frontiers in Molecular Neuroscience

MINI REVIEW
published: 28 March 2019
doi: 10.3389/fnmol.2019.00072

The Functional Role of Spontaneously Opening $GABA_A$ Receptors in Neural Transmission

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Ionotropic type of γ-aminobutyric acid receptors ($GABA_A$Rs) produce two forms of inhibitory signaling: phasic inhibition generated by rapid efflux of neurotransmitter GABA into the synaptic cleft with subsequent binding to $GABA_A$Rs, and tonic inhibition generated by persistent activation of extrasynaptic and/or perisynaptic $GABA_A$Rs by GABA continuously present in the extracellular space. It is widely accepted that phasic and tonic GABAergic inhibition is mediated by receptor groups of distinct subunit composition and modulated by different cytoplasmic mechanisms. Recently, however, it has been demonstrated that spontaneously opening $GABA_A$Rs (s-$GABA_A$Rs), which do not need GABA binding to enter an active state, make a significant input into tonic inhibitory signaling. Due to GABA-independent action mode, s-$GABA_A$Rs promise new safer options for therapy of neural disorders (such as epilepsy) devoid of side effects connected to abnormal fluctuations of GABA concentration in the brain. However, despite the potentially important role of s-$GABA_A$Rs in neural signaling, they still remain out of focus of neuroscience studies, to a large extent due to technical difficulties in their experimental research. Here, we summarize present data on s-$GABA_A$Rs functional properties and experimental approaches that allow isolation of s-$GABA_A$Rs effects from those of conventional (GABA-dependent) $GABA_A$Rs.

Keywords: GABA-A receptor, GABA-independent inhibition, phasic conductance, tonic conductance, G-proteins

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Received: *22 January 2019*
Accepted: *08 March 2019*
Published: *28 March 2019*

Citation:
O'Neill N and Sylantyev S (2019) The Functional Role of Spontaneously Opening $GABA_A$ Receptors in Neural Transmission. Front. Mol. Neurosci. 12:72. doi: 10.3389/fnmol.2019.00072

INTRODUCTION

Ionotropic receptors of γ-aminobutyric acid (GABA receptors of type A, $GABA_A$Rs) are the main receptor type that generates inhibitory interneuronal signaling in the brain. The classical form of $GABA_A$R-induced inhibitory signal is phasic inhibition: a short synchronized opening of $GABA_A$Rs in a synapse, generated by the binding of GABA released from a presynaptic terminal. However, there is an alternative form of inhibition: charge transfer through continuously active $GABA_A$Rs, or tonic inhibition, detected in peripheral nervous system in the 1970s (Brown, 1979) but documented for the central nervous system only in the 1990s (Otis et al., 1991; Brickley et al., 1996). The classical view is that tonic inhibition is generated in response to GABA, which is continuously present in the extracellular space of neural tissue due to spillover from synapses or release from astroglia and/or neurogliaform cells (Farrant and Nusser, 2005; Kozlov et al., 2006; Oláh et al., 2009). This implies the generation of a continuous inhibitory tone mainly by perisynaptic and extrasynaptic $GABA_A$Rs, since the vast majority of transporters which perform reverse uptake of GABA are localized in synapses or in their immediate vicinity (Minelli et al., 1996; Chiu et al., 2002; Conti et al., 2004). Hence, the magnitude of tonic $GABA_A$Rs-delivered current is considered to be regulated by the

availability of extracellular GABA, and by the quantity of $GABA_A$Rs at an extrasynaptic surface of a given neuron (Glykys and Mody, 2007). Later research, however, revealed that a significant part of tonic inhibition mediated by $GABA_A$Rs is independent of GABA binding, i.e., it is delivered by spontaneously opening $GABA_A$Rs (s-$GABA_A$Rs). s-$GABA_A$Rs in that study were shown to be insensitive to the competitive GABA antagonist SR-95531 (SR), but could be suppressed by the $GABA_A$R open channel blocker picrotoxin (PTX), and, to the less extent, by competitive GABA antagonist bicuculline (BIC; McCartney et al., 2007).

In the last few decades, studies of $GABA_A$Rs-mediated tonic currents have attracted a considerable interest, and have described a functional role of this form of inhibition in a number of brain areas; in particular, its important input into neural excitability, synaptic plasticity, neurogenesis and network oscillations (Mody and Pearce, 2004; Farrant and Nusser, 2005; Glykys and Mody, 2007). Since our understanding of underlying mechanisms is still far from excellent, the newly discovered type of tonic conductance delivered *via* s-$GABA_A$Rs promises a conceptual breakthrough in the field. Nevertheless, despite the phenomenon of GABA-independent gating of $GABA_A$Rs being reported in numerous publications (Neelands et al., 1999; Birnir et al., 2000; Maksay et al., 2003; Miko et al., 2004), until recently the functional role of s-$GABA_A$Rs in living neural tissue has remained beyond the focus of neuroscience research.

In this article, we try to summarize the data available to date on s-$GABA_A$Rs function in neural transmission and to discuss perspective directions for further studies which should clarify the role of s-$GABA_A$Rs under normal conditions and in pathology.

FUNCTIONAL PROPERTIES OF s-GABARs

s-GABARs: Problem of the Isolation of GABA-Independent Effects

One of the main factors which prevent a detailed study of s-$GABA_A$Rs functioning is a lack of specific pharmacological tools: the independence of s-$GABA_A$Rs gating from GABA binding makes impossible the use of competitive GABA antagonists for selective s-$GABA_A$Rs silencing, whereas allosteric modulators such as benzodiazepines display a lack of specificity, tuning both GABA-dependent and GABA-independent effects (Bianchi and Macdonald, 2001; McCartney et al., 2007; Gerak, 2009).

Hence, to clarify the input of s-$GABA_A$Rs into a given effect, differences in molecular mechanisms of SR- and PTX-induced $GABA_A$Rs silencing have been used. SR is a competitive antagonist and thus negates $GABA_A$R activity induced by GABA binding (i.e., it acts on conventional $GABA_A$Rs); in contrast, PTX binds inside the $GABA_A$R ion channel, and thus blocks all open channels, independently of the presence of GABA binding (i.e., it acts on both conventional $GABA_A$Rs and s-$GABA_A$Rs). Therefore, conventional $GABA_A$R activity can be assessed as the change in the given effect obtained in the control vs. after application of SR, whereas s-$GABA_A$R activity can be measured as the change in the effect obtained after SR application vs. after subsequent application of SR+PTX (Wlodarczyk et al., 2013)—see **Figure 1**. SR is a "silent" competitor for the GABA-binding site, i.e., it does not display inverse agonist properties. Obviously, competitive antagonists such as BIC, which display inverse agonism, cannot be used for the quantitative assessment of s-$GABA_A$Rs effects: BIC was shown not only to suppress synaptic events as SR does but also to induce an outward shift of holding current (Wlodarczyk et al., 2013).

s-GABARs Single-Channel Properties

The obvious step in the biophysical characterization of different subgroups of ionotropic receptors is a dissection of single-channel properties, such as electrical conductance, opening frequency and average open time. Single-channel recordings have repeatedly demonstrated similar or very close conductance values for s-$GABA_A$Rs and conventional $GABA_A$Rs (Mathers, 1985; Neelands et al., 1999; Birnir et al., 2000;

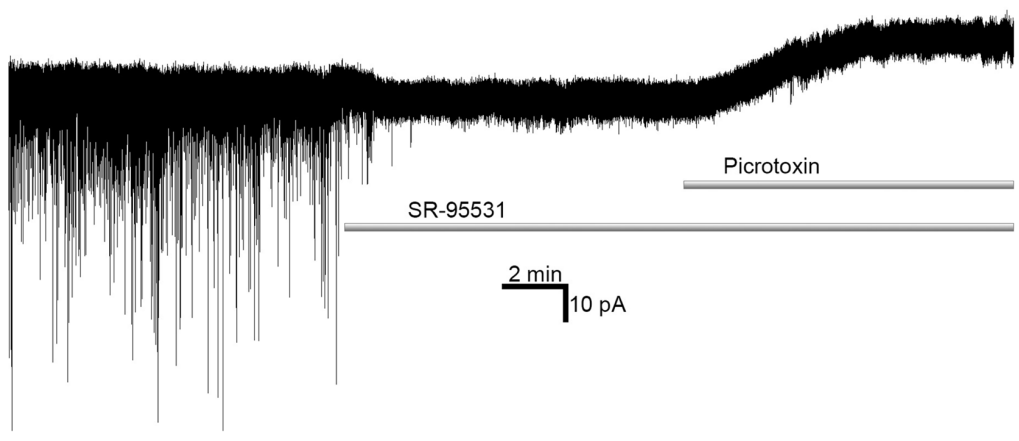

FIGURE 1 | Competitive γ-aminobutyric acid (GABA) antagonist SR-95531 suppresses spontaneous GABA-ergic synaptic signaling, but does not affect tonic conductance; on the contrary, open-channel blocker picrotoxin applied after SR-95531 shuts spontaneously opening GABA-receptors (s-$GABA_A$Rs), revealing the amount of inhibitory current passing through s-$GABA_A$Rs independently of GABA binding.

O'Neill and Sylantyev, 2018a,b) thus making this parameter hardly applicable for distinguishing between two receptor subtypes. Similarly, the dependence of $GABA_A$Rs opening frequency on the concentration of GABA, makes this parameter inapplicable for discrimination of effects of s-$GABA_A$Rs and conventional $GABA_A$Rs in single-channel recordings. In contrast, the average open time was found to be significantly lower for s-$GABA_A$Rs than for conventional $GABA_A$Rs. This generates a two-peak distribution of opening time values under physiological conditions when free GABA is present in extracellular space (O'Neill and Sylantyev, 2018a). Earlier observations demonstrated that the two-peak Gaussian distribution of average open times is a characteristic feature of $GABA_A$Rs of at least three different subunit compositions (Mortensen et al., 2010). It is important to note that the mode values for shorter durations in that work were found to be similar, irrespective of the agonist's type and concentration, thus representing an agonist-independent input. This suggests that: (i) s-$GABA_A$Rs activity is a common element of integral $GABA_A$R response; and (ii) that s-$GABA_A$Rs represent a functionally similar receptor subgroup composed of receptors of various subunit compositions.

Another method of distinguishing between s-$GABA_A$Rs and conventional $GABA_A$Rs at a level of single-channel effects may potentially develop from the recent observation about the ability of benzodiazepine flurazepam to modulate GABA-dependent and GABA-independent $GABA_A$R gating *via* different molecular mechanisms (Jatczak-Śliwa et al., 2018).

s-GABARs Input Into Tonic Conductance

Overall, charge transfer with phasic events mediated by $GABA_A$Rs (and induced by GABA binding) compared to that delivered by tonic conductance through $GABA_A$Rs, displays a ratio of more than 9/1 (Cope et al., 2005; O'Neill and Sylantyev, 2018a). Taking into account that GABA-induced tonic current was found to be negligible under physiological concentrations of extracellular GABA, whereas under these conditions s-$GABA_A$Rs generated a significant amount of tonic current (Wlodarczyk et al., 2013), s-$GABA_A$Rs should be considered as a potential key element in the generation of lasting inhibitory tone and, in a wider context, in inter-neuronal crosstalk.

Tonic inhibition has been widely accepted to be a strong modulator of action potential (AP) generation (Hamann et al., 2002; Bonin et al., 2007), AP firing patterns (Häusser and Clark, 1997) and the coincidence detection time window for synaptic inputs (Tang et al., 2011). Experiments on s-$GABA_A$Rs have readily confirmed their significant input into the regulation of the following phenomena: the modulation of AP generation (O'Neill and Sylantyev, 2018b), firing patterns (Botta et al., 2015; O'Neill and Sylantyev, 2018a), neurons' rheobase, and the time window of coincidence detection of excitatory inputs (O'Neill and Sylantyev, 2018a).

s-$GABA_A$Rs Input Into Phasic Conductance

Several classical studies have demonstrated that $GABA_A$Rs of specific subunit compositions (e.g., δ-$GABA_A$Rs) which may be responsible for a lion's share of tonic current (Nusser and Mody, 2002; Stell et al., 2003; Mortensen et al., 2010) are localized exclusively at the extrasynaptic membrane (Nusser et al., 1998; Wei et al., 2003). However, if s-$GABA_A$Rs are a functionally similar group of receptors of different subunit composition (see "s-GABARs Single-Channel Properties" section), their absence in synapses would be highly doubtful. This, in turn, raises a question as to how (and whether) s-$GABA_A$Rs modify synaptic (phasic) GABA-ergic inhibitory responses (inhibitory post-synaptic currents, IPSCs). In truth, recent studies have demonstrated their significant input into IPSC decay kinetics: s-$GABA_A$Rs introduced a slow element of decay profile (O'Neill and Sylantyev, 2018a), probably due to their higher potency to GABA (Yeung et al., 2003) and/or modified receptor efficacy.

It was shown earlier that $GABA_A$R-generated IPSC may contain fast and slow components with different sensitivities to GABA competitive antagonists, which resembles the functional profile of s-$GABA_A$Rs (Kapur et al., 1997). In this research, the generation of fast and slow components of whole-cell IPSC was attributed to different cell regions: dendritic and somatic, respectively. On the other hand, later direct recordings of s-$GABA_A$Rs activity confirmed a significant input of this receptor subtype into both whole-cell IPSCs (which are generated in synapses), and into IPSCs evoked in nucleated membrane patches, i.e., generated by $GABA_A$Rs localized at a neural cell soma (O'Neill and Sylantyev, 2018a). On top of that, a significant input of δ-$GABA_A$Rs into IPSCs was recently demonstrated (Sun et al., 2018), which confirms once again both the synaptic and extrasynaptic localization of $GABA_A$Rs which display high tonic activity.

Intracellular Regulatory Mechanisms of s-$GABA_A$Rs Activity

The particular intracellular mechanisms which are used by neural cells to modulate the activity of $GABA_A$Rs are still far from being completely understood; however, it has long been established that direct phosphorylation is of major importance (Brandon et al., 2002). It was shown that $GABA_A$Rs functions can be modulated differentially (potentiated or suppressed) depending on the receptor subunit composition, the type of neuron, et cetera by cAMP-dependent protein kinase A (PKA), tyrosine kinase Src and PKC: refer to Brandon et al. (2002) for review. In particular, $GABA_A$R-mediated tonic inhibitory currents were shown to be downregulated by PKC Bright and Smart, 2013, whereas PKA was found to enhance this type of inhibition (Carlson et al., 2016). In addition, $GABA_A$Rs effects were repeatedly shown to be modulated by G-protein-coupled receptors *via* G-proteins of different types (Cai et al., 2002; Wang et al., 2002) which are, in turn, tightly connected to the regulation of PKC and PKA activity (Neves et al., 2002). Hence, the clarification of impact on s-$GABA_A$Rs function delivered by intracellular regulatory factors (specifically, by various kinases and G-proteins), is one of the key steps needed for understanding and predicting s-$GABA_A$Rs functional input into a neural transmission.

To date, there is little data on this. It has been demonstrated that in dentate gyrus granule cells of hippocampus PKC regulates tonic GABA-dependent inhibitory conductance but has no significant impact on the GABA-independent effects of s-$GABA_A$Rs (O'Neill and Sylantyev, 2018b). However, at a longer time scale it was repeatedly shown that PKC and Ca^{2+}/calmodulin-dependent protein kinase II increase tonic inhibition in hippocampus and amygdala due to enhanced phosphorylation and membrane insertion of β3-containing $GABA_A$Rs (Saliba et al., 2012; Modgil et al., 2017) and α4-containing $GABA_A$Rs; this PKC action can be potentiated by neurosteroids such as THDOC (Abramian et al., 2010, 2014; Romo-Parra et al., 2015). In turn, s-$GABA_A$Rs-mediated tonic inhibition in dentate gyrus granule cells is controlled by G-proteins: non-specific block of G-proteins by pertussis toxin decreases the tonic current *via* the reduction of the s-$GABA_A$Rs opening frequency (O'Neill and Sylantyev, 2018b).

In contrast to PKC, activation of PKA was found to increase the tonic current through α4β3δ and, to a lesser extent, α4β3γ2L-$GABA_A$Rs in absence of GABA due to upregulation of single-channel opening frequency. Addition of GABA to an ambient solution, however, gradually decreased the sensitivity of $GABA_A$Rs of both subunit compositions to modulation by PKA; such a modulation became insignificant when GABA concentration reached micromolar values (Tang et al., 2010).

It is important to note, however, that a significant part of GABA-independent s-$GABA_A$Rs activity was found to be out of the control of any soluble cytoplasmic factors. GABA-independent openings of $GABA_A$Rs were recorded from outside-out patches excised from dentate gyrus granule cells somata: in this preparation, all cytoplasmic signaling chains are surely destroyed (O'Neill and Sylantyev, 2018b). However, anchored kinases that modulate ionotropic receptors (Brandon et al., 2003; Carnegie and Scott, 2003) may still be responsible for at least a part of the s-$GABA_A$Rs activity observed in outside-out patches.

CONCLUSIONS AND FURTHER RESEARCH DIRECTIONS

To date, there have been only a few publications highlighting the functional properties of s-$GABA_A$Rs in living neurons. This imposes obvious limitations on conclusions in terms of the applicability for different brain regions and types of neurons. Nevertheless, the significant input of s-$GABA_A$Rs into the modulation of output signal generation and into the integration of input signaling in a given neuron, suggests that s-$GABA_A$R activity is one of the key actors that regulate neural inhibition.

Indeed, the relative importance of GABA-independent s-$GABA_A$Rs signaling in a given region of the brain depends critically on the native concentration of GABA in the extracellular space. Different groups report *in vivo* concentrations varying by more than an order of magnitude: from less than 100 (Wlodarczyk et al., 2013) or 200 (Glaeser and Hare, 1975) nM to units of micromoles (Tossman et al., 1986; Takagi et al., 1993). Moreover, there may be local inhomogeneities of GABA concentrations due to cell-specific differences in the distribution and/or activity of GABA transporters and the elements of the GABA synthesis system. This was indirectly confirmed by the observation that the silencing of GAD-65 activity reduces tonic inhibitory currents in interneurons, but not in the pyramidal neurons of the hippocampal CA1 area (Song et al., 2011). A recent study on the hippocampus has demonstrated that at a GABA concentration of ∼100 nM, the amount of GABA-induced tonic current (which can be suppressed by SR) is close to statistical noise (see example at **Figure 1**), and negligible when compared to that through GABA-independent openings of s-$GABA_A$Rs (Wlodarczyk et al., 2013); on the contrary, SR has been shown to reveal a huge amount of tonic GABA-dependent current in thalamus (Cope et al., 2005). These data suggest that the relative impact of s-$GABA_A$Rs into neural signaling varies widely, depending on the particular brain region and cell type. To the best of our knowledge, previous articles that discuss lower EC_{50} values (i.e., higher potency) of extrasynaptic $GABA_A$Rs *in vivo* do not consider spontaneous channels and how they influence such measurements. This fact enforces the importance of the work on s-$GABA_A$Rs pharmacology for an understanding of biophysical phenomena in living neurons.

The important question regarding s-$GABA_A$Rs is whether or not these receptors represent a convergent group with similar functional properties, or if they share common receptor subunit(s). Numerous studies have attributed the majority (up to 75%) of $GABA_A$R-delivered tonic inhibition to δ-containing $GABA_A$Rs (Stell et al., 2003), which are abundant at extrasynaptic membranes (Nusser et al., 1998) but have been also found in synapses where they make a significant input into phasic inhibition (Sun et al., 2018), and in perisynaptic loci (Wei et al., 2003). The remaining portion of tonic inhibition is, to a large extent but not fully, produced by receptors containing the α5-subunit (Farrant and Nusser, 2005). Furthermore, the agonist-independent $GABA_A$R openings were observed under similar conditions for receptors of three different subunit compositions (Mortensen et al., 2010). In addition, the observation that mutations in α1 and β2 subunits modulate spontaneous $GABA_A$Rs gating (Baptista-Hon et al., 2017) prevents us from ruling out these subunits as potential alternative candidates to be involved in the formation of s-$GABA_A$Rs. Combined with the facts of the GABA-independent tonic activity of α4-$GABA_A$Rs (Tang et al., 2010) and spontaneous openings of α2β1ε-$GABA_A$Rs which contribute to the baseline currents in whole-cell recordings (Wagner et al., 2005), the abovementioned data on GABA-independent activity suggest that GABA-independent inhibition is of poly-subtype origin, with a substantial part inherent in the non-δ- and non-α5-containing receptors.

In view of numerous subunits and subunit compositions of $GABA_A$R which demonstrate spontaneous gating, the obvious question is: are there $GABA_A$Rs subtype(s) which do not demonstrate GABA-independent activity? The existence of such $GABA_A$Rs was suggested by the study showing that, in contrast to the α2α1ε receptor, responses of α2β1 and α2β1γ2-$GABA_A$Rs do not produce a "baseline overshoot" associated with spontaneous openings (Wagner et al., 2005).

Therefore, data collected to date suggest revision of two traditional views, now common in fundamental neuroscience: (i) that tonic inhibitory conductance is generated by ambient GABA (due to proven significance of s-$GABA_A$Rs input); and (ii) that tonic and phasic inhibition are mediated by different $GABA_A$Rs subtypes (due to growing evidence that typical extrasynaptic $GABA_A$Rs can make a significant contribution into IPSCs *via* a synaptic and/or perisynaptic presence).

It has been demonstrated that a scarcity of α1 subunit is correlated with resistance to anti-epileptic drugs (Bethmann et al., 2008), whereas increased α1-$GABA_A$R expression in the hippocampus suppresses the development of temporal lobe epilepsy (TLE; Raol et al., 2006). Apart from that, it was shown that phasic GABA-ergic inhibition is lowered in TLE, whereas tonic GABA-ergic conductance remains intact (Palma et al., 2007; Pavlov et al., 2011), making tonic GABA-ergic current a perspective target for TLE treatment. The classical paradigm, where extracellular GABA triggers tonic GABA-ergic current, implies that the most effective therapeutic approach is to increase the concentration of GABA in the cerebrospinal fluid, and thus augment inhibitory conductance. However, this approach was repeatedly found to be ineffective (Cohen et al., 2002; Glykys et al., 2009) or even one that leads to epileptogenesis (Palma et al., 2006; Cope et al., 2009) due to various side effects. These side effects impose limitations on the clinical use of specific antiepileptic drugs that increase the concentration of GABA in cerebrospinal fluid (Sander and Hart, 1990; Leppik, 1995). In contrast, the modulation of s-$GABA_A$Rs in GABA-independent manner promises an alternative for TLE treatment through the regulation of tonic conductance without the need to interfere with extracellular GABA concentration, thus avoiding the afore mentioned side effects.

Apart from the potential of α1-$GABA_A$Rs for TLE treatment, α5-$GABA_A$Rs (which also display GABA-independent activity) were found to be a perspective target for schizophrenia treatment (Lodge and Grace, 2011). Taking into account similar concentration of GABA found *in vivo* in the brains of schizophrenic patients and of a control group (Tayoshi et al., 2010), and the well-established fact that changes in tonic GABA-ergic inhibition are involved in the generation of schizophrenia symptoms (Damgaard et al., 2011), these data suggest a potentially important role of drugs targeting s-$GABA_A$Rs in the suppression of schizophrenia development, since action through s-$GABA_A$Rs in GABA-independent manner eliminates the need to modify GABA concentration in cerebrospinal fluid.

Another clinical implication of s-$GABA_A$Rs rises from the fact that sedative and analgesic effects of gaboxadol (THIP) are mediated exclusively by α4-containing $GABA_A$Rs (Chandra et al., 2006), that demonstrate GABA-independent activity.

AUTHOR CONTRIBUTIONS

NO and SS contributed to the conception and design of the article. NO received data displayed at the figure and analyzed literature connected to the topic, contributed to manuscript revision. SS wrote the manuscript.

FUNDING

This work was supported by the Rosetrees Research Grant A-1066.

REFERENCES

Abramian, A. M., Comenencia-Ortiz, E., Vithlani, M., Tretter, E. V., Sieghart, W., Davies, P. A., et al. (2010). Protein kinase C phosphorylation regulates membrane insertion of $GABA_A$ receptor subtypes that mediate tonic inhibition. *J. Biol. Chem.* 285, 41795–41805. doi: 10.1074/jbc.m110.149229

Abramian, A. M., Comenencia-Ortiz, E., Modgil, A., Vien, T. N., Nakamura, Y., Moore, Y. E., et al. (2014). Neurosteroids promote phosphorylation and membrane insertion of extrasynaptic $GABA_A$ receptors. *Proc. Natl. Acad. Sci. U S A* 111, 7132–7137. doi: 10.1073/pnas.1403285111

Baptista-Hon, D. T., Gulbinaite, S., and Hales, T. G. (2017). Loop G in the $GABA_A$ receptor α1 subunit influences gating efficacy. *J. Physiol.* 595, 1725–1741. doi: 10.1113/jp273752

Bethmann, K., Fritschy, J. M., Brandt, C., and Löscher, W. (2008). Antiepileptic drug resistant rats differ from drug responsive rats in $GABA_A$ receptor subunit expression in a model of temporal lobe epilepsy. *Neurobiol. Dis.* 31, 169–187. doi: 10.1016/j.nbd.2008.01.005

Bianchi, M. T., and Macdonald, R. L. (2001). Agonist trapping by $GABA_A$ receptor channels. *J. Neurosci.* 21, 9083–9091. doi: 10.1523/jneurosci.21-23-09083.2001

Birnir, B., Everitt, A. B., Lim, M. S., and Gage, P. W. (2000). Spontaneously opening $GABA_A$ channels in CA1 pyramidal neurones of rat hippocampus. *J. Membr. Biol.* 174, 21–29. doi: 10.1007/s002320001028

Bonin, R. P., Martin, L. J., MacDonald, J. F., and Orser, B. A. (2007). α5$GABA_A$ receptors regulate the intrinsic excitability of mouse hippocampal pyramidal neurons. *J. Neurophysiol.* 98, 2244–2254. doi: 10.1152/jn.00482.2007

Botta, P., Demmou, L., Kasugai, Y., Markovic, M., Xu, C., Fadok, J. P., et al. (2015). Regulating anxiety with extrasynaptic inhibition. *Nat. Neurosci.* 18, 1493–1500. doi: 10.1038/nn.4102

Brandon, N., Jovanovic, J., and Moss, S. (2002). Multiple roles of protein kinases in the modulation of gamma-aminobutyric $acid_{(A)}$ receptor function and cell surface expression. *Pharmacol. Ther.* 94, 113–122. doi: 10.1016/s0163-7258(02)00175-4

Brandon, N. J., Jovanovic, J. N., Colledge, M., Kittler, J. T., Brandon, J. M., Scott, J. D., et al. (2003). A-kinase anchoring protein 79/150 facilitates the phosphorylation of $GABA_A$ receptors by cAMP-dependent protein kinase via selective interaction with receptor β subunits. *Mole. Cell. Neurosci.* 22, 87–97. doi: 10.1016/s1044-7431(02)00017-9

Brickley, S. G., Cull-Candy, S. G., and Farrant, M. (1996). Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of $GABA_A$ receptors. *J Physiol.* 497, 753–759. doi: 10.1113/jphysiol.1996.sp021806

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

Brown, D. A. (1979). Extrasynaptic GABA systems. *Trends Neurosci.* 2, 271–273. doi: 10.1016/0166-2236(79)90107-3

Cai, X., Flores-Hernandez, J., Feng, J., and Yan, Z. (2002). Activity-dependent bidirectional regulation of $GABA_A$ receptor channels by the $5\text{-}HT_{(4)}$ receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. *J. Physiol.* 540, 743–759. doi: 10.1113/jphysiol.2001.013391

Carlson, S. L., Bohnsack, J. P., Patel, V., and Morrow, A. L. (2016). Regulation of extrasynaptic $GABA_A$ α4 receptors by ethanol-induced protein kinase, A, but not protein kinase C activation in cultured rat cerebral cortical neurons. *J. Pharmacol. Exp. Ther.* 356, 148–156. doi: 10.1124/jpet.115.228056

Carnegie, G. K., and Scott, J. D. (2003). A-kinase anchoring proteins and neuronal signaling mechanisms. *Genes Dev.* 17, 1557–1568. doi: 10.1101/gad.1095803

Chandra, D., Jia, F., Liang, J., Peng, Z., Suryanarayanan, A., Werner, D. F., et al. (2006). $GABA_A$ receptor α4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc. Natl. Acad. Sci. U S A* 103, 15230–15235. doi: 10.1073/pnas.0604304103

Chiu, C. S., Jensen, K., Sokolova, I., Wang, D., Li, M., Deshpande, P., et al. (2002). Number, density and surface/cytoplasmic distribution of GABA transporters at presynaptic structures of knock-in mice carrying GABA transporter subtype 1-Green fluorescent protein fusions. *J. Neurosci.* 22, 10251–10266. doi: 10.1523/jneurosci.22-23-10251.2002

Cohen, I., Navarro, V., Clemenceau, S., Baulac, M., and Miles, R. (2002). On the origin of interictal activity in human temporal lobe epilepsy *in vitro*. *Science* 298, 1418–1421. doi: 10.1126/science.1076510

Conti, F., Minelli, A., and Melone, M. (2004). GABA transporters in the mammalian cerebral cortex: localization, development and pathological implications. *Brain Res. Rev.* 45, 196–212. doi: 10.1016/j.brainresrev.2004.03.003

Cope, D. W., Hughes, S. W., and Crunelli, V. (2005). $GABA_A$ receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci.* 25, 11553–11563. doi: 10.1523/jneurosci.3362-05.2005

Cope, D. W., Di Giovanni, G., Fyson, S. J., Orbán, G., Errington, A. C., Lorincz, M. L., et al. (2009). Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. *Nat. Med.* 15, 1392–1398. doi: 10.1038/nm.2058

Damgaard, T., Plath, N., Neill, J. C., and Hansen, S. L. (2011). Extrasynaptic $GABA_A$ receptor activation reverses recognition memory deficits in an animal model of schizophrenia. *Psychopharmacology* 214, 403–413. doi: 10.1007/s00213-010-2039-9

Farrant, M., and Nusser, Z. (2005). Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. *Nat. Rev. Neurosci.* 6, 215–229. doi: 10.1038/nrn1625

Gerak, L. R. (2009). Selective changes in sensitivity to benzodiazepines, and not other positive $GABA_A$ modulators, in rats receiving flunitrazepam chronically. *Psychopharmacology* 204, 667–677. doi: 10.1007/s00213-009-1497-4

Glaeser, B. S., and Hare, T. A. (1975). Measurement of GABA in human cerebrospinal fluid. *Biochem. Med.* 12, 274–282. doi: 10.1016/0006-2944(75)90129-5

Glykys, J., and Mody, I. (2007). Activation of $GABA_A$ receptors: views from outside the synaptic cleft. *Neuron* 56, 763–770. doi: 10.1016/j.neuron.2007.11.002

Glykys, J., Dzhala, V. I., Kuchibhotla, K. V., Feng, G., Kuner, T., Augustine, G., et al. (2009). Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. *Neuron* 63, 657–672. doi: 10.1016/j.neuron.2009.08.022

Hamann, M., Rossi, D. J., and Attwell, D. (2002). Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron* 33, 625–633. doi: 10.1016/s0896-6273(02)00593-7

Häusser, M., and Clark, B. A. (1997). Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron* 19, 665–678. doi: 10.1016/s0896-6273(00)80379-7

Jatczak-Śliwa, M., Terejko, K., Brodzki, M., Michaowski, M. A., Czyzewska, M. M., Nowicka, J. M., et al. (2018). Distinct modulation of spontaneous and GABA-evoked gating by flurazepam shapes cross-talk between agonist-free and liganded $GABA_A$ receptor activity. *Front. Cell. Neurosci.* 12:237. doi: 10.3389/fncel.2018.00237

Kapur, A., Pearce, R. A., Lytton, W. W., and Haberly, L. B. (1997). $GABA_A$-mediated IPSCs in piriform cortex have fast and slow components with different properties and locations on pyramidal cells. *J. Neurophysiol.* 78, 2531–2545. doi: 10.1152/jn.1997.78.5.2531

Kozlov, A. S., Angulo, M. C., Audinat, E., and Charpak, S. (2006). Target cell-specific modulation of neuronal activity by astrocytes. *Proc. Natl. Acad. Sci. U S A* 103, 10058–10063. doi: 10.1073/pnas.0603741103

Leppik, I. E. (1995). Tiagabine: the safety landscape. *Epilepsia* 36, S10–S13. doi: 10.1111/j.1528-1157.1995.tb06009.x

Lodge, D. J., and Grace, A. A. (2011). Hippocampal dysregulation of dopamine system function and the pathophysiology of schizophrenia. *Trends Pharmacol. Sci.* 32, 507–513. doi: 10.1016/j.tips.2011.05.001

Maksay, G., Thompson, S. A., and Wafford, K. A. (2003). The pharmacology of spontaneously open α1 β3 ε $GABA_A$ receptor-ionophores. *Neuropharmacology* 44, 994–1002. doi: 10.1016/s0028-3908(03)00116-3

Mathers, D. A. (1985). Spontaneous and GABA-induced single channel currents in cultured murine spinal cord neurons. *Can. J. Physiol. Pharmacol.* 63, 1228–1233. doi: 10.1139/y85-203

McCartney, M. R., Deeb, T. Z., Henderson, T. N., and Hales, T. G. (2007). Tonically active $GABA_A$ receptors in hippocampal pyramidal neurons exhibit constitutive GABA-independent gating. *Mol. Pharmacol.* 71, 539–548. doi: 10.1124/mol.106.028597

Miko, A., Werby, E., Sun, H., Healey, J., and Zhang, L. (2004). A TM2 residue in the β1 subunit determines spontaneous opening of homomeric and heteromeric γ-aminobutyric acid-gated ion channels. *J. Biol. Chem.* 279, 22833–22840. doi: 10.1074/jbc.m402577200

Minelli, A., DeBiasi, S., Brecha, N. C., Zuccarello, L. V., and Conti, F. (1996). GAT-3, a high-affinity GABA plasma membrane transporter, is localized to astrocytic processes, and it is not confined to the vicinity of GABAergic synapses in the cerebral cortex. *J. Neurosci.* 16, 6255–6264. doi: 10.1523/jneurosci.16-19-06255.1996

Modgil, A., Parakala, M. L., Ackley, M. A., Doherty, J. J., Moss, S. J., and Davies, P. A. (2017). Endogenous and synthetic neuroactive steroids evoke sustained increases in the efficacy of GABAergic inhibition via a protein kinase C-dependent mechanism. *Neuropharmacology* 113, 314–322. doi: 10.1016/j.neuropharm.2016.10.010

Mody, I., and Pearce, R. A. (2004). Diversity of inhibitory neurotransmission through $GABA_A$ receptors. *Trends Neurosci.* 27, 569–575. doi: 10.1016/j.tins.2004.07.002

Mortensen, M., Ebert, B., Wafford, K., and Smart, T. G. (2010). Distinct activities of GABA agonists at synaptic- and extrasynaptic-type $GABA_A$ receptors. *J. Physiol.* 588, 1251–1268. doi: 10.1113/jphysiol.2009.182444

Neelands, T. R., Fisher, J. L., Bianchi, M., and Macdonald, R. L. (1999). Spontaneous and γ-aminobutyric acid (GABA)-activated $GABA_A$ receptor channels formed by ε subunit-containing isoforms. *Mol. Pharmacol.* 55, 168–178. doi: 10.1124/mol.55.1.168

Neves, S. R., Ram, P. T., and Iyengar, R. (2002). G protein pathways. *Science* 296, 1636–1639. doi: 10.1126/science.1071550

Nusser, Z., and Mody, I. (2002). Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J. Neurophysiol.* 87, 2624–2628. doi: 10.1152/jn.2002.87.5.2624

Nusser, Z., Sieghart, W., and Somogyi, P. (1998). Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J. Neurosci.* 18, 1693–1703. doi: 10.1523/jneurosci.18-05-01693.1998

O'Neill, N., and Sylantyev, S. (2018a). Spontaneously opening $GABA_A$ receptors play a significant role in neuronal signal filtering and integration. *Cell Death Dis.* 9:813. doi: 10.1038/s41419-018-0856-7

O'Neill, N., and Sylantyev, S. (2018b). Feature Article: selective modulation of tonically active $GABA_A$ receptor functional subgroups by G-proteins and protein kinase C. *Exp. Biol. Med.* 243, 1046–1055. doi: 10.1177/1535370218800980

Oláh, S., Füle, M., Komlósi, G., Varga, C., Báldi, R., Barzó, P., et al. (2009). Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. *Nature* 461, 1278–1281. doi: 10.1038/nature08503

Otis, T. S., Staley, K. J., and Mody, I. (1991). Perpetual inhibitory activity in mammalian brain slices generated by spontaneous GABA release. *Brain Res.* 545, 142–150. doi: 10.1016/0006-8993(91)91280-e

Palma, E., Amici, M., Sobrero, F., Spinelli, G., Di Angelantonio, S., Ragozzino, D., et al. (2006). Anomalous levels of Cl- transporters in the hippocampal subiculum from temporal lobe epilepsy patients make GABA excitatory. *Proc. Natl. Acad. Sci. U S A* 103, 8465–8468. doi: 10.1073/pnas.0602979103

Palma, E., Roseti, C., Maiolino, F., Fucile, S., Martinello, K., Mazzuferi, M., et al. (2007). $GABA_A$-current rundown of temporal lobe epilepsy is associated with repetitive activation of $GABA_A$ "phasic" receptors. *Proc. Natl. Acad. Sci. U S A* 104, 20944–20948. doi: 10.1073/pnas.0710522105

Pavlov, I., Huusko, N., Drexel, M., Kirchmair, E., Sperk, G., Pitkänen, A., et al. (2011). Progressive loss of phasic, but not tonic, $GABA_A$ receptor-mediated inhibition in dentate granule cells in a model of post-traumatic epilepsy in rats. *Neuroscience* 194, 208–219. doi: 10.1016/j.neuroscience.2011.07.074

Raol, Y. H., Lund, I. V., Bandyopadhyay, S., Zhang, G., Roberts, D. S., Wolfe, J. H., et al. (2006). Enhancing $GABA_A$ receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal

lobe epilepsy. *J. Neurosci.* 26, 11342–11346. doi: 10.1523/jneurosci.3329-06.2006

Romo-Parra, H., Blaesse, P., Sosulina, L., and Pape, H. C. (2015). Neurosteroids increase tonic GABAergic inhibition in the lateral section of the central amygdala in mice. *J. Neurophysiol.* 113, 3421–3431. doi: 10.1152/jn.00045.2015

Saliba, R. S., Kretschmannova, K., and Moss, S. J. (2012). Activity-dependent phosphorylation of $GABA_A$ receptors regulates receptor insertion and tonic current. *EMBO J.* 31, 2937–2951. doi: 10.1038/emboj.2012.109

Sander, J. W., and Hart, Y. M. (1990). Vigabatrin and behaviour disturbances. *Lancet* 335:57. doi: 10.1016/0140-6736(90)90190-g

Song, I., Savtchenko, L., and Semyanov, A. (2011). Tonic excitation or inhibition is set by $GABA_A$ conductance in hippocampal interneurons. *Nat. Commun.* 2:376. doi: 10.1038/ncomms1377

Stell, B. M., Brickley, S. G., Tang, C. Y., Farrant, M., and Mody, I. (2003). Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing $GABA_A$ receptors. *Proc. Natl. Acad. Sci. U S A* 100, 14439–14444. doi: 10.1073/pnas.2435457100

Sun, M. Y., Shu, H. J., Benz, A., Bracamontes, J., Akk, G., Zorumski, C. F., et al. (2018). Chemogenetic isolation reveals synaptic contribution of δ $GABA_A$ receptors in mouse dentate granule neurons. *J. Neurosci.* 38, 8128–8145. doi: 10.1523/jneurosci.0799-18.2018

Takagi, K., Ginsberg, M. D., Globus, M. Y., Dietrich, W. D., Martinez, E., Kraydieh, S., et al. (1993). Changes in amino acid neurotransmitters and cerebral blood flow in the ischemic penumbral region following middle cerebral artery occlusion in the rat: correlation with histopathology. *J. Cereb. Blood Flow Metab.* 13, 575–585. doi: 10.1038/jcbfm.1993.75

Tang, X., Hernandez, C. C., and Macdonald, R. L. (2010). Modulation of spontaneous and GABA-evoked tonic α4β3δ and α4β3γ2L $GABA_A$ receptor currents by protein kinase A. *J. Neurophysiol.* 103, 1007–1019. doi: 10.1152/jn.00801.2009

Tang, Z. Q., Dinh, E. H., Shi, W., and Lu, Y. (2011). Ambient GABA-activated tonic inhibition sharpens auditory coincidence detection via a depolarizing shunting mechanism. *J. Neurosci.* 31, 6121–6131. doi: 10.1523/jneurosci.4733-10.2011

Tayoshi, S., Nakataki, M., Sumitani, S., Taniguchi, K., Shibuya-Tayoshi, S., Numata, S., et al. (2010). GABA concentration in schizophrenia patients and the effects of antipsychotic medication: A proton magnetic resonance spectroscopy study. *Schizophr. Res.* 117, 83–91. doi: 10.1016/j.schres.2009.11.011

Tossman, U., Jonsson, G., and Ungerstedt, U. (1986). Regional distribution and extracellular levels of amino acids in rat central nervous system. *Acta Physiol. Scand.* 127, 533–545. doi: 10.1111/j.1748-1716.1986.tb07938.x

Wagner, D. A., Goldschen-Ohm, M. P., Hales, T. G., and Jones, M. V. (2005). Kinetics and spontaneous open probability conferred by the ε subunit of the $GABA_A$ receptor. *J. Neurosci.* 25, 10462–10468. doi: 10.1523/jneurosci.1658-05.2005

Wang, X., Zhong, P., and Yan, Z. (2002). Dopamine D4 receptors modulate GABAergic signaling in pyramidal neurons of prefrontal cortex. *J. Neurosci.* 22, 9185–9193. doi: 10.1523/jneurosci.22-21-09185.2002

Wei, W., Zhang, N., Peng, Z., Houser, C. R., and Mody, I. (2003). Perisynaptic localization of delta subunit-containing $GABA_A$ receptors and their activation by GABA spillover in the mouse dentate gyrus. *J. Neurosci.* 23, 10650–10661. doi: 10.1523/jneurosci.23-33-10650.2003

Wlodarczyk, A. I., Sylantyev, S., Herd, M. B., Kersanté, F., Lambert, J. J., Rusakov, D. A., et al. (2013). GABA-independent $GABA_A$ receptor openings maintain tonic currents. *J. Neurosci.* 33, 3905–3914. doi: 10.1523/jneurosci.4193-12.2013

Yeung, J. Y., Canning, K. J., Zhu, G., Pennefather, P., MacDonald, J. F., and Orser, B. A. (2003). Tonically activated $GABA_A$ receptors in hippocampal neurons are high-affinity, low-conductance sensors for extracellular GABA. *Mol. Pharmacol.* 63, 2–8. doi: 10.1124/mol.63.1.2

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.