegle, J. H., Ritt, J. T., Ting, J. T., Feng, G., and Moore, C. I. (2011). Selective optical drive of thalamic reticular nucleus generates thalamic bursts and cortical spindles. *Nat. Neurosci.* 14, 1118–1120. doi: 10.1038/nn.2880
- Huguenard, J. R., and Prince, D. A. (1992). A novel T-type current underlies prolonged Ca^{2+} -dependent burst firing in GABAergic neurons of rat thalamic reticular nucleus. *J. Neurosci.* 12, 3804–3817.
- Kaller, C. P., Loosli, S. V., Rahm, B., Gossel, A., Schieting, S., Hornig, T., et al. (2014). Working memory in schizophrenia: behavioral and neural evidence for reduced susceptibility to item-specific proactive interference. *Biol. Psychiatry* 76, 486–494. doi: 10.1016/j.biopsych.2014.03.012
- Kim, A., Latchoumane, C., Lee, S., Kim, G. B., Cheong, E., Augustine, G. J., et al. (2012). Optogenetically induced sleep spindle rhythms alter sleep architectures in mice. *Proc. Natl. Acad. Sci. U.S.A.* 109, 20673–20678. doi: 10.1073/pnas.1217897109
- Lee, S. H., and Dan, Y. (2012). Neuromodulation of brain states. *Neuron* 76, 209–222. doi: 10.1016/j.neuron.2012.09.012
- Lee, S. H., Govindaiah, G., and Cox, C. L. (2007). Heterogeneity of firing properties among rat thalamic reticular nucleus neurons. *J. Physiol.* 582, 195–208. doi: 10.1113/jphysiol.2007.134254
- Limoges, E., Mottron, L., Bolduc, C., Berthiaume, C., and Godbout, R. (2005). Atypical sleep architecture and the autism phenotype. *Brain* 128, 1049–1061. doi: 10.1093/brain/awh425
- Llinas, R. R., and Steriade, M. (2006). Bursting of thalamic neurons and states of vigilance. *J. Neurophysiol.* 95, 3297–3308. doi: 10.1152/jn.00166.2006
- Lorincz, M. L., Kékesi, K. A., Juhász, G., Crunelli, V., and Hughes, S. W. (2009). Temporal framing of thalamic relay-mode firing by phasic inhibition during the alpha rhythm. *Neuron* 63, 683–696. doi: 10.1016/j.neuron.2009.08.012
- Martínez, M. P., Miro, E., Sanchez, A. I., Diaz-Piedra, C., Caliz, R., Vlaeyen, J. W., et al. (2014). Cognitive-behavioral therapy for insomnia and sleep hygiene in fibromyalgia: a randomized controlled trial. *J. Behav. Med.* 37, 683–697. doi: 10.1007/s10865-013-9520-y
- McAlonan, K., Cavanaugh, J., and Wurtz, R. H. (2006). Attentional modulation of thalamic reticular neurons. *J. Neurosci.* 26, 4444–4450. doi: 10.1523/JNEUROSCI.5602-05.2006
- Mitra, P., and Bokil, H. (2008). *Observed Brain Dynamics*. New York, NY: Oxford University Press.
- Neumann, D., Spezio, M. L., Piven, J., and Adolphs, R. (2006). Looking you in the mouth: abnormal gaze in autism resulting from impaired top-down modulation of visual attention. *Soc. Cogn. Affect. Neurosci.* 1, 194–202. doi: 10.1093/scan/nsi030
- Pinault, D. (2004). The thalamic reticular nucleus: structure, function and concept. *Brain Res. Brain Res. Rev.* 46, 1–31. doi: 10.1016/j.brainresrev.2004.04.008
- Saalmann, Y. B., Pinsk, M. A., Wang, L., Li, X., and Kastner, S. (2012). The pulvinar regulates information transmission between cortical areas based on attention demands. *Science* 337, 753–756. doi: 10.1126/science.1223082
- Samaha, J., and Postle, B. R. (2015). The speed of alpha-band oscillations predicts the temporal resolution of visual perception. *Curr. Biol.* 25, 2985–2990. doi: 10.1016/j.cub.2015.10.007
- Sato, Y., Fukuoka, Y., Minamitani, H., and Honda, K. (2007). Sensory stimulation triggers spindles during sleep stage 2. *Sleep* 30, 511–518. Available online at: <http://www.journalsleep.org/ViewAbstract.aspx?pid=26789>
- Sheroziya, M., and Timofeev, I. (2014). Global intracellular slow-wave dynamics of the thalamocortical system. *J. Neurosci.* 34, 8875–8893. doi: 10.1523/JNEUROSCI.4460-13.2014
- Siegel, J. M. (2009). Sleep viewed as a state of adaptive inactivity. *Nat. Rev. Neurosci.* 10, 747–753. doi: 10.1038/nrn2697
- Slézia, A., Hangya, B., Ulbert, I., and Acsády, L. (2011). Phase advancement and nucleus-specific timing of thalamocortical activity during slow cortical oscillation. *J. Neurosci.* 31, 607–617. doi: 10.1523/JNEUROSCI.3375-10.2011
- Steriade, M., Deschênes, M., Domich, L., and Mulle, C. (1985). Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. *J. Neurophysiol.* 54, 1473–1497.
- Steriade, M., Domich, L., Oakson, G., and Deschênes, M. (1987). The deafferented reticular thalamic nucleus generates spindle rhythmicity. *J. Neurophysiol.* 57, 260–273.
- Timofeev, I., and Chauvette, S. (2013). The spindles: are they still thalamic? *Sleep* 36, 825–826. doi: 10.5665/sleep.2702
- Walsh, J. K., Muehlbach, M. J., and Schweitzer, P. K. (1995). Hypnotics and caffeine as countermeasures for shiftwork-related sleepiness and sleep disturbance. *J. Sleep Res.* 4, 80–83. doi: 10.1111/j.1365-2869.1995.tb00233.x
- Wamsley, E. J., Shinn, A. K., Tucker, M. A., Ono, K. E., McKinley, S. K., Ely, A. V., et al. (2013). The effects of eszopiclone on sleep spindles and memory consolidation in schizophrenia: a randomized placebo-controlled trial. *Sleep* 36, 1369–1376. doi: 10.5665/sleep.2968
- Wimmer, R. D., Astori, S., Bond, C. T., Rovó, Z., Chatton, J. Y., Adelman, J. P., et al. (2012). Sustaining sleep spindles through enhanced SK2-channel activity consolidates sleep and elevates arousal threshold. *J. Neurosci.* 32, 13917–13928. doi: 10.1523/JNEUROSCI.2313-12.2012
- Wimmer, R. D., Schmitt, L. I., Davidson, T. J., Nakajima, M., Deisseroth, K., and Halassa, M. M. (2015). Thalamic control of sensory selection in divided attention. *Nature* 526, 705–709. doi: 10.1038/nature15398
- Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., et al. (2013). Sleep drives metabolite clearance from the adult brain. *Science* 342, 373–377. doi: 10.1126/science.1241224
- Xu, J. Q., Hui, C. L., Longenecker, J., Lee, E. H., Chang, W. C., Chan, S. K., et al. (2014). Executive function as predictors of persistent thought disorder in first-episode schizophrenia: a one-year follow-up study. *Schizophr. Res.* 159, 465–470. doi: 10.1016/j.schres.2014.08.022
- Zhao, S., Ting, J. T., Atallah, H. E., Qiu, L., Tan, J., Gloss, B., et al. (2011). Cell type-specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function. *Nat. Methods* 8, 745–752. doi: 10.1038/nmeth.1668
- Zisapel, N. (2007). Sleep and sleep disturbances: biological basis and clinical implications. *Cell. Mol. Life Sci.* 64, 1174–1186. doi: 10.1007/s00018-007-6529-9

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Thalamic Circuit Diversity: Modulation of the Driver/Modulator Framework

Martha E. Bickford *

Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY, USA

The idea that dorsal thalamic inputs can be divided into “drivers”, which provide the primary excitatory drive for the relay of information to cortex, and “modulators”, which alter the gain of signal transmission, has provided a valuable organizing principle for the study of thalamic function. This view further promoted the identification of “first order” and “higher order” thalamic nuclei, based on the origin of their driving inputs. Since the introduction of this influential terminology, a number of studies have revealed the existence of a wide variety of thalamic organizational schemes. For example, some thalamic nuclei are not innervated by typical driver inputs, but instead receive input from terminals which exhibit features distinct from those of either classic drivers or modulators. In addition, many thalamic nuclei contain unique combinations of convergent first order, higher order, and/or other “driver-like” inputs that do not conform with the driver/modulator framework. The assortment of synaptic arrangements identified in the thalamus are reviewed and discussed from the perspective that this organizational diversity can dramatically increase the computational capabilities of the thalamus, reflecting its essential roles in sensory, motor, and sensory-motor circuits.

Keywords: dorsal lateral geniculate nucleus, pulvinar nucleus, corticothalamic, thalamocortical, lateral posterior nucleus, retinogeniculate, tectothalamic

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University of California, Davis, USA

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Laszlo Acsady,
Institute of Experimental Medicine,
Hungary
Henry Joseph Alitto,
University of California, Berkeley, USA

*Correspondence:

Martha E. Bickford
martha.bickford@louisville.edu

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UNIVERSAL FEATURES OF THALAMIC CIRCUITS

Early electron microscopic studies of the dorsal thalamus revealed a number of similarities across sensory-related nuclei. Studies of the dorsal lateral geniculate nucleus (dLGN; Szentagothai, 1963; Guillery, 1969; Pasik et al., 1973), ventrobasal nucleus (VB; Ralston and Herman, 1969), medial geniculate nucleus (MGN; Majorossy and Réthelyi, 1968), and pulvinar nucleus (Majorossy et al., 1965; Mathers, 1972a) demonstrated the presence of complex (glomerular, **Figure 1A**) synaptic arrangements in which large synaptic terminals that contain round vesicles (RL profiles, **Figures 1A,B**, green) contact the proximal dendrites of thalamocortical relay cells (**Figures 1A,B**, blue), as well as the dendritic terminals of interneurons which contain sparsely distributed flattened or pleomorphic vesicles (F2 profiles, **Figure 1A**, yellow). RL profiles were identified as arising from the retina (axons traveling in the optic tract to the dLGN; Szentagothai, 1963), trigeminal nucleus (medial lemniscus to the VB; Ralston, 1969), inferior colliculus (lateral lemniscus to the MGN; Majorossy and Réthelyi, 1968) or cortex (internal capsule to the pulvinar; Mathers, 1972b). Two additional terminal types were identified across thalamic nuclei: small terminals that contained round vesicles (RS profiles, **Figures 1C,D**, red) that primarily contact the more distal portions of relay cell dendrites (**Figures 1C,D**, blue), and terminals that contained a high density of flattened vesicles (F1 profiles, **Figure 1A**, purple).

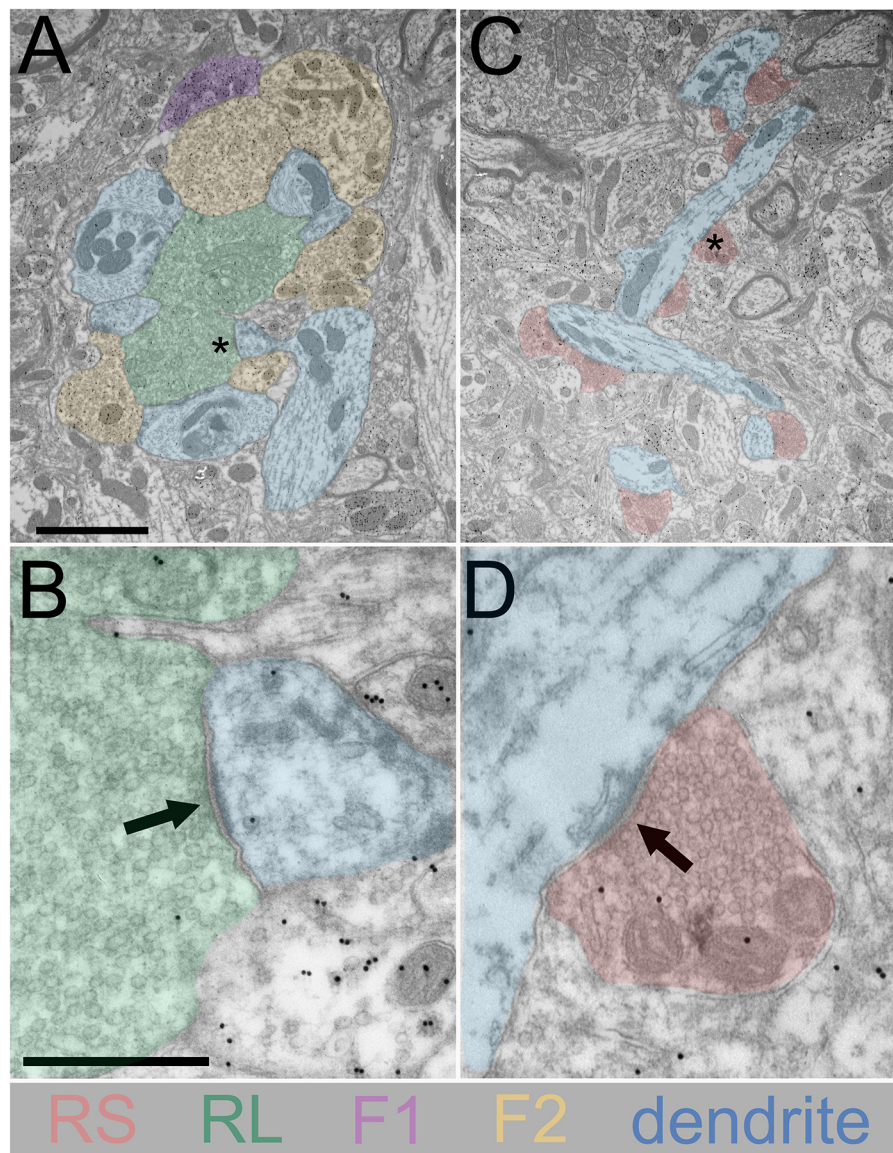


FIGURE 1 | Synaptic terminal types in the dorsal thalamus defined using electron microscopy. Electron microscopic images of the cat dorsal lateral geniculate nucleus (dLGN) are shown. Tissue was obtained from a previous study (Bickford et al., 2008); postembedding immunocytochemical techniques were used to reveal the presence of gamma amino butyric acid, GABA, with gold particles). **(A)** A dLGN glomerulus is illustrated which contains a large profile with round vesicles (RL, green), GABAergic dendritic terminals (F2, yellow, high density of gold particles), and relay cell dendrites (blue). A GABAergic axon terminal (F1, purple, high density of gold particles) is located at the periphery of the glomerulus. The asterisk indicates the location of a synapse shown at higher magnification in **(B)**. **(B)** The arrow indicates a synaptic contact of the RL profile (green) onto a relay cell dendrite (blue). **(C)** A non-glomerular region of the dLGN is illustrated which contains small profiles with round vesicles (RS, pink) that synapse on relay cell dendrites (blue). The asterisk indicates the location of a synapse shown at higher magnification in **(D)**. **(D)** The arrow indicates the synaptic contact of an RS profile (pink) onto a relay cell dendrite (blue). Scale in **(A)** = 2 μm and also applies to **(C)**. Scale in **(B)** = 0.5 μm and also applies to **(D)**.

THE DRIVER/MODULATOR CONCEPT

The identified similarities in sensory thalamus circuits led Sherman and Guillery (1998) to propose an organizing framework of thalamic circuitry that has inspired numerous studies and greatly advanced our understanding of thalamic function. Based on the finding that the receptive field properties of dLGN neurons are nearly identical to that of their retinal

inputs (Cleland et al., 1971), as well as the finding that each dLGN cell is innervated by only a few retinal ganglion cell axons (Hamos et al., 1987). Sherman and Guillery (1998) proposed that the receptive field properties of each thalamic nucleus are determined by RL inputs that originate from a single source. In the dLGN, although retinal input comprises only 5–10% of the synapses (Van Horn et al., 2000), it is nevertheless the primary determinate of geniculate activity, and is therefore aptly

named the driving input. Within this framework, RL inputs across the thalamus are proposed to drive activity patterns (i.e., determine receptive field properties), while the remaining inputs to each nucleus are considered modulators, which can alter the transmission of sensory-driven activity in a state-dependent manner.

The prime examples of modulating inputs are the RS profiles, which in the dLGN, are either glutamatergic inputs that originate from layer VI of the striate cortex (Gilbert and Kelly, 1975), or cholinergic/nitroergic terminals that originate from the pedunculopontine tegmentum (PPT; Bickford et al., 1993; Erişir et al., 1997a,b). Both of these RS inputs have been found to influence the responsiveness of geniculate neurons, without dramatically changing their receptive field properties. Stimulation of the PPT increases the responsiveness of geniculate neurons to their driving retinal inputs (Lu et al., 1993), providing a mechanism for the global regulation of visual signal transfer during different states of arousal. Corticothalamic inputs may additionally tune activity patterns to enhance the responsiveness of restricted populations of thalamic neurons to their driving inputs, thereby aligning the actions of the thalamus and cortex (Briggs and Usrey, 2008).

BIOPHYSICAL FEATURES OF DRIVERS AND MODULATORS

RL profiles are approximately 10 times larger than RS profiles (Li et al., 2003b; Bickford et al., 2010, 2015), and each RL bouton establishes numerous synaptic contacts (Budisantoso et al., 2012; Hammer et al., 2014, 2015), whereas RS profiles typically form single synapses with their postsynaptic partners (Jones and Powell, 1969; Erişir et al., 1997b). *In vitro* studies of responses elicited by activation of retinogeniculate or corticothalamic terminals in brain slices revealed that RL and RS profiles evoke very distinct types of postsynaptic responses. RL terminals exhibit a high probability of neurotransmitter release and their stimulation initially elicits large amplitude, fast, primarily ionotropic, glutamatergic responses; repetitive stimulation of RL profiles depletes synaptic vesicles and desensitizes postsynaptic receptors so that the amplitudes of postsynaptic responses rapidly decrease in a frequency-dependent manner (**Figure 2 class II, RL profile, driver, red traces**; Turner and Salt, 1998; Chen and Regehr, 2003; Li et al., 2003a; Reichova and Sherman, 2004; Groh et al., 2008; Budisantoso et al., 2012). In contrast, stimulation of RS corticothalamic terminals initially elicits smaller amplitude, inotropic glutamatergic responses. These terminals exhibit a low probability of glutamate release, but their repetitive stimulation rapidly increases the amplitudes of postsynaptic responses in a frequency-dependent manner (**Figure 2 class I, RS profile, modulator, gray traces**; Turner and Salt, 1998; Granseth et al., 2002; Kielland et al., 2006; Jurgens et al., 2012). Repetitive stimulation of corticothalamic terminals can also activate metabotropic glutamate receptors (McCormick and von Krosigk, 1992). Finally, electrical stimulation of layer VI corticothalamic axons with increasing current levels results in a graded increase in the amplitude of postsynaptic responses, demonstrating that many RS terminals converge on postsynaptic

neurons (**Figure 2 class I, RS profile, modulator, gray**; Li et al., 2003a,b; Masterson et al., 2009, 2010). In contrast, electrical stimulation of RL axons with increasing current levels results in “all or none” changes in the amplitude of postsynaptic responses, demonstrating that each postsynaptic neuron receives input from only a few RL axons (**Figure 2 class II, RL profile, driver, red**; Li et al., 2003a,b; Ziburkus and Guido, 2006).

FIRST AND HIGHER ORDER THALAMIC NUCLEI

A further organizing principal that grew from the driver/modulator framework of thalamic function was the ability to categorize nuclei based on the origin of their driving input. Sherman and Guillery (1998) defined first order nuclei as those that receive their driving input from sources that relay information from peripheral sensory receptors, such as the retinal input to the dLGN, or the lemniscal inputs to the VB and MGN (**Figure 2, first order, red neuron**). Higher order nuclei are defined as those that receive their driving input from the cortex, specifically from neurons in layer V (**Figure 2, higher order, yellow neuron**). The chief example of a higher order nucleus is the pulvinar nucleus, which receives very little ascending subcortical input (Rovó et al., 2012), but receives abundant input from corticothalamic cells located in both layer V and layer VI. In particular, the striate-recipient zones of the pulvinar nucleus (or lateral posterior nucleus, LPN, of carnivores and rodents) are the best examples of higher order thalamic nuclei (Mathers, 1972b; Ogren and Hendrickson, 1979; Abramson and Chalupa, 1985; Guillery et al., 2001; Li et al., 2003b; Huppé-Gourgues et al., 2006).

The idea that each thalamic nucleus is driven by a single primary input suggested that the function of higher order thalamic nuclei may be to transfer information from one cortical area to another. In other words, it has been suggested that the receptive field properties of pulvinar neurons are driven by layer V input from one cortical area, and these signals are transferred via the pulvinar to other cortical areas (Guillery and Sherman, 2002; Sherman and Guillery, 2002). While this hypothesis has not been fully tested with *in vivo* experiments, the existence of cortical-thalamo-cortical signal transmission has been demonstrated *in vitro* (Theyel et al., 2010).

TECTORECIPIENT THALAMIC NUCLEI AND SPATIAL INTEGRATION

Although many thalamic nuclei can be categorized as first or higher order, it is now apparent that this nomenclature must be modified in order to include the wide variety of “non-canonical” thalamic circuits that have been identified in more recent years. For example, thalamic nuclei that are innervated by the superior colliculus cannot be classified as either first or higher order because, although tectothalamic synaptic terminals are not archetypal drivers, they are larger than all other synaptic terminals within these nuclei. Tectothalamic inputs can be considered “driver-like” in that they are medium-sized terminals that contain round vesicles (RM profiles) that innervate

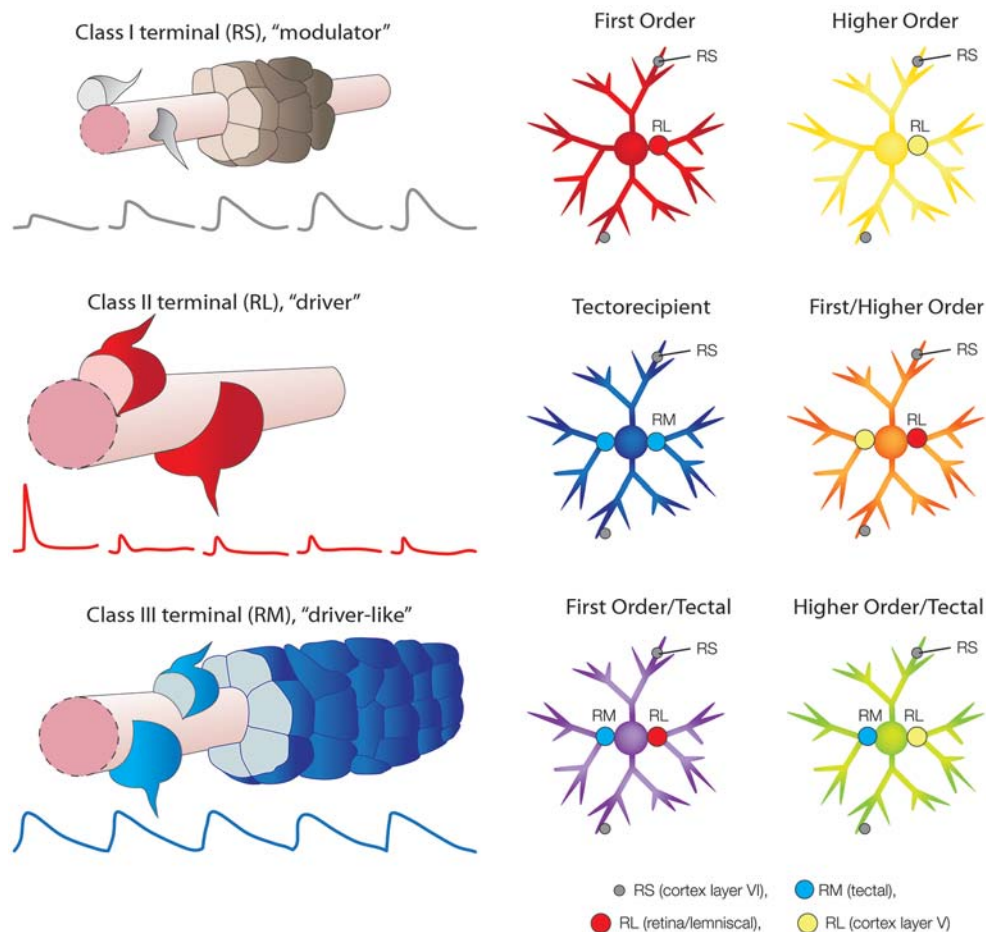


FIGURE 2 | Schematic summary of synaptic terminal types and their arrangements in the dorsal thalamus. *Class I* axons (Guillery, 1966) form small terminals with round vesicles (RS; Guillery, 1969) that are defined as modulators (Sherman and Guillery, 1998). RS terminals that originate from cortex layer VI converge on small caliber (distal) dendrites (depicted by the gray terminals surrounding a section of dendrite, pink, modified from Robson and Hall, 1977). Repetitive stimulation of layer VI corticothalamic terminals results in a frequency-dependent facilitation of excitatory postsynaptic potentials (EPSPs; depicted by gray traces, from Li et al., 2003a). *Class II* axons (Guillery, 1966) form large terminals that contain round vesicles (RL; Guillery, 1969) that are defined as drivers (Sherman and Guillery, 1998). RL terminals that originate from the retina, medial or lateral lemniscus, or cortex layer V, form relatively few synapses on large caliber (proximal) dendrites (depicted by the red terminals surrounding a section of dendrite, pink). Repetitive stimulation of RL terminals results in a frequency-dependent depression of excitatory postsynaptic potentials (depicted by red traces, from Li et al., 2003a). *Class III* terminals form medium size terminals that contain round vesicles (RM; Robson and Hall, 1977), here referred to as “driver-like”. RM terminals that originate from the superior colliculus (tectal) converge on large caliber (proximal) dendrites (depicted by the blue terminals surrounding a section of dendrite, pink, modified from Robson and Hall, 1977). Repetitive stimulation of tectothalamic terminals results in little change in the amplitude of EPSPs (depicted by blue traces, from Masterson et al., 2010). *First order* nuclei (red neuron, modified from Bickford et al., 2015) receive a small number of RL inputs on their proximal dendrites that originate from a single subcortical source (red terminal). *Higher order* nuclei (yellow neuron) receive a small number of RL inputs on their proximal dendrites that originate from cortex layer V (yellow terminal). *Tectorecipient* nuclei (dark blue neuron) receive convergent RM inputs on their proximal dendrites (light blue terminals). As discussed in the text, a variety of combinations of first order, higher order and tectal inputs have been identified which may result in emergent receptive field properties (depicted by the orange, purple and green neurons).

proximal dendrites (**Figure 2 class III, RM profile, driver-like, blue**; Robson and Hall, 1977; Kelly et al., 2003; Chomsung et al., 2008; Masterson et al., 2009) and release glutamate to activate ionotropic glutamate receptors on postsynaptic neurons (Masterson et al., 2010). However, unlike typical driver inputs, many tectothalamic inputs can converge on individual neurons, and in nuclei where this convergence occurs, stimulation of tectothalamic inputs at frequencies of up to 20 Hz elicits postsynaptic responses that maintain stable amplitudes (**Figure 2 class III, RM profile, driver-like, blue traces**). That is, tectothalamic inputs exhibit neither frequency-dependent

depression, nor facilitation. However, stimulation at 100 Hz can elicit the release of substance P from these terminas which, through activation of neurokinin one receptors, can boost tectothalamic responses (Masterson et al., 2010). Finally, tectothalamic terminals contain a different complement of presynaptic proteins than those found in classic drivers or modulators (Wei et al., 2011). Thus, tectorecipient nuclei (**Figure 2, tectorecipient, blue neuron**) are distinct from either first or higher order nuclei, which both contain RL profiles.

The absence of RL inputs has been described in other thalamic nuclei (Smith et al., 2007; Rovó et al., 2012). In the paralaminar

region of the MGN, inputs originating from the superior and inferior colliculi, were described as “integrators” (Smith et al., 2007). The idea behind this nomenclature is that within nuclei that lack typical driver inputs, the collective activity of many convergent inputs may determine the receptive field properties of thalamic neurons. Support for this concept was provided by Chalupa et al. (1983), who found that the receptive field sizes of neurons in the tectorecipient zone of the cat LPN were much larger than those of neurons in the superficial layers of the superior colliculus. This suggests that, in some regions of the thalamus, the convergence of multiple inputs onto individual neurons provides spatial integration to create unique, emergent, receptive field properties.

FIRST AND HIGHER ORDER CONVERGENCE AND TEMPORAL INTEGRATION

Groh et al. (2014) clearly demonstrated the convergence of both first and higher order driver inputs onto single neurons in the somatosensory thalamus (**Figure 2**, *first order/higher order*, orange neuron). Using anatomical techniques, they demonstrated that large synaptic terminals from both the trigeminal nucleus and layer V of the barrel cortex innervated the proximal dendrites of single neurons in the medial subdivision of the mouse posterior nucleus. They then established that when activated simultaneously, these two inputs combine in a supralinear fashion. Such convergence therefore provides a mechanism for the synergistic amplification of signals within a narrow temporal window. In this case the convergence of two driver inputs may report the relative timing between sensory events and ongoing cortical activity.

FIRST ORDER AND TECTAL “DRIVER-LIKE” CONVERGENCE: SENSORY/MOTOR INTEGRATION?

Even within the first order dLGN, where the synaptic arrangements originally inspired the driver/modulator framework, there are restricted regions that contain unique circuits. In the dorsolateral shell of the mouse dLGN, inputs from the superior colliculus and the retina were demonstrated to converge on single neurons using both anatomical and physiological approaches (Bickford et al., 2015; **Figure 2**, *first order/tectal*, purple neuron). In this case, such convergence may be used to integrate visual and motor signals. For example the convergence of retinal and tectal inputs in the dLGN may be necessary to calculate the trajectory of visual stimuli in relation to movement of the body.

CONVERGENCE OF HIGHER ORDER AND “DRIVER-LIKE” INPUTS?

There are a number of thalamic regions that are innervated by large driver terminals that originate from the cortex, as well as ascending driver-like terminals. One region is the rodent LPN, where large terminals that originate from the

primary visual cortex overlap the distribution of terminals that originate from the superior colliculus (Li et al., 2003b; Masterson et al., 2009). Another example is the cat pulvinar nucleus where large terminals that originate from cortical area 7 overlap the distribution of large terminals that originate from the pretectum (Baldauf et al., 2005a,b). Many other possible combinations have been revealed by the distributions of the type 1 and type 2 vesicular glutamate transporters, which are found in cortical and subcortical inputs respectively (Rovó et al., 2012). While the convergence of tectal/pretectal and higher order inputs onto single neurons has not yet been definitively demonstrated, the variety of terminal patterns found across the thalamus suggest that novel spatial and temporal receptive field properties can potentially be constructed via the integration of first order, higher order and/or other driver-like inputs.

ADDITIONAL THALAMIC DIVERSITY

This short review highlights just a few of the variations of the driver/modulator framework, by focusing on thalamic nuclei related to audition, somatosensation and vision. When the full complement of thalamic nuclei is considered, a host of additional synaptic arrangements can be identified. For example, nuclei of the motor thalamus receive convergent input from the cortex, cerebellum and basal ganglia, and have been described as “super integrators” (Bosch-Bouju et al., 2013). Finally, in addition to the various arrangements of glutamatergic inputs, a wide variety of inhibitory circuits have been identified that can provide potent suppression of thalamic activity (Barthó et al., 2002; Bokor et al., 2005; Bodor et al., 2008; Giber et al., 2015).

SUMMARY AND FUTURE DIRECTIONS

The detailed study of thalamic circuits has unveiled a wide range of potential computational capabilities. Receptive field properties in both first and higher order nuclei are likely driven by a single input, and modulated in a state dependent manner. In contrast, receptive field properties in tectorecipient nuclei may be created by the integration of multiple convergent inputs. A wide array of additional thalamic receptive field properties may be created, dependent on the degree of convergence and relative timing of first order, higher order, and/or other driver-like inputs.

Correlations between the diversity of thalamic circuits and thalamocortical circuits may be a particularly fruitful avenue for furthering our understanding of thalamic function. As recently reviewed by Harris and Shepherd (2015), the division of the thalamus into “core” and “matrix” nuclei based on their thalamocortical projection patterns (Jones, 1998, 2001) is a useful starting point, in that the core and matrix categories roughly correlate with first and higher order nuclei. In primary sensory areas of cortex, the thalamocortical axons originating from core nuclei primarily target layer IV (e.g., core/first order dLGN projections to V1; Winfield and Powell, 1976; Winfield et al., 1982; Raczkowski and Fitzpatrick, 1990; Nahmani and Erisir, 2005; Familtsev et al., 2015),

whereas thalamocortical axons originating from matrix nuclei target layers I and V (e.g., matrix/higher order pulvinar/LPN projections to V1; Ogren and Hendrickson, 1977; Carey et al., 1979; Herkenham, 1980; Abramson and Chalupa, 1985).

However, as stated by Harris and Shepherd (2015), “the concepts of core- and matrix-type projections may need to be extended to manage the full complexity of thalamic projections to higher order cortex”. Toward this end, Clascá et al. (2012) have described four categories of thalamic nuclei (core, matrix-focal, matrix-interareal, and intralaminar) to incorporate the diversity of thalamocortical projection patterns, as well as the subcortical projections of the thalamus to the striatum and amygdala. Within this framework, the matrix-focal category is typified by neurons in the koniocellular layers (primate), or shell (rodent) of the dLGN, which project to the superficial layers of V1 (Hendry and Reid, 2000; Shostak et al., 2002; Cruz-Martín et al., 2014; Bickford et al., 2015). The matrix-intrareal category is correlated with nuclei such as the tectorecipient pulvinar or LPN, where neurons project to multiple visual areas, as well as the striatum and amygdala (Chomsung et al., 2010; Day-Brown et al., 2010; Nakamura et al., 2015).

REFERENCES

- Abramson, B. P., and Chalupa, L. M. (1985). The laminar distribution of cortical connections with the tecto- and cortico-recipient zones in the cat's lateral posterior nucleus. *Neuroscience*. 15, 81–95. doi: 10.1016/0306-4522(85)90125-3
- Baldauf, Z. B., Chomsung, R. D., Carden, W. B., May, P. J., and Bickford, M. E. (2005a). Ultrastructural analysis of projections to the pulvinar nucleus of the cat. I: Middle suprasylvian gyrus (areas 5 and 7). *J. Comp. Neurol.* 485, 87–107. doi: 10.1002/cne.20480
- Baldauf, Z. B., Wang, S., Chomsung, R. D., May, P. J., and Bickford, M. E. (2005b). Ultrastructural analysis of projections to the pulvinar nucleus of the cat. II: Pretectum. *J. Comp. Neurol.* 485, 108–126. doi: 10.1002/cne.20487
- Barthó, P., Wang, S., Chomsung, R. D., Freund, T. F., and Acsády, L. (2002). Selective GABAergic innervation of thalamic nuclei from zona incerta. *Eur. J. Neurosci.* 16, 999–1014. doi: 10.1046/j.1460-9568.2002.02157.x
- Bickford, M. E., Günlük, A. E., Guido, W., and Sherman, S. M. (1993). Evidence that cholinergic axons from the parabrachial region of the brainstem are the exclusive source of nitric oxide in the lateral geniculate nucleus of the cat. *J. Comp. Neurol.* 334, 410–430. doi: 10.1002/cne.903340307
- Bickford, M. E., Slusarczyk, A., Dilger, E. K., Krahe, T. E., Kucuk, C., Guido, W., et al. (2010). Synaptic development of the mouse dorsal lateral geniculate nucleus. *J. Comp. Neurol.* 518, 622–635. doi: 10.1002/cne.22223
- Bickford, M. E., Wei, H., Eisenback, M. A., Chomsung, R. D., Slusarczyk, A. S., and Dankowski, A. B. (2008). Synaptic organization of thalamocortical axon collaterals in the perigeniculate nucleus and dorsal lateral geniculate nucleus. *J. Comp. Neurol.* 508, 264–285. doi: 10.1002/cne.21671
- Bickford, M. E., Zhou, N., Krahe, T. E., Govindaiah, G., and Guido, W. (2015). Retinal and Tectal “Driver-Like” Inputs Converge in the Shell of the Mouse Dorsal Lateral Geniculate Nucleus. *J. Neurosci.* 35, 10523–10534. doi: 10.1523/JNEUROSCI.3375-14.2015
- Bodor, A. L., Giber, K., Rovó, Z., Ulbert, I., and Acsády, L. (2008). Structural correlates of efficient GABAergic transmission in the basal ganglia-thalamus pathway. *J. Neurosci.* 28, 3090–3102. doi: 10.1523/jneurosci.5266-07.2008
- Bokor, H., Frère, S. G. A., Eyre, M. D., Slézia, A., Ulbert, I., Lüthi, A., et al. (2005). Selective GABAergic Control of Higher-Order Thalamic Relays. *Neuron*. 45, 929–940. doi: 10.1016/j.neuron.2005.01.048
- Bosch-Bouju, C., Hyland, B. I., and Parr-Brownlie, L. C. (2013). Motor thalamus integration of cortical, cerebellar and basal ganglia information: implications for normal and parkinsonian conditions. *Front. Comput. Neurosci.* 7:163. doi: 10.3389/fncom.2013.00163
- Briggs, F., and Usrey, W. M. (2008). Emerging views of corticothalamic function. *Curr. Opin. Neurobiol.* 18, 403–407. doi: 10.1016/j.conb.2008.09.002
- Budisantoso, T., Matsui, K., Kamasawa, N., Fukazawa, Y., and Shigemoto, R. (2012). Mechanisms underlying signal filtering at a multisynapse contact. *J. Neurosci.* 32, 2357–2376. doi: 10.1523/jneurosci.5243-11.2012
- Carey, R. G., Fitzpatrick, D., and Diamond, I. T. (1979). Layer I of striate cortex of Tupaia glis and Galago senegalensis: projections from thalamus and claustrum revealed by retrograde transport of horseradish peroxidase. *J. Comp. Neurol.* 186, 393–437. doi: 10.1002/cne.901860306
- Chalupa, L. M., Williams, R. W., and Hughes, M. J. (1983). Visual response properties in the tectorecipient zone of the cat's lateral posterior-pulvinar complex: a comparison with the superior colliculus. *J. Neurosci.* 3, 2587–2596.
- Chen, C., and Regehr, W. G. (2003). Presynaptic modulation of the retinogeniculate synapse. *J. Neurosci.* 23, 3130–3135. doi: 10.3410/f.1013375.190301
- Chomsung, R. D., Petry, H. M., and Bickford, M. E. (2008). Ultrastructural examination of diffuse and specific tectopulvinar projections in the tree shrew. *J. Comp. Neurol.* 510, 24–46. doi: 10.1002/cne.21763
- Chomsung, R. D., Wei, H., Day-Brown, J. D., Petry, H. M., and Bickford, M. E. (2010). Synaptic organization of connections between the temporal cortex and pulvinar nucleus of the tree shrew. *Cereb. Cortex*. 20, 997–1011. doi: 10.1093/cercor/bhp162
- Clascá, F., Rubio-Garrido, P., and Jabaudon, D. (2012). Unveiling the diversity of thalamocortical neuron subtypes. *Eur. J. Neurosci.* 35, 1524–1532. doi: 10.1111/j.1460-9568.2012.08033.x
- Cleland, B. G., Dubin, M. W., and Levick, W. R. (1971). Simultaneous recording of input and output of lateral geniculate neurones. *Nat. New. Biol.* 231, 191–192. doi: 10.1038/newbio231191a0
- Cruikshank, S. J., Ahmed, O. J., Stevens, T. R., Patrick, S. L., Gonzalez, A. N., Elmaleh, M., et al. (2012). Thalamic control of layer 1 circuits in prefrontal cortex. *J. Neurosci.* 32, 17813–17823. doi: 10.1523/JNEUROSCI.3231-12.2012
- Cruikshank, S. J., Urabe, H., Nurmikko, A. V., and Connors, B. W. (2010). Pathway-specific feedforward circuits between thalamus and neocortex

Recent anatomical and optogenetic studies have demonstrated that thalamic axons can target a wide array of cortical cell types, dependent on the cortical area, cortical lamina, and thalamic nucleus of origin (Petreanu et al., 2009; Cruikshank et al., 2010, 2012; Hooks et al., 2013; Kloc and Maffei, 2014; Shigematsu et al., 2015). Thus a challenge for future studies will be the documentation and classification of thalamocortical microcircuits. As evidenced by the advancements achieved since the introduction of the driver/modulator framework, identification of canonical microcircuits is a key component in deciphering nervous system function. The subsequent identification of variations in standard circuit modules allows us then to build and expand upon these conceptual frameworks, driving the field forward.

AUTHOR CONTRIBUTIONS

MEB wrote the manuscript.

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- revealed by selective optical stimulation of axons. *Neuron*. 65, 230–245. doi: 10.1016/j.neuron.2009.12.025
- Cruz-Martín, A., El-Danaf, R. N., Osakada, F., Sriram, B., Dhande, O. S., Nguyen, P. L., et al. (2014). A dedicated circuit links direction-selective retinal ganglion cells to the primary visual cortex. *Nature* 507, 358–361. doi: 10.1038/nature12989
- Day-Brown, J. D., Wei, H., Chomsung, R. D., Petry, H. M., and Bickford, M. E. (2010). Pulvinar projections to the striatum and amygdala in the tree shrew. *Front. Neuroanat.* 4:143. doi: 10.3389/fnana.2010.00143
- Erişir, A., Van Horn, S. C., and Sherman, S. M. (1997a). Relative numbers of cortical and brainstem inputs to the lateral geniculate nucleus. *Proc. Natl. Acad. Sci. U S A* 94, 1517–1520. doi: 10.1073/pnas.94.4.1517
- Erişir, A., Van Horn, S. C., Bickford, M. E., and Sherman, S. M. (1997b). Immunocytochemistry and distribution of parabrachial terminals in the lateral geniculate nucleus of the cat: a comparison with corticogeniculate terminals. *J. Comp. Neurol.* 377, 535–549. doi: 10.1002/(sici)1096-9861(19970127)377:4<535::aid-cne5>3.3.co;2-q
- Familtsev, D., Quiggins, R., Masterson, P. S., Dang, W., Slusarczyk, A. S., Petry, H. M., et al. (2015). The ultrastructure of geniculocortical synaptic connections in the tree shrew striate cortex. *J. Comp. Neurol.* doi: 10.1002/cne.23907 [Epub ahead of print].
- Giber, K., Diana, M. A., Plattner, V. M., Dugué, G. P., Bokor, H., Rousseau, C. V., et al. (2015). A subcortical inhibitory signal for behavioral arrest in the thalamus. *Nat. Neurosci.* 18, 562–568. doi: 10.1038/nn.3951
- Gilbert, C. D., and Kelly, J. P. (1975). The projections of cells in different layers of the cat's visual cortex. *J. Comp. Neurol.* 163, 81–105. doi: 10.1002/cne.901630106
- Granseth, B., Ahlstrand, E., and Lindström, S. (2002). Paired pulse facilitation of corticogeniculate EPSCs in the dorsal lateral geniculate nucleus of the rat investigated *in vitro*. *J. Physiol.* 544, 477–486. doi: 10.1111/j.2002.00477.x
- Groh, A., Bokor, H., Mease, R. A., Plattner, V. M., Hangya, B., Stroth, A., et al. (2014). Convergence of cortical and sensory driver inputs on single thalamocortical cells. *Cereb. Cortex* 24, 3167–3179. doi: 10.1093/cercor/bht173
- Groh, A., de Kock, C. P. J., Wimmer, V. C., Sakmann, B., and Kuner, T. (2008). Driver or coincidence detector: modal switch of a corticothalamic giant synapse controlled by spontaneous activity and short-term depression. *J. Neurosci.* 28, 9652–9663. doi: 10.1523/jneurosci.1554-08.2008
- Guillery, R. W. (1966). A study of Golgi preparations from the dorsal lateral geniculate nucleus of the adult cat. *J. Comp. Neurol.* 128, 21–50. doi: 10.1002/cne.901280104
- Guillery, R. W. (1969). The organization of synaptic interconnections in the laminae of the dorsal lateral geniculate nucleus of the cat. *Z. Zellforsch. Mikrosk. Anat.* 96, 1–38. doi: 10.1007/bf00321474
- Guillery, R. W., Feig, S. L., and Van Lieshout, D. P. (2001). Connections of higher order visual relays in the thalamus: a study of corticothalamic pathways in cats. *J. Comp. Neurol.* 438, 66–85. doi: 10.1002/cne.1302
- Guillery, R. W., and Sherman, S. M. (2002). Thalamic relay functions and their role in corticocortical communication: generalizations from the visual system. *Neuron*. 33, 163–175. doi: 10.1016/s0896-6273(01)00582-7
- Hammer, S., Carrillo, G. L., Govindaiah, G., Monavarfeshani, A., Bircher, J. S., Su, J., et al. (2014). Nuclei-specific differences in nerve terminal distribution, morphology and development in mouse visual thalamus. *Neural. Dev.* 9:16. doi: 10.1186/1749-8104-9-16
- Hammer, S., Monavarfeshani, A., Lemon, T., Su, J., and Fox, M. A. (2015). Multiple retinal axons converge onto relay cells in the adult mouse thalamus. *Cell. Rep.* 12, 1575–1583. doi: 10.1016/j.celrep.2015.08.003
- Hamos, J. E., Van Horn, S. C., Raczkowski, D., and Sherman, S. M. (1987). Synaptic circuits involving an individual retinogeniculate axon in the cat. *J. Comp. Neurol.* 259, 165–192. doi: 10.1002/cne.902590202
- Harris, K. D., and Shepherd, G. M. G. (2015). The neocortical circuit: themes and variations. *Nat. Neurosci.* 18, 170–181. doi: 10.1038/nn.3917
- Hendry, S. H., and Reid, R. C. (2000). The koniocellular pathway in primate vision. *Annu. Rev. Neurosci.* 23, 127–153. doi: 10.1146/annurev.neuro.23.1.127
- Herkenham, M. (1980). Laminar organization of thalamic projections to the rat neocortex. *Science*. 207, 532–535. doi: 10.1126/science.7352263
- Hooks, B. M., Mao, T., Gutnisky, D. A., Yamawaki, N., Svoboda, K., and Shepherd, G. M. G. (2013). Organization of cortical and thalamic input to pyramidal neurons in mouse motor cortex. *J. Neurosci.* 33, 748–760. doi: 10.1523/JNEUROSCI.4338-12.2013
- Huppé-Gourgues, F., Bickford, M. E., Boire, D., Ptito, M., and Casanova, C. (2006). Distribution, morphology and synaptic targets of corticothalamic terminals in the cat lateral posterior-pulvinar complex that originate from the posteromedial lateral suprasylvian cortex. *J. Comp. Neurol.* 497, 847–863. doi: 10.1002/cne.21024
- Jones, E. G. (1998). A new view of specific and nonspecific thalamocortical connections. *Adv. Neurol.* 77, 49–71.
- Jones, E. G. (2001). The thalamic matrix and thalamocortical synchrony. *Trends. Neurosci.* 24, 595–601. doi: 10.1016/s0166-2236(00)01922-6
- Jones, E. G., and Powell, T. P. (1969). An electron microscopic study of the mode of termination of cortico-thalamic fibres within the sensory relay nuclei of the thalamus. *Proc. R. Soc. Lond. B Biol. Sci.* 172, 173–185. doi: 10.1098/rspb.1969.0018
- Jurgens, C. W. D., Bell, K. A., McQuiston, A. R., and Guido, W. (2012). Optogenetic stimulation of the corticothalamic pathway affects relay cells and GABAergic neurons differently in the mouse visual thalamus. *PLoS One* 7:e45717. doi: 10.1371/journal.pone.0045717
- Kelly, L. R., Li, J., Carden, W. B., and Bickford, M. E. (2003). Ultrastructure and synaptic targets of tectothalamic terminals in the cat lateral posterior nucleus. *J. Comp. Neurol.* 464, 472–486. doi: 10.1002/cne.10800
- Kielland, A., Erisir, A., Walaas, S. I., and Heggelund, P. (2006). Synapsin utilization differs among functional classes of synapses on thalamocortical cells. *J. Neurosci.* 26, 5786–5793. doi: 10.1523/jneurosci.4631-05.2006
- Kloc, M., and Maffei, A. (2014). Target-specific properties of thalamocortical synapses onto layer 4 of mouse primary visual cortex. *J. Neurosci.* 34, 15455–15465. doi: 10.1523/jneurosci.2595-14.2014
- Li, J., Guido, W., and Bickford, M. E. (2003a). Two distinct types of corticothalamic EPSPs and their contribution to short-term synaptic plasticity. *J. Neurophysiol.* 90, 3429–3440. doi: 10.1152/jn.00456.2003
- Li, J., Wang, S., and Bickford, M. E. (2003b). Comparison of the ultrastructure of cortical and retinal terminals in the rat dorsal lateral geniculate and lateral posterior nuclei. *J. Comp. Neurol.* 460, 394–409. doi: 10.1002/cne.10646
- Lu, S. M., Guido, W., and Sherman, S. M. (1993). The brain-stem parabrachial region controls mode of response to visual stimulation of neurons in the cat's lateral geniculate nucleus. *Vis. Neurosci.* 10, 631–642. doi: 10.1017/s0952523800005332
- Majorossy, K., and Réthelyi, M. (1968). Synaptic architecture in the medial geniculate body (ventral division). *Exp. brain. Res.* 6, 306–323. doi: 10.1007/bf00233182
- Majorossy, K., Réthelyi, M., and Szentágothai, J. (1965). The large glomerular synapse of the pulvinar. *J. Hirnforsch.* 7, 415–432.
- Masterson, S. P., Li, J., and Bickford, M. E. (2009). Synaptic organization of the tectorecipient zone of the rat lateral posterior nucleus. *J. Comp. Neurol.* 515, 647–663. doi: 10.1002/cne.22077
- Masterson, S. P., Li, J., and Bickford, M. E. (2010). Frequency-dependent release of substance P mediates heterosynaptic potentiation of glutamatergic synaptic responses in the rat visual thalamus. *J. Neurophysiol.* 104, 1758–1767. doi: 10.1152/jn.00010.2010
- Mathers, L. H. (1972a). Ultrastructure of the pulvinar of the squirrel monkey. *J. Comp. Neurol.* 146, 15–42. doi: 10.1002/cne.901460103
- Mathers, L. H. (1972b). The synaptic organization of the cortical projection to the pulvinar of the squirrel monkey. *J. Comp. Neurol.* 146, 43–60. doi: 10.1002/cne.901460104
- McCormick, D. A., and von Krosigk, M. (1992). Corticothalamic activation modulates thalamic firing through glutamate “metabotropic” receptors. *Proc. Natl. Acad. Sci. U S A* 89, 2774–2778. doi: 10.1073/pnas.89.7.2774
- Nahmani, M., and Erisir, A. (2005). VGluT2 immunocytochemistry identifies thalamocortical terminals in layer 4 of adult and developing visual cortex. *J. Comp. Neurol.* 484, 458–473. doi: 10.1002/cne.20505
- Nakamura, H., Hioki, H., Furuta, T., and Kaneko, T. (2015). Different cortical projections from three subdivisions of the rat lateral posterior thalamic nucleus: a single-neuron tracing study with viral vectors. *Eur. J. Neurosci.* 41, 1294–1310. doi: 10.1111/ejn.12882
- Ogren, M. P., and Hendrickson, A. E. (1977). The distribution of pulvinar terminals in visual areas 17 and 18 of the monkey. *Brain Res.* 137, 343–350. doi: 10.1016/0006-8993(77)90344-4

- Ogren, M. P., and Hendrickson, A. E. (1979). The morphology and distribution of striate cortex terminals in the inferior and lateral subdivisions of the Macaca monkey pulvinar. *J. Comp. Neurol.* 188, 179–199. doi: 10.1002/cne.901880113
- Pasik, T., Pasik, P., Hamori, J., and Szentagothai, J. (1973). “Triadic” synapses and other articulations of interneurons in the lateral geniculate nucleus of rhesus monkeys. *Trans. Am. Neurol. Assoc.* 98, 293–295.
- Petreanu, L., Mao, T., Sternson, S. M., and Svoboda, K. (2009). The subcellular organization of neocortical excitatory connections. *Nature* 457, 1142–1145. doi: 10.1038/nature07709
- Raczkowski, D., and Fitzpatrick, D. (1990). Terminal arbors of individual, physiologically identified geniculocortical axons in the tree shrew’s striate cortex. *J. Comp. Neurol.* 302, 500–514. doi: 10.1002/cne.903020307
- Ralston, H. J., III (1969). The synaptic organization of lemniscal projections to the ventrobasal thalamus of the cat. *Brain Res.* 14, 99–115. doi: 10.1016/0006-8993(69)90033-x
- Ralston, H. J., III, and Herman, M. M. (1969). The fine structure of neurons and synapses in ventrobasal thalamus of the cat. *Brain Res.* 14, 77–97. doi: 10.1016/0006-8993(69)90032-8
- Reichova, I., and Sherman, S. M. (2004). Somatosensory corticothalamic projections: distinguishing drivers from modulators. *J. Neurophysiol.* 92, 2185–2197. doi: 10.1152/jn.00322.2004
- Robson, J. A., and Hall, W. C. (1977). The organization of the pulvinar in the grey squirrel (*Sciurus carolinensis*). II. Synaptic organization and comparisons with the dorsal lateral geniculate nucleus. *J. Comp. Neurol.* 173, 389–416. doi: 10.1002/cne.901730211
- Rovó, Z., Ulbert, I., and Acsády, L. (2012). Drivers of the primate thalamus. *J. Neurosci.* 32, 17894–17908. doi: 10.1523/jneurosci.2815-12.2012
- Sherman, S. M., and Guillery, R. W. (1998). On the actions that one nerve cell can have on another: distinguishing “drivers” from “modulators”. *Proc. Natl. Acad. Sci. U S A* 95, 7121–7126. doi: 10.1073/pnas.95.12.7121
- Sherman, S. M., and Guillery, R. W. (2002). The role of the thalamus in the flow of information to the cortex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 357, 1695–1708. doi: 10.1098/rstb.2002.1161
- Shigematsu, N., Ueta, Y., Mohamed, A. A., Hatada, S., Fukuda, T., Kubota, Y., et al. (2015). Selective Thalamic Innervation of Rat Frontal Cortical Neurons. *Cereb. Cortex*. doi: 10.1093/cercor/bhv124 [Epub ahead of print].
- Shostak, Y., Ding, Y., Mavity-Hudson, J., and Casagrande, V. A. (2002). Cortical synaptic arrangements of the third visual pathway in three primate species: *Macaca mulatta*, *Saimiri sciureus* and *Aotus trivirgatus*. *J. Neurosci.* 22, 2885–2893.
- Smith, P. H., Bartlett, E. L., and Kowalkowski, A. (2007). Cortical and collicular inputs to cells in the rat paralamina thalamic nuclei adjacent to the medial geniculate body. *J. Neurophysiol.* 98, 681–695. doi: 10.1152/jn.00235.2007
- Szentagothai, J. (1963). The structure of the synapse in the lateral geniculate body. *Acta. Anat. (Basel)*. 55, 166–185. doi: 10.1159/000142468
- Theyel, B. B., Llano, D. A., and Sherman, S. M. (2010). The corticothalamic circuit drives higher-order cortex in the mouse. *Nat. Neurosci.* 13, 84–88. doi: 10.1038/nn.2449
- Turner, J. P., and Salt, T. E. (1998). Characterization of sensory and corticothalamic excitatory inputs to rat thalamocortical neurones *in vitro*. *J. Physiol.* 510, 829–843. doi: 10.1111/j.1469-7793.1998.829bj.x
- Van Horn, S. C., Erişir, A., and Sherman, S. M. (2000). Relative distribution of synapses in the A-laminae of the lateral geniculate nucleus of the cat. *J. Comp. Neurol.* 416, 509–520. doi: 10.1002/(sici)1096-9861(20000124)416:4<509::aid-cne7>3.0.co;2-h
- Wei, H., Masterson, S. P., Petry, H. M., and Bickford, M. E. (2011). Diffuse and specific tectopulvinar terminals in the tree shrew: synapses, synapses and synaptic potentials. *PLoS One* 6:e23781. doi: 10.1371/journal.pone.0023781
- Winfield, D. A., and Powell, T. P. (1976). The termination of thalamo-cortical fibres in the visual cortex of the cat. *J. Neurocytol.* 5, 269–281. doi: 10.1007/bf01175115
- Winfield, D. A., Rivera-Dominguez, M., and Powell, T. P. (1982). The termination of geniculocortical fibres in area 17 of the visual cortex in the macaque monkey. *Brain Res.* 231, 19–32. doi: 10.1016/0006-8993(82)90004-x
- Ziburkus, J., and Guido, W. (2006). Loss of binocular responses and reduced retinal convergence during the period of retinogeniculate axon segregation. *J. Neurophysiol.* 96, 2775–2784. doi: 10.1152/jn.01321.2004

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Central Thalamic Deep-Brain Stimulation Alters Striatal-Thalamic Connectivity in Cognitive Neural Behavior

Hui-Ching Lin^{1,2†}, Han-Chi Pan^{3†}, Sheng-Huang Lin^{4,5}, Yu-Chun Lo⁶, Elise Ting-Hsin Shen⁵, Lun-De Liao^{7,8}, Pei-Han Liao⁹, Yi-Wei Chien⁹, Kuei-Da Liao¹⁰, Fu-Shan Jaw⁵, Kai-Wen Chu¹, Hsin-Yi Lai^{11*} and You-Yin Chen^{9*}

¹ Department and Institute of Physiology, School of Medicine, National Yang Ming University, Taipei, Taiwan, ² Brain Research Center, National Yang Ming University, Taipei, Taiwan, ³ Institute of Neuroscience, National Yang Ming University, Taipei, Taiwan, ⁴ Department of Neurology, Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan, ⁵ Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan, ⁶ Institute of Medical Device and Imaging, National Taiwan University College of Medicine, Taipei, Taiwan, ⁷ Centre for Life Sciences, Singapore Institute for Neurotechnology, National University of Singapore, Singapore, Singapore, ⁸ Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Miaoli, Taiwan, ⁹ Department of Biomedical Engineering, National Yang Ming University, Taipei, Taiwan, ¹⁰ Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan, ¹¹ Interdisciplinary Institute of Neuroscience and Technology, Qushi Academy for Advanced Studies, Zhejiang University, Hangzhou, China

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Edited by:

Vincenzo Crunelli,
Cardiff University, UK

Reviewed by:

Thomas DeMarse,
University of Florida, USA
Ya-tang Li,
California Institute of Technology, USA

*Correspondence:

Hsin-Yi Lai
laihy@zju.edu.cn;
You-Yin Chen
irradiance@so-net.net.tw

[†]These authors have contributed
equally to this work.

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Central thalamic deep brain stimulation (CT-DBS) has been proposed as an experimental therapeutic approach to produce consistent sustained regulation of forebrain arousal for several neurological diseases. We investigated local field potentials (LFPs) induced by CT-DBS from the thalamic central lateral nuclei (CL) and the striatum as potential biomarkers for the enhancement of lever-pressing skill learning. LFPs were simultaneously recorded from multiple sites in the CL, ventral striatum (Vstr), and dorsal striatum (Dstr). LFP oscillation power and functional connectivity were assessed and compared between the CT-DBS and sham control groups. The theta and alpha LFP oscillations were significantly increased in the CL and striatum in the CT-DBS group. Furthermore, interhemispheric coherences between bilateral CL and striatum were increased in the theta band. Additionally, enhancement of *c-Fos* activity, dopamine D2 receptor (Drd2), and α 4-nicotinic acetylcholine receptor (α 4-nAChR) occurred after CT-DBS treatment in the striatum and hippocampus. CT-DBS strengthened thalamic-striatal functional connectivity, which demonstrates that the inter-regional connectivity enhancement might contribute to synaptic plasticity in the striatum. Altered dopaminergic and cholinergic receptors resulted in modulation of striatal synaptic plasticity's ability to regulate downstream signaling cascades for higher brain functions of lever-pressing skill learning.

Keywords: deep brain stimulation, reward-associated learning, thalamus, local field potentials, functional connectivity

INTRODUCTION

Deep brain stimulation (DBS) is a potent therapeutic approach of electrical stimulation through electrodes implanted in specific regions to modulate abnormal neuronal activities that contribute to neurological diseases and psychiatric disorders (Kolb et al., 1983; Overbeek et al., 2013; Williams and Okun, 2013; Schlaepfer and Bewernick, 2014). Several studies demonstrated DBS can modulate the firing patterns of neurons through changes in subregional synchronization and low-frequency rhythmic oscillation (Bergman et al., 1998; Vitek and Giroux, 2000; Deuschl et al., 2001). Studies have shown that DBS mediates neurological changes and behavioral improvement, and interval stimulation of the medial temporal lobe and the memory formation-related region with specific frequencies and critical timing is important for memory processing (Suthana et al., 2012; Fell et al., 2013; Lee et al., 2013).

Recently, several animal experiments and clinical trials have indicated that DBS contributed to enhanced learning and memory (Suthana and Fried, 2014). Applications are being developed for memory impairment due to Alzheimer disease, traumatic brain injury, temporal lobe epilepsy, stroke, and encephalitis. Many studies (Halgren et al., 1985; Lacruz et al., 2010; Stone et al., 2011) found that electrical stimulation to the hippocampal entorhinal cortex circuit (Squire et al., 2004), that has been shown to improve spatial learning. However, hippocampal DBS has been found to disrupt memory (Ego-Stengel and Wilson, 2010) and decline learning (Leung and Shen, 2006) due to electrical stimulation induced seizures.

Central thalamus (CT) nuclei contained densely populated with neurons that widely project to striatum as well as cortical targets and collectively provide the largest thalamic efference to the striatum, which are hypothesized to synchronize activity in neural networks that underlie cognitive functions (Mengual et al., 1999; Jones, 2009). Meanwhile, CT nuclei regulated arousal and awareness, influencing activity in distributed neural networks that give widespread effects on cortical and subcortical functions (Parikh and Sarter, 2008; Robbins and Arnsten, 2009). Alternatively, CT of investigation is whether DBS may be a potent therapeutics for disorders of learning and memory. Several studies have demonstrated that thalamic DBS is a safe and efficacious treatment for essential tremor (Flora et al., 2010; Baizabal Carvallo et al., 2011). It has also been reported that DBS at CT (CT-DBS) enhanced exploratory motor behaviors and cognitive performance through neocortical and hippocampal neuronal activation by specific regulation of *c-Fos* and immediate-early gene-encoded protein *Egr-1* (zif268) expressions in normal rats (Shirvalkar et al., 2006). Moreover, Schiff (Schiff et al., 2007) showed that bilateral CT-DBS could restore consciousness in patients in a coma by changing the arousal state. Thus, it has been proposed that CT-DBS could be an available treatment for remediation of learning and memory deficits.

An important anatomical specialization of the CT that supports an overall role in shifting levels of activity across broad cerebral networks is their strong efference to the striatum. Deschenes et al.'s research demonstrated the neuronal projections

of CT to the striatum and cortical layers, defined by biocytin anterograde labeling (Deschenes et al., 1996). The striatum is associated with numerous cognitive processes, that plays an important role in motor control (Yin and Knowlton, 2006) and reward cue-reward association tasks (Atallah et al., 2007; Jacquet et al., 2013). Striatum has been implicated in the modulation of motor control and learning ability by receiving neural signals from thalamus and transmitting to the motor cortex (Yin and Knowlton, 2006). In addition, Atallah et al. (2007) demonstrated that, the ventral striatum (Vstr) is critical for skill learning, and the dorsal striatum (Dstr) is important for skill performance but not for learning.

Based on the anatomical connections of CT with the striatum, we were interested in the direct electrical stimulation of CT that altered the changes in functional connectivity for the targeted Vstr and Dstr, the stimulation site of CT, which improved the skill learning process. Therefore, spontaneous fluctuations in the local field potentials (LFPs) were used to investigate the full functional connectivity pattern of the paired brain areas. Synchronization of regional neuronal activity due to post-synaptic activation gave rise to LFP oscillations, and it played a major role in functional communication related to memory, integrative functions (Basar et al., 2001), information transfer, perception, and motor control (Fries, 2005).

In this study, we designed a water reward-related skill-learning task to explore the CT-DBS influence on cognitive performance. We performed simultaneous multi-site LFP recordings and investigate the functional connectivity in awake rats to assess the effects of CT-DBS and sham. LFP activities were recorded from bilateral CT, Vstr, and Dstr, and the stimulation sites were in bilateral CT. In addition, we identified possible molecular mechanisms by examining the protein level of dopamine and acetylcholine receptors. We hypothesized that functional connectivity could be enhanced by CT-DBS treatment in the reward and skill learning-related brain areas. Regulation of the synaptic dopaminergic and cholinergic systems are required for lever-pressing skill learning.

MATERIALS AND METHODS

Animal Preparation

Twenty male adult Sprague-Dawley rats (250–300 g) were maintained on a 12-h light-dark cycle (light from 7.00 h to 19.00 h) at a constant temperature of $22 \pm 3^\circ\text{C}$ in the experimental animal center of National Yang Ming University. All experiments were performed in accordance with the approved guidelines and regulations, and were approved by the Institutional Animal Care and Use Committee of the National Yang Ming University. All animals were equally divided into the DBS group ($N = 10$) and sham control group ($N = 10$) to investigate the effect of CT-DBS on the animal behavioral tasks.

Animal Surgical Procedures for Neural Implantation

The animals were anesthetized with intramuscular tiletamin and zolazepam (Zoletil 50, Virbac, Carros, France), 6 mg/kg

each, suspended in 8 µg/kg Dexdomitor (Orion Pharma, Esbo, Finland). The anesthetized rats were placed in a stereotaxic frame (Model 962, Kopf Instruments, Tujunga, CA), and a craniotomy was performed over the location of electrode implantation.

In this study, an 8-channel stainless microwire electrode array (product # M177390, 30-µm diameter, California Fine Wire Co., Grover Beach, CA, USA), combined with two 1 × 4 arrays (not pictured), was used to perform CT-DBS and multi-site recording. One 1 × 4 array was geometrically designed from two pairs of microwires that were implanted bilaterally into the CL (AP: -3.5 mm; ML: ±1.4 mm; VD: 5 mm) to perform both bipolar CT-DBS and LFP recordings. The spacing between each pair of the microwires is 200 µm (Figure S1A, Supplementary Note 1). The other 1 × 4 array also was designed pair two o microwires, that was implanted into the bilateral Vstr (AP: 0.8 mm; ± ML: 2.2 mm; VD: 6.2 mm) and Dstr (AP: 0.8 mm; ± ML: 2.5 mm; VD: 3.5 mm) for LFP recordings, respectively. The spacing between each pair of microwires is 400 µm (Figure S1B, Supplementary Note 1). The spacing between the two 1 × 4 arrays was 4.3 mm in the anterior-posterior direction. A stainless steel screw was secured to the skull over the cerebellum as a reference electrode. The microwire electrode array was secured in the skull using dental acrylic and was covered with a small amount of 2% agar. One week of recovery after the implantation, we performed the behavioral tasks combined with CT-DBS and LFP recording. The implantation sites of the electrodes were confirmed and examined by Nissl staining (Figure S1C, Supplementary Note 1).

Behavioral Training

The implanted rats were single housed and deprived of water for 8 h before lever-pressing training. The lab-designed Plexiglas testing box (Figures 1A,B) used in the present study was based on *Skinner box module* (Skinner, 1992) which is known to be related to instrumental conditioning (operant conditioning; Balleine et al., 2003; Atallah et al., 2007). All implanted animals underwent LFP recording for 30 min as a baseline before the 1st reward training. Before each daily reward training, rats in the CT-DBS group received 100-Hz biphasic stimulus (0.4 mA, 25 µs per phase pulse) or the sham rats without DBS were placed in another plastic cage (30 cm diameter, 38 cm height) for 30 min. Following 30-min CT-DBS (or sham), each animal was individually introduced into the Plexiglas testing box for training the associated lever-pressing (appetitive behavior) and water-reward (instrumental skill). The cumulative time to reach the successful instrumental skill for each rat was analyzed offline by a video camera, which was placed above the Plexiglas testing box during the training sessions. In this study, the water-deprived rats had to press the lever to obtain the water conducted in a lab-designed Plexiglas testing box, that they learn the associated lever-pressing (appetitive behavior) and water-reward (consummatory behavior). We have defined the criterion for the successful skill learning was to consecutively repeat the lever-pressing and water-drinking for five times during daily 5-h sessions (9:00–14:00), for 4 days at the most. Once reaching the criterion or end of the daily training time period, LFPs were recorded for 30 min to evaluate the changes in LFP spectrum and coherence between groups. Each group was equally divided into

two subgroups, and the animals were sacrificed 2 h after DBS (or sham) for further immunohistochemistry and Western blot studies.

Neural Recording and Data Analysis

Many studies provided evidences that the rat Vstr and Dstr play distinct roles in instrumental conditioning (skill learning; Atallah et al., 2007; Yin et al., 2008) and CT has distinct afferent and efferent connections that appear organized to project to anatomically related targets in the cerebral cortex and basal ganglia (striatum; Chen et al., 2014). In this study, multi-site LFPs were recorded bilaterally in the CL, Vstr, and Dstr using the Cerebus data acquisition system (Blackrock Microsystems, Salt Lake City, UT, USA) to explore changes of neural oscillation and functional connectivity. Neural signals were amplified, filtered at cut-off frequencies of 0.3 and 250 Hz, and sampled at 1 kHz. All data analysis was post-processed with MATLAB (R12, Mathworks Inc., Natick, MA, USA). The comparison of spectral power of LFP oscillations and coherence between DBS and sham control group were further analyzed.

LFP data for delta (1–4 Hz), theta (4–7 Hz), alpha (7–13 Hz), and beta (13–20 Hz) bands were calculated from the power spectral density (PSD), which was computed via Welch's method (see Supplementary Note 2). The coherence, the principal measure of functional connectivity used in this study (see Supplementary Note 3), provides a frequency-domain measurement of the linear magnitude and phase relationships between each channel pair of LFPs (Srinath and Ray, 2014). Intrahemispheric coherence in each hemisphere was examined for adjacent microwire electrode pairings. Interhemispheric coherence was examined for electrode pairings across the hemispheres. For comparison of functional connectivity between groups, the intrahemispheric coherence and interhemispheric coherence of each spectrum band was normalized to the percent coherence change ($\Delta\text{Coh}^{\text{intra}}$ (site A–site B) % and $\Delta\text{Coh}^{\text{inter}}$ (site A–site B) %), (1) subtracting the baseline coherence, and then (2) dividing the baseline coherence. The baseline was chosen as the LFP of coherence at each frequency band from before behavioral training.

Immunohistochemistry

Ten anesthetized rats (DBS group: $N = 5$; sham control group: $N = 5$) were perfused with phosphate-buffered saline (PBS) with 0.05% heparin and 4% paraformaldehyde (PFA, Sigma-Aldrich, St. Louis, MO, USA). The rat brain was extracted from the skull and soaked in a mixture of 4% PFA and 30% sucrose (J.T. Baker, Center Valley, PA) at 4°C for 72 h, sliced on a freezing microtome at 30 µm, and stored in PBS at 4°C. The brain sections (30 µm) were washed with PBS, permeated with 0.2% triton X-100, and incubated with 3% H₂O₂ and 10% MeOH. Then, the brain sections were blocked with 3% normal goat serum (NGS) and hybridized with anti-*c-Fos* antibody (rabbit, 1:10000; Novus Biologicals, Littleton, CA, USA). The sections were then washed, hybridized with biotinylated goat anti-rabbit IgG antibody (1:500; Vector Laboratories, Burlingame, CA, USA), and incubated with horseradish peroxidase (HRP)-conjugated avidin complex (Vector Laboratories, Burlingame, CA, USA). In

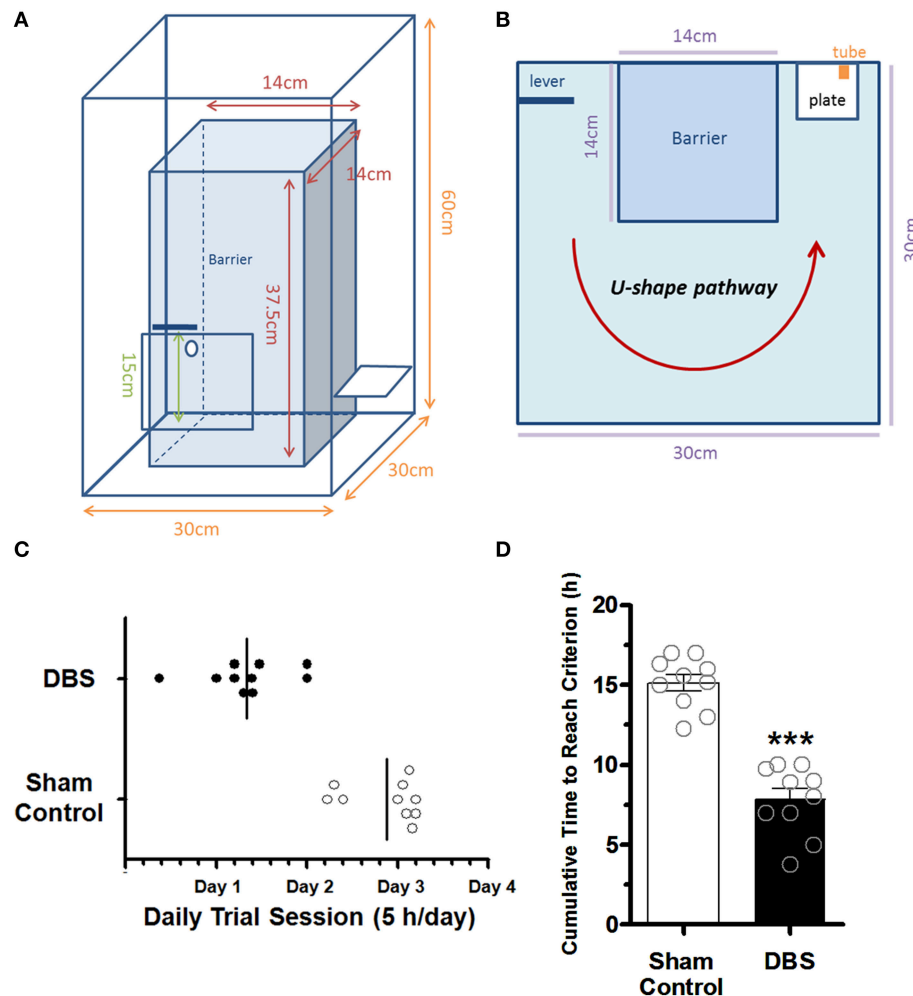


FIGURE 1 | The Plexiglas testing box and comparison of the time to reach the criterions between the sham control and CT-DBS-treated group. The side view (A) and top view (B) of the schematics of the Plexiglas testing box. (C) The lotted time necessary for the rats to reach the criteria set for the water reward-related lever-pressing learning within daily trial session. (D) The cumulative time to reach the criterion for the water reward-related lever-pressing learning. The symbol *** indicates significant difference as compared with the sham control ($P < 0.001$, Wilcoxon two-sample tests, $N = 10$).

addition, the sections were incubated with 2% diaminobenzidine (DAB; Sigma-Aldrich, St. Louis, MO, USA) and 4% ammonium nickel sulfate (Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M Na-K PB and developed with 0.004% H_2O_2 .

The quantification of *c-Fos* positive immunoreactivity was performed bilaterally for 30 slices ranging from 1.2 to -3.5 mm to the bregma for each rat for each selected brain region using freeware Image Processing and Analysis in Java (ImageJ, National Institute of Health, Bethesda, MD, USA). Cell counts/mm² were analyzed for the bilateral CL, primary motor cortex (M1), anterior cingulate cortex (ACC), caudate-putamen in dorsal striatum (CPu), accumbens nucleus in ventral striatum (NAc), retrosplenial cortex (Rsc), parietal association cortex (PtA), and hippocampus (CA1, CA3, and DG; Paxinos and Watson, 2005). The density of *c-Fos* positive cells for each brain area in the CT-DBS group was normalized using the mean values of the control group.

Western Blots

The striatum or hippocampus was dissected from the brain tissues of the other ten rats (DBS group: $N = 5$; sham control group: $N = 5$). Protein samples were extracted in ice-cold lysis buffer (50 mM Tris-HCl, pH = 7.5, 0.3 M sucrose, 5 mM EDTA, 2 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM PMSF, 20 μ g/ml leupeptin, and 4 μ g/ml aprotinin) and then separated (30 μ g) by SDS-PAGE, and trans-blotted onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were hybridized with anti-dopamine D2 receptor (Drd2; 1:1000; ADR-002-50UL, Alomone Labs, Jerusalem, Israel) or anti-nicotinic acetylcholine α_4 receptor (α_4 -nAChR; 1:1000; ANC-004-50UL, Alomone Labs, Jerusalem, Israel) antibodies. Then, the blots were washed and incubated with HRP-conjugated goat anti-rabbit IgG antibody (1:1000 dilution; Jackson ImmunoResearch Inc., West Grove, PA, USA), and developed by Luminata Forte Western HRP substrate

(Millipore, Billerica, MA, USA). The images were recorded using the luminescence imaging system (LAS-4000, Fujifilm, Tokyo, Japan). A gel analysis plug-in for the ImageJ software was used to quantify the intensity of the protein bands.

Statistical Analysis of Grouped Data

Non-parametric statistical analyses between groups were tested using a Wilcoxon two-sample test. To assess performance with the water reward-related lever-pressing learning, we analyzed the effect of DBS on the changes in LFP PSD in multiple areas by averaging the power over each frequency point in multiple spectral bands, and then performing a Wilcoxon signed-rank test ($N = 10$) on PSD in each frequency band as compared with those before behavioral training.

After the water reward-related lever-pressing learning, the Wilcoxon two-sample test was used to compare the differences in LFP PSD and the changes in synchronization (coherence) between the DBS and sham control groups. The significance level was corrected to $P < 0.0125$ using a Bonferroni correction for the comparison of four bands. The comparison of *c-Fos* expression and Drd2 and $\alpha 4$ -nAChR protein expression (Western blotting) between the groups was explored using the Wilcoxon two-sample test. A probability value of $P < 0.05$ was used as the criterion to determine statistical significance. The resulting mean values and standard error (mean $\pm$ SEM) for the data, including cumulative time to reach the successful instrumental skill, LFP spectrum and coherence, and expression of *c-Fos*, Drd2, and $\alpha 4$ -nAChR proteins, are presented in the text.

RESULTS

Behavioral Task Comparison: CT-DBS vs. Sham Control

To examine whether CT-DBS has an effect on cognitive function, we developed a water reward-related lever-pressing learning for the rats. The trained rats had to press the lever on the left side (1st action) of the box and then went along the U-shaped path to a water port on the right side of the box within 3 s to receive a reward (2nd action; Supplementary Video 1). Behavioral data showed the animals in CT-DBS group completed the lever-pressing task in 2th or 3th day while the animals in sham control group completed the lever-pressing task in 3th or 4th day as shown in **Figure 1C**. The learning criterion was defined by consecutively repeating the lever-press—water association more than five times during the study. Behavioral data showed animals in the DBS-treated group (7.84 ± 0.7 h) had a significantly shorter cumulative time to reach the criterion ($*P < 0.001$, Wilcoxon two-sample tests, $N = 10$) as compared with sham control animals (15.13 ± 0.5 h, $N = 10$), as shown in **Figure 1D**. The data suggested that the rats treated with CT-DBS had an enhanced rate of acquisition of the task of performing lever-pressing learning in comparison to sham control rats.

Neural Oscillation Comparison: CT-DBS vs. Sham Control

The neuronal activities of the stimulated brain regions might have directly participated in the enhancement of reward-related

lever-pressing learning, so we also recorded the LFP signals from the CL, Vstr, and Dstr, which have been shown to be associated with reward-related learning in rats after behavioral tasks. The oscillations, including delta, theta, alpha and beta were examined. There were no significant PSD differences in the CL, Vstr, and Dstr between before and after the reward-related lever-pressing learning in the sham control group as shown in the upper row of **Figure 2** ($*P > 0.0125$, Wilcoxon signed-rank tests with a Bonferroni correction, $N = 10$).

In the DBS-treated group, the statistical analysis revealed that the theta and alpha bands in the CL robustly increased to a level of $236 \pm 42\%$ ($*P < 0.0125$, Wilcoxon signed-rank tests with a Bonferroni correction, $N = 10$) and $260 \pm 88\%$ ($*P < 0.0125$, Wilcoxon signed-rank tests with a Bonferroni correction, $N = 10$), respectively, compared with those data before reward-related lever-pressing learning (the lower row of **Figure 2**). Therefore, enhancement of theta and alpha oscillations in the CL, Vstr, and Dstr might be highly associated with reward-related lever-pressing behavior (instrumental skill learning). Detailed PSD traces from the sham control and CT-DBS groups are shown in the Figure S2.

For comparison of LFP PSD differences between groups after the completion of behavioral testing, statistical analysis of the group data, shown in **Figure 3**, revealed that DBS treatment altered the amplitude of PSD peaks, and elevated the significantly higher spectral density over the theta band in the CL ($*P < 0.0125$, Wilcoxon two-sample tests with a Bonferroni correction, $N = 10$), and theta and alpha bands in the ventral striatum ($*P < 0.0125$, Wilcoxon two-sample tests with a Bonferroni correction, $N = 10$). There was significant elevation in the theta power after DBS treatment in the dorsal striatum compared with the sham control group ($*P < 0.0125$, Wilcoxon two-sample tests with a Bonferroni correction, $N = 10$). Our results indicate that CT-DBS drastically increases oscillations, especially for the theta band, and might contribute to cognition related learning ability.

Functional Connectivity Comparison: CT-DBS vs. Sham Control

To compare the effect of CT-DBS on functional connectivity among local populations of neurons in the CL, Vstr, and Dstr, we used the coherence between aggregated neuronal activities as an index of functional connectivity. Correlation matrices with delta, theta, alpha, and beta bands, respectively, are shown in Figure S3.

Further, we examined the changes in inter- and intra-hemispheric coherences across six brain areas (**Figures 4A,B**), where normalized synchronization changes were quantified between LFP channel-pairs at various frequency bands. The comparison shown in **Figure 4C** illustrated that the coherence changes of the CT-DBS group in delta band were significantly increased in the right hemispheric $\Delta\text{Coh}^{\text{intra}}$ (CL – Vstr) %, $\Delta\text{Coh}^{\text{intra}}$ (CL – Dstr) %, and $\Delta\text{Coh}^{\text{intra}}$ (Vstr – Dstr) %, as compared with the sham control group. In addition, most of the coherence changes in the theta band were largely increased except for the left hemispheric $\Delta\text{Coh}^{\text{intra}}$ (CL – Vstr). Moreover, the coherence changes in the alpha band were significantly increased in the right hemispheric $\Delta\text{Coh}^{\text{intra}}$ (CL – Vstr) %

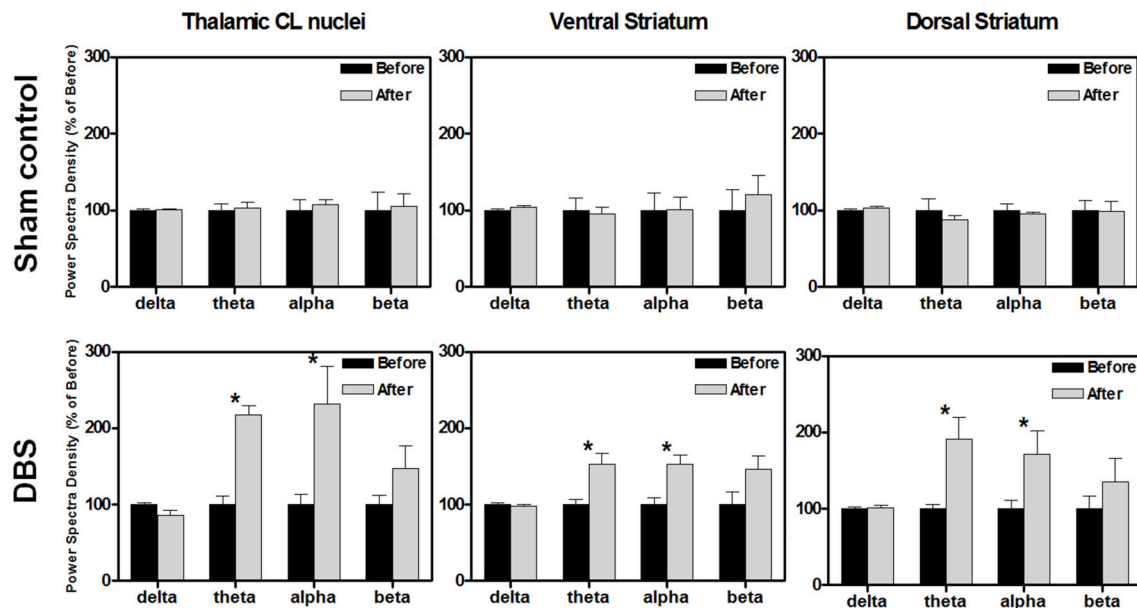


FIGURE 2 | The comparison % of the power spectral density (PSD) changes of the central lateral thalamic nucleus (CL), ventral striatum (Vstr), and dorsal striatum (Dstr), before and after the water reward-related lever-pressing learning in the sham control group (upper row) and DBS group (lower row), respectively. The calculated PSD changes of the CL, Vstr, and Dstr were the average of bilateral-channel recordings. The symbol * indicates significant different means with $P < 0.0125$ compared with the respective sham control, and analyzed by Bonferroni correction for multiple comparisons, $N = 10$. Mean $\pm$ SEM%.

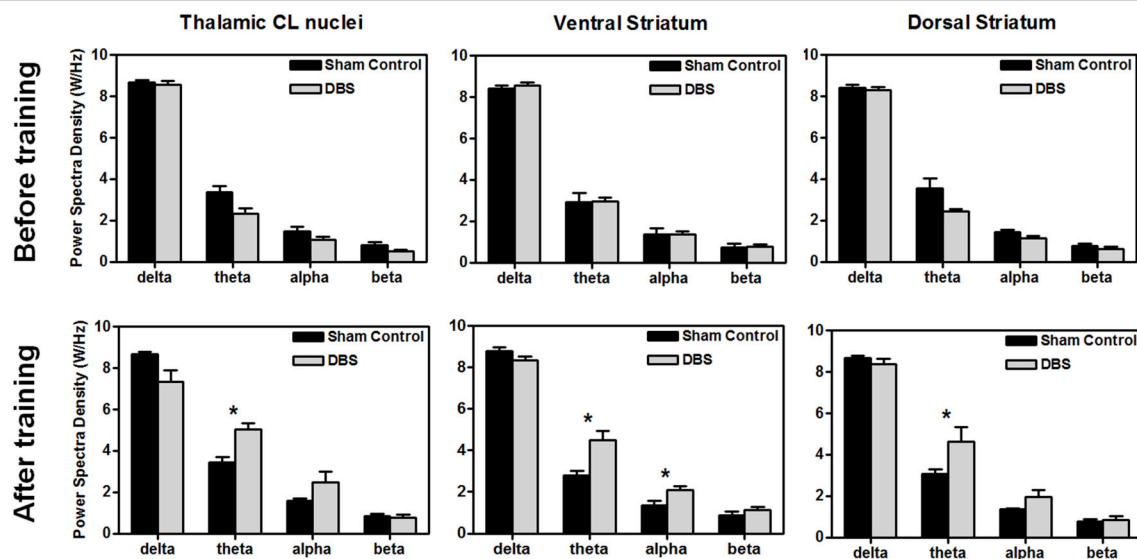


FIGURE 3 | Comparison of power spectral density (PSD) between groups before (upper row) and after (lower row) the water reward-related lever-pressing learning, respectively. The calculated PSDs of the central lateral thalamic nucleus (CL), ventral striatum (Vstr), and dorsal striatum (Dstr) were the average of bilateral-channel recordings. The symbol * indicates significant different means with $P < 0.0125$ compared with respect to the sham control (Bonferroni correction for multiple comparisons, $N = 10$). Mean $\pm$ SEM.

and inter-hemispheric $\Delta\text{Coh}^{\text{inter}}$ (CL – CL). However, there was no significant coherence change in the beta band. The more detailed description for the comparison of functional connectivity of the paired brain areas was in the Supplementary Note.4.

C-Fos Expression Comparison: CT-DBS vs. Sham Control

The expression of neuronal *c-Fos* is a well-known marker of neuronal activity. After completing the behavioral training, animals treated with CT-DBS (or sham) were held for an

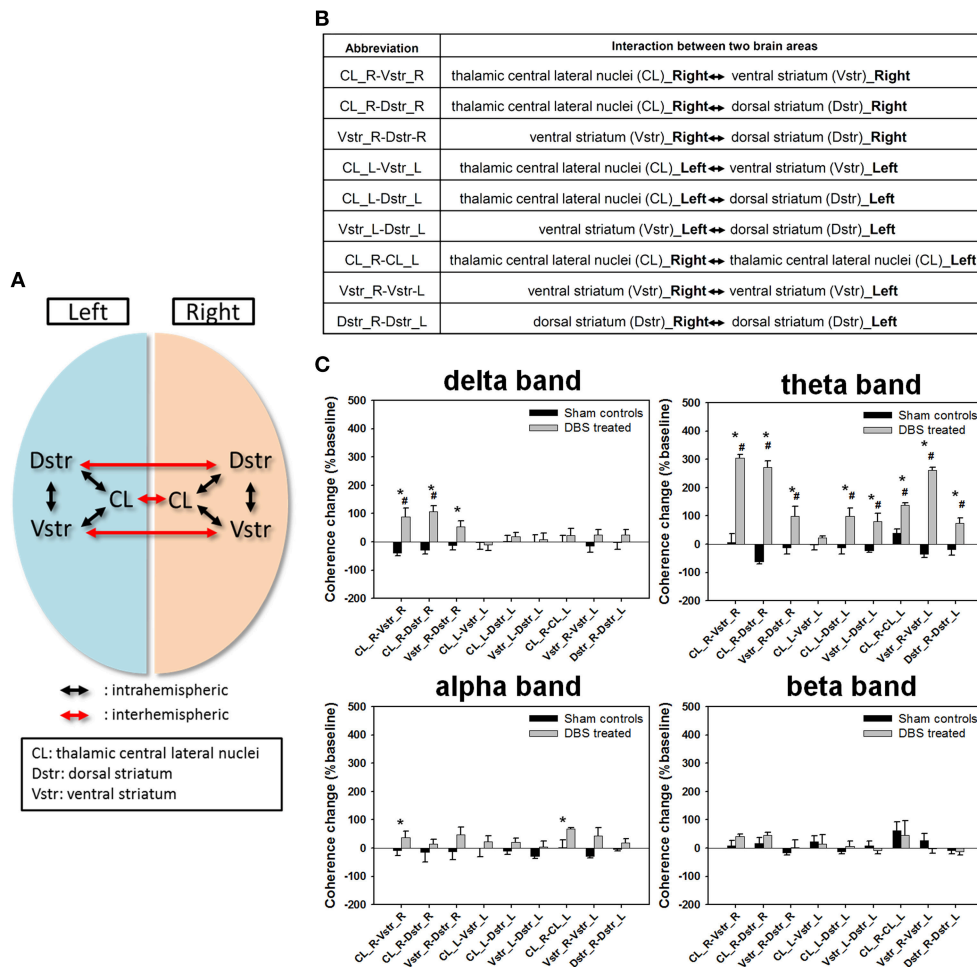


FIGURE 4 | (A) The schematics of intrahemispheric and interhemispheric coherences measured between two different brain areas. **(B)** Abbreviation table of intrahemispheric and interhemispheric coherences between two different brain areas. **(C)** Statistical comparison of the coherence changes of the functional connectivity between two recording regions (bilateral central lateral thalamic nucleus [CL], ventral striatum [Vstr], and dorsal striatum [Dstr]). Data are expressed as means $\pm$ SEM%. * $P < 0.0125$ significant differences in coherence changes between the sham control and DBS-treated groups with the Bonferroni correction for multiple comparisons. # $P < 0.0125$ significant coherence changes compared to baseline between same brain areas with the Bonferroni correction for multiple comparisons.

additional 30 min and then single housed for 2 h for the peak expression of the *c-Fos* protein after cell activation in the isolated environment (Waters et al., 1997). In the comparison of neuronal activation distribution between CT-DBS and sham control groups, we found enhancement of *c-Fos*-positive cells in the M1, ACC, CPu, Nac, Rsc, PtA and hippocampal CA1, CA3, and DG as illustrated in **Figure 5**.

c-Fos expression in the analyzed brain areas was normalized and expressed as % compared with the sham control group, as shown in **Figure 6**. The statistical analysis revealed a significant increase in *c-Fos*-positive neurons in the CT-DBS group relative to the sham control group, observed in the M1 ($347 \pm 39\%$; *** $P < 0.001$, Wilcoxon two-sample tests, $N = 5$), ACC ($256 \pm 46\%$; ** $P < 0.01$, Wilcoxon two-sample tests, $N = 5$), CPu ($207 \pm 39\%$; * $P < 0.05$, Wilcoxon two-sample tests, $N = 5$), NAc ($270 \pm 32\%$; *** $P < 0.001$, Wilcoxon two-sample tests, $N = 5$), Rsc ($184 \pm 24\%$; * $P < 0.05$, Wilcoxon two-sample tests, $N = 5$),

PtA ($221 \pm 35\%$; * $P < 0.05$, Wilcoxon two-sample tests, $N = 5$), hippocampal CA1 ($322 \pm 25\%$; *** $P < 0.001$, Wilcoxon two-sample tests, $N = 5$), CA3 ($167 \pm 35\%$; * $P < 0.05$, Wilcoxon two-sample tests, $N = 5$), and DG ($150 \pm 27\%$; * $P < 0.05$, Wilcoxon two-sample tests, $N = 5$). Taken together, our results show that CT-DBS significantly increases widespread neuronal activity, however the mechanism of interaction in the activated brain regions, which conveys the cognitive enhancement, needs to be further characterized.

Dopamine And Acetylcholine Receptors are Up-Regulated by CT-DBS

The striatal neural circuits are composed of dopaminergic and cholinergic synapses that receive signals from the cerebral cortex and propagate them to the basal ganglia to modulate reward-related learning. Thus, we examined the protein levels of Drd2 and $\alpha 4$ -nAChR in the striatum by Western blot analysis. In

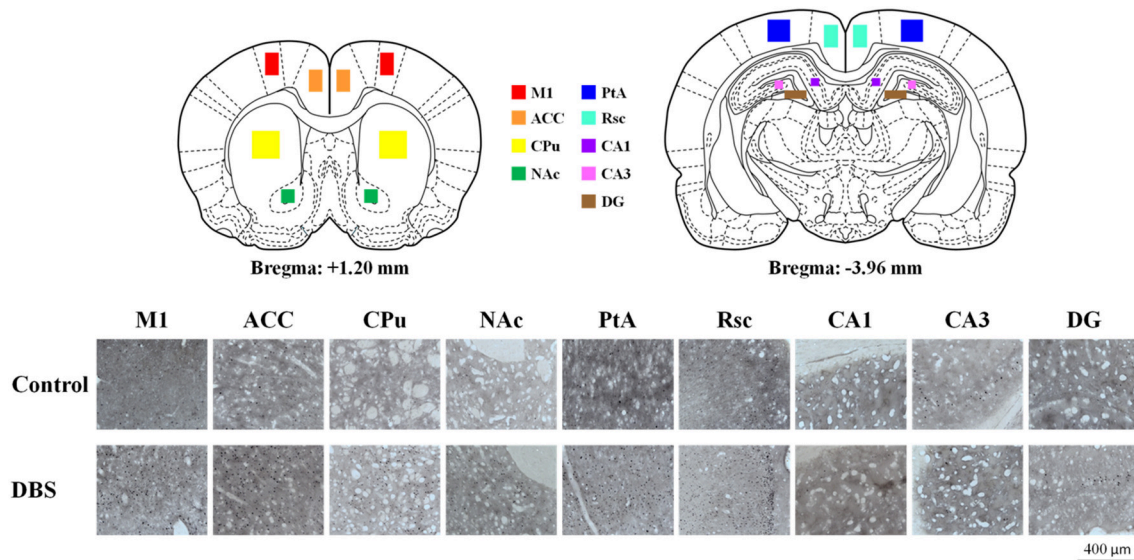


FIGURE 5 | C-Fos expression was up-regulated by CT-DBS. The cartoon depicts a representative area of *c-Fos* staining quantification from the nine brain regions where *c-Fos* was counted (primary motor cortex [M1], anterior cingulate cortex [ACC], caudate-putamen in dorsal striatum [CPu], accumbens nucleus in ventral striatum [NAc], retrosplenial cortex [Rsc], parietal association cortex [PtA], and hippocampus [CA1, CA3, and DG]). Representative photomicrographs show the immunostaining for *c-Fos* expression in each brain region from sham control and CT-DBS samples.

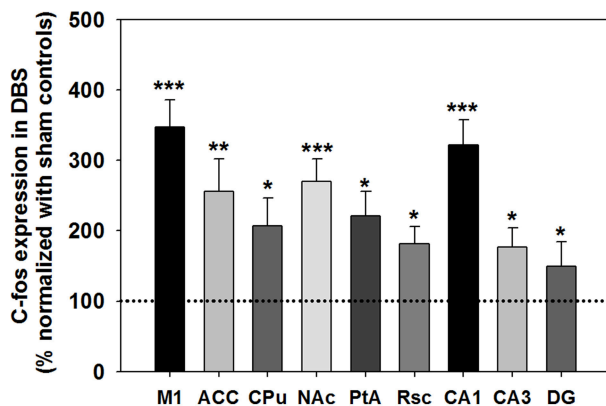


FIGURE 6 | The *c-Fos* expression after DBS was assessed and normalized to sham control (primary motor cortex [M1], anterior cingulate cortex [ACC], caudate-putamen in the dorsal striatum [CPu], accumbens nucleus in the ventral striatum [NAc], retrosplenial cortex [Rsc], parietal association cortex [PtA], and hippocampus [CA1, CA3 and DG]). *, **, and *** indicate significant *c-Fos* expression with $P < 0.05$, $P < 0.01$, and $P < 0.001$ compared with the sham control, analyzed by Wilcoxon two-sample tests (Mean $\pm$ SEM). The dotted line is the baseline of *c-Fos* expression (100%) for the sham control group.

the top row of **Figure 7A**, the results revealed up-regulation of Drd2 and $\alpha 4$ -nAChR in the striatum after DBS treatment compared with sham controls. The statistical analysis indicated that the striatal levels of Drd2 and $\alpha 4$ -nAChR protein were significantly increased to 120.3 ± 9.9 and $124.9 \pm 10.1\%$, respectively, compared with the respective sham controls, $N = 5$ (**Figure 7B**). The levels of hippocampal Drd2 and $\alpha 4$ -nAChR

protein, as shown in the bottom row of **Figure 7A**, were also significantly increased to 135.0 ± 5.0 and $152.0 \pm 8.8\%$ compared to the respective sham controls, $N = 5$ (**Figure 7B**). The data demonstrate CT-DBS-induced up-regulation of Drd2 and $\alpha 4$ -nAChR receptor expression in the striatum and hippocampus.

DISCUSSION

Our study demonstrated that electrical stimulation of CT produced a novel enhancement of the water-reward lever-pressing behavior associated with instrumental learning (operant conditioning), the increasing in neural theta oscillations, and the strength of connections of CT with the striatum. Meanwhile, CT-DBS induced widespread *c-Fos* expressions in cortical and subcortical areas since CT widely projected to striatum as well as cortical targets. Significant both Drd2 and $\alpha 4$ -nAChR expressions were found in hippocampus and striatum as well.

CT-DBS Enhanced Theta LFP Oscillation as the Biomarker for Correlated to Instrumental Learning

CT-DBS increased both theta and alpha LFP oscillations in CT, Vstr, and Dstr, and largely enhanced water-reward lever-pressing associated with instrumental learning (operant conditioning) as well. Our findings are consistent with a series of studies that alpha and theta LFP oscillations has been potentiated in learning and memory processing of cortical and subcortical regions (Klimesch et al., 1997; Buzsaki, 2002; Knyazev, 2007; Kirov et al., 2009). Consistent with the above proposed neural oscillations for the instrumental learning regulation, Shirvalkar

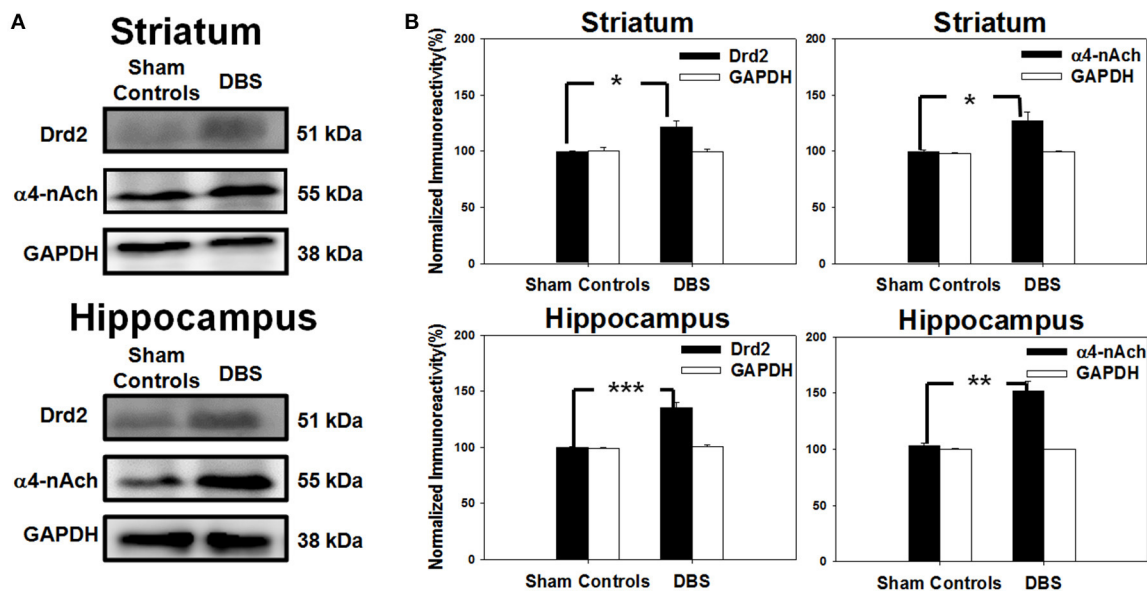


FIGURE 7 | Western blot protein analysis of cells excised individually from striatal and hippocampal tissues. (A) The SDS-PAGE blots show the expression of dopamine D2 receptor (Drd2) and $\alpha 4$ -nicotinic acetylcholine receptor ($\alpha 4$ -nAChR) in the striatum (top) and hippocampus (bottom). **(B)** Results from the quantitative analysis of Drd2 and $\alpha 4$ -nAChR expression (mean $\pm$ SEM; expressed as ratio to GAPDH) in the striatum (top) and hippocampus (bottom). Striatal and Drd2 and $\alpha 4$ -nAChR protein expressions were significantly increased relative to sham control group. Significant increases in Drd2 and $\alpha 4$ -nAChR protein expressions in the hippocampus also were found. *, **, and *** indicate significant protein expression with $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, relative to sham control group.

et al. (2006) demonstrated CT-DBS increased generalized arousal and recognition memory performance, such as untrained goal-directed seeking behavior, exploratory motor activity, grooming, and object recognition memory through selective network activation in intact rats. Therefore, CT-DBS enhancement for the water-reward lever-pressing learning was characterized by the prominent theta LFP oscillation, as a reliable biomarker correlated to animal skill learning, recorded throughout the thalamic-striatal neural circuit.

CT-DBS Contribution of Connections of CT with the Striatum

Our results showed that the significantly increasing intrahemispheric and interhemispheric theta-band coherences in the paired brain areas of CT–Vstr, CT–Dstr in the CT-DBS treated animals. The functional connectivity between the CT and striatum consistent with other reports, including the neuroanatomical mechanisms (Deschenes et al., 1996) and neural signal processing (Yin and Knowlton, 2006) for the modulation of motor control and learning ability. The CT-DBS enhanced local theta-band activities synchronized between distant areas in the thalamic-striatal circuit indicating that excitatory long-range projections functionally coupled CT and striatum connections in this study (Womelsdorf et al., 2010). Meanwhile, our results showed that the CT-DBS modulated the connectivity of Vstr – Dstr with significantly increasing the theta-band coherence, indicating that the Vstr (motor) and Dstr (associative) were both strongly activated simultaneously during the water reward-related skill-learning (Cardinal and Cheung, 2005; Thorn and Graybiel, 2014; Nagel et al., 2015).

In addition, our observation that the alpha- and beta- band coherences in sham control group were slightly decreased without significance in water-reward lever-pressing learning task. During the behavioral task, many factors might influence the changes in strength of the functional connectivity including how thirsty the animal is, how desirable the reward is, or how familiar the animal is with the environment. From animal behavioral video recording in the sham control group, we found animals less actively exploring their environment after reaching the criterion of the behavioral task when compared to the exploratory activity before training. Less rats' exploratory behavior in the late phase could hypothetically reflect any or all of the several internal factors, including most obviously lack of thirst (others include frustration and fatigue). Therefore, the slight decreases in alpha- and beta- band coherences might be due to less reward-motivated behavior toward the tasks in the rats (Sturman and Moghaddam, 2011; Neale et al., 2015). Furthermore, many studies have reported striatal LFPs modulation in the theta band during exploratory behavior (Tort et al., 2008; Lepski et al., 2012). Oscillatory activity was also observed in the delta and beta bands (Hasselmo et al., 2002; Lepski et al., 2012) as well. For functional connectivity, striatal LFPs were also oscillated in strong coherence with the theta rhythm in the prefrontal cortex and thalamus (Ishii et al., 1999; McCracken and Grace, 2009). In the sham control group, animals performed less exploration after the behavioral task, which might be the causes of the slight decrease in the theta-, delta-, and beta- band coherences when compared to the animals before training.

One of the unanticipated findings in our study was that the bilateral CT-DBS increased the coherence change at the

delta band in the intra-hemisphere lead to enhancing the lateralized motor skill leaning (the water reward-related lever-pressing learning). To our knowledge, this is the first report of lateralization effect in coherence by bilateral CT-DBS. This effect might be due to that the rats preferred to press the lever by forelimb (handedness; data not shown). However, the function of hemispheric lateralization or asymmetry effect related to CT-DBS increased skill learning needs to be further investigated. Taken together, our results provided evidence that the neuronal activities, especially theta oscillations, in CT and striatum are highly correlated to animal skill learning and modulated by CT-DBS treatment.

CT-DBS Molecularly Widespread Neuromodulation

DBS is an established therapeutic approach to modulating abnormal neuronal firing of the subthalamic nucleus or internal segments of the globus pallidus for Parkinson's disease patients, whereas several findings have revealed that DBS also functions as a stimulation device to activate the neural network for cognitive functions and alter underlying molecular modification, such as gene expression (Shirvankar et al., 2006). Accordingly, a well-known activity-dependent neuronal marker, *c-Fos*, is largely up-regulated in several brain regions of CT-DBS rats, especially the Dstr, Vstr, hippocampus, Rsc and PtA. The *c-Fos* up-regulation in the CPu and Dstr, and the NAc in the Vstr demonstrates that CT-DBS activates and increases connectivity between Vstr and Dstr to enhance motor ability and reward-related skill learning. We observed *c-Fos* expression was also elevated among the hippocampus (CA3 and CA1), ACC, Rsc, PtA, and M1. Hippocampus has been demonstrated that it is involved control and process of learning and memory signals (Kesner, 2013). One study revealed that the ACC might regulate cognitive and emotional processing, even motor and sensory functions (Bush et al., 2000). In addition, Rsc and PtA have emerged as key regions of the brain network that supports spatial navigation and short term memory (Maguire, 2001; Vann et al., 2009). Recently finding indicated that the ACC projects axons to the Rsc and PtA, and should be considered an important component related to reward anticipation, decision-making, impulse control, and emotion (Ragozzino and Rozman, 2007). Taken together, the results suggest that CT-DBS causes widespread cerebral network activation that is associated with cognition and motor function by up-regulation of immediate early gene expression.

CT-DBS Enhanced Functional Synaptic Connections Associated with Drd2 and $\alpha 4$ -nAChR

We further examined the synaptic neurotransmitter mechanisms underlie CT-DBS induced the enhancement of functional connectivity and water-reward level pressing learning. Our data showed the expression of Drd2 and $\alpha 4$ -nAChR was increased in striatum and hippocampus following CT-DBS. Previous findings suggested striatum is the main component mediating various inputs of the basal ganglia neural circuit, and the interaction of dopaminergic and cholinergic signaling is crucial for cognitive functions, motor activity, and reward-related

information (Calabresi et al., 2006; Cragg, 2006; Calabresi and Di Filippo, 2008). The Drd2 participates in modulating local motor activity, schizophrenia, and working memory in the prefrontal cortex (Luciana et al., 1992; Baik et al., 1995; Wang et al., 2004). Dorsolateral striatum is involved in the initial discrimination of exercise-associated tasks mediated by up-regulation of striatal Drd2 (Eddy et al., 2014). Additionally, activation of $\alpha 4$ -nAChR in striatum and hippocampus increased following CT-DBS. Meanwhile, the nicotinic cholinergic system plays a pivotal role in working memory and attention in the hippocampal and prefrontal regions (Levin and Simon, 1998; Ross et al., 2000; Labarca et al., 2001). Thus, we mentioned that dopaminergic and cholinergic systems are the major neurotransmission system in water reward-related skill-learning. Applied of CT-DBS evoked an up-regulation of Drd2 in the stratum, indicating that the enhancement of reward-associated learning might be due to increase activity of the Drd2. This suggests that the inter-regional connectivity enhancement might contribute to synaptic plasticity, at least in the striatum, by altering expression of dopaminergic and cholinergic receptors that modulate striatal synaptic plasticity to regulate downstream signaling cascades for higher brain reward-related skill learning process.

This study suggested that DBS at CT modulates a cortical network for reward-related skill-learning behavior. CT-DBS is seen to produce increase of oscillation patterns and functional connectivity of thalamus-striatum network, especially in the theta band. We also delineated a possible underlying molecular mechanism that involves activation of neuronal projections from the CL to striatal dopaminergic neurons and up-regulation of the *c-Fos*, Drd2, and $\alpha 4$ -nAChR of the striatum to modulate higher cognitive learning function. Our future studies will further explore the function of these circuits in small animal model with neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

HCL, HYL, and YYC designed the project, organized the entire research. SL, HP, and YL conceived the experiments. HP, YL, ES, LL, PL, YWC, and YYC conducted the experiments. HCP, YL, YYC, KL, and FJ analyzed the results. HCL, HYL, and KC performed the immunohistochemistry and Western blot studies. HCL, ES, HYL, and YYC wrote the manuscript. All authors discussed the results and reviewed on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fncir.2015.00087>

REFERENCES

- Atallah, H. E., Lopez-Paniagua, D., Rudy, J. W., and O'Reilly, R. C. (2007). Separate neural substrates for skill learning and performance in the ventral and dorsal striatum. *Nat. Neurosci.* 10, 126–131. doi: 10.1038/nn1817
- Baik, J. H., Picetti, R., Saiardi, A., Thiriet, G., Dierich, A., Depaulis, A., Borrelli, E. (1995). Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. *Nature* 377, 424–428. doi: 10.1038/377424a0
- Baizabal Carvallo, J. F., Simpson, R., and Jankovic, J. (2011). Diagnosis and treatment of complications related to deep brain stimulation hardware. *Mov. Disord.* 26, 1398–1406. doi: 10.1002/mds.23800
- Balleine, B. W., Killcross, A. S., and Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *J. Neurosci.* 23, 666–675.
- Basar, E., Basar-Eroglu, C., Karakas, S., and Schürmann, M. (2001). Gamma, alpha, delta, and theta oscillations govern cognitive processes. *Int. J. Psychophysiol.* 39, 241–248. doi: 10.1016/S0167-8760(00)00145-8
- Bergman, H., Feingold, A., Nini, A., Raz, A., Slovin, H., Abeles, M., et al. (1998). Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. *Trends Neurosci.* 21, 32–38.
- Bush, G., Luu, P., and Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn. Sci.* 4, 215–222. doi: 10.1016/S1364-6613(00)01483-2
- Buzsáki, G. (2002). Theta oscillations in the hippocampus. *Neuron* 33, 325–340. doi: 10.1016/S0896-6273(02)00586-X
- Calabresi, P., and Di Filippo, M. (2008). ACh/dopamine crosstalk in motor control and reward: a crucial role for alpha 6-containing nicotinic receptors? *Neuron* 60, 4–7. doi: 10.1016/j.neuron.2008.09.031
- Calabresi, P., Picconi, B., Parnetti, L., and Di Filippo, M. (2006). A convergent model for cognitive dysfunctions in Parkinson's disease: the critical dopamine-acetylcholine synaptic balance. *Lancet Neurol.* 5, 974–983. doi: 10.1016/S1474-4422(06)70600-7
- Cardinal, R. N., and Cheung, T. H. C. (2005). Nucleus accumbens core lesions retard instrumental learning and performance with delayed reinforcement in the rat. *BMC Neurosci.* 6:9. doi: 10.1186/1471-2202-6-9
- Chen, C. H., Fremont, R., Arteaga-Bracho, E. E., and Khodakhah, K. (2014). Short latency cerebellar modulation of the basal ganglia. *Nat. Neurosci.* 17, 1767–1775. doi: 10.1038/nn.3868
- Cragg, S. J. (2006). Meaningful silences: how dopamine listens to the ACh pause. *Trends Neurosci.* 29, 125–131. doi: 10.1016/j.tins.2006.01.003
- Deschenes, M., Bourassa, J., and Parent, A. (1996). Striatal and cortical projections of single neurons from the central lateral thalamic nucleus in the rat. *Neuroscience* 72, 679–687.
- Deuschl, G., Raethjen, J., Lindemann, M., and Krack, P. (2001). The pathophysiology of tremor. *Muscle Nerve* 24, 716–735. doi: 10.1002/mus.1063
- Eddy, M. C., Stansfield, K. J., and Green, J. T. (2014). Voluntary exercise improves performance of a discrimination task through effects on the striatal dopamine system. *Learn. Mem.* 21, 334–337. doi: 10.1101/lm.034462.114
- Ego-Stengel, V., and Wilson, M. A. (2010). Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. *Hippocampus* 20, 1–10. doi: 10.1002/hipo.20707
- Fell, J., Staesina, B. P., Do Lam, A. T., Widman, G., Helmstaedter, C., Elger, C. E., et al. (2013). Memory modulation by weak synchronous deep brain stimulation: a pilot study. *Brain Stimul.* 6, 270–273. doi: 10.1016/j.brs.2012.08.001
- Flora, E. D., Perera, C. L., Cameron, A. L., and Madder, G. J. (2010). Deep brain stimulation for essential tremor: a systematic review. *Mov. Disord.* 25, 1550–1559. doi: 10.1002/mds.23195
- Fries, P. (2005). A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. *Trends Cogn. Sci.* 9, 474–480. doi: 10.1016/j.tics.2005.08.011
- Halgren, E., Wilson, C. L., and Stapleton, J. M. (1985). Human medial temporal-lobe stimulation disrupts both formation and retrieval of recent memories. *Brain Cogn.* 4, 287–295. doi: 10.1016/0278-2626(85)90022-3
- Hasselmo, M. E., Hay, J., Ilyn, M., and Gorchetnikov, A. (2002). Neuromodulation, theta rhythm and rat spatial navigation. *Neural Netw.* 15, 689–707. doi: 10.1016/S0893-6080(02)00057-6
- Ishii, R., Shinosaki, K., Ukai, S., Inouye, T., Ishihara, T., Yoshimine, T., et al. (1999). Medial prefrontal cortex generates frontal midline theta rhythm. *Neuroreport* 10, 675–679.
- Jacquet, M., Lecourtier, L., Cassel, R., Loureiro, M., Cosquer, B., Escoffier, G., et al. (2013). Dorsolateral striatum and dorsal hippocampus: a serial contribution to acquisition of cue-reward associations in rats. *Behav. Brain Res.* 239, 94–103. doi: 10.1016/j.bbr.2012.10.061
- Jones, E. G. (2009). Synchrony in the interconnected circuitry of the thalamus and cerebral cortex. *Ann. N. Y. Acad. Sci.* 1157, 10–23. doi: 10.1111/j.1749-6632.2009.04534.x
- Kesner, R. P. (2013). A process analysis of the CA3 subregion of the hippocampus. *Front. Cell. Neurosci.* 7:78. doi: 10.3389/fncel.2013.00078
- Kirov, R., Weiss, C., Siebner, H. R., Born, J., and Marshall, L. (2009). Slow oscillation electrical brain stimulation during waking promotes EEG theta activity and memory encoding. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15460–15465. doi: 10.1073/pnas.0904438106
- Klimesch, W., Doppelmayr, M., Pachinger, T., and Ripper, B. (1997). Brain oscillations and human memory: EEG correlates in the upper alpha and theta band. *Neurosci. Lett.* 238, 9–12.
- Knyazev, G. G. (2007). Motivation, emotion, and their inhibitory control mirrored in brain oscillations. *Neurosci. Biobehav. Rev.* 31, 377–395. doi: 10.1016/j.neubiorev.2006.10.004
- Kolb, B., Sutherland, R. J., and Whishaw, I. Q. (1983). A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats. *Behav. Neurosci.* 97, 13–27.
- Labarca, C., Schwarz, J., Deshpande, P., Schwarz, S., Nowak, M. W., Fonck, C., et al. (2001). Point mutant mice with hypersensitive alpha 4 nicotinic receptors show dopaminergic deficits and increased anxiety. *Proc. Natl. Acad. Sci. U.S.A.* 98, 2786–2791. doi: 10.1073/pnas.041582598
- Lacruz, M. E., Valentin, A., Seoane, J. J. G., Morris, R. G., Selway, R. P., and Alarcón, G. (2010). Single pulse electrical stimulation of the hippocampus is sufficient to impair human episodic memory. *Neuroscience* 170, 623–632. doi: 10.1016/j.neuroscience.2010.06.042
- Lee, H., Fell, J., and Axmacher, N. (2013). Electrical engram: how deep brain stimulation affects memory. *Trends Cogn. Sci.* 17, 574–584. doi: 10.1016/j.tics.2013.09.002
- Lepski, G., Arévalo, A., do Valle, A. C., Ballester, G., and Gharabaghi, A. (2012). Increased coherence among striatal regions in the theta range during attentive wakefulness. *Braz. J. Med. Biol. Res.* 45, 763–770. doi: 10.1590/S0100-879X2012007500104
- Leung, L. S., and Shen, B. (2006). Hippocampal CA1 kindling but not long-term potentiation disrupts spatial memory performance. *Learn. Mem.* 13, 18–26. doi: 10.1101/lm.66106
- Levin, E. D., and Simon, B. B. (1998). Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology (Berl)* 138, 217–230.
- Luciana, M., Depue, R. A., Arbisi, P., and Leon, A. (1992). Facilitation of working memory in humans by a d2 dopamine receptor agonist. *J. Cogn. Neurosci.* 4, 58–68. doi: 10.1162/jocn.1992.4.1.58
- Maguire, E. A. (2001). The retrosplenial contribution to human navigation: a review of lesion and neuroimaging findings. *Scand. J. Psychol.* 42, 225–238. doi: 10.1111/1467-9450.00233
- McCracken, C. B., and Grace, A. A. (2009). Nucleus accumbens deep brain stimulation produces region-specific alterations in local field potential oscillations and evoked responses *in vivo*. *J. Neurosci.* 29, 5354–5363. doi: 10.1523/JNEUROSCI.0131-09.2009
- Mengual, E., de las Heras, S., Erro, E., Lanciego, J. L., and Giménez-Amaya, J. M. (1999). Thalamic interaction between the input and the output systems of the basal ganglia. *J. Chem. Neuroanat.* 16, 187–200. doi: 10.1016/S0891-0618(99)00010-1
- Nagel, S. J., Machado, A. G., Gale, J. T., Lobel, D. A., and Pandya, M. (2015). Preserving cortico-striatal function: deep brain stimulation in Huntington's disease. *Front. Syst. Neurosci.* 9:32. doi: 10.3389/fnsys.2015.00032
- Neale, C., Johnston, P., Hughes, M., and Scholey, A. (2015). Functional activation during the rapid visual information processing task in a middle aged cohort: an fMRI study. *PLoS ONE* 10:e0138994. doi: 10.1371/journal.pone.0138994
- Overbeek, J. M., de Koning, P., Luigjes, J., van den Munckhof, P., Schuurman, P. R., and Denys, D. (2013). Deep brain stimulation for psychiatric disorders. *Ned. Tijdschr. Geneesk.* 157, A7015.
- Parikh, V., and Sarter, M. (2008). Cholinergic mediation of attention: contributions of phasic and tonic increases in prefrontal cholinergic activity. *Ann. N. Y. Acad. Sci.* 1129, 225–235. doi: 10.1196/annals.1417.021

- Paxinos, G., and Watson, C. (2005). *The Rat Brain in Stereotaxic Coordinates*. Amsterdam; Boston, MA: Elsevier Academic Press.
- Ragozzino, M. E., and Rozman, S. (2007). The effect of rat anterior cingulate inactivation on cognitive flexibility. *Behav. Neurosci.* 121, 698–706. doi: 10.1037/0735-7044.121.4.698
- Robbins, T. W., and Arnsten, A. F. (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annu. Rev. Neurosci.* 32, 267–287. doi: 10.1146/annurev.neuro.051508.135535
- Ross, S. A., Wong, J. Y., Clifford, J. J., Kinsella, A., Massalas, J. S., Horne, M. K., et al. (2000). Phenotypic characterization of an alpha 4 neuronal nicotinic acetylcholine receptor subunit knock-out mouse. *J. Neurosci.* 20, 6431–6441.
- Schiff, N. D., Giacino, J. T., Kalmar, K., Victor, J. D., Baker, K., Gerber, M., et al. (2007). Behavioural improvements with thalamic stimulation after severe traumatic brain injury. *Nature* 448, 600–603. doi: 10.1038/nature06041
- Schlaepfer, T. E., and Bewernick, B. H. (2014). Neuromodulation for treatment resistant depression: state of the art and recommendations for clinical and scientific conduct. *Brain Topogr.* 27, 12–19. doi: 10.1007/s10548-013-0315-9
- Shirvalkar, P., Seth, M., Schiff, N. D., and Herrera, D. G. (2006). Cognitive enhancement with central thalamic electrical stimulation. *Proc. Natl. Acad. Sci. U.S.A.* 103, 17007–17012. doi: 10.1073/pnas.0604811103
- Skinner, B. F. (1992). 'Superstition' in the pigeon. 1948. *J. Exp. Psychol. Gen.* 121, 273–274.
- Squire, L. R., Stark, C. E., and Clark, R. E. (2004). The medial temporal lobe. *Annu. Rev. Neurosci.* 27, 279–306. doi: 10.1146/annurev.neuro.27.070203.144130
- Srinath, R., and Ray, S. (2014). Effect of amplitude correlations on coherence in the local field potential. *J. Neurophysiol.* 112, 741–751. doi: 10.1152/jn.00851.2013
- Stone, S. S., Teixeira, C. M., Devito, L. M., Zaslavsky, K., Josselyn, S. A., Lozano, A. M., et al. (2011). Stimulation of entorhinal cortex promotes adult neurogenesis and facilitates spatial memory. *J. Neurosci.* 31, 13469–13484. doi: 10.1523/JNEUROSCI.3100-11.2011
- Sturman, D. A., and Moghaddam, B. (2011). Reduced neuronal inhibition and coordination of adolescent prefrontal cortex during motivated behavior. *J. Neurosci.* 31, 1471–1478. doi: 10.1523/JNEUROSCI.4210-10.2011
- Suthana, N., and Fried, I. (2014). Deep brain stimulation for enhancement of learning and memory. *Neuroimage* 85(Pt 3), 996–1002. doi: 10.1016/j.neuroimage.2013.07.066
- Suthana, N., Haneef, Z., Stern, J., Mukamel, R., Behnke, E., Knowlton, B., et al. (2012). Memory enhancement and deep-brain stimulation of the entorhinal area. *N. Engl. J. Med.* 366, 502–510. doi: 10.1056/NEJMoa1107212
- Thorn, C. A., and Graybiel, A. M. (2014). Differential entrainment and learning-related dynamics of spike and local field potential activity in the sensorimotor and associative striatum. *J. Neurosci.* 34, 2845–2859. doi: 10.1523/JNEUROSCI.1782-13.2014
- Tort, A. B., Kramer, M. A., Thorn, C., Gibson, D. J., Kubota, Y., Graybiel, A. M., et al. (2008). Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20517–20522. doi: 10.1073/pnas.0810524105
- Vann, S. D., Aggleton, J. P., and Maguire, E. A. (2009). What does the retrosplenial cortex do? *Nat. Rev. Neurosci.* 10, 792–802. doi: 10.1038/nrn2733
- Vitek, J. L., and Giroux, M. (2000). Physiology of hypokinetic and hyperkinetic movement disorders: model for dyskinesia. *Ann. Neurol.* 47(4 Suppl. 1), S131–S140.
- Wang, M., Vijayraghavan, S., and Goldman-Rakic, P. S. (2004). Selective D2 receptor actions on the functional circuitry of working memory. *Science* 303, 853–856. doi: 10.1126/science.1091162
- Waters, N. S., Klintsova, A. Y., and Foster, T. C. (1997). Insensitivity of the hippocampus to environmental stimulation during postnatal development. *J. Neurosci.* 17, 7967–7973.
- Williams, N. R., and Okun, M. S. (2013). Deep brain stimulation (DBS) at the interface of neurology and psychiatry. *J. Clin. Invest.* 123, 4546–4556. doi: 10.1172/JCI68341
- Womelsdorf, T., Vinck, M., Leung, L. S., and Everling, S. (2010). Selective theta-synchronization of choice-relevant information subserves goal-directed behavior. *Front. Hum. Neurosci.* 4:210. doi: 10.3389/fnhum.2010.00210
- Yin, H. H., and Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nat. Rev. Neurosci.* 7, 464–476. doi: 10.1038/nrn1919
- Yin, H. H., Ostlund, S. B., and Balleine, B. W. (2008). Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. *Eur. J. Neurosci.* 28, 1437–1448. doi: 10.1111/j.1460-9568.2008.06422.x

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The Slow Oscillation in Cortical and Thalamic Networks: Mechanisms and Functions

Garrett T. Neske^{1,2*}

¹ Department of Neuroscience, Division of Biology and Medicine, Brown University, Providence, RI, USA, ² Department of Neurobiology, Yale University, New Haven, CT, USA

During even the most quiescent behavioral periods, the cortex and thalamus express rich spontaneous activity in the form of slow (<1 Hz), synchronous network state transitions. Throughout this so-called slow oscillation, cortical and thalamic neurons fluctuate between periods of intense synaptic activity (Up states) and almost complete silence (Down states). The two decades since the original characterization of the slow oscillation in the cortex and thalamus have seen considerable advances in deciphering the cellular and network mechanisms associated with this pervasive phenomenon. There are, nevertheless, many questions regarding the slow oscillation that await more thorough illumination, particularly the mechanisms by which Up states initiate and terminate, the functional role of the rhythmic activity cycles in unconscious or minimally conscious states, and the precise relation between Up states and the activated states associated with waking behavior. Given the substantial advances in multineuronal recording and imaging methods in both *in vivo* and *in vitro* preparations, the time is ripe to take stock of our current understanding of the slow oscillation and pave the way for future investigations of its mechanisms and functions. My aim in this Review is to provide a comprehensive account of the mechanisms and functions of the slow oscillation, and to suggest avenues for further exploration.

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*Correspondence:

Garrett T. Neske
garrett_neske@brown.edu

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INTRODUCTION

The mammalian neocortex is a massively interconnected synaptic network. The vast majority of excitatory synapses onto cortical excitatory neurons come from other cortical excitatory neurons (Braitenberg and Shüz, 1998; Binzegger et al., 2004; Douglas and Martin, 2004). One consequence of the vast recurrent connectivity of the neocortex is the ability to initiate and sustain patterned network activity, even in the virtual absence of sensory stimulation, such as during quiescent sleep and anesthesia. During these quiescent periods, the entire neocortex undergoes slow, synchronized transitions between vigorous synaptic activity (Up states) and relative silence (Down states). This cycling (<~1 Hz) between Up and Down states constitutes the *slow oscillation*.

The occurrence of the slow oscillation has been well-documented from intracellular and extracellular recording and imaging in various experimental preparations, including anesthetized, naturally sleeping, and quiescent waking animals. Great strides have been made in uncovering the cellular and network mechanisms involved in this widespread phenomenon. Several mechanistic

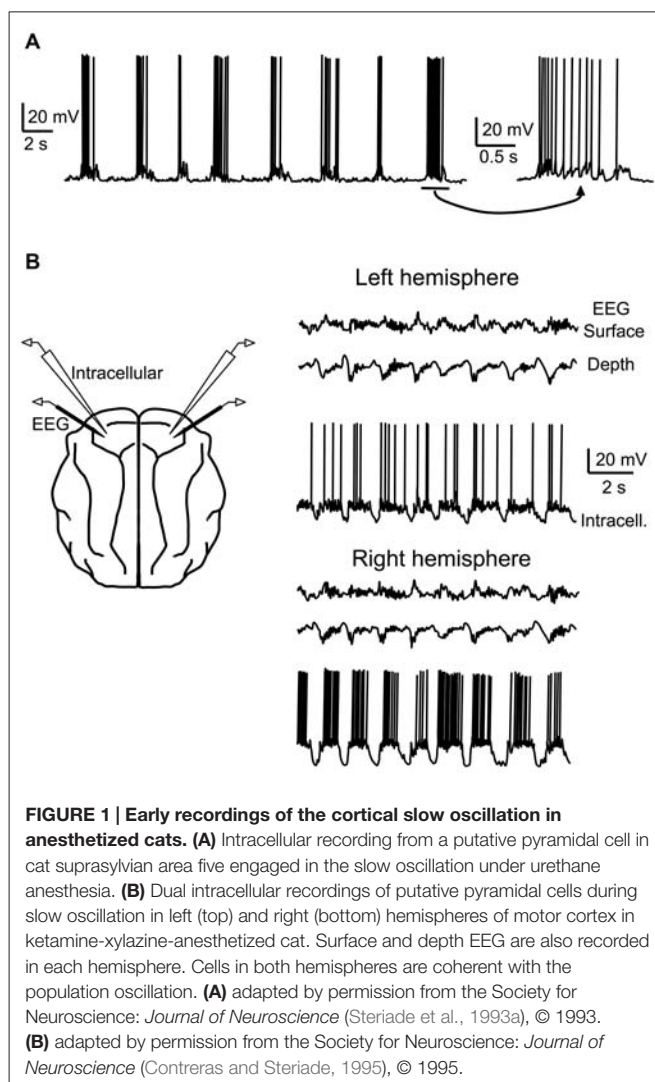
features of the slow oscillation, however, remain to be explored, especially the initiation and termination of Up states and the roles of subcortical structures in sustaining and pacing the slow oscillation in the cortex. Furthermore, very little is known about the functional roles of the slow oscillation and the exact relation between Up states and activated states of the awake cortex.

In this Review, I provide a comprehensive account of the mechanisms and functions of the slow oscillation in the cortex and thalamus and also indicate areas requiring further investigation. I first consider the phenomenology of the slow oscillation in the cortex and thalamus. I then discuss the cellular and network mechanisms thought to be involved in the initiation, persistence, and termination of Up states. I then consider the involvement of subcortical structures in either modulating or mediating the slow oscillation in cortex. In the latter half of the Review, I discuss the putative functional roles of the slow oscillation, concluding with a consideration of how Up states may be a manifestation of dynamic routing of information flow in cortical networks.

THE DISCOVERY OF THE SLOW OSCILLATION AND ITS CHARACTERIZATION

While first described in the striatum of anesthetized rats (Wilson and Groves, 1981), in a series of three articles published in the *Journal of Neuroscience* in 1993, Steriade et al. (1993a,b,c) provided the first characterization of the slow oscillation in cortical and thalamic networks using intracellular and EEG recordings in anesthetized cats. During the slow oscillation, most neurons showed periods of suprathreshold depolarization, interspersed with periods of relative inactivity (“Up” and “Down” states in the later literature, respectively; **Figure 1A**). The depolarizing periods were associated with barrages of synaptic inputs, while the silent periods showed a marked withdrawal of these inputs. Importantly, the cortical slow oscillation persisted after thalamic and callosal lesions (Steriade et al., 1993b), suggesting that the cortical network itself is sufficient for the generation of the oscillation (but see “Contribution of the thalamus” Section). Later work demonstrated that the slow oscillation could also be expressed in deafferented cortical slabs of a certain size (Timofeev et al., 2000), as well as in cortical slice preparations *in vitro* under certain conditions (Sanchez-Vives and McCormick, 2000; see “The slow oscillation *in vitro*” Section).

The slow oscillation is a global and synchronized network phenomenon, engaging neurons throughout the cortex (**Figure 1B**), and also involving neurons in several subcortical areas, including the thalamus (see “Contribution of the thalamus” Section), striatum (see above), and the cerebellum (Ros et al., 2009). Within the local cortical network (within a few tens of millimeters), cortical neurons synchronously depolarize and hyperpolarize during the slow oscillation, with phase delays less than an order of magnitude of the oscillation period (<100 ms; Amzica and Steriade, 1995a;



Volgushev et al., 2006). The long-range coherency of the slow oscillation likely depends upon horizontal axon collaterals of cortical pyramidal cells (Amzica and Steriade, 1995b), though diffusely projecting thalamocortical neurons from higher-order and intralaminar thalamic nuclei may also play a role in synchronizing the cortical population (Sherozhiya and Timofeev, 2014; see also “Contribution of the thalamus” Section).

The spatiotemporal evolution of the slow oscillation often exhibits greater complexity than a simultaneous activation of all neurons in the local cortical network. Recordings from high-density EEG (Massimini et al., 2004) and extracellular arrays (Luczak et al., 2007) indicate that the slow oscillation *in vivo* propagates as a traveling wave, often in the anteroposterior direction. The slow oscillation also activates neurons in particular, stereotyped sequences (Luczak et al., 2007). The complex spatiotemporal architecture of the slow oscillation may provide a mechanism for essential computations during slow-wave sleep, such as those related to memory consolidation (see also “Synaptic plasticity and the slow oscillation” Section).

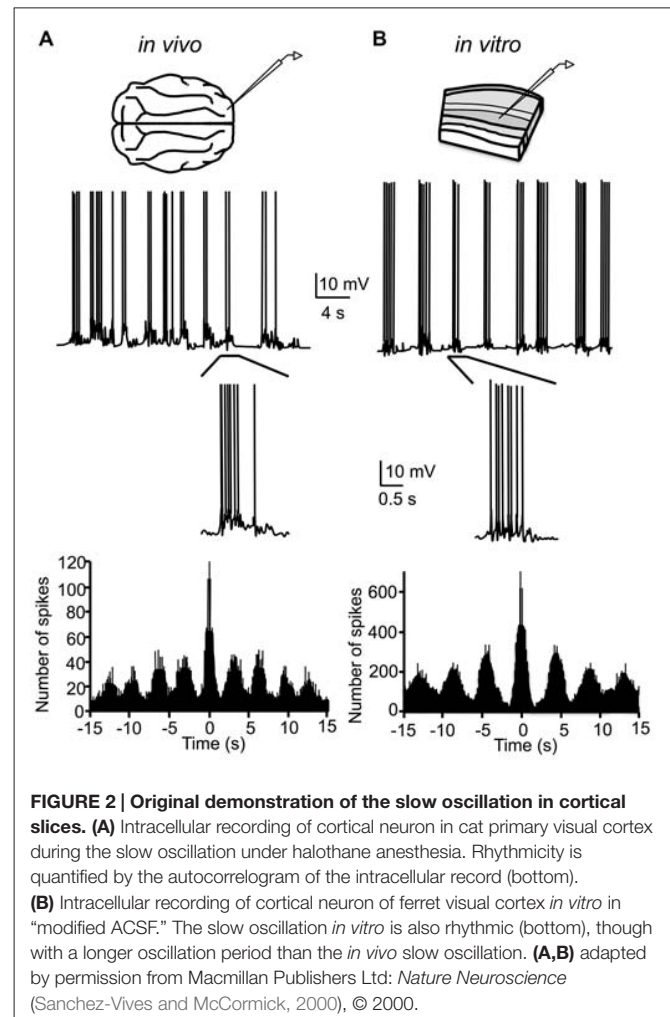
THE SLOW OSCILLATION *IN VITRO*

The genesis of the slow oscillation seems largely endemic to cortex, since, as discussed in the previous section, the oscillation can persist in the absence of thalamic input. The extent of the ability of the local cortical network to generate slow oscillations is perhaps most exemplified by the fact the cortical slice preparation *in vitro* can also exhibit this activity.

In the process of determining why short-term synaptic depression is often higher *in vitro* compared to *in vivo* (Sanchez-Vives, 2007), Sanchez-Vives and McCormick (2000) discovered that by slightly modifying the ionic composition of the artificial cerebrospinal fluid (ACSF) bathing slices of ferret visual and prefrontal cortex, rhythmic spontaneous network activity occurring at ~ 0.3 Hz could be recorded both intracellularly and in the multi-unit activity. Specifically, by reducing the concentrations of Mg^{2+} and Ca^{2+} from (in mM) 2 and 2–1 and 1.2, respectively, and increasing the concentration of K^+ from 2.5–3.5, slow oscillatory activity arose in the slice that was largely indistinguishable from the slow oscillation *in vivo*, albeit with a lower frequency (Figure 2). Notably, the reduced Mg^{2+} and Ca^{2+} concentrations are actually closer to those measured *in situ* (Somjen, 2004). The effect of these changes in ionic concentrations is to increase the overall excitability of neurons, either through direct depolarization of the resting membrane potential or through shifts in the activation curves of various voltage-dependent conductances. The increased K^+ concentration presumably depolarizes the resting membrane potential via a less negative K^+ Nernst potential; the resting neuronal membrane is primarily permeable to K^+ . The reduced concentration of Mg^{2+} and Ca^{2+} may enhance excitability through reduced charge screening of the neuronal membrane, resulting in a negative shift in the activation curves for voltage-dependent conductances (Frankenhaeuser and Hodgkin, 1957; McLaughlin et al., 1971). These ionic concentrations afford a critical level of excitability in the *in vitro* recurrent cortical network such that reverberant activity can be sustained in rhythmic cycles.

The ability of relatively small cortical circuits to exhibit the slow oscillation seems to be a generalizable feature across cortical areas and species. After the slow oscillation was first demonstrated in ferret visual and prefrontal cortex *in vitro*, various groups showed that similar behavior could be expressed in slices from other cortical areas and species, including: ferret piriform cortex (Sanchez-Vives et al., 2008), mouse barrel cortex (MacLean et al., 2005; Rigas and Castro-Alamancos, 2007; Watson et al., 2008; Rigas and Castro-Alamancos, 2009; Fanselow and Connors, 2010; Yassin et al., 2010; Favero et al., 2012; Favero and Castro-Alamancos, 2013; Sippy and Yuste, 2013; Neske et al., 2015), rat barrel cortex (Wester and Contreras, 2012, 2013), mouse entorhinal cortex (Tahvildari et al., 2012; Craig et al., 2013; Neske et al., 2015; Salkoff et al., 2015), rat entorhinal cortex (Cunningham et al., 2006; Mann et al., 2009; Mayne et al., 2013), and rat prefrontal cortex (Wang et al., 2010).

Acute cortical slice preparations provide numerous experimental benefits, such as control of the composition



of the extracellular environment and ease of recording and imaging from particular neuron types throughout all layers of cortex. These benefits have been harnessed to study the slow oscillation in exquisite detail, revealing the synaptic and intrinsic currents involved in the spatiotemporal evolution of network activity. Intracellular and extracellular recording, as well as calcium and voltage-sensitive dye imaging of cortical slices exhibiting the slow oscillation have provided important details about the cellular and network mechanisms involved, especially when combined with pharmacological isolation of particular conductances.

CELLULAR AND NETWORK MECHANISMS OF THE SLOW OSCILLATION

The entire cortical network is engaged by the slow oscillation. While it seems almost inevitable that such a highly interconnected system as the cortex would generate such activity, there are many aspects of the slow oscillation that deserve attention. How does it initiate? How does it persist? What terminates it? Why is it rhythmic? Such questions concerning

the cellular and network mechanisms of the slow oscillation arose even from the very first observation of its presence in the cortex by Steriade and colleagues (see “The discovery of the slow oscillation and its characterization” Section). Since that time, considerable progress has been made toward a detailed understanding of the slow oscillation.

Up State Initiation

How does synchronous network activation arise from a relatively silent network? Current thought about the initiation of cortex-wide activity during the slow oscillation is largely divided between two mechanisms: initiation by persistently active, pacemaker-like cortical neurons and stochastic initiation by temporal summation of spontaneous synaptic activity. In both cases, pyramidal cells in cortical layer 5 are considered to be the key players.

There is substantial evidence that cortical layer 5 is instrumental in the initiation of Up states. Early current-source-density analysis *in vivo* revealed that the polarity of the extracellular potential recorded during the slow oscillation reversed from source to sink toward the middle cortical layers (Steriade and Amzica, 1996). The first *in vitro* recordings of the slow oscillation (see “The slow oscillation *in vitro*” Section) showed that multi-unit activity was strongest and earliest in layer 5 (Sanchez-Vives and McCormick, 2000). Furthermore, when synaptic connections between the upper and lower layers of cortex were severed via a horizontal cut through layer 4, the lower cortical layers could still generate the slow oscillation, while the upper cortical layers could generate this activity either infrequently or not at all. The importance of the layer 5 cortical network in generating Up states has also been demonstrated in later *in vitro* work (Wester and Contreras, 2012) and through optogenetic manipulation of layer 5 and layer 2/3 pyramidal cells *in vivo* (Beltramo et al., 2013).

There are several possible reasons why layer 5 is critical for the initiation of the slow oscillation. Many layer 5 pyramidal cells exhibit intrinsic rhythmicity, with resonant firing frequencies <15 Hz following short depolarizing or hyperpolarizing current pulses (Agmon and Connors, 1989; Silva et al., 1991). Such resonance at low frequencies could facilitate the emergence of the slow oscillation in the entire cortical network. Within layer 5, there is also a subtype of pyramidal cell that displays bursts of action potentials upon depolarizing current injection (Connors et al., 1982). This subtype of pyramidal cell was originally suggested to play the dominant role in the initiation of epileptiform activity in the cortex (Connors, 1984; Chagnac-Amitai and Connors, 1989). In addition to signaling through bursts of action potentials, these pyramidal cells have other features that are conducive to initiating network activity, such as wide axonal arborization within layer 5 (Chagnac-Amitai et al., 1990) and high spine density on their dendrites (DeFelipe and Fariñas, 1992), implying an especially high degree of divergence and convergence from and onto these cells. As a consequence of this synaptic architecture, horizontal propagation of epileptiform discharges occurs preferentially via

pathways in layer 5 (Telfeian and Connors, 1998; Connors et al., 2001; Pinto et al., 2005). Comparably to their putative role in the initiation of seizure-like activity, intrinsic bursting pyramidal cells in layer 5 may also play a role in the initiation of Up states (Lörincz et al., 2015). Indeed, it could be argued that the paroxysmal activity associated with certain epileptic seizures are actually dysregulated Up states (Žiburkus et al., 2013). Consistent with this proposed role for intrinsic-bursting pyramidal cells in Up state initiation are intracellular recordings during the slow oscillation in anesthetized and sleeping cats, showing that these cells often fire before all other recorded neuron types preceding Up state onset (Chauvette et al., 2010).

The intrinsic and synaptic properties of pyramidal cells in layer 5 establish these cells as well-primed to initiate the Up states of the slow oscillation, but how does activity emerge in these cells in the first place during the quiescent Down state? There are at least two plausible scenarios for the intracortical origination of the Up state.

In one scenario, spontaneous, action potential-independent excitatory synaptic potentials (i.e., miniature EPSPs; Fatt and Katz, 1952) temporally summate in a critical number of layer 5 pyramidal neurons, driving these neurons to spike. These neurons then tip the entire cortical network into the Up state. Evidence for this hypothesis of Up state generation comes from both computational models (Timofeev et al., 2000; Bazhenov et al., 2002) and intracellular recordings during the slow oscillation in sleeping and anesthetized cats (Chauvette et al., 2010). In the latter study, cells that were active earliest in the Down to Up state transition exhibited a slow ramp of depolarization crowned by putative EPSPs, whereas cells active later in the transition depolarized more rapidly, without the presence of visibly discrete EPSPs.

In another scenario for Up state generation, layer 5 pyramidal cells that fire persistently during the Down state initiate the Up state after the cellular and network refractory mechanisms associated with the previous Up state have subsided (see “Up state termination” Section). The observation of persistently firing layer 5 pyramidal cells during the Down state is variable in the literature. Neither intracellular nor extracellular recordings in most of the studies of the slow oscillation in anesthetized and sleeping cats have shown the presence of spiking activity during the Down state in any layer of cortex. During slow oscillations *in vitro*, however, pyramidal cells in layer 5 often exhibit spontaneous firing during the Down state (Sanchez-Vives and McCormick, 2000), many of which might be of the intrinsic-bursting subtype (Neske et al., 2015). As expected due to its occurrence during the Down state, the spontaneous firing of these layer 5 pyramidal cells seems to be independent of synaptic activity, since it persists under blockade of fast excitatory and inhibitory synaptic transmission (Le Bon-Jego and Yuste, 2007). The spontaneous firing of certain layer 5 pyramidal neurons during the Down state, or at least the presence of a signal in the multi-unit activity, has also been reported during the slow oscillation in rodents *in vivo* (Hasenstaub et al., 2007; Sakata and Harris, 2009; Crunelli et al., 2012).

The oscillation period of the slow oscillation depends upon the interplay between Up state initiation mechanisms and the refractory mechanisms associated with the Down state (see “Up state termination” Section), with a possible contribution from the intrinsic rhythmicity of certain layer 5 pyramidal cells (Lörincz et al., 2015). After an Up state terminates, a sufficient amount of synaptic activity, either due to action-potential-independent synaptic release or persistently firing pyramidal cells, must accumulate in the network to ignite the next Up state. The potential for synaptic activity during the Down state to trigger another Up state depends first and foremost on when this activity occurs during the network refractory period, which is most likely set by the level of activation and inactivation of activity-dependent K^+ conductances that were opened during the Up state (see “Up state termination” Section). Akin to the absolute and relative refractory periods associated with single action potentials, as well as epileptiform discharges (Gutnick et al., 1982), there appears to be a time period following an Up state during which another Up state cannot be elicited (Sanchez-Vives and McCormick, 2000). This “absolute” network refractory period sets a lower bound on the oscillation period of the slow oscillation. While “absolute” in the sense that an Up state cannot be elicited even with a high degree of synaptic activity (e.g., via electrical stimulation or glutamate puff), this refractory period is likely regulable to the extent that activity-dependent K^+ conductances are regulable (e.g., via neuromodulatory tone). The next factor that determines the slow oscillation period is the level of synaptic activity during the Down state. If the amount of synaptic activity exceeds some critical threshold after enough K^+ conductances have inactivated, another Up state will initiate. As discussed above, the origin of this synaptic activity may be either the persistent firing of certain layer 5 pyramidal cells, miniature EPSPs, or both. At the most basic level, the amount of synaptic activity contributed by either of these sources will depend upon the size of the network; with more synapses impinging on a given post-synaptic cell, the higher the probability of EPSPs (miniature or evoked) temporally summing to a critical level to initiate an Up state. This is presumably the major reason why the slow oscillation often has a lower frequency *in vitro* than *in vivo*, in which in the former case, the number of synapses is significantly truncated. The amount of synaptic activity during the Down state likely has multiple points of regulation, and consequently multiple mechanisms by which the period of the slow oscillation can be adjusted. If Down-state synaptic activity depends significantly on the persistent firing of certain layer 5 pyramidal cells, this property may be subject to multiple types of neuromodulatory control (Dembrow and Johnston, 2014; see also “Contribution of the basal forebrain and brainstem nuclei” Section). If the temporal summation of miniature EPSPs is the driver of Up state initiation, there are multiple mechanisms for the regulation of this process as well, many of which affect either miniature EPSP size or frequency independently of action-potential-dependent synaptic release (Ramirez and Kavalali, 2011).

Another issue regarding Up state initiation is the fine-scale specificity of the neuronal sub-networks that first engage the

rest of the network: can an Up state in principle initiate in any sub-network or does the same sub-network initiate the Up state every time in a given cortex, whether slice, slab, or intact brain? Evidence from calcium imaging in cortical slices (Mao et al., 2001; Cossart et al., 2003; Ikegaya et al., 2004; MacLean et al., 2005) and laminar extracellular probes *in vivo* (Luczak et al., 2007) suggest that Up states initiate in the same groups of neurons and engage the rest of the cortical network in the same sequence on each cycle. Occasionally, however, Up states can initiate in different sequences of cells or travel in different directions either spontaneously or due to stimulation of a region distinct from the “default” region of Up state initiation (Sanchez-Vives and McCormick, 2000; Luczak et al., 2007). Thus, while Up state initiation appears to depend upon the stereotypical activation of specific sub-networks, this is likely not an immutable property.

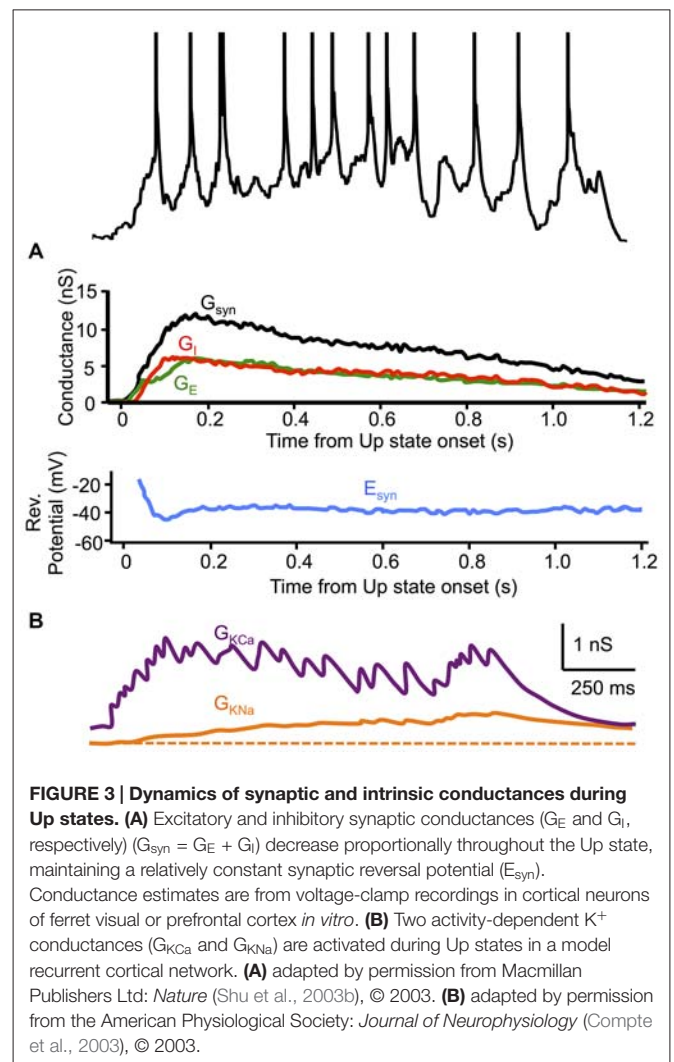
Up State Persistence

Up states of the slow oscillation are persistent network events, sustained for hundreds of milliseconds to a few seconds. Persistent action potential output in neurons in the absence of a stimulus or following the termination of a stimulus is a prevalent phenomenon in most central nervous system (CNS) structures. One important mechanistic issue concerning persistent neuronal activity is the relative contribution of synaptic vs. intrinsic membrane properties in sustaining such activity (Marder et al., 1996; Major and Tank, 2004). Purely intrinsic mechanisms for bi- or multistability of neuronal activity have been documented for certain cells under certain conditions in the mammalian CNS. Motor neurons of the spinal cord *in vitro* exhibit intrinsic persistent activity in the form of plateau potentials mediated by L-type Ca^{2+} channels (Alaburda et al., 2002). Plateau potentials are also a prominent feature of cerebellar Purkinje cells *in vitro* (Llinás and Sugimori, 1980) and *in vivo* (Loewenstein et al., 2005). Persistent neuronal activity due to intrinsic membrane mechanisms has been demonstrated to a lesser degree in cerebral cortex, though there are some notable examples. Pyramidal cells of the entorhinal cortex *in vitro* in the presence of carbachol exhibit graded persistent activity, in which depolarizing intracellular current stimuli lead to progressively more intense persistent firing outlasting the stimulus (Egorov et al., 2002; Fransén et al., 2006). This phenomenon may depend upon Ca^{2+} -dependent nonspecific cation currents (I_{CAN}).

Does activity during the Up state persist primarily as a consequence of cell-intrinsic mechanisms or synaptic mechanisms? While a certain role for intrinsic mechanisms cannot be entirely ruled out (particularly in the layer 5 pyramidal cells discussed earlier), most evidence suggests that persistent activity during the Up state crucially depends on recurrent excitatory synaptic activity, balanced by synaptic inhibition. Several features of the Up state from intracellular recordings suggest a predominately synaptic basis for persistent activity. First, injection of current to either depolarize or hyperpolarize the membrane potential of recorded cells does not affect the duration of the Up state or its rhythmicity (Steriade et al., 1993a; Contreras et al., 1996b; Sanchez-Vives and McCormick, 2000; McCormick et al., 2003; Shu et al., 2003a), contrary

to the expectation if voltage-dependent conductances were involved. Second, both membrane potential variance and irregularity of interspike intervals are high in cortical neurons during Up states, consistent with a role of excitatory and inhibitory synaptic barrages sustaining persistent activity. Third, Up states are completely abolished in cortical slices during application of antagonists of fast glutamatergic transmission (i.e., α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA or N-Methyl-D-aspartate, NMDA receptor antagonists; Sanchez-Vives and McCormick, 2000; McCormick et al., 2003; Shu et al., 2003a). Regarding the last point, while both AMPA and NMDA receptor activation appear to be necessary for the natural persistence of the Up state, NMDA receptors may play a paramount role (Major et al., 2013). Blockade of NMDA receptors by supplemental doses of ketamine to pre-existing urethane anesthesia greatly reduced Up state duration (by more than half) in the original *in vivo* characterization of the cortical slow oscillation (Steriade et al., 1993a). Additionally, bath-application of NMDA receptor antagonists in slices exhibiting the slow oscillation virtually abolishes this activity (Sanchez-Vives and McCormick, 2000; Favero and Castro-Alamancos, 2013; Castro-Alamancos and Favero, 2015), while bath-application of AMPA receptor antagonists can unmask Up states that are entirely dependent on NMDA receptors for fast glutamatergic signaling (Favero and Castro-Alamancos, 2013). The role of NMDA receptors in the persistence of the Up state is evocative of its purported role in the persistent activity underlying working memory in prefrontal cortex (Lisman et al., 1998; Wang et al., 2013). Activation of NMDA receptors also sustains certain paroxysmal oscillations originating in layer 5 (Silva et al., 1991; Flint and Connors, 1996). Interestingly, in rat entorhinal cortex *in vitro*, only selective blockade of kainate receptors abolished the slow oscillation (Cunningham et al., 2006).

Synaptic inhibition also plays a critical role in the sustenance of Up state activity by maintaining the membrane potential at a level near spike threshold where synaptic noise can transiently cause firing. Neurons secure this stable region of membrane potential dynamics as a consequence of a precise balance between synaptic excitation and inhibition. During Up states in ferret cortex *in vitro* (Shu et al., 2003b) and *in vivo* (Haider et al., 2006), excitatory and inhibitory conductances change proportionally, often with an approximately unity ratio, at least as measured from the soma (Figure 3A). The result of this balance is that, even as the total synaptic conductance changes during the Up state, the synaptic reversal potential remains essentially fixed, supporting a steady level of the membrane potential near spike threshold (≈ 37 mV). During Up states in other cortical areas and other species, while excitatory and inhibitory conductances vary mostly concomitantly, there is divergence in both the reported ratios of these conductances and their precise time-courses. As mentioned above, estimated excitatory and inhibitory conductances in the deep layers of ferret prefrontal and visual cortex *in vitro* (Shu et al., 2003b) and the deep layers of prefrontal cortex in ketamine-xylazine-anesthetized ferrets (Haider et al., 2006) were markedly similar in magnitude throughout the duration of the Up state,



with the exception of the very beginning and end of the Up state, where excitation dominated. In the deep layers of parietal association cortex of naturally sleeping cats, however, the estimated inhibitory conductance strongly dominated the excitatory conductance, by $\sim 10:1$, throughout the majority of the Up state (Rudolph et al., 2007). While not as pronounced as the latter study, inhibition also dominated excitation, by $\sim 2:1$, in the estimated synaptic conductance during Up states in both the deep and superficial layers of mouse barrel cortex *in vitro*, though primarily during the first half of the Up state (Neske et al., 2015). In one study of the superficial layers of barrel cortex of urethane-anesthetized rats, Up states were overwhelmingly dominated by excitation, with an estimated excitatory-to-inhibitory conductance ratio of $\sim 10:1$ (Waters and Helmchen, 2006).

Up State Termination

The central contention regarding the termination of Up states is its dependance on synaptic vs. intrinsic cellular mechanisms, notably a similar debate with regard to the termination of seizure activity (McCormick and Contreras, 2001). More specifically,

Up state termination may result either from enhanced activity of inhibitory interneurons near the end of the Up state (or synaptic depression of excitatory synapses) or from the activation of activity-dependent hyperpolarizing conductances. Of course, intrinsic and synaptic mechanisms of Up state termination are likely not mutually exclusive. In principle, any of these mechanisms would lead to disfacilitation (i.e., removal of synaptic inputs) in excitatory cells and an eventual failure of the network's ability to sustain the Up state.

In the original characterization of the cortical slow oscillation (Steriade et al., 1993a), the authors considered Ca^{2+} -dependent K^+ (K_{Ca}) conductances as candidates for the termination of Up states based upon the long-lasting afterhyperpolarizations (AHPs) they effect in pyramidal cells (Schwindt et al., 1992). Shortly after the initial characterization of the cortical slow oscillation, Steriade et al. (1993d) showed that Up-Down transitions were abolished by stimulation of brainstem nuclei originating cholinergic projections to cortex. This effect was dependent on muscarinic, but not nicotinic signaling. These results suggested that activity-dependent K^+ channels, such as K_{Ca} , might be crucial for the termination of Up states, since such channels are blocked by acetylcholine via muscarinic receptors (McCormick and Williamson, 1989). Both experimental (Sanchez-Vives and McCormick, 2000) and computational modeling studies (Compte et al., 2003) have suggested that activation of Na^+ -dependent K^+ (K_{Na}) conductances may also play a role in the termination of Up states since these conductances also cause an activity-dependent slow AHP in cortical neurons, which is also blocked by acetylcholine (Schwindt et al., 1989; Sanchez-Vives et al., 2000). Additional K^+ conductances that may play a role in Up state termination include those activated by extracellular adenosine (Phillis et al., 1975) or intracellular ATP (Ashford et al., 1988), whose concentrations increase with enhanced spiking activity due to the increased metabolic load of synchronous network activity. The role of increased extracellular adenosine in terminating Up states through blockade of a K^+ conductance has been hypothesized (Steriade et al., 1994; Amzica and Steriade, 1995a; Contreras and Steriade, 1995; Contreras et al., 1996b), while the role of K^+ conductances activated by increased intracellular ATP in Up state termination was suggested by the increased duration of cortical Up states *in vitro* under non-specific pharmacological blockade of these conductances (Cunningham et al., 2006).

Activation of activity-dependent K^+ conductances during Up states would decrease the excitability of neurons in which these conductances are expressed (i.e., predominately cortical pyramidal cells), decreasing their firing rates, thus leading to a generalized disfacilitation in cortical networks and Up state termination (**Figure 3B**). Disfacilitation can also result from short-term synaptic depression, a feature of intracortical excitatory synapses (Thomson and Deuchars, 1994). The relative importance of synaptic depression vs. the activation of K^+ conductances for disfacilitation during the Up state is unresolved. Measurements of input resistance of neurons during the slow oscillation show a minimum at the beginning of the Up state, which steadily increases toward a maximum at the

point of Up state termination (Contreras et al., 1996b). This temporal profile of input resistance is consistent with a role for synaptic depression in terminating the Up state, since a role for the activation of K^+ conductances might predict a decrease in input resistance as the Up state progresses. Changes in input resistance, however, would be due to both the closing of synaptic conductances and the opening of K^+ conductances. If the synaptic conductance decrease is greater than the K^+ conductance increase, then the overall effect would be an increase in input resistance, though the K^+ conductance increase might still be contributing via hyperpolarization. Indeed, it might be this hyperpolarizing effect that contributes to decreased firing in excitatory cells, leading to decreased synaptic conductance. Thus, it is difficult to determine from input resistance changes alone whether synaptic depression or K^+ conductance increase is the primary cause of Up state termination. Nevertheless, if activity-dependent K^+ conductances contribute to Up state termination, a prediction might be that a slight decrease in input resistance occurs after the synaptic conductance decays completely at the end of the Up state but while the K^+ conductance is still active. Input resistance measurements during the Down state in Sanchez-Vives and McCormick (2000) are consistent with this notion, as is the temporal profile of input resistance in a cortical network model of the slow oscillation (Compte et al., 2003), though this particular model did not include synaptic depression. One model including both activity-dependent K^+ conductances and synaptic depression concluded that both are important for Up state termination, but did not manipulate either variable alone to examine their differential effects (Bazhenov et al., 2002), while another model, with either variable changed in isolation, showed that the absence of activity-dependent K^+ conductances virtually abolished transitions to the Down state, while absence of synaptic depression only diminished the synchrony of these transitions, suggesting a paramount role for K^+ conductances (Hill and Tononi, 2005).

Proposed mechanisms for Up state termination must explain the high level of synchrony of this event across cortical neurons. The transition to the Down state may be the most synchronous event during the slow oscillation (Volgushev et al., 2006; Mochol et al., 2015). It might seem that a terminating mechanism such as the activation of intrinsic hyperpolarizing currents or the depression of synaptic inputs is incompatible with such a high level of synchrony; intrinsic currents and synaptic depression vary in expression and magnitude from cell to cell and from synapse to synapse. However, given that Up states also *initiate* with a high level of synchrony due to the preferential activation of a certain number of highly excitable or well connected cells (see "Up state initiation" Section), it is also conceivable that Up states can *terminate* synchronously due to decreased excitability in these same cells; the failure of activity in the rest of the network would rapidly follow the decreased excitability of this critical sub-network. The prediction of this account of Up state termination synchrony is that not only the initiation of the Up state but also the termination of the Up state should be a spatiotemporally stereotypical event. That is, activation of a given sub-network should lead to the

rapid recruitment of the rest of the network into an Up state, while decreased excitability in this same sub-network should lead to the rapid failure of wider network activity, leading to the Down state. In contrast to Up state onset, however, there is little evidence that Down state onset exhibits a stereotypical neuronal sequence (Volgushev et al., 2006; Luczak et al., 2007). Thus, it is unclear if the termination of the Up state is due to the decreased excitability of the same sub-network responsible for initiating the Up state. Furthermore, such a mechanism for Up state termination does not account for the higher degree of synchrony compared to initiation, unless activity in the critical sub-network is somehow halted more synchronously than it began. Such a scenario is conceivable if, for instance, all of the neurons in this sub-network express an activity-dependent K^+ conductance to a similar degree, thereby extinguishing activity with smaller temporal lags than are associated with the various synaptic delays within the sub-network. It is important to note that, while the transition to the Down state is a highly synchronous event, the degree of synchrony likely varies greatly with neuromodulatory tone during natural slow-wave sleep and with anesthesia in experimental preparations (Chauvette et al., 2011). Indeed, in the limit, highly local Down states emerging from the “activated” cortical network during wakefulness have been reported (Vyazovskiy et al., 2011). These local Down states, which can be selective enough to involve particular groups of cells within a cortical area, increase in frequency, duration, and spatial extent with time awake. While the mechanisms of these local Down states are unknown, their locality is likely due to the high neuromodulatory tone associated with waking.

Early investigations of the cellular basis of the slow oscillation suggested that the transition to the Down state was likely not precipitated by synaptic inhibition because hardly any cells discharged preferentially in relation to this transition, including putative GABAergic aspiny cells (Contreras and Steriade, 1995), and input resistance monotonically increased during the Up state, suggesting a withdrawal of both excitatory and inhibitory inputs (Contreras et al., 1996b). Estimates of excitatory and inhibitory conductances and their temporal profile during Up states *in vivo* (Haider et al., 2006; Rudolph et al., 2007) and *in vitro* (Shu et al., 2003b; Neske et al., 2015) also support the view that both types of synaptic inputs decrease during the Up state. Thus, the lack of an observed increase in inhibitory cell firing relative to Up state termination and the fact that inhibitory conductances decrease as the Up state progresses has suggested that synaptic inhibition is not an important factor for Up state termination. In fact, progressive pharmacological blockade of fast, GABA_A-mediated inhibition actually results in a continuous *decrease* in Up state duration *in vitro* (Mann et al., 2009; Sanchez-Vives et al., 2010), perhaps attributable to the enhanced activation of activity-dependent K^+ conductances due to increased pyramidal cell firing in the disinhibited network (Sanchez-Vives et al., 2010; Igelström, 2013). At the same time, however, slow GABA_B-mediated inhibition appears to play some role in terminating Up states, since progressive pharmacological blockade of this inhibition results in a continuous *increase* in Up state duration *in vitro* (Mann et al., 2009), an effect that depends on presynaptic

GABA_B receptors (Craig et al., 2013). Thus, while the firing of inhibitory cells does not seem to occur preferentially in relation to Up state termination, a slower, non-phasic inhibition, due to both the slower kinetics of GABA_B receptors and perhaps also the more prolonged GABAergic signaling associated with the extrasynaptic localization of these receptors (Kulik et al., 2003), may play a role. This slow GABAergic process may supplement the similarly slow intrinsic inhibitory process associated with activity-dependent K^+ conductances.

While, at the population level, inhibitory cells do not increase their firing rate as the Up state progresses and inhibitory synaptic conductance accordingly does not increase, it is still possible that some fraction of inhibitory cells discharge preferentially toward the end of Up states. Consequently, a small, but perhaps significant fraction of excitatory cells would experience an increase in inhibitory synaptic input in the latter portions of an Up state. If this fraction of “late-firing” inhibitory cells has a sufficient degree of axonal spread, then it is possible that synaptic inhibition, while not necessarily apparent at the level of population data, contributes significantly to Up state termination and its characteristic synchrony. One possible candidate for such a “late-firing” inhibitory cell is the somatostatin (SOM)-positive, Martinotti cell, which receives strongly facilitating excitatory synaptic input (Reyes et al., 1998; Gibson et al., 1999; Beierlein et al., 2003; Silberberg and Markram, 2007). In paired recordings of connected SOM-positive inhibitory cells and pyramidal cells during Up states *in vitro*, the spike-triggered average membrane potential of the SOM-positive inhibitory cell was greatest during the last third of the Up state (Fanselow and Connors, 2010), suggesting that this particular inhibitory cell may be most excited near the point of Up state termination. Two cortical network models incorporating facilitating excitatory synapses onto SOM-positive inhibitory cells have suggested that the late firing of these cells during simulated slow oscillations is decisive in terminating Up states (Melamed et al., 2008; Krishnamurthy et al., 2012). The relatively low firing rates (<20 Hz) of individual pyramidal cells during Up states, however, are unlikely to permit effective excitatory facilitation onto postsynaptic SOM-positive inhibitory cells. Indeed, when the temporal profile of firing of these cells during Up states has been characterized, the result has been either a decrease in firing with progression of the Up state (Neske et al., 2015) or a relatively constant level of firing (Fanselow and Connors, 2010). One study has reported a class of fast-spiking inhibitory cells in rat prefrontal cortex that discharges preferentially near the termination of the Up state (Puig et al., 2008), though the source (cortical vs. subcortical) and synaptic basis (excitation vs. disinhibition) for the increased firing of this particular cell during the late portions of the Up state is unclear, as is the generalizability of this firing pattern across cortical areas and species.

Recent evidence from intracellular recordings in anesthetized and sleeping cats combined with computational network models suggest that, while not necessarily apparent in the population firing rate of particular inhibitory cells or the population inhibitory conductance of excitatory cells, synchronized inhibition in a small number of excitatory cells may occur

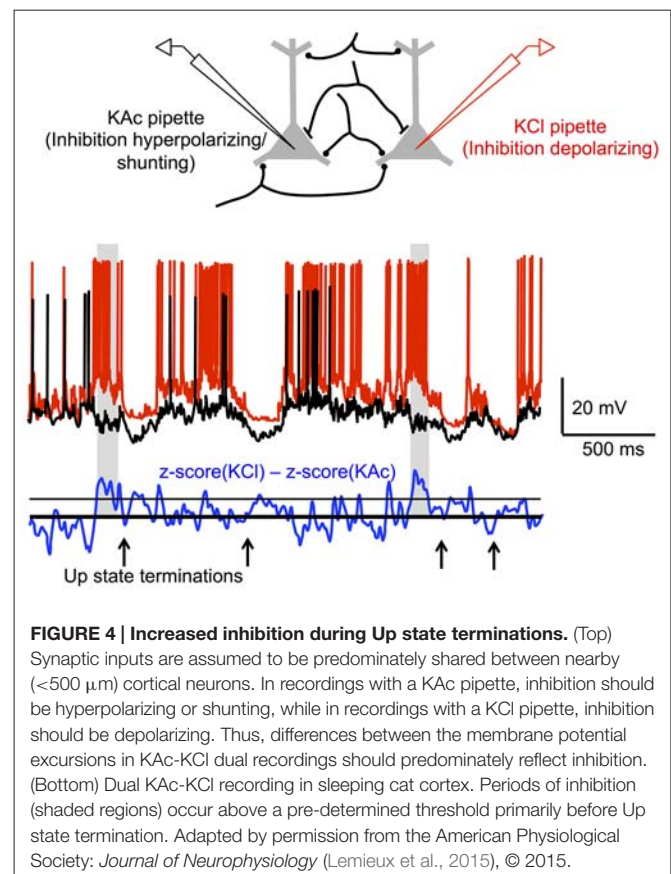
prior to Up state termination. Impelled by their observation that Up state termination is often more synchronized among cortical neurons than initiation (Volgushev et al., 2006), Igor Timofeev and colleagues have reconsidered the possibility that synaptic inhibition increases in certain cortical neurons at the end of Up states. In a computational model of the cortico-thalamocortical network, they showed that either enhancing the intrinsic excitability of cortical inhibitory cells or increasing the strength of excitatory-to-inhibitory cell synapses improved the synchrony of Up state terminations and shortened Up state durations (Chen et al., 2012). Conversely, when the strength of excitatory-to-inhibitory cell synapses was decreased, the duration of Up states increased until reaching a point at which inhibition was so meager that intense excitatory cell firing activated activity-dependent K^+ conductances, which abruptly terminated network activity. These simulations suggested that synaptic inhibition is critical for the synchronous termination of Up states, while activity-dependent K^+ conductances might play a more important role in a disinhibited network.

Contrary to the modeling results in Chen et al. (2012), progressive pharmacological blockade of fast synaptic inhibition during Up states *in vitro* progressively decreased Up state duration (Mann et al., 2009; Sanchez-Vives et al., 2010; see also above). While the reasons for these discrepancies are unclear, they might be attributed to the differential degree of afferentation of the systems queried in these studies. In Chen et al. (2012), the model includes both the local cortical circuit as well as cortico-thalamocortical loops encompassing both core and matrix thalamic nuclei. In the *in vitro* studies of Mann et al. (2009) and Sanchez-Vives et al. (2010), these loops are not present. It is possible that thalamocortical projections in the model from Chen et al. (2012) have a synchronizing influence on cortical inhibitory cells beyond what the local cortical circuit is capable of achieving (see also “Contribution of the thalamus” Section). It would be interesting to dissect the precise contribution of the cortico-thalamocortical loop to the synchronizing effect of synaptic inhibition on Up state termination by systematically removing components of this loop and examining possible changes in the relative contributions of synaptic inhibition vs. activity-dependent K^+ conductances in termination.

Most recently, Timofeev and colleagues have studied the possible contribution of synaptic inhibition to Up state termination with intracellular recordings in anesthetized or sleeping cats. Specifically, by performing dual recordings of nearby ($<500\ \mu\text{m}$) neurons, with one pipette filled with potassium acetate (KAc) and the other filled with KCl, the authors quantified the contribution of synaptic inhibition to the membrane potential during different periods of the Up state (Lemieux et al., 2015). GABA_A-mediated responses in the cell recorded with KCl would be depolarizing, whereas these same responses would be hyperpolarizing or shunting in the cell recorded with KAc. Since cells separated by $<500\ \mu\text{m}$ receive many common synaptic inputs, subtracting the membrane potential excursions in a cell recorded with KAc from those of a nearby cell simultaneously recorded with KCl will primarily reflect GABA_A-mediated responses. Lemieux et al. (2015) found

that when the differences in membrane potential excursions between KAc- and KCl-recorded cells exceeded a particular threshold, they mostly did so during the last few hundred milliseconds prior to Up state termination (Figure 4). These periods of “long-duration inhibition” prior to Up state termination occurred above chance level in 35% of paired recordings and during 10–19% of Up state terminations in these recordings. Thus, at the population level, this implies that synaptic inhibition dominates 4–7% of cortical neurons prior to Up state termination. Whether this elevated synaptic inhibition in a small proportion of cells is decisive in terminating Up states is still unclear. Furthermore, it is still unknown whether this inhibition reflects the synchronized firing of a particular group of inhibitory cells near the end of the Up state, as in Puig et al. (2008). If so, this implies that a very small and selective proportion of inhibitory cells increases their firing rate during the Up state, with the cellular or network mechanisms for such an increase to be determined.

Taking the various *in vivo*, *in vitro*, and *in silico* results discussed above into account, the principal mechanism by which the cortical network transitions from activity to silence during the slow oscillation is likely the activation of activity-dependent K^+ conductances. A modulatory influence on Up state duration may also be provided by the activation of GABA_B receptors due to the extrasynaptic accumulation of GABA resulting from the high activity of inhibitory cells during the Up state. While fast, GABA_A-mediated inhibitory signaling does not appear to occur



preferentially near the end of the Up state at the population level, a role for this type of inhibition in a small group of neurons cannot be discounted. Whether fast synaptic inhibition in a small population of neurons is critical for the termination of the Up state and its characteristic synchronization, or is ancillary to slower inhibitory mechanisms, remains to be determined. Unequivocal evidence for such a prime role for fast synaptic inhibition in a small population of cells would require the identification of these cells, or more desirably the population of presynaptic inhibitory cells whose firing rate presumably increase toward the end of the Up state, and the experimental manipulation of their activity. This is a challenging task, but may be possible at least in *in vitro* preparations with some of the newest techniques for cell-specific optical control of neuronal activity (Packer et al., 2013).

CONTRIBUTION OF THE THALAMUS

Since its discovery, the slow oscillation has generally been considered a predominantly cortical phenomenon. The sufficiency of the cortex for the generation of the slow oscillation was suggested based on its survival following thalamic lesions (Steriade et al., 1993b). While expressed at a lower frequency, the slow oscillation can also be recorded from cortical slabs *in vivo* (Timofeev et al., 2000) and cortical slices maintained *in vitro* (Sanchez-Vives and McCormick, 2000; see also “The slow oscillation *in vitro*” Section). Thus, the tendency to oscillate at <1 Hz appears to be a fundamental feature of even quite local cortical circuits. It is possible, however, that these observations have led to an underestimation of the role of subcortical structures in shaping the slow oscillation, in particular the thalamus and brainstem neuromodulatory nuclei. Earlier work on the slow oscillation had suggested at least a modulatory role for these subcortical structures, and recent evidence has suggested an even more active role.

The role of the thalamus in regulating the properties of the slow oscillation has been increasingly acknowledged in the ensuing two decades since the original work of Steriade and colleagues. To understand the role of the thalamus during the slow oscillation, it is first important to consider the firing properties of excitatory thalamic relay cells and inhibitory cells of the thalamic reticular nucleus (TRN) during this activity. Steriade and colleagues provided intracellular recordings from these cells in their initial studies, primarily from the ventral lateral nucleus of the thalamus (VL) and the reciprocally connected rostral lateral sector of the TRN (Steriade et al., 1993c; Contreras and Steriade, 1995; Timofeev and Steriade, 1996). Following the Down state, thalamocortical neurons fire a post-inhibitory rebound spike-burst, which is followed by an Up state in the cortex. Corticothalamic input depolarizes TRN cells, causing them to discharge in bursts or tonically at high frequencies. Due to the high level of spiking activity in the TRN, thalamocortical neurons, after their initial spike burst, are quickly overcome by massive synaptic inhibition from the TRN, mostly preventing spiking output. Thus, if thalamocortical projections play a role in the slow oscillation, it would seem that this role is primarily the initiation of Up states and consequently, the determination

of the oscillation period. The phasic burst-firing behavior of thalamocortical neurons at the onset of the Up state, which often preceded the firing of cortical neurons by a few tens of milliseconds, indeed prompted Steriade and colleagues to propose that the activity of thalamocortical neurons might provide the trigger for Up states on each cycle of the slow oscillation (Contreras and Steriade, 1995) and contribute to their synchronization across the cortex (Amzica and Steriade, 1995a).

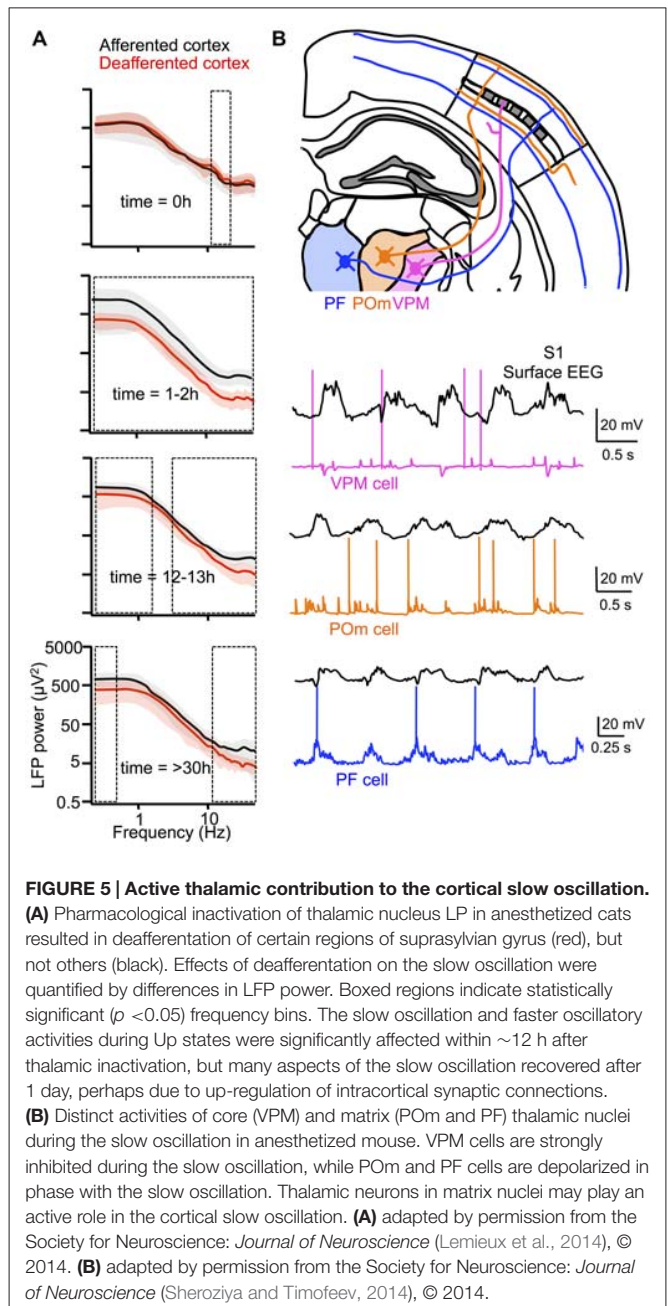
The potential for thalamocortical cells to initiate Up states rhythmically during the slow oscillation can be appreciated when considering the powerful effect of sensory stimulation or direct thalamic stimulation in evoking Up states. In anesthetized animals, both temporally prolonged sensory stimuli, such as drifting gratings (Anderson et al., 2000; Jia et al., 2010) and punctate stimuli, such as brief whisker deflections in rodents (Petersen et al., 2003; Hasenstaub et al., 2007) are effective initiators of Up states in the respective sensory cortices. In slice preparations that preserve axons of thalamocortical neurons (Agmon and Connors, 1991; Cruikshank et al., 2002), electrical or optogenetic stimulation of the thalamus is also capable of triggering Up states (Metherate and Cruikshank, 1999; MacLean et al., 2005; Rigas and Castro-Alamancos, 2007; Watson et al., 2008; Wester and Contreras, 2012; Favero and Castro-Alamancos, 2013; Wester and Contreras, 2013). A thalamic mechanism for Up state initiation could also be manifested during the slow oscillation.

The burst-firing of thalamocortical neurons prior to the discharge of cortical neurons during the slow oscillation is suggestive of a thalamic contribution to Up state initiation, yet this possibility has to a large extent been ignored due to the endurance of the oscillation following thalamic lesions, as well as its presence in cortical slabs and *in vitro* slice preparations. Recent work, however, has revealed a fundamental role for the thalamus in the full expression of the slow oscillation. In mouse barrel cortex *in vitro*, severing thalamocortical axons significantly decreased the frequency of the slow oscillation, indicating that spontaneous thalamic activity in this preparation contributes to the initiation of spontaneous Up states (Rigas and Castro-Alamancos, 2007). Recent *in vivo* studies have also demonstrated an important contribution of thalamic activity to the pacing of the slow oscillation. Acute pharmacological blockade of action potentials in thalamic neurons in anesthetized and naturally sleeping rats decreased the frequency of the slow oscillation (David et al., 2013). Interestingly, selective blockade of T-type Ca^{2+} channels also significantly reduced slow oscillation frequency (David et al., 2013), consistent with the idea that the post-inhibitory rebound spike burst in thalamocortical neurons is an initiation signal for Up states.

In a seemingly marked deviation from the original work of Steriade and colleagues, Timofeev and colleagues, using the same *in vivo* preparation (i.e., the anesthetized cat), recently showed that thalamic inactivation reduced both the slow oscillation frequency and the occurrence of fast oscillations during Up states (see “Beta/gamma oscillations” Section), and furthermore diminished the synchronization of Up states in simultaneously

recorded cortical neurons (Lemieux et al., 2014). In some recordings, slow oscillations were virtually abolished following thalamic lesions. This is in striking contrast to the results of Steriade et al. (1993b) who reported that thalamectomy does not affect the properties of the slow oscillation. The difference between the results of this study and those of the earlier work by Steriade and colleagues is likely attributable to the time after thalamic inactivation that slow oscillations were recorded. In Steriade et al. (1993b), recordings of the slow oscillation were performed 2 days following experimental lesions of the thalamus, whereas in Lemieux et al. (2014), recordings were performed continuously before and after thalamic inactivation. These continuous recordings revealed that while the slow oscillation frequency remained significantly lower up to 12 h following thalamic inactivation, the cortex attained a slow oscillation frequency comparable to its afferented state within 30 h (Figure 5A). This recovery was also evident in a cortical slab preparation. The authors attributed the recovery of the normal slow oscillation frequency to compensatory changes in excitatory synaptic connections, based on the increase of spontaneous EPSPs recorded during the Down state and the recovery of a normal slow oscillation in a “deafferented” thalamocortical network model following the up-scaling of excitatory synaptic connections. Thus, the results from Lemieux et al. (2014) not only demonstrate a major contribution of the thalamus to the full expression of the slow oscillation, but also suggest that this oscillation is of particular importance to cortical networks, since active modifications of local recurrent circuitry appear to compensate for the absence of a thalamic signal for Up state initiation (see also “Functions of the slow oscillation” Section).

Recordings from various thalamic nuclei reveal a possible role for thalamocortical neurons beyond that of a cue for the beginning of each slow oscillation cycle. In particular, whereas inhibition strongly dominates the Up state in relay cells from sensory thalamic nuclei [e.g., ventral posterior medial nucleus (VPM) and lateral geniculate nucleus (LGN)], thus largely preventing spiking at all times except the very onset of the Up state, excitation dominates in non-sensory thalamic nuclei [e.g., posterior nucleus (PO) and intralaminar nuclei] allowing constituent neurons to spike throughout the duration of the Up state (Sherozia and Timofeev, 2014; Figure 5B). The basis for this dichotomy in firing pattern probably lies in the intense inhibition of sensory thalamic nuclei by the TRN and the lack of this inhibition in non-sensory thalamic nuclei, which receive the majority of their inhibitory input not from the TRN, but from the zona incerta (Barthó et al., 2002). Notably, while TRN neurons with projections to sensory thalamic nuclei are highly active during the slow oscillation, TRN neurons with projections to limbic thalamic nuclei exhibit low levels of activity (Halassa et al., 2014). Given the preponderance of excitation in non-sensory thalamic nuclei, thalamocortical neurons in these regions are in a position to play an active role not only in Up state initiation, but also in its persistence and perhaps termination. Moreover, a feature of the projections of thalamocortical neurons from many non-sensory thalamic nuclei is a diffuse targeting of multiple cortical territories, in contrast to sensory thalamic nuclei, whose projections are more focal. These



distinct projection patterns are often denoted as “matrix” or “core,” respectively (Jones, 1998). Taking into account both the prolonged excitation of thalamocortical neurons of non-sensory thalamic nuclei during Up states and their diffuse projections to cortex, these neurons might not only play an active role during multiple phases of the slow oscillation, but might also be key to the synchronization of the slow oscillation across the cortex. This might explain why in Lemieux et al. (2014) not only did thalamic inactivation impair the slow oscillation, but also the higher-frequency oscillations occurring during Up states; the absence of thalamocortical synaptic activity might also have impaired the synchronization of local cortical network activity.

Thalamic neurons are also endowed with intrinsic ionic currents whose interactions can give rise to slow (<1 Hz), rhythmic oscillations, perhaps serving as at least a partial pacemaker for the cortical slow oscillation. While thalamic neurons *in vitro* generally do not exhibit any spontaneous rhythmic membrane potential fluctuations or spiking, activation of metabotropic glutamate receptors (mGluRs; e.g., through bath-application of appropriate agonists or stimulation of corticothalamic axons) causes a rhythmic slow oscillation in these neurons that is insensitive to blockade of synaptic transmission and spiking activity (Hughes et al., 2002). The mechanism for this cell-intrinsic slow oscillation in thalamic neurons is an mGluR-mediated reduction in the outward current associated with the leak conductance (g_{Leak} ; McCormick and von Krosigk, 1992), which leads to membrane potential bistability due to the interaction of this current with the inward current associated with the non-inactivating portion of T-type Ca^{2+} channel conductance (i.e., T_{Window}). That is, when g_{Leak} is low, there are two stable membrane potentials where the outward current mediated by g_{Leak} is balanced by the inward current mediated by T_{Window} , one hyperpolarized and one depolarized (Williams et al., 1997; Hughes et al., 1999). If these were the only conductances activated in these voltage ranges, the membrane potential of thalamic neurons would not oscillate, but rather remain at either of the stable levels until external stimulation toggled between them. This is not the case, however, because the hyperpolarization-activated current (h-current) is also expressed in these neurons, which depolarizes them to the level at which they generate a low-threshold Ca^{2+} spike (mediated by T-type Ca^{2+} channels) and subsequently, due to the stable interaction between the leak current and the current mediated by T_{Window} , the membrane potential remains depolarized. The Ca^{2+} -dependent nonspecific cation current (I_{CAN}) also plays a role in maintaining the depolarized membrane potential of thalamic neurons (Hughes et al., 2002). This depolarized membrane potential is not sustained indefinitely, however, because the h-current inactivates and I_{CAN} decreases, repolarizing the cell to the other (hyperpolarized) stable membrane potential, before the cycle recommences. The same intrinsic slow oscillation is also expressed in TRN neurons (Blethyn et al., 2006). The importance of the h-current for exhibiting this slow oscillation is apparent when it is blocked pharmacologically; short current pulses can then switch thalamic neurons between two stable membrane potential values, where they remain until the another current pulse is applied (Williams et al., 1997; Hughes et al., 1999).

The recent *in vivo* studies demonstrating a dramatic effect of thalamic inactivation on the rhythmicity and synchrony of the cortical slow oscillation, in addition to the observation of an autonomous slow oscillation in thalamic and TRN neurons *in vitro*, has challenged the cortico-centric view of its generation. A complete understanding of the mechanisms of the slow oscillation requires a consideration of the synaptic interactions among all of these components (Crunelli and Hughes, 2010; Crunelli et al., 2015). The potential to be enforced by multiple sites entails the robustness of the slow oscillation, since any deficiencies (e.g., deviations from rhythmicity or synchrony) are likely to be quickly amended due to the redundant expression of

the oscillation. This redundancy might be an indication of the importance of the slow oscillation for normal brain function.

CONTRIBUTION OF THE BASAL FOREBRAIN AND BRAINSTEM NUCLEI

Other subcortical structures that may actively contribute to the slow oscillation include various brainstem nuclei sending cholinergic, noradrenergic, or serotonergic axons to cortex and thalamus. Cortically projecting cholinergic neurons in the basal forebrain are also in a position to modulate the slow oscillation. While the slow-wave sleep state in which the slow oscillation occurs is associated with generally low neuromodulatory tone, neurons in these subcortical nuclei are not completely silent. Neurons firing with distinct preference for either the Up or Down state have been found in the basal forebrain (Détári et al., 1997; Manns et al., 2000), pedunculopontine tegmentum (PPT; Mena-Segovia et al., 2008), locus coeruleus (LC; Eschenko et al., 2012), and dorsal raphe nucleus (DRN; Schweimer et al., 2011). Whether the phasic or tonic firing properties of neurons in these various subcortical areas are a signature of an active role in shaping the slow oscillation or merely a transfer of activity from presynaptic cortical neurons that project to these areas still must be resolved.

The causal role of brainstem nuclei as “activating systems” (i.e., extinguishers of slow-wave EEG patterns) has been well-documented since the pioneering work of Moruzzi and Magoun (1949). A causal role for brainstem nuclei in the control of slow waves themselves, however, has been less studied, likely due to the assumption that the contribution of these nuclei to cortical or thalamic activity is negligible except in states of cortical activation. Additionally, in slice preparations, application of agonists for neuromodulator receptors often abolishes spontaneous and evoked Up states (Hsieh et al., 2000; Favero et al., 2012; Wester and Contreras, 2013), likely due to the suppression of release probability in intracortical synapses (Gil et al., 1997). Nevertheless, a functional, while small, level of neuromodulatory tone may exist in cortical and thalamic networks during the slow oscillation. One of the first indications that neuromodulatory signals arising from subcortical structures could be important for the slow oscillation came from Steriade and colleagues shortly after their initial characterization of the oscillation. They determined that while electrical stimulation of the PPT and LC caused “activation” of the cortical EEG (i.e., abolition of slow waves; see “Up state termination” Section), blockade of muscarinic cholinergic signaling during the baseline, slow-wave state reduced the duration of Up and Down states (i.e., shorter Up states occurring at higher frequency; Steriade et al., 1993d). A recent *in vitro* study of mouse sensory and frontal cortex has proposed a role for cholinergic afferents specifically in maintaining the rhythmicity of the slow oscillation (Lörincz et al., 2015). An *in vivo* component of this study also replicated the result of Steriade et al. (1993d) that the slow oscillation depends on intact cholinergic tone. Similar to other neuromodulators, the cellular and synaptic effects of acetylcholine are quite complex, being dependent on receptor subtype, pre- vs. postsynaptic expression of receptor, neurotransmitter concentration, and

differential expression of receptors in different excitatory and inhibitory neuron subtypes (McCormick, 1992; Muñoz and Rudy, 2014). Thus, it will be challenging to decipher the precise mechanisms of cholinergic modulation of the slow oscillation.

Serotonergic signaling has a long and controversial history in relation to the slow oscillation and sleep. In the 1960's, studies employing lesions of the DRN and chemical inhibition of serotonin synthesis resulted in strong and long-lasting insomnia in cats, leading to the hypothesis that serotonin was a crucial, if not the sufficient neuromodulator for initiating slow-wave sleep (Jouvet, 1972, 1999). Subsequent recordings from the DRN during the sleep-wake cycle, however, showed that neurons substantially decreased their firing from behavioral arousal to slow-wave sleep (McGinty and Harper, 1976; Trulsson and Jacobs, 1979), a conspicuous inconsistency with the serotonergic hypothesis of sleep. While probably not the nexus of sleep promotion it was once proposed to be, the serotonergic system of the DRN may play a role in the slow oscillation in several ways. First, while DRN neurons as a population considerably decrease their firing from waking to sleep, a heterogeneity of firing patterns exists among different types of DRN neurons, with 12% of cat DRN neurons actually exhibiting an increase in firing rate during slow-wave sleep compared to waking (Sakai and Crochet, 2001). In anesthetized rats, most serotonergic DRN neurons fired phasically with the cortical slow oscillation, though interestingly with a preference for the Down state (Schweimer et al., 2011). Thus, at least a fraction of DRN neurons can influence their synaptic targets during the slow oscillation. Second, serotonin differentially modulates the excitability of thalamocortical and TRN neurons, in the same direction as these cells are affected during the slow oscillation. That is, while serotonin depolarizes TRN neurons (McCormick and Wang, 1991), it hyperpolarizes thalamocortical neurons (Monckton and McCormick, 2002). Although the pronounced excitation in TRN neurons and pronounced inhibition in thalamocortical neurons during the slow oscillation is no doubt predominately due to the stronger unitary excitation of TRN neurons than of thalamocortical neurons by corticothalamic afferents (Golshani et al., 2001), serotonergic tone in the thalamus may augment the divergent excitability of TRN and thalamocortical neurons during the slow oscillation. Lastly, the high activity of DRN neurons during waking may *indirectly* affect the properties of the slow oscillation through a homeostatic up-regulation of sleep-promoting neurotransmitters. According to the homeostatic hypothesis of sleep regulation, the length of time spent in the waking period is proportional to the length of subsequent slow-wave sleep due to the exponential, but saturating increase in some homeostatic process during waking that decreases exponentially during sleep (Borbély, 1980). There is evidence that the serotonergic system engaged during waking plays a role in such a homeostatic process. For instance, chemical inhibition of serotonin synthesis throughout, but not at the end of sleep deprivation, abolishes slow-wave sleep, suggesting that the onset of sleep requires the serotonin-dependent accumulation of hypnogenic factors during waking (Sallanon et al., 1983). Thus, while mostly inactive

during the slow oscillation, DRN neurons may contribute to the properties of the oscillation by virtue of the biochemical changes they bring about through high levels of activity during waking.

FUNCTIONS OF THE SLOW OSCILLATION

On first consideration, it is curious that the slow oscillation, a recurrence of robust and synchronized activity, is associated with deep sleep, a condition in which the cortex is largely disconnected from the sensory environment. Why is a behavioral state bereft of cognitive or sensory processing associated with such rich spontaneous activity? Indeed, the neuronal activity during the slow oscillation that has been detailed in cortical and thalamic networks over the past two decades would undoubtedly have surprised early psychologists and neurophysiologists who viewed sleep as a manifestation of behavioral inhibition (Pavlov, 1923) or “abject mental annihilation” (Eccles, 1961). While the network-wide cycling between activity and quiescence during the slow oscillation is highly suggestive of a functional role, it could be argued that the salience of this phenomenon alone is not sufficient to afford it functionality. It could be that Up and Down states are simply default activity patterns that unavoidably follow from the recurrent network architecture of the cortex. As much is suggested by the fact that this activity occurs even in cortical slices *in vitro* under conditions of slightly enhanced excitability. Nevertheless, even if the slow oscillation primarily owes itself to the basic architecture of the cortex, a functional role for the slow oscillation may still exist.

When slow-wave sleep is viewed not as a mere idling of the brain, but as an active period in its own right, associated with the functional reorganization of neuronal networks across wide cortical territories, the possible functions of the slow oscillation become more clear. These possible functions include the synchronization of higher-frequency oscillations, the consolidation of memory traces acquired during waking, and basic biochemical maintenance in neurons during Down states.

Grouping of Higher-Frequency Oscillations by the Slow Oscillation

The cerebral cortex generates a panoply of oscillations distinguished by their different frequency bands (Buzsáki, 2006). Except in artificial circumstances, cortical oscillations of one frequency band are rarely expressed alone. Rather, oscillations of different frequencies interact in various ways through relations between their amplitudes and phases. For instance, one of the earliest demonstrations of the interactions between two different oscillations was the modulation of the amplitude of gamma (30–80 Hz)-band activity by the phase of slower theta (4–10 Hz)-band activity in the rat hippocampus (Buzsáki et al., 1983). Such phase-amplitude coupling could provide a mechanism for integrating the results of neural computation across multiple spatial and temporal scales (Canolty and Knight, 2010) and allow for information to be processed in a hierarchical manner (Buzsáki, 2010). During slow-wave sleep, the slow oscillation

serves as a “base frequency” that groups the two other major oscillations associated with slow-wave sleep, spindle and delta oscillations. The Up states of the slow oscillation are furthermore often associated with an increase in beta/gamma-band activity, frequencies that are often considered hallmarks of the waking, information-processing state.

Spindle Oscillations

Spindle oscillations (7–14 Hz) are one of the distinctive features of slow-wave sleep. Rhythmogenesis of spindles is due to the discharge of spike-bursts by GABAergic neurons of the TRN, which impart rhythmic IPSPs onto thalamocortical neurons. At sufficiently hyperpolarized potentials, as is the case during slow-wave sleep, thalamocortical neurons can fire rebound spike-bursts after each IPSP, imparting spindle-frequency excitatory input to cortex. In many respects, the TRN is a pacemaker for spindles, as evidenced by, for instance, the persistence of spindles when the TRN is separated from the thalamus (Steriade et al., 1987) and the sufficiency of optogenetic excitation of TRN cells to generate spindles (Halassa et al., 2011).

While the TRN may be sufficient for the rhythmicity of spindles, it is not sufficient for their characteristic synchrony across the thalamus and cortex. The synchronous occurrence of spindles requires active corticothalamic input impinging on TRN cells, since the removal of cortex results in a temporal disorganization of spindles across thalamic recording sites (Contreras et al., 1996a). Furthermore, spindles occurring *in vitro* do not occur synchronously, but propagate as a traveling wave (Kim et al., 1995), likely due to the absence of the synchronizing action of corticothalamic inputs. The origin of the synchronizing corticothalamic activity is the spiking of cortical cells during the Up state. Thus, when they occur synchronously (to the extent that they can be recorded with EEG), spindles quickly follow the initial depolarization of the Up state in cortical neurons (Steriade et al., 1993c), a sequence known as a “K-complex” in the clinical EEG literature (Amzica and Steriade, 1997). Thus, there is a consistent phase relationship between Up states and spindles.

Delta Oscillations

Delta oscillations (1–4 Hz) are most common during the deepest stages of slow-wave sleep. There appear to be at least two rhythmogenic mechanisms for the delta oscillation, one relying solely on the intrinsic properties of thalamocortical neurons, and the other likely relying on the intrinsic properties of layer 5 pyramidal neurons. The ionic currents underlying the delta oscillation arising from thalamocortical neurons have been well-characterized. At membrane potentials more hyperpolarized than those associated with spindles, interactions between the h-current and the T-current (see “Contribution of the thalamus” Section) produce delta rhythmicity: the h-current is activated and the T-current is de-inactivated, which leads to the activation of the T-current and the emergence of a low-threshold calcium spike (often crowned by sodium spikes), which deactivates the h-current and inactivates the T-current, repolarizing the cell such that the h-current can begin the cycle again (McCormick and Pape, 1990). The requisite hyperpolarization of thalamocortical cells for the interaction of the h-current and T-current to give

rise to delta oscillations is caused by the even further decrease in firing of brainstem cholinergic neurons during deep slow-wave sleep (Steriade et al., 1991b).

The existence of a separate cortical generator of the delta oscillation was suggested after its persistence following removal of the thalamus (Steriade et al., 1993b). The ionic basis for the cortically generated delta oscillation is not known at the same level of detail as the thalamically generated one, but it may incorporate many of the same mechanisms involved in the alternation of Up and Down states during the slow oscillation (i.e., the activation of intrinsic bursting pyramidal neurons in layer 5 and the subsequent activation of long-lasting K^+ conductances; see “Up state initiation” and “Up state termination” Section). Indeed, recent observations in quiet-waking rodents call into question the notion that delta oscillations, at least the cortically generated variety, are only associated with slow-wave sleep. Petersen et al. (2003) first reported ~2 Hz oscillations in the membrane potential of cortical neurons in quiet-waking mice and rats. Carl Petersen’s group has subsequently recorded these synchronized membrane potential oscillations in waking mice in several reports (e.g., Crochet and Petersen, 2006; Poulet and Petersen, 2008). Delta-frequency oscillations during waking cannot originate from thalamus because thalamocortical neurons are too depolarized to activate the h-current and de-inactivate the T-current. Instead, these oscillations occurring at traditional delta frequencies during the waking state are probably mechanistically indistinguishable from cortical Up and Down states. Such a high-frequency “slow oscillation” might be attributable to a level of K^+ conductance as high as that during slow-wave sleep, yet perhaps in concert with cortical activity levels or synaptic transmission properties comparable to those associated with waking. Indeed, cholinergic signaling in cortex increases from quiet wakefulness to active behavior (Eggermann et al., 2014), which would be associated with the closing of K^+ conductances that perhaps promote delta-frequency membrane potential oscillations.

During deep slow-wave sleep, cortical Up states synchronize thalamically generated delta oscillations in a manner similar to their synchronization of spindles during lighter slow-wave sleep. While thalamocortical neurons can generate delta oscillations autonomously, there is no intrathalamic mechanism to synchronize the oscillation among thalamocortical neurons since these cells primarily lack any recurrent collaterals. The synchronization of the thalamic delta oscillation is attributable to monosynaptic excitatory input from corticothalamic cells that are depolarized during the Up state, as well as disinaptic inhibition from TRN cells (Steriade et al., 1991b). The cortical enforcement of delta-band synchrony among bursting thalamocortical neurons has the same functional consequence as in spindles: a massive postsynaptic effect in the dendrites of cortical neurons, perhaps promoting signaling cascades associated with plasticity (see “Synaptic plasticity and the slow oscillation” Section).

Beta/Gamma Oscillations

During states of cortical activation, which include REM sleep and the waking state, slow and globally synchronized oscillations

are replaced with faster and more locally synchronized oscillations, such as in the beta (15–30 Hz) and gamma (30–80 Hz) range. While usually associated with sensory processing and higher-level mental activity, synchronized fast oscillations also occur during the Up states of the slow oscillation (Steriade and Amzica, 1996; Steriade et al., 1996a,b). This observation led Steriade and colleagues to suggest that synchronized fast oscillations are not a special quality of the waking, information-processing state, but a general characteristic of depolarization in cortical and thalamic neurons. This notion was based on the ability of cortical and thalamic neurons to exhibit sub- and suprathreshold fast oscillations under appropriate depolarizing current injection (Llinás et al., 1991; Steriade et al., 1991a; Nuñez et al., 1992; Gray and McCormick, 1996). Fast oscillations in cortical networks are also critically dependent on the synchronized firing of fast-spiking inhibitory interneurons, which is a phenomenon associated with Up states of the slow oscillation (Hasenstaub et al., 2005). The suggested functional roles of synchronized fast oscillations in cortical processing during waking are myriad (Fries, 2009; Bosman et al., 2014; Pritchett et al., 2015), though their roles in slow-wave sleep, if any, are uncertain. The fast oscillations associated with REM sleep have been proposed to be a correlate of dreaming mentation (Llinás and Ribary, 1993). While dreams have primarily been considered the province of REM sleep, they also occur during slow-wave sleep, though with different content (Hobson et al., 2000; McNamara et al., 2010). Fast oscillations during Up states of the slow oscillation during slow-wave sleep may provide the framework for dreaming mentation during this stage of sleep.

Synaptic Plasticity and the Slow Oscillation

The globally synchronized nature of the slow oscillation promotes the synchronization of faster sleep oscillations, particularly those generated in the thalamus, as discussed above. Thus, a major role of the slow oscillation is to modulate other oscillations. Yet, this explanation essentially displaces to another level the original question: is the slow oscillation functional or is it a byproduct of the basic architecture of the cortex? Are thalamic spindle and delta oscillations, potentiated by the cortical slow oscillation, also simply epiphenomena that arise when the brain is disconnected from the sensory environment?

One of the most prominent hypotheses regarding the function of slow-wave sleep, during which the slow oscillation is the cardinal form of neural activity, is that this state allows for the consolidation of memories. While the association of increased synaptic plasticity with sleep was first articulated by Moruzzi (1966) and Steriade and Timofeev (2003), behavioral and physiological experiments testing the dependence of learning and memory on sleep did not begin in earnest until the early 1990's. At that time, increasing research on a link between sleep and memory consolidation was largely catalyzed by two articles (see comment in Barinaga, 1994): one demonstrating a positive relationship between REM sleep and performance on a learned visual discrimination task in humans (Karni

et al., 1994), and another reporting the replay of waking patterns of hippocampal place cell activity during slow-wave sleep in rats (Wilson and McNaughton, 1994). Since the publication of these two articles, there has been a wealth of studies examining relations between sleep and memory in various model organisms and at levels of analysis spanning the molecular to the behavioral (reviewed in Walker and Stickgold, 2004; Waters and Helmchen, 2006; Stickgold, 2005; Massimini et al., 2009; Diekelmann and Born, 2010; Tononi and Cirelli, 2014). Nevertheless, the interpretation of the results of these various studies as conclusive demonstrations of the dependence of learning and memory processes on sleep remains controversial (Vertes, 2004; Frank and Benington, 2006).

As alluded to in “Grouping of higher-frequency oscillations by the slow oscillation” Section, the synchronous burst-firing of thalamocortical neurons during slow-wave sleep, suggests a recruitment of signaling cascades involved in synaptic plasticity in cortical neurons.

Thalamocortical neurons firing synchronous bursts would strongly depolarize the dendrites of postsynaptic pyramidal cells, leading to a massive dendritic Ca^{2+} influx (Yuste and Tank, 1996). Ca^{2+} , acting as a second messenger (Ghosh and Greenberg, 1995), can subsequently set into motion signaling cascades associated with increased synaptic plasticity, such as those involving Ca^{2+} /calmodulin-dependent kinase II (CaMKII) (Soderling and Derkach, 2000).

A role for the cortical slow oscillation in memory consolidation is also suggested based on its coordination with hippocampal activity during slow-wave sleep. The promotion of synaptic plasticity in the cortex via Ca^{2+} -dependent processes coupled with the entrainment of hippocampal activity by cortical Up states may permit the transfer of memory traces from their more labile form in hippocampal networks to their consolidated form in the neocortex. The initial discovery of hippocampal replay of waking patterns of activity during slow-wave sleep (Wilson and McNaughton, 1994) suggested a possible dialogue between hippocampus and neocortex during this period. Since then, there have been many demonstrations of temporal coordination in firing between cortical and hippocampal rhythms during slow wave sleep, with a particular emphasis on the occurrence of sharp-wave ripples (~200 Hz) in hippocampus shortly after the initiation of cortical Up states (Siapas and Wilson, 1998; Sirota et al., 2003; Isomura et al., 2006). Furthermore, hippocampal replay of a sensory experience is coordinated with replay in the associated sensory cortex during slow-wave sleep, with the onset of replay in the cortex apparently driving that in the hippocampus (Ji and Wilson, 2007). Thus, a current working hypothesis for slow-wave-dependent memory consolidation is that cortical Up states, traversing the entorhinal cortex, bias the occurrence of sharp-wave ripples in the hippocampus, which repeatedly transfer hippocampal activity patterns, acquired during learning, to the cortical network in the midst of an Up state, during which the disposition for cortical plasticity is high (Sirota and Buzsáki, 2005).

The Slow Oscillation as a Period of Cellular Restoration

Down states, periods of synchronously enforced neuronal quiescence, are arguably the most conspicuous feature of the slow oscillation, since network-wide hyperpolarizing periods are rare during waking. While the hypothesis that slow-wave sleep promotes synaptic plasticity and memory consolidation primarily emphasizes the occurrence of Up states, another leading hypothesis of the function of slow-wave sleep focuses on the occurrence of Down states: during these slow-wave-defining periods of network silence, neurons can engage in various restorative and cellular maintenance functions. The notion that slow-wave sleep serves functions besides memory consolidation is conveyed by the various cognitive impairments associated with sleep deprivation (Goel et al., 2009; Killgore, 2010; McCoy and Strecker, 2011; Van Dongen et al., 2011). Indeed, in the most extreme case of sleep deprivation, fatal familial insomnia (Cortelli et al., 1999), slow-wave sleep is impossible, inevitably leading to death. Thus, slow-wave sleep seems to be a period associated with basic repair after the high metabolic demand of the waking state, and perhaps prophylactic measures that counteract further cellular attrition accompanying sustained neuronal activity. Such basic restorative roles during the slow oscillation are likely to be associated with the Down state (Vyazovskiy and Harris, 2013).

THE RELATION BETWEEN UP STATES AND ACTIVATED STATES

During the Up states of the slow oscillation, cortical neurons exhibit membrane potential dynamics and spiking activity nearly indistinguishable from those properties during the “cortical activation” of REM sleep and waking. In addition, the fast oscillations that typify the activated cortex are also present during Up states (see “Beta/gamma oscillations” Section). Thus, in many respects, Up states resemble abbreviated periods of cortical activation. It can consequently be argued that Up states trigger network operations that are very similar to the essence of waking cortical behavior. From this standpoint, the Up state can be considered a model of the cortex in the active, information-processing state (see “Up states as a model of cortical gain control” Section).

The similarities between the Up states of the slow oscillation during natural sleep or anesthesia and cortical activation during waking span multiple spatiotemporal scales, often as measured in the same preparation. For instance, waking activity and slow-oscillation Up states are characterized by similar relations between multiunit discharges and LFP and by similar spatiotemporal coherence of fast oscillatory activity (Destexhe et al., 1999). The similarity between Up states during slow-wave sleep and waking cortical activity also extends to the relative contribution of excitatory and inhibitory synaptic conductances that drive membrane potential fluctuations: during both waking activity and Up states, the magnitude and variance of inhibitory conductance dominates that of excitatory conductance (Rudolph et al., 2007). One difference between Up states of the slow

oscillation and waking activity is the input resistance of cortical neurons. While both types of activity are high-conductance states, in which synaptic activity decreases input resistance by several times the value associated with quiescent periods (i.e., during Down states or *in vitro*; Paré et al., 1998; though, see Waters and Helmchen, 2006), input resistances are higher during waking activity than during Up states (Steriade et al., 2001), perhaps due to the blockade of K^+ conductances by muscarinic acetylcholine receptors during waking (see “Up state termination” Section).

While the cortex is largely disconnected from the sensory environment when the slow oscillation occurs, the presence of waking-like segments of network activity during this period suggests the cortex is processing internally generated signals, perhaps related to memory consolidation (see “Synaptic plasticity and the slow oscillation” Section) or dreaming mentation (see “Beta/gamma oscillations” Section). Of course, the fragments of cortical activation during Up states do not lead to conscious awareness, so there must be distinguishing factors of veritable waking activity in the cortex that are not reflected during Up states. These factors may include: dynamic variables that cannot be detected with the current spatiotemporal resolution of current electrophysiological or imaging methods, the brevity of cortical activation during Up states (conscious awareness may require longer periods of activation), or diminished long-range effective cortical connectivity during the slow oscillation (Massimini et al., 2005; Destexhe et al., 2007).

CORTICAL RESPONSIVENESS DURING UP AND DOWN STATES

Compared with the Down state, characterized by the almost complete absence of action-potential-dependent synaptic activity in the cortex, the Up state is characterized by vigorous synaptic activity in many cortical neurons. Synaptic activity can have diverse effects on the ability of cortical neurons to respond to afferent inputs. The diversity of the effects of synaptic activity on neuronal responsiveness stems from the various cellular and network properties altered by this activity. The combination of these changes can have complex effects on the ability of cortical neurons to respond to incoming signals. The effects of Up states on neuronal responsiveness have been studied in both *in vitro* and *in vivo* preparations, using various intracellular, sensory, or afferent-pathway stimulation protocols.

Up States Affect Intrinsic Responsiveness

At the single-neuron level, synaptic activity associated with the Up state causes three primary changes: membrane potential depolarization, increased membrane conductance, and increased membrane potential variance. Each of these changes modulates the neuronal input-output function differently. The neuronal input-output function is defined as the relation between stimulus strength and action potential output. Combining the *in vitro* slow oscillation with the application of dynamic clamp, McCormick et al. (2003) and Shu et al. (2003a) dissected the contribution to the neuronal input-output function of each of the three

primary changes associated with Up states. Up states themselves enhance the sensitivity of neurons to synaptic inputs, shifting the input-output function to the left, and selectively enhance the responsiveness to small inputs that are normally subthreshold during the Down state, which is associated with a decreased slope of the input-output function. The leftward shift in the input-output function is primarily due to membrane potential depolarization, which overcomes the rightward shift due to increased membrane conductance. The selective enhancement of responsiveness to small inputs is caused by increased membrane potential noise. An additional effect of the Up state is an overall enhancement of the fidelity of signal transmission: compared to Down states, Up states decrease the latency between the arrival of a synaptic input and the firing of an action potential, enhance spike-timing precision, and improve the cross-correlation between action potential output and complex stimulus waveforms.

Up States Affect Synaptic Properties of Thalamocortical and Corticothalamic Afferents

The intrinsic membrane properties of cortical neurons constitute one dimension of responsiveness that Up states can modulate. The release properties and synaptic targets of different afferents onto the cortical network are other variables potentially modulable by Up state activity, sometimes in the opposite direction from changes in intrinsic excitability. The effects of Up states on the responsiveness of cortical neurons to stimulation of either thalamocortical or corticocortical afferents has primarily been studied in the somatosensory thalamocortical slice preparation *in vitro* (Agmon and Connors, 1991).

Combining calcium imaging and intracellular recording in thalamocortical slices, MacLean et al. (2005) and Watson et al. (2008) determined that not only did thalamic stimulation elicit Up states that were spatiotemporally indistinguishable from those occurring spontaneously, but also that this stimulation did not perturb spontaneous Up states. These *in vitro* results were consistent with earlier reports in either anesthetized or quiet-waking rodent barrel cortex, in which whisker-stimulation-evoked cortical responses were suppressed by Up states, compared to Down states (Petersen et al., 2003; Sachdev et al., 2004; see also “Up states affect sensory-evoked responses” Section). There are several reasons why Up states might suppress thalamocortical responses: decreased driving force for thalamocortical excitation and increased driving force for thalamocortical feedforward/intracortical inhibition, shunting of thalamocortical PSPs by increased membrane conductance, increased spike threshold, and lower release probability at thalamocortical synapses. Thus, while neurons may be intrinsically more excitable during Up states, the effect of Up states on the response to synaptic input can be suppressive if, for instance, afferent synapses are tonically depressed due to ongoing activity (Reig et al., 2006) or the reversal potential of the thalamocortical synaptic response is close to the level of membrane potential depolarization during the Up state. Another *in vitro* study, however, showed that Up states enhanced

cortical responses to thalamic stimulation, but suppressed cortical responses to local cortical stimulation (Rigas and Castro-Alamancos, 2009). The authors of this study attributed the enhanced thalamocortical response primarily to depolarization during the Up state and the suppressed intracortical response to reduced release probability resulting from ongoing activity in the cortex, which is mostly lacking in the thalamus in slice.

Up States Affect Sensory-Evoked Responses

The results reviewed so far suggest that the interaction between Up states and afferent synaptic input is not straightforward. While depolarization and increased membrane potential variance generally enhance cellular excitability, especially when inputs are small, the ongoing dynamics of the presynaptic afferents that are stimulated and the precise mixture of excitation and inhibition that make up the synaptic response will also determine the direction in which the occurrence of an Up state affects the probability of spiking upon afferent stimulation. If the afferents are already tonically depressed from ongoing activity and if the mixture of excitation and inhibition composing the synaptic response causes the reversal potential of this response to lie near the mean value of Up state depolarization, Up states will suppress neuronal responsiveness compared to Down states. Additionally, while the enhancing effect of depolarization during the Up state has been shown to overcome the accompanying suppressing effect of increased membrane conductance (Shu et al., 2003a), it is conceivable that this relationship might not hold for every Up state or for the entire time course of any given Up state. Diverse effects of Up states on cortical responses to sensory stimuli have been reported *in vivo*, as discussed below.

Early studies investigating the dependance of evoked cortical responses on spontaneous network activity demonstrated a linear relationship between the spontaneous level of the membrane potential in cortical neurons and the magnitude of their evoked response. Such a linear relationship between spontaneous membrane potential and evoked response was described in anesthetized cat motor cortex while stimulating pre-thalamic afferents onto VL (Timofeev et al., 1996) and in anesthetized cat visual cortex while presenting drifting gratings (Arieli et al., 1996; Azouz and Gray, 1999). Later work in anesthetized cat visual cortex also showed a linear relationship between Up-state membrane potential and visually evoked spiking output, with a further demonstration that Up-state depolarization multiplicatively scales the contrast-response function (Haider et al., 2007).

Results from rodent somatosensory (barrel) cortex, however, have generally contradicted those in the visual system. VSD imaging of cortical responses to whisker deflection have revealed lower-amplitude and more spatially confined responses during Up states compared with Down states (Petersen et al., 2003; Civillico and Contreras, 2012). A quenching effect of Up states on whisker-evoked responses in barrel cortex is also evident from the reduced spiking output of single cortical neurons compared to the Down state (Sachdev et al., 2004;

Hasenstaub et al., 2007; **Figure 6**). Discrepancies between visual and somatosensory cortex regarding the influence of Up states in sensory responsiveness could have a number of explanations. As suggested above, the relation between the reversal potential of the sensory-evoked synaptic response and the average membrane potential during the Up state is one factor that could determine the direction in which the Up state affects responsiveness. If the sensory-evoked synaptic reversal potential is comparable to the membrane potential during the Up state, there will hardly be any driving force for the sensory response during the Up state. The relative combinations of excitatory and inhibitory conductances recruited by sensory stimulation determine the sensory-evoked synaptic reversal potential. It could be that the precise combination of these conductances leads to a more hyperpolarized reversal potential in barrel cortex compared to visual cortex. In support of the notion that low driving force during the Up state accounts for the reduced effectiveness of sensory stimulation compared to the Down state, sensory-evoked PSPs in barrel cortex neurons were significantly larger during Down states compared to Up states (Sachdev et al., 2004); in visual cortex, no significant difference was found between PSPs evoked in the Up vs. the Down state (Haider et al., 2007). In contrast, injection of depolarizing current during the Down state in barrel-cortical neurons to maintain the membrane potential at the level encountered during Up states enhanced spiking output to whisker stimulation (Hasenstaub et al., 2007; **Figure 6**), which is inconsistent with the notion that a reduction of driving force alone accounts for reduced sensory-evoked responses during the Up state. Additionally, consistent with *in vitro* work (Shu et al., 2003a; see “Up states affect intrinsic responsiveness” Section), injection of artificial PSPs into barrel-cortical neurons more readily elicited spiking during Up states than during Down states (Hasenstaub et al., 2007).

Thus, the diminished response in barrel cortex to whisker deflection during Up states does not seem attributable to properties intrinsic to neurons, such as their depolarization, spike threshold, or membrane conductance, but rather to network effects. Network-level mechanisms for decreased responsiveness to whisker stimulation during Up states in barrel cortex likely involve an overall decreased stimulus-evoked synaptic conductance compared to the Down state, most likely due to depression of thalamo- and corticocortical synapses during network activity, and a relative increase of the contribution of inhibition over excitation to the stimulus-evoked synaptic conductance during the Up state compared to the Down state. Both of these possibilities were suggested via estimates of stimulus-evoked synaptic conductance and the contributions of excitation and inhibition to this conductance in Up vs. Down states in barrel cortex (Hasenstaub et al., 2007). One would therefore predict that in visual cortex, where Up states enhance responses to sensory stimuli, stimulus-evoked synaptic conductances are not significantly smaller in the Up state compared to the Down state, or that evoked inhibition is not comparatively larger in the Up state compared to the Down state. Whether there is a difference between barrel cortex and visual cortex in these regards is not known. Interestingly, whereas the response to brief whisker deflections is diminished by the

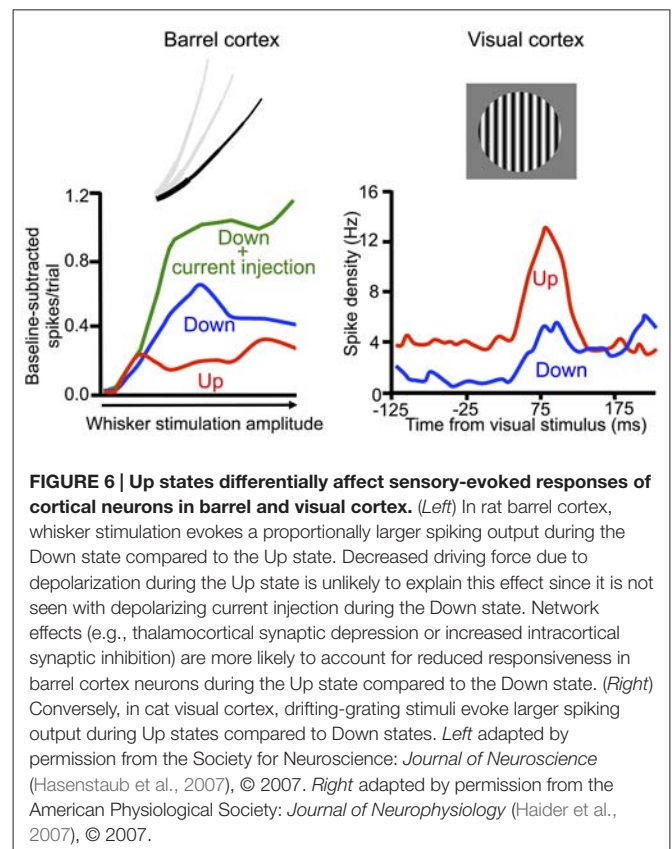


FIGURE 6 | Up states differentially affect sensory-evoked responses of cortical neurons in barrel and visual cortex. (Left) In rat barrel cortex, whisker stimulation evokes a proportionally larger spiking output during the Down state compared to the Up state. Decreased driving force due to depolarization during the Up state is unlikely to explain this effect since it is not seen with depolarizing current injection during the Down state. Network effects (e.g., thalamocortical synaptic depression or increased intracortical synaptic inhibition) are more likely to account for reduced responsiveness in barrel cortex neurons during the Up state compared to the Down state. (Right) Conversely, in cat visual cortex, drifting-grating stimuli evoke larger spiking output during Up states compared to Down states. *Left* adapted by permission from the Society for Neuroscience: *Journal of Neuroscience* (Hasenstaub et al., 2007), © 2007. *Right* adapted by permission from the American Physiological Society: *Journal of Neurophysiology* (Haider et al., 2007), © 2007.

occurrence of Up states, responses to temporally prolonged whisker stimulation are enhanced (Hasenstaub et al., 2007). Thus, the effect of Up states on sensory-evoked responses in cortex can also depend on the dynamics of the stimulus.

Intensity of the sensory stimulus is another possible factor that determines the direction in which Up states modulate sensory responses in cortex. This issue was recently addressed in rat auditory cortex, in which a range of sound stimulus intensities was used, and whose effects on cortical responses were compared between Up and Down states (Reig et al., 2015). In this study, the direction in which Up states modulated neuronal responsiveness during sensory stimulation depended on stimulus strength: responses to weak stimuli were potentiated and responses to strong stimuli were diminished by Up states. The authors attributed the stimulus-intensity-dependent effect of Up states on cortical responses to the competition between thalamocortical network excitability and neuronal membrane conductance, both of which increase during Up states. In essence, high network excitability, associated with Up states, favors low-intensity stimuli, whereas low membrane conductance, associated with Down states, favors high-intensity stimuli. Another possible mechanism for the stimulus-intensity-dependent effect of Up states on neuronal responsiveness (i.e., potentiation at low intensities and suppression at high intensities) was suggested by Shu et al. (2003a), who described an identical effect using artificial EPSPs as stimuli during both natural Up states and simulated Up states via the injection of conductance noise. In this case, noise facilitates spiking

probability to small synaptic inputs (those that would cause spiking <50% of the time during the Down state) due to a floor effect (only the depolarizing components of the noise can affect spiking probability), yet diminishes spiking probability to large synaptic inputs (those that would cause spiking >50% of the time during the Down state) due to a ceiling effect (only the hyperpolarizing components of the noise can affect spiking probability). Thus, stimulus-intensity-dependent effects of Up states on neuronal responsiveness could be due solely to the effects of membrane potential variance on individual cortical neurons.

UP STATES AS A MODEL OF CORTICAL GAIN CONTROL

Since the onset and termination of Up states is quite rapid (a few tens of milliseconds), such periods of enhanced cortical network activity could provide a substrate for the moment-to-moment changes in the routing of information by the cortex, such as is required for processing the context of sensory stimuli, maintaining objects in working memory, or attention. While veritable “Up states,” as distinguished from intervening hyperpolarized periods, have only been unequivocally observed during slow-wave sleep, under certain types of anesthesia, and to some degree to during quiet wakefulness, the rapid increases in synaptic barrages in the cortical network that characterize Up states might also characterize the rapid changes in excitability that would be required for the flexible processing of information (McCormick et al., 2004; McCormick and Yuste, 2006; Haider and McCormick, 2009). In essence, the same neuronal machinery that is spontaneously engaged during sleep may also be adapted by the waking cortex to adaptively route signals on a fast time-scale according to behavioral demands. Consequently, investigation of the cellular and network characteristics of Up states not only provides an understanding of the nature of cortical activity during sleep, but may also provide insight into the mechanisms associated with rapid changes in functional connectivity during waking behavior.

A fundamental operation in neuronal networks is gain modulation, defined as multiplicative or divisive transformations of the neuronal input-output function. Changes in gain amplify or reduce neuronal responsiveness with minimal effects on stimulus selectivity or receptive field structure (Sclar and Freeman, 1982; Carandini and Heeger, 1994). Such a transformation facilitates the interaction among multiple variables in the spiking output of a single neuron. One of the earliest demonstrations of the computational role of gain modulation was the multiplicative increase in visual responses in posterior parietal cortex by eye position (Andersen and Mountcastle, 1983). In addition to implementing sensorimotor transformations, gain modulation has also been shown to play a role in the enhancement of sensory-evoked responses by spatial or feature-based attention (Connor et al., 1996, 1997; McAdams and Maunsell, 1999; Treue and Martinez-Trujillo, 1999; Williford and Maunsell, 2006).

Changes in membrane potential depolarization, membrane potential variance, and membrane conductance, all features of cortical Up states, are ideal candidate mechanisms for rapid gain modulation. Yet, since many basic changes of the neuronal membrane, such as depolarization and shunting, in either model neurons or neurons *in vitro* only effect additive or subtractive shifts in the neuronal input-output function (Holt and Koch, 1997), pure or nearly pure changes in neuronal gain based on fast synaptic signaling (i.e., via ionotropic glutamate and GABA_A receptors) have historically been rejected. Many models of gain modulation have relied on more complex mechanisms (Srinivasan and Bernard, 1976; Salinas and Abbott, 1996; Hahnloser et al., 2000). When neurons are embedded in a recurrently active network, however, the presence of synaptic noise fundamentally alters the ability of membrane potential, membrane potential variance, and membrane conductance to modulate gain (Hô and Destexhe, 2000; Chance et al., 2002; Fellous et al., 2003; Mitchell and Silver, 2003; Murphy and Miller, 2003; Shu et al., 2003a; Kuhn et al., 2004). In the presence of synaptic noise, the relation between membrane potential and firing rate is not a linear function with a threshold, as is the case in quiescent networks, but a power law, in which firing rate relates to membrane potential by raising the latter by an exponent (Miller and Troyer, 2002). When the relation between membrane potential and firing rate obeys a power-law function, simple manipulations of the neuronal membrane, such as depolarization and hyperpolarization, can change neuronal gain (Murphy and Miller, 2003). Indeed, such multiplicative modulations due to depolarization alone have been described during Up states in visual cortex *in vivo* (Haider et al., 2007). Thus, enhanced barrages of synaptic inputs, which occur on a rapid time-scale in local cortical networks, could produce immediate changes in neuronal gain in the subset of neurons responsible for momentary behavioral demands. In another model of gain modulation by synaptic inputs, neuronal gain is increased by the *removal* of these inputs (Chance et al., 2002). In this model, EPSPs and IPSPs must be removed in precise proportion to keep membrane potential constant while decreasing membrane conductance. Which model of synaptic-input-dependent gain control is employed by the cortex during active behavior is unclear, but each provides testable predictions: in one model, behaviorally relevant gain control is associated with depolarization, while in the other model, gain control is associated with a decrease in membrane conductance and a constant membrane potential.

CONCLUSIONS

The slow oscillation is a fundamental feature of the corticothalamic system; during behavioral periods associated with effective disconnection from the sensory environment, it is essentially the default activity pattern of the entire cortical mantle. Through the use of various recording and imaging techniques in *in vivo* and *in vitro* preparations, considerable progress has been made in elucidating the cellular

and network mechanisms involved in the synchronous cycling of Up and Down states in both local and disparate cortical networks.

There are many further avenues for investigation of the mechanisms and functions of the slow oscillation. Among the most crucial are the causal roles of thalamic activity in the initiation, persistence, and termination of Up states, the causal roles of inhibitory interneurons vs. intrinsic hyperpolarizing conductances in Up state termination, the functions of slow rhythmic activity in quiescent behavioral states, and the precise relation between network activity during the Up state and network activity associated with active sensory and cognitive processing. While these are all quite ambitious questions, the necessary technologies for addressing them at the

single-neuron, microcircuit, and large-scale network levels are emerging.

AUTHOR CONTRIBUTION

GTN wrote the manuscript.

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REFERENCES

- Agmon, A., and Connors, B. W. (1989). Repetitive burst-firing neurons in the deep layers of mouse somatosensory cortex. *Neurosci. Lett.* 99, 137–141. doi: 10.1016/0304-3940(89)90278-4
- Agmon, A., and Connors, B. W. (1991). Thalamic cortical responses of mouse somatosensory (barrel) cortex *in vitro*. *Neuroscience* 41, 365–379. doi: 10.1016/0306-4522(91)90333-j
- Alaburda, A., Perrier, J. F., and Hounsgaard, J. (2002). Mechanisms causing plateau potentials in spinal motoneurons. *Adv. Exp. Med. Biol.* 508, 219–226. doi: 10.1007/978-1-4615-0713-0_27
- Amzica, F., and Steriade, M. (1995a). Short- and long-range neuronal synchronization of the slow (<1 Hz) cortical oscillation. *J. Neurophysiol.* 73, 20–38.
- Amzica, F., and Steriade, M. (1995b). Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. *J. Neurosci.* 15, 4658–4677.
- Amzica, F., and Steriade, M. (1997). The K-complex: its slow (<1-Hz) rhythmicity and relation to delta waves. *Neurology* 49, 952–959. doi: 10.1212/wnl.49.4.952
- Andersen, R. A., and Mountcastle, V. B. (1983). The influence of the angle of gaze upon the excitability of the light-sensitive neurons of the posterior parietal cortex. *J. Neurosci.* 3, 532–548.
- Anderson, J., Lampl, I., Reichova, I., Carandini, M., and Ferster, D. (2000). Stimulus dependence of two-state fluctuations of membrane potential in cat visual cortex. *Nat. Neurosci.* 3, 617–621. doi: 10.1038/75797
- Arieli, A., Sterkin, A., Grinvald, A., and Aertsen, A. (1996). Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. *Science* 273, 1868–1871. doi: 10.1126/science.273.5283.1868
- Ashford, M. L., Sturgess, N. C., Trout, N. J., Gardner, N. J., and Hales, C. N. (1988). Adenosine-5'-triphosphate-sensitive ion channels in neonatal rat cultured central neurones. *Pflugers. Arch.* 412, 297–304. doi: 10.1007/bf00582512
- Azouz, R., and Gray, C. M. (1999). Cellular mechanisms contributing to response variability of cortical neurons. *J. Neurosci.* 19, 2209–2223.
- Barinaga, M. (1994). Neuroscience. To sleep, perchance to... learn? New studies say yes. *Science* 265, 603–604. doi: 10.1126/science.8036508
- Barthó, P., Freund, T. F., and Acsády, L. (2002). Selective GABAergic innervation of thalamic nuclei from zona incerta. *Eur. J. Neurosci.* 16, 999–1014. doi: 10.1046/j.1460-9568.2002.02157.x
- Bazhenov, M., Timofeev, I., Steriade, M., and Sejnowski, T. J. (2002). Model of thalamocortical slow-wave sleep oscillations and transitions to activated states. *J. Neurosci.* 22, 8691–8704.
- Beierlein, M., Gibson, J. R., and Connors, B. W. (2003). Two dynamically distinct inhibitory networks in layer 4 of the neocortex. *J. Neurophysiol.* 90, 2987–3000. doi: 10.1152/jn.00283.2003
- Beltramini, R., D'Urso, G., Dal Maschio, M., Farisello, P., Bovetti, S., Clovis, Y., et al. (2013). Layer-specific excitatory circuits differentially control recurrent network dynamics in the neocortex. *Nat. Neurosci.* 16, 227–234. doi: 10.1038/nn.3306
- Binzegger, T., Douglas, R. J., and Martin, K. A. C. (2004). A quantitative map of the circuit of cat primary visual cortex. *J. Neurosci.* 24, 8441–8453. doi: 10.1523/jneurosci.1400-04.2004
- Blethyn, K. L., Hughes, S. W., Tóth, T. I., Cope, D. W., and Crunelli, V. (2006). Neuronal basis of the slow (<1 Hz) oscillation in neurons of the nucleus reticularis thalami. *J. Neurosci.* 26, 2474–2486. doi: 10.1523/jneurosci.3607-05.2006
- Borbély, A. A. (1980). "Sleep: circadian rhythm versus recovery process," in *Functional States of the Brain: Their Determinants*, eds M. Koukoku, D. Lehmann and J. Angst (Amsterdam: Elsevier), 151–161.
- Bosman, C. A., Lansink, C. S., and Pennartz, C. M. (2014). Functional of gamma-band synchronization in cognition: from single circuits to functional diversity across cortical and subcortical systems. *Eur. J. Neurosci.* 39, 1982–1999. doi: 10.1111/ejn.12606
- Braitenberg, V., and Shüz, A. (1998). *Cortex: Statistics and Geometry of Neuronal Connectivity*. New York: Springer.
- Buzsáki, G. (2006). *Rhythms of the Brain*. New York: Oxford University Press.
- Buzsáki, G. (2010). Neural syntax: cell assemblies, synapsembles and readers. *Neuron* 68, 362–385. doi: 10.1016/j.neuron.2010.09.023
- Buzsáki, G., Leung, L. W., and Vanderwolf, C. H. (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Res.* 287, 139–171. doi: 10.1016/0165-0173(83)90037-1
- Canolty, R. T., and Knight, R. T. (2010). The functional role of cross-frequency coupling. *Trends Cogn. Sci.* 14, 506–515. doi: 10.1016/j.tics.2010.09.001
- Carandini, M., and Heeger, D. J. (1994). Summation and division by neurons in primate visual cortex. *Science* 264, 1333–1336. doi: 10.1126/science.8191289
- Castro-Alamancos, M. A., and Favero, M. (2015). NMDA receptors are the basis for persistent network activity in neocortex slices. *J. Neurophysiol.* 113, 3816–3826. doi: 10.1152/jn.00090.2015
- Chagnac-Amitai, Y., and Connors, B. W. (1989). Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. *J. Neurophysiol.* 62, 1149–1162.
- Chagnac-Amitai, Y., Luhmann, H. J., and Prince, D. A. (1990). Burst generating and regular spiking layer 5 pyramidal neurons of rat neocortex have different morphological features. *J. Comp. Neurol.* 296, 598–613. doi: 10.1002/cne.902960407
- Chance, F. S., Abbott, L. F., and Reyes, A. D. (2002). Gain modulation from background synaptic input. *Neuron* 35, 773–782. doi: 10.1016/S0896-6273(02)00820-6
- Chauvette, S., Crochet, S., Volgushev, M., and Timofeev, I. (2011). Properties of slow oscillation during slow-wave sleep and anesthesia in cats. *J. Neurosci.* 31, 14998–15008. doi: 10.1523/JNEUROSCI.2339-11.2011
- Chauvette, S., Volgushev, M., and Timofeev, I. (2010). Origin of active states in local neocortical networks during slow sleep oscillation. *Cereb. Cortex* 20, 2660–2674. doi: 10.1093/cercor/bhq009
- Chen, J. Y., Chauvette, S., Skorheim, S., Timofeev, I., and Bazhenov, M. (2012). Interneuron-mediated inhibition synchronizes neuronal activity during

- slow oscillation. *J. Physiol.* 590, 3987–4010. doi: 10.1113/jphysiol.2012.227462
- Civillico, E. F., and Contreras, D. (2012). Spatiotemporal properties of sensory responses *in vivo* are strongly dependent on network context. *Front. Syst. Neurosci.* 6:25. doi: 10.3389/fnsys.2012.00025
- Compte, A., Sanchez-Vives, M. V., McCormick, D. A., and Wang, X. J. (2003). Cellular and network mechanisms of slow oscillatory activity (<1 Hz) and wave propagations in a cortical network model. *J. Neurophysiol.* 89, 2707–2725. doi: 10.1152/jn.00845.2002
- Connor, C. E., Gallant, J. L., Preddie, D. C., and Van Essen, D. C. (1996). Responses in area V4 depend on the spatial relationship between stimulus and attention. *J. Neurophysiol.* 75, 1306–1308.
- Connor, C. E., Preddie, D. C., Gallant, J. L., and Van Essen, D. C. (1997). Spatial attention effects in macaque area V4. *J. Neurosci.* 17, 3201–3214.
- Connors, B. W. (1984). Initiation of synchronized neuronal bursting in neocortex. *Nature* 310, 685–687. doi: 10.1038/310685a0
- Connors, B. W., Gutnick, M. J., and Prince, D. A. (1982). Electrophysiological properties of neocortical neurons *in vitro*. *J. Neurophysiol.* 48, 1302–1320.
- Connors, B. W., Pinto, D. J., and Telfeian, A. E. (2001). Local pathways of seizure propagation in neocortex. *Int. Rev. Neurobiol.* 45, 527–546. doi: 10.1016/s0074-7742(01)45027-6
- Contreras, D., and Steriade, M. (1995). Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J. Neurosci.* 15, 604–622.
- Contreras, D., Destexhe, A., Sejnowski, T. J., and Steriade, M. (1996a). Control of spatiotemporal coherence of a thalamic oscillation by corticothalamic feedback. *Science* 274, 771–774. doi: 10.1126/science.274.5288.771
- Contreras, D., Timofeev, I., and Steriade, M. (1996b). Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *J. Physiol.* 494, 251–264. doi: 10.1113/jphysiol.1996.sp021488
- Cortelli, P., Gambetti, P., Montagna, P., and Lugaresi, E. (1999). Fatal familial insomnia: clinical features and molecular genetics. *J. Sleep Res.* 8(Suppl. 1), 23–29. doi: 10.1046/j.1365-2869.1999.00005.x
- Cossart, R., Aronov, D., and Yuste, R. (2003). Attractor dynamics of network UP states in the neocortex. *Nature* 423, 283–288. doi: 10.1038/nature01614
- Craig, M. T., Mayne, E. W., Bettler, B., Paulsen, O., and McBain, C. (2013). Distinct roles of GABA_{B1a}- and GABA_{B1b}-containing GABA_B receptors in spontaneous and evoked termination of persistent cortical activity. *J. Physiol.* 591, 835–843. doi: 10.1113/jphysiol.2012.248088
- Crochet, S., and Petersen, C. C. (2006). Correlating whisker behavior with membrane potential in barrel cortex of awake mice. *Nat. Neurosci.* 9, 608–610. doi: 10.1038/nn1690
- Cruikshank, S. J., Rose, H. J., and Metherate, R. (2002). Auditory thalamocortical synaptic transmission *in vitro*. *J. Neurophysiol.* 87, 361–384. doi: 10.1152/jn.00549.2001
- Crunelli, V., David, F., Lörincz, M. L., and Hughes, S. W. (2015). The thalamocortical network as a single slow wave-generating unit. *Curr. Opin. Neurobiol.* 31, 72–80. doi: 10.1016/j.conb.2014.09.001
- Crunelli, V., and Hughes, S. W. (2010). The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat. Neurosci.* 13, 9–17. doi: 10.1038/nn.2445
- Crunelli, V., Lörincz, M. L., Errington, A. C., and Hughes, S. W. (2012). Activity of cortical and thalamic neurons during the slow (<1 Hz) rhythm in the mouse. *Pflugers. Arch.* 463, 73–88. doi: 10.1007/s00424-011-1011-9
- Cunningham, M. O., Pervouchine, D. D., Racca, C., Kopell, N. J., Davies, C. H., Jones, R. S. G., et al. (2006). Neuronal metabolism governs cortical network response state. *Proc. Natl. Acad. Sci. U S A* 103, 5597–5601. doi: 10.1073/pnas.060604103
- David, F., Schmiedt, J. T., Taylor, H. L., Orban, G., Di Giovanni, G., Uebele, V. N., et al. (2013). Essential thalamic contribution to slow waves of natural sleep. *J. Neurosci.* 33, 19599–19610. doi: 10.1523/JNEUROSCI.3169-13.2013
- DeFelipe, J., and Fariñas, I. (1992). The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. *Prog. Neurobiol.* 39, 563–607. doi: 10.1016/0301-0082(92)90015-7
- Dembrow, N., and Johnston, D. (2014). Subcircuit-specific neuromodulation in the prefrontal cortex. *Front. Neural Circuits* 8:54. doi: 10.3389/fncir.2014.00054
- Destexhe, A., Contreras, D., and Steriade, M. (1999). Spatiotemporal analysis of local field potentials and unit discharges in cat cerebral cortex during natural wake and sleep states. *J. Neurosci.* 19, 4595–4608.
- Destexhe, A., Hughes, S. W., Rudolph, M., and Crunelli, V. (2007). Are corticothalamic ‘up’ states fragments of wakefulness? *Trends Neurosci.* 30, 334–342. doi: 10.1016/j.tins.2007.04.006
- Détári, L., Rasmusson, D. D., and Semba, K. (1997). Phasic relationship between the activity of basal forebrain neurons and cortical EEG in urethane-anesthetized rat. *Brain Res.* 759, 112–121. doi: 10.1016/s0006-8993(97)00252-7
- Diekelmann, S., and Born, J. (2010). The memory function of sleep. *Nat. Rev. Neurosci.* 11, 114–126. doi: 10.1038/nrn2762
- Douglas, R. J., and Martin, K. A. C. (2004). Neuronal circuits of the neocortex. *Annu. Rev. Neurosci.* 27, 419–451. doi: 10.1146/annurev.neuro.27.070203.144152
- Eccles, J. C. (1961). “Chairman’s opening remarks,” in *CIBA Foundation Symposium on the Nature of Sleep*, eds G. E. W. Wolstenholme, M. O’Connor (Boston: Little, Brown and Co), 1–3.
- Eggermann, E., Kremer, Y., Crochet, S., and Petersen, C. C. (2014). Cholinergic signals in mouse barrel cortex during active whisker sensing. *Cell Rep.* 9, 1654–1660. doi: 10.1016/j.celrep.2014.11.005
- Egorov, A. V., Hamam, B. N., Fransén, E., Hasselmo, M. E., and Alonso, A. A. (2002). Graded persistent activity in entorhinal cortex neurons. *Nature* 420, 173–178. doi: 10.1038/nature01171
- Eschenko, O., Magri, C., Panzeri, S., and Sara, S. J. (2012). Noradrenergic neurons of the locus coeruleus are phase locked to cortical up-down states during sleep. *Cereb. Cortex* 22, 426–435. doi: 10.1093/cercor/bhr121
- Fanselow, E. E., and Connors, B. W. (2010). The roles of somatostatin-expressing (GIN) and fast-spiking inhibitory interneurons in UP-DOWN states of mouse neocortex. *J. Neurophysiol.* 104, 596–606. doi: 10.1152/jn.00206.2010
- Fatt, P., and Katz, B. (1952). Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* 117, 109–128.
- Favero, M., and Castro-Alamancos, M. A. (2013). Synaptic cooperativity regulates persistent network activity in neocortex. *J. Neurosci.* 33, 3151–3163. doi: 10.1523/JNEUROSCI.4424-12.2013
- Favero, M., Varghese, G., and Castro-Alamancos, M. A. (2012). The state of somatosensory cortex during neuromodulation. *J. Neurophysiol.* 108, 1010–1024. doi: 10.1152/jn.00256.2012
- Fellous, J. M., Rudolph, M., Destexhe, A., and Sejnowski, T. J. (2003). Synaptic background noise controls the input/output characteristic of single cells in an *in vitro* model of *in vivo* activity. *Neuroscience* 122, 811–829. doi: 10.1016/j.neuroscience.2003.08.027
- Flint, A. C., and Connors, B. W. (1996). Two types of network oscillations in neocortex mediated by distinct glutamate receptor subtypes and neuronal populations. *J. Neurophysiol.* 75, 951–957.
- Frank, M. G., and Benington, J. H. (2006). The role of sleep in memory consolidation and brain plasticity: dream of reality? *Neuroscientist* 12, 477–488. doi: 10.1177/1073858406293552
- Frankenhaeuser, B., and Hodgkin, A. L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.* 137, 218–244. doi: 10.1113/jphysiol.1957.sp005808
- Fransén, E., Tahvildari, B., Egorov, A. V., Hasselmo, M. E., and Alonso, A. A. (2006). Mechanism of graded persistent cellular activity of entorhinal cortex layer v neurons. *Neuron* 49, 735–746. doi: 10.1016/j.neuron.2006.01.036
- Fries, P. (2009). Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annu. Rev. Neurosci.* 32, 209–224. doi: 10.1146/annurev.neuro.051508.135603
- Ghosh, A., and Greenberg, M. E. (1995). Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* 268, 239–247. doi: 10.1126/science.7716515
- Gibson, J. R., Beierlein, M., and Connors, B. W. (1999). Two networks of electrically coupled inhibitory interneurons in neocortex. *Nature* 402, 75–79. doi: 10.1038/47035
- Gil, Z., Connors, B. W., and Amitai, Y. (1997). Differential regulation of neocortical synapses by neuromodulators and activity. *Neuron* 19, 679–686. doi: 10.1016/s0896-6273(00)80380-3
- Goel, N., Rao, H., Durmer, J. S., and Dinges, D. F. (2009). Neurocognitive consequences of sleep deprivation. *Semin. Neurol.* 29, 320–339. doi: 10.1055/s-0029-1237117
- Golshani, P., Liu, X. B., and Jones, E. G. (2001). Differences in quantal amplitude reflect GluR4-subunit number at corticothalamic synapses on two populations

- of thalamic neurons. *Proc. Natl. Acad. Sci. U S A* 98, 4172–4177. doi: 10.1073/pnas.061013698
- Gray, C. M., and McCormick, D. A. (1996). Chattering cells: superficial pyramidal neurons contributing to the generation of synchronous oscillations in the visual cortex. *Science* 274, 109–113. doi: 10.1126/science.274.5284.109
- Gutnick, M. J., Connors, B. W., and Prince, D. A. (1982). Mechanisms of neocortical epileptogenesis *in vitro*. *J. Neurophysiol.* 48, 1321–1335.
- Hahnloser, R. H., Sarpeshkar, R., Mahowald, M. A., Douglas, R. J., and Seung, H. S. (2000). Digital selection and analogue amplification coexist in a cortex-inspired silicon circuit. *Nature* 405, 947–951. doi: 10.1038/35016072
- Haider, B., Duque, A., Hasenstaub, A. R., and McCormick, D. A. (2006). Neocortical network activity *in vivo* is generated through a dynamic balance of excitation and inhibition. *J. Neurosci.* 26, 4535–4545. doi: 10.1523/jneurosci.5297-05.2006
- Haider, B., Duque, A., Hasenstaub, A. R., Yu, Y., and McCormick, D. A. (2007). Enhancement of visual responsiveness by spontaneous local network activity *in vivo*. *J. Neurophysiol.* 97, 4186–4202. doi: 10.1152/jn.01114.2006
- Haider, B., and McCormick, D. A. (2009). Rapid neocortical dynamics: cellular and network mechanisms. *Neuron* 62, 171–189. doi: 10.1016/j.neuron.2009.04.008
- Halassa, M. M., Chen, Z., Wimmer, R. D., Brunetti, P. M., Zhao, S., Zikopoulos, B., et al. (2014). State-dependent architecture of thalamic reticular subnetworks. *Cell* 158, 808–821. doi: 10.1016/j.cell.2014.06.025
- Halassa, M. M., Siegle, J. H., Ritt, J. T., Ting, J. T., Feng, G., and Moore, C. I. (2011). Selective optical drive of thalamic reticular nucleus generates thalamic bursts and cortical spindles. *Nat. Neurosci.* 14, 1118–1120. doi: 10.1038/nn.2880
- Hasenstaub, A., Sachdev, R. N., and McCormick, D. A. (2007). State changes rapidly modulate cortical responsiveness. *J. Neurosci.* 27, 9607–9622. doi: 10.1523/jneurosci.2184-07.2007
- Hasenstaub, A., Shu, Y., Haider, B., Kraushaar, U., Duque, A., and McCormick, D. A. (2005). Inhibitory postsynaptic potentials carry synchronized frequency information in active cortical networks. *Neuron* 47, 423–435. doi: 10.1016/j.neuron.2005.06.016
- Hill, S., and Tononi, G. (2005). Modeling sleep and wakefulness in the thalamocortical system. *J. Neurophysiol.* 93, 1671–1698. doi: 10.1152/jn.00915.2004
- Hô, N., and Destexhe, A. (2000). Synaptic background activity enhances the responsiveness of neocortical pyramidal neurons. *J. Neurophysiol.* 84, 1488–1496.
- Hobson, J. A., Pace-Schott, E. F., and Stickgold, R. (2000). Dreaming and the brain: toward a cognitive neuroscience of conscious states. *Behav. Brain. Sci.* 23, 793–842; discussion 904–1121. doi: 10.1017/S0140525X00003976
- Holt, G. R., and Koch, C. (1997). Shunting inhibition does not have a divisive effect on firing rates. *Neural Comput.* 9, 1001–1013. doi: 10.1162/neco.1997.9.5.1001
- Hsieh, C. Y., Cruikshank, S. J., and Metherate, R. (2000). Differential modulation of auditory thalamocortical and intracortical synaptic transmission by cholinergic agonist. *Brain Res.* 880, 51–64. doi: 10.1016/S0006-8993(00)02766-9
- Hughes, S. W., Cope, D. W., Blethyn, K. L., and Crunelli, V. (2002). Cellular mechanisms of the slow (<1 Hz) oscillation in thalamocortical neurons *in vitro*. *Neuron* 33, 947–958. doi: 10.1016/S0896-6273(02)00623-2
- Hughes, S. W., Cope, D. W., Tóth, T. I., Williams, S. R., and Crunelli, V. (1999). All thalamocortical neurones possess a T-type Ca^{2+} “window” current that enables the expression of bistability-mediated activities. *J. Physiol.* 517, 805–815. doi: 10.1111/j.1469-7793.1999.0805s.x
- Igelström, K. M. (2013). Is slack an intrinsic seizure terminator? *Neuroscientist* 19, 248–254. doi: 10.1177/1073858412446311
- Ikegaya, Y., Aaron, G., Cossart, R., Aronov, D., Lampl, I., Ferster, D., et al. (2004). Synfire chains and cortical songs: temporal modules of cortical activity. *Science* 304, 559–564. doi: 10.1126/science.1093173
- Isomura, Y., Sirota, A., Ozen, S., Montgomery, S., Mizuseki, K., Henze, D. A., et al. (2006). Integration and segregation of activity in entorhinal-hippocampal subregions by neocortical slow oscillations. *Neuron* 52, 871–882. doi: 10.1016/j.neuron.2006.10.023
- Ji, D., and Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nat. Neurosci.* 10, 100–107. doi: 10.1038/nn1825
- Jia, H., Rochefort, N. L., Chen, X., and Konnerth, A. (2010). Dendritic organization of sensory input to cortical neurons *in vivo*. *Nature* 464, 1307–1312. doi: 10.1038/nature08947
- Jones, E. G. (1998). Viewpoint: the core and matrix of thalamic organization. *Neuroscience* 85, 331–345. doi: 10.1016/S0306-4522(97)00581-2
- Jouvet, M. (1972). The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergebn. Physiol.* 64, 166–307.
- Jouvet, M. (1999). Sleep and serotonin: an unfinished story. *Neuropsychopharmacology* 21, 24S–27S. doi: 10.1038/sj.npp.1395333
- Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J., and Sagi, D. (1994). Dependence on REM sleep of overnight improvement of a perceptual skill. *Science* 265, 679–682. doi: 10.1126/science.8036518
- Killgore, W. D. (2010). Effects of sleep deprivation on cognition. *Prog. Brain Res.* 185, 105–129. doi: 10.1016/B978-0-444-53702-7.00007-5
- Kim, U., Bal, T., and McCormick, D. A. (1995). Spindle waves are propagating synchronized oscillations in the ferret LGNd *in vitro*. *J. Neurophysiol.* 74, 1301–1323.
- Krishnamurthy, P., Silberberg, G., and Lansner, A. (2012). A cortical attractor network with Martinotti cells driven by facilitating synapses. *PLoS One* 7:e30752. doi: 10.1371/journal.pone.0030752
- Kulik, A., Vida, I., Luján, R., Haas, C. A., López-Bendito, G., Shigemoto, R., et al. (2003). Subcellular localization of metabotropic GABA_B receptor subunits GABA_{B1a/b} and GABA_{B2} in the rat hippocampus. *J. Neurosci.* 23, 11026–11035.
- Kuhn, A., Aertsen, A., and Rotter, S. (2004). Neuronal integration of synaptic input in the fluctuation-driven regime. *J. Neurosci.* 24, 2345–2356. doi: 10.1523/jneurosci.3349-03.2004
- Le Bon-Jego, M., and Yuste, R. (2007). Persistently active, pacemaker-like neurons in neocortex. *Front. Neurosci.* 1, 123–129. doi: 10.3389/neuro.01.1.1.009.2007
- Lemieux, M., Chauvette, S., and Timofeev, I. (2015). Neocortical inhibitory activities and long-range afferents contribute to the synchronous onset of silent states of the neocortical slow oscillation. *J. Neurophysiol.* 113, 768–779. doi: 10.1152/jn.00858.2013
- Lemieux, M., Chen, J. Y., Lonjers, P., Bazhenov, M., and Timofeev, I. (2014). The impact of cortical deafferentation on the neocortical slow oscillation. *J. Neurosci.* 34, 5689–5703. doi: 10.1523/JNEUROSCI.1156-13.2014
- Lisman, J. E., Fellous, J. M., and Wang, X. J. (1998). A role for NMDA-receptor channels in working memory. *Nat. Neurosci.* 1, 273–275. doi: 10.1038/1086
- Llinás, R. R., Grace, A. A., and Yarom, Y. (1991). *In vitro* neurons in mammalian cortical layer 4 exhibit intrinsic oscillatory activity in the 10-to 50-Hz frequency range. *Proc. Natl. Acad. Sci. U S A* 88, 897–901. doi: 10.1073/pnas.88.8.3510-c
- Llinás, R., and Ribary, U. (1993). Coherent 40-Hz oscillation characterizes dream state in humans. *Proc. Natl. Acad. Sci. U S A* 90, 2078–2081. doi: 10.1073/pnas.90.5.2078
- Llinás, R., and Sugimori, M. (1980). Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *J. Physiol.* 305, 197–213. doi: 10.1113/jphysiol.1980.sp013358
- Loewenstein, Y., Mahon, S., Chadderton, P., Kitamura, K., Sompolsky, H., Yarom, Y., et al. (2005). Bistability of cerebellar Purkinje cells modulated by sensory stimulation. *Nat. Neurosci.* 8, 202–211. doi: 10.1038/nn1393
- Lörincz, M. L., Gunner, D., Bao, Y., Connelly, W. M., Isaac, J. T. R., Hughes, S. W., et al. (2015). A distinct class of slow (~0.2–2 Hz) intrinsically bursting layer 5 pyramidal neurons determines UP/DOWN state dynamics in the neocortex. *J. Neurosci.* 35, 5442–5458. doi: 10.1523/JNEUROSCI.3603-14.2015
- Luczak, A., Barthó, P., Marguet, S. L., Buzsáki, G., and Harris, K. D. (2007). Sequential structure of neocortical spontaneous activity. *Proc. Natl. Acad. Sci. U S A* 104, 347–352. doi: 10.1073/pnas.0605643104
- MacLean, J. N., Watson, B. O., Aaron, G. B., and Yuste, R. (2005). Internal dynamics determine the cortical response to thalamic stimulation. *Neuron* 48, 811–823. doi: 10.1016/j.neuron.2005.09.035
- Major, G., Larkum, M. E., and Schiller, J. (2013). Active properties of neocortical pyramidal neuron dendrites. *Annu. Rev. Neurosci.* 36, 1–24. doi: 10.1146/annurev-neuro-062111-150343
- Major, G., and Tank, D. (2004). Persistent neural activity: prevalence and mechanisms. *Curr. Opin. Neurobiol.* 14, 675–684. doi: 10.1016/j.conb.2004.10.017
- Mann, E. O., Kohl, M. M., and Paulsen, O. (2009). Distinct roles of GABA_A and GABA_B receptors in balancing and terminating persistent cortical activity. *J. Neurosci.* 29, 7513–7518. doi: 10.1523/JNEUROSCI.6162-08.2009
- Manns, I. D., Alonso, A., and Jones, B. E. (2000). Discharge properties of juxtacellularly labeled and immunohistochemically identified cholinergic basal

- forebrain neurons recorded in association with electroencephalogram in anesthetized rats. *J. Neurosci.* 20, 1505–1518.
- Mao, B. Q., Hamzei-Sichani, F., Aronov, D., Froemke, R. C., and Yuste, R. (2001). Dynamics of spontaneous activity in neocortical slices. *Neuron* 32, 883–898. doi: 10.1016/s0896-6273(01)00518-9
- Marder, E., Abbott, L. F., Turrigiano, G. G., Liu, Z., and Golowasch, J. (1996). Memory from the dynamics of intrinsic currents. *Proc. Natl. Acad. Sci. U S A* 93, 13481–13486. doi: 10.1073/pnas.93.24.13481
- Massimini, M., Ferrarelli, F., Huber, R., Esser, S. K., Singh, H., and Tononi, G. (2005). Breakdown of cortical effective connectivity during sleep. *Science* 309, 2228–2232. doi: 10.1126/science.1117256
- Massimini, M., Huber, R., Ferrarelli, F., Hill, S., and Tononi, G. (2004). The sleep slow oscillation as a traveling wave. *J. Neurosci.* 24, 6862–6870. doi: 10.1523/jneurosci.1318-04.2004
- Massimini, M., Tononi, G., and Huber, R. (2009). Slow waves, synaptic plasticity and information processing: insights from transcranial magnetic stimulation and high-density EEG experiments. *Eur. J. Neurosci.* 29, 1761–1770. doi: 10.1111/j.1460-9568.2009.06720.x
- Mayne, E. W., Craig, M. T., McBain, C. J., and Paulsen, O. (2013). Dopamine suppresses persistent network activity via D(1)-like dopamine receptors in rat medial entorhinal cortex. *Eur. J. Neurosci.* 37, 1242–1247. doi: 10.1111/ejn.12125
- McAdams, C. J., and Maunsell, J. H. (1999). Effects of attention on orientation-tuning functions of single neurons in macaque cortical area V4. *J. Neurosci.* 19, 431–441.
- McCormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog. Neurobiol.* 39, 337–388. doi: 10.1016/0301-0082(92)90012-4
- McCormick, D. A., and Contreras, D. (2001). On the cellular and network bases of epileptic seizures. *Annu. Rev. Physiol.* 63, 815–846. doi: 10.1146/annurev.physiol.63.1.815
- McCormick, D. A., and Pape, H. C. (1990). Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurons. *J. Physiol.* 431, 291–318. doi: 10.1113/jphysiol.1990.sp018331
- McCormick, D. A., Shu, Y., and Hasenstaub, A. (2004). “Balanced recurrent excitation and inhibition in local cortical networks,” in *Excitatory-Inhibitory Balance: Synapses, Circuits, Systems*, eds T. K. Hensch and M. Fagioli (New York: Kluwer), 113–122.
- McCormick, D. A., Shu, Y., Hasenstaub, A., Sanchez-Vives, M., Badoual, M., and Bal, T. (2003). Persistent cortical activity: mechanisms of generation and effects on neuronal excitability. *Cereb. Cortex* 13, 1219–1231. doi: 10.1093/cercor/bhg104
- McCormick, D. A., and von Krosigk, M. (1992). Corticothalamic activation modulates thalamic firing through glutamate “metabotropic” receptors. *Proc. Natl. Acad. Sci. U S A* 89, 2774–2778. doi: 10.1073/pnas.89.7.2774
- McCormick, D. A., and Wang, Z. (1991). Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. *J. Physiol.* 442, 235–255. doi: 10.1113/jphysiol.1991.sp018791
- McCormick, D. A., and Williamson, A. (1989). Convergence and divergence of neurotransmitter action in human cerebral cortex. *Proc. Natl. Acad. Sci. U S A* 86, 8098–8102. doi: 10.1073/pnas.86.20.8098
- McCormick, D. A., and Yuste, R. (2006). “UP states and cortical dynamics,” in *Microcircuits: The Interface between Neurons and Global Brain Function*, eds S. Grillner and A. M. Graybiel (Cambridge: MIT Press), 327–346.
- McCoy, J. G., and Strecker, R. E. (2011). The cognitive cost of sleep lost. *Neurobiol. Learn. Mem.* 96, 564–582. doi: 10.1016/j.nlm.2011.07.004
- McGinty, D. J., and Harper, R. M. (1976). Dorsal raphe neurons: depression of firing during sleep in cats. *Brain Res.* 101, 569–575. doi: 10.1016/0006-8993(76)90480-7
- McLaughlin, S. G., Szabo, G., and Eisenman, G. (1971). Divalent ions and the surface potential of charged phospholipid membranes. *J. Gen. Physiol.* 58, 667–687. doi: 10.1085/jgp.58.6.667
- McNamara, P., Johnson, P., McLaren, D., Harris, E., Beauharnais, C., and Auerbach, S. (2010). REM and NREM sleep mentation. *Int. Rev. Neurobiol.* 92, 69–86. doi: 10.1016/S0074-7742(10)92004-7
- Melamed, O., Barak, O., Silberberg, G., Markram, H., and Tsodyks, M. (2008). Slow oscillations in neural networks with facilitating synapses. *J. Comput. Neurosci.* 25, 308–316. doi: 10.1007/s10827-008-0080-z
- Mena-Segovia, J., Sims, H. M., Magill, P. J., and Bolam, J. P. (2008). Cholinergic brainstem neurons modulate cortical gamma activity during slow oscillations. *J. Physiol.* 586, 2947–2960. doi: 10.1113/jphysiol.2008.153874
- Metherate, R., and Cruikshank, S. J. (1999). Thalamocortical inputs trigger a propagating envelope of gamma-band activity in auditory cortex. *Exp. Brain Res.* 126, 160–174. doi: 10.1007/s002210050726
- Miller, K. D., and Troyer, T. W. (2002). Neural noise can explain expansive, power-law nonlinearities in neural response functions. *J. Neurophysiol.* 87, 653–659. doi: 10.1152/jn.00425.2001
- Mitchell, S. J., and Silver, R. A. (2003). Shunting inhibition modulates neuronal gain during synaptic excitation. *Neuron* 38, 433–445. doi: 10.1016/s0896-6273(03)00200-9
- Mochol, G., Hermoso-Mendizabal, A., Sakata, S., Harris, K. D., and de la Rocha, J. (2015). Stochastic transitions into silence cause noise correlations in cortical circuits. *Proc. Natl. Acad. Sci. U S A* 112, 3529–3534. doi: 10.1073/pnas.1410509112
- Monckton, J. E., and McCormick, D. A. (2002). Neuromodulatory role of serotonin in the ferret thalamus. *J. Neurophysiol.* 87, 2124–2136. doi: 10.1152/jn.00650.2001
- Moruzzi, G. (1966). “The functional significance of sleep with particular regard to the brain mechanisms underlying consciousness,” in *Brain and Conscious Experience*, ed. J. C. Eccles (New York: Springer), 345–379.
- Moruzzi, G., and Magoun, H. W. (1949). Brain stem reticular formation and activation of the EEG. *Electroencephalogr. Clin. Neurophysiol.* 1, 455–473. doi: 10.1016/0013-4694(49)90219-9
- Muñoz, W., and Rudy, B. (2014). Spatiotemporal specificity in cholinergic control of neocortical function. *Curr. Opin. Neurobiol.* 26, 149–160. doi: 10.1016/j.conb.2014.02.015
- Murphy, B. K., and Miller, K. D. (2003). Multiplicative gain changes are induced by excitation or inhibition alone. *J. Neurosci.* 23, 10040–10051.
- Neske, G. T., Patrick, S. L., and Connors, B. W. (2015). Contributions of diverse excitatory and inhibitory neurons to recurrent network activity in cerebral cortex. *J. Neurosci.* 35, 1089–1105. doi: 10.1523/JNEUROSCI.2279-14.2015
- Núñez, A., Amzica, F., and Steriade, M. (1992). Voltage-dependent fast (20–40 Hz) oscillations in long-axonated neocortical neurons. *Neuroscience* 51, 7–10. doi: 10.1016/0306-4522(92)90464-d
- Packer, A. M., Roska, B., and Häusser, M. (2013). Targeting neurons and photons for optogenetics. *Nat. Neurosci.* 16, 805–815. doi: 10.1038/nn.3427
- Paré, D., Shink, E., Gaudreau, H., Destexhe, A., and Lang, E. J. (1998). Impact of spontaneous synaptic activity on the resting properties of cat neocortical pyramidal neurons *in vivo*. *J. Neurophysiol.* 79, 1450–1460.
- Pavlov, I. P. (1923). Address on “the identity of inhibition with hypnosis and sleep.” *Q. J. Exp. Physiol.* 13, 39–43. doi: 10.1113/expphysiol.1923.sp000307
- Petersen, C. H., Hahn, T. T. G., Mehta, M., Grinvald, A., and Sakmann, B. (2003). Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. *Proc. Natl. Acad. Sci. U S A* 100, 13638–13643. doi: 10.1073/pnas.2235811100
- Phillips, J. W., Kostopoulos, G. K., and Limacher, J. J. (1975). A potent depressant action of adenine derivatives on cerebral cortical neurons. *Eur. J. Pharmacol.* 30, 125–129. doi: 10.1016/0014-2999(75)90214-9
- Pinto, D. J., Patrick, S. L., Huang, W. C., and Connors, B. W. (2005). Initiation, propagation and termination of epileptiform activity in rodent neocortex *in vitro* involve distinct mechanisms. *J. Neurosci.* 25, 8131–8140. doi: 10.1523/jneurosci.2278-05.2005
- Poulet, J. F., and Petersen, C. C. (2008). Internal brain state regulates membrane potential synchrony in barrel cortex of behaving mice. *Nature* 454, 881–885. doi: 10.1038/nature07150
- Pritchett, D. L., Siegle, J. H., Deister, C. A., and Moore, C. I. (2015). For things needing your attention: the role of neocortical gamma in sensory perception. *Curr. Opin. Neurobiol.* 31, 254–263. doi: 10.1016/j.conb.2015.02.004
- Puig, M. V., Ushimaru, M., and Kawaguchi, Y. (2008). Two distinct activity patterns of fast-spiking interneurons during neocortical UP states. *Proc. Natl. Acad. Sci. U S A* 105, 8428–8433. doi: 10.1073/pnas.0712219105
- Ramirez, D. M., and Kavalali, E. T. (2011). Differential regulation of spontaneous and evoked neurotransmitter release at central synapses. *Curr. Opin. Neurobiol.* 21, 275–282. doi: 10.1016/j.conb.2011.01.007

- Reig, R., Gallego, R., Nowak, L. G., and Sanchez-Vives, M. V. (2006). Impact of cortical network activity on short-term synaptic depression. *Cereb. Cortex* 16, 688–695. doi: 10.1093/cercor/bhj014
- Reig, R., Zerlaut, Y., Vergara, R., Destexhe, A., and Sanchez-Vives, M. V. (2015). Gain modulation of synaptic inputs by network state in auditory cortex *in vivo*. *J. Neurosci.* 35, 2689–2702. doi: 10.1523/JNEUROSCI.2004-14.2015
- Reyes, A., Lujan, R., Rozov, A., Burnashev, N., Somogyi, P., and Sakmann, B. (1998). Target-cell-specific facilitation and depression in neocortical circuits. *Nat. Neurosci.* 1, 279–285. doi: 10.1038/1092
- Rigas, P., and Castro-Alamancos, M. A. (2007). Thalamocortical Up states: differential effects of intrinsic and extrinsic cortical inputs on persistent activity. *J. Neurosci.* 27, 4261–4272. doi: 10.1523/jneurosci.0003-07.2007
- Rigas, P., and Castro-Alamancos, M. A. (2009). Impact of persistent cortical activity (Up states) on intracortical and thalamocortical synaptic inputs. *J. Neurophysiol.* 102, 119–131. doi: 10.1152/jn.00126.2009
- Ros, H., Sachdev, R. N., Yu, Y., Sestan, N., and McCormick, D. A. (2009). Neocortical networks entrain neuronal circuits in cerebellar cortex. *J. Neurosci.* 29, 10309–10320. doi: 10.1523/JNEUROSCI.2327-09.2009
- Rudolph, M., Pospischil, M., Timofeev, I., and Destexhe, A. (2007). Inhibition determines membrane potential dynamics and controls action potential generation in awake and sleeping cat cortex. *J. Neurosci.* 27, 5280–5290. doi: 10.1523/jneurosci.4652-06.2007
- Sachdev, R. N. S., Ebner, F. F., and Wilson, C. J. (2004). Effect of subthreshold up and down states on the whisker-evoked response in somatosensory cortex. *J. Neurophysiol.* 92, 3511–3521. doi: 10.1152/jn.00347.2004
- Sakai, K., and Crochet, S. (2001). Differentiation of presumed serotonergic dorsal raphe neurons in relation to behavior and wake-sleep states. *Neuroscience* 104, 1141–1155. doi: 10.1016/s0306-4522(01)00103-8
- Sakata, S., and Harris, K. D. (2009). Laminar structure of spontaneous and sensory-evoked population activity in auditory cortex. *Neuron* 64, 404–418. doi: 10.1016/j.neuron.2009.09.020
- Salinas, E., and Abbott, L. F. (1996). A model of multiplicative neural responses in parietal cortex. *Proc. Natl. Acad. Sci. U S A* 93, 11956–11961. doi: 10.1073/pnas.93.21.11956
- Salkoff, D. B., Zagha, E., Yüzgeç, Ö., and McCormick, D. A. (2015). Synaptic mechanisms of tight spike synchrony at gamma frequency in cerebral cortex. *J. Neurosci.* 35, 10236–10251. doi: 10.1523/JNEUROSCI.0828-15.2015
- Sallanon, M., Buda, C., Janin, M., and Jouvet, M. (1983). Serotonergic mechanisms and sleep rebound. *Brain Res.* 268, 95–104. doi: 10.1016/0006-8993(83)90393-1
- Sanchez-Vives, M. V. (2007). “An active cortical network *in vitro*,” in *Mechanisms of Spontaneous Active States in the Neocortex*, ed. I. Timofeev (Kerala, India: Research Signpost), 23–44.
- Sanchez-Vives, M. V., Descalzo, V. F., Reig, R., Figueroa, N. A., Compte, A., and Gallego, R. (2008). Rhythmic spontaneous activity in the piriform cortex. *Cereb. Cortex* 18, 1179–1192. doi: 10.1093/cercor/bhm152
- Sanchez-Vives, M. V., Mattia, M., Compte, A., Perez-Zabalza, M., Winograd, M., Descalzo, V. F., et al. (2010). Inhibitory modulation of cortical up states. *J. Neurophysiol.* 104, 1314–1324. doi: 10.1152/jn.00178.2010
- Sanchez-Vives, M. V., and McCormick, D. A. (2000). Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat. Neurosci.* 3, 1027–1034. doi: 10.1038/79848
- Sanchez-Vives, M. V., Nowak, L. G., and McCormick, D. A. (2000). Cellular mechanisms of long-lasting adaptation in visual cortical neurons. *J. Neurosci.* 20, 4286–4299.
- Schweimer, J. V., Mallet, N., Sharp, T., and Ungless, M. A. (2011). Spike-timing relationship of neurochemically-identified dorsal raphe neurons during cortical slow oscillations. *Neuroscience* 196, 115–123. doi: 10.1016/j.neuroscience.2011.08.072
- Schwindt, P. C., Spain, W. J., and Crill, W. E. (1989). Long-lasting reduction of excitability by a sodium-dependent potassium current in cat neocortical neurons. *J. Neurophysiol.* 61, 233–244.
- Schwindt, P. C., Spain, W. J., and Crill, W. E. (1992). Calcium-dependent potassium currents in neurons from cat sensorimotor cortex. *J. Neurophysiol.* 67, 216–226.
- Sclar, G., and Freeman, R. D. (1982). Orientation selectivity in the cat's striate cortex is invariant with stimulus contrast. *Exp. Brain Res.* 46, 457–461. doi: 10.1007/BF00238641
- Sheroziya, M., and Timofeev, I. (2014). Global intracellular slow-wave dynamics of the thalamocortical system. *J. Neurosci.* 34, 8875–8893. doi: 10.1523/JNEUROSCI.4460-13.2014
- Shu, Y., Hasenstaub, A., Badoual, M., Bal, T., and McCormick, D. A. (2003a). Barrages of synaptic activity control the gain and sensitivity of cortical neurons. *J. Neurosci.* 23, 10388–10401.
- Shu, Y., Hasenstaub, A., and McCormick, D. A. (2003b). Turning on and off recurrent balanced cortical activity. *Nature* 423, 288–293. doi: 10.1038/nature01616
- Siapas, A. G., and Wilson, M. A. (1998). Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron* 21, 1123–1128. doi: 10.1016/s0896-6273(00)80629-7
- Silberberg, G., and Markram, H. (2007). Disynaptic inhibition between neocortical pyramidal cells mediated by Martinotti cells. *Neuron* 53, 735–746. doi: 10.1016/j.neuron.2007.02.012
- Silva, L. R., Amitai, Y., and Connors, B. W. (1991). Intrinsic oscillations of neocortex generated by layer 5 pyramidal neurons. *Science* 251, 432–435. doi: 10.1126/science.1824881
- Sippy, T., and Yuste, R. (2013). Decorrelating action of inhibition in neocortical networks. *J. Neurosci.* 33, 9813–9830. doi: 10.1523/JNEUROSCI.4579-12.2013
- Sirota, A., and Buzsáki, G. (2005). Interaction between neocortical hippocampal networks via slow oscillations. *Thalamus Relat. Syst.* 3, 245–259. doi: 10.1017/s1472928807000258
- Sirota, A., Csicsvari, J., Buhl, D., and Buzsáki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. *Proc. Natl. Acad. Sci. U S A* 100, 2065–2069. doi: 10.1073/pnas.0437938100
- Soderling, T. R., and Derkach, V. A. (2000). Postsynaptic protein phosphorylation and LTP. *Trends Neurosci.* 23, 75–80. doi: 10.1016/S0166-2236(99)01490-3
- Somjen, G. G. (2004). *Ions in the Brain: Normal Function, Seizures and Stroke*. Oxford: Oxford University Press.
- Srinivasan, M. V., and Bernard, G. D. (1976). A proposed mechanism for the multiplication of neural signals. *Biol. Cybern.* 21, 227–236. doi: 10.1007/bf00344168
- Steriade, M., and Amzica, F. (1996). Intracortical and corticothalamic coherency of fast spontaneous oscillations. *Proc. Natl. Acad. Sci. U S A* 93, 2533–2538. doi: 10.1073/pnas.93.6.2533
- Steriade, M., Amzica, F., and Contreras, D. (1996a). Synchronization of fast (30–40 Hz) spontaneous cortical rhythms during brain activation. *J. Neurosci.* 16, 392–417.
- Steriade, M., Contreras, D., Amzica, F., and Timofeev, I. (1996b). Synchronization of fast (30–40 Hz) spontaneous oscillations in intrathalamic and thalamocortical networks. *J. Neurosci.* 16, 2788–2808.
- Steriade, M., Contreras, D., and Amzica, F. (1994). Synchronized sleep oscillations and their paroxysmal developments. *Trends Neurosci.* 17, 199–208. doi: 10.1016/0166-2236(94)90105-8
- Steriade, M., Domich, L., Oakson, G., and Deschênes, M. (1987). The deafferented reticularis thalami nucleus generates spindle rhythmicity. *J. Neurophysiol.* 57, 260–273.
- Steriade, M., Dossio, R. C., Paré, D., and Oakson, G. (1991a). Fast oscillations (20–40 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat. *Proc. Natl. Acad. Sci. U S A* 88, 4396–4400. doi: 10.1073/pnas.88.10.4396
- Steriade, M., Dossi, R. C., and Nuñez, A. (1991b). Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortical potentiation and brainstem cholinergic suppression. *J. Neurosci.* 11, 3200–3217.
- Steriade, M., Nuñez, A., and Amzica, F. (1993a). A novel (<1 Hz) oscillation of neocortical neurons *in vivo*: depolarizing and hyperpolarizing components. *J. Neurosci.* 13, 3252–3265.
- Steriade, M., Nuñez, A., and Amzica, F. (1993b). Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *J. Neurosci.* 13, 3266–3283.
- Steriade, M., Contreras, D., Curró Dossi, R., and Nuñez, A. (1993c). The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J. Neurosci.* 13, 3284–3299.

- Steriade, M., Amzica, F., and Nuñez, A. (1993d). Cholinergic and noradrenergic modulation of the slow (~0.3 Hz) oscillation in neocortical cells. *J. Neurophysiol.* 70, 1385–1400.
- Steriade, M., Timofeev, I., and Grenier, F. (2001). Natural waking and sleep states: a view from inside neocortical neurons. *J. Neurophysiol.* 85, 1969–1985.
- Steriade, M., and Timofeev, I. (2003). Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron* 37, 563–576. doi: 10.1016/s0896-6273(03)00065-5
- Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature* 437, 1272–1278. doi: 10.1038/nature04286
- Tahvildari, B., Wölfl, M., Duque, A., and McCormick, D. A. (2012). Selective functional interactions between excitatory and inhibitory cortical neurons and differential contribution to persistent activity of the slow oscillation. *J. Neurosci.* 32, 12165–12179. doi: 10.1523/JNEUROSCI.1181-12.2012
- Telfeian, A. E., and Connors, B. W. (1998). Layer-specific pathways for the horizontal propagation of epileptiform discharges in neocortex. *Epilepsia* 39, 700–708. doi: 10.1111/j.1528-1157.1998.tb01154.x
- Thomson, A. M., and Deuchars, J. (1994). Temporal and spatial properties of local circuits in neocortex. *Trends Neurosci.* 17, 119–126. doi: 10.1016/0166-2236(94)90121-x
- Timofeev, I., Contreras, D., and Steriade, M. (1996). Synaptic responsiveness of cortical and thalamic neurones during various phases of slow sleep oscillation in cat. *J. Physiol.* 494, 265–278. doi: 10.1113/jphysiol.1996.sp021489
- Timofeev, I., Grenier, F., Bazhenov, M., Sejnowski, T. J., and Steriade, M. (2000). Origin of slow cortical oscillations in deafferented cortical slabs. *Cereb. Cortex* 10, 1185–1199. doi: 10.1093/cercor/10.12.1185
- Timofeev, I., and Steriade, M. (1996). Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. *J. Neurophysiol.* 76, 4152–4168.
- Tononi, G., and Cirelli, C. (2014). Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron* 81, 12–34. doi: 10.1016/j.neuron.2013.12.025
- Treue, S., and Martinez-Trujillo, J. C. (1999). Feature-based attention influences motion processing gain in macaque visual cortex. *Nature* 399, 575–579. doi: 10.1038/21176
- Trulsson, M. E., and Jacobs, B. L. (1979). Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. *Brain Res.* 163, 135–150. doi: 10.1016/0006-8993(79)90157-4
- Van Dongen, H. P., Belenky, G., and Krueger, J. M. (2011). A local, bottom-up perspective on sleep deprivation and neurobehavioral performance. *Curr. Top. Med. Chem.* 11, 2414–2422. doi: 10.2174/156802611797470286
- Vertes, R. P. (2004). Memory consolidation in sleep: dream or reality. *Neuron* 44, 135–148. doi: 10.1016/j.neuron.2004.08.034
- Volgushev, M., Chauvette, S., Mukovski, M., and Timofeev, I. (2006). Precise long-range synchronization of activity and silence during slow-wave sleep. *J. Neurosci.* 26, 5665–5672. doi: 10.1523/jneurosci.0279-06.2006
- Vyazovskiy, V. V., and Harris, K. D. (2013). Sleep and the single neuron: the role of global slow oscillations in individual cell rest. *Nat. Rev. Neurosci.* 14, 443–451. doi: 10.1038/nrn3494
- Vyazovskiy, V. V., Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., and Tononi, G. (2011). Local sleep in awake rats. *Nature* 472, 443–447. doi: 10.1038/nature10009
- Walker, M. P., and Stickgold, R. (2004). Sleep-dependent learning and memory consolidation. *Neuron* 44, 121–133. doi: 10.1016/j.neuron.2004.08.031
- Wang, Y., Neubauer, F. B., Lüscher, H. R., and Thurley, K. (2010). GABA_B receptor-dependent modulation of network activity in the rat prefrontal cortex *in vitro*. *Eur. J. Neurosci.* 31, 1582–1594. doi: 10.1111/j.1460-9568.2010.07191.x
- Wang, M., Yang, Y., Wang, C. J., Gamo, N. J., Jin, L. E., Mazer, J. A., et al. (2013). NMDA receptors subserve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. *Neuron* 77, 736–749. doi: 10.1016/j.neuron.2012.12.032
- Waters, J., and Helmchen, F. (2006). Background synaptic activity is sparse in neocortex. *J. Neurosci.* 26, 8267–8277. doi: 10.1523/jneurosci.2152-06.2006
- Watson, B. O., MacLean, J. N., and Yuste, R. (2008). UP states protect ongoing cortical activity from thalamic inputs. *PLoS One* 3:e3971. doi: 10.1371/journal.pone.0003971
- Wester, J. C., and Contreras, D. (2012). Columnar interactions determine horizontal propagation of recurrent network activity in neocortex. *J. Neurosci.* 32, 5454–5471. doi: 10.1523/JNEUROSCI.5006-11.2012
- Wester, J. C., and Contreras, D. (2013). Differential modulation of spontaneous and evoked thalamocortical network activity by acetylcholine level *in vitro*. *J. Neurosci.* 33, 17951–17966. doi: 10.1523/JNEUROSCI.1644-13.2013
- Williams, S. R., Turner, J. P., Tóth, T. I., Hughes, S. W., and Crunelli, V. (1997). The “window” component of the low threshold Ca²⁺ current produces input signal amplification and bistability in cat and rat thalamocortical neurones. *J. Physiol.* 505, 689–705. doi: 10.1111/j.1469-7793.1997.689ba.x
- Williford, T., and Maunsell, J. H. (2006). Effects of spatial attention on contrast response functions in macaque area V4. *J. Neurophysiol.* 96, 40–54. doi: 10.1152/jn.01207.2005
- Wilson, C. J., and Groves, P. M. (1981). Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. *Brain Res.* 220, 67–80. doi: 10.1016/0006-8993(81)90211-0
- Wilson, M. A., and McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science* 265, 676–679. doi: 10.1126/science.8036517
- Yassin, L., Benedetti, B. L., Jouhanneau, J. S., Wen, J. A., Poulet, J. F. A., and Barth, A. L. (2010). An embedded subnetwork of highly active neurons in the neocortex. *Neuron* 68, 1043–1050. doi: 10.1016/j.neuron.2010.11.029
- Yuste, R., and Tank, D. W. (1996). Dendritic integration in mammalian neurons, a century after Cajal. *Neuron* 16, 701–716. doi: 10.1016/s0896-6273(00)80091-4
- Žiburkus, J., Cressman, J. R., and Schiff, S. J. (2013). Seizures as imbalanced up states: excitatory and inhibitory conductances during seizure-like events. *J. Neurophysiol.* 109, 1296–1306. doi: 10.1152/jn.00232.2012

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The Thalamus as a Low Pass Filter: Filtering at the Cellular Level does Not Equate with Filtering at the Network Level

William M. Connelly^{1,2*}, Michael Laing³, Adam C. Errington³ and Vincenzo Crunelli^{1,4}

¹ Division of Neuroscience, School of Biosciences, Cardiff University, Cardiff, UK, ² The John Curtin School of Medical Research, Eccles Institute of Neuroscience, The Australian National University, Canberra, ACT, Australia, ³ School of Medicine, Neuroscience and Mental Health Research Institute, Cardiff University, Cardiff, UK, ⁴ Department of Physiology and Biochemistry, University of Malta, Msida, Malta

In the mammalian central nervous system, most sensory information passes through primary sensory thalamic nuclei, however the consequence of this remains unclear. Various propositions exist, likening the thalamus to a gate, or a high pass filter. Here, using a simple leaky integrate and fire model based on physiological parameters, we show that the thalamus behaves akin to a low pass filter. Specifically, as individual cells in the thalamus rely on consistent drive to spike, stimuli that is rapidly and continuously changing over time such that it activates sensory cells with different receptive fields are unable to drive thalamic spiking. This means that thalamic encoding is robust to sensory noise, however it induces a lag in sensory representation. Thus, the thalamus stabilizes encoding of sensory information, at the cost of response rate.

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*Correspondence:

William M. Connelly
connelly.bill@gmail.com

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INTRODUCTION

The thalamus lies at a cross roads between the external world and the cerebral cortex (Steriade, 2003). All senses excluding olfaction pass through the thalamus, where they are subjected to some kind of processing, before being routed to the cortex. The action of the thalamus in this context has been likening to a gate, that is to say, only letting through information as dictated by higher areas (Wang et al., 2006; McAlonan et al., 2008; Saalmann and Kastner, 2011). It has also been proposed that the thalamus acts as a frequency-sensitive filter, generating spikes more easily when some time-dependent component of the sensory stimuli is correct (Heggelund et al., 2003).

The proposition that the thalamus acts as a high-pass filter has been most thoroughly explored in the vibrotactile thalamocortical system: the thalamic ventroposterior medial (VPm) nucleus and the barrel cortex. Here there is clear evidence that when the whisker is driven by a sinusoidal deflection, cortical, and thalamic spiking rates are higher with increasing stimulus frequency (Arabzadeh et al., 2003; Khatri et al., 2004). However, this effect cannot be completely ascribed to thalamic filtering, due to the velocity sensitive nature of the sensory organ (Hartings et al., 2003; Gerdjikov et al., 2010). Conversely, both the neurons of the lateral geniculate nucleus (LGN) and the primary visual cortex have most often been shown to produce low pass or band pass behavior (Hawken et al., 1996; Van Hooser et al., 2013). Again, however, this result is confounded by the fact that the retinal processing itself acts as either a low pass or band pass filter (Shapley and Victor, 1978).

The fact that individual neurons in the thalamus act as high-pass filters in terms of input rate vs. output rate is a necessary consequence of the neural membrane being a leaky capacitor. Specifically, a certain amount of charge must be delivered into a neuron in a given time to bring it to the threshold for firing and this can only be achieved when the neuron's inputs fire above certain rate. However, filtering in the context of firing rate must be considered separately from filtering in terms of what data the neural circuit is extracting from its input. That is to say, one can imagine a hypothetical neural system that codes for a particular property of a sensory stimuli, where increasing the rate of change of that property causes a significant increase in the firing rate of neurons in the system, but the system itself loses the ability to encode that property. For instance, consider a stimuli that can be in two states, and two populations of neurons coding for these two states: when one population is active the neural system is encoding one state, when the other population is active the system is encoding the other state. Finally, if both populations are active at the same time, the system encoding is ambiguous. When the stimuli is slowly changing between its two states, the neurons in each population might fire slowly, but so long as the two populations are not active at the same time, then the neural system should be able to encode the two stimuli. However, when the stimuli is rapidly switching between these two states, the neurons in our hypothetical system begin firing more rapidly. If the stimuli is switching rapidly enough the two cell populations will become active at the same time and the neural system lose encoding ability. Thus, when considered on a single cell basis, the network appears to behave as a high-pass filter (that is, the neurons fire faster as the stimulus property changes faster), but when considered from a population standpoint, the network is in fact behaving as a low-pass filter (the network loses the ability to encode the stimulus as the stimulus property changes faster).

Here we report, using a computational approach, based on a highly simplified, yet biologically reasonable model that the thalamus necessarily works to stabilize sensory representations in the presence of noise in a manner akin to a low pass filter.

METHODS

In vitro Electrophysiology

Wistar rats, of either sex, at postnatal days 20–30 were anesthetized with isoflurane and decapitated in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 and local ethical committee approval. As described previously by Turner and Salt (1998) brains were rapidly removed and 300 μm -thick slices containing the dorsal lateral geniculate nuclei and an intact retinogeniculate pathway were cut in continuously oxygenated sucrose aCSF (Errington et al., 2010). Slices were incubated for at least 1 h before being transferred to the recording chamber where they were continuously perfused ($\sim 2\text{ ml/min}$) with warmed ($32\text{--}34^\circ\text{C}$) oxygenated recording aCSF containing the following (in mM) 125 NaCl, 5 KCl, 25 NaHCO_3 , 1.25 NaH_2PO_4 , 1 MgCl_2 , 2 CaCl_2 , 25 glucose. Whole-cell patch-clamp recordings were made using pipettes (resistance, 2–4 $\text{M}\Omega$) containing the following (in mM): 135

K-methylsulfonate, 10 HEPES, 10 Na-phosphocreatine, 4 MgCl_2 , 4 Na-ATP, 0.4 mM Na-GTP, pH 7.3, 300 mOsm. Current clamp was performed with a Multiclamp 700B preamplifier (Molecular Devices). Experimental data were filtered at 6 kHz, digitized at 20 kHz (Digidata 1322A; Molecular Devices), and acquired using pClamp 10 software (Molecular Devices). Electrical stimulation was evoked with a constant current stimulus isolator (DS3, Digitimer) through a bipolar tungsten electrode (Frederick Haer) in the presence of the GABA_A receptor antagonist gabazine (10 μM), and the GABA_B receptor antagonist 55845 (1 μM ; Tocris).

Computational Modeling

The network model was realized in Python 2.7. All cells were simple leaky integrate-and-fire neurons with no explicitly enforced refractory period. When V_m reached threshold, a 1 ms long spike was generated, after which V_m was reset to the resting membrane potential. The model consisted of two separate layers, a sensory layer that responded to a stimuli referred to as the retinal ganglion cell (RGC) layer, and an integrative layer, referred to as the thalamocortical cell (TCC) layer, that received input from the RGC layer (Figure 1).

For RGCs, membrane current was calculated as a sum of two currents, a noisy current and a sensory current. The noisy current was an Ornstein–Uhlenbeck process with a time constant ($1/k_{OU}$) of 5 ms and an amplitude (σ_{ou}) that was varied between 0 and 120 pA (Figures 1A,B,C; Destexhe et al., 2001). The sensory current was designed to model vision, and was a current whose amplitude varied in a Gaussian fashion with distance from the visual stimuli $[D(n,t)]$. The maximum this current reached was 200 pA, which meant the maximum visually evoked firing rate for a RGC was $\sim 80\text{ Hz}$ (Figures 1A,E; Croner and Kaplan, 1995). This Gaussian current had a standard deviation (σ_D) of 2.5% of visual space. Visual space was defined as the maximum field within which RGCs could code, with 0 representing one end, and 1 representing the other. With 200 RGCs, the center of each RGC's receptive field $[p(n)]$ was centered at 0.5 % of visual space from its neighbors. RGCs had a membrane resistance of 260 $\text{M}\Omega$ and a membrane capacitance of 96 pF, giving them a membrane time constant of 25 ms (O'Brien et al., 2002). RGCs had a threshold of 20 mV depolarized to rest. It should be noted that these properties intentionally give the RGCs a relatively flat frequency-response profile, allowing us to resolve the filtering properties of the TCC layer without being confounded by filtering at the retinal level.

TCCs had a membrane resistance of 70 $\text{M}\Omega$ and a membrane capacitance of 160 pF, giving them a membrane time constant of 11 ms (Crunelli et al., 1987; White and Sur, 1992). Excitatory input to TCCs was modeled as a current that synaptic input caused to instantaneously increase by 750 pA and which decayed with first-order kinetics and a time constant of 1.6 ms (Chen and Regehr, 2000). This produced a unitary excitatory postsynaptic potential (EPSP) 3.5 mV in amplitude. Spiking threshold was set at 9 mV (see Section Results).

In order to decode the information in a given layer at a given time $[P(t)]$, we sought to use a maximum likelihood estimator (MLE) approach, that is to calculate the value of $D(t)$ that

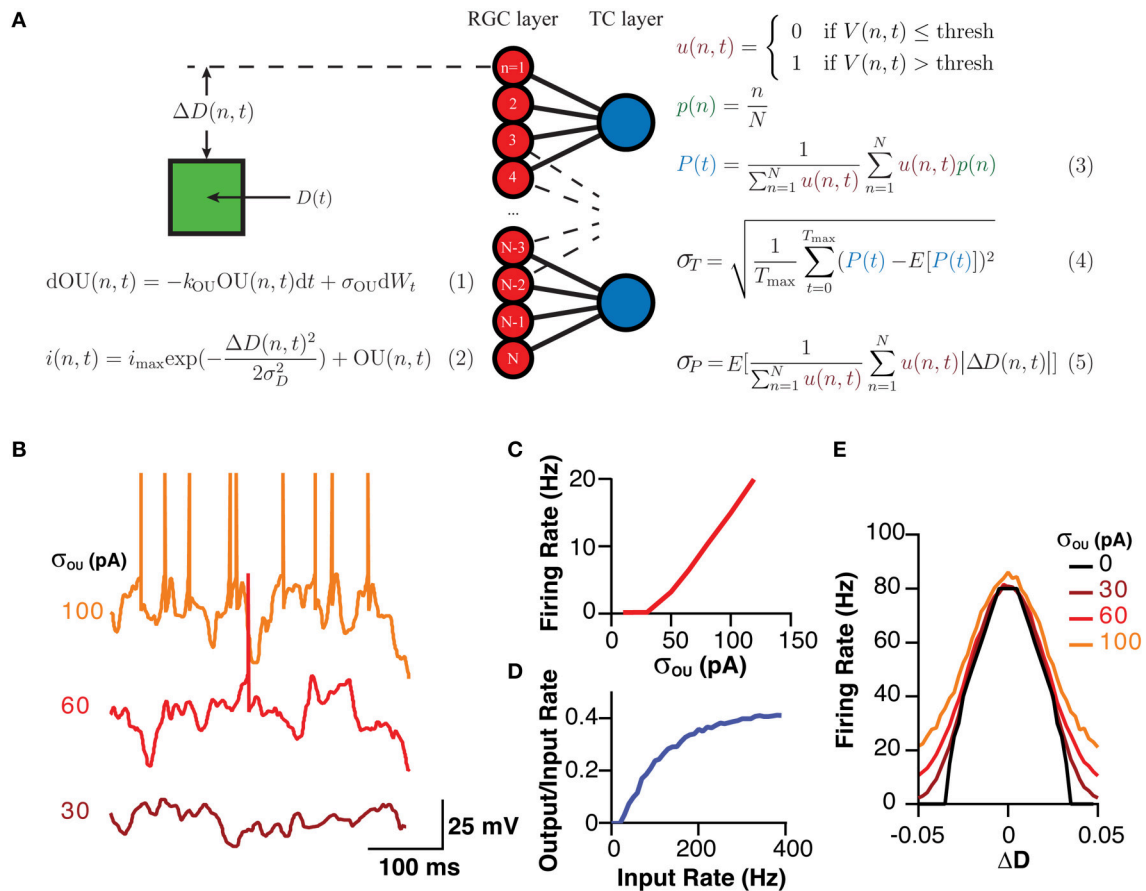


FIGURE 1 | A schematic of the model, the equations governing its behavior and its analysis and the model's typical behavior. (A) A Schematic of the model and the fundamental equations dictating its behavior and analysis. Equation (1) produces the current noise while Equation (2) shows that the total current the membrane receives is the sum of the OU noise and a “sensory” current that is a Gaussian function of the difference between the cells position and stimuli. Equation (3) demonstrates how the estimator for the position of the stimuli was calculated. Equation (4) shows how σ_T , which was used as a measure of the stability of the encoding over time, was calculated as the standard deviation of $P(t)$. Equation (5) shows how σ_P , a measure of the spatial accuracy of the encoding in the layer, was calculated. Note, color of equations is used to link similar terms. $E[X]$ represented the expectation value of the expression X . **(B)** Representative examples of the membrane potential of RGCs in response to various amplitudes of OU noise. **(C)** The mean firing rate of an RGC in response to various amplitudes of OU noise. **(D)** The transfer function of TCCs, represented as the rate of retinogeniculate EPSPs against the ratio of the output (spiking rate) to the input (EPSP rate), showing the high-pass behavior of TCCs. **(E)** The sensory behavior of RGCs showing their firing rate as a function of the distance to the stimuli (ΔD) and the amplitude of the OU noise.

maximizes the probability of getting a particular pattern of neural activity. However, under the assumption that the probability of any neuron in a given layer firing was an arbitrary Gaussian function of its distance to the stimuli, we were left with an expression that was mathematically uncooperative, and in our hands at least, could only be solved by numerical methods. Given this, we attempted a much simpler approach, where the position information encoded in any layer at time t , was simply the average $p(n)$ of all spiking neurons at time t , given by the equation.

$$P(t) = \frac{1}{\sum_{n=1}^N u(n, t)} \sum_{n=1}^N u(n, t)p(n)$$

where $u(n, t)$ is either 1 or 0 depending on whether neuron n is firing at time t , or not. Fortunately, the MLE of $P(t)$ calculated

numerically for all possible patterns of a small population of neurons ($n = 24$), produces values almost identical to that calculated via the mean approach (Linear regression: Slope = 0.95, $R^2 = 0.96$, $n = 8388608$). Thus, the mean approach was used. In order to give some measure of the stability of the encoding over time we calculated the standard deviation of $P(t)$ over time (σ_T). We also sought to capture some measure of spatial accuracy of the encoding. Thus, we used the mean over time of the average distance between $p(n)$ of all spiking neuron and the target (σ_P), that is

$$\sigma_P = E[\frac{1}{\sum_{n=1}^N u(n, t)} \sum_{n=1}^N u(n, t)|\Delta D(n, t)|]$$

Simulations were performed with a fixed time-step of 0.5 ms. Phase (φ) information was extracted by performing a Fast Fourier

Transform on $P(t)$, and the results are presented as the phase in the cell layer minus the phase of the target. Phase responses were fit with the following function:

$$\varphi = \tan^{-1}(-2\pi f\tau)$$

where $1/2\pi\tau$ gives the corner frequency of the filter, or $\pi/4$ point. Statistical tests were performed using linear regression via Matlab R2014b (Mathworks).

RESULTS

In order to make a reasonable model of thalamic behavior, the native properties of retinogeniculate transmission was studied *in vitro*. TCCs ($n = 26$) in the dLGN were patch clamped and input from the optic tract was evoked using a ramping stimulation intensity to recruit minimal events and to investigate the threshold for action potential generation (Figures 2A,B,C). The evoked EPSPs had the hallmarks of retinogeniculate EPSPs in that they had all-or-none responses as opposed to the graded recruitment typical for corticothalamic EPSPs (Turner and Salt, 1998). Across all cells and stimulation intensities, the distribution of EPSPs amplitudes ($n = 461$ events) clearly formed two peaks, one at 3.2 mV and one at 5.9 mV (95% confidence interval 3.1–3.2 mV and 5.7–6.0 mV, respectively) and the largest subthreshold EPSP recorded was

7.9 mV (Figure 2D). We believe these peaks represent the recruitment of one and two retinogeniculate axons, respectively. As no subthreshold EPSP was ever seen with an amplitude that matched three times the single axon event, we believe this demonstrates that dLGN TCCs at rest require, on average, the coincident input of three retinal EPSPs to be driven to spike. These results fit with others who reported that one or two subthreshold unitary events could be recruited in dLGN neurons, and the development evidence that dLGN TCCs receive input from 3 to 5 RGCs (Turner and Salt, 1998; Chen and Regehr, 2000; Tavazoie and Reid, 2000; Hong and Chen, 2011).

Based on these data we developed a simple model (see Section Methods), involving 120 integrate-and-fire neurons representing a simplified RGC layer, connected to either 60, 120, or 240 TCCs, consistent with the ratio of RGC to LGN neurons (Spear et al., 1996). Cells in the RGC layer were connected in a visuotopic manner to neurons in the TCC layer, such that each TCC received input from the four (unless stated otherwise) “nearest” RGCs, while needing three coincident EPSPs to spike (Figure 1A). RGCs were driving by a sum of two currents. The first component was membrane noise modeled as an Ornstein–Uhlenbeck process, whose parameter σ_{OU} dictated the magnitude of the noise and subsequent noise induced firing rate (Figures 1B,C; Equation 1). The second current had a Gaussian receptive field with a full-width at half-maximum

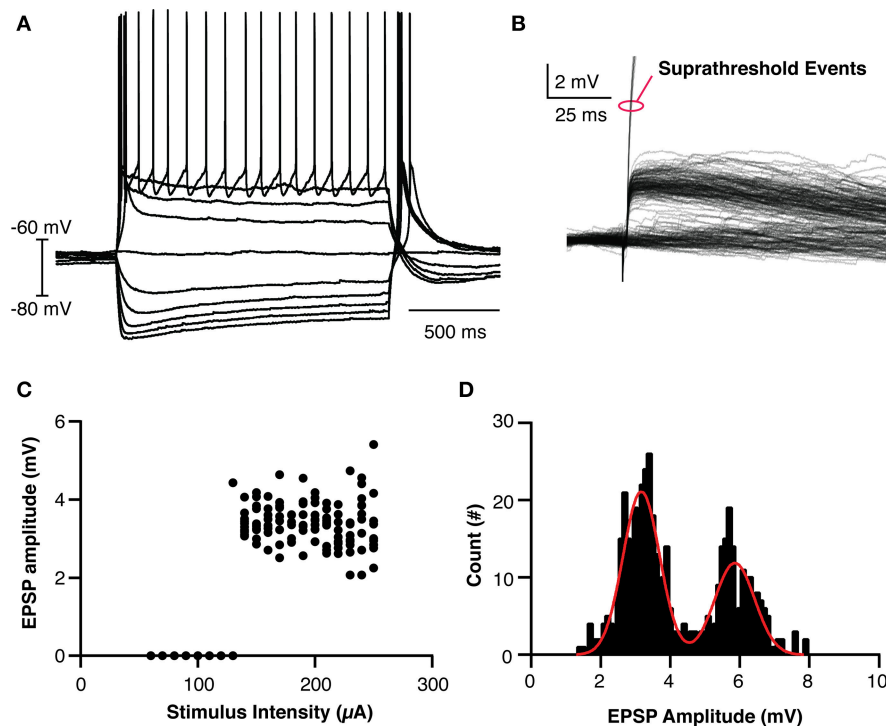


FIGURE 2 | Basic Properties of LGN thalamocortical neurons and their retinogeniculate EPSPs. (A) Overlaid traces showing the response of an LGN cell to current injection. **(B)** Overlaid traces showing the recruited retinogeniculate EPSPs in response to optic nerve stimulation. Events marked with red oval are truncated spikes. **(C)** Calculated EPSP size vs. stimulation intensity for the cell shown in **(B)**, clearly showing the lack of graded recruitment of EPSP amplitude. **(D)** The histogram of EPSP amplitudes across all cells and events, clearly showing two peaks, where the amplitude of the larger peak is essentially twice the amplitude of the smaller peak. The cell in **(C)** had events only from the smaller amplitude peak.

of $\sim 6\%$ of the visual field such that during complete visual activation RGCs would fire at a maximum of ~ 80 Hz (**Figure 1E**; Equation 1; Croner and Kaplan, 1995). As expected for a simple leaky integrate-and-fire neuron, when one considered the rate of EPSP input against the ratio of the output spiking rate to the input EPSP rate, each individual cell functioned as a high pass filter (**Figure 1D**). It is important to note, that while this model is explicitly of the visual system, it should not be assumed that we have tried to make this model replicate all the features of the visual system. Indeed, we have purposefully kept the model simple to allow it to generalize to other sensory system. Thus, while we have called the sensory layer the RGC layer, it should more be thought of as a set of sensory cells who have some arbitrary receptive field, and hence could just as well be called the “trigeminal nucleus layer” with each cell having a preferred direction of whisker deflection (Minnery et al., 2003).

The position information at any time for each cell layer [$P(t)$] was calculated when the simulation was exposed to a single stationary visual stimuli taking up 20% of the visual space (**Figure 1**; Equation 3). The standard deviation of $P(t)$, σ_T , acted as a measure of the stability of the representation over time (**Figure 1**; Equation 4). Higher values of σ_T are seen as an indicator of poorer network performance due to the reasonable assumption that information encoded in a network should not change if the input does not change. As the membrane noise of the RGC layer was increased, σ_T increased in both RGC and TCC layers ($P < 1 \times 10^{-28}$) (**Figures 3A–C**). Given that in this model TCCs receive their sole input from RGCs, it might seem reasonable to assume that the thalamic layer could only report the stimulus position as accurately as the TCC layer (receptive field half widths were almost identical between individual RGC and TCC, at 2.5 and 2%, respectively). However, apart from when RGCs had a subthreshold level of noise and the thalamic layer was made up of less cells than the RGC layer, the thalamic layer had significantly lower values of σ_T , ($P < 1 \times 10^{-12}$). Furthermore, when considering σ_T in just TCCs, increasing the ratio of cells in the TCC layer to RGC layer (while maintaining the number of inputs that each TCC receives, meaning that each RGC projects to more TCCs) caused the values of σ_T to drop ($P = 0.003$), presumably due to the simple fact that there were more TCCs available to be directly driven by visually stimulate RGCs. More importantly however, the result that σ_T was lower in the TCC layer than the RGC layer was independent of the ratio of TCCs to RGCs over this range (**Figure 3C**). This clearly shows that integration by the TCC layer enhances the stability of the visual representation over time, however, how does it affect the spatial accuracy? Therefore, to measure how integration by the TCC layer affects spatial accuracy, we calculated σ_P , a measure which was in essence the average distance between each active cell and the center of the visual target (**Figure 1**; Equation 5).

Increasing the amplitude of the RGC membrane noise significantly increased σ_P in both layers ($P < 1 \times 10^{-30}$), but unlike σ_T , altering the ratio of RGCs to TCCs have no effect on σ_P ($P = 0.9$). Again, however, the TCC layer proved to be more accurate, having a significantly lower σ_P ($P < 5 \times 10^{-18}$;

Figure 3D). Simply put, these data show that integration by the TCC layer allows the TCC layer to provide a more temporally stable and spatially accurate representation than the RGC layer, by filtering out “occasional” noise-driven spikes from the RGC layer. One could consider occasional spikes as high frequency input, not in the sense of high frequency EPSPs, but as a high frequency change in which position information that is being supplied to the TCC layer. That is, if the stimuli rapidly moved to a new position and then back to its original position, it could create spike patterns in the RGC layer very similar to noise: one or two spikes in an otherwise silent spike history. If the TCC layer does not respond to these high frequency changes in the stimuli, then we could see the TCC layer as acting like a low pass filter.

If the TCC layer is in fact acting as a low pass filter for sensory information, then like all low pass filters, it must come at a cost in terms of response rate. This effect could be clearly seen when the visual stimuli was instantly stepped from one part of the visual space to another, as the RGC layer took only approximately 4 ms to encode the new position, while the TCC layer took 8 ms (**Figure 4A**). This effect was investigated in a more quantitative fashion by moving the visual stimuli across the visual space in a sinusoid at increasing frequencies up to 50 Hz (**Figure 4B**). The RGC layer was able to accurately follow the visual stimuli, and at no point did it encode beyond $\pi/4$ radians behind the stimuli (**Figure 4C**). The TCC layer, on the other hand, fell behind to this extent at less than 20 Hz (**Figure 4D**). Increasing the noise the RGC layer allowed the TCC to follow at higher frequencies ($\sigma_{OU} = 30$ pA: $\pi/4$ point: 12 ± 0.5 Hz; $\sigma_{OU} = 100$ pA: $\pi/4$ point: 36 ± 0.3 Hz, $P < 1 \times 10^{-10}$). The fact that increasing noise in the RGC layer allows the TCC layer to respond more rapidly to RGC input is presumably due to the increased synaptic drive the TCC layer receives due to noise driven retinal spikes. This brings TCCs closer to threshold, meaning they need to integrate less stimulus driven input to fire. Thus, the TCC layer fails to produce an accurate representation of sensory input that changes above ~ 10 Hz.

We have already demonstrated that the filtering effect of the thalamic layer is robust to changes in the numerical ratio of RGC to TCCs, however, we have not investigated how changes in the convergence onto TCCs effects their ability to filter input noise. In order to investigate this we altered the convergence of RGCs onto TCCs in tandem with changing the amplitude of retinal EPSCs. We did this in such a way that an individual TCC might receive, for instance, input from two times as many RGCs but with each one having one half the synaptic weight, thus keeping the total synaptic drive approximately equal. By increasing the convergence of RGCs onto TCCs, the TCC layer became increasingly robust to noise in the RGC cells, both in terms of σ_T ($P = 0.0004$) and σ_P ($P < 1 \times 10^{-7}$; **Figures 5A,B**). However, this came at the cost of increasingly slow frequency response (TC:RGC = 1:1, $\sigma_{OU} = 30$ pA, EPSC $\times 0.5$: $\pi/4$ point: 6.0 ± 0.5 Hz; EPSC $\times 4$: $\pi/4$ point: 60 ± 0.3 Hz; $P < 1 \times 10^{-16}$; **Figures 5C,D**). Therefore, across a variety circuit configurations, including ones where single RGC spikes are capable of driving TCC

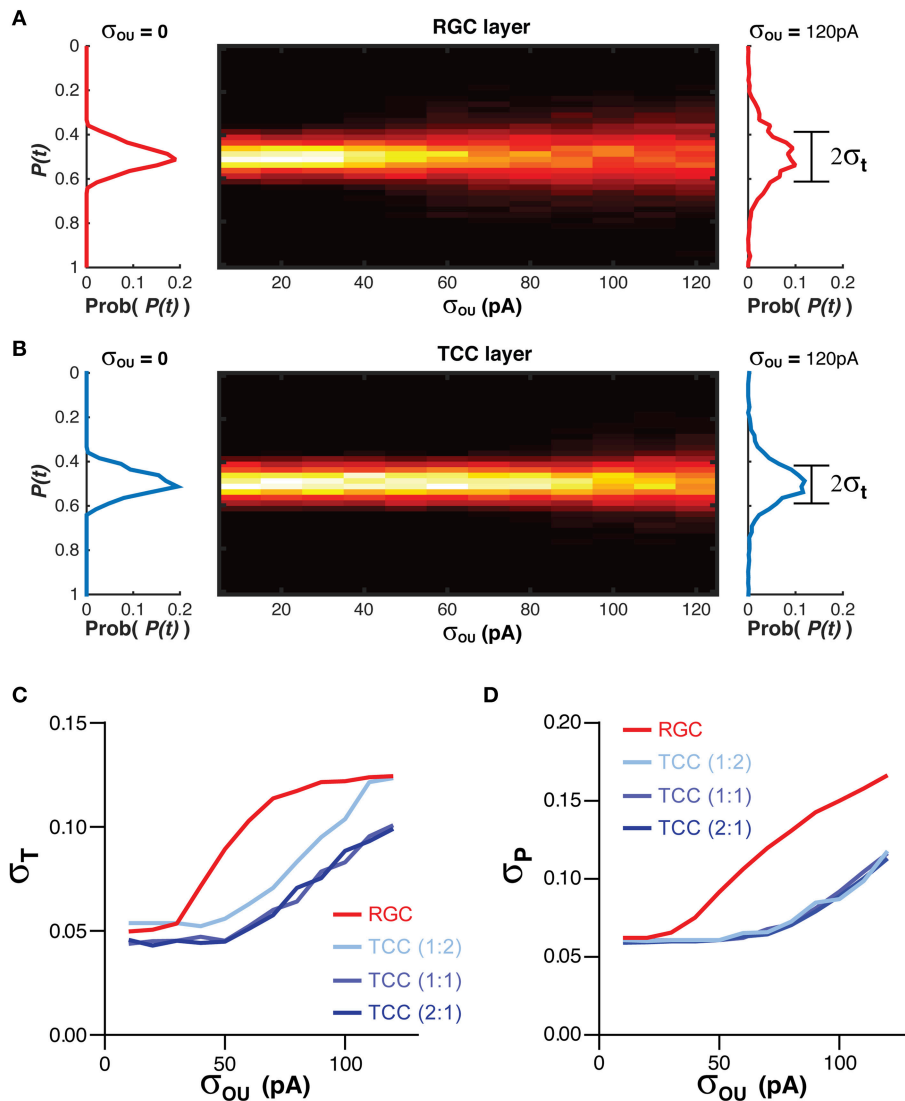


FIGURE 3 | Integration by TCCs increases the accuracy of the sensory coding in the presence of noise. (A) To the left and right of the heat map are histograms showing the distribution of the decoded position of the stimuli $P(t)$ in the RGC layer, in the presence of no retinal noise ($\sigma_{OU} = 0$) and a large degree of retinal noise ($\sigma_{OU} = 120$ pA). The central heat map shows the distribution of $P(t)$ across a range of retinal noise. Note the broadening of the distribution of $P(t)$ as the amplitude of the retinal noise increases. **(B)** The same data as presented in **(A)**, but for the TCC layer. Again, as the noise in the RGC layer increases, the distribution of $P(t)$ broadens, but to lesser degree. **(C)** The effect of network architecture (the ratio of TCCs to RGCs) and retinal noise on the distribution of $P(t)$ (σ_T). As retinal noise increases, in both the RGC and TCC layers, σ_T increases, but over most of the parameter space, the TCC layer has a significantly lower value of σ_T , that is, the encoding of position is more stable over time. **(D)** The effect of network architecture and retinal noise on the accuracy of encoding (σ_P). Again, retinal noise causes worse performance in both layers but the thalamic layer performs better at all but the lowest levels of retinal noise. However, the ratio of TCCs to RGCs has no effect on performance.

spikes ($\text{EPSC} \times 4$), the thalamic layer still acts as a low-pass filter.

DISCUSSION

Visual acuity is as high, or higher, than the density of RGCs predict (Wässle et al., 1981; Gauthier et al., 2009; Rossi and Roorda, 2010), a fact that may be surprising, given the inherent noise of RGC discharges and the fact that RGCs converge onto TCCs (Croner and Kaplan, 1995). Here we show that TCCs act as

a low-pass filter, reducing the consequence of noise driven spikes in the RGCs. However, this behavior comes at a cost: slowing the rate of response.

While this model was explicitly a model of visual system, there is little to suggest the same effect will not be seen in other sensory pathways, as the other sensory pathways share numerous similarities. For instance, input from the trigeminal nuclei to the VPM is also mediated by very large unitary events and that each TCC in the VPM is probably contacted by a small number of trigeminal neurons (Spacek and Lieberman,

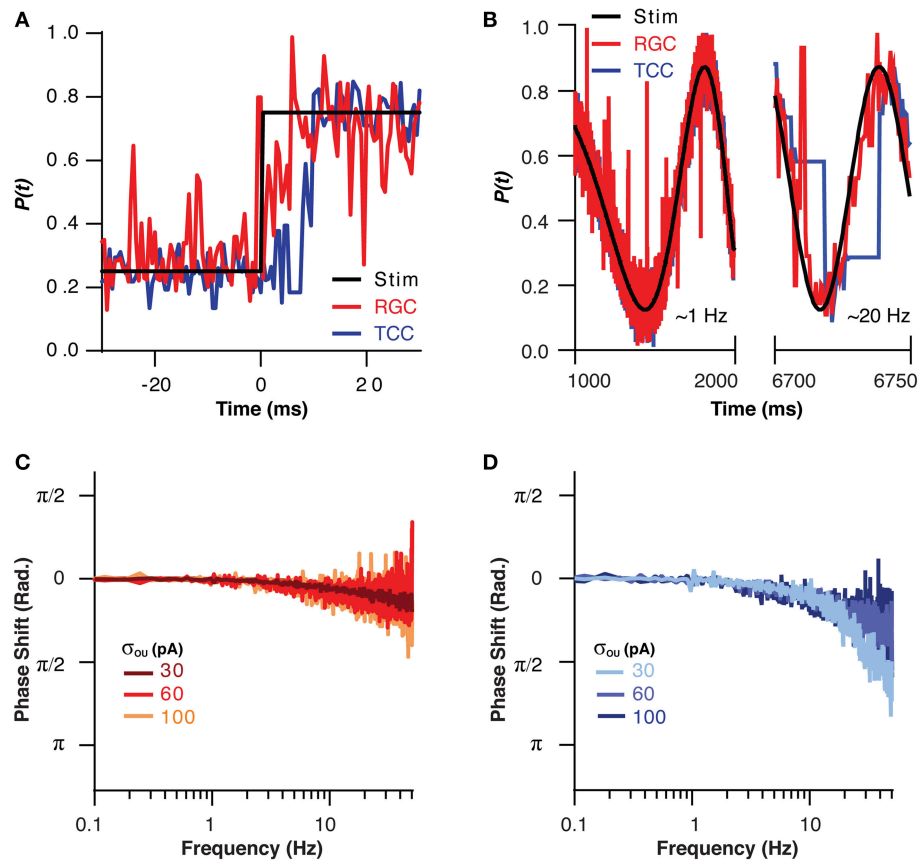


FIGURE 4 | The filtering effect of the thalamic layer comes at the cost of response rate. (A) The position encoded by the RGC and TCC layers in response to a step change in the position of the stimuli, note the response of the TCC layer is significantly delayed relative to the response of the RGC layer. **(B)** Two sections of the response of the RGC and TCC layers to a sinusoidal input of increasing spatial frequency ($\sigma_{ou} = 0.03$). **(C)** The phase of the RGC layer response with respect to the stimuli, showing the minimal phase shift and general independence from the magnitude of retinal noise. **(D)** The phase of the TCC response with respect to the stimuli, showing the large phase shift than that seen in the RGC layer, and the effect that increasing retinal noise decreases the phase shift seen in the TCC layer.

1974; Castro-Alamancos, 2002). Furthermore, we have shown that the filtering effect is robust to changes in the underlying connectivity.

Despite the fact that this result was consistent even when model parameters were pushed outside of what has been demonstrated in the native system, one needs to ask whether these results fit with published data. The response of LGN and VPM cells are known to be phase lagged relative to input from RGCs and trigeminal nucleus cells, respectively. However, the reported latency between the RGC and LGN activation (measured as the slope of the phase lag in cycles against frequency) is larger *in vivo* (~ 15 ms) than reported here (~ 5 ms), though similar to that reported for trigeminal to VPM (~ 3 – 6 ms; Lee et al., 1981; Hartings et al., 2003). Furthermore, there is evidence that thalamic cells do filter sensory noise. Hartings et al. (2003) demonstrated that the noisy (non-modulated) component of the spiking rate of trigeminal nucleus increases almost by a factor of 10 as the rate of whisker stimulation increases, however, the noisy component of the firing rate in VPM neurons is almost unchanged.

We have shown that the TCC layer of our model accurately encodes the information in the RGC layer up to approximately 10 Hz. While this may seem far from optimal, it is worth considering the nature of the information the RGC can provide. Studies in the cat have shown that the retina cannot encode changing information up to arbitrarily high frequencies, and in fact RGC discharges are significant phase shifted relative to visual stimuli above 10 Hz, and have a $\pi/4$ point of ~ 3 Hz (Shapley and Victor, 1978). Thus, the phase shift cause by the TCC may well be minimal in the scope of the visual pathway.

One needs to consider the terminology use in this paper. If we define a channel as the collection of inputs to a network that code for nearly identical stimulus features, then we are describing the thalamic layer as behaving as a low-pass filter in the domain of the rate channel change and not in the domain of rate of input in a given channel. Concretely, a channel could be a collection of trigeminal nucleus neurons that response to a whisker being deflected in a given direction, while another channel would be a collection of neurons who respond when the whisker is deflected in a direction perpendicular to the first

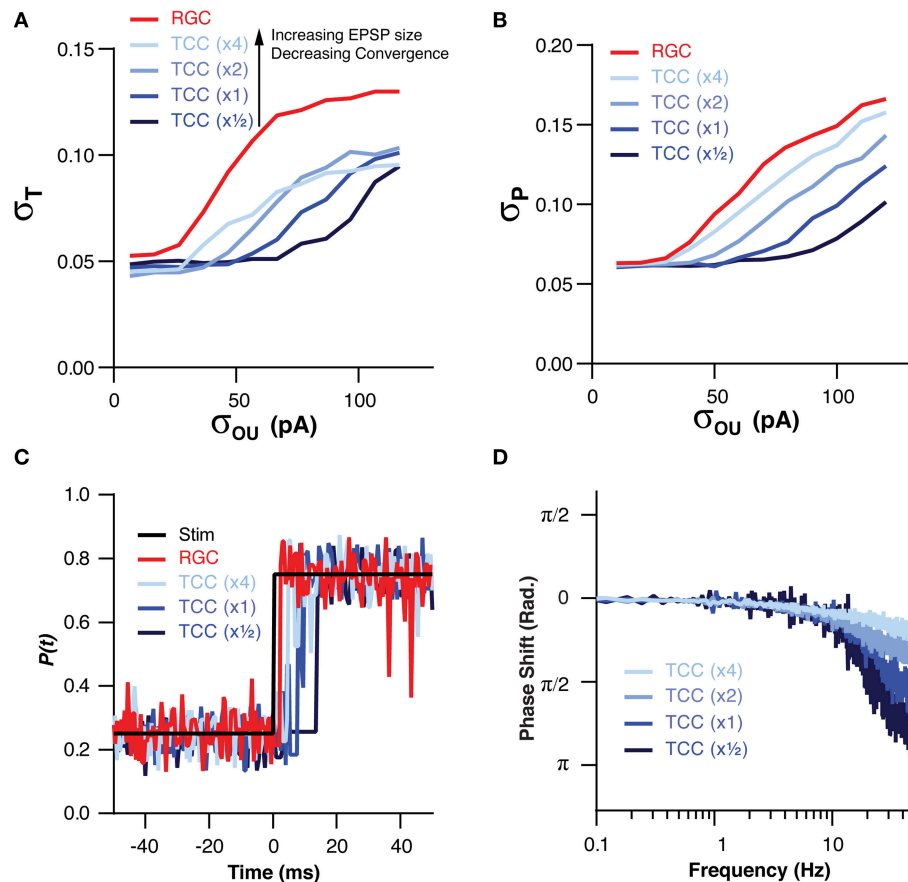


FIGURE 5 | The low pass effect of the TCC layer is robust to changes in the convergence of RGCs onto TCCs and EPSP size. (A) Across a range of convergence/EPSP sizes, the stability of encoding in the TCC layer was more robust to retinal noise than the RGC layer. Furthermore, increasing the convergence/decreasing the EPSP size produced a greater degree of robustness to noise. **(B)** The accuracy of encoding was higher in the TCC layer than the RGC layer across a range of convergence/EPSP sizes. Again, the higher the convergence/the lower the EPSP size, the greater the robustness to noise was. **(C)** Impulse response of the RGC and TCC layers across a range of convergence/EPSP sizes, showing that response delay increased as the convergence became higher/EPSP size became smaller. **(D)** The phase of the TCC response in relation to the stimuli across a range of convergence/EPSP sizes, clearly showing the slower response of networks with larger convergence and smaller EPSPs.

direction. Then we are describing the thalamus as limiting the rate at which information on how rapidly the whisker is shifting between moving in those two directions can be transferred, while providing noise immunity and stability of encoding. Indeed, if we consider the domain of the rate of channel change (e.g., the rate of the whisker shifting between moving in two perpendicularly oriented directions), and the domain of the rate of input in a given channel (e.g., mechanosensory current), then these two domains can be thought of almost as inverses, as a stimuli that causes only one channel to be active (low frequency channel change) should cause a consistent drive to a particular set of cells (high frequency input within a given channel). Conversely, a stimuli that is changing rapidly which channels is being driven will drive infrequent input in any given channel. This means that the low-pass behavior of the thalamic layer in the domain of the rate of channel change is a direct consequence of the high-pass behavior of the individual cells in the layer.

The decoding algorithm used here, which was the mean of the centers of the receptive fields of all active cells, and is essentially equivalent to a MLE, was very simple and did not take into account the temporal structure of the neural discharge, or correlation between cells (Usrey et al., 1998; Reinagel and Reid, 2000). This method was chosen because of its simplicity, and the ease of interpreting the results it generates. Moreover, we doubt using a different method would change the fundamental nature of the result: that the thalamic layer will not spike in response to rare input, thereby filtering out noise. This result has been demonstrated in another model (Martinez et al., 2014). However, due to being much closer to the *in vivo* case, this model was far more complex and is applicable to only the visual system.

In conclusion, we have shown that over a wide range of circuit parameters, in terms of changes in the nature of a stimuli, thalamic neurons act as a low pass filter for sensory information. This behavior comes as a consequence of the

fact that the cells in the thalamus act as high pass filters in terms of the rate at which they are driven. By only spiking during sustained high frequency input, thalamic cells filter out noise from their inputs allowing higher accuracy and higher stability decode of the stimuli. This however, comes at a cost, in that it produces a delay in transmission of information. We do not propose that this is the only function of the thalamus, but simply that it is a necessary consequence of the fact that TCCs must integrate charge before spiking.

REFERENCES

- Arabzadeh, E., Petersen, R. S., and Diamond, M. E. (2003). Encoding of whisker vibration by rat barrel cortex neurons: implications for texture discrimination. *J. Neurosci.* 23, 6011–6020.
- Castro-Alamancos, M. A. (2002). Properties of primary sensory (lemniscal) synapses in the ventrobasal thalamus and the relay of high-frequency sensory inputs. *J. Neurophysiol.* 87, 946–953. doi: 10.1013/jphysiol.2001.013283
- Chen, C., and Regehr, W. G. (2000). Developmental remodeling of the retinogeniculate synapse. *Neuron* 28, 955–966. doi: 10.1016/S0896-6273(00)00166-5
- Croner, L. J., and Kaplan, E. (1995). Receptive fields of P and M ganglion cells across the primate retina. *Vision Res.* 35, 7–24. doi: 10.1016/0042-6989(94)E0066-T
- Crunelli, V., Leresche, N., and Parnavelas, J. G. (1987). Membrane properties of morphologically identified X and Y cells in the lateral geniculate nucleus of the cat *in vitro*. *J. Physiol.* 390, 243–256. doi: 10.1113/jphysiol.1987.sp.016697
- Destexhe, A., Rudolph, M., Fellous, J. M., and Sejnowski, T. J. (2001). Fluctuating synaptic conductances recreate *in vivo*-like activity in neocortical neurons. *Neuroscience* 107, 13–24. doi: 10.1016/S0306-4522(01)00344-X
- Errington, A. C., Renger, J. J., Uebele, V. N., and Crunelli, V. (2010). State-dependent firing determines intrinsic dendritic Ca²⁺ signaling in thalamocortical neurons. *J. Neurosci.* 30, 14843–14853. doi: 10.1523/JNEUROSCI.2968-10.2010
- Gauthier, J. L., Field, G. D., Sher, A., Greschner, M., Shlens, J., Litke, A. M., et al. (2009). Receptive fields in primate retina are coordinated to sample visual space more uniformly. *PLoS Biol.* 7:e1000063. doi: 10.1371/journal.pbio.1000063
- Gerdjikov, T. V., Bergner, C. G., Stüttgen, M. C., Waiblinger, C., and Schwarz, C. (2010). Discrimination of vibrotactile stimuli in the rat whisker system: behavior and neurometrics. *Neuron* 65, 530–540. doi: 10.1016/j.neuron.2010.02.007
- Hartings, J. A., Temereanca, S., and Simons, D. J. (2003). Processing of periodic whisker deflections by neurons in the ventroposterior medial and thalamic reticular nuclei. *J. Neurophysiol.* 90, 3087–3094. doi: 10.1152/jn.00469.2003
- Hawken, M. J., Shapley, R. M., and Grosof, D. H. (1996). Temporal-frequency selectivity in monkey visual cortex. *Vis. Neurosci.* 13, 477–492. doi: 10.1017/S0952523800008154
- Heggelund, P., Buracas, G. T., Ruksenas, O., Boynton, G. M., and Thomas, D. A. (2003). *Signal Processing in the Lateral Geniculate Nucleus*. Amsterdam: IOS Press.
- Hong, Y. K., and Chen, C. (2011). Wiring and rewiring of the retinogeniculate synapse. *Curr. Opin. Neurobiol.* 21, 228–237. doi: 10.1016/j.conb.2011.02.007
- Khatri, V., Hartings, J. A., and Simons, D. J. (2004). Adaptation in thalamic barreloid and cortical barrel neurons to periodic whisker deflections varying in frequency and velocity. *J. Neurophysiol.* 92, 3244–3254. doi: 10.1152/jn.00257.2004
- Lee, B. B., Elepand, A., and Virsu, V. (1981). Phase of responses to moving sinusoidal gratings in cells of cat retina and lateral geniculate nucleus. *J. Neurophysiol.* 45, 807–817.
- Martinez, L. M., Molano-Mazón, M., Wang, X., Sommer, F. T., and Hirsch, J. A. (2014). Statistical wiring of thalamic receptive fields optimizes spatial sampling of the retinal image. *Neuron* 81, 943–956. doi: 10.1016/j.neuron.2013.12.014
- McAlonan, K., Cavanaugh, J., and Wurtz, R. H. (2008). Guarding the gateway to cortex with attention in visual thalamus. *Nature* 456, 391–394. doi: 10.1038/nature07382
- Minnery, B. S., Bruno, R. M., and Simons, D. J. (2003). Response transformation and receptive-field synthesis in the lemniscal trigeminothalamic circuit. *J. Neurophysiol.* 90, 1556–1570. doi: 10.1152/jn.00111.2003
- O'Brien, B. J., Isayama, T., Richardson, R., and Berson, D. M. (2002). Intrinsic physiological properties of cat retinal ganglion cells. *J. Physiol.* 538, 787–802. doi: 10.1113/jphysiol.2001.013009
- Reinagel, P., and Reid, R. C. (2000). Temporal coding of visual information in the thalamus. *J. Neurosci.* 20, 5392–5400.
- Rossi, E. A., and Roorda, A. (2010). The relationship between visual resolution and cone spacing in the human fovea. *Nat. Neurosci.* 13, 156–157. doi: 10.1038/nn.2465
- Saalmann, Y. B., and Kastner, S. (2011). Cognitive and perceptual functions of the visual thalamus. *Neuron* 71, 209–223. doi: 10.1016/j.neuron.2011.06.027
- Shapley, R. M., and Victor, J. D. (1978). The effect of contrast on the transfer properties of cat retinal ganglion cells. *J. Physiol.* 285, 275–298. doi: 10.1113/jphysiol.1978.sp012571
- Spacek, J., and Lieberman, A. R. (1974). Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus. *J. Anat.* 117, 487–516.
- Spear, P. D., Kim, C. B., Ahmad, A., and Tom, B. W. (1996). Relationship between numbers of retinal ganglion cells and lateral geniculate neurons in the rhesus monkey. *Vis. Neurosci.* 13, 199–203. doi: 10.1017/S0952523800007239
- Steriade, M. (2003). “Thalamus,” in *Encyclopedia of Life Sciences* (John Wiley & Sons, Ltd.), 1–8. doi: 10.1038/npg.els.0000217
- Tavazoie, S. F., and Reid, R. C. (2000). Diverse receptive fields in the lateral geniculate nucleus during thalamocortical development. *Nat. Neurosci.* 3, 608–616. doi: 10.1038/75786
- Turner, J. P., and Salt, T. E. (1998). Characterization of sensory and corticothalamic excitatory inputs to rat thalamocortical neurones *in vitro*. *J. Physiol.* 510 (Pt 3), 829–843.
- Usrey, W. M., Reppas, J. B., and Reid, R. C. (1998). Paired-spike interactions and synaptic efficacy of retinal inputs to the thalamus. *Nature* 395, 384–387. doi: 10.1038/26487
- Van Hooser, S. D., Roy, A., Rhodes, H. J., Culp, J. H., and Fitzpatrick, D. (2013). Transformation of receptive field properties from lateral geniculate nucleus to superficial V1 in the tree shrew. *J. Neurosci.* 33, 11494–11505. doi: 10.1523/JNEUROSCI.1464-13.2013

AUTHOR CONTRIBUTIONS

WC performed simulations and data analysis. ML and AE performed and designed *in vitro* experiments. WC, ML, AE, and VC wrote the manuscript.

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- Wang, W., Jones, H. E., Andolina, I. M., Salt, T. E., and Sillito, A. M. (2006). Functional alignment of feedback effects from visual cortex to thalamus. *Nat. Neurosci.* 9, 1330–1336. doi: 10.1038/nn1768
- Wässle, H., Peichl, L., and Boycott, B. B. (1981). Dendritic territories of cat retinal ganglion cells. *Nature* 292, 344–345. doi: 10.1038/292344a0
- White, C. A., and Sur, M. (1992). Membrane and synaptic properties of developing lateral geniculate nucleus neurons during retinogeniculate axon segregation. *Proc. Natl. Acad. Sci. U.S.A.* 89, 9850–9854. doi: 10.1073/pnas.89.20.9850

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Stimulus Contrast and Retinogeniculate Signal Processing

Daniel L. Rathbun^{1,2}, Henry J. Alitto^{1,3}, David K. Warland^{1,3} and W. Martin Usrey^{1,3*}

¹ Center for Neuroscience, University of California, Davis, Davis, CA, USA, ² Institute for Ophthalmology and Center for Integrative Neuroscience, University of Tübingen, Tübingen, Germany, ³ Department of Neurobiology, Physiology, and Behavior, University of California, Davis, Davis, CA, USA

Neuronal signals conveying luminance contrast play a key role in nearly all aspects of perception, including depth perception, texture discrimination, and motion perception. Although much is known about the retinal mechanisms responsible for encoding contrast information, relatively little is known about the relationship between stimulus contrast and the processing of neuronal signals between visual structures. Here, we describe simultaneous recordings from monosynaptically connected retinal ganglion cells and lateral geniculate nucleus (LGN) neurons in the cat to determine how stimulus contrast affects the communication of visual signals between the two structures. Our results indicate that: (1) LGN neurons typically reach their half-maximal response at lower contrasts than their individual retinal inputs and (2) LGN neurons exhibit greater contrast-dependent phase advance (CDPA) than their retinal inputs. Further analyses suggests that increased sensitivity relies on spatial convergence of multiple retinal inputs, while increased CDPA is achieved, in part, on temporal summation of arriving signals.

Keywords: retina, LGN, coding, vision, thalamus

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New York University, USA

*Correspondence:

W. Martin Usrey
wmusrey@ucdavis.edu

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INTRODUCTION

All visual information leaving the eye is conveyed in the spiking activity of retinal ganglion cells. Given the limited dynamic range of these cells and the dramatically varying statistics of visual stimuli in the natural world, efficient encoding of visual information requires processing that responds to the statistics of the visual input. Contrast gain control is a prominent mechanism used by the visual system to meet the challenge of encoding visual information in diverse visual environments. Contrast gain control refers to the nonlinear receptive field property whereby a neuron's gain and temporal dynamics are dependent upon stimulus contrast. Specifically, the response gain of neurons in the early visual system, including the retina, LGN, and V1 decreases as stimulus contrast increases, causing contrast response functions to saturate at contrasts below 100%. Additionally, as stimulus contrast increases, these same visual neurons become more responsive to stimuli with high temporal frequencies, and they exhibit a contrast-dependent phase advance (CDPA) in their responses to periodic stimuli. Although contrast gain control is first established within the retina, an open and unresolved question is how contrast gain control is enhanced between the retina and the LGN.

The axons of retinal ganglion cells target several central structures, including the lateral geniculate nucleus (LGN) of the thalamus which in turn provides monosynaptic excitation to primary visual cortex (V1). Although the response properties of retinal ganglion cells and LGN neurons are generally quite similar (Hubel and Wiesel, 1961; Cleland et al., 1971; Levick et al., 1972;

So and Shapley, 1981; Lee et al., 1983; Cleland and Lee, 1985; Kaplan et al., 1987; Mastronarde, 1987, 1992; Usrey et al., 1999; Rathbun et al., 2010), there are significant differences, and one of the most prominent of these involves the relationship between stimulus contrast and neuronal activity. In particular, LGN neurons display greater contrast gain control than their retinal inputs (Kaplan et al., 1987; Scholl et al., 2012; but see Sclar, 1987).

Factors that influence the feedforward communication of retinal signals to LGN neurons include the convergence of retinal inputs onto individual LGN neurons and the temporal summation of arriving signals. Studies in the cat indicate that LGN neurons typically receive convergent input from 2 to 5 retinal ganglion cells (Cleland et al., 1971; Cleland, 1986; Hamos et al., 1987; Mastronarde, 1992; Usrey et al., 1999; Reid and Usrey, 2004; Martinez et al., 2014). Likewise, the excitatory postsynaptic potentials (EPSPs) evoked from the spikes of individual retinal axons interact over interspike intervals (ISIs) of up to ~30 ms to increase the likelihood of bringing an LGN neuron to spike threshold (Mastronarde, 1987; Usrey et al., 1998; Levine and Cleland, 2001; Rowe and Fischer, 2001; Carandini et al., 2007; Sincich et al., 2007; Weyand, 2007; Rathbun et al., 2010). The goal of this study was to determine whether and how convergence and temporal summation contribute to the transmission and processing of contrast information between the retina and LGN.

To determine the influence of stimulus contrast on retinogeniculate communication and visual processing in the retina and LGN, we made simultaneous recordings from monosynaptically connected retinal ganglion cells and LGN neurons in the anesthetized cat and measured neuronal responses to drifting sinusoidal gratings that varied in stimulus contrast. Consistent with predictions from past studies (Kaplan et al., 1987; Scholl et al., 2012), our results demonstrate that the contrast needed to evoke a half-maximal response (C_{50}) is lower for LGN neurons than for their individual retinal afferents. Further analysis suggests that this effect relies on the integration of multiple retinal inputs by individual LGN neurons. Our results also reveal that CDPA—a hallmark of contrast gain control—is significantly greater for LGN neurons than for their individual retinal afferents. To probe the underlying mechanism responsible for the CDPA changes, we applied a model of ISI-based filtering to recorded retinal spike trains. Results from this effort reveal that an ISI-based filtering mechanism of retinal spikes can produce CDPA in target neurons. Taken together, these results indicate that the LGN is more than a simple relay station, as it adjusts both the sensitivity and timing of visual signals en route from retina to cortex.

MATERIALS AND METHODS

Animal Preparation

Six adult cats of both sexes were used in this study. All surgical and experimental procedures conformed to NIH guidelines and were carried out with the approval of the Animal Care and Use Committee at the University of California, Davis. Anesthesia was induced with ketamine (10 mg/kg, IM) and

thiopental sodium (10 mg/kg, IV; supplemented as needed). Animals received a tracheotomy and were placed in a stereotaxic apparatus where the temperature, electrocardiogram (ECG), electroencephalogram (EEG), and expired CO_2 were monitored continuously for the duration of the experiment. All wound margins were infused with lidocaine and anesthesia was maintained with a continuous infusion of thiopental sodium (2–3 mg/kg/h, IV). If physiological monitoring indicated a low level of anesthesia, additional thiopental was given and the rate of continuous infusion was increased. A midline scalp incision was made and the cortical surface above the LGN was exposed through a craniotomy which was filled with agarose. The lateral margin of each eye was dissected and each sclera was glued to a rigid post mounted on the stereotaxic frame. These posts secured the eyes and facilitated the introduction of a trans-scleral guide tube for retinal recordings. The nictitating membranes were retracted with 10% phenylephrine and flurbiprofen sodium drops were administered (1.5 mg/h) to prevent miosis. The eyes were refracted, fitted with appropriate contact lenses, and focused on a tangent screen located 172 cm in front of the animal. The positions of area centralis and the optic disk were plotted by back-projecting the retinal vasculature of each eye onto the tangent screen. Once all surgical procedures were complete, animals were paralyzed with vecuronium bromide (0.2 mg/kg/h, IV) and mechanically respired.

Electrophysiological Recording and Visual Stimuli

A multielectrode array (Thomas Recording, Marburg, Germany) was used to record from individual LGN neurons from seven independently positioned microelectrodes. The locations of receptive fields measured using the array were used to guide the placement of the retinal electrode. To record from retinal ganglion cells, a tungsten-in-glass microelectrode was introduced into the posterior chamber of the eye through a guide tube and positioned using a custom-made manipulator. Neural responses were amplified, filtered and recorded to a personal computer with a Power 1401 data acquisition interface and the Spike 2 software package (Cambridge Electronic Design, Cambridge, UK). The spikes from individual neurons were isolated using template matching and parametric clustering.

Visual stimuli were created with a VSG 2/5 visual stimulus generator (Cambridge Research Systems, Rochester, UK) and presented on a gamma-calibrated Sony monitor with a mean luminance of 38 cd/m^2 . Receptive fields were mapped in space and time using a binary white-noise stimulus and reverse-correlation analysis (Reid et al., 1997; Rathbun et al., 2007). To examine the influence of stimulus contrast on the timing and strength of neuronal responses and the efficacy of retinogeniculate communication, recordings were made while neurons were excited with drifting sinusoidal gratings (4 Hz, optimal spatial frequency). Gratings were shown for 4 s, followed by 4 s of mean gray, at 10 different contrast levels (random order), spaced logarithmically from 1 to 100%. The complete stimulus set was presented 100–300 times, as permitted by recording stability.

X and Y cells were distinguished on the basis of receptive field size, response latency, and time course of response (Usrey et al., 1999). Although recordings were made from both X and Y cells in the LGN, there was a heavy sampling bias for Y-type cells in the retina (see also Rathbun et al., 2010). Consequently, only Y-cell pairs were examined in this study. It is worth noting that Y-type cells are well suited for studying contrast-dependent processing, as they generally exhibit stronger contrast gain control and CDPA than X cells (Shapley and Victor, 1978).

Data Analysis

Cross Correlation Analysis

Cross-correlation analysis was used to identify monosynaptically connected retinogeniculate cell pairs (**Figure 1**). A cross-correlogram was generated by creating a histogram of LGN spikes relative to each retinal spike. The presence of a sharp, short-latency peak in the cross-correlogram was taken as evidence of a monosynaptically-connected pair of cells (Cleland et al., 1971). For quantitative analysis, cross-correlation bins contributing to the peak were identified using a bin size of 0.1 ms. The peak bin was first identified and all neighboring bins greater than three standard deviations above the baseline mean were considered part of the peak; where the baseline consisted of bins ranging from 30 to 50 ms on either side of the peak bin. Because each count in the cross-correlogram peak represents a single retinal spike that was relayed by the LGN neuron to cortex, these retinal spikes were termed “relayed” spikes whereas the remaining retinal spikes were termed “non-relayed” spikes. Likewise, LGN spikes that contributed to the cross-correlogram peak were termed “triggered” spikes, indicating that they were evoked by the simultaneously recorded retinal ganglion cell; and the remaining LGN spikes were termed “non-triggered” spikes (i.e., spontaneous spikes or spikes evoked from a source

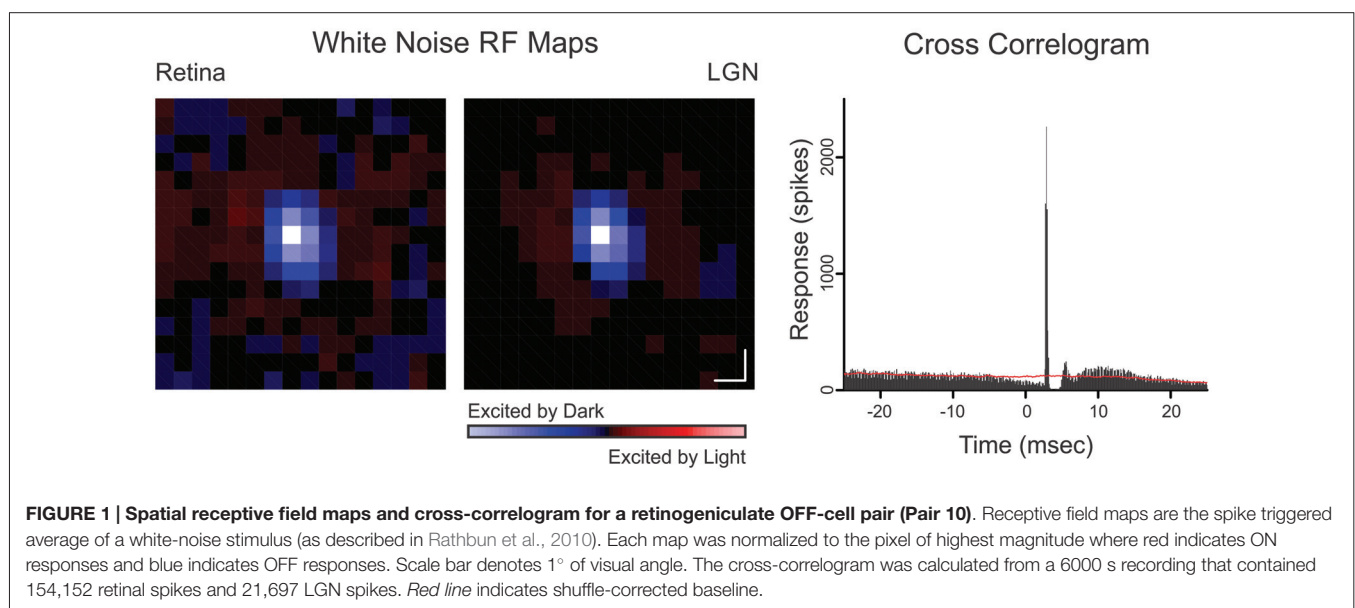
other than the simultaneously recorded retinal ganglion cell). Two values used to quantify the strength of communication between a simultaneously recorded retinal ganglion cell and LGN neuron are efficacy and contribution (Levick et al., 1972), where efficacy is the percentage of retinal spikes that evoked an LGN spike (i.e., relayed spikes) and contribution is the percentage of LGN spikes evoked from a particular retinal input (i.e., triggered spikes).

Response Curve Fitting

To determine the amplitude and phase of responses to drifting gratings, spike times were expressed relative to the phase of the sinusoid cycle, producing a cyclic histogram for each contrast. A constrained nonlinear optimization procedure (MATLAB function: *nlinfit*; The Mathworks, Natick, MA, USA) was used to fit each cyclic histogram with the positive-only rectification of the following sinusoidal equation:

$$R(t) = A * \sin(\omega * t + \theta) + b \quad (1)$$

where $R(t)$ is the magnitude of the cyclic histogram at time t , A is the response amplitude at the modulation frequency, ω is angular frequency of the drifting stimulus in radians per second, t is time in seconds, θ is the response phase determined by the vector sum of phases for all spikes in the cyclic histogram, and the baseline (b) indicates the value below which the sinusoid is rectified. The baseline was constrained to range between $-A$ and $2 * A$. For each spike train, cyclic histograms were fitted sequentially from low to high contrast with the additional constraint that b was monotonically decreasing. This procedure was found to produce more useful estimates of the modulated response amplitude and response phase, independent of contrast-induced changes in rectification, than a standard Fourier decomposition algorithm available in MATLAB (*fft*), and is analogous to estimation of the F1.



For each spike train, the contrast response function was fitted with a hyperbolic ratio (Albrecht and Hamilton, 1982; MATLAB function: *fminunc*; The Mathworks, Natick, MA, USA):

$$R(C) = \frac{R_{\max} * C^n}{C^n + C_{50}^n} + b \quad (2)$$

where $R(C)$ is the response for a given contrast C , $R_{\max}$ is the maximal response amplitude across contrasts, the exponential n reflects the sensitivity of the response function, C_{50} is the contrast corresponding to 50% of the maximal response, and b denotes baseline and was set as the response to the lowest contrast (1%).

As contrast increased, response phase was often observed to advance progressively earlier in the stimulus cycle. This phenomenon will be referred to as CDPA to distinguish it from absolute phase advance relative to the stimulus. In order to quantify CDPA, a first-order polynomial was fit to the curve of phase vs. log (contrast) over the middle six contrasts presented (range: 2.78–35.94% contrast; see **Figure 4**). The resultant slope quantifies CDPA magnitude in units of degree/octave. In earlier reports, CDPA has sometimes been expressed as the amount of phase advance over the eightfold range from 1.25 to 10% contrast (Shapley and Victor, 1978; Sclar, 1987). Because, we found that phase estimates were often unreliable at very low levels of contrast, we chose to exclude response values from contrasts less than 2.78%. The upper contrast limit was chosen to exclude saturation effects and falls near or below the C_{50} for all curves.

Modeling Contrast-Dependent Phase Advance

Results from previous studies show that retinal spikes following short ISIs are more effective in evoking LGN responses than retinal spikes following longer ISIs (Mastronarde, 1987; Usrey et al., 1998; Levine and Cleland, 2001; Rowe and Fischer, 2001; Carandini et al., 2007; Sincich et al., 2007; Weyand, 2007; Rathbun et al., 2010). Given that the mean firing rate of retinal ganglion cells typically increases as contrast increases, there will necessarily be a shift in the distribution of ISIs as a function of contrast. To determine the extent to which the augmentation of CDPA measured in the LGN relative to the retina could be accounted for by the contrast-dependent shift in ISI distribution, we generated simulated LGN spike trains based on weighting actual retinal spikes in experimentally recorded data according to the ISI vs. spike efficacy relationship curve calculated from responses to white-noise stimulation (Rathbun et al., 2010). For example, if 30% of retinal spikes following an ISI of 10–15 ms were found to evoke an LGN spike compared to only 15% of retinal spikes following an ISI of 15–20 ms, then these two groups of retinal spikes were assigned weights of 0.3 and 0.15, respectively (Alitto and Usrey, 2015) as their contributions to the simulated LGN spike train. This process was repeated for every retinal spike and simulated spike trains were analyzed in exactly the same manner as those from real LGN neurons, as described above.

Statistical Analysis

Unless otherwise indicated, population data is summarized by the mean and standard error of the mean. Wilcoxon's

signed-rank test (MATLAB function: *signrank*; The Mathworks, Natick, MA, USA) was used to determine p values for all pairwise statistical tests.

RESULTS

Comparing Contrast Response Functions in Retina and LGN

Simultaneous recordings were made from 10 pairs of monosynaptically-connected retinal ganglion cells and LGN neurons in order to study the influence of stimulus contrast on neuronal responses across the retinogeniculate synapse (see “Materials and Methods” Section; **Figure 1**). Responses to contrast-variant drifting grating stimuli (4 Hz, optimal spatial frequency) were used to determine the C_{50} , defined as the contrast to evoke 50% of maximum response, for each neuron in our sample ($n = 19$; all Y cells). The C_{50} is therefore a good metric for contrast gain control as it tends to be lowest for neurons that exhibit greater contrast saturation. Across our sample of connected cells, LGN neurons typically had significantly lower C_{50} values than their simultaneously recorded retinal input (**Figures 2A,B**; $p = 0.02$), indicating that LGN neurons display greater contrast gain control than their retinal counterparts.

To determine whether the decrease in C_{50} that occurred between pre- and postsynaptic neurons was the result of a selective filtering of retinal spikes, we compared C_{50} values for relayed and non-relayed retinal spikes (see “Materials and Methods” Section). As shown in **Figures 2C,D**, there was not a significant difference in C_{50} between the two classes of retinal spikes ($p = 0.23$). Thus, it seems unlikely that the difference in C_{50} values between retina and LGN can be attributed to the selective filtering of spikes generated by the simultaneously recorded retinal ganglion cells.

Estimates indicate that individual LGN neurons in the cat typically receive monosynaptic input from approximately 2–5 retinal ganglion cells (Cleland et al., 1971; Cleland, 1986; Hamos et al., 1987; Mastronarde, 1992; Usrey et al., 1999; Reid and Usrey, 2004; Martinez et al., 2014). To address the possibility that this convergence contributes to the shift in C_{50} between retinal ganglion cells and LGN neurons, we divided the spikes generated by each LGN neuron in our sample into two categories: those evoked (or “triggered”) by the simultaneously recorded retinal ganglion cell, and those evoked from other sources (“non-triggered”), including other retinal ganglion cells. Across our sample of cells, C_{50} values were significantly lower for non-triggered LGN spikes compared to triggered spikes (**Figures 2E,F**; $p < 0.05$). This finding is consistent with the idea that an LGN cell's contrast response function is shifted in the direction of its most sensitive input which, because of convergence, is likely to be an input other than the simultaneously recorded retinal ganglion cell.

We next examined whether the exponent “ n ” from the equation used to fit the contrast response functions differed between connected retinal and LGN neurons (see “Materials and Methods” Section). In general, this exponent can be taken

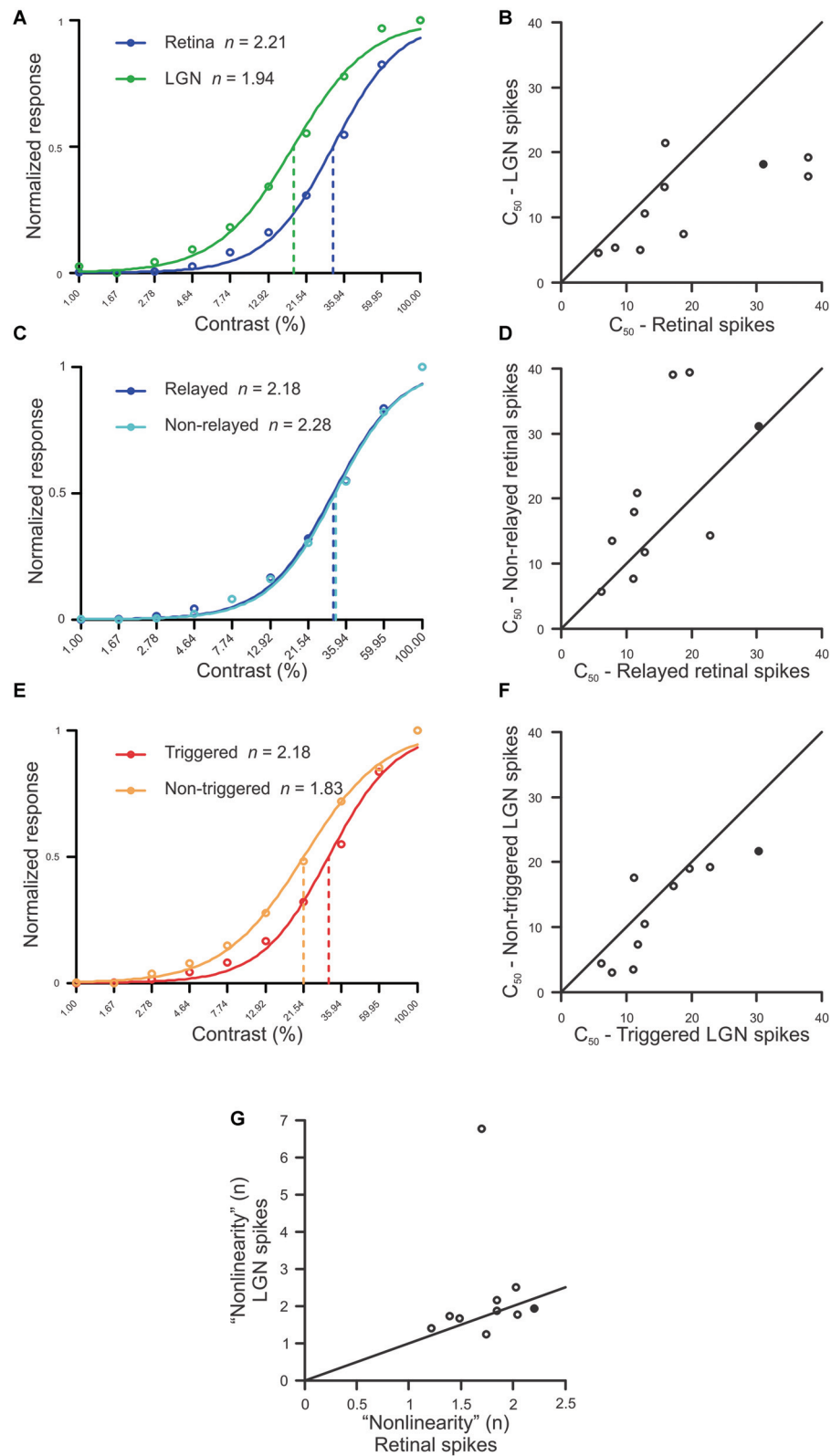


FIGURE 2 | (Continued)

FIGURE 2 | Continued**Comparison between spike classes for hyperbolic ratio fit parameters.**

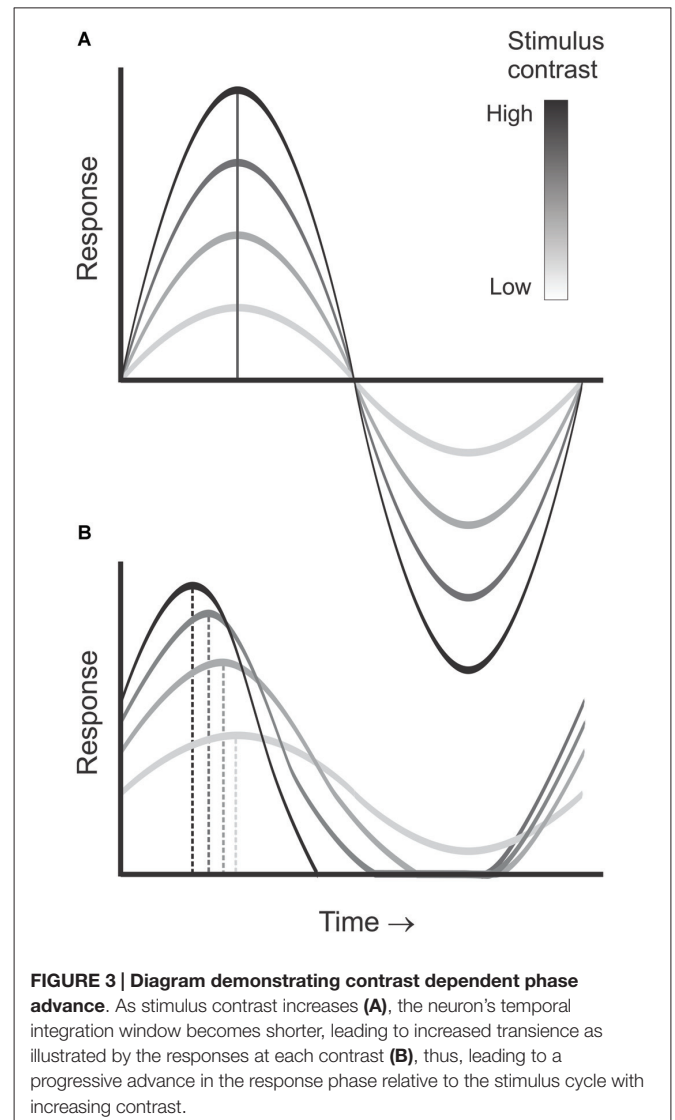
(A,C,E) Contrast response functions for a single example pair (Pair 16, filled symbol in B,D,F,G) where raw data is plotted with circles, solid lines denote the hyperbolic ratio fit, and dashed lines indicate the C_{50} . (B,D,F) Scatterplots of C_{50} for retinal and LGN spikes (B), relayed and non-relayed retinal spikes (D), and triggered and non-triggered LGN spikes (F). (G) Scatterplot comparing the exponent n between retinal and LGN spike trains. In all scatterplots, solid diagonal line denotes unity.

to quantify the linearity of the contrast response function, with small exponents indicating a relatively linear curve, and larger exponents indicating nonlinear expansion below the inflection point and compression above it. Consistent with Duong and Freeman (2008), we found that the expansive nonlinearity at low contrasts ($n > 1$) was present for all of the Y-type retinal ganglion cells and LGN neurons in our sample. However, we did not find a significant difference in the exponent term n between pre- and postsynaptic neurons, suggesting that this feature of the contrast response function is passed on, unaltered, from retina to LGN (Figure 2G; $p = 0.375$). It is worth noting that the single outlier in Figure 2G corresponds to an LGN neuron which exhibited a significantly stronger F2/F1 ratio than any other cell in this study. This pair of cells also represents the only pair in the sample in which the classification of the retinal ganglion cell was not clear (the LGN neuron was Y type). With that said, this cell pair did not differ from the rest of the sample in all of the other analyses performed.

Comparing Contrast-Dependent Phase Advance in Retina and LGN

CDPA is another hallmark of nonlinear processing in the early visual system. As contrast increases, neurons in the retina, LGN and visual cortex are reported to respond progressively earlier relative to the phase of the stimulus (Shapley and Victor, 1978; Dean and Tolhurst, 1986; Sclar, 1987; Alitto and Usrey, 2004). While this effect is partly due to a decrease in latency with increasing contrast, it is also believed to result from an increase in transience that is induced by contrast gain control mechanisms (Figure 3, Shapley and Victor, 1978). Importantly, models of geniculocortical processing often incorporate an increase in the CDPA from LGN to cortex (Kayser et al., 2001). While a similar increase from retina to LGN has been hypothesized, this increase has yet to be demonstrated directly. One study that examined this question through a meta-analysis of the existing literature found no difference between the two structures (Kayser et al., 2001). Paired-cell recording, however, provide a particularly sensitive tool to examine directly subtle differences in the responses of LGN neurons and their retinal inputs.

Consistent with previous reports, our sample of Y-type retinal ganglion cells exhibited an average phase advance of $\sim 55^\circ$ over the 2.78–35.94% contrast ranges (Shapley and Victor, 1978 reported $\sim 50^\circ$). More importantly, a comparison of CDPA between simultaneously recorded retinal ganglion cells and LGN neurons revealed that CDPA is significantly greater for LGN neurons than their recorded retinal inputs



($p = 0.027$; Figures 4A,B). This finding confirms the hypothesis that the influence of contrast gain control progressively increases throughout the early visual system. In examining differences between spike classes, we found that relayed retinal spikes exhibited significantly greater CDPA values than non-relayed retinal spikes (Figures 4C,D; $p = 0.004$), suggesting that the increase in CDPA from retina to LGN relies, in part, on a selective filtering of retinal spikes. Across our sample of cell pairs, there was not a significant difference between CDPA values calculated from triggered and non-triggered LGN spikes (Figures 4E,F; $p = 0.777$), suggesting that signals from simultaneously recorded and non-recorded retinal ganglion cells experienced comparable levels of CDPA.

Modeling Changes in Contrast-Dependent Phase Advance

Previous research has shown that the retinal interspike interval (ISI) has a strong influence on the generation of postsynaptic

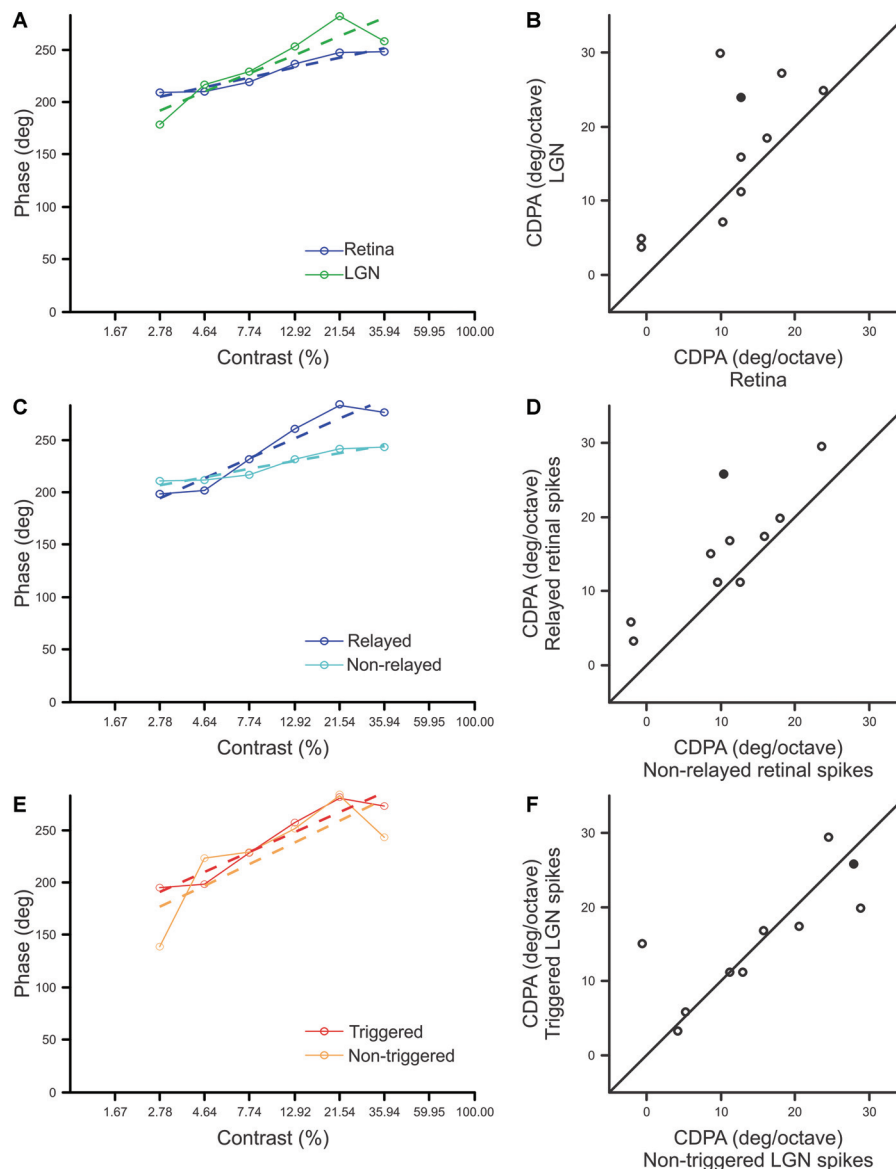


FIGURE 4 | Comparison of contrast-dependent phase advance (CDPA) between spike classes. (A,C,E) Response phase at each contrast for a single example pair (Pair 15, filled symbol in **B,D,F**) where *dashed lines* indicate best fit line to the data, *solid lines*, over the range of 2.78–35.94% contrast. The phase of responses to 1% contrast are omitted to improve graph legibility. CDPA is given by the slope of the best fit line in degree/octave. **(B,D,F)** Scatterplots of CDPA for retinal and LGN spikes **(B)**, relayed and non-relayed retinal spikes **(D)**, and triggered and non-triggered LGN spikes **(F)**.

responses in the LGN, as retinal spikes following short ISIs (<30 ms) are significantly more likely to evoke a postsynaptic spike than retinal spikes following longer ISIs (Carandini et al., 2007). Given this, we asked whether an ISI-based filter for retinogeniculate communication could underlie the increase in CDPA, described above. As a first step, we determined the influence of stimulus contrast on the distribution of ISIs in retinal spike trains. Across our sample of retinal ganglion cells, the distribution of ISIs was shifted toward lower ISIs as contrast increased (**Figures 5A,B**), consistent with the expected inverse relationship between firing rate and ISI.

We next quantified the relationship between ISI and the efficacy of retinal spikes in evoking postsynaptic responses for each retinogeniculate pair of simultaneously recorded neurons in our sample. Similar to previous results (Rathbun et al., 2010), retinal spikes with the shortest preceding ISIs were most effective in evoking postsynaptic responses (**Figure 5C**). Finally, we combined the ISI distributions that were determined for each contrast with the ISI filter to determine the extent to which the filter could reproduce the CDPA effect (see “Materials and Methods” Section). This model tests the hypothesis that simple, temporal filtering of retinal spikes, as estimated by the

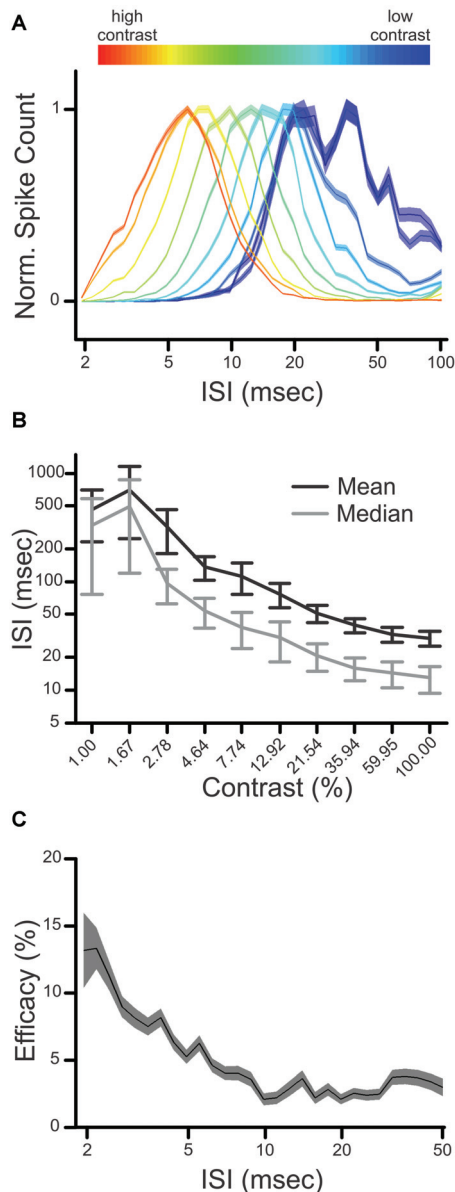


FIGURE 5 | Influence of contrast on interspike interval (ISI) distribution and ISI-based filtering. (A) Normalized ISI distributions of a single retinal neuron (Pair 2). The influence of contrast on ISI distribution is indicated by color (cold to hot), where the ISI distribution produced by the lowest contrast is plotted in *blue*, and that of the highest contrast is in *red*. **(B)** influence of contrast on mean and median ISI for all Y-class retinal ganglion cells. **(C)** ISI-based efficacy filter for the sample pair. Shaded regions in **(A,C)** denote error bars.

ISI-spike efficacy curve, can directly contribute to the increased CDPA of LGN neurons. For the modeled data, as with the original data, we found that relayed spikes had significantly greater CDPA when compared to non-relayed spikes (**Figure 6**; $p = 0.014$). While the magnitude of this effect was less than what was found in the original data (2.0 ± 0.1 vs. 5.0 ± 1.6 degree/octave, respectively; see “Discussion” Section), this result suggests that temporal filtering indeed plays a significant role

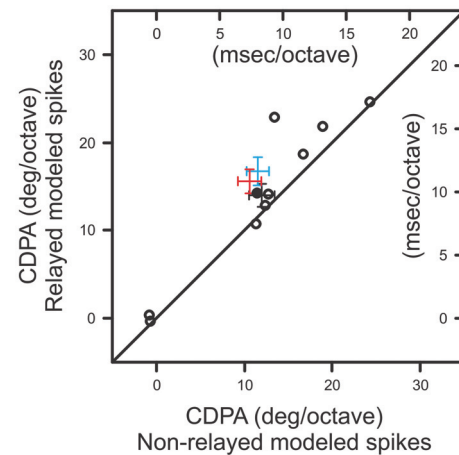


FIGURE 6 | Scatterplot showing CDPA of modeled relayed and non-relayed spikes. Black diagonal denotes unity. Population means and SEM from **Figures 4B,D are indicated in *blue* and *red*, respectively.**

in the increased CDPA that occurs across the retinogeniculate synapse.

DISCUSSION

Stimulus contrast is one of the most salient features encoded in the activity of neurons in the retina and LGN. Indeed, the center/surround organization of retinal and LGN receptive fields is ideal for detecting local changes in contrast. Although the spatial organization of retinal and LGN receptive fields are quite similar, past studies indicate that LGN neurons display greater contrast gain control, on average, than retinal ganglion cells. Given the significance of stimulus contrast for nearly all aspects of visual processing, it is surprising that relatively little attention has been paid to how contrast responses are transformed as they pass from retina to LGN (Kaplan et al., 1987; Cheng et al., 1995).

Here, we compared the responses of monosynaptically connected Y-type retinal ganglion cells and LGN neurons in the cat as a function of stimulus contrast. Consistent with the view that nearly all spikes in the LGN are triggered by retinal action potentials (Kaplan and Shapley, 1984; Sincich et al., 2007), we found the overall shape of contrast response functions to be similar for simultaneously recorded retinal ganglion cells and LGN neurons. However, our results also demonstrate that the semisaturation contrast (C_{50}) is significantly lower for LGN neurons compared to their simultaneously recorded retinal input, indicating that contrast gain control is enhanced by the LGN and that LGN responses begin to saturate at lower contrasts than their retinal inputs. Further analysis suggests that this decrease in C_{50} relies, at least in part, on convergence of multiple retinal inputs onto individual LGN neurons. With convergence, LGN neurons receive input from an ensemble of retinal ganglion cells that have a range of sensitivity profiles, and the most sensitive of these inputs will be those that influence LGN activity

under low contrast conditions, thereby shifting C_{50} values toward lower contrasts. Because individual LGN neurons are estimated to receive convergent input from approximately 2–5 retinal ganglion cells, our recording configuration was unlikely to include the most sensitive retinal input, a view consistent with the finding that non-triggered retinal spikes (spikes evoked from other sources) have lower C_{50} values than spikes triggered by the simultaneously recorded retinal ganglion cell.

Past efforts have shown that CDPA is most robust for Y cells (Shapley and Victor, 1978). Our investigation of CDPA in monosynaptically connected Y-type retinal ganglion cells and LGN neurons reveals that LGN neurons exhibit greater CDPA than their retinal inputs. Our results also show that relayed retinal spikes exhibit greater CDPA than non-relayed spikes, suggesting that contrast gain control exerts an influence on spike transmission at the retinogeniculate synapse. To gain insight into the possible mechanisms that underlie the increase in CDPA between the retina and LGN, we developed a model to examine whether temporal filtering of incoming retinal spikes could produce CDPA. In this model, retinal spikes following a short ISI (<30 ms) are more effective in driving a postsynaptic spike than retinal spikes following longer ISIs. Results from this effort revealed that relayed spikes had greater CDPA than non-relayed spikes, although the magnitude of this effect was less than that measured between retinal ganglion cells and LGN neurons *in vivo*. While additional mechanisms, including polysynaptic interactions and local inhibition, likely contribute to increased transience and the increased CDPA of LGN neurons, ISI filtering provides a simple feed-forward

mechanism by which gain control can be augmented in the visual pathway.

In summary, simultaneous recordings of monosynaptically connected retinal ganglion cells and LGN neurons showed enhancement of contrast gain control mechanisms by the LGN. Two measures of contrast gain control: (1) increased sensitivity to low-contrast stimuli and (2) CDPA, were both greater for individual LGN neurons compared to their simultaneously recorded retinal inputs. Further analyses suggests that increased sensitivity is achieved via spatial convergence of multiple retinal inputs, while increased CDPA is achieved, in part, on temporal summation of arriving signals. Taken together, these results reveal a breadth of processing strategies, both spatial and temporal, employed by the thalamus to transform visual signals en route from retina to cortex.

AUTHOR CONTRIBUTIONS

DLR contributed to experimental design, data collection, data analysis, and manuscript preparation. HJA contributed to data analysis and manuscript preparation. DKW contributed to data analysis and manuscript preparation. WMU contributed to experimental design, data collection and manuscript preparation.

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REFERENCES

- Albrecht, D. G., and Hamilton, D. B. (1982). Striate cortex of monkey and cat: contrast response function. *J. Neurophysiol.* 48, 217–237.
- Alitto, H. J., and Usrey, W. M. (2004). Influence of contrast on orientation and temporal frequency tuning in ferret primary visual cortex. *J. Neurophysiol.* 91, 2797–2808. doi: 10.1152/jn.00943.2003
- Alitto, H. J., and Usrey, W. M. (2015). Surround suppression and temporal processing of visual signals. *J. Neurophysiol.* 113, 2605–2617. doi: 10.1152/jn.00480.2014
- Carandini, M., Horton, J. C., and Sincich, L. C. (2007). Thalamic filtering of retinal spike trains by postsynaptic summation. *J. Vis.* 7, 20.1–20.11. doi: 10.1167/7.14.20
- Cheng, H., Chino, Y. M., Smith, E. L. III, Hamamoto, J., and Yoshida, K. (1995). Transfer characteristics of lateral geniculate nucleus X neurons in the cat: effects of spatial frequency and contrast. *J. Neurophysiol.* 74, 2548–2557.
- Cleland, B. G. (1986). “The dorsal lateral geniculate nucleus of the cat,” in *Visual Neuroscience*, eds J. D., Pettigrew, K. S., Sanderson, and W. R., Levick (London: Cambridge University Press), 111–120.
- Cleland, B. G., and Lee, B. B. (1985). A comparison of visual responses of cat lateral geniculate nucleus neurones with those of ganglion cells afferent to them. *J. Physiol.* 369, 249–268. doi: 10.1113/jphysiol.1985.sp015899
- Cleland, B. G., Dubin, M. W., and Levick, W. R. (1971). Sustained and transient neurones in the cat's retina and lateral geniculate nucleus. *J. Physiol.* 217, 473–496. doi: 10.1113/jphysiol.1971.sp009581
- Dean, A. F., and Tolhurst, D. J. (1986). Factors influencing the temporal phase of response to bar and grating stimuli for simple cells in the cat striate cortex. *Exp. Brain Res.* 62, 143–151. doi: 10.1007/bf00237410
- Duong, T., and Freeman, R. D. (2008). Contrast sensitivity is enhanced by expansive nonlinear processing in the lateral geniculate nucleus. *J. Neurophysiol.* 99, 367–372. doi: 10.1152/jn.00873.2007
- Hamos, J. E., Van Horn, S. C., Raczkowski, D., and Sherman, S. M. (1987). Synaptic circuits involving an individual retinogeniculate axon in the cat. *J. Comp. Neurol.* 259, 165–192. doi: 10.1002/cne.902590202
- Hubel, D. H., and Wiesel, T. N. (1961). Integrative action in the cat's lateral geniculate body. *J. Physiol.* 155, 385–398. doi: 10.1113/jphysiol.1961.sp006635
- Kaplan, E., Purpura, K., and Shapley, R. M. (1987). Contrast affects the transmission of visual information through the mammalian lateral geniculate nucleus. *J. Physiol.* 391, 267–288. doi: 10.1113/jphysiol.1987.sp016737
- Kaplan, E., and Shapley, R. (1984). The origin of the S (slow) potential in the mammalian lateral geniculate nucleus. *Exp. Brain Res.* 55, 111–116. doi: 10.1007/bf00240504
- Kayser, A., Priebe, N. J., and Miller, K. D. (2001). Contrast-dependent nonlinearities arise locally in a model of contrast-invariant orientation tuning. *J. Neurophysiol.* 85, 2130–2149.
- Lee, B. B., Virsu, V., and Creutzfeldt, O. D. (1983). Linear signal transmission from prepotentials to cells in the macaque lateral geniculate nucleus. *Exp. Brain Res.* 52, 50–56. doi: 10.1007/bf00237148
- Levick, W. R., Cleland, B. G., and Dubin, M. W. (1972). Lateral geniculate neurons of cat: retinal inputs and physiology. *Invest. Ophthalmol.* 11, 302–311.
- Levine, M. W., and Cleland, B. G. (2001). An analysis of the effect of retinal ganglion cell impulses upon the firing probability of neurons in the dorsal lateral geniculate nucleus of the cat. *Brain Res.* 902, 244–254. doi: 10.1016/s0006-8993(01)02411-8
- Martinez, L. M., Molano-Mazón, M., Wang, X., Sommer, F. T., and Hirsch, J. A. (2014). Statistical wiring of thalamic receptive fields optimizes spatial sampling of the retinal image. *Neuron* 81, 943–956. doi: 10.1016/j.neuron.2013.12.014

- Mastronarde, D. N. (1987). Two classes of single-input X-cells in cat lateral geniculate nucleus. II. Retinal inputs and the generation of receptive-field properties. *J. Neurophysiol.* 57, 381–413.
- Mastronarde, D. N. (1992). Nonlagged relay cells and interneurons in the cat lateral geniculate nucleus: receptive-field properties and retinal inputs. *Vis. Neurosci.* 8, 407–441. doi: 10.1017/s0952523800004934
- Rathbun, D. L., Alitto, H. J., Weyand, T. G., and Usrey, W. M. (2007). Interspike interval analysis of retinal ganglion cell receptive fields. *J. Neurophysiol.* 98, 911–919. doi: 10.1152/jn.00802.2006
- Rathbun, D. L., Warland, D. K., and Usrey, W. M. (2010). Spike timing and information transmission at retinogeniculate synapses. *J. Neurosci.* 30, 13558–13566. doi: 10.1523/JNEUROSCI.0909-10.2010
- Reid, R. C., and Usrey, W. M. (2004). “Functional connectivity in the pathway from retina to visual cortex,” in *The Visual Neurosciences*, eds L. M., Chalupa, and J. S., Werner (Cambridge, MA: MIT Press), 673–679.
- Reid, R. C., Victor, J. D., and Shapley, R. M. (1997). The use of m-sequences in the analysis of visual neurons: linear receptive field properties. *Vis. Neurosci.* 16, 1015–1027. doi: 10.1017/s0952523800011743
- Rowe, M. H., and Fischer, Q. (2001). Dynamic properties of retinogeniculate synapses in the cat. *Vis. Neurosci.* 18, 219–231. doi: 10.1017/s0952523801182076
- Scholl, B., Latimer, K. W., and Priebe, N. J. (2012). A retinal source of spatial contrast gain control. *J. Neurosci.* 32, 9824–9830. doi: 10.1523/JNEUROSCI.0207-12.2012
- Sclar, G. (1987). Expression of “retinal” contrast gain control by neurons of the cat’s lateral geniculate nucleus. *Exp. Brain Res.* 66, 589–596. doi: 10.1007/bf00270692
- Shapley, R., and Victor, J. (1978). The effect of contrast on the transfer properties of cat retinal ganglion cells. *J. Physiol.* 285, 275–298. doi: 10.1113/jphysiol.1978.sp012571
- Sincich, L. C., Adams, D. L., Economides, J. R., and Horton, J. C. (2007). Transmission of spike trains at the retinogeniculate synapse. *J. Neurosci.* 27, 2683–2692. doi: 10.1523/jneurosci.5077-06.2007
- So, Y. T., and Shapley, R. M. (1981). Spatial tuning of cells in and around lateral geniculate nucleus of the cat: X and Y relay cells and perigeniculate interneurons. *J. Neurophysiol.* 45, 107–120.
- Usrey, W. M., Reppas, J. B., and Reid, R. C. (1998). Paired-spike interactions and synaptic efficacy of retinal inputs to the thalamus. *Nature* 395, 384–387. doi: 10.1038/26487
- Usrey, W. M., Reppas, J. B., and Reid, R. C. (1999). Specificity and strength of retinogeniculate connections. *J. Neurophysiol.* 82, 3527–3540.
- Weyand, T. G. (2007). Retinogeniculate transmission in wakefulness. *J. Neurophysiol.* 98, 769–785. doi: 10.1152/jn.00929.2006

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Presynaptic Adenosine Receptor-Mediated Regulation of Diverse Thalamocortical Short-Term Plasticity in the Mouse Whisker Pathway

Giovanni Ferrati¹, Francisco J. Martini¹ and Miguel Maravall^{1,2*}

¹ Instituto de Neurociencias de Alicante UMH-CSIC, Sant Joan d'Alacant, Spain, ² School of Life Sciences, Sussex Neuroscience, University of Sussex, Brighton, UK

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Ponce School of Medicine and
Health Sciences, Puerto Rico

*Correspondence:

Miguel Maravall
m.maravall@sussex.ac.uk

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Short-term synaptic plasticity (STP) sets the sensitivity of a synapse to incoming activity and determines the temporal patterns that it best transmits. In “driver” thalamocortical (TC) synaptic populations, STP is dominated by depression during stimulation from rest. However, during ongoing stimulation, lemniscal TC connections onto layer 4 neurons in mouse barrel cortex express variable STP. Each synapse responds to input trains with a distinct pattern of depression or facilitation around its mean steady-state response. As a result, in common with other synaptic populations, lemniscal TC synapses express diverse rather than uniform dynamics, allowing for a rich representation of temporally varying stimuli. Here, we show that this STP diversity is regulated presynaptically. Presynaptic adenosine receptors of the A1R type, but not kainate receptors (KARs), modulate STP behavior. Blocking the receptors does not eliminate diversity, indicating that diversity is related to heterogeneous expression of multiple mechanisms in the pathway from presynaptic calcium influx to neurotransmitter release.

Keywords: vibrissae, somatosensory, tactile, whole-cell, short-term plasticity, patch clamp, *in vitro*

INTRODUCTION

Visual, auditory and somatosensory information reaches the neocortex by way of thalamocortical (TC) synapses. Moment-to-moment changes in the size and reliability of these synaptic connections, termed short-term synaptic plasticity (STP), shape the information that reaches the cortex and determine how the response of a thalamic neuron to a sensory event is transmitted. STP acts as a filter of presynaptic patterns, preferentially transmitting particular frequencies or events (e.g., bursts as compared to single spikes; Fortune and Rose, 2001; Abbott and Regehr, 2004; Buonomano and Maass, 2009). When stimulated from rest, “driver” synapses

Abbreviations: STP, short-term plasticity; TC, thalamocortical; ISI, inter-stimulus interval; PSP, post-synaptic potential; VPM, ventral posterior medial thalamic nucleus; TCS, slope of instantaneous tuning curve; ACSF, artificial cerebrospinal fluid; DNDS, dinitrostilbene-2,2'-disulfonic acid; DPCPX, 8-Cyclopentyl-1,3-dipropylxanthine; CPT or 8-CPT, 8-Cyclopentyl-1,3-dimethylxanthine; UBP 310, (S)-1-(2-Amino-2-carboxyethyl)-3-(2-carboxy-thiophene-3-yl-methyl)-5-methylpyrimidine-2,4-dione; NS-102, 6,7,8,9-Tetrahydro-5-nitro-1H-benz[g]indole-2,3-dione 3-oxime.

from TC neurons onto excitatory neurons in layer 4 show STP dominated by strong depression (Stratford et al., 1996; Gil et al., 1997, 1999; Chung et al., 2002; Bruno and Sakmann, 2006; Sherman and Guillery, 2006; Lee and Sherman, 2008; Viaene et al., 2011). However, stimulation from rest does not reproduce the physiologically relevant *in vivo* situation in which sensory information does not arrive against a background of perfect silence. Rather, thalamic spiking as delivered to the cortex consists of ongoing sensory and contextual activity (Slézia et al., 2011; Poulet et al., 2012; Ollerenshaw et al., 2014; Bale et al., 2015; Crunelli et al., 2015; McCormick et al., 2015; Urbain et al., 2015). Prior activity sets the amount of TC synaptic depression: active synapses are effectively “pre-depressed” (Castro-Alamancos and Oldford, 2002; Castro-Alamancos, 2004; Boudreau and Ferster, 2005; Reig et al., 2006). The ongoing STP state of synapses determines the regime of operation of the TC network and conditions how information is transmitted (Buonomano and Maass, 2009).

We recently determined the population-level variability of STP in TC connections during ongoing stimulation (Díaz-Quesada et al., 2014). Recording responses of layer 4 excitatory neurons in acute TC slices, we found that although different TC connections share prominent depression during stimulation from rest, STP during ongoing stimulation is highly heterogeneous across connections. Some TC connections are strongly depressing and respond more weakly to shorter inter-stimulus intervals, while others facilitate and show an enhanced response to shorter intervals. A given temporal stimulus pattern can facilitate some synapses while depressing others, implying that different TC synapses are strong at distinct times during ongoing activity. This range of behaviors does not define separate categories of STP; instead, connections form a continuum. STP variability applies across identified spiny stellate neurons, and occurs even for different recordings carried out in the same slice (Díaz-Quesada et al., 2014).

The mechanisms governing the diversity of STP across excitatory lemniscal TC synapses are unknown and could potentially include pre- and postsynaptic loci. Here, we used whole-cell patch clamp recordings in acute slices to uncover mechanisms whose expression covaries with the amount and tendency of STP and localize them pre- or postsynaptically.

MATERIALS AND METHODS

Slice Preparation

All procedures were performed in accordance with national and European Union policies for the care and use of animals in research. The study was approved by the Instituto de Neurociencias and CSIC Ethical Review Committees. TC slices (Agmon and Connors, 1991) were obtained from male and female ICR mice between 14–25 postnatal days of age. This age is later than the established critical period for TC synaptic plasticity (Crair and Malenka, 1995) and the period when sensory responses have been described as facilitating (Borgdorff et al., 2007); over this range of ages, the distribution of STP values does not depend on age (Díaz-Quesada et al., 2014).

Slices (350 μm thickness) were prepared with conventional methods (Díaz-Quesada and Maravall, 2008): after killing the animal, the brain was placed in ice-cold cutting solution bubbled with carbogen (95% O_2 , 5% CO_2) and containing (in mM): 110 Cl-choline, 25 NaHCO_3 , 25 D-glucose, 11.6 Na-aspartate, 7 MgSO_4 , 3.1 Na-pyruvate, 2.5 KCl, 1.25 NaH_2PO_4 , 0.5 CaCl_2 . The brain was split at the midline and each hemisphere placed on a custom-made wedge at a slope of 50° . Hemispheres were placed lying on their medial face at a tilt of 10° on the sloped surface of the wedge, with the left hemisphere glued onto the right side of the wedge with its rostral edge facing down and the right hemisphere arranged symmetrically. Around three TC slices were collected per hemisphere. Slices were cut on a vibratome (Campden Instruments Integraslice 7550M; Leica VT1000S) and transferred to a chamber containing artificial cerebrospinal fluid (ACSF) continuously perfused with carbogen and incubated at 34°C for ~ 30 min. They were then kept at room temperature until used. ACSF composition was usually (in mM): 127 NaCl, 25 NaHCO_3 , 25 D-glucose, 2.5 KCl, 1.25 NaH_2PO_4 , 2 MgCl_2 , 1 CaCl_2 unless otherwise noted. However, to examine the effects of $[\text{Ca}^{2+}]$ on STP diversity, the ACSF composition was modified by increasing CaCl_2 concentration to 2 or 4 mM while reducing $[\text{MgCl}_2]$ to 1 or 0.5 mM respectively. All chemicals were from Sigma-Aldrich unless otherwise noted.

Recordings

Patch electrodes were pulled from borosilicate glass (1.5 mm outer diameter, 0.86 mm inner; 3–6 M Ω) and filled with internal solution containing (in mM) 130 K-methylsulfonate, 10 Na-phosphocreatine, 10 HEPES, 4 MgCl_2 , 4 $\text{Na}_2\text{-ATP}$, 3 Na-ascorbate, and 0.4 $\text{Na}_2\text{-GTP}$; pH 7.33, 287–303 mOsm. To ensure that measured STP was purely monosynaptic, the internal solution incorporated the intracellular GABA $_A$ antagonist dinitrostilbene-2,2'-disulfonic acid (DNDS), a chloride channel blocker (Dudek and Friedlander, 1996; Covic and Sherman, 2011). DNDS (1 mM; Tocris) worked effectively in TC connections (Díaz-Quesada et al., 2014). Kynurenic acid, a blocker of ionotropic glutamate receptors, was tested at various concentrations and found to provide reliable partial blockade at 150 μM . To manipulate adenosine receptor activation, we used the receptor (A1R) agonist adenosine (9- β -D-Ribofuranosyladenine, Adenine riboside, Adenine-9- β -D-ribofuranoside) and two different antagonists, DPCPX and 8-CPT (all from Tocris). For kainate receptor manipulation we used antagonists UBP 310 (Tocris) and NS-102. Recordings were performed at room temperature (24°C). Earlier work found no evidence that temperature (24°C vs. 33°C) influences STP during ongoing stimulation (Díaz-Quesada et al., 2014).

Neurons were selected based on morphological criteria using infrared differential interference contrast optics and patched in the whole-cell mode. Cells with small spherical cell bodies (~ 10 – 15 μm in diameter) and dendrites confined to L4, typical of spiny stellate neurons, were chosen. Recordings were not corrected for liquid junction potential. Neuronal responses were

measured while stimulating with depolarizing square pulses of 500 ms duration and increasing intensity; only neurons displaying a regular spiking phenotype (McCormick et al., 1985), clearly distinct from fast spiking or low threshold spiking cells, were included in the analyzed data set. Input resistance was 150–500 M Ω and access resistance was under 10% of input resistance; recordings were discarded if access resistance was unstable or the resting membrane potential drifted by more than 10 mV. Data were acquired with an Axon Multiclamp 700-B amplifier (Molecular Devices), filtered at 4–10 kHz, and sampled at 20 kHz (PCI 6040-E; National Instruments) under the control of software custom-written in Matlab (The Mathworks; Pologrueto et al., 2003).

Electrical Stimulation

TC fibers were stimulated with a Pt-Ir concentric bipolar electrode (FHC; outer pole diameter 200 μ m, inner pole diameter 25 μ m) located in the white matter; a stimulus isolator generated monophasic pulses (Iso Flex; A.M.P.I.). All stimuli were generated in Matlab. To restrict stimulation to a reduced number of fibers (putatively down to a single fiber), we first searched for a stimulus amplitude at which a clear PSP was seen in a fraction of trials. We then further increased amplitude to a level such that PSP size remained stable but each temporally isolated single stimulus evoked a successful response in almost all trials (Díaz-Quesada et al., 2014). This approach ensured that failures of stimulation were negligible, but kept low the number of stimulated fibers. At this stimulation intensity, successful PSPs maintained their stereotypical shape throughout a train of repetitive stimulation, suggesting that the fibers contributing to the response remained stable. Experiments with unstable success probability or response characteristics (latency, shape) were discarded. Stimulus amplitudes were 1–15 μ A, towards the lower end of previously reported thresholds for TC activation and an order of magnitude lower than thresholds for antidromic activation of corticothalamic neurons (Rose and Metherate, 2001).

Stimulation protocols were adapted from Díaz-Quesada et al. (2014). In brief, they consisted of sequences of regular and irregular pulse trains. A regular train was followed by an irregular train, both with the same average frequency (4.59 Hz) and duration (21 pulses, 4.36 s). The regular train had a constant ISI and the irregular train consisted of pulses at different interspersed intervals in the range 13–806 ms. A single specific irregular train was used. Each sequence (9 s long) was repeated 10–15 times per recording; each trial lasted 10 s, including periods of silence during which baseline properties were monitored. Additionally, there was a stimulation pause between trials >5 s (trial start corresponded to condition “from rest”). All protocols were applied with the same stimulation intensity.

Analysis

To compute PSP amplitude, we searched for the first membrane potential peak in the window extending from 0.5 to 12 ms after the stimulation pulse, averaged the membrane potential

over five data samples (from –0.1 to 0.1 ms relative to the raw peak), and subtracted a baseline averaged over 2 ms immediately preceding the stimulation pulse. This short baseline effectively compensated for depolarization caused by earlier PSPs. Mean PSP amplitude was computed separately for each stimulus in a train after removing stimulus artifacts with median filtering. The steady-state response level was assessed by discarding the first five PSPs from stimulation onset (i.e., approximately the first second of stimulation), and computing the mean amplitude over all remaining PSPs.

We quantified STP magnitude using two measures, as follows. First, for each connection we constructed a tuning curve, plotting PSP magnitude as a function of the preceding ISI during ongoing irregular stimulation. Tuning curves were constructed only from steady-state PSPs, discarding the first few responses from rest. We computed the slope of the tuning curve by linear regression over the range of intervals up to 218 ms. This tuning curve slope (TCS) provided a simple measure of whether a connection tended to respond more to shorter or to longer ISIs. Connections with smaller responses to short intervals (i.e., to high instantaneous frequencies) had positive TCS, while connections with larger responses to short intervals had negative TCS. This simple quantification of response tuning disregards effects on timescales longer than a single ISI. We also computed each connection's relative response upon transitioning from stimulation at a constant frequency to irregular stimulation at a higher frequency, hereafter referred to as “facilitation index”. To obtain the facilitation index, we took the ratio of the average PSP amplitude evoked after the first two intervals after the switch from regular to irregular stimulation, to the steady-state PSP amplitude just before the switch. The resulting index was <1 when the mean response amplitude was reduced upon transitioning to higher-frequency irregular stimulation, and >1 when amplitude was increased. The facilitation index was potentially sensitive to timescales longer than a single ISI; its goal was to quantify the degree of context-dependent facilitation or depression during ongoing stimulation.

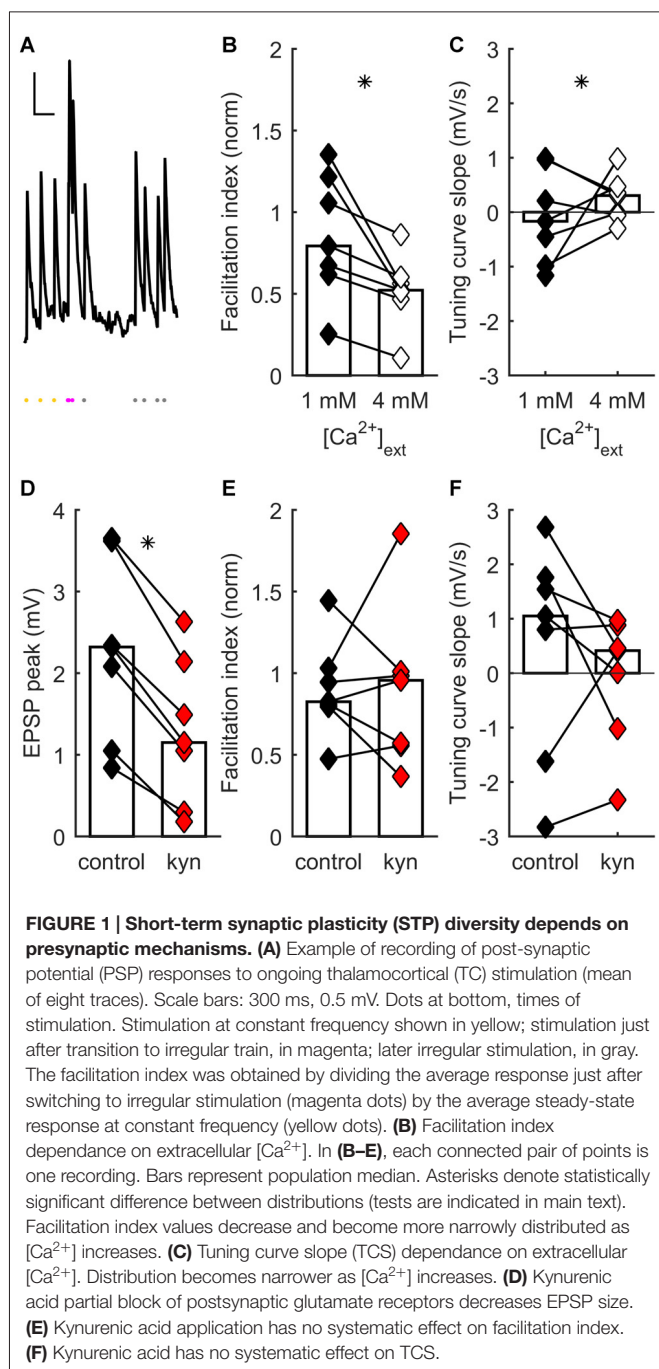
All analyses were conducted in Matlab (The Mathworks).

RESULTS

STP During Ongoing Stimulation Depends on Presynaptic Mechanisms

To search for mechanisms regulating differences in STP across TC synapses, we performed whole-cell patch clamp recordings of postsynaptic potentials (PSPs) from visually identified regular spiking neurons located in layer 4 of mouse TC slices (Figure 1A).

The observed diversity of STP across different synapses could result from the differential contribution of disynaptic inhibition to the overall synaptic response. Responses of layer 4 neurons to TC stimulation have a strong disynaptic inhibitory component (Agmon and Connors, 1991; Porter et al., 2001; Gabernet et al., 2005; Wilent and Contreras, 2005; Sun et al., 2006; Cruikshank et al., 2007; Daw et al., 2007). An observed short-term facilitation



of the PSP response could potentially arise from faster short-term depression of the inhibitory component relative to the excitatory component (Beierlein et al., 2003; Gabernet et al., 2005; Higley and Contreras, 2006; Heiss et al., 2008). However, in previous work we showed that disynaptic inhibition is not necessary for STP diversity by conducting experiments under intracellular blockade of GABA_A receptors: a similar range of behaviors, encompassing depression to facilitation, is found in recordings with intact inhibition and recordings where GABAergic inputs are blocked (Díaz-Quesada et al., 2014). Thus monosynaptic TC

connections to cortical layer 4 display STP diversity, which must originate in differences in the properties of those connections.

STP diversity could be pre- or postsynaptically regulated. We conducted a series of experiments to establish the locus of regulation, as follows. Earlier results had shown that extracellular $[Ca^{2+}]$ influences STP, because there was significantly less depression at $[Ca^{2+}] = 1$ mM than at 2 mM (Díaz-Quesada et al., 2014). This suggested a presynaptic locus for STP regulation (Zucker and Regehr, 2002; Fioravante and Regehr, 2011). We reasoned that, if the main locus is presynaptic, saturating presynaptic terminals with a much higher extracellular $[Ca^{2+}]$ (4 mM) should further bias results towards depression, possibly decreasing STP variability across the recorded population. To test this, we recorded TC synaptic responses in a set of neurons while switching extracellular $[Ca^{2+}]$ from 1 to 4 mM (see “Materials and Methods” Section). As expected, increasing $[Ca^{2+}]$ to 4 mM induced an increase in onset PSP response (because of an enhanced initial probability of neurotransmitter release) followed by faster depression. This was reflected in a significant change towards lower values of the facilitation index ($p = 0.016$, Wilcoxon signed-rank test, $n = 7$ recordings; **Figure 1B**). Moreover there was a significant reduction in the heterogeneity of STP across neurons, such that synapses became depressing ($p = 0.0026$, 2-dimensional Kolmogorov-Smirnov two-sample test, $n = 7$; **Figures 1B,C**). These results show that STP diversity is influenced by $[Ca^{2+}]$ in a manner consistent with presynaptic regulation of neurotransmitter release.

As well as presynaptic mechanisms, did postsynaptic mechanisms play a significant role in setting each connection's effective STP? One such contribution could come from differences across synapses in postsynaptic summation: for example, broader PSPs might lead to greater effective facilitation. However, differences in PSP width do not influence a connection's facilitation index and thus do not contribute to STP diversity (Díaz-Quesada et al., 2014). We decided to test specifically for an effect of differences in glutamate receptor activation on STP. We reasoned that any effects of NMDA receptor (NMDAR)-mediated summation, or of modulation in postsynaptic receptor activation (e.g., in saturation or desensitization), would be reduced as a result of partial receptor blockade. We thus partially blocked ionotropic glutamate receptors by adding kynurenic acid to the extracellular ACSF (Elmslie and Yoshikami, 1985). At a concentration of 150 μ M, kynurenic acid significantly decreased steady-state PSP magnitude ($p = 0.016$, Wilcoxon signed-rank test, $n = 7$; **Figure 1D**), consistent with a reduced postsynaptic response to neurotransmitter release. However, kynurenic acid had no effect either on facilitation index ($p = 0.94$, Wilcoxon signed-rank test, $n = 7$; **Figure 1E**) or on TCS ($p = 0.38$, Wilcoxon signed-rank test, $n = 7$; **Figure 1F**). Thus, STP diversity remained unaffected by manipulation of postsynaptic ionotropic glutamate receptors.

STP is Regulated by Presynaptic Adenosine Receptor Activation

Which presynaptic mechanisms could modulate STP differentially across synapses? Several mechanisms in the

pathway leading from Ca^{2+} entry to neurotransmitter release could potentially contribute. One prominent mechanism modulating synaptic release involves the action of local neurotransmitters through presynaptically expressed receptors (Zucker and Regehr, 2002). We hypothesized that one or several such types of release modulation could contribute to the regulation of STP.

Adenosine reduces synaptic excitation through the action of presynaptic receptors that inhibit glutamate release (Lupica et al., 1992; Scanziani et al., 1992; Shen and Johnson, 2003; Nicoll and Schmitz, 2005). In TC synapses, application of adenosine decreases EPSCs and increases paired-pulse facilitation (Fontanez and Porter, 2006). To test whether release probability and STP in TC connections can be differentially modulated by adenosine receptors, we recorded responses to TC stimulation before and after adding 100 μM of adenosine to the bath. Consistent with a presynaptic site of action, application of adenosine significantly increased paired-pulse ratios ($p = 0.024$, Wilcoxon signed-rank test, $n = 11$; **Figure 2A**) but caused no significant change in membrane potential ($p = 0.24$, Wilcoxon signed-rank test, $n = 11$; median depolarization 0.97 mV). Adenosine increased the facilitation index during ongoing stimulation ($p = 0.0049$, Wilcoxon signed-rank test, $n = 11$; **Figure 2B**), shifting the distribution of STP behaviors expressed in the data set. Median TCS was unchanged ($p = 0.97$, Wilcoxon signed-rank test, $n = 11$; **Figure 2C**), but variability in this parameter decreased ($p = 0.0009$, F -test). This decrease in variability was accounted for by the subset of synapses which, in the absence of added adenosine, had the highest release probability and depressed most strongly: only this minority of synapses had their release probability significantly dampened by adenosine. The dissociation of effects on facilitation index and TCS indicates that adenosine tonically downregulated release probability across the range of intervals (Moore et al., 2003), because the slope relating probability to interval duration was unchanged for the majority of synapses.

Which receptor mediated the modulatory action of adenosine? A1 receptors appear to underpin the inhibitory effects of adenosine on glutamate release (Wu and Saggau, 1994; Dunwiddie and Masino, 2001), including in TC synapses (Fontanez and Porter, 2006). We evaluated the impact of A1 receptors on STP by using the antagonists DPCPX and CPT. We recorded responses to TC stimulation in control ACSF and after addition of 1 μM DPCPX or, in a separate set of experiments, 2 μM CPT. Inhibiting A1 receptors caused a significant change in TCS towards more positive values: i.e., synapses became relatively more responsive to longer rather than shorter intervals (DPCPX: $p = 0.0011$, Wilcoxon signed-rank test, $n = 16$; CPT: $p = 0.0078$, Wilcoxon signed-rank test, $n = 6$; **Figure 2E**). We interpret this as indicating that A1 receptor inhibition prevented adenosine from limiting glutamate release, leading to a greater tendency towards depression, and more substantial recovery after longer intervals. Conversely, neither DPCPX nor CPT caused a significant difference in facilitation index (DPCPX: $p = 0.61$, Wilcoxon signed-rank test, $n = 16$; CPT: $p = 0.078$, Wilcoxon signed-rank test, $n = 6$;

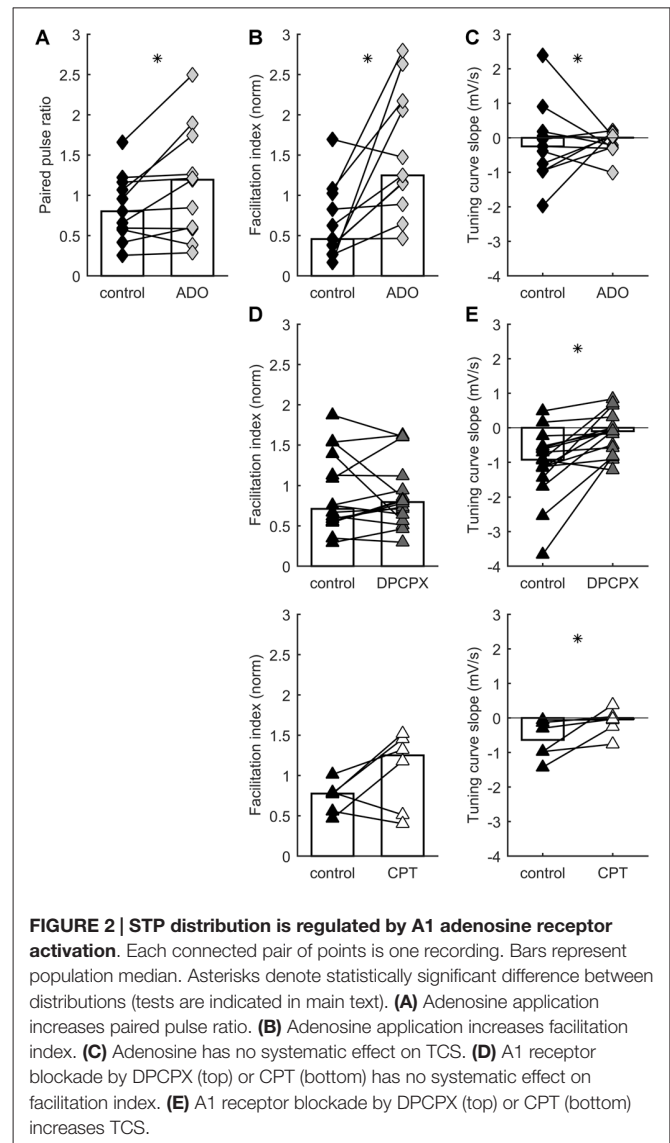


Figure 2D). These effects again shifted the distribution of STP behaviors as compared to control conditions, but did so in the opposite direction to the experiments described above involving application of adenosine. In conclusion, activation of A1 adenosine receptors modulates STP of TC synapses.

Absence of Evidence for a Role of Kainate Receptors in Regulation of STP

Our experiments demonstrated that activation and manipulation of A1 receptors shifts STP behavior but does not eliminate its diversity. Thus, multiple mechanisms act together to determine STP in each synapse. A possible additional mechanism for regulating STP through neurotransmitter action is modulation by presynaptic KARs. KARs can be powerful presynaptic regulators of synaptic efficacy and STP (Lerma and Marques, 2013). In developing TC synapses, KARs containing GluK1–3 subunits are expressed presynaptically

and regulate neurotransmission (Kidd et al., 2002; Urbano and Lerma, 2006; Jouhanneau et al., 2011). We therefore hypothesized that KARs could help modulate release probability and set the level of STP. To test this idea, we compared STP of responses to TC stimulation in layer 4 neurons before and after application of either the selective GluK1 antagonist UBP 310 (10 μ M) or the GluK2 antagonist NS-102 (20 μ M). We found no consistent effect of UBP 310 across recordings, suggesting that the operation of GluK1 receptors does not systematically modulate STP in TC synapses ($p = 0.57$ for facilitation index, $p = 0.73$ for TCS, Wilcoxon signed-rank test, $n = 9$; **Figure 3A**). Similarly, we found no consistent effect of NS-102 application ($p = 0.57$ for facilitation index, $p = 0.054$ for TCS, Wilcoxon signed-rank test, $n = 9$; **Figure 3B**). In conclusion, these experiments found no consistent evidence for a role of these receptor subunits in regulating STP under ongoing stimulation.

DISCUSSION

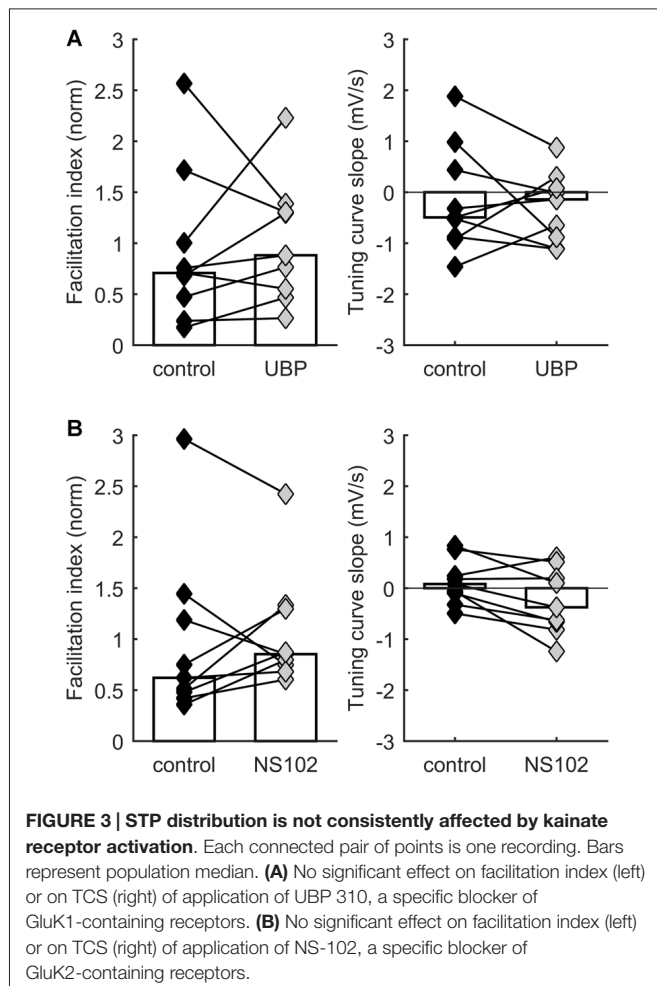
Recent work from our laboratory has shown that TC connections do not constitute a population with uniform dynamics. Rather, lemniscal TC connections onto layer 4 spiny stellate neurons

respond with diverse STP when stimuli arrive against a background of ongoing activity (Díaz-Quesada et al., 2014). A continuum of STP behaviors is represented across the population. This heterogeneity implies that each synapse is preferentially tuned to certain stimulation intervals, and potentially allows TC pathways to convey rich information about temporal patterns at the population level (Abbott and Regehr, 2004; Buonomano and Maass, 2009; Lee and Buonomano, 2012; David and Shamma, 2013; Chabrol et al., 2015). For example, TC synapses with specific dynamics could act as channels preferentially conveying certain information (e.g., facilitating synapses could act as burst detectors and depressing synapses as “wake-up” signals). Here, we addressed the mechanisms that underpin the diversity of STP. We found evidence for presynaptic regulation of diversity. Modulation of A1 adenosine receptor activation shifts the STP set point.

Many presynaptic mechanisms mediate the complex process from Ca^{2+} entry to exocytosis (Zucker and Regehr, 2002; Fioravante and Regehr, 2011). Diversity of STP within a single population of synapses indicates variability in the expression of this machinery. We found that activation and manipulation of A1 receptors shifts STP behavior but does not eliminate its diversity across the synaptic population (Kerr et al., 2013). This implies that several mechanisms act together to set the overall type and level of STP for each synapse. In our earlier results, the progression of STP during stimulation from rest comprised an initial phase dominated by depression, superimposed with steady-state behavior that could incorporate a varying degree of facilitation (Díaz-Quesada et al., 2014); accounting for this behavior requires phenomena beyond uniform resource depletion (Beck et al., 2005; Kandaswamy et al., 2010; Müller et al., 2010; Hennig, 2013).

We have explored one additional potential mechanism for regulating STP—modulation by KARs. Presynaptically expressed KARs regulate synaptic efficacy (Lerma and Marques, 2013) and are expressed in TC synapses (Kidd et al., 2002; Urbano and Lerma, 2006; Jouhanneau et al., 2011). However, our recordings in the presence of subunit-specific pharmacological blockers of GluK1 and GluK2 failed to find evidence for a role of these receptor subunits in STP under ongoing stimulation. We also performed experiments in slices from knock-out mice for the GluK1 subunit and double knock-outs for the GluK1 and GluK2 subunits (Mulle et al., 1998, 2000; courtesy of Juan Lerma laboratory): these also failed to find a shift in STP behavior across TC synapses (data not shown).

Key questions for future work concern the impact of different mechanisms linking Ca^{2+} entry to triggering of release. STP diversity could result from dynamical regulation of STP at each synapse (Sippy et al., 2003; Cheetham et al., 2007; Branco et al., 2008; Pfister et al., 2010; Carvalho and Buonomano, 2011; Yang and Xu-Friedman, 2012). Multiple steps in the pathway from action potential to exocytosis can be subject to modulation (terminal size; Ca^{2+} influx, distribution and sensing; determination of vesicle availability and fusion; e.g., Sippy et al., 2003; Mochida et al., 2008; Welzel et al., 2011;



Zhao et al., 2011; Ermolyuk et al., 2012; Leal et al., 2012; Sylwestrak and Ghosh, 2012; Baden et al., 2014; Fioravante et al., 2014; Calloway et al., 2015; Körber et al., 2015; reviewed in Branco and Staras, 2009; Fioravante and Regehr, 2011; de Jong and Fioravante, 2014). Moreover, additional mechanisms sited postsynaptically as well as presynaptically, and not ruled out by the present study (e.g., metabotropic glutamate receptors, GABA_B receptors) could also help tune each connection's particular STP behavior. Is diversity essentially random (Ribault et al., 2011), or regulated via modulation of a specific subset of mechanisms? Are these parameters regulated locally at the synapse level or are they set at a cell-wide level (Armbruster and Ryan, 2011; Ermolyuk et al., 2012; Ariel et al., 2013)? Supporting the latter possibility, there is evidence from other pathways that STP properties cluster postsynaptically, i.e., different synapses onto the same neuron can share STP of a similar nature (Branco et al., 2008; Yang and Xu-Friedman, 2012), consistent with postsynaptic regulation by the target cell (Blackman et al., 2013). However, in our present TC data set, preliminary analysis shows no evidence of postsynaptic clustering (data not shown).

REFERENCES

- Abbott, L. F., and Regehr, W. G. (2004). Synaptic computation. *Nature* 431, 796–803. doi: 10.1038/nature03010
- Agmon, A., and Connors, B. W. (1991). Thalamocortical responses of mouse somatosensory (barrel) cortex *in vitro*. *Neuroscience* 41, 365–379. doi: 10.1016/0306-4522(91)90333-j
- Ariel, P., Hoppa, M. B., and Ryan, T. A. (2013). Intrinsic variability in Pv, RRP size, Ca²⁺ channel repertoire and presynaptic potentiation in individual synaptic boutons. *Front. Synaptic Neurosci.* 4:9. doi: 10.3389/fnsyn.2012.00009
- Armbruster, M., and Ryan, T. A. (2011). Synaptic vesicle retrieval time is a cell-wide rather than individual-synapse property. *Nat. Neurosci.* 14, 824–826. doi: 10.1038/nn.2828
- Baden, T., Nikolaev, A., Esposti, F., Dreosti, E., Odermatt, B., and Lagnado, L. (2014). A synaptic mechanism for temporal filtering of visual signals. *PLoS Biol.* 12:e1001972. doi: 10.1371/journal.pbio.1001972
- Bale, M. R., Ince, R. A., Santagata, G., and Petersen, R. S. (2015). Efficient population coding of naturalistic whisker motion in the ventro-posterior medial thalamus based on precise spike timing. *Front. Neural Circuits* 9:50. doi: 10.3389/fncir.2015.00050
- Beck, O., Chistiakova, M., Obermayer, K., and Volgushev, M. (2005). Adaptation at synaptic connections to layer 2/3 pyramidal cells in rat visual cortex. *J. Neurophysiol.* 94, 363–376. doi: 10.1152/jn.01287.2004
- Beierlein, M., Gibson, J. R., and Connors, B. W. (2003). Two dynamically distinct inhibitory networks in layer 4 of the neocortex. *J. Neurophysiol.* 90, 2987–3000. doi: 10.1152/jn.00283.2003
- Blackman, A. V., Abrahamsson, T., Costa, R. P., Lalanne, T., and Sjöström, P. J. (2013). Target-cell-specific short-term plasticity in local circuits. *Front. Synaptic Neurosci.* 5:11. doi: 10.3389/fnsyn.2013.00011
- Borgdorff, A. J., Poulet, J. F., and Petersen, C. C. (2007). Facilitating sensory responses in developing mouse somatosensory barrel cortex. *J. Neurophysiol.* 97, 2992–3003. doi: 10.1152/jn.00013.2007
- Boudreau, C. E., and Ferster, D. (2005). Short-term depression in thalamocortical synapses of cat primary visual cortex. *J. Neurosci.* 25, 7179–7190. doi: 10.1523/JNEUROSCI.1445-05.2005
- Branco, T., and Staras, K. (2009). The probability of neurotransmitter release: variability and feedback control at single synapses. *Nat. Rev. Neurosci.* 10, 373–383. doi: 10.1038/nrn2634
- Branco, T., Staras, K., Darcy, K. J., and Goda, Y. (2008). Local dendritic activity sets release probability at hippocampal synapses. *Neuron* 59, 475–485. doi: 10.1016/j.neuron.2008.07.006
- Bruno, R. M., and Sakmann, B. (2006). Cortex is driven by weak but synchronously active thalamocortical synapses. *Science* 312, 1622–1627. doi: 10.1126/science.1124593
- Buonomano, D. V., and Maass, W. (2009). State-dependent computations: spatiotemporal processing in cortical networks. *Nat. Rev. Neurosci.* 10, 113–125. doi: 10.1038/nrn2558
- Calloway, N., Gouzer, G., Xue, M., and Ryan, T. A. (2015). The active-zone protein Munc13 controls the use-dependence of presynaptic voltage-gated calcium channels. *elife* 4:e07728. doi: 10.7554/elife.07728
- Carvalho, T. P., and Buonomano, D. V. (2011). A novel learning rule for long-term plasticity of short-term synaptic plasticity enhances temporal processing. *Front. Integr. Neurosci.* 5:20. doi: 10.3389/fnint.2011.00020
- Castro-Alamancos, M. A. (2004). Absence of rapid sensory adaptation in neocortex during information processing states. *Neuron* 41, 455–464. doi: 10.1016/s0896-6273(03)00853-5
- Castro-Alamancos, M. A., and Oldford, E. (2002). Cortical sensory suppression during arousal is due to the activity-dependent depression of thalamocortical synapses. *J. Physiol.* 541, 319–331. doi: 10.1113/jphysiol.2002.016857
- Chabrol, F. P., Arenz, A., Wiechert, M. T., Margrie, T. W., and DiGregorio, D. A. (2015). Synaptic diversity enables temporal coding of coincident multisensory inputs in single neurons. *Nat. Neurosci.* 18, 718–727. doi: 10.1038/nn.3974
- Cheetham, C. E., Hammond, M. S., Edwards, C. E., and Finnerty, G. T. (2007). Sensory experience alters cortical connectivity and synaptic function site specifically. *J. Neurosci.* 27, 3456–3465. doi: 10.1523/JNEUROSCI.5143-06.2007
- Chung, S., Li, X., and Nelson, S. B. (2002). Short-term depression at thalamocortical synapses contributes to rapid adaptation of cortical sensory responses *in vivo*. *Neuron* 34, 437–446. doi: 10.1016/s0896-6273(02)00659-1
- Covic, E. N., and Sherman, S. M. (2011). Synaptic properties of connections between the primary and secondary auditory cortices in mice. *Cereb. Cortex* 21, 2425–2441. doi: 10.1093/cercor/bhr029
- Crair, M. C., and Malenka, R. C. (1995). A critical period for long-term potentiation at thalamocortical synapses. *Nature* 375, 325–328. doi: 10.1038/375325a0
- Cruikshank, S. J., Lewis, T. J., and Connors, B. W. (2007). Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. *Nat. Neurosci.* 10, 462–468. doi: 10.1038/nn1861
- Crunelli, V., David, F., Lorincz, M. L., and Hughes, S. W. (2015). The thalamocortical network as a single slow wave-generating unit. *Curr. Opin. Neurobiol.* 31, 72–80. doi: 10.1016/j.conb.2014.09.001

AUTHOR CONTRIBUTIONS

GF and FJM performed experiments. FJM and MM wrote code for data analysis. GF, FJM and MM analyzed and interpreted the data. MM drafted the article; all authors read and approved the final manuscript.

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- David, S. V., and Shamma, S. A. (2013). Integration over multiple timescales in primary auditory cortex. *J. Neurosci.* 33, 19154–19166. doi: 10.1523/JNEUROSCI.2270-13.2013
- Daw, M. I., Ashby, M. C., and Isaac, J. T. (2007). Coordinated developmental recruitment of latent fast spiking interneurons in layer IV barrel cortex. *Nat. Neurosci.* 10, 453–461. doi: 10.1038/nn1866
- de Jong, A. P., and Fioravante, D. (2014). Translating neuronal activity at the synapse: presynaptic calcium sensors in short-term plasticity. *Front. Cell. Neurosci.* 8:356. doi: 10.3389/fncel.2014.00356
- Díaz-Quesada, M., and Maravall, M. (2008). Intrinsic mechanisms for adaptive gain rescaling in barrel cortex. *J. Neurosci.* 28, 696–710. doi: 10.1523/JNEUROSCI.4931-07.2008
- Díaz-Quesada, M., Martini, F. J., Ferrati, G., Bureau, I., and Maravall, M. (2014). Diverse thalamocortical short-term plasticity elicited by ongoing stimulation. *J. Neurosci.* 34, 515–526. doi: 10.1523/JNEUROSCI.2441-13.2014
- Dudek, S. M., and Friedlander, M. J. (1996). Intracellular blockade of inhibitory synaptic responses in visual cortical layer IV neurons. *J. Neurophysiol.* 75, 2167–2173.
- Dunwiddie, T. V., and Masino, S. A. (2001). The role and regulation of adenosine in the central nervous system. *Annu. Rev. Neurosci.* 24, 31–55. doi: 10.1146/annurev.neuro.24.1.31
- Elmslie, K. S., and Yoshikami, D. (1985). Effects of kynurenate on root potentials evoked by synaptic activity and amino acids in the frog spinal cord. *Brain Res.* 330, 265–272. doi: 10.1016/0006-8993(85)90685-7
- Ermolyuk, Y. S., Alder, F. G., Henneberger, C., Rusakov, D. A., Kullmann, D. M., and Volynski, K. E. (2012). Independent regulation of basal neurotransmitter release efficacy by variable Ca^{2+} influx and bouton size at small central synapses. *PLoS Biol.* 10:e1001396. doi: 10.1371/journal.pbio.1001396
- Fioravante, D., Chu, Y., De Jong, A. P., Leitges, M., Kaeser, P. S., and Regehr, W. G. (2014). Protein kinase C is a calcium sensor for presynaptic short-term plasticity. *elife* 3:e03011. doi: 10.7554/eLife.03011
- Fioravante, D., and Regehr, W. G. (2011). Short-term forms of presynaptic plasticity. *Curr. Opin. Neurobiol.* 21, 269–274. doi: 10.1016/j.conb.2011.02.003
- Fontanez, D. E., and Porter, J. T. (2006). Adenosine A1 receptors decrease thalamic excitation of inhibitory and excitatory neurons in the barrel cortex. *Neuroscience* 137, 1177–1184. doi: 10.1016/j.neuroscience.2005.10.022
- Fortune, E. S., and Rose, G. J. (2001). Short-term synaptic plasticity as a temporal filter. *Trends Neurosci.* 24, 381–385. doi: 10.1016/s0166-2236(00)01835-x
- Gabernet, L., Jadhav, S. P., Feldman, D. E., Carandini, M., and Scanziani, M. (2005). Somatosensory integration controlled by dynamic thalamocortical feed-forward inhibition. *Neuron* 48, 315–327. doi: 10.1016/j.neuron.2005.09.022
- Gil, Z., Connors, B. W., and Amitai, Y. (1997). Differential regulation of neocortical synapses by neuromodulators and activity. *Neuron* 19, 679–686. doi: 10.1016/s0896-6273(00)80380-3
- Gil, Z., Connors, B. W., and Amitai, Y. (1999). Efficacy of thalamocortical and intracortical synaptic connections: quanta, innervation and reliability. *Neuron* 23, 385–397. doi: 10.1016/s0896-6273(00)80788-6
- Heiss, J. E., Katz, Y., Ganmor, E., and Lampl, I. (2008). Shift in the balance between excitation and inhibition during sensory adaptation of S1 neurons. *J. Neurosci.* 28, 13320–13330. doi: 10.1523/JNEUROSCI.2646-08.2008
- Hennig, M. H. (2013). Theoretical models of synaptic short term plasticity. *Front. Comput. Neurosci.* 7:154. doi: 10.3389/fncom.2013.00154
- Higley, M. J., and Contreras, D. (2006). Balanced excitation and inhibition determine spike timing during frequency adaptation. *J. Neurosci.* 26, 448–457. doi: 10.1523/JNEUROSCI.3506-05.2006
- Jouhanneau, J. S., Ball, S. M., Molnár, E., and Isaac, J. T. (2011). Mechanisms of bi-directional modulation of thalamocortical transmission in barrel cortex by presynaptic kainate receptors. *Neuropharmacology* 60, 832–841. doi: 10.1016/j.neuropharm.2010.12.023
- Kandaswamy, U., Deng, P. Y., Stevens, C. F., and Klyachko, V. A. (2010). The role of presynaptic dynamics in processing of natural spike trains in hippocampal synapses. *J. Neurosci.* 30, 15904–15914. doi: 10.1523/JNEUROSCI.4050-10.2010
- Kerr, M. I., Wall, M. J., and Richardson, M. J. (2013). Adenosine A1 receptor activation mediates the developmental shift at layer 5 pyramidal cell synapses and is a determinant of mature synaptic strength. *J. Physiol.* 591, 3371–3380. doi: 10.1113/jphysiol.2012.244392
- Kidd, F. L., Coumis, U., Collingridge, G. L., Crabtree, J. W., and Isaac, J. T. (2002). A presynaptic kainate receptor is involved in regulating the dynamic properties of thalamocortical synapses during development. *Neuron* 34, 635–646. doi: 10.1016/s0896-6273(02)00699-2
- Körber, C., Horstmann, H., Venkataramani, V., Herrmannsdorfer, F., Kremer, T., Kaiser, M., et al. (2015). Modulation of presynaptic release probability by the vertebrate-specific protein mover. *Neuron* 87, 521–533. doi: 10.1016/j.neuron.2015.07.001
- Leal, K., Mochida, S., Scheuer, T., and Catterall, W. A. (2012). Fine-tuning synaptic plasticity by modulation of $\text{Ca}_v2.1$ channels with Ca^{2+} sensor proteins. *Proc. Natl. Acad. Sci. U S A* 109, 17069–17074. doi: 10.1073/pnas.1215172109
- Lee, T. P., and Buonomano, D. V. (2012). Unsupervised formation of vocalization-sensitive neurons: a cortical model based on short-term and homeostatic plasticity. *Neural Comput.* 24, 2579–2603. doi: 10.1162/NECO_a_00345
- Lee, C. C., and Sherman, S. M. (2008). Synaptic properties of thalamic and intracortical inputs to layer 4 of the first- and higher-order cortical areas in the auditory and somatosensory systems. *J. Neurophysiol.* 100, 317–326. doi: 10.1152/jn.90391.2008
- Lerma, J., and Marques, J. M. (2013). Kainate receptors in health and disease. *Neuron* 80, 292–311. doi: 10.1016/j.neuron.2013.09.045
- Lupica, C. R., Proctor, W. R., and Dunwiddie, T. V. (1992). Presynaptic inhibition of excitatory synaptic transmission by adenosine in rat hippocampus: analysis of unitary EPSP variance measured by whole-cell recording. *J. Neurosci.* 12, 3753–3764.
- McCormick, D. A., Connors, B. W., Lighthall, J. W., and Prince, D. A. (1985). Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. *J. Neurophysiol.* 54, 782–806.
- McCormick, D. A., Mcginley, M. J., and Salkoff, D. B. (2015). Brain state dependent activity in the cortex and thalamus. *Curr. Opin. Neurobiol.* 31, 133–140. doi: 10.1016/j.conb.2014.10.003
- Mochida, S., Few, A. P., Scheuer, T., and Catterall, W. A. (2008). Regulation of presynaptic $\text{Ca}_v2.1$ channels by Ca^{2+} sensor proteins mediates short-term synaptic plasticity. *Neuron* 57, 210–216. doi: 10.1016/j.neuron.2007.11.036
- Moore, K. A., Nicoll, R. A., and Schmitz, D. (2003). Adenosine gates synaptic plasticity at hippocampal mossy fiber synapses. *Proc. Natl. Acad. Sci. U S A* 100, 14397–14402. doi: 10.1073/pnas.1835831100
- Mulle, C., Sailer, A., Perez-Otano, I., Dickinson-Anson, H., Castillo, P. E., Bureau, I., et al. (1998). Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. *Nature* 392, 601–605. doi: 10.1038/33408
- Mulle, C., Sailer, A., Swanson, G. T., Brana, C., O’Gorman, S., Bettler, B., et al. (2000). Subunit composition of kainate receptors in hippocampal interneurons. *Neuron* 28, 475–484. doi: 10.1016/s0896-6273(00)00126-4
- Müller, M., Goutman, J. D., Kochubey, O., and Schneggenburger, R. (2010). Interaction between facilitation and depression at a large CNS synapse reveals mechanisms of short-term plasticity. *J. Neurosci.* 30, 2007–2016. doi: 10.1523/JNEUROSCI.4378-09.2010
- Nicoll, R. A., and Schmitz, D. (2005). Synaptic plasticity at hippocampal mossy fibre synapses. *Nat. Rev. Neurosci.* 6, 863–876. doi: 10.1038/nrn1786
- Ollerenshaw, D. R., Zheng, H. J., Millard, D. C., Wang, Q., and Stanley, G. B. (2014). The adaptive trade-off between detection and discrimination in cortical representations and behavior. *Neuron* 81, 1152–1164. doi: 10.1016/j.neuron.2014.01.025
- Pfister, J. P., Dayan, P., and Lengyel, M. (2010). Synapses with short-term plasticity are optimal estimators of presynaptic membrane potentials. *Nat. Neurosci.* 13, 1271–1275. doi: 10.1038/nn.2640
- Pologruto, T. A., Sabatini, B. L., and Svoboda, K. (2003). ScanImage: flexible software for operating laser scanning microscopes. *Biomed. Eng. Online* 2:13. doi: 10.1186/1475-925X-2-13
- Porter, J. T., Johnson, C. K., and Agmon, A. (2001). Diverse types of interneurons generate thalamus-evoked feedforward inhibition in the mouse barrel cortex. *J. Neurosci.* 21, 2699–2710.
- Poulet, J. F., Fernandez, L. M., Crochet, S., and Petersen, C. C. (2012). Thalamic control of cortical states. *Nat. Neurosci.* 15, 370–372. doi: 10.1038/nn.3035
- Reig, R., Gallego, R., Nowak, L. G., and Sanchez-Vives, M. V. (2006). Impact of cortical network activity on short-term synaptic depression. *Cereb. Cortex* 16, 688–695. doi: 10.1093/cercor/bhj014

- Ribault, C., Sekimoto, K., and Triller, A. (2011). From the stochasticity of molecular processes to the variability of synaptic transmission. *Nat. Rev. Neurosci.* 12, 375–387. doi: 10.1038/nrn3025
- Rose, H. J., and Metherate, R. (2001). Thalamic stimulation largely elicits orthodromic, rather than antidromic, cortical activation in an auditory thalamocortical slice. *Neuroscience* 106, 331–340. doi: 10.1016/s0306-4522(01)00284-2
- Scanziani, M., Capogna, M., Gähwiler, B. H., and Thompson, S. M. (1992). Presynaptic inhibition of miniature excitatory synaptic currents by baclofen and adenosine in the hippocampus. *Neuron* 9, 919–927. doi: 10.1016/0896-6273(92)90244-8
- Shen, K. Z., and Johnson, S. W. (2003). Presynaptic inhibition of synaptic transmission by adenosine in rat subthalamic nucleus *in vitro*. *Neuroscience* 116, 99–106. doi: 10.1016/s0306-4522(02)00656-5
- Sherman, S. M., and Guillery, R. W. (2006). *Exploring the Thalamus and its Role in Cortical Function*. Cambridge, MA: MIT Press.
- Sippy, T., Cruz-Martin, A., Jeromin, A., and Schweizer, F. E. (2003). Acute changes in short-term plasticity at synapses with elevated levels of neuronal calcium sensor-1. *Nat. Neurosci.* 6, 1031–1038. doi: 10.1038/nn1117
- Slézia, A., Hangya, B., Ulbert, I., and Acsády, L. (2011). Phase advancement and nucleus-specific timing of thalamocortical activity during slow cortical oscillation. *J. Neurosci.* 31, 607–617. doi: 10.1523/JNEUROSCI.3375-10.2011
- Stratford, K. J., Tarczy-Hornoch, K., Martin, K. A., Bannister, N. J., and Jack, J. J. (1996). Excitatory synaptic inputs to spiny stellate cells in cat visual cortex. *Nature* 382, 258–261. doi: 10.1038/382258a0
- Sun, Q. Q., Huguenard, J. R., and Prince, D. A. (2006). Barrel cortex microcircuits: thalamocortical feedforward inhibition in spiny stellate cells is mediated by a small number of fast-spiking interneurons. *J. Neurosci.* 26, 1219–1230. doi: 10.1523/JNEUROSCI.4727-04.2006
- Sylwestrak, E. L., and Ghosh, A. (2012). Elfn1 regulates target-specific release probability at CA1-interneuron synapses. *Science* 338, 536–540. doi: 10.1126/science.1222482
- Urbain, N., Salin, P. A., Libourel, P. A., Comte, J. C., Gentet, L. J., and Petersen, C. C. (2015). Whisking-related changes in neuronal firing and membrane potential dynamics in the somatosensory thalamus of awake mice. *Cell Rep.* 13, 647–656. doi: 10.1016/j.celrep.2015.09.029
- Urbano, F., and Lerma, J. (2006). “Tonic activity of presynaptic receptors modulates synaptic transfer at thalamocortical synapses”, in *Poster at FENS Forum Abstract, Vienna*.
- Viaene, A. N., Petrof, I., and Sherman, S. M. (2011). Synaptic properties of thalamic input to layers 2/3 and 4 of primary somatosensory and auditory cortices. *J. Neurophysiol.* 105, 279–292. doi: 10.1152/jn.00747.2010
- Welzel, O., Henkel, A. W., Stroebel, A. M., Jung, J., Tischbirek, C. H., Ebert, K., et al. (2011). Systematic heterogeneity of fractional vesicle pool sizes and release rates of hippocampal synapses. *Biophys. J.* 100, 593–601. doi: 10.1016/j.bpj.2010.12.3706
- Wilent, W. B., and Contreras, D. (2005). Dynamics of excitation and inhibition underlying stimulus selectivity in rat somatosensory cortex. *Nat. Neurosci.* 8, 1364–1370. doi: 10.1038/nn1545
- Wu, L. G., and Saggau, P. (1994). Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. *Neuron* 12, 1139–1148. doi: 10.1016/0896-6273(94)90321-2
- Yang, H., and Xu-Friedman, M. A. (2012). Emergence of coordinated plasticity in the cochlear nucleus and cerebellum. *J. Neurosci.* 32, 7862–7868. doi: 10.1523/JNEUROSCI.0167-12.2012
- Zhao, C., Dreosti, E., and Lagnado, L. (2011). Homeostatic synaptic plasticity through changes in presynaptic calcium influx. *J. Neurosci.* 31, 7492–7496. doi: 10.1523/JNEUROSCI.6636-10.2011
- Zucker, R. S., and Regehr, W. G. (2002). Short-term synaptic plasticity. *Annu. Rev. Physiol.* 64, 355–405. doi: 10.1146/annurev.physiol.64.092501.114547

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Dynamic Analysis of the Conditional Oscillator Underlying Slow Waves in Thalamocortical Neurons

François David^{1, 2, 3, 4, 5, 6, 7*}, Vincenzo Crunelli^{1, 8}, Nathalie Leresche^{5, 6, 7} and Régis C. Lambert^{5, 6, 7*}

¹ Neuroscience Division, School of Biosciences, Cardiff University, Cardiff, UK, ² Lyon Neuroscience Research Center, Centre National de la Recherche Scientifique UMR 5292, Lyon, France, ³ Lyon Neuroscience Research Center, Institut National de la Santé et de la Recherche Médicale U1028, Lyon, France, ⁴ Faculté de Médecine, Université Claude Bernard, Lyon, France, ⁵ Sorbonne Universités, UPMC Université Paris 06, UMR 119, Neuroscience Paris Seine, Paris, France, ⁶ Centre National de la Recherche Scientifique, UMR 8246, Neuroscience Paris Seine, Paris, France, ⁷ Institut National de la Santé et de la Recherche Médicale, U1130, Neuroscience Paris Seine, Paris, France, ⁸ Department of Physiology and Biochemistry, University of Malta, Msida, Malta

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Miles A. Whittington,
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Roger D. Traub,
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USA

Heiko J. Luhmann,
Institut für Physiologie und
Pathophysiologie, Germany

*Correspondence:

François David
francois.david@inserm.fr;
Régis C. Lambert
regis.lambert@upmc.fr

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During non-REM sleep the EEG shows characteristic waves that are generated by the dynamic interactions between cortical and thalamic oscillators. In thalamic neurons, low-threshold T-type Ca^{2+} channels play a pivotal role in almost every type of neuronal oscillations, including slow (<1 Hz) waves, sleep spindles and delta waves. The transient opening of T channels gives rise to the low threshold spikes (LTSs), and associated high frequency bursts of action potentials, that are characteristically present during sleep spindles and delta waves, whereas the persistent opening of a small fraction of T channels, (i.e., $I_{T\text{window}}$) is responsible for the membrane potential bistability underlying sleep slow oscillations. Surprisingly thalamocortical (TC) neurons express a very high density of T channels that largely exceed the amount required to generate LTSs and therefore, to support certain, if not all, sleep oscillations. Here, to clarify the relationship between T current density and sleep oscillations, we systematically investigated the impact of the T conductance level on the intrinsic rhythmic activities generated in TC neurons, combining *in vitro* experiments and TC neuron simulation. Using bifurcation analysis, we provide insights into the dynamical processes taking place at the transition between slow and delta oscillations. Our results show that although stable delta oscillations can be evoked with minimal T conductance, the full range of slow oscillation patterns, including groups of delta oscillations separated by *Up* states (“grouped-delta slow waves”) requires a high density of T channels. Moreover, high levels of T conductance ensure the robustness of different types of slow oscillations.

Keywords: thalamus, sleep slow wave, delta waves, T-type calcium channels, bifurcation, computational modeling

INTRODUCTION

Sleep is characterized by the regular appearance of stereotyped sequences of EEG waves (Achermann and Borbély, 1997; Steriade, 2006; Crunelli et al., 2014) that are generated by the dynamic interaction between, and require the integrity of both cortical and thalamic oscillators (Steriade et al., 1993b; Crunelli and Hughes, 2010; David et al., 2013; Lemieux et al., 2014). The various cellular activities that are expressed by thalamocortical (TC) neurons during sleep

oscillations tightly depend on the expression of low-threshold T-type Ca^{2+} channels (T channels; Leresche et al., 1991; Williams et al., 1997a; Crunelli et al., 2014). In fact, while these channels are almost fully inactivated in the range of membrane potentials associated to the wake state (but see Lambert et al., 2014), during non-REM sleep the progressive reduction in the depolarizing tone exerted by modulatory afferents onto both cortical and thalamic neurons (McCormick, 1992) allows T channel de-inactivation. As a consequence, the recruitment of de-inactivated T channels generates large inward currents resulting in transient depolarizations, called low-threshold spike (LTS). Thus, rhythmic LTSs, often crowned by bursts of high-frequency (>200 Hz) action potentials, are present in TC neurons during sleep spindles (7–14 Hz; Steriade et al., 1993b; Contreras and Steriade, 1996; David et al., 2013) and delta waves (0.5–4 Hz; Steriade et al., 1993a), and an LTS is almost invariably present at the start of each *Up* state of sleep slow oscillations in TC neurons (Figure 2A; Steriade et al., 1993a). *Up* states interspersed with periods of hyperpolarization (i.e., *Down* states) are the thalamic cellular hallmarks of sleep slow (<1 Hz) waves (Figure 2A). Moreover, slow waves group together periods of sleep spindle and delta waves (Steriade, 2006), and these periods of delta oscillations that are visible during the *Down* state of the cellular counterpart of sleep slow waves in TC neurons have been named “grouped-delta slow waves” (Figure 2A; Steriade et al., 1993a; Hughes et al., 2002; Crunelli et al., 2015). Importantly, the interaction of the leak current with a small number of de-inactivated T channels opening with a low (but non-zero) probability in a narrow range of membrane potentials around -60 mV (i.e., $I_{T\text{window}}$; Perez-Reyes, 2003; Dreyfus et al., 2010) is necessary for the generation of the membrane potential bistability that in TC neuron underlies the expression of the *Up* and *Down* state dynamics of sleep slow waves (Williams et al., 1997a; Toth et al., 1998; Hughes et al., 2002; Dreyfus et al., 2010).

Despite these key roles for T channels in sleep waves, it is still not known how the density of the T-type Ca^{2+} current (I_T) affects each sleep oscillation. We previously demonstrated that robust LTSs can be evoked even when up to 70% of the T channel population is pharmacologically blocked (Dreyfus et al., 2010), suggesting that the high T channel expression that is present in TC neurons is not required for LTS generation during delta and slow oscillations. A high T channel expression in TC neurons, however, may be crucial to provide a level of $I_{T\text{window}}$ sufficient for the generation of the *Up* and *Down* state dynamics underlying slow oscillations in this type of thalamic neurons.

Here, using both *in vitro* experiments and TC neuron simulation, we systematically investigated the impact of the T conductance level on the various sleep oscillations intrinsically generated in TC neurons. Since I_T can be controlled by various modulatory mechanisms (Lambert et al., 2006; Huc et al., 2009), we also investigated the effects of the ATP- and voltage-dependent regulation that potentiates the amplitude of I_T in sensory TC neurons (Leresche et al., 2004). Our results show that although stable delta oscillations can be evoked with minimal T conductance, the full range of slow oscillation patterns, including simple *Up* and *Down* state transitions and the more complex “grouped-delta slow waves,” requires a high density of T channels

or a potentiation of the current. Moreover, high levels of I_T ensure the robustness of different slow wave oscillations over a larger range of leak conductance values.

MATERIALS AND METHODS

Slice Preparation and Recordings

All procedures involving experimental animals were carried out in accordance with the UK Animals (Scientific Procedure) Act, 1986 and Cardiff Ethical Review Committee guidelines. Thalamic slices from a 3-year old cat were prepared as described previously (Hughes et al., 2002). Briefly, the cat was deeply anesthetized with a mixture of O_2 and NO_2 (2:1) and 5% isoflurane, a wide craniotomy was performed to remove the brain and coronal slices of the thalamus (300–400 μm) that contain the dorsal lateral geniculate nucleus (LGN), were prepared and incubated at 35°C for 1 h before being maintained at room temperature. For recording, slices were perfused with a warmed ($35 \pm 1^\circ\text{C}$) continuously oxygenated (95% O_2 , 5% CO_2) artificial CSF (ACSF) containing the following (in mM): 134 NaCl, 2 KCl, 1.25 KH_2PO_4 , 1 MgSO_4 , 2 CaCl_2 , 16 NaHCO_3 , and 10 glucose.

Intracellular recordings, using the current clamp technique, were performed with standard-wall glass microelectrodes filled with 1 M potassium acetate (resistance, 80–120 M Ω) and connected to an Axoclamp-2A amplifier (Molecular Devices, Sunnyvale, CA) operating in bridge mode. Membrane potentials were digitized at 25 kHz using pClamp 9 (Molecular Devices). All recordings in the LGN were obtained from lamina A. Impaled cells were identified as TC neurons using established criteria (Pirchio et al., 1997; Turner et al., 1997). Sleep oscillations (including slow oscillations <1 Hz) were induced by bath application of 50 μM ($\pm$)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) followed by changes in steady-state current injections to allow neurons to express different slow oscillations, as previously shown (Hughes et al., 2004). SR95531 (gabazine, 10 μM), CGP54626 (20 μM), D-APV (50 μM), and CNQX (10 μM) were included in the bath solution to block both GABA-A and GABA-B as well as NMDA and AMPA glutamatergic synaptic inputs onto TC neurons, respectively. The T channel antagonist, TTA-P2 (kindly provided by Merck Inc, USA), was made up as a 10 mM stock solution in dimethylsulfoxide and kept at -20°C until use at a final concentration of 500 nM.

Simulations

All simulations were performed using the Matlab based programs (Mathworks, Natick, MA) or xppaut continuation application developed by Ermentrout (2002), and were run with a fixed time step of 0.02 ms using the Euler integration method. For simulations, the system was initiated at a point close to the *Up* state and the simulation results were analyzed only after stabilization of the simulation result (i.e., 50 s after the start of the simulation).

The single-compartment TC neuron model based on (Williams et al., 1997a; Hughes et al., 2002), expressed the essential physiological properties of these neurons (Figure 2).

Ionic currents were simulated following Hodgkin-Huxley formalism.

The membrane potential (V) was described by the following equation:

$$C_m dV/dt = -I_{Leak} - I_T - I_{TP} - I_h - I_{CAN} - I_{Na} - I_{Kir},$$

where C_m (50 pF) is the membrane capacitance, I_{Leak} is a potassium leak current (reversal potential = -95 mV), I_T is the T current, I_{TP} is the potentiated component of the T current, I_h is the hyperpolarization-activated nonspecific cationic current, I_{CAN} is the Ca^{2+} activated non-selective cation current, I_{Na} is the voltage-dependent Na^+ current and I_{Kir} is K^+ current which includes the inward and delayed rectifier components. All current units are pA. Each current was simulated as follows:

I_T :

$$I_T = g_T \cdot m^3(V) \cdot h(V) \cdot (V(t) - E_T),$$

where g_T is the maximal conductance and $E_T = 180$ mV is the reversal potential for Ca^{2+} flux. m and h are activation and inactivation variables, respectively, which are defined as follows:

$$m_{\infty,T} = \frac{1}{1 + \exp\left(-\frac{v+63}{7.8}\right)},$$

$$\tau_{m,T} = 0.612 + \frac{1}{\exp\left(\frac{V+16.8}{18.2}\right) + \exp\left(-\frac{V+131.6}{16.7}\right)}$$

$$h_{\infty,T} = \frac{1}{1 + \exp\left(\frac{v+83.5}{6.3}\right)},$$

$$\text{if } V < -80 \text{ } \tau_{h,T} = \exp\left(\frac{V+467}{66.6}\right)$$

$$\text{otherwise } \tau_{h,T} = \left[28 + \exp\left(\frac{V+21.88}{10.2}\right)\right]$$

I_{TP} :

The potentiated component of the T current was modeled by multiplying the T current by a voltage-dependent coefficient P representing the fraction of phosphorylated ("potentiated") channels (see Leresche et al., 2004 for details)

$$I_{TP} = g_{TP} \cdot m^3(V) \cdot h(V) \cdot P(V) \cdot (V(t) - E_T)$$

The voltage dependence of P is related to the steady-state inactivation of I_T as followed (Figure 1A):

$$P_{\infty,T} = 1 - h_{\infty,T} \quad \tau_{p,T} = 3000 - \frac{2700}{(1 + \exp(-(V+65)))}$$

I_h :

$$I_h = g_h \cdot m^3(V) \cdot (V(t) - E_h)$$

$g_h = 8$ nS

$$m_{\infty,h} = \frac{1}{1 + \exp\left(\frac{v+75}{5.5}\right)}, \text{ if } V < -77.57,$$

$$\tau_h = \frac{120819.5}{\exp(-0.0614 \cdot V)} \text{ otherwise } \tau_h = \frac{29.54}{\exp(0.0458 \cdot V)}$$

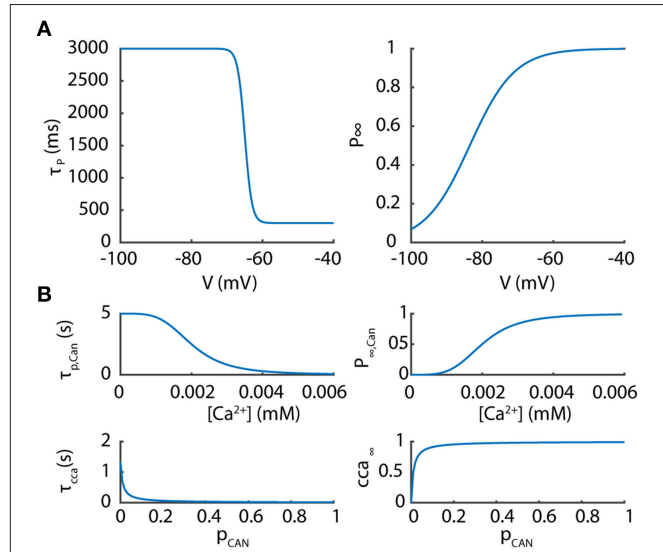


FIGURE 1 | Parameters of the potentiated component of the T current and the Ca^{2+} activated non-selective cation current. (A) The potentiated component of the T current is controlled by a voltage-dependent coefficient P whose kinetics and steady values are presented according to membrane reversal potentials. **(B)** The Ca^{2+} activated non-selective cation channel (CAN) is controlled by two variables p_{CAN} and cca depending upon Ca^{2+} concentration as plotted.

I_{CAN} (Figure 1B):

$$I_{CAN} = g_{CAN} \cdot cca \cdot (V(t) - E_{CAN})$$

$g_{CAN} = 12$ nS

$$cca_{\infty,CAN} = \frac{a_{cca}}{a_{cca} + b_{cca}} \quad \tau_{cca,CAN} = \frac{1}{a_{cca} + b_{cca}}$$

$$a_{cca} = 0.075 \cdot p_{CAN} \quad b_{cca} = 0.00075$$

$$p_{\infty,CAN} = \frac{1.25 \cdot 10^{-7} \cdot [Ca^{2+}]^4}{1.25 \cdot 10^{-7} \cdot [Ca^{2+}]^4 + 0.2} \quad \tau_{p,CAN} = \frac{1}{1.25 \cdot 10^{-7} \cdot [Ca^{2+}]^4 + 0.2}$$

The calcium concentration ($[Ca^{2+}]$, in mM) is governed by the Ca^{2+} influx through T channels and a Ca^{2+} pump that controls intracellular Ca^{2+} levels.

$$[Ca^{2+}] = -(I_T + I_{TP}) \frac{0.0052}{Area \cdot Depth} - 5 \cdot [Ca^{2+}]$$

where Area is $5000 \mu m^2$ and Depth is $0.1 \mu m$.

I_{Na} :

$$I_{Na} = g_{Na} m(V)^3 \cdot h(V) \cdot (V - E_{Na+})$$

$$g_{Na} = 1320$$
 nS

$$a_{m,Na} = 0.32 \frac{-V - 49.3}{(e^{(-V-49.3)/4} - 1)}$$

$$b_{m,Na} = 0.28 \frac{-V - 22.3}{1 - e^{(-V-22.3)/5}}$$

$$m_{\infty,Na} = \frac{a_{m,Na}}{a_{m,Na} + b_{m,Na}} \quad \tau_{m,Na} = \frac{1}{a_{m,Na} + b_{m,Na}}$$

$$a_{h,Na} = 0.128 e^{\frac{-V-45.4}{18}} \quad b_{h,Na} = \frac{4}{e^{\frac{-V-22.4}{5}} + 1}$$

$$h_{\infty,Na} = \frac{a_{h,Na}}{a_{h,Na} + b_{h,Na}} \quad \tau_{h,Na} = \frac{1}{a_{h,Na} + b_{h,Na}}$$

I_{Kir} :

$$I_{Kir} = g_K \cdot n^4(V) \cdot (V - E_K)$$

$$g_K = 600 \text{ nS}$$

$$a_{n,K} = 9.93 \cdot 0.016 \cdot \frac{-V - 57.2 + 35.1/9.93}{(e^{(-V-57.2+35.1/9.93)/5} - 1)}$$

$$b_{n,K} = 0.25 \cdot 9.93 \cdot e^{\frac{-V-57.2+20/9.93}{40}}$$

$$n_{\infty,K} = \frac{a_{n,K}}{a_{n,K} + b_{n,K}} \quad \tau_{n,K} = \frac{1}{a_{n,K} + b_{n,K}}$$

Data Analysis

Numerical integrations of the equations without g_{Na} were performed with the software package XPPAUT (Ermentrout, 2002) to compute the periodic and steady-state solutions as a function of a given parameter (either g_{Leak} or g_T). The orbits (or periodic solutions) were detected by continuation of the equation system i.e., by computing the equilibrium solutions of the differential equations of the membrane potential and of other variables by the forward and backward temporal integration of these equations starting from the bifurcation fixed points with Xppaut (<http://www.math.pitt.edu/~bard/xpp/xpp.html>). The bifurcation parameter (g_{Leak}) was varied on adaptive step size between 0.0001 and 0.1 nS and a discretization interval number for periodic orbit of 50. g_{Na} was not used on a first approximation as this fast component easily prevents the system from converging to a stable orbit solution on a slow temporal scale. Stable solutions found without g_{Na} were nonetheless confirmed or infirmed in the system that included g_{Na} in the following steps of the analysis. For *Up* and *Down* state detection, the membrane potential was down-sampled at 1 kHz. *Up* states were defined as the proportion of simulated time where the membrane potential was > -65 mV. An *Up* state episode during slow oscillation was defined as a finite temporal continuous sequence during which the membrane potential remained > -65 mV for more than 500 ms. A *Down* state during slow oscillation was defined as a continuous temporal sequence where the membrane potential remained below the -65 mV threshold. The average membrane potential during an *Up* state was estimated by averaging all membrane potential values belonging to the *Up* state. The number of LTSs per slow oscillation was estimated as the number of *Down* states (which always precede a LTS) divided by the number of *Up* states. Slow oscillation frequency was estimated by averaging instantaneous frequencies measured for each slow oscillation cycle that was defined as starting and finishing with the LTS that is invariably present at the start of each *Up* state.

RESULTS

In slices, TC neurons of sensory (lateral and medial geniculate, VB), motor (ventrolateral), and intralaminar (centrolateral)

thalamic nuclei recorded in the presence of *trans*-ACPD exhibit stereotypical firing patterns and oscillations when submitted to steady hyperpolarizing currents of increasing amplitudes, as we previously described (Hughes et al., 2002; Zhu et al., 2006; Crunelli et al., 2012, 2014): from stable *UP* states, at times showing tonic firing, to slow *Up* and *Down* state oscillations, “grouped-delta slow waves” (i.e., slow oscillations with delta oscillations during the *DOWN* state), pure delta oscillations (1–4 Hz) and stable silent *DOWN* states (Figure 2A). These activities result from the interplay of intrinsic TC neuron conductances, including the T-type Ca^{2+} current (I_T), with both its transient and window ($I_{Twindow}$) components, the hyperpolarization activated Na^+ - K^+ current (I_h), the Ca^{2+} activated non-selective cation current (I_{CAN}), the inward rectifying potassium current (I_{Kir}) and the leak K^+ current (I_{leak}) (Williams et al., 1997b; Hughes et al., 2002). In order to investigate how I_T density affects the expression of these various oscillations, we compared in LGN TC neurons the range of injected steady hyperpolarizing current required to observe the distinct patterns of oscillations in control conditions and when I_T was partially blocked by the selective antagonist TTA-P2 (Dreyfus et al., 2010). As shown in Figure 2B, the range of steady hyperpolarizing currents where slow oscillations could occur under control condition (355 ± 31 pA, $n = 5$) was clearly smaller in the presence of TTA-P2 (198 ± 28 pA, $n = 5$), indicating that a reduction in I_T drastically weakens the generation of the slow oscillation.

To thoroughly analyze the relationship between the T conductance and the ability of TC neuron to generate various sleep-related oscillations, we constructed a minimal single compartment model of a TC neuron that, upon g_{Leak} variation, satisfactorily reproduced the activities observed *in vitro* in response to different steady hyperpolarizing currents (Figure 2C). Although not strictly equivalent, we chose to vary g_{Leak} instead of simulating a hyperpolarizing current injection in order to mimic the natural changes observed across various sleep stages. Using the bifurcation analysis of this dynamic system (without I_{Na} to facilitate analysis, see Materials and Methods), we first calculated the extent of stable *Up* and *Down* states as a function of g_T and g_{Leak} . As shown in Figure 3A, increasing g_T favors a stable *Up* state and larger g_{Leak} values are required to switch the system to a stable *DOWN* state. As already mentioned, $I_{Twindow}$ contributes to the resting membrane potential around -60 mV (Dreyfus et al., 2010). Since departure from the stable *Up* state occurs around this potential, a stronger I_{Leak} is required to counteract the depolarizing drive resulting from a large g_T and a consequently greater $I_{Twindow}$. The graph also shows the presence of a region of membrane potential instability (delineated by the green and yellow dashed lines in Figure 3A) which occurs for a range of g_{Leak} and g_T values. This area of instability can be associated to particular oscillatory dynamics: slow oscillations and continuous delta oscillations (Figure 3B). As already observed experimentally (see Figure 9 in Soltesz and Crunelli, 1992), for some g_T values where the system has a subcritical Hopf bifurcation point (Wang and Rinzel, 2002; Amarillo et al., 2015), oscillatory regimes and a stable *Down* state can theoretically occur in the same g_{Leak} domain.

For a small g_T (Figure 3Ca), the one-dimension bifurcation diagram of the model system as a function of g_{Leak} remains simple

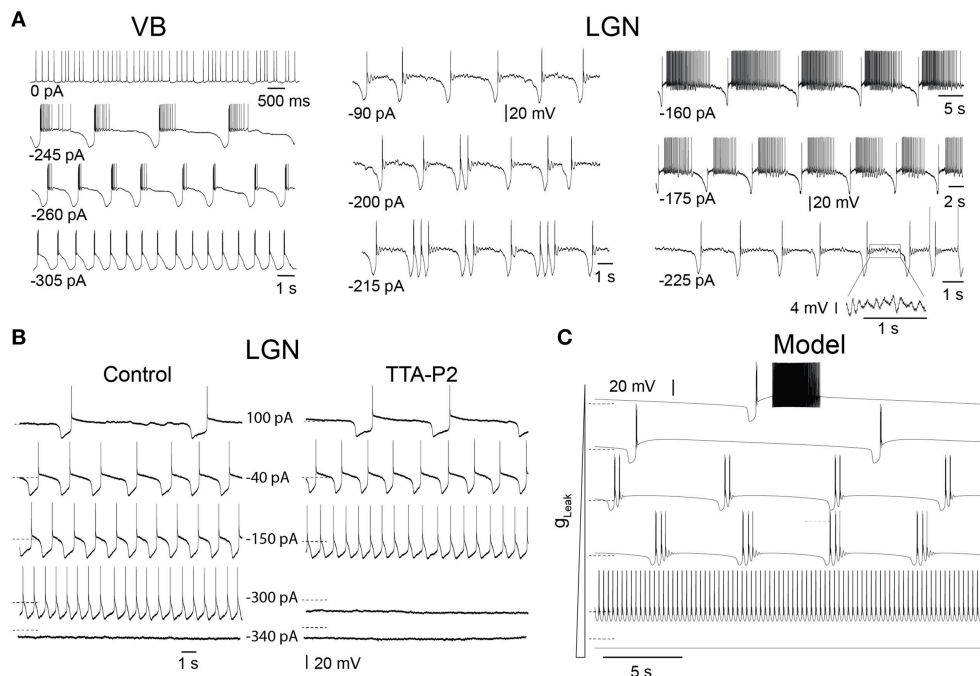


FIGURE 2 | Different intrinsic oscillations in TC neurons and the effect of low doses of TTA-P2. (A) Typical membrane potential oscillations recorded *in vitro* in a ventrobasal nucleus (VB) neuron and two lateral geniculate nucleus (LGN) neurons in response to injection of steady hyperpolarizing current of increasing amplitude in the presence of *trans*-ACPD (adapted, with permission from Zhu et al. (2006)). With little current injection, oscillations exhibited Up states associated to periods of tonic firing. Increasing the hyperpolarizing current induced slow oscillations comprising quiescent Up states followed by isolated LTSs or short episode of delta oscillations. In the VB neuron, continuous delta oscillation was observed with large current injection. Note also the periods of small amplitude, 5–7 Hz oscillations during Up states in the LGN neurons (see enlargement of a section of the bottom right trace). **(B)** Membrane potential oscillations recorded *in vitro* in a cat LGN TC neuron in the continuous presence of *trans*-ACPD before and during perfusion of the slice with the selective T channel blocker TTA-P2. The reduction in I_T leads to a narrowing of the range of hyperpolarizing currents that triggers slow oscillations. **(C)** Membrane potential oscillations in a model of TC neuron. As observed in experiments, enhancing the hyperpolarizing drive by increasing g_{Leak} values from 1.5 to 4.2 nS induced (from top to bottom) a transition from slowly alternating Up and Down states to “group-delta slow waves,” continuous delta oscillations and stable Down state. Dotted lines: -65 mV.

with departure from the stable Up or Down states involving Hopf bifurcations. The stable periodic orbits correspond to pure delta oscillations (red lines in Figure 3Ca) that do not overlap with the regions where stable Up or Down states exist. This indicates that small g_T values allow only 3 robust exclusive activity patterns in TC neurons: stable Up state, pure delta oscillations and stable Down state. However, when g_T is increased, the bifurcation diagram becomes more complex (Figures 3Cb,c). At departure from the stable Up-state, small periodic orbits involving membrane potential oscillations of a few millivolts in amplitude (Figure 3B left) at 6 Hz (or higher frequency) are present for a very narrow range of g_{Leak} (green line in Figures 3Cb,c). Such low-amplitude oscillations that occur close to -60 mV are consistently present in our simulations. Although these oscillations cannot be easily related to any physiologically defined membrane potential waveform of TC neurons, they resemble oscillations that occasionally appear in the Up state of slow oscillations in these neurons (Figure 2A; see Hughes et al., 2002; Zhu et al., 2006), and have been suggested to represent the intrinsic dynamic contribution of TC neurons to synaptically generated spindle oscillations (Wang, 1994). As g_{Leak} further increases, unstable orbit cycles (blue dots in Figures 3Cb,c), corresponding to the complex “grouped-delta

slow waves” (Figure 3B middle), occur for a large range of g_{Leak} before the stable periodic orbits corresponding to pure delta oscillations (Figure 3B right) could develop (red lines in Figures 3Cb,c). Therefore, although delta oscillations are already present with small g_T values, only a larger g_T allows the occurrence of the full dynamics observed in TC neurons, including “grouped-delta slow waves.”

In order to more precisely describe the different slow wave patterns present for a given g_T value, simulations were then run while systematically varying g_T and g_{Leak} (in the presence of g_{Na} ; Figure 4). Confirming the conclusions of the bifurcation diagrams, analysis of the membrane potential dynamics as a function of g_{Leak} indicates that only delta oscillations occur for the smallest g_T value (10 nS, Figures 4B,C). When g_T is increased, in addition to continuous delta oscillations, slow oscillations with Up states that always start with a LTS are observed in a narrow range of g_{Leak} (Figures 4A–C). For larger values of g_T , “grouped-delta slow waves” are present and this firing pattern can be observed in a large range of g_{Leak} values that expands as g_T increases (Figures 4A–C). Further quantification of the slow oscillation parameters indicates that for g_T values associated with a robust slow oscillation pattern ($g_T \geq 30$ nS), the ranges of Up-state duration and slow wave frequencies remain

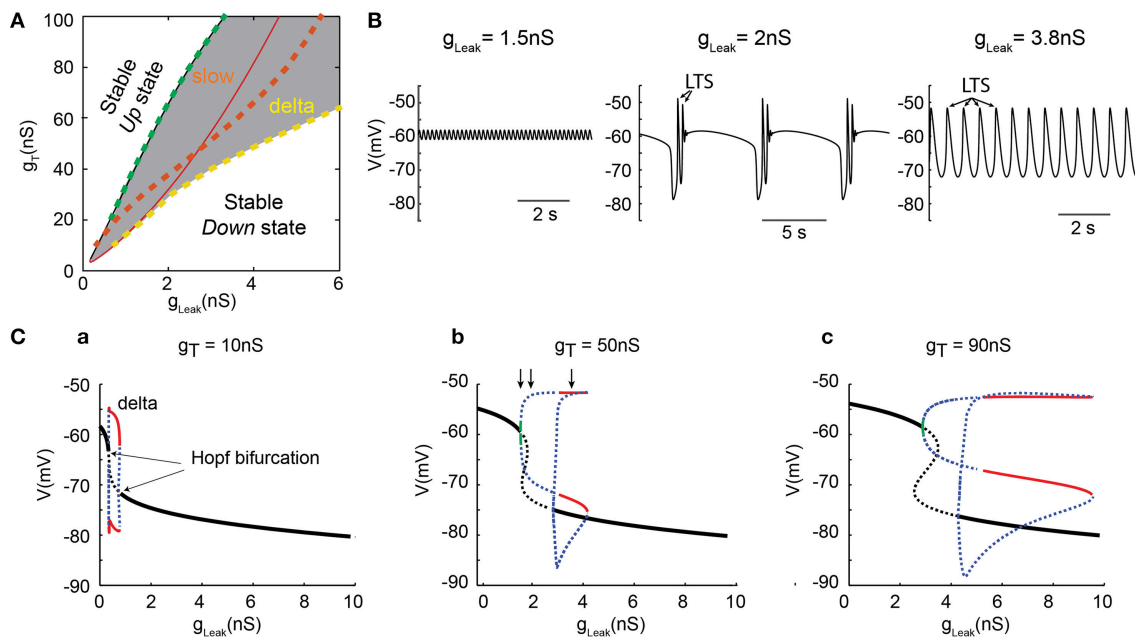


FIGURE 3 | Dynamical analysis of different oscillations in the TC model cell. (A) Two-parameter bifurcation diagram indicating the lines of bifurcation of the system in the g_{Leak} – g_T plane: (i) black line (almost overlapping with the dashed green line), the (supercritical Hopf) bifurcations from the stable *Up* states (left side) to oscillatory regimes (right side); (ii) dashed green line, frontier from the small amplitude 6 Hz oscillations to slow oscillations (right side); (iii) red line, the (subcritical Hopf) bifurcations from oscillatory regime (left side) to stable *Down* state (right side); (iv) dashed orange line, bifurcations from the slow oscillation regime (left side) to continuous delta oscillation (right side); (v) dashed yellow line, limit of continuous delta oscillation. The gray zone indicates the domains of oscillations. **(B)** Example of small amplitude 6 Hz oscillations (left), slow oscillations (middle) and delta oscillations (right) observed in the model for $g_T = 50$ nS with $g_{Leak} = 1.5, 2$, and 3.8 nS, respectively (corresponding to the three vertical arrows in Cb, respectively). Some of the LTSs are indicated by arrows. **(C)** One-parameter (g_{Leak}) bifurcation diagrams for 3 increasing values of g_T . Maximum and minimum membrane potential values (V_m) of small amplitude 6 Hz oscillations (green line), continuous delta oscillations (red lines), unstable orbits (blue dashed lines), and fixed-point equilibria (black lines) for stable *Up* and *Down* states. The dashed black lines indicate the unstable static equilibria. As g_T increases, the range of g_{Leak} that allows delta and slow oscillations is drastically increased. These analyses were performed without g_{Na} to simplify computation of the bifurcation diagrams.

stable (**Figures 5A,B**), but the maximal value of the *Up* state membrane potential increases proportionally to g_T (**Figure 5C**). These increasingly more depolarized *Up* states may result from both a stronger $I_{Twindow}$ directly linked to the larger g_T and the consequently stronger I_{CAN} due to the larger Ca^{2+} entry occurring during the T channel activation that generates the LTS at the beginning of each *Up* state.

Surprisingly, for the highest g_T values (>70 nS), although long sequences of “grouped-delta slow waves” are present (**Figure 4C**), our model cell did not anymore display continuous delta oscillations but abruptly switched from “grouped-delta slow waves” to stable *Down* states when g_{Leak} increased. While the bifurcation diagrams previously calculated for large g_T values (**Figures 3A,Cc**) predicted an increase of the range of g_{Leak} values where stable periodic delta orbits corresponding to continuous delta oscillations may develop, stable *Down* states were the only solutions observed in our simulations (**Figure 4C**). Such dominance of the stable *Down* state over continuous delta oscillations was due to the presence of action potentials on top of the LTS which elicit large high K^+ rectifying currents. Indeed, in simulations performed without g_{Na} continuous delta oscillations were observed for some g_{Leak} values (data not shown).

Although the prominent I_T in TC neurons mainly results from a high channel expression, we previously demonstrated

that in neurons of sensory thalamic nuclei, I_T amplitude is also transiently potentiated by a phosphorylation (ATP-dependent) mechanism, which exclusively occurs when the channels are inactivated, i.e., it increases with membrane depolarization (Leresche et al., 2004). To study how this additional mechanism that drastically controls the T current amplitude in this population of TC neurons contributes to their firing dynamics a new set of simulations was run where part of the total I_T was due to a “potentiated” T conductance (g_{TP}). The kinetics, amplitude and voltage-dependence of this g_{TP} mimic the effect and properties of the described phosphorylation mechanism (see Materials and Methods for further details; Leresche et al., 2004). When I_T was increased by introducing g_{TP} in the model, a strengthening of the slow oscillation which occurred in a larger range of g_{Leak} values is once again observed (**Figure 6C**; also compare **Figures 6A,B** with **Figures 4A,B**). However, for a given value of T conductance, negligible differences in the bifurcation diagrams are observed when comparing the dynamical behaviors supported either by non-potentiated currents or by a combination of non-potentiated and potentiated currents (**Figures 6D–G**). Hence, the peculiar biophysical properties of the potentiated T conductance do not significantly modify the membrane potential dynamics of slow oscillations. Nevertheless a close examination of the oscillatory

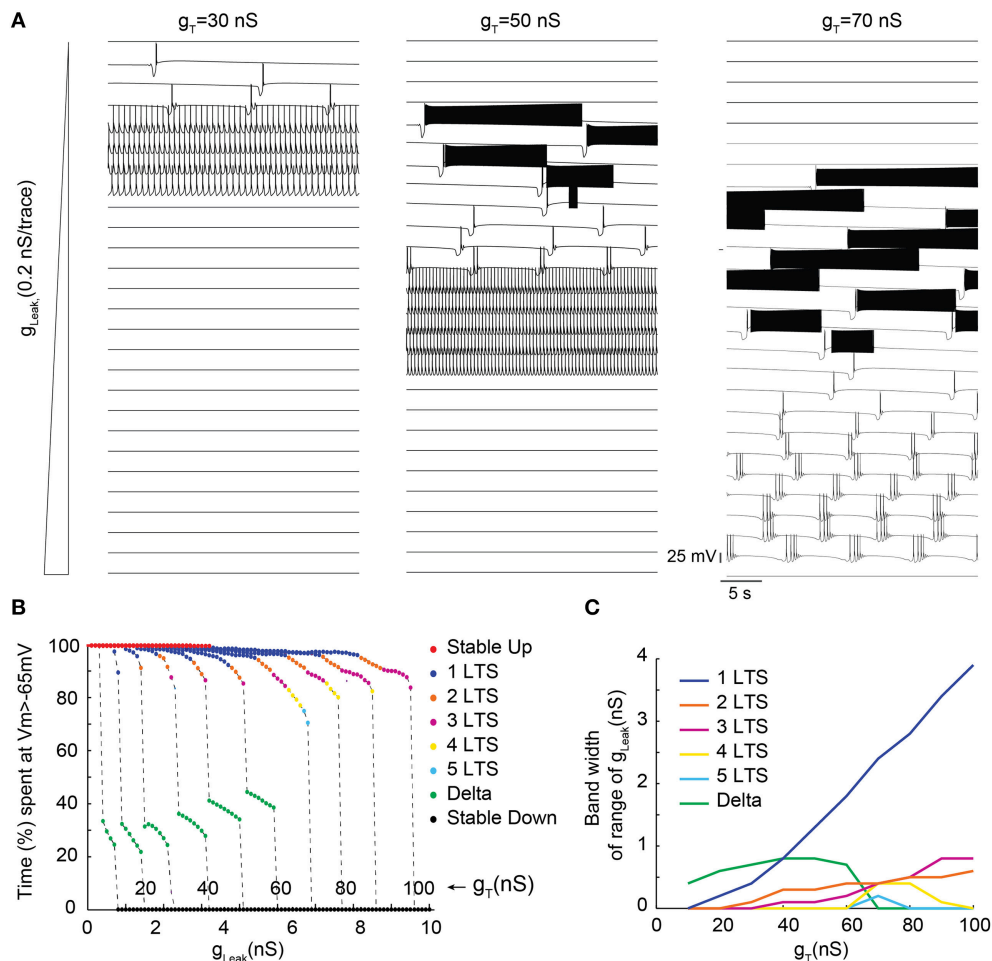


FIGURE 4 | Membrane potential dynamics as a function of g_{Leak} of TC model cells with increasing g_T . (A) Examples of membrane potential dynamics for $g_T = 30, 50$, and 70 nS and increasing values of g_{Leak} (from top to bottom). (B) Proportion of time spent at depolarized membrane potential ($V_m > -65$ mV) as a function of g_{Leak} for TC model cells with increasing g_T values (from 10 to 100 nS). Dynamic regimes are categorized by color coding according to the number of LTSs per slow oscillation period. (C) Range of g_{Leak} , in which a given dynamic regime is observed as a function of g_T .

regimes shows that for T conductance values where both “grouped-delta slow waves” and continuous delta developed (Figure 6B, $g_T = 60$ nS), the continuous delta disappeared upon introduction of the voltage-dependent potentiation (Figure 6B, $g_T = 30$ nS + $g_{TP} = 30$ nS). This suggests that compared to a simple increase in g_T , this ATP-dependent T channel regulation can selectively enhance the occurrence of slow oscillations of TC neurons at the expenses of delta oscillations.

DISCUSSION

Since their first development (Rose and Hindmarsh, 1989), TC neuron models have gained in precision and completeness (Destexhe et al., 1998), thus allowing detailed analysis of the dynamical processes that are intrinsic to these neurons (Destexhe and Sejnowski, 2003; Amarillo et al., 2015). Our current model adds to this knowledge by providing for the first time insights into the dynamical processes that take place at the transition

between slow and delta oscillations. In particular, our results strongly suggest that the high g_T of TC neurons, either due to channel expression or regulation, is not required to generate full-blown LTSs during delta and slow oscillations but is necessary for the generation of the *Up* and *Down* state dynamics underlying the slow oscillation of these neurons (David et al., 2013; Crunelli et al., 2014).

Contrary to the interpretation of the original *in vitro* and *in vivo* studies (see Crunelli et al., 2015), it is now well established that the full expression of slow oscillations requires both cortical and thalamic activities. In particular, combining ensemble recordings of single TC neurons and reverse microdialysis, we recently showed that slow wave frequency is strongly reduced following intrathalamic application of either TTX or TTA-P2 in both anesthetized and naturally sleeping rats (David et al., 2013). In agreement with these data, mice with a Cav3.1 deletion in the thalamus (but not in the cortex) experience frequent arousals during sleep (Anderson et al., 2005), supporting the importance of thalamic T channels in stabilizing sleep rhythms. Moreover,

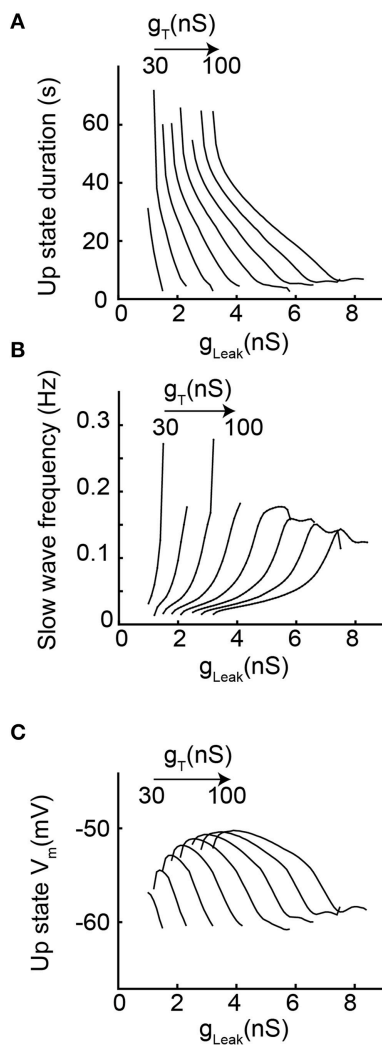


FIGURE 5 | Slow oscillation properties as a function of increasing g_T and g_{Leak} . (A) Each line represents the duration of Up state episodes during slow oscillation as a function of g_{Leak} , for a given g_T (from 20 to 100 nS). (B) Slow oscillation frequency for the same data set. (C) Up state average membrane potential values (see text and Materials and Methods for further details).

the biophysical mechanisms underlying the conditional thalamic oscillator responsible in TC neurons for the full manifestation of different types of slow oscillation depends on the membrane potential bistability that is created by the interaction between $I_{Twindow}$ and I_{Leak} (Williams et al., 1997a; Toth et al., 1998; Crunelli et al., 2006). TC neurons present a small $I_{Twindow}$ (a few tens of pA; Dreyfus et al., 2010) and any decrease in this current may drastically impact its ability to play a significant physiological role. Our simulations results indicate that the high T channel expression in TC neurons is crucial to generate a large enough $I_{Twindow}$ capable of supporting the Up and Down states dynamics of slow oscillations over a large range of g_{Leak} values. This was clearly confirmed by our *in vitro* recordings showing that partial block of the I_T by TTA-P2 drastically reduces

the range of steady hyperpolarizing currents that can generate intrinsic slow oscillations in TC neurons.

In addition, our simulations have indicated that a large g_T is also essential for the appearance of “grouped-delta slow waves.” As indicated above, during slow waves the voltage-dependence of $I_{Twindow}$ creates the membrane potential bistability but the rhythmic occurrence of Up and Down states relies on the dynamics of I_{CAN} (Hughes et al., 2002). Indeed, upon Ca^{2+} entry via the T channels these mixed cationic channels generates a transient depolarizing current that adds to $I_{Twindow}$ to set the membrane potential of the Up state. As intracellular Ca^{2+} slowly return to its basal level, the progressive decrease of I_{CAN} reduces this membrane potential up to the point where the stable Up state equilibrium disappears and the membrane potential switches to the Down-state. With medium g_T values, Ca^{2+} entry during LTS is moderate and I_{CAN} activation, together with $I_{Twindow}$, is not strong enough to counteract a strong I_{Leak} and thus to generate the stable equilibrium necessary for an Up-state. Consequently, TC neurons go into continuous delta oscillations. However, with higher g_T values, Ca^{2+} accumulation after a few delta oscillation cycles is sufficient to maximally activate I_{CAN} and thus set an Up state equilibrium that terminates a delta oscillation episode.

Importantly, when our simulations included g_{Na} we did not observe continuous delta oscillations for high or potentiated T channel conductances. Indeed, during natural sleep, thalamic delta oscillations appear to occur mostly in discrete groups during the down state of slow oscillations in both TC and nucleus reticularis thalami neurons (Steriade et al., 1993c; Timofeev and Steriade, 1996) and there is no evidence supporting the presence of continuous delta oscillations in TC neurons *in vivo*. Therefore, one can hypothesize that as suggested in Figure 3A strong T channel expression may prevent the appearance of continuous intrinsic rhythmicity at delta frequency in TC neurons. Interestingly, we previously showed that the maximal amplitude of I_T is highly variable across neurons of different thalamic nuclei and even in different TC neurons within a nucleus (Leresche et al., 2004). Therefore, further modeling studies should aim to investigate how this heterogeneity in T channel density among TC neurons interacts with other intrinsic conductance expression such as I_{CAN} or I_h to impact oscillatory dynamics.

Finally, one has to consider that the slow oscillation modeled here is generated intrinsically by single TC neurons (i.e., recorded in the presence of both glutamate and GABA blockers) and thus without the influence of either excitatory cortical and inhibitory inputs. Although this should represent the basic cellular mechanism explaining the conditional role played by the thalamus in sleep slow waves generation (Crunelli and Hughes, 2010), the precise interactions between this intrinsic mechanism and the complex thalamocortical activities (Sherozhiya and Timofeev, 2014) remain to be clarified. Along the same line our simulations cannot inform on the firing dynamics of TC neurons during the expression of absence seizures since this abnormal activity requires the integrity of the thalamocortical network (Crunelli and Leresche, 2002). However the present results may help to better understand firing patterns observed during general anesthesia where the EEG mostly includes spindle and delta waves (Franks, 2008). In particular, phase 2 of the

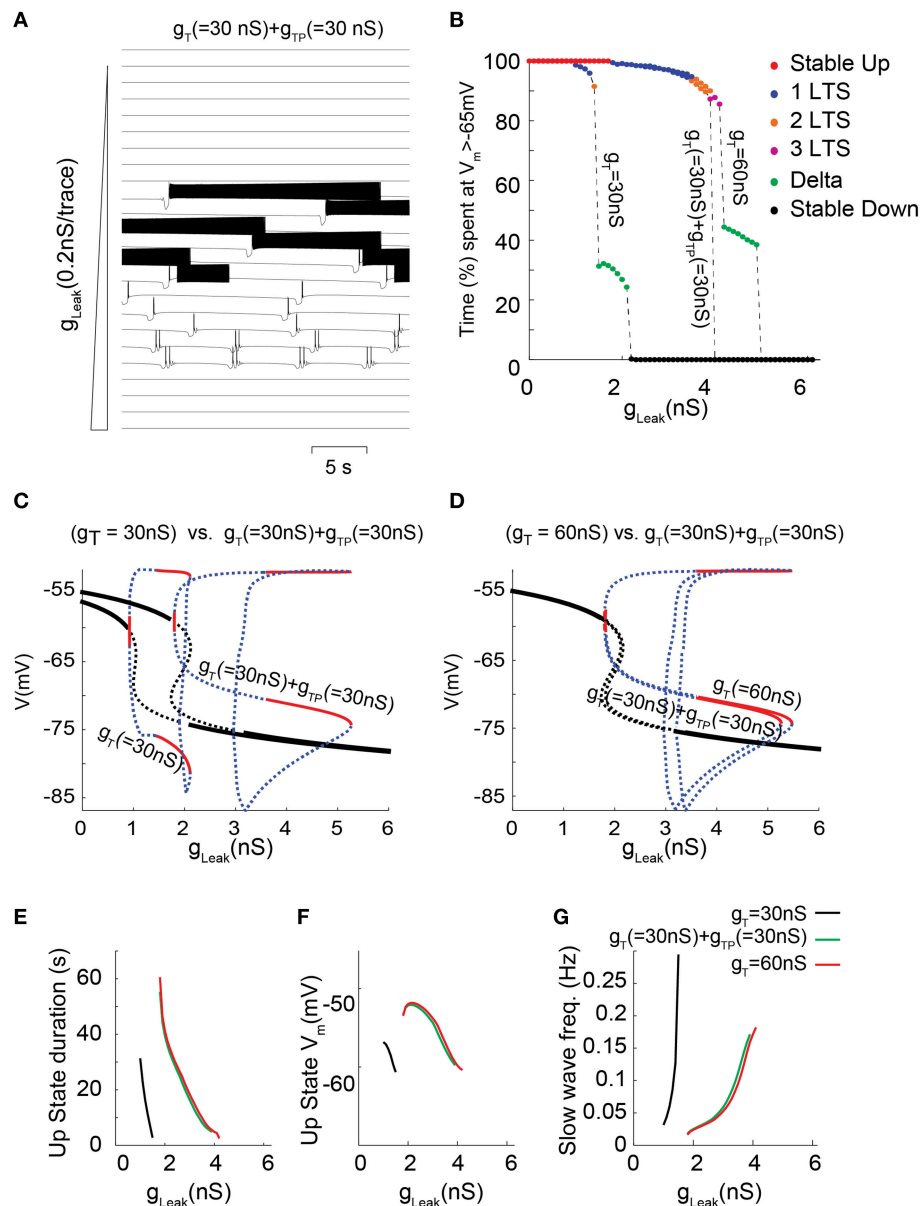


FIGURE 6 | Membrane potential dynamics as a function of g_{Leak} for a TC model cell that incorporates I_T potentiation. (A) Typical example of membrane potential dynamics for $g_T = 30 \text{ nS} + g_{TP} = 30 \text{ nS}$ for increasing g_{Leak} values. **(B)** Proportion of time spent at depolarized membrane potential ($V_m > -65 \text{ mV}$) as a function of g_{Leak} for TC model cells with $g_T = 30 \text{ nS}$; $g_T = 30 \text{ nS} + g_{TP} = 30 \text{ nS}$; and $g_T = 60 \text{ nS}$ (same color code as in **Figure 4B** to indicate dynamical regimes). **(C,D)** Effect on the one-parameter (g_{Leak} on x-axis) bifurcation diagrams of either adding a potentiated T conductance (C, $g_T = 30 \text{ nS}$ vs. $g_T = 30 \text{ nS} + g_{TP} = 30 \text{ nS}$) or replacing half of the T conductance by a potentiated T conductance (D, $g_T = 60 \text{ nS}$ vs. $g_T = 30 \text{ nS} + g_{TP} = 30 \text{ nS}$). Same legend as in **Figure 3C**. **(E–G)** Slow oscillation characteristics observed in model TC cells with either $g_T = 30 \text{ nS}$ (black); $g_T = 30 \text{ nS} + g_{TP} = 30 \text{ nS}$ (green) or $g_T = 60 \text{ nS}$ (red). **(E)** Each line represents the duration of Up state episodes during slow oscillation as a function of g_{Leak} , for a given g_T and g_{TP} . **(F)** Slow oscillation frequency for the same data set. **(G)** Up state average membrane potential values.

maintenance period is characterized by an increase in delta (0–4 Hz) activity (Brown et al., 2010). At clinically relevant concentrations, a number of volatile general anesthetics have been shown to inhibit both recombinant and native T channels (Orestes and Todorovic, 2010; Eckle et al., 2012), a result which was considered at first glance in contradiction with the occurrence of delta oscillations. However, (Eckle et al., 2012)

showed that anesthetic doses of isoflurane only inhibits 20 to 60% of I_T but induce a marked decrease of $I_{T\text{window}}$ in TC neurons. In this respect, our simulations clearly indicate that such partial inhibition of I_T should indeed favor the occurrence of continuous delta oscillations, at least in TC neurons. In agreement with this view, a partial block of I_T by *in vivo* administration of TTA-related compounds also produce sedative

effects in behaving mice (Uebele et al., 2009; Kraus et al., 2010), further illustrating the complex relationship between the amount of g_T and various sleep-related activities.

AUTHOR CONTRIBUTIONS

FD, VC, NL, RL contribute to the design of the work, the acquisition, analysis and interpretation of data. FD, VC, NL, RL contribute to the manuscript and approve the final version.

REFERENCES

- Achermann, P., and Borbely, A. A. (1997). Low-frequency (< 1 Hz) oscillations in the human sleep electroencephalogram. *Neuroscience* 81, 213–222.
- Amarillo, Y., Mato, G., and Nadal, M. S. (2015). Analysis of the role of the low threshold currents I_T and I_h in intrinsic delta oscillations of thalamocortical neurons. *Front. Comput. Neurosci.* 9:52. doi: 10.3389/fncom.2015.00052
- Anderson, M. P., Mochizuki, T., Xie, J., Fischler, W., Manger, J. P., Talley, E. M., et al. (2005). Thalamic Cav3.1 T-type Ca^{2+} channel plays a crucial role in stabilizing sleep. *Proc. Natl. Acad. Sci. U.S.A.* 102, 1743–1748. doi: 10.1073/pnas.0409644102
- Brown, E. N., Lydic, R., and Schiff, N. D. (2010). General anesthesia, sleep, and coma. *N. Engl. J. Med.* 363, 2638–2650. doi: 10.1056/NEJMra0808281
- Contreras, D., and Steriade, M. (1996). Spindle oscillation in cats: the role of corticothalamic feedback in a thalamically generated rhythm. [published erratum appears in *J. Physiol. (Lond)* 1996 Mar 15;491(Pt 3):889]. *J. Physiol. (Lond)*. 490, 159–179.
- Crunelli, V., Cope, D. W., and Hughes, S. W. (2006). Thalamic T-type Ca^{2+} channels and NREM sleep. *Cell Calcium* 40, 175–190. doi: 10.1016/j.ceca.2006.04.022
- Crunelli, V., David, F., Leresche, N., and Lambert, R. C. (2014). Role for T-type Ca^{2+} channels in sleep waves. *Pflügers Archiv.* 466, 735–745. doi: 10.1007/s00424-014-1477-3
- Crunelli, V., David, F., Lorincz, M. L., and Hughes, S. W. (2015). The thalamocortical network as a single slow wave-generating unit. *Curr. Opin. Neurobiol.* 31, 72–80. doi: 10.1016/j.conb.2014.09.001
- Crunelli, V., and Hughes, S. W. (2010). The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat. Neurosci.* 13, 9–17. doi: 10.1038/nn.2445
- Crunelli, V., and Leresche, N. (2002). Childhood absence epilepsy: genes, channels, neurons and networks. *Nat. Rev. Neurosci.* 3, 371–382. doi: 10.1038/nrn811
- Crunelli, V., Lorincz, M., and Hughes, S. W. (2012). Activity of cortical and thalamic neurons during the slow (<1Hz) oscillation in the mouse *in vivo*. *Pflügers Arch.* 463, 73–88. doi: 10.1007/s00424-011-1011-9
- David, F., Schmiedt, J. T., Taylor, H. L., Orban, G., Di Giovanni, G., Uebele, V. N., et al. (2013). Essential thalamic contribution to slow waves of natural sleep. *J. Neurosci.* 33, 19599–19610. doi: 10.1523/JNEUROSCI.3169-13.2013
- Destexhe, A., Neubig, M., Ulrich, D., and Huguenard, J. (1998). Dendritic low-threshold calcium currents in thalamic relay cells. *J. Neurosci.* 18, 3574–3588.
- Destexhe, A., and Sejnowski, T. J. (2003). Interactions between membrane conductances underlying thalamocortical slow-wave oscillations. *Physiol. Rev.* 83, 1401–1453. doi: 10.1152/physrev.00012.2003
- Dreyfus, F. M., Tscherter, A., Errington, A. C., Renger, J. J., Shin, H. S., Uebele, V. N., et al. (2010). Selective T-type calcium channel block in thalamic neurons reveals channel redundancy and physiological impact of $I(T)$ window. *J. Neurosci.* 30, 99–109. doi: 10.1523/JNEUROSCI.4305-09.2010
- Eckle, V. S., Digrucio, M. R., Uebele, V. N., Renger, J. J., and Todorovic, S. M. (2012). Inhibition of T-type calcium current in rat thalamocortical neurons by isoflurane. *Neuropharmacology* 63, 266–273. doi: 10.1016/j.neuropharm.2012.03.018
- Ermentrout, B. (2002). *Simulating, Analyzing and Animating Dynamical Systems: A Guide to Xppaut for Researchers and Student*. 1st Edn. Philadelphia, PA: Society for Industrial and Applied Mathematics.

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- Franks, N. P. (2008). General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat. Rev. Neurosci.* 9, 370–386. doi: 10.1038/nrn2372
- Huc, S., Monteil, A., Bidaud, I., Barbara, G., Chemin, J., and Lory, P. (2009). Regulation of T-type calcium channels: signalling pathways and functional implications. *Biochim. Biophys. Acta* 1793, 947–952. doi: 10.1016/j.bbamcr.2008.11.003
- Hughes, S. W., Cope, D. W., Blethyn, K. L., and Crunelli, V. (2002). Cellular mechanisms of the slow (1 Hz) oscillation in thalamocortical neurons *in vitro*. *Neuron* 33, 947–958. doi: 10.1016/S0896-6273(02)00623-2
- Hughes, S. W., Lorincz, M., Cope, D. W., Blethyn, K. L., Kekesi, K. A., Parri, H. R., et al. (2004). Synchronized oscillations at alpha and theta frequencies in the lateral geniculate nucleus. *Neuron* 42, 253–268. doi: 10.1016/S0896-6273(04)00191-6
- Kraus, R. L., Li, Y., Gegan, Y., Gotter, A. L., Uebele, V. N., Fox, S. V., et al. (2010). *In vitro* characterization of T-type calcium channel antagonist TTA-A2 and *in vivo* effects on arousal in mice. *J. Pharmacol. Exp. Ther.* 335, 409–417. doi: 10.1124/jpet.110.171058
- Lambert, R. C., Bessaih, T., Crunelli, V., and Leresche, N. (2014). The many faces of T-type calcium channels. *Pflügers Archiv.* 466, 415–423. doi: 10.1007/s00424-013-1353-6
- Lambert, R. C., Bessaih, T., and Leresche, N. (2006). Modulation of neuronal T-type calcium channels. *CNS Neurol. Disord. Drug Targets* 5, 611–627. doi: 10.2174/187152706779025544
- Lemieux, M., Chen, J. Y., Lonjers, P., Bazhenov, M., and Timofeev, I. (2014). The impact of cortical deafferentation on the neocortical slow oscillation. *J. Neurosci.* 34, 5689–5703. doi: 10.1523/JNEUROSCI.1156-13.2014
- Leresche, N., Hering, J., and Lambert, R. C. (2004). Paradoxical potentiation of neuronal T-type Ca^{2+} current by ATP at resting membrane potential. *J. Neurosci.* 24, 5592–5602. doi: 10.1523/JNEUROSCI.1038-04.2004
- Leresche, N., Lightowler, S., Soltesz, I., Jassik-Gerschenfeld, D., and Crunelli, V. (1991). Low-frequency oscillatory activities intrinsic to rat and cat thalamocortical cells. *J. Physiol. (Lond)* 441, 155–174.
- McCormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog. Neurobiol.* 39, 337–388.
- Orestes, P., and Todorovic, S. M. (2010). Are neuronal voltage-gated calcium channels valid cellular targets for general anesthetics? *Channels* 4, 518–522. doi: 10.4161/chan.4.6.12873
- Perez-Reyes, E. (2003). Molecular physiology of low-voltage-activated t-type calcium channels. *Physiol. Rev.* 83, 117–161. doi: 10.1152/physrev.00018.2002
- Pirchio, M., Turner, J. P., Williams, S. R., Asprodingi, E., and Crunelli, V. (1997). Postnatal development of membrane properties and delta oscillations in thalamocortical neurons of the cat dorsal lateral geniculate nucleus. *J. Neurosci.* 17, 5428–5444.
- Rose, R. M., and Hindmarsh, J. L. (1989). The assembly of ionic currents in a thalamic neuron. I. The three-dimensional model. *Proceedings of the Royal Society of London. Ser. B Biol. Sci.* 237, 267–288.
- Sheroziya, M., and Timofeev, I. (2014). Global intracellular slow-wave dynamics of the thalamocortical system. *J. Neurosci.* 34, 8875–8893. doi: 10.1523/JNEUROSCI.4460-13.2014

- Soltesz, I., and Crunelli, V. (1992). A role for low-frequency, rhythmic synaptic potentials in the synchronization of cat thalamocortical cells. *J. Physiol. (Lond)*. 457, 257–276.
- Steriade, M. (2006). Grouping of brain rhythms in corticothalamic systems. *Neuroscience* 137, 1087–1106. doi: 10.1016/j.neuroscience.2005.10.029
- Steriade, M., Contreras, D., Curro Dossi, R., and Nunez, A. (1993a). The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J. Neurosci.* 13, 3284–3299.
- Steriade, M., McCormick, D. A., and Sejnowski, T. J. (1993b). Thalamocortical oscillations in the sleeping and aroused brain. *Science* 262, 679–685.
- Steriade, M., Nunez, A., and Amzica, F. (1993c). Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *J. Neurosci.* 13, 3266–3283.
- Timofeev, I., and Steriade, M. (1996). Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. *J. Neurophysiol.* 76, 4152–4168.
- Toth, T. I., Hughes, S. W., and Crunelli, V. (1998). Analysis and biophysical interpretation of bistable behaviour in thalamocortical neurons [In Process Citation]. *Neuroscience* 87, 519–523.
- Turner, J. P., Anderson, C. M., Williams, S. R., and Crunelli, V. (1997). Morphology and membrane properties of neurones in the cat ventrobasal thalamus *in vitro*. *J. Physiol. (Lond)*. 505, 707–726.
- Uebele, V. N., Nuss, C. E., Fox, S. V., Garson, S. L., Cristescu, R., Doran, S. M., et al. (2009). Positive allosteric interaction of structurally diverse T-type calcium channel antagonists. *Cell Biochem. Biophys.* 55, 81–93. doi: 10.1007/s12013-009-9057-4
- Wang, X. J. (1994). Multiple dynamical modes of thalamic relay neurons: rhythmic bursting and intermittent phase-locking. *Neuroscience* 59, 21–31.
- Wang, X. J., and Rinzel, J. (2002). *The Handbook of Brain Theory and Neural Networks*. Cambridge, MA: The MIT Press.
- Williams, S. R., Toth, T. I., Turner, J. P., Hughes, S. W., and Crunelli, V. (1997a). The ‘window’ component of the low threshold Ca^{2+} current produces input signal amplification and bistability in cat and rat thalamocortical neurones. *J. Physiol. (Lond)*. 505, 689–705.
- Williams, S. R., Turner, J. P., Hughes, S. W., and Crunelli, V. (1997b). On the nature of anomalous rectification in thalamocortical neurones of the cat ventrobasal thalamus *in vitro*. *J. Physiol. (Lond)*. 505, 727–747.
- Zhu, L., Blethyn, K. L., Cope, D. W., Tsomaia, V., Crunelli, V., and Hughes, S. W. (2006). Nucleus- and species-specific properties of the slow (<1 Hz) sleep oscillation in thalamocortical neurons. *Neuroscience* 141, 621–636. doi: 10.1016/j.neuroscience.2006.04.069

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On Parallel Streams through the Mouse Dorsal Lateral Geniculate Nucleus

Daniel J. Denman¹ and Diego Contreras^{2*}

¹ Allen Institute for Brain Science, Seattle, WA, USA, ² Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

The mouse visual system is an emerging model for the study of cortical and thalamic circuit function. To maximize the usefulness of this model system, it is important to analyze the similarities and differences between the organization of all levels of the murine visual system with other, better studied systems (e.g., non-human primates and the domestic cat). While the understanding of mouse retina and cortex has expanded rapidly, less is known about mouse dorsal lateral geniculate nucleus (dLGN). Here, we study whether parallel processing streams exist in mouse dLGN. We use a battery of stimuli that have been previously shown to successfully distinguish parallel streams in other species: electrical stimulation of the optic chiasm, contrast-reversing stationary gratings at varying spatial phase, drifting sinusoidal gratings, dense noise for receptive field reconstruction, and frozen contrast-modulating noise. As in the optic nerves of domestic cats and non-human primates, we find evidence for multiple conduction velocity groups after optic chiasm stimulation. As in so-called “visual mammals”, we find a subpopulation of mouse dLGN cells showing non-linear spatial summation. However, differences in stimulus selectivity and sensitivity do not provide sufficient basis for identification of clearly distinct classes of relay cells. Nevertheless, consistent with presumptively homologous status of dLGNs of all mammals, there are substantial similarities between response properties of mouse dLGN neurons and those of cats and primates.

Keywords: LGN, parallel processing, mouse vision, cell types, mouse models

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*Correspondence:

Diego Contreras
diegoc@upenn.edu

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INTRODUCTION

In carnivores and primates, processing of visual information is carried out in parallel streams from the retina to the cerebral cortex (Stone, 1983; Livingstone and Hubel, 1988; Merigan and Maunsell, 1993; Wässle, 2004; Nassi and Callaway, 2009). Different types of visual information remain segregated in these pathways, and are later combined in cortex for different visual processing tasks.

The first evidence that visual pathways are organized into parallel streams was the recording of early and late components in the compound action potential triggered by electrical stimulation of the optic nerve of frog and rabbit (Bishop, 1933). The discovery of the presence in the optic nerve of groups of axons with different conduction velocities and different diameters followed the seminal discovery of a correspondence between axon caliber and sensory modality in the somatosensory system (Gasser and Erlanger, 1929; Heinbecker et al., 1933) and prompted the question of whether the different conduction velocity groups in the optic nerve

also underpin parallel streams of visual information (see Stone, 1983). The presence of distinct groups of optic nerve fibers with different conduction velocities in response to electrical stimulation was later confirmed in the cat (Bishop and O'Leary, 1938). Subsequently, recording from within the dorsal lateral geniculate nucleus (dLGN), Bishop and McLeod (1954) showed that volleys arrived at different times (which they called t_1 and t_2) and each led to the generation of local field potential (LFPs), interpreted as corresponding postsynaptic responses of dLGN neurons (r_1 and r_2 ; Bishop and McLeod, 1954). The work of Stone and Hoffmann (1971) established a correspondence between dLGN neuron orthodromic latency and the antidromic latency to electrical stimulation of visual cortex (V1), thus demonstrating the segregation of pathways according to conduction velocity (i.e., axonal diameter) from retina to V1.

Parallel pathways can be distinguished by a number of visual response properties. In cat, linear of spatial summation of contrast over their receptive fields is the hallmark of X cells, while Y cells display non-linear spatial summation (Shapley and Hochstein, 1975; Shapley et al., 1981). In addition, X cells show sustained responses with high-spatial and low-temporal selectivity and low-contrast sensitivity, while Y cells group at the other end of the spectrum of these response properties (Cleland et al., 1971, 1973). X and Y cells can also be differentiated by the precision and reliability of their responses (Reinagel and Reid, 2000; Kumbhani et al., 2007) and the size of their receptive fields at matching eccentricities (Saul and Humphrey, 1990; Usrey and Reid, 2000; Xu et al., 2001; Weng et al., 2005).

Further parallel pathways exist in the magnocellular and parvocellular layers primate dLGN (Nassi and Callaway, 2009). Cells in the parvo and magnocellular layers of the LGN show unimodal distribution of visual response properties that are very similar to cat X and Y cells, respectively (Dreher et al., 1976; Sherman et al., 1976; Merigan and Maunsell, 1993; Levitt et al., 2001). Even though the strict application of linear spatial summation tests suggests the existence of two populations (linear and non-linear cells) in the magnocellular layers of primates (Shapley et al., 1981; Kaplan and Shapley, 1982), other studies have shown that the distribution of linearity is unimodal within magnocellular or parvocellular neurons (Levitt et al., 2001). In primates, processing is separated into at least one pathway for depth and motion and one for space and detail: the magnocellular and parvocellular pathways, respectively (Livingstone and Hubel, 1988; Callaway, 1998). Within the parvocellular population, color processing is also "parallelized" (Dacey, 2000; Nassi and Callaway, 2009).

Similar organization into parallel pathways has also been found in ferret, squirrel, and rat (reviewed in Van Hooser, 2007). However, little physiological evidence for parallel streams in mouse dLGN exists (Grubb and Thompson, 2003; Piscopo et al., 2013), despite evidence for multiple dLGN morphological populations (Krahe et al., 2011) and the clustering of visual response properties in cells of mouse primary V1 that suggests a parallel organization (Gao et al., 2010). Given the rising prominence of the mouse visual system as a tool

for understanding visual processing (Niell and Stryker, 2008, 2010; Liu et al., 2010, 2011; Huberman and Niell, 2011; Niell, 2011; Polack et al., 2013), cortical structure and function (Sohya et al., 2007; Cardin et al., 2009; Sohal et al., 2009; Marshel et al., 2011; Adesnik et al., 2012; Bock et al., 2012; Olsen et al., 2012), and visually-guided behavior (Dombeck et al., 2007; Andermann et al., 2010; Busse et al., 2011; Lee et al., 2012; Carandini and Churchland, 2013; Saleem et al., 2013), it is important to characterize the output of mouse dLGN, if that output is organized into parallel streams when projecting to V1, and how that organization compares to what is known in other species. Towards this end, recent studies have identified direction and orientation selective cells (Marshel et al., 2012; Cruz-Martin et al., 2014) that may be analogous to koniocellular or W cell pathways and a diversity of response properties (Piscopo et al., 2013) in mouse dLGN.

Here, we present evidence for the existence of parallel streams in the retinogeniculate pathway of the mouse, however, the clustering of visual response properties of mouse dLGN neurons suggest a less segregated relay of visual information to primary V1. We recorded the spiking responses of single dLGN cells to electrical stimulation of the optic chiasm and to visual stimuli including spatiotemporal noise, drifting sinusoidal gratings, counterphased sinusoidal gratings, and a spatially-uniform flicker sequence. We first classified our cells based on the linearity of spatial summation to a counterphased modulating sinusoidal grating, and observed approximately 9:1 more X-like than Y-like cells, which we called linear and non-linear. However, these classes showed little difference in spatial and temporal and contrast response properties as well as in their receptive field parameters. Finally, with the possible exception of a subset of slower-responding linear cells, most cells recorded in mouse dLGN responded with approximately equal precision and reliability (Kumbhani et al., 2007).

MATERIALS AND METHODS

Animal Preparation and Surgery

All procedures were done within the guidelines of the National Institutes of Health and were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Adult C57/B6 mice (8–24 weeks) were anesthetized with a high concentration of isoflurane (5%) and maintained with continuous inhaled isoflurane (0.8–1.2%). The depth of anesthesia was monitored using heart rate (maintained between 300 and 600 beats/min), pupil size, pinch reflex, and following the opening of the craniotomy, by the level of synchronous activity in the LFP. After placement in a stereotactic apparatus, eye moisture was maintained by application of a transparent lubricant and body temperature was maintained at 37°C by rectal monitoring and a heating pad (FHC Inc., Bowdoin, ME, USA). A 2-by-3 mm craniotomy was opened over dLGN. Following surgery, the entire stereotactic apparatus was rotated 60° to position the contralateral eye in front of the display screen.

Electrophysiology

An array of four to six tetrodes (Thomas Recording GmbH, Giessen, Germany) arranged concentrically was inserted perpendicularly relative to the cortical surface. In both configurations, the tip-to-tip space between neighboring tetrodes was 254 μm . Individual tetrodes were 100 μm in diameter with a central contact at the tip 40 μm below three concentrically arranged contacts around the shaft 20 μm from each other. Signals were preamplified by the tetrode drive and amplified, individually filtered, and acquired at 30 kHz using a Cheetah 32 acquisition system (Neuralynx, Bozeman, MT, USA). High-frequency spiking activity was isolated at each contact by filtering between 600 and 6000 Hz. A single channel from each tetrode was duplicated and filtered 0.1–375 Hz to record an LFP. Following a rest period of at least 30 min, each tetrode was lowered through the neocortex and hippocampus until audible modulation of background activity to a test stimulus was apparent. Tetrodes were further lowered until at least one isolatable unit appeared.

Visual Stimuli

All visual stimuli were generated using the ViSaGe stimulus generation hardware (Cambridge Research Systems, Cambridge, UK) and a custom software package utilizing the accompanying MATLAB (Mathworks, Natick, MA, USA) toolbox. Stimuli were displayed on a 19-inch cathode ray tube monitor configured to refresh at 100 Hz at 600 $\times$ 800 resolution. This monitor was gamma-corrected using a luminometer and ViSaGe configuration software and placed 30 cm from the eye contralateral to the craniotomy. Full-screen stimuli covered approximately 70° of visual field. After tetrode insertion, the screen was set to a background of 50% luminance. Stimuli consisted of drifting sinusoidal gratings, stationary contrast-reversing gratings (i.e., counterphased), two-dimensional ternary white noise, and a spatially-uniform contrast modulating flicker stimulus. Counterphased sine-wave gratings were the size of the display ($\sim 70^\circ$), had variable spatial phase, and their contrast was reversed at 2 Hz following a square wave. Ternary white noise and spatially uniform flicker updated at 50 Hz. For ternary white noise the contrast each 50 $\times$ 50 pixel square was chosen for each frame independently of the previous frames and other pixels in that frame. Flicker stimuli consisted of a repeated sequence of contrasts; this sequence was generated by choosing randomly from a flat distribution of contrasts.

Electrical Stimulation

Electrical stimuli were delivered through a bipolar stimulating electrode inserted proximal to the optic chiasm through independent burrhole craniotomies made with 500 μm of bregma. Each lead was connected to a stimulus isolation unit controlled by a Master-8 pulse stimulator (A.M.P.I., Jerusalem, Israel). Stimulation was monophasic and the duration was 50 μs . Initially large stimulus intensities (2 mA) were stepped down in ~ 0.1 mA increments in order

to determine the sensitivity of components of the dLGN response.

Spike Clustering and Data Analysis

Spike waveforms from each tetrode were clustered into individual units offline using a mixture of algorithmic and manual sorting (Spike- Sort3D, Neuralynx). Waveforms were initially sorted using KlustaKwik and subsequently manually refined. All clusters with spikes in the 0–1 ms bin of the interspike interval histogram were strictly rejected. To assess the quality of separation of the identified single units, we measured isolation distance and the L-ratio for each cluster, which indicate the distance of the center of the cluster from the noise and the quality of the moat around the cluster, respectively (Schmitzer-Torbert et al., 2005). Linearity of spatial summation was measured using the frequency components of the response to counterphased, stationary sinusoidal gratings (Shapley and Hochstein, 1975). The analyses of responses to drifting gratings and ternary white noise were performed as elsewhere (Denman and Contreras, 2014).

RESULTS

In order to investigate the organization of the mouse retinogeniculate pathway in relation to functional parallel streams, we recorded LFPs and single units from the dLGN using an array of independently-positionable tetrodes in isoflurane-anesthetized mice ($n = 18$). We studied responses to electrical stimulation of the optic chiasm and to a battery of visual stimuli. These recordings yielded 311 single units and 24 multi-unit clusters consisting of a mixture of spikes from several cells. Unless otherwise noted, the analyses described below were all performed on isolated single units.

Electrical Stimulation of the Optic Chiasm

In the dLGN of cat (Bishop and McLeod, 1954) and primate (Reese and Cowey, 1990), the afferent fibers from retinal ganglion cells have a non-unimodal distribution of conduction velocities, which corresponds to the non-unimodal distribution of retinal axon diameters in the optic tract (Bishop, 1933, 1946; Bishop et al., 1953; Bishop and Clare, 1955; Guillery et al., 1982). Detailed anatomical characterization of C57/B6 retinal axons suggest a bimodal distribution of axonal diameters (Seecharan et al., 2003), but to our knowledge no study of mouse dLGN activity evoked by electrical stimulation of the optic nerve has been published.

To test for the presence of functionally distinct neuronal populations in mouse dLGN, we first analyzed the responses to electrical stimulation of the optic chiasm. We estimated the distance along the optic tract from the stimulating electrode to the dLGN to be 4.5 mm, according to the placement of the stimulating electrode just caudal to bregma (**Figure 1A**), the online 3D mouse brain atlas (Allen Institute 3D Connectivity Atlas) and published measurements of the mouse optic nerve (Kurimoto et al., 2010). We also verified the placement of

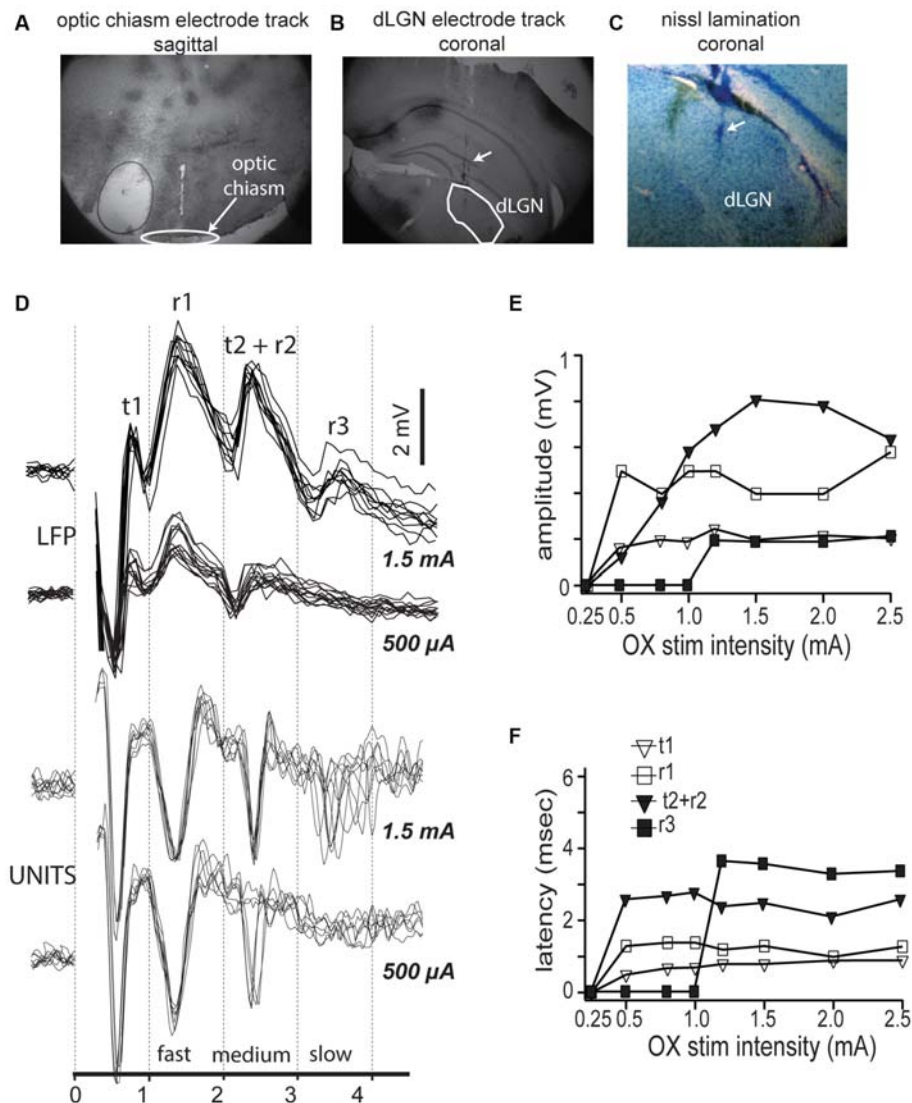
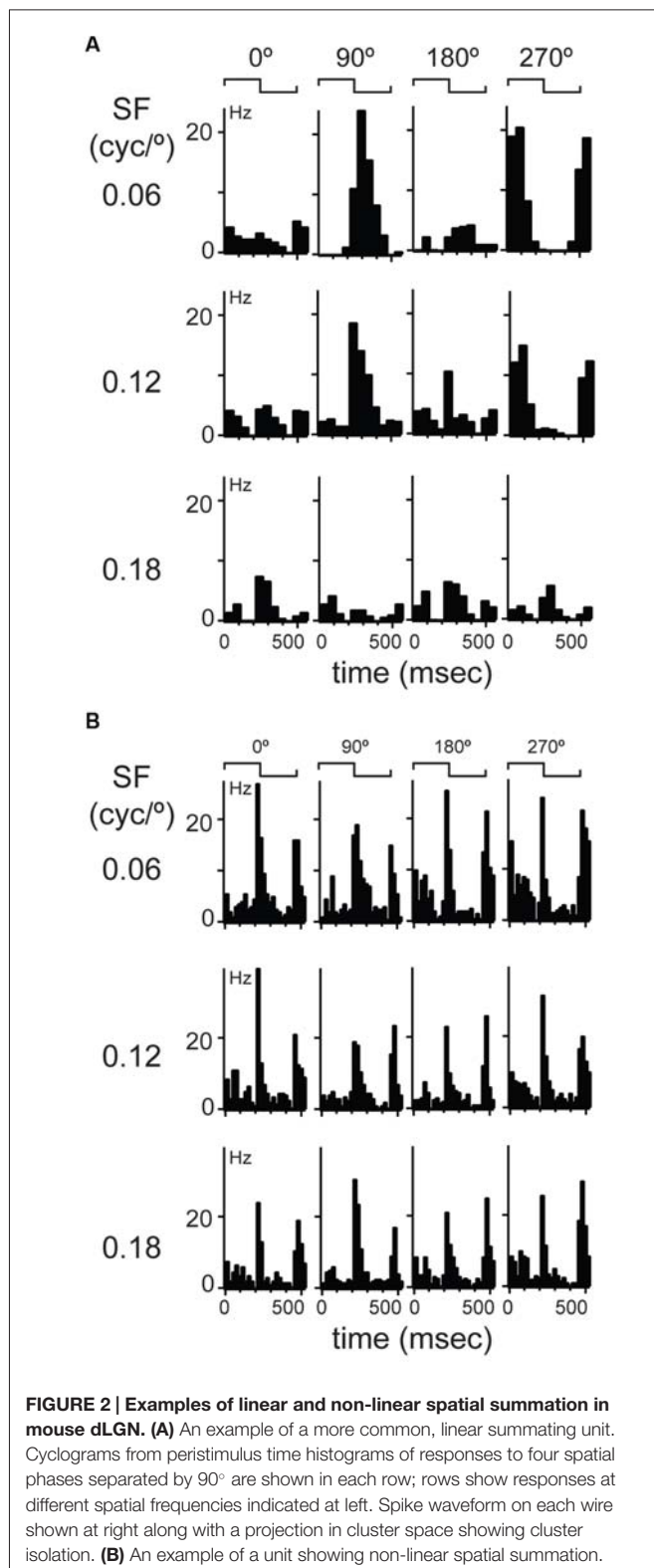


FIGURE 1 | Optic chiasm stimulation reveals three compound field responses and corresponding spike responses in mouse dorsal lateral geniculate nucleus (dLGN). (A) Electrode track showing positioning of stimulating electrodes at optic chiasm fibers. (B) Electrode tracks showing multiple tetrode placement into dLGN. (C) Nissl stain of dLGN showing lack of obvious lamination. (D) Top traces, Local field activity in mouse dLGN following dLGN stimulation, at two stimulus intensities: 500 μ A and 1.5 mA. Local field potential (LFP) shows four response components. Bottom traces, spikes recorded in LGN in response to same two intensities, overlaid traces to repeated stimuli show three latencies corresponding with the three LFP components. (E,F) Measurement of response component amplitudes (E) and latencies (F).

our electrodes into dLGN histologically after each experiment (Figure 1B). We saw no obvious lamination in mouse V1 (Nissl stain, Figure 1C). Based on our distance estimation and the conduction velocities for the two primary axonal populations measured in other species (Gouras, 1969), 3.8 and 1.8 m/s, we expected to see electrically evoked responses with latencies of 1.2 and 2.5 ms. Bipolar LFP responses showed four overlapping peaks similar to those described in cat (Bishop and McLeod, 1954; their Figure 2A; here we follow their original nomenclature). An early positive-negative peak that corresponds with the arriving volley of fast conducting fibers, the t_1 component, followed by the synchronous postsynaptic potential

in dLGN neurons, the negative-positive potential r_1 . The subsequent large and less precise negative field (r_2) corresponds with the postsynaptic potential triggered by the slower fibers, which in cats lead to a visible tract volley (t_2 ; Figure 1D, bottom). Thus, both in mice and rats (Sefton and Swinburn, 1964), r_2 and t_2 appeared fused. Finally, at longer delays we see r_3 , which corresponds with postsynaptic potential of slower conducting fibers. The peak r_1 reliably followed the presynaptic volley t_1 at low stimulation intensities (Figure 1D, top), but t_2 , r_2 and r_3 were only present at higher stimulation intensities (Figure 1D, top), an intensity dependence relationship similar to that described by Bishop and McLeod (1954) and Bishop et al. (1959). This



activation sequence was not modified by switching the stimulus polarity (not shown). Strength response curves for the different components revealed that the fastest component, t_1 had the lowest stimulation intensity threshold (**Figure 1E**), and that r_1

only occurred after t_1 , and t_2 after r_2 , and r_3 only occurred at higher intensities (**Figure 1F**).

X and Y cells in cat (Cleland et al., 1971; Stone and Hoffmann, 1971), tree shrew (Sherman et al., 1975), as well as parvo and magnocellular cells in primate dLGN (Dreher et al., 1976; Sherman et al., 1976; Levitt et al., 2001), have statistically different mean response latencies to electrical stimulation of retinal axons. We examined the latency of dLGN spike responses to optic chiasm electrical stimulation (**Figure 1D**, bottom). We saw fast (~ 700 μ s) spikes occurring at delays of 1.2, 2.2, and 3.5–4.0 ms after optic chiasm stimulation, consistent with spike latencies reported in cat (Hoffmann and Stone, 1971) and rat (Fukuda et al., 1979; Hale et al., 1979; Crunelli et al., 1987). Reversing the polarity of the bipolar stimulus changed the stimulus intensities necessary to elicit spike responses (not shown) but the responses had the same latencies and amplitudes.

Classification of Units with the Modified Null Test

The observation of consistent response components in LFP and multiunit recordings in mouse dLGN suggests distinct populations of relay cells, in agreement with a previous anatomical study (Krahe et al., 2011). To classify dLGN units physiologically, we first utilized the modified null test (Enroth-Cugell and Robson, 1966; Cleland et al., 1971; Shapley and Hochstein, 1975). We presented stationary gratings with a sinusoidal modulation of contrast across space and a square modulation in time (counterphased, period = 0.5 s; contrast = 100%). The gratings were presented at 11 spatial phases, with 30° phase increments, and at four spatial frequencies (0.06 cycles/°, 0.12 cycles/°, 0.18 cycles/°, and 0.24 cycles/°). In cats, a dLGN cell is classified as an X-cell by the presence of at least one spatial phase that elicits no response to the temporal modulation of the grating. The presence of such null-phase indicates that the cell sums contrast inputs linearly over space. The majority of our cells had at least one null-phase at one of the tested spatial frequencies (277/311). We called these cells linear cells. We resisted the temptation of calling them X-like because, as will be shown below, their response properties were not clustered uniformly around expected values characteristic of X cells. The example dLGN neuron shown in **Figure 2A** showed the largest response at 0.06 cycles/° and at a 90° phase. This cell had two null-phases 90° away from the maximum, at 0 and 180°. Responses were robust at the 0.06 and 0.12 cycles/° but this cell did not respond at the highest spatial frequency of 0.18 cycles/°.

A subset of dLGN cells (34/311) did not display a null-phase, such as the example shown in **Figure 2B**, indicating that these cells do not perform linear summation of their inputs over space. Furthermore, at all spatial phases and all spatial frequencies, these cells responded to both contrast reversals during a stimulus cycle, thus leading to a response at twice the temporal frequency of the grating (**Figure 2B**). In cats (So and Shapley, 1979) and primates (Shapley et al., 1981; Kaplan and Shapley, 1982) these LGN cells are called Y cells. Here, we called them non-linear cells because, as will be shown below,

their visual response properties were not uniformly consistent with this category in other species. The extracellular waveforms of putative linear and non-linear cells were not significantly different in amplitude of the rising phase, ratio of peak-to-trough, or the slope of the repolarization phase (data not shown).

Linearity of Spatial Summation

X cells respond to the contrast reversal of a sinusoidal grating at the modulation frequency of the grating (Shapley and Hochstein, 1975; So and Shapley, 1979; Kaplan and Shapley, 1982), so that their response is dominated by the fundamental frequency (F1, or first harmonic) of the stimulus. This modulation at F1 is greater than mean firing rate (F0 or DC). Furthermore, the F1 component of an X cell response is modulated sinusoidally as a function of the spatial phase of the stimulus. The example linear cell shown in **Figure 2A** had a sinusoidal modulation of its response F1 as a function of spatial phase (**Figure 3A**, filled circles), with a much smaller change in mean firing rate (**Figure 3A**, DC, red symbols). This unit's response had a small F2 component (**Figure 3A**, open symbols) also modulated by the spatial phase, but overall the response was dominated by the F1 component.

Y cells respond to contrast reversal of the grating at twice its modulation frequency (Shapley and Hochstein, 1975; So and Shapley, 1979; Kaplan and Shapley, 1982). This leads to a response dominated by the second harmonic (the F2 component) of the modulation frequency of the grating. Furthermore, the F2 component is independent of spatial phase. For example, the non-linear unit illustrated in **Figure 2B** showed a response dominated by the F2 component (**Figure 3B**, open symbols), which was larger than both the F1 (**Figure 3B**, filled symbols) and DC (**Figure 3B**, red symbols) components and remained constant across spatial phases. The DC component of the response was also constant across spatial phases.

We measured the linearity of spatial summation for all units as the peak of the F2/F1 ratio across all spatial frequencies (Van Hooser et al., 2003). A ratio above 1 indicates non-linear spatial summation (**Figure 3C**, dotted line) and a ratio below 1 indicates linear summation. The linear unit in **Figure 4A** had a linearity index of 0.24 (**Figure 3C**, "A") and the non-linear unit in **Figure 4B** had a linearity index of 2.0 (**Figure 3C**, "B"). Our population showed a unimodal distribution and was dominated by linear cells (277/311); we identify for the first time a population of non-linear cells in mouse dLGN (34/311).

Response Properties of Linear and Non-linear Cells

In addition to the distinction based on response latency and linearity of spatial summation, functionally distinct dLGN populations in other species show differences in their contrast sensitivity and their selectivity to spatial and temporal frequency (Cleland et al., 1971; Sherman et al., 1975, 1976; Dreher et al., 1976; Derrington and Lennie, 1984; Price and Morgan, 1987; Livingstone and Hubel, 1988; Levitt et al., 2001). Typically, X cells

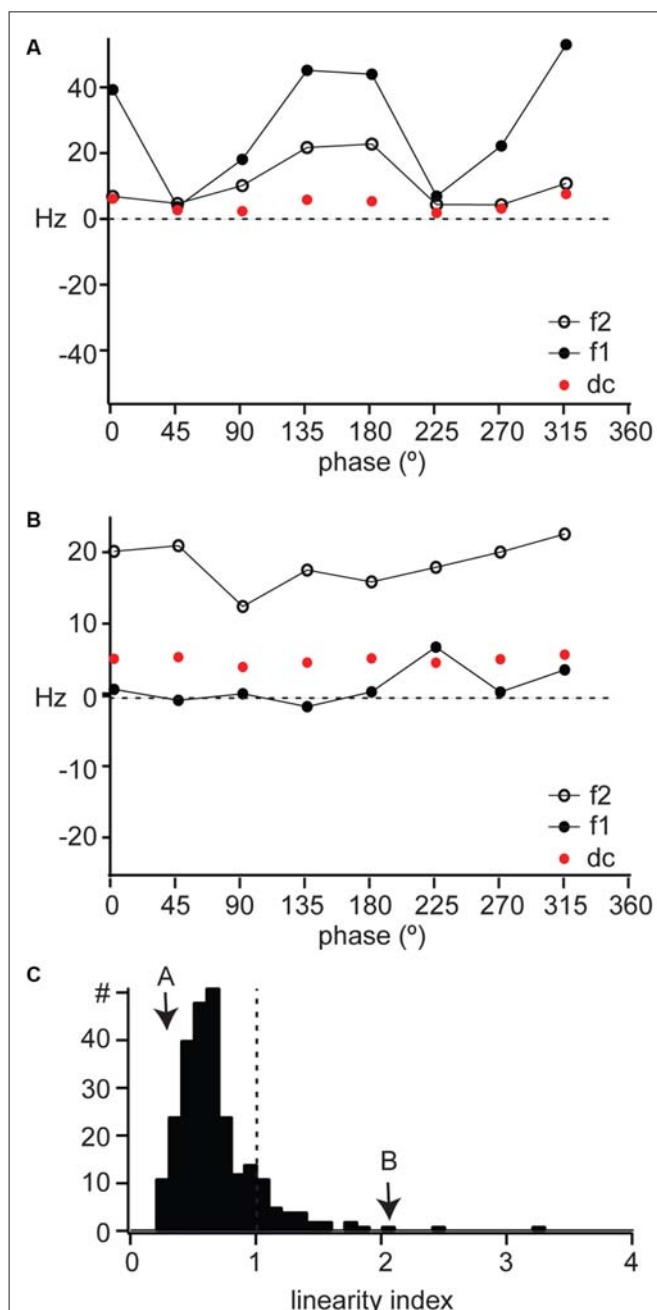


FIGURE 3 | Linearity of spatial summation in mouse dLGN. (A,B) The DC, F1, and F2 components of the response to counterphased gratings across all spatial phases for the example cells shown in **Figure 2**. **(C)** The distribution of linearity index across our population of dLGN single units, with the examples in parts **(A)** and **(B)** indicated with arrows. A linearity index above 1 indicated non-linear spatial summation.

respond better to higher spatial and lower temporal frequencies and have high contrast sensitivity, while Y cells prefer higher temporal and lower spatial frequencies and have high contrast sensitivity, though significant overlap between these pathways has also been reported (Bullier and Norton, 1979). We probed single units in mouse dLGN with a battery of gratings that

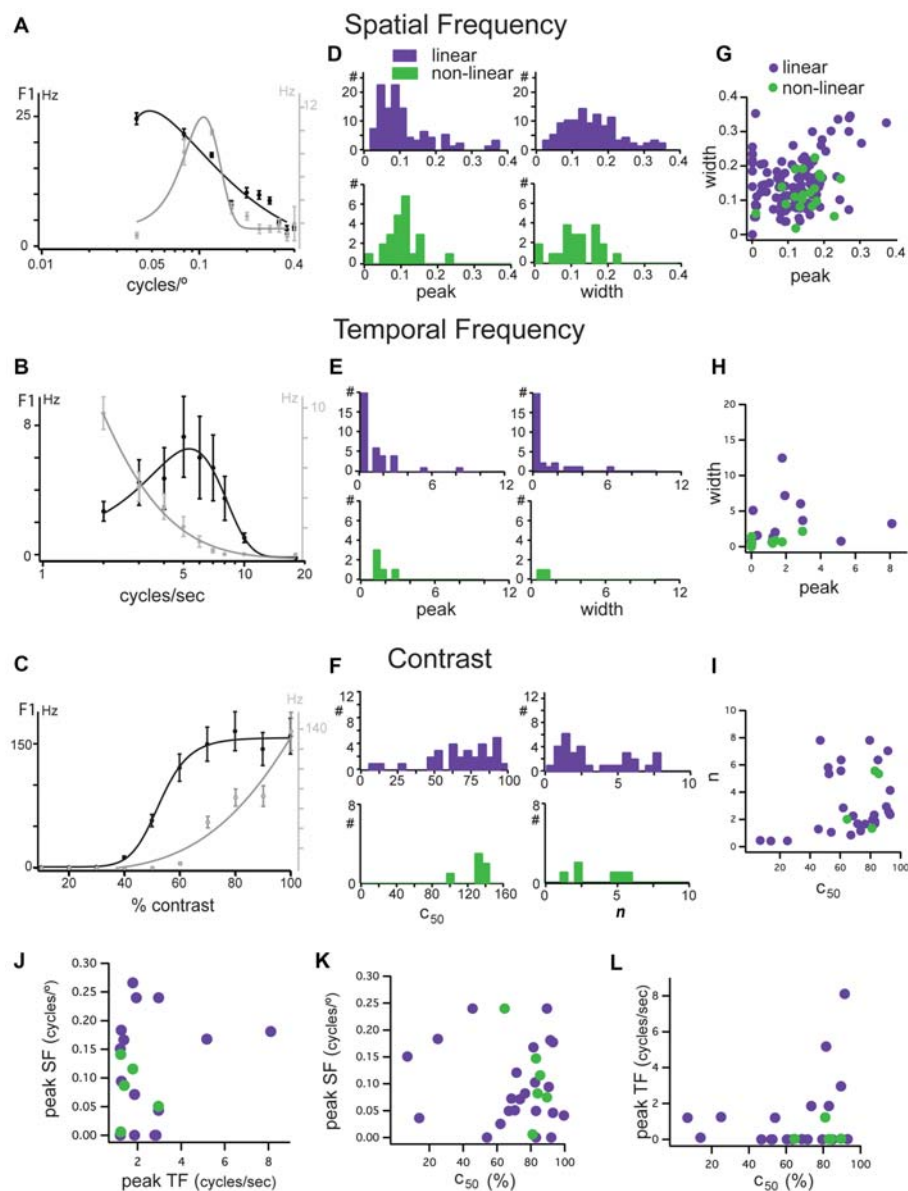


FIGURE 4 | Tuning characteristics of linear and non-linear units in mouse dLGN. (A–C) Examples of opposing spatial frequency, temporal frequency, and contrast tuning. Two units shown, one a low-pass unit in black and bandpass in gray. Same two units in each panel. **(D)** Distributions of peak spatial frequency (left) and width of spatial frequency tuning (right) for cells classified as linear (purple bars) and non-linear (green bars) using the modified null test, measured from fits to spatial frequency tuning plots. **(E)** Distributions of peak temporal frequency (left) and width of temporal frequency tuning (right) for cells classified as linear (purple bars) and non-linear (green bars) using the modified null test, measured from fits to temporal frequency tuning plots. **(F)** Distributions of C_{50} (left) and n parameters (right) of contrast response functions for cells classified as linear (purple bars) and non-linear (green bars) using the modified null test. **(G)** Correlation of spatial frequency tuning width with peak spatial frequency, taken from fit parameters. **(H)** Correlation of temporal frequency tuning width with peak spatial frequency, taken from fit parameters. **(I)** Correlation of the slope and C_{50} of hyperbolic ratio fits of contrast response functions. **(J)** Correlation of peak spatial frequency with peak temporal frequency. **(K)** Correlation of peak spatial frequency with C_{50} . **(L)** Correlation of peak temporal frequency with C_{50} .

varied in spatial frequency, temporal frequency, and contrast (Figure 4).

In some cells, we observed a clustering of response properties such as those exemplified by the two linear neurons in Figures 4A–C. The linear neuron depicted in black had a low-pass selectivity for spatial frequency with a peak response at 0.05 cycles/° (Figure 4A), a high temporal frequency preference

with a peak at 5 cycles/s (Figure 5B) and high contrast sensitivity ($C_{50} = 54\%$; Figure 4C). The linear neuron depicted in gray was band-pass for spatial frequency, with a higher peak spatial frequency of 0.12 cycles/° (Figure 4A), low-pass for temporal frequency with a peak response at 2 cycles/s (Figure 4B), and a higher contrast sensitivity ($C_{50} = 89\%$; Figure 4C). While these cells seem to match X-like (the gray

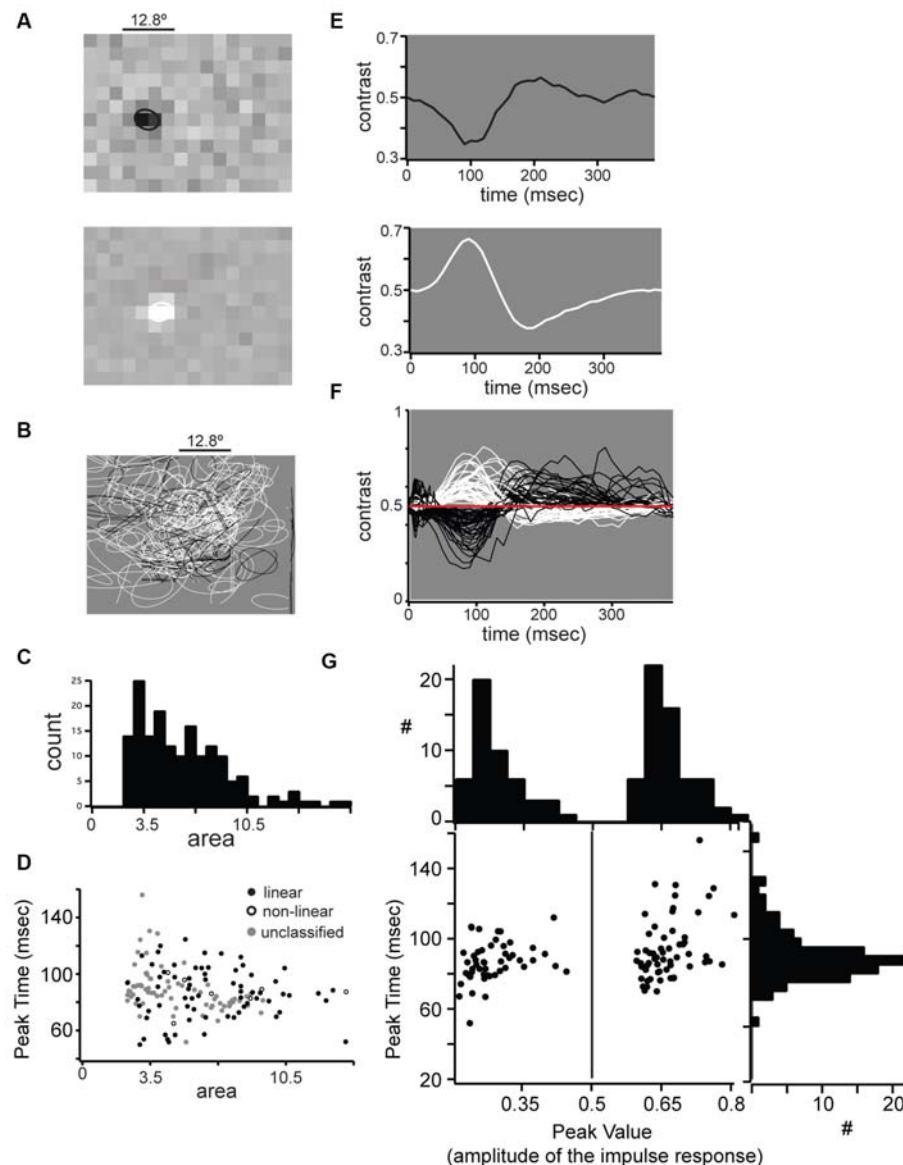


FIGURE 5 | Receptive field properties of single units in mouse dLGN. (A) Example spatial receptive fields from an OFF-center (top) and ON-center (bottom) cell. **(B)** All spatial receptive fields from our population. For each cell the 0.5 level contour from a two-dimensional Gaussian fit is shown. **(C)** Distribution of receptive field areas in mouse dLGN, calculated from two-dimensional Gaussian fit parameter. **(D)** No difference in linear, non-linear, and unclassified receptive field areas. **(E)** Impulse response function from the center of the example receptive fields shown in part **(A)**. **(F)** All impulse responses from our population. **(G)** Bimodal distribution of impulse response absolute maxima, but unimodal distribution of maximum time.

cell) and Y-like (the black cell) properties, both had null spatial phases to counterphased gratings and were classified as linear.

Analysis of the population revealed three important features: (1) In mouse dLGN, stimulus preferences were not correlated with null-test based classification. Distributions of peak spatial frequency (Figure 4D, left), spatial frequency bandwidth (Figure 4D, right), peak temporal frequency (Figure 4E, left), temporal frequency bandwidth (Figure 4E, right), the mid-saturation point (c_{50} ; Figure 4F, left), and the slope (n)

of the contrast response function (Figure 4F, right) were not statistically different between linear and non-linear cells ($p > 0.05$, Wilcoxon rank test). Furthermore, unlike in other species, we found that both linear and non-linear cells span a broad range of stimulus preferences; (2) We did not observe a consistent correlation between the peak and the width of the spatial (Figure 4G) or temporal (Figure 4H) frequency selectivity, nor between the mid contrast and slope of the contrast response functions (Figure 4I), meaning that cells with high spatial and/or temporal frequency did not

necessarily have narrower tuning curves; and (3) We did not observe a clustering of visual response properties, as shown by the lack of correlation between selectivity to spatial and temporal frequency or contrast sensitivity (Figures 4J–L). Due to the high degree of overlap in stimulus preferences between linear and non-linear cells it is likely that each of postulated parallel channels in mouse V1 (Gao et al., 2010) receive their thalamic inputs from a mixture of linear and non-linear dLGN cells.

Receptive Field Properties

We mapped the RF of dLGN units with reverse correlation on spikes elicited by dense ternary noise (Figure 5). To quantify RF size we fit the RFs with a 2-dimensional Gaussian and used the square of σ as a measure of RF area; the example ON-center and OFF-center linear units in Figure 5A had RF center areas of 7.8 and 7.6°², respectively. The areas of RF centers for the population span from 3.5 to 20.4°² (Figure 5B) with a unimodal distribution and a median of 8.3°² (Figure 5C). Thus, in mouse dLGN, linear and non-linear units could not be distinguished from each other, nor from unclassified units, based on RF size (Figure 5D).

The time course of the responses of ON- and OFF- cells was similar as shown by the impulse response of two example linear cells (Figure 5E) and the superimposed impulse responses of the population (Figure 5F). We compared the impulse response functions of ON- and OFF-center cells by plotting the peak amplitude vs. peak time (Figure 5G). While ON and OFF cells were distinguished by the polarity of the peak amplitude (along the x -axis), the distribution of peak times (along the y -axis) was unimodal with a mean of 83.9 ± 11.8 ms, showing that ON and OFF cells show similar response time course. Unlike previous reports (Piscopo et al., 2013), we observed transient and sustained temporal profiles from both ON and OFF cells. Furthermore, the time course of the impulse response did not distinguish between linear, non-linear or unclassified neurons (Figure 5G, distribution along the y -axis).

Precision and Reliability

In cats, Y cells have slightly higher precision and reliability than X-cells when tested with a stimulus with rapidly changing contrast (Reinagel and Reid, 2000; Kumbhani et al., 2007). Such differences are in part attributed to the higher temporal resolution of Y-cells in that species. We used a full screen flicker stimulus consisting of spatially homogeneous stimulus whose contrast varies rapidly (50 Hz), drawing from an even distribution of contrasts. Linear units responded robustly to repeated presentations of the same stimulus sequence (Figure 6A, raster plots), giving rise to clear distinguishable events in the accumulated PSTH (Figure 6A, bottom row, 1 ms bins). These events were at much higher firing rates than the background as seen by the period before time zero in the PSTH.

To estimate precision and reliability we identified events from the PSTH based on a threshold and fit each event with

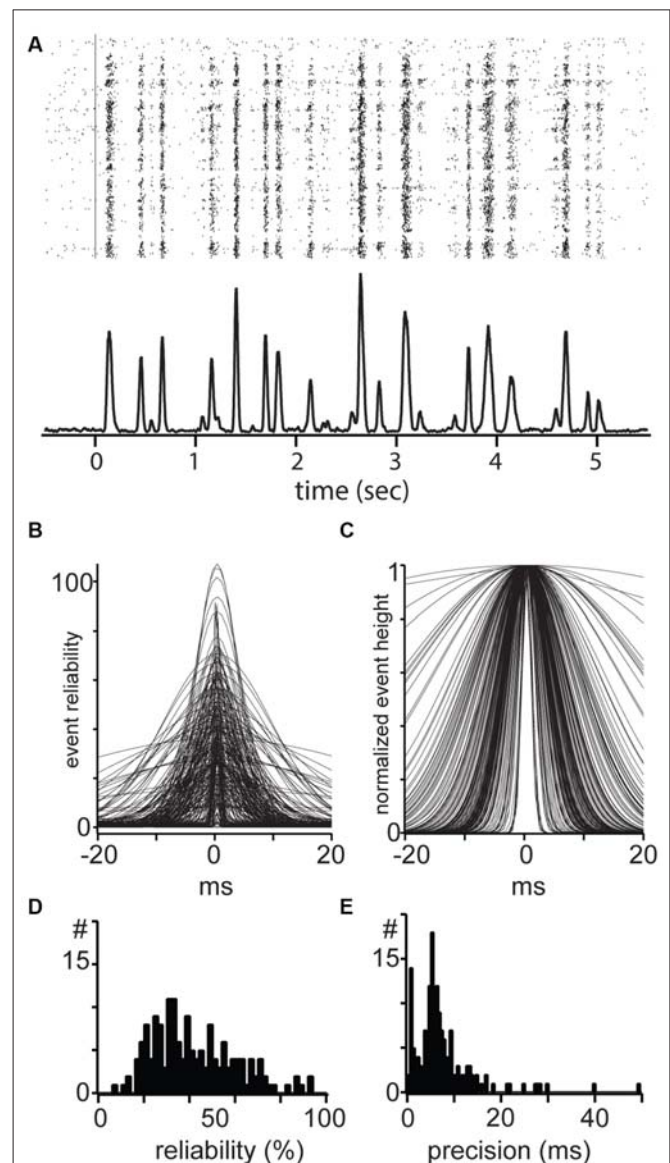


FIGURE 6 | Reliability and temporal precision of mouse dLGN single units. (A) Example response of a mouse dLGN cell to spatially uniform flicker. **(B)** All events from all recorded mouse dLGN units to flicker stimulus; each identified event has been fit with a Gaussian, aligned, and overlaid. **(C)** All events, normalized to the maximum of each event to show the temporal precision of each event. **(D)** Distribution of event reliability. **(E)** Distribution of width from the Gaussian fit to each event.

a Gaussian. These fits for all the events and from all cells are shown in Figure 6. Here, event reliability is measured as the height of the fit, which represents the percent of trials in which a spike occurred (Figure 6B). After normalizing for differences in reliability (Figure 6C), we used the width of the events as an estimate of spike precision of the total spikes in the event.

For each cell, we took the percent of stimulus presentations in which there was at least one spike in the event window (± 30 ms around the peak). This measure of reliability quantifies

the reproducibility of the entire spike train in the response, independent of variability within each event. The population of dLGN cells was distributed along a range of reliability from 3% to 94%, with a median of 31.6% and mean of $35.5 \pm 23.0\%$ (**Figure 6D**). The distribution of precision of all events in all cells spanned the range of 0.5–50 ms and showed a unimodal distribution with a median of 7.9 ms and a mean of 8.8 ± 6.6 ms (**Figure 6E**). In conclusion, we did not observe a difference in precision or reliability between linear and non-linear cells.

DISCUSSION

In this study, we have used electrical and visual stimulation to study parallel processing in mouse dLGN and find evidence for parallel pathways in only partial homology with cats and monkeys. We find that in the mouse, three distinct populations of dLGN neurons could be distinguished on the basis of the latency of response to electrical stimulation of the optic chiasm. We find that mouse dLGN is dominated by neurons that perform linear spatial summation, though we do observe a subpopulation with non-linear spatial summation properties and frequency doubling. Unlike in cats and primates, linearity of spatial summation did not correlate with RF size, response precision, spatial and temporal selectivity, or contrast sensitivity.

Retinal Basis of Mouse dLGN Parallel Streams

In both primates and cats, parallel processing streams are established in the retina. In the macaque, retinal ganglion cells can be distinguished morphologically; the predominant class is the “midget” (or type III or B-type) ganglion cell, which is smaller than the “parasol” (or type II or A-type) ganglion cell (Levanthal et al., 1981; Watanabe and Rodieck, 1989). These cells are also distinguished by their response properties: midget cells tend to have sustained responses to flashed spots, whereas parasol cells display transient responses (De Monasterio and Gouras, 1975). In cats, X and Y type retinal ganglion cells are distinguished by morphology, and by the linearity of spatial summation (Enroth-Cugell and Robson, 1966) and the transient or sustained nature of their responses (Cleland et al., 1971). Like primate and cat retinae, mouse retina contains >20 retinal ganglion cell types (Sun et al., 2002; Völgyi et al., 2009). Morphometric analyses of soma size and dendritic field shape suggest that these types include homologs of A and B type primate retinal ganglion cells and X and Y type cat cell ganglion cells. Recordings from mouse retina validate this morphological evidence: sustained and transient ganglion cells have been observed in the mouse retina (Balkema and Pinto, 1982). In addition, non-linear spatial summation is observed in a subset of mouse retinal ganglion cells (Stone and Pinto, 1993). It is therefore reasonable to hypothesize the continuation of parallel streams into mouse dLGN. Indeed, the percentage of X-like ganglion cells reported by Stone and Pinto (1993); 87% agrees well with percentage

of linearly summing cells in mouse dLGN (89%; present study).

Similarities between Mouse dLGN and those of other Mammals

We noticed several similarities between dLGN of mouse and dLGN of domestic cat. Multiple component field responses were first observed following optic chiasm stimulation in the cat (Bishop and McLeod, 1954; Bishop et al., 1959). Similarly, we observed compound responses in the field potential in mouse dLGN. While we were unable to measure spike latencies from isolated single units, we did see multiple compound high-frequency spikes with distinct latencies. Direct comparison of the observed mouse potentials with those from other species (Bishop and McLeod, 1954; Hale et al., 1979) is complicated by the small size of structures in mouse and the arrangement of cat and primate dLGN in distinct horizontal layers with vertical optic tract input. Stimulation of mouse optic chiasm may spread to the nearby optic nerve or optic tract and the volleys may arrive from both sides with slightly different delays. In the mouse, the lack of lamination may result in different arrangement of inputs and differences in waveforms compared to those of cat and monkey.

Furthermore, in cat retina (Enroth-Cugell and Robson, 1966) and dLGN (Shapley and Hochstein, 1975) cells have been classified on the basis of linearity vs. non-linearity of spatial summation within their receptive fields. We were able to identify 11% of mouse dLGN units as non-linear. The encounter rate of non-linear cells in the mouse dLGN (and retina) is thus much lower than encounter rate of Y cells (defined on other grounds than non-linearity of spatial summation) in dLGN of cats (48%; Sireteanu and Hoffmann, 1979), substantially lower than encounter rate of Y cells (defined on the basis of non-linearity of spatial summation) in dLGN of ferrets (23%; Price and Morgan, 1987) as well as substantially lower than encounter rate of Y-like cells (defined on other grounds than non-linearity of spatial summation) in dLGN of rats (27%; Hale et al., 1979).

Both of these factors, conduction velocity and linearity of spatial summation, can also distinguish streams in the macaque retinogeniculate pathway. In macaque a 25% subset of magnocellular cells are non-linear (Kaplan and Shapley, 1982), making the total percentage of non-linear cells ~8%, a number closer to the observed frequency in mouse. Like the percentage of linear cells we observe in mouse dLGN, and the number of B-type ganglion cells in mouse, the percentage of midget cells in the macaque retina is ~90% (Dacey, 1994). The great majority of macaque magnocellular and virtually all macaque parvocellular cells are reported to exhibit linear spatial summation (e.g., Kaplan and Shapley, 1982). Nevertheless, macaque's magnocellular dLGN neurons could be easily distinguished from the parvocellular neurons on the basis of their spatial frequency preferences and contrast sensitivity (Livingstone and Hubel, 1988). By contrast, we did not see obvious separation of spatiotemporal profiles

within the mouse dLGN linear population. However, it should be noted that the clear-cut separation in spatial frequency preferences between the magnocellular and parvocellular cells in macaque's dLGN applies only when comparisons are made between magnocellular and parvocellular cells with receptive field at the same eccentricities. Mice, unlike virtually all primates, do not have fovea and there is very shallow center-periphery gradient in the density of their retinal ganglion cells density (Dräger and Olsen, 1981). Furthermore, in mouse, unlike in primates, neither somal sizes nor dendritic tree sizes of retinal ganglion cells increase substantially with lowering ganglion cell density (Sun et al., 2002). Thus, it is very unlikely that in the mouse, eccentricity differences in the retinal ganglion cell density would create substantial differences in spatio-temporal profiles of dLGN cells with receptive fields in different parts of the visual field.

Whether the ~90% of linearly responding cells in mouse dLGN more closely resemble magno or parvocellular cells is not immediately clear. Unlike macaque magnocellular and parvocellular populations, which are linear in spatial summation (Kaplan and Shapley, 1982) but identifiable on the basis of spatial frequency preference and contrast sensitivity (Livingstone and Hubel, 1988), we saw no obvious separation of spatiotemporal profiles within the mouse dLGN linear population. However, it should be noted that this separation in macaque depends strongly on eccentricity, which we did not account for here. Although mice do not have a fovea, there are differences in retinal ganglion density that may create differences in tuning across visual space (Bleckert et al., 2014) and very recently, Zhang et al. (2015) reported that the spatial frequency cut-offs of mouse V1 neurons with receptive fields in the upper visual field (where mouse aerial predators are likely to lurk) is substantially higher than that of V1 neurons with receptive fields in the lower visual field. It should further be noted that overlap in the spatial and temporal tuning properties has also been reported in the visual pathways of squirrels (Van Hooser et al., 2003), rats (Hale et al., 1979), rabbits (Swadlow and Weyand, 1985), cats (Bullier and Norton, 1979), galagos (Irvin et al., 1993) and even macaques (Hicks et al., 1983). We did not explicitly measure transience, but the qualitative transience of responses in our population (e.g., **Figure 2**) leads us to hypothesize that mouse dLGN is dominated by magnocellular-like cells.

Our results in mouse dLGN are similar to those seen in rat dLGN (Hale et al., 1979), where three groups of cells can be distinguished by conduction velocities of their retinal afferents but not the sizes of their receptive fields. It has been argued that this homogeneity indicates a lack of an X-like pathway and dominance of Y-like and W-like pathways. Further, some anatomical evidence points to the lack of an X-like pathway in rats (Reese, 1988). It is possible that our population of linear cells is an entirely W-like population, though based on the receptive field shape and response dynamics to gratings we believe it more likely our linear cells are comprised of a mix of X-like and W-like cells.

W-like and Koniocellular-like Responses in Mouse dLGN

In both cat and macaque a third, somewhat catch-all, class of geniculate cells contains a diversity of response properties including orientation selective responses (LeVay and Ferster, 1977; Hendry and Reid, 2000). Here, we see some evidence for orientation biased responses in mouse dLGN, but more complete and convincing descriptions of orientation selective responses in mouse dLGN have been published elsewhere (Huberman et al., 2009; Krahe et al., 2011; Piscopo et al., 2013; Scholl et al., 2013; Cruz-Martin et al., 2014). The shell region of mouse dLGN appears to be somewhat analogous to koniocellular and W cell pathways in other species; most notably, it relays direction selectivity circuit from the retina to superficial layers V1 (Cruz-Martin et al., 2014). The mouse does seem to contain a third pathway, how much of these mixed koniocellular and W streams are apparent in mouse dLGN is far from resolved by this or any other mouse studies.

Major Differences between the Mouse dLGN and those of Cats and Macaques

While the organization of mouse dLGN contains several homologies to the macaque system, it must also be noted that there are also several major differences. The most obvious difference is the gross laminar organization: while in macaque's dLGN cells cluster in several distinct layers with smaller numbers distributed in the interlaminar zones, mouse dLGN, like dLGN of rat (Reese, 1988) is not layered (Paxinos and Franklin, 2004). There is some organization in mouse dLGN, with W-like dendritic morphologies in a dorsal shell and X-like morphologies in a core (Krahe et al., 2011), but this pales in comparison to the organization of both cat and macaque dLGN.

We saw little difference in receptive field size between linear and non-linear cells in mouse dLGN, whereas Y and magnocellular cells tend toward larger receptive fields than X and parvocellular cells, respectively (Saul and Humphrey, 1990; Usrey and Reid, 2000; Xu et al., 2001; Weng et al., 2005). Several factors may contribute to our inability to see differences in receptive field size. Here, we have combined cells from across retinotopic positions; as receptive field size is correlated with eccentricity, different eccentricities with both the linear and non-linear samples could be a factor. In addition, the large spatial scale of the mouse system could limit our ability to fully stimulate very large receptive fields because of the limits of our stimulus monitor. To resolve this, measurement of eye position for display position and very large displays corrected for distortions may be required. Finally, color based parallel pathways within the parvocellular system are well described (Dacey, 2000), and while the mouse expresses two cone opsins and possesses color-based circuitry in the retina (Brueninger et al., 2011; Baden et al., 2013), little is known about parallel color pathways mouse dLGN. Further work investigating transience and opsin-specific responses should help identify a parvocellular-like pathway in mouse dLGN, if one exists.

AUTHOR CONTRIBUTIONS

DJD designed experiments, performed research and data analysis, and wrote the manuscript. DC designed experiments and wrote the manuscript.

REFERENCES

- Adesnik, H., Bruns, W., Taniguchi, H., Huang, Z. J., and Scanziani, M. (2012). A neural circuit for spatial summation in visual cortex. *Nature* 490, 226–231. doi: 10.1038/nature11526
- Andermann, M. L., Kerlin, A. M., and Reid, R. C. (2010). Chronic cellular imaging of mouse visual cortex during operant behavior and passive viewing. *Front. Cell. Neurosci.* 4:3. doi: 10.3389/fncel.2010.00003
- Baden, T., Schubert, T., Chang, L., Wei, T., Zaichuk, M., Wissinger, B., et al. (2013). A tale of two retinal domains: near-optimal sampling of achromatic contrasts in natural scenes through asymmetric photoreceptor distribution. *Neuron* 80, 1206–1217. doi: 10.1016/j.neuron.2013.09.030
- Balkema, G. W. Jr., and Pinto, L. H. (1982). Electrophysiology of retinal ganglion cells in the mouse: a study of a normally pigmented mouse and a congenic hypopigmentation mutant, pearl. *J. Neurophysiol.* 48, 968–980.
- Bishop, G. H. (1933). Fiber groups in the optic nerve. *Am. J. Physiol.* 106, 460–470.
- Bishop, G. H. (1946). Nerve and synaptic conduction. *Annu. Rev. Physiol.* 8, 355–374. doi: 10.1146/annurev.ph.08.030146.002035
- Bishop, P. O., Burke, W., and Davis, R. (1959). Activation of single lateral geniculate cells by stimulation of either optic nerve. *Science* 130, 506–507. doi: 10.1126/science.130.3374.506
- Bishop, G. H., and Clare, M. H. (1955). Organization and distribution of fibers in the optic tract of the cat. *J. Comp. Neurol.* 103, 269–304. doi: 10.1002/cne.901030204
- Bishop, P. O., Jeremy, D., and Lance, J. W. (1953). The optic nerve; properties of a central tract. *J. Physiol.* 121, 415–432. doi: 10.1113/jphysiol.1953.sp004955
- Bishop, P. O., and McLeod, J. G. (1954). Nature of potentials associated with synaptic transmission in lateral geniculate of cat. *J. Neurophysiol.* 17, 387–414.
- Bishop, G. H., and O'Leary, J. (1938). Potential records from the optic cortex of the cat. *J. Neurophysiol.* 1, 391–404.
- Bleckert, A., Schwartz, G. W., Turner, M. H., Rieke, F., and Wong, R. O. L. (2014). Visual space is represented by non-matching topographies of distinct mouse retinal ganglion cell types. *Curr. Biol.* 24, 310–315. doi: 10.1016/j.cub.2013.12.020
- Bock, D. D., Lee, W.-C. A., Kerlin, A. M., Andermann, M. L., Hood, G., Wetzel, A. W., et al. (2012). Network anatomy and *in vivo* physiology of visual cortical neurons. *Nature* 471, 177–182. doi: 10.1038/nature09802
- Brueninger, T., Puller, C., Haverkamp, S., and Euler, T. (2011). Chromatic bipolar cell pathways in the mouse retina. *J. Neurosci.* 31, 6504–6517. doi: 10.1523/JNEUROSCI.0616-11.2011
- Bullier, J., and Norton, T. T. (1979). X and Y relay cells in cat lateral geniculate nucleus: quantitative analysis of receptive-field properties and classification. *J. Neurophysiol.* 42, 244–273.
- Busse, L., Ayaz, A., Dhruv, N. T., Katzner, S., Saleem, A. B., Schölvinck, M. L., et al. (2011). The detection of visual contrast in the behaving mouse. *J. Neurosci.* 31, 11351–11361. doi: 10.1523/JNEUROSCI.6689-10.2011
- Callaway, E. M. (1998). Local circuits in primary visual cortex of the macaque monkey. *Annu. Rev. Neurosci.* 21, 47–74. doi: 10.1146/annurev.neuro.21.1.47
- Carandini, M., and Churchland, A. K. (2013). Probing perceptual decisions in rodents. *Nat. Neurosci.* 16, 824–831. doi: 10.1038/nn.3410
- Cardin, J. A., Carlén, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., et al. (2009). Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 459, 663–667. doi: 10.1038/nature08002
- Cleland, B. G., Dubin, M. W., and Levick, W. R. (1971). Sustained and transient neurones in the cat's retina and lateral geniculate nucleus. *J. Physiol.* 217, 473–496. doi: 10.1113/jphysiol.1971.sp009581
- Cleland, B. G., Levick, W. R., and Sanderson, K. J. (1973). Properties of sustained and transient ganglion cells in the cat retina. *J. Physiol.* 228, 649–680. doi: 10.1113/jphysiol.1973.sp010105
- Crunelli, V., Kelly, J. S., Leresche, N., and Pirchio, M. (1987). On the excitatory post-synaptic potential evoked by stimulation of the optic tract in the rat lateral geniculate nucleus. *J. Physiol.* 385, 603–618. doi: 10.1113/jphysiol.1987.sp016472
- Cruz-Martin, A., El-Danaf, R. N., Osakada, F., Sriram, B., Dhande, O. S., Nguyen, P. L., et al. (2014). A dedicated circuit linking direction selective retinal ganglion cells to primary visual cortex. *Nature* 507, 358–361. doi: 10.1038/nature12989
- Dacey, D. M. (1994). Physiology, morphology and spatial densities of identified ganglion cell types in primate retina. *Ciba Found. Symp.* 184, 12–28; discussion 28–34, 63–70.
- Dacey, D. M. (2000). Parallel pathways for spectral coding in primate retina. *Annu. Rev. Neurosci.* 23, 743–775. doi: 10.1146/annurev.neuro.23.1.743
- De Monasterio, F. M., and Gouras, P. (1975). Functional properties of ganglion cells of the rhesus monkey retina. *J. Physiol.* 251, 167–195. doi: 10.1113/jphysiol.1975.sp011086
- Denman, D. J., and Contreras, D. (2014). The structure of pairwise correlation in mouse primary visual cortex reveals functional organization in the absence of an orientation map. *Cereb. Cortex* 24, 2707–2720. doi: 10.1093/cercor/bht128
- Derrington, A. M., and Lennie, P. (1984). Spatial and temporal contrast sensitivities of neurones in lateral geniculate nucleus of macaque. *J. Physiol.* 357, 219–240. doi: 10.1113/jphysiol.1984.sp015498
- Dombeck, D. A., Khabbazi, A. N., Collman, F., Adelman, T. L., and Tank, D. W. (2007). Imaging large-scale neural activity with cellular resolution in awake, mobile mice. *Neuron* 56, 43–57. doi: 10.1016/j.neuron.2007.08.003
- Dräger, U., and Olsen, J. (1981). Ganglion cell distribution in the retina of the mouse. *Invest. Ophthalmol. Vis. Sci.* 20, 285–293.
- Dreher, B., Fukada, Y., and Rodieck, R. W. (1976). Identification, classification and anatomical segregation of cells with X-like and Y-like properties in the lateral geniculate nucleus of old-world primates. *J. Physiol.* 258, 433–452. doi: 10.1113/jphysiol.1976.sp011429
- Enroth-Cugell, C., and Robson, J. G. (1966). The contrast sensitivity of retinal ganglion cells of the cat. *J. Physiol.* 187, 517–552. doi: 10.1113/jphysiol.1966.sp008107
- Fukuda, Y., Sumitomo, I., Sugitani, M., and Iwama, K. (1979). Receptive-field properties of cells in the dorsal part of the albino rat's lateral geniculate nucleus. *Jpn. J. Physiol.* 29, 283–307. doi: 10.2170/jjphysiol.29.283
- Gao, E., DeAngelis, G. C., and Burkhalter, A. (2010). Parallel input channels to mouse primary visual cortex. *J. Neurosci.* 30, 5912–5926. doi: 10.1523/JNEUROSCI.6456-09.2010
- Gasser, H. S., and Erlanger, J. (1929). The role of fiber size in the establishment of a nerve block by pressure or cocaine. *Am. J. Physiol.* 88, 581–591.
- Gouras, P. (1969). Antidromic responses of orthodromically identified ganglion cells in monkey retina. *J. Physiol.* 204, 407–419. doi: 10.1113/jphysiol.1969.sp008920
- Grubb, M. S., and Thompson, I. D. (2003). Quantitative characterization of visual response properties in the mouse dorsal lateral geniculate nucleus. *J. Neurophysiol.* 90, 3594–3607. doi: 10.1152/jn.00699.2003
- Guillery, R. W., Polley, E. H., and Torrealba, F. (1982). The arrangement of axons according to fiber diameter in the optic tract of the cat. *J. Neurosci.* 2, 714–721.
- Hale, P. T., Sefton, A. J., and Dreher, B. (1979). A correlation of receptive field properties with conduction velocity of cells in the rat's retino-geniculate-cortical pathway. *Exp. Brain Res.* 35, 425–442. doi: 10.1007/bf00236762
- Heinbecker, P., Bishop, G. H., and O'Leary, J. (1933). Pain and touch fibers in peripheral nerves. *Arch. Neurol. Psychiatry* 29, 771–789. doi: 10.1001/archneurpsyc.1933.02240100090008
- Hendry, S. H., and Reid, R. C. (2000). The koniocellular pathway in primate vision. *Annu. Rev. Neurosci.* 23, 127–153. doi: 10.1146/annurev.neuro.23.1.127
- Hicks, T. P., Lee, B. B., and Vidyasagar, T. R. (1983). The responses of cells in macaque lateral geniculate nucleus to sinusoidal gratings. *J. Physiol.* 337, 183–200. doi: 10.1113/jphysiol.1983.sp014619

- Hochstein, S. and Shapley, R. M. (1976). Linear and nonlinear spatial subunits in Y cat retinal ganglion cells. *J. Physiol.* 262, 265–284. doi: 10.1113/jphysiol.1976.sp011595
- Hoffmann, K. P., and Stone, J. (1971). Conduction velocity of afferents to cat visual cortex: a correlation with cortical receptive field properties. *Brain Res.* 32, 460–466. doi: 10.1016/0006-8993(71)90340-4
- Huberman, A. D., and Niell, C. M. (2011). What can mice tell us about how vision works? *Trends Neurosci.* 34, 464–473. doi: 10.1016/j.tins.2011.07.002
- Huberman, A. D., Wei, W., Elstrott, J., Stafford, B. K., Feller, M. B., and Barres, B. A. (2009). Genetic identification of an On-Off direction-selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. *Neuron* 62, 327–334. doi: 10.1016/j.neuron.2009.04.014
- Irvin, G. E., Casagrande, V. N., and Norton, T. T. (1993). Center/surround relationships of magnocellular, parvocellular and koniocellular relay cells in primate lateral geniculate nucleus. *Vis. Neurosci.* 10, 363–373. doi: 10.1017/s0952523800003758
- Kaplan, E., and Shapley, R. M. (1982). X and Y cells in the lateral geniculate nucleus of macaque monkeys. *J. Physiol.* 330, 125–143. doi: 10.1113/jphysiol.1982.sp014333
- Krahe, T. E., El-Danaf, R. N., Dilger, E. K., Henderson, S. C., and Guido, W. (2011). Morphologically distinct classes of relay cells exhibit regional preferences in the dorsal lateral geniculate nucleus of the mouse. *J. Neurosci.* 31, 17437–17448. doi: 10.1523/JNEUROSCI.4370-11.2011
- Kumbhani, R. D., Nolt, M. J., and Palmer, L. A. (2007). Precision, reliability and information-theoretic analysis of visual thalamocortical neurons. *J. Neurophysiol.* 98, 2647–2663. doi: 10.1152/jn.00900.2006
- Kurimoto, T., Yin, Y., Omura, K., Gilbert, H. Y., Kim, D., Cen, L. P., et al. (2010). Long-distance axon regeneration in the mature optic nerve: contributions of oncomodulin, cAMP, and *pten* gene deletion. *J. Neurosci.* 30, 15654–15663. doi: 10.1523/JNEUROSCI.4340-10.2010
- Lee, S.-H., Kwan, A. C., Zhang, S., Phoumthipphavong, V., Flannery, J. G., Masmanidis, S. C., et al. (2012). Activation of specific interneurons improves V1 feature selectivity and visual perception. *Nature* 488, 379–383. doi: 10.1038/nature11312
- Levanthal, A. G., Rodieck, R. W., and Dreher, B. (1981). Retinal ganglion cell classes in the old world monkey: morphology and central projections. *Science* 213, 1139–1142. doi: 10.1126/science.7268423
- LeVay, S., and Ferster, D. (1977). Relay cell classes in the lateral geniculate nucleus of the cat and the effects of visual deprivation. *J. Comp. Neurol.* 172, 563–584. doi: 10.1002/cne.901720402
- Levitt, J. B., Schumer, R. A., Sherman, S. M., Spear, P. D., and Movshon, J. A. (2001). Visual response properties of neurons in the LGN of normally reared and visually deprived macaque monkeys. *J. Neurophysiol.* 85, 2111–2129.
- Liu, B.-H., Li, Y.-T., Ma, W.-P., Pan, C.-J., Zhang, L. I., and Tao, H. W. (2011). Broad inhibition sharpens orientation selectivity by expanding input dynamic range in mouse simple cells. *Neuron* 71, 542–554. doi: 10.1016/j.neuron.2011.06.017
- Liu, B.-H., Li, P., Sun, Y. J., Li, Y.-T., Zhang, L. I., and Tao, H. W. (2010). Intervening inhibition underlies simple-cell receptive field structure in visual cortex. *Nat. Neurosci.* 13, 89–96. doi: 10.1038/nn.2443
- Livingstone, M., and Hubel, D. (1988). Segregation of form, color, movement and depth: anatomy, physiology and perception. *Science* 240, 740–749. doi: 10.1126/science.3283936
- Marshall, J. H., Garrett, M. E., Nauhaus, I., and Callaway, E. M. (2011). Functional specialization of seven mouse visual cortical areas. *Neuron* 72, 1040–1054. doi: 10.1016/j.neuron.2011.12.004
- Marshall, J. H., Kaye, A. P., Nauhaus, I., and Callaway, E. M. (2012). Anterior-Posterior direction opponency in the superficial mouse lateral geniculate nucleus. *Neuron* 76, 713–720. doi: 10.1016/j.neuron.2012.09.021
- Merigan, W. H., and Maunsell, J. H. (1993). How parallel are the primate visual pathways? *Annu. Rev. Neurosci.* 16, 369–402. doi: 10.1146/annurev.neuro.16.1.369
- Nassi, J. J., and Callaway, E. M. (2009). Parallel processing strategies of the primate visual system. *Nat. Rev. Neurosci.* 10, 360–372. doi: 10.1038/nrn2619
- Niell, C. M. (2011). Exploring the next frontier of mouse vision. *Neuron* 72, 889–892. doi: 10.1016/j.neuron.2011.12.011
- Niell, C. M., and Stryker, M. P. (2008). Highly selective receptive fields in mouse visual cortex. *J. Neurosci.* 28, 7520–7536. doi: 10.1523/JNEUROSCI.0623-08.2008
- Niell, C. M., and Stryker, M. P. (2010). Modulation of visual responses by behavioral state in mouse visual cortex. *Neuron* 65, 472–479. doi: 10.1016/j.neuron.2010.01.033
- Olsen, S. R., Bortone, D. S., Adesnik, H., and Scanziani, M. (2012). Gain control by layer six in cortical circuits of vision. *Nature* 483, 47–52. doi: 10.1038/nature10835
- Paxinos, G., and Franklin, K. B. J. (2004). *The Mouse Brain in Stereotaxic Coordinates*. Oxford: Academic Press.
- Piscopo, D. M., El-Danaf, R. N., Huberman, A. D., and Niell, C. M. (2013). Diverse visual features encoded in mouse lateral geniculate nucleus. *J. Neurosci.* 33, 4642–4656. doi: 10.1523/JNEUROSCI.5187-12.2013
- Polack, P.-O., Friedman, J., and Golshani, P. (2013). Cellular mechanisms of brain state-dependent gain modulation in visual cortex. *Nat. Neurosci.* 16, 1331–1339. doi: 10.1038/nn.3464
- Price, D. J., and Morgan, J. E. (1987). Spatial properties of neurones in the lateral geniculate nucleus of the pigmented ferret. *Exp. Brain Res.* 68, 28–36. doi: 10.1007/bf00255231
- Reese, B. E. (1988). ‘Hidden lamination’ in the dorsal lateral geniculate nucleus: the functional organization of this thalamic region in the rat. *Brain Res. Rev.* 13, 119–137. doi: 10.1016/0165-0173(88)90017-3
- Reese, B. E., and Cowey, A. (1990). Fibre organization of the monkey’s optic tract: I. Segregation of functionally distinct optic axons. *J. Comp. Neurol.* 295, 385–400. doi: 10.1002/cne.902950304
- Reinagel, P., and Reid, R. C. (2000). Temporal coding of visual information in the thalamus. *J. Neurosci.* 20, 5392–5400.
- Saleem, A. B., Ayaz, A., Jeffery, K. J., Harris, K. D., and Carandini, M. (2013). Integration of visual motion and locomotion in mouse visual cortex. *Nat. Neurosci.* 16, 1864–1869. doi: 10.1038/nn.3567
- Saul, A. B., and Humphrey, A. L. (1990). Spatial and temporal response properties of lagged and nonlagged cells in cat lateral geniculate nucleus. *J. Neurophysiol.* 64, 206–224.
- Schmitzer-Torbert, N., Jackson, J., Henze, D., Harris, K., and Redish, A. D. (2005). Quantitative measures of cluster quality for use in extracellular recordings. *Neuroscience* 131, 1–11. doi: 10.1016/j.neuroscience.2004.09.066
- Scholl, B., Tan, A. Y. Y., Corey, J., and Priebe, N. J. (2013). Emergence of orientation selectivity in the mammalian visual pathway. *J. Neurosci.* 33, 10616–10624. doi: 10.1523/JNEUROSCI.0404-13.2013
- Seecharan, D. J., Kulkarni, A. L., Lu, L., Rosen, G. D., and Williams, R. W. (2003). Genetic control of interconnected neuronal populations in the mouse primary visual system. *J. Neurosci.* 23, 11178–11188.
- Sefton, A. J., and Swinburn, M. (1964). Electrical activity of lateral geniculate nucleus and optic tract of the rat. *Vision Res.* 4, 315–328. doi: 10.1016/0042-6989(64)90020-3
- Shapley, R., and Hochstein, S. (1975). Visual spatial summation in two classes of geniculate cells. *Nature* 256, 411–413. doi: 10.1038/256411a0
- Shapley, R., Kaplan, E., and Soodak, R. (1981). Spatial summation and contrast sensitivity of X and Y cells in the lateral geniculate nucleus of the macaque. *Nature* 292, 543–545. doi: 10.1038/292543a0
- Sherman, S. M., Norton, T. T., and Casagrande, V. A. (1975). X-and Y-cells in the dorsal lateral geniculate nucleus of the tree shrew (*Tupaia glis*). *Brain Res.* 93, 152–157. doi: 10.1016/0006-8993(75)90294-2
- Sherman, S. M., Wilson, J. R., Kaas, J. H., and Webb, S. V. (1976). X- and Y-cells in the dorsal lateral geniculate nucleus of the owl monkey (*Aotus trivirgatus*). *Science* 192, 475–477. doi: 10.1126/science.816006
- Sireteanu, R., and Hoffmann, K. P. (1979). Relative frequency and visual resolution of X- and Y-cells in the LGN of normal and monocularly deprived cats: interlaminar differences. *Exp. Brain Res.* 34, 591–603. doi: 10.1007/bf00239151
- So, Y. T., and Shapley, R. (1979). Spatial properties of X and Y cells in the lateral geniculate nucleus of the cat and conduction velocities of their inputs. *Exp. Brain Res.* 36, 533–550. doi: 10.1007/bf00238521
- Sohal, V. S., Zhang, F., Yizhar, O., and Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* 459, 698–702. doi: 10.1038/nature07991
- Sohya, K., Kameyama, K., Yanagawa, Y., Obata, K., and Tsumoto, T. (2007). GABAergic neurons are less selective to stimulus orientation than excitatory neurons in layer II/III of Visual cortex, as revealed by *in vivo* functional Ca²⁺ imaging in transgenic mice. *J. Neurosci.* 27, 2145–2149. doi: 10.1523/jneurosci.4641-06.2007

- Stone, J. (1983). *Parallel Processing in the Visual System: The Classification of Retinal Ganglion Cells and its Impact on the Neurobiology of Vision*. New York, NY: Plenum Press.
- Stone, J., and Hoffmann, K. P. (1971). Conduction velocity as a parameter in the organisation of the afferent relay in the cat's lateral geniculate nucleus. *Brain Res.* 32, 454–459. doi: 10.1016/0006-8993(71)90339-8
- Stone, C., and Pinto, L. H. (1993). Response properties of ganglion cells in the isolated mouse retina. *Vis. Neurosci.* 10, 31–39. doi: 10.1017/s0952523800003205
- Sun, W., Li, N., and He, S. (2002). Large-scale morphological survey of mouse retinal ganglion cells. *J. Comp. Neurol.* 451, 115–126. doi: 10.1002/cne.10323
- Swadlow, H. A., and Weyand, T. G. (1985). Receptive-field and axonal properties of neurons in the dorsal lateral geniculate nucleus of awake unparalyzed rabbits. *J. Neurophysiol.* 54, 168–183.
- Usrey, W. M., and Reid, R. C. (2000). Visual physiology of the lateral geniculate nucleus in two species of new world monkey: *Saimiri sciureus* and *Aotus trivirgatus*. *J. Physiol.* 523, 755–769. doi: 10.1111/j.1469-7793.2000.00755.x
- Van Hooser, S. D. (2007). Similarity and diversity in visual cortex: is there a unifying theory of cortical computation? *Neuroscientist* 13, 639–656. doi: 10.1177/1073858407306597
- Van Hooser, S. D., Heimel, J. A., and Nelson, S. B. (2003). Receptive field properties and laminar organization of lateral geniculate nucleus in the gray squirrel (*Sciurus carolinensis*). *J. Neurophysiol.* 90, 3398–3418. doi: 10.1152/jn.00474.2003
- Völgyi, B., Chheda, S., and Bloomfield, S. A. (2009). Tracer coupling patterns of the ganglion cell subtypes in the mouse retina. *J. Comp. Neurol.* 512, 664–687. doi: 10.1002/cne.21912
- Wässle, H. (2004). Parallel processing in the mammalian retina. *Nat. Rev. Neurosci.* 5, 747–757. doi: 10.1038/nrn1497
- Watanabe, M., and Rodieck, R. W. (1989). Parasol and midget ganglion cells of the primate retina. *J. Comp. Neurol.* 289, 434–454. doi: 10.1002/cne.902890308
- Weng, C., Yeh, C.-I., Stoelzel, C. R., and Alonso, J.-M. (2005). Receptive field size and response latency are correlated within the cat visual thalamus. *J. Neurophysiol.* 93, 3537–3547. doi: 10.1152/jn.00847.2004
- Xu, X., Ichida, J. M., Allison, J. D., Boyd, J. D., and Bonds, A. B. (2001). A comparison of koniocellular, magnocellular and parvocellular receptive field properties in the lateral geniculate nucleus of the owl monkey (*Aotus trivirgatus*). *J. Physiol.* 531, 203–218. doi: 10.1111/j.1469-7793.2001.0203j.x
- Zhang, X., An, X., Liu, H., Peng, J., Cai, S., Wang, W., et al. (2015). The topographical arrangement of cutoff spatial frequencies across lower and upper visual fields in mouse V1. *Sci. Rep.* 5:7734. doi: 10.1038/srep07734

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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