



# Postnatal maturation of somatostatin-expressing inhibitory cells in the somatosensory cortex of GIN mice

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Postnatal inhibitory neuron development affects mammalian brain function, and failure of this maturation process may underlie pathological conditions such as epilepsy, schizophrenia, and depression. Furthermore, understanding how physiological properties of inhibitory neurons change throughout development is critical to understanding the role(s) these cells play in cortical processing. One subset of inhibitory neurons that may be affected during postnatal development is somatostatin-expressing (SOM) cells. A subset of these cells is labeled with green-fluorescent protein (GFP) in a line of mice known as the GFP-positive inhibitory neurons (GIN) line. Here, we studied how intrinsic electrophysiological properties of these cells changed in the somatosensory cortex of GIN mice between postnatal ages P11 and P32+. GIN cells were targeted for whole-cell current-clamp recordings and ranges of positive and negative current steps were presented to each cell. The results showed that as the neocortical circuitry matured during this critical time period multiple intrinsic and firing properties of GIN inhibitory neurons, as well as those of excitatory (regular-spiking [RS]) cells, were altered. Furthermore, these changes were such that the output of GIN cells, but not RS cells, increased over this developmental period. We quantified changes in excitability by examining the input–output relationship of both GIN and RS cells. We found that the firing frequency of GIN cells increased with age, while the rheobase current remained constant across development. This created a multiplicative increase in the input–output relationship of the GIN cells, leading to increases in gain with age. The input–output relationship of the RS cells, on the other hand, showed primarily a subtractive shift with age, but no substantial change in gain. These results suggest that as the neocortex matures, inhibition coming from GIN cells may become more influential in the circuit and play a greater role in the modulation of neocortical activity.

**Keywords:** somatostatin, inhibition, somatosensory cortex, postnatal development

## INTRODUCTION

The second and third postnatal weeks are a period of massive developmental change within the rodent neocortex. This is the time of sensory onset for multiple sensory modalities, including the visual, auditory, and somatosensory systems (Welker, 1964; Ehret, 1976; Hensch, 2005). This time window represents a “critical period” for the brain, as the organization of neural circuitry that develops within this time remains relatively fixed throughout adulthood. Diseases such as schizophrenia, which do not appear until late adolescence in humans, are thought to be a manifestation of pathological alterations in developmental processes, potentially in inhibitory neurons (Lewis et al., 2005). Understanding the development of cortical circuits during this period is therefore an important component to understanding both normal and pathological cortical function.

Maturation of inhibitory circuitry is of particular importance for shaping cortical activity during postnatal development (Hensch, 2005). Investigation of inhibitory interneuron development in multiple cortical areas reveals that during the second to third postnatal weeks, inhibitory cells undergo changes in their

physiological, molecular, and anatomical characteristics and do not reach maturation until the third week of postnatal development or later (Goldberg et al., 2011; Lazarus and Huang, 2011; Oswald and Reyes, 2011). In mice and rats, the animals first begin actively using their whiskers around postnatal day 14, signaling the onset of active sensory processing within the somatosensory system (Welker, 1964; Mosconi et al., 2010). During the first few weeks of postnatal development, inhibition onto pyramidal neurons in somatosensory cortex increases in amplitude and frequency (Kobayashi et al., 2008), and alterations in sensory experience, such as whisker removal, cause substantial disruptions to the intrinsic and synaptic properties of inhibitory interneurons (Jiao et al., 2006, 2011; Daw et al., 2007; Lee et al., 2007; Chittajallu and Isaac, 2010).

Understanding maturation of inhibitory circuits is important for determining how neurons control synaptic integration and communication within and amongst cortical areas during development. Most studies investigating how development of inhibitory neurons affects cortical circuitry have focused on one type of inhibitory neuron, known as the fast-spiking (FS) or

parvalbumin-expressing (PV) cells. These cells represent a major subtype of inhibitory neurons, but only constitute approximately half of inhibitory neurons as a whole (Markram et al., 2004). A second major class of inhibitory neurons is the somatostatin-expressing (SOM) inhibitory cells. However, the developmental trajectory of SOM cells in the somatosensory system is largely unknown. One recent study found that the intrinsic properties of SOM cells in the visual cortex change during the third postnatal week and suggested SOM cells may be involved in ocular dominance plasticity (Lazarus and Huang, 2011).

The role of SOM inhibitory cells in the neocortex has yet to be fully understood. Because the properties of these cells differ significantly from other inhibitory cells, such as PV cells, it is thought that they could play a unique role in neocortical processing. In contrast to PV cells, which target the perisomatic regions of their downstream cells, SOM cells primarily target the dendrites of pyramidal neurons. They may, therefore, be important for regulating and modulating incoming synaptic inputs. Recent *in vivo* recordings from superficial SOM cells in mouse somatosensory cortex have found that activity in SOM cells is suppressed during both passive whisker deflection and during active whisking states (Gentet et al., 2012). This loss of dendritic inhibition may function to allow excitatory inputs on the distal dendrites to summate and propagate to the soma more effectively. This may be particularly relevant during awake states when activity in the dendrites is enhanced (Murayama and Larkum, 2009). Activation of SOM cells has also been shown to effectively prevent pyramidal neurons from producing “bursts” of action potentials that are generated in the apical dendrite through active dendritic currents (Larkum et al., 1999; Gentet et al., 2012; Lovett-Barron et al., 2012). In addition, SOM cells are extensively coupled to each other through gap junctions (Gibson et al., 1999; Fanselow et al., 2008). Alterations in intrinsic properties with age could change how SOM cells act as an electrically coupled network, such as by changing their tendency to correlate or synchronize their activity (Amitai et al., 2002; Long et al., 2005; de la Rocha et al., 2007).

Across the SOM interneuron population there exists a degree of variability in the morphology, physiological characteristics, and co-expression of additional neuropeptides. Whether this reflects variability within a single class or is sufficiently diverse to warrant multiple subtypes is unclear (Ma et al., 2006; Sugino et al., 2006; McGarry et al., 2010). This issue has been partially addressed by the creation of transgenic mouse lines that express a fluorescent molecule, such as green fluorescent protein (GFP), in particular subsets of inhibitory neurons. Here, we used such a mouse line to study SOM inhibitory cells by utilizing a line of mice that expresses GFP in approximately one-third of SOM cells (Oliva et al., 2000). In these mice, the GFP-positive inhibitory neurons (“GIN”) express the neuropeptide, SOM, exhibit adapting responses to intracellular current steps, and are often of Martinotti morphology with an axon traveling up to layer 1 and ramifying extensively (Oliva et al., 2000; Halabisky et al., 2006; Ma et al., 2006; Fanselow et al., 2008).

Understanding the normal trajectory of GIN cell maturation serves several purposes. First, it helps clarify the physiological role(s) these cells can play during different stages of postnatal

development by indicating how intrinsic properties of GIN cells change over age, how readily these cells are excited and what their firing characteristics are once activated. Second, it will help us learn how to best distinguish between types and subtypes of inhibitory neurons even as their physiological characteristics, which are often used as factors to distinguish between cell types (Kawaguchi, 1995; Kawaguchi and Kubota, 1996; Kawaguchi and Kondo, 2002; Markram et al., 2004; Ascoli et al., 2008), change systematically with age. Finally, the way circuitry of excitatory and inhibitory neurons changes at different stages of maturation may help us determine causes and mechanisms of abnormal cortical development (Dani et al., 2005; Lewis et al., 2005; Yizhar et al., 2011).

Here, we characterized the intrinsic physiological properties of GIN cells and regular-spiking (RS) excitatory cells in the mouse primary somatosensory cortex during a developmental period (P11–P32+) that encompasses changes in multiple intrinsic cellular properties in somatosensory cortex (Maravall et al., 2004; Jiao et al., 2006, 2011; Goldberg et al., 2011) as well as whisking onset. We found that changes in sub- and supra-threshold properties occurred in both cell types, collectively resulting in an increase in GIN cell excitability and no change in RS cell excitability as a function of age. These results suggest that as the neocortical circuit develops, GIN cells may increasingly participate in sensory processing within somatosensory cortex.

## MATERIALS AND METHODS

### SLICING PROCEDURE

All experiments were carried out in compliance with the University of Pittsburgh School of Medicine animal use policies and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Thalamocortical slices from mouse somatosensory cortex were prepared as previously described (Agmon and Connors, 1991). In addition, a subset of cells was recorded from coronal sections of the same brain region. No systematic differences between these two slicing angles were observed, so the data were pooled.

### RECORDING PROCEDURES

Whole-cell current-clamp recordings were performed using micropipettes (4–7 M $\Omega$ ) filled with internal solution containing (in mM): 135 K-gluconate, 4 KCl, 2 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, and 14 phosphocreatine-Tris (pH 7.25, 280–290 mOsm). Membrane potentials reported here were not corrected for the liquid junction potential. Recordings were conducted at 32°C. When patching, cell-attached seal resistances were 1 G $\Omega$  or greater and series resistance after achieving whole-cell configuration was 5–20 M $\Omega$ . Data were collected using a Multiclamp 700B amplifier and pClamp10 software (Molecular Devices). Data were analyzed using in-house programs written in Matlab (The MathWorks, Natick, MA; E. Fanselow).

### CELL VISUALIZATION AND IDENTIFICATION

Cells were viewed under infrared-differential interference contrast illumination using a Nikon FN-1 microscope and a Dage IR-1000 CCD camera. GIN cells were identified by visualization

of GFP under epifluorescence illumination. RS pyramidal cells did not express GFP in these mice and were, therefore, targeted for recording based on having a triangular-shaped soma. During recording, RS pyramidal cells were positively distinguished from RS inhibitory interneurons as having strongly adapting spike firing rates during suprathreshold current steps, small amplitude afterhyperpolarization (AHPs), and during a train of action potentials the first AHP was smaller than subsequent AHPs (Porter et al., 2001; Beierlein et al., 2003). All cells were recorded in layers 2 and 3 of somatosensory cortex. Layer 2/3 cells were defined as those between the bottom of layer 1, identified by its lack of cell bodies, and the tops of the barrels, which demarcate layer 4.

### CELLS RECORDED

Recordings from a total of 269 GIN cells and 200 RS cells from a total of 170 mice were analyzed for this study. Numbers of cells for each cell type and age can be found in **Table 1**. All mice were of the GIN strain [Jackson Labs, Bar Harbor, ME; (Oliva et al., 2000)] and were aged P11–P50. Cells from those aged >P31 were combined and are identified here as P32+. In addition, we combined P18 and P19 cells into one group, identified as P19 in the text and on our plots. Both male and female mice were used. The morphologies of GIN cells have been described extensively in other publications, and these cells have been shown to express the peptide, SOM; (Oliva et al., 2000; Halabisky et al., 2006; Ma et al., 2006; Fanselow et al., 2008).

### MEASURES OF CELLULAR PROPERTIES

Once patched, we presented each cell with a series of negative and positive current steps, ranging from –100 to 300 pA in 10 or 20 pA steps. The following measures were made from the voltage traces recorded during these current steps.

*Adaptation ratio*: inverse of mean of the last three interspike intervals during a 200 pA step, divided by the inverse of the initial interspike interval in that step.

*Action potential threshold (mV)*: voltage at the time of the peak of the third derivative of the voltage trace for the first action potential in a given current step.

*AHP magnitude (mV)*: voltage at the most negative trough of the AHP (within 4 ms of the action potential peak) minus the action potential threshold voltage.

*AHP slope (mV/ms)*: slope of the AHP for 8 ms following the most negative trough in the AHP.

*Firing frequency, initial (Fi; Hz)*: inverse of the first interspike interval during a 200 pA current step.

*Firing frequency, steady state (Fss; Hz)*: average of the inverse of the last three interspike intervals in a 200 pA current step.

*Half-width (ms)*: width of the action potential halfway between the threshold voltage and the peak voltage.

*Input-output gain (F–I slope; Hz/pA)*: slope of the linear portion of each frequency-current (F–I) curve calculated from responses to suprathreshold current steps. Frequency was defined as the inverse of the interspike intervals throughout the entire current step.

*Input resistance (MΩ)*: magnitude of voltage deflection (mV)/magnitude of current step (pA); calculated as the slope of the V/I plot for positive and negative voltage deflections between ±8 mV.

*Membrane time constant (τ<sub>m</sub>; ms)*: calculated from a mono-exponential curve best fitting the falling phase of the voltage deflection in response to negative current steps. Only voltage deflections <8 mV negative to the resting membrane potential were used.

*Rebound depolarization (mV)*: The height of the voltage peak within 200 ms of the end of a current step. Current steps with voltage deflections from –70 to –15 mV were used.

*Resting membrane potential (V<sub>rest</sub>; mV)*: average voltage in the 20 ms prior to a current step.

*Rheobase current (pA)*: We plotted the number of action potentials as a function of input current and extrapolated to find the current required to elicit a single action potential.

*Sag magnitude (mV)*: calculated from negative-going current steps as the difference between the peak negative voltage deflection and the average voltage for the last 50 ms of the 600 ms current step. Only traces in which the maximum voltage deflection was >15 mV more negative than the resting membrane potential were analyzed.

### STATISTICAL ANALYSES

For quantification of RS and GIN cell properties, measurements were made from negative or positive current steps as described above. When results were plotted as a function of age, the resulting data were fit with linear, exponential, second degree polynomial or sigmoidal functions, as appropriate, using SigmaPlot (San Jose, CA). P11 and P32+ values were compared for each type of measurement using a two-tailed *t*-test and results are presented in **Table 2**.

### RESULTS

In this study, we quantified intrinsic physiological properties of GIN and RS pyramidal cells in layer 2/3 of somatosensory cortex

**Table 1 | Cell counts.**

Cell type	Postnatal Age (days)									Total
	P11	P12	P13	P14	P15	P16	P17	P18–19	P32+	
GIN	16	14	41	66	48	46	10	6	22	269
RS	11	4	42	37	39	20	22	12	13	200

**Table 2 | Cell properties at P11 and P32+.**

Property	GIN			RS		
	P11	P32+	<i>p</i> -value	P11	P32+	<i>p</i> -value
Adaptation Ratio	0.6 ± 0.03	0.5 ± 0.03	*	0.4 ± 0.03	0.4 ± 0.06	0.162
AHP magnitude (mV)	-9.6 ± 0.5	-14.7 ± 0.7	***	-6.4 ± -1.1	-11.8 ± -1.5	*
AHP slope (mV/ms)	0.7 ± 0.1	1.9 ± 0.15	***	0.4 ± 0.03	0.8 ± 0.2	0.189
Action potential threshold, 1st spike (mV)	-40 ± 0.5	-41 ± 0.6	0.511	-40 ± 0.9	-40 ± 1.3	0.633
Firing frequency, initial (Hz)	61 ± 2	96 ± 2.6	***	59 ± 2.6	60 ± 5.1	0.856
Firing frequency, steady state (Hz)	38 ± 3	42.4 ± 3.2	**	20 ± 1	25 ± 1.9	0.11
Gain (Hz/pA)	0.2 ± 0.02	0.44 ± 0.02	***	0.14 ± 0.02	0.28 ± 0.05	*
Half-width (ms)	1.4 ± 0.05	0.8 ± 0.05	***	1.8 ± 0.08	0.96 ± 0.09	***
Input-output gain (Hz/pA)	0.2 ± 0.02	0.4 ± 0.02	***	0.14 ± 0.02	0.28 ± 0.05	0.037
Input resistance (MΩ)	379 ± 32	244 ± 12.1	***	326 ± 35	160 ± 9.7	***
Membrane time constant (ms)	56 ± 4.6	23.2 ± 1.7	***	32 ± 4.9	14 ± 1.9	0.009
Rebound depolarization (mV)	2 ± 0.8	4.4 ± 0.4	*	3.1 ± 0.7	1.4 ± 0.4	0.033
Rheobase current (pA)	14 ± 2	28 ± 5	*	30 ± 4	90 ± 21	*
Sag magnitude (mV)	3 ± 0.6	5.6 ± 0.5	**	2.7 ± 0.6	1.02 ± 0.3	*
$V_{rest}$ (mV)	-57.2 ± 1	-59.2 ± 0.8	0.135	-64.9 ± 1.3	-64.9 ± 2.1	0.978

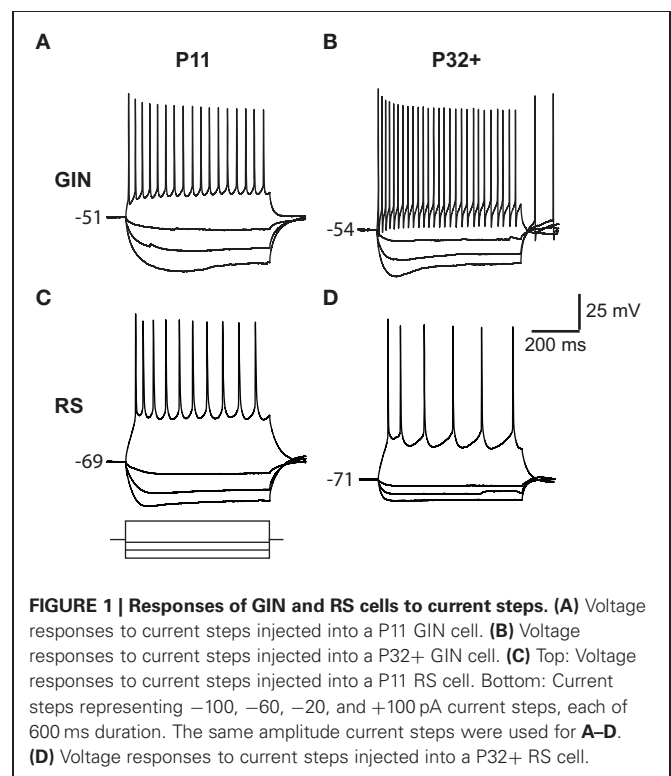
\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

ranging in age from P11 to P32+. We recorded from local pyramidal neurons (RS cells) in order to directly compare how GIN and RS cells develop relative to one another within the same network. The development of intrinsic properties of pyramidal cells has been reported previously in multiple brain regions (McCormick and Prince, 1987; Maravall et al., 2004; Frick et al., 2007; Oswald and Reyes, 2008), and the results we present here for RS cells are largely in agreement with these previous studies. In contrast, the changes in GIN properties we observed have not been reported for the somatosensory cortex and differed somewhat from those described for visual cortex (Lazarus and Huang, 2011).

### SUBTHRESHOLD INTRINSIC PROPERTIES

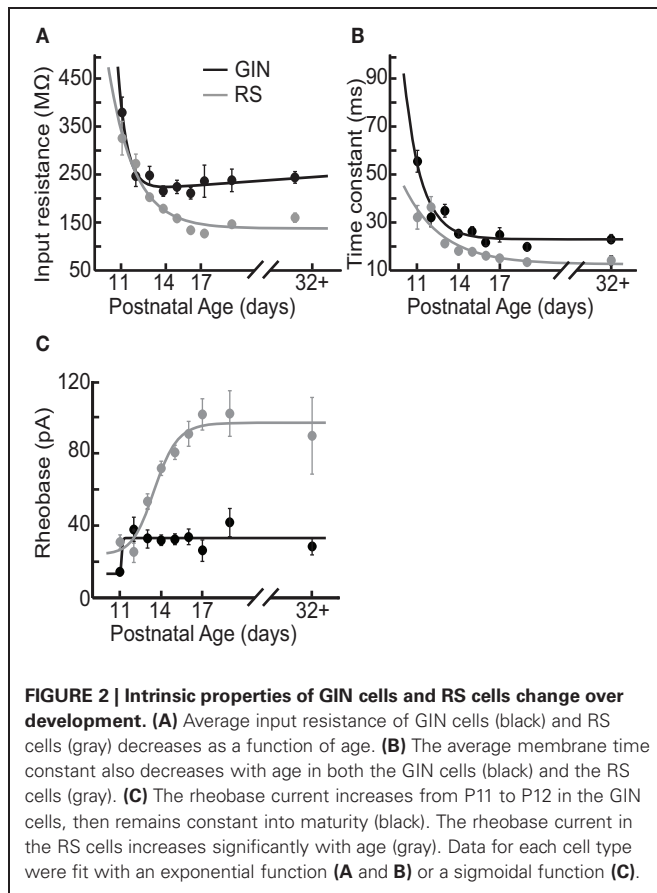
We observed that multiple subthreshold characteristics of GIN cells developed over the time window examined in this study, P11–P32+ (**Figure 1**). First, we examined several intrinsic properties of these cells: the input resistance ( $R_{in}$ ), membrane time constant ( $\tau_m$ ), rheobase current, resting membrane potential ( $V_{rest}$ ), and the threshold voltage for action potential generation ( $V_{thresh}$ ). We found that several, but not all, of these features changed over this age range (**Figure 2**). The  $R_{in}$  of GIN cells decreased significantly from P11 to P32+ (P11:  $379 \pm 32$  MΩ; P32+:  $244 \pm 12.1$  MΩ,  $p < 0.001$ ). This change occurred rapidly in GIN cells, as  $R_{in}$  decreased mainly between P11 ( $379 \pm 32$  MΩ) and P12 ( $247 \pm 22$  MΩ) then remained steady until P32+ (**Figure 2A**). The  $\tau_m$  in GIN cells also decreased from P11 to P12 then remained steady until P32+ (P11:  $56 \pm 4.6$  ms; P12:  $32 \pm 4$  ms; P32+:  $23.2 \pm 1.7$  ms,  $p < 0.001$ ; **Figure 2B**). The rheobase current in GIN cells increased from P11 to P12 and then remained constant (P11:  $14 \pm 2$  pA; P12:  $37 \pm 7$  pA, P32+:  $28 \pm 5$  pA,  $p < 0.05$ ; **Figure 2C**).

In RS cells, the  $R_{in}$  and  $\tau_m$  also decreased during this period, but did so more gradually than in GIN cells ( $R_{in}$ : P11,  $326 \pm 35$  MΩ; P32+,  $160 \pm 9.7$  MΩ,  $p < 0.001$ ;  $\tau_m$ : P11,  $32 \pm 4.9$  ms; P32+,  $14 \pm 1.9$  ms,  $p = 0.009$ ; **Figures 2A,B**). The rheobase



**FIGURE 1 | Responses of GIN and RS cells to current steps. (A)** Voltage responses to current steps injected into a P11 GIN cell. **(B)** Voltage responses to current steps injected into a P32+ GIN cell. **(C)** Top: Voltage responses to current steps injected into a P11 RS cell. Bottom: Current steps representing -100, -60, -20, and +100 pA current steps, each of 600 ms duration. The same amplitude current steps were used for **A–D**. **(D)** Voltage responses to current steps injected into a P32+ RS cell.

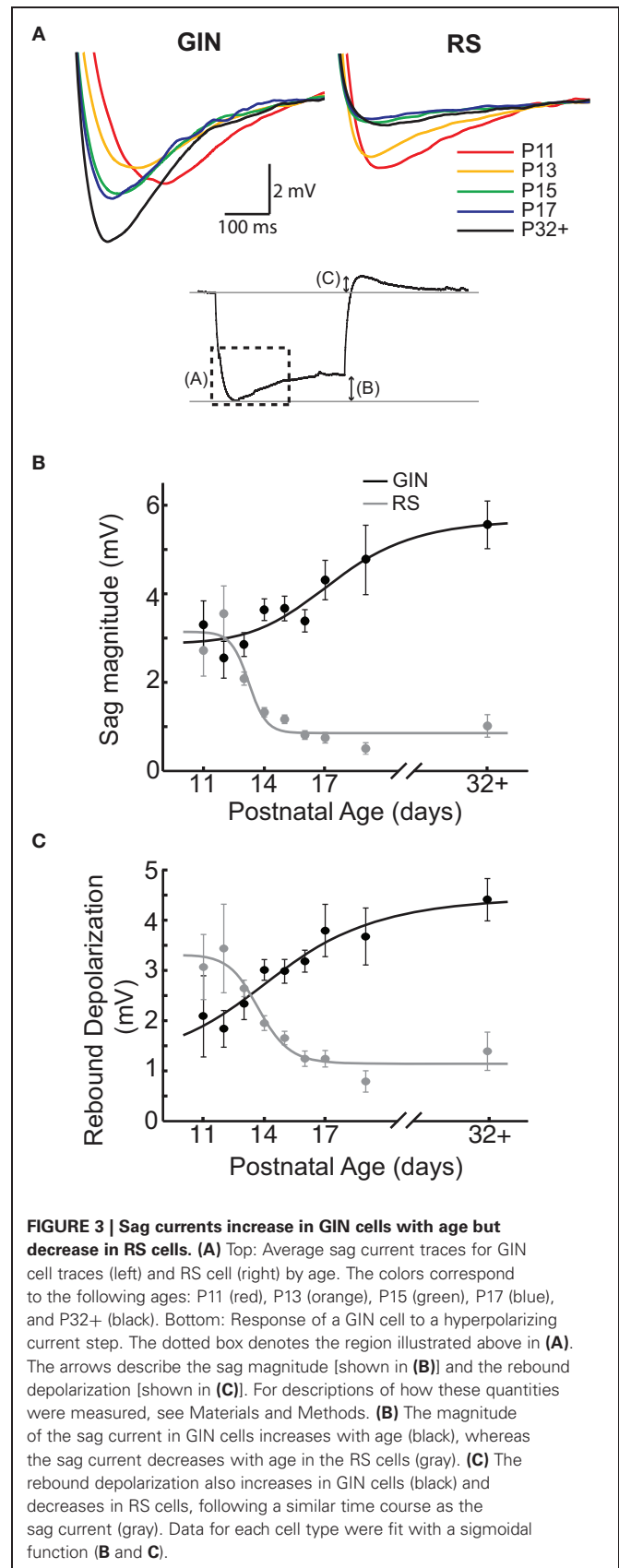
current in RS cells increased threefold, from  $30 \pm 4$  pA at P11 to  $90 \pm 21$  pA at P32+ (**Figure 2C**). This increase in rheobase current also occurred more gradually over the second postnatal week than in GIN cells.  $V_{rest}$  did not change significantly throughout development in GIN cells (P11:  $-57.2 \pm 1$  mV; P32+:  $-59.2 \pm 0.8$  mV,  $p = 0.135$ ; **Table 2**) or RS cells (P11:  $-64.9 \pm 1.3$  mV; P32+:  $-64.9 \pm 2.1$  mV;  $p = 0.978$ ; **Table 2**). In addition,  $V_{thresh}$



remained constant across the age range investigated here in both GIN cells (P11:  $-40 \pm 0.5$  mV; P32+:  $-41 \pm 0.6$  mV,  $p = 0.511$ ; **Table 2**) and RS cells (P11:  $-40 \pm 0.9$  mV; P32+:  $-40 \pm 1.3$ ,  $p = 0.633$ ; **Table 2**).

Collectively, these results suggest that during the age range investigated, the intrinsic excitability of both GIN and RS cells decreased. But it should be noted that within the same age range, GIN cells remained more excitable than RS cells, exhibiting higher input resistance, higher  $\tau_m$ , lower rheobase current, and more depolarized  $V_{rest}$  than RS cells across this developmental period.

Another characteristic feature of GIN cells is the presence of a pronounced “sag current” in response to hyperpolarizing current steps (Oliva et al., 2000; Halabisky et al., 2006; Ma et al., 2006) **Figures 1B and 3A**). In GIN cells, sag currents are mediated by  $I_h$  currents (Ma et al., 2006). We observed a strong increase in the magnitude of the sag current with age (P11:  $3 \pm 0.6$  mV; P32+:  $5.6 \pm 0.5$  mV,  $p < 0.01$ ; **Figures 3A,B**). A related feature observed in GIN cells is the presence of a rebound depolarization when the cell is released from hyperpolarization, which in some cases results in the generation of a rebound spike (**Figures 1B and 3A**). This depolarization is also mediated by  $I_h$  currents in GIN cells (Ma et al., 2006). We observed that the amplitude of rebound depolarization also increased with age (P11:  $2 \pm 0.8$  mV; P32+:  $4.4 \pm 0.4$  mV,  $p < 0.05$ ; **Figure 3C**), and showed a similar developmental time course as the change in sag current magnitude.



RS cells also showed a small  $I_h$ -mediated sag current (Maravall et al., 2004), but the magnitude of the sag current and rebound depolarization decreased with age in RS cells (Figures 3A–C), consistent with previous results (Maravall et al., 2004). Together, these findings suggest that  $I_h$  currents increased in GIN cells and decreased in RS cells from ages P11 to P32+.

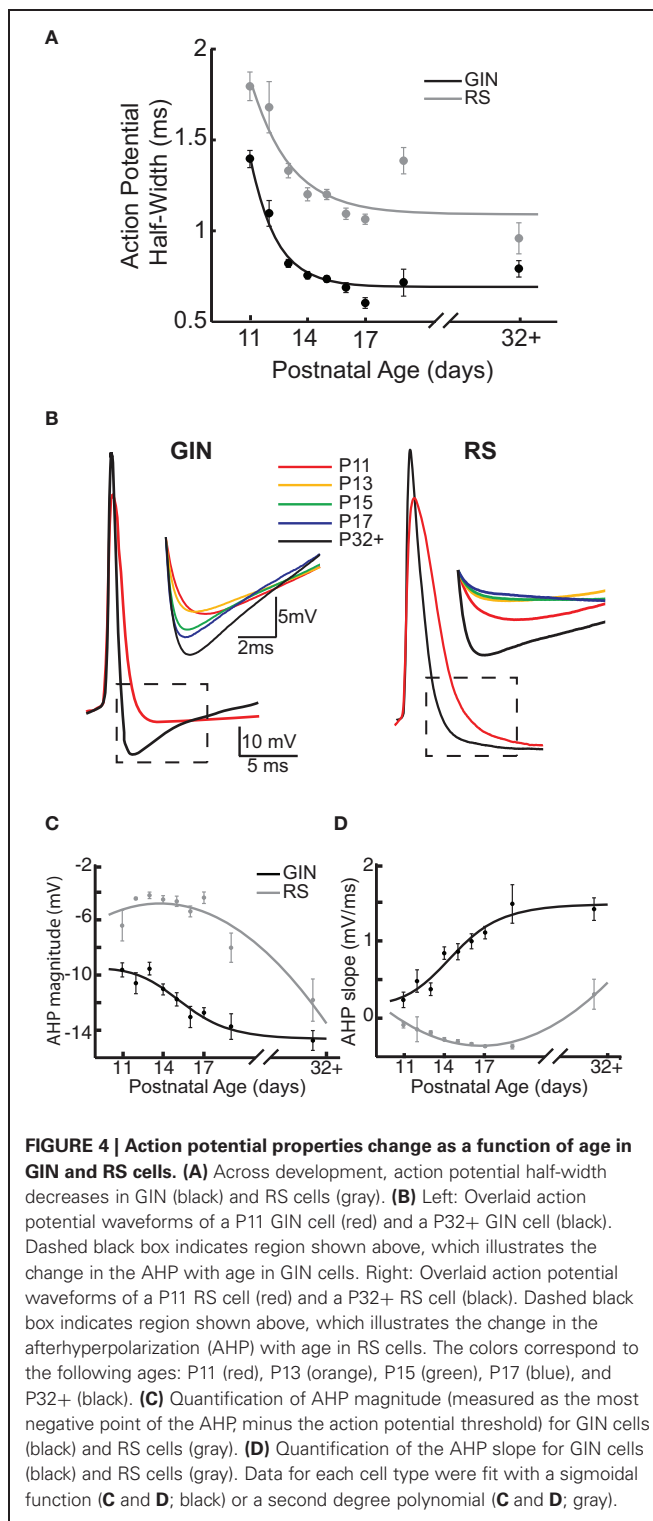
### ACTION POTENTIAL PROPERTIES

GIN cells exhibit a distinct action potential and AHP waveform, which is likely due to expression of several types of potassium currents (Zhang and McBain, 1995; Rudy and McBain, 2001; Halabisky et al., 2006; Ma et al., 2006). We therefore sought to determine whether action potential properties of GIN cells changed with age, which would suggest differential expression of these currents across development. We measured three properties related to the shape of the action potential: action potential half-width, the AHP magnitude, and the slope of the voltage depolarization following the AHP. The average action potential half-width in GIN cells decreased with age, from  $1.4 \pm 0.05$  ms at P11 to  $0.8 \pm 0.05$  ms at P32+ ( $p < 0.001$ ; Figure 4A). Narrowing of the action potential with age was also observed in RS cells (P11:  $1.8 \pm 0.08$  ms; P32+:  $0.96 \pm 0.09$  ms,  $p < 0.001$ ; Figure 4A), but at all ages recorded the half-width of GIN cells was shorter than for RS cells of the same age.

GIN cells can display a complex “triphasic” AHP shape following an action potential that consists of both a rapid “early” component, followed by a slower “late” component (Halabisky et al., 2006; Ma et al., 2006). We found that both of these features were almost completely absent at P11 in the GIN cells but developed throughout the second and third postnatal weeks (Figures 1A,B and 4B). Specifically, the trough of the AHP became increasingly negative with age (P11:  $-9.6 \pm 0.5$  mV; P32+:  $-14.7 \pm 0.7$  mV,  $p < 0.001$ ; Figures 4B,C). We then quantified the repolarization of the voltage following the AHP and found that the slope increased almost threefold from P11 ( $0.7 \pm 0.1$  mV/ms) to P32+ ( $1.9 \pm 0.15$  mV/ms,  $p < 0.001$ ; Figures 4B,D). Therefore, in GIN cells, although the AHP became increasingly negative with age, the voltage repolarized more quickly so that by  $\sim 8$  ms following the AHP the voltage was more depolarized in mature animals than younger animals (Figures 4B,D). In contrast, RS cells had a smaller AHP magnitude across all ages than GIN cells (Figure 4C). We did observe a significant increase in the AHP magnitude with age in RS cells (P11:  $-6.4 \pm -1.1$  mV; P32+  $-11.8 \pm -1.5$  mV,  $p < 0.05$ ), but no significant change in AHP slope for RS cells (P11:  $0.4 \pm 0.03$  mV/ms; P32+:  $0.8 \pm 0.2$  mV/ms;  $p = 0.189$ ).

### FIRING PROPERTIES

Finally, we examined how the propensity for GIN cells to fire in response to a current step changed as a function of age. We started by quantifying the firing of the GIN cells throughout the duration of suprathreshold current steps across each age (Figure 5A). GIN cells across all ages exhibited adaptation throughout the current steps, such that the initial firing rate was higher than the steady state firing rate (Figure 5A). Both the initial (P11:  $61 \pm 2$  Hz; P32+:  $96 \pm 2.6$  Hz,  $p < 0.001$ ) and the steady state firing rate (P11:  $38 \pm 3$  Hz; P32+:  $42.4 \pm 3.2$  Hz,



$p < 0.01$ ) increased significantly with age. The ratio of the two values, the adaptation ratio, decreased across development (P11:  $0.6 \pm 0.03$ ; P32+:  $0.5 \pm 0.03$ ,  $p < 0.05$ ; Figure 5C). This is likely due to the fact that the initial firing rate increased more with age than the steady state firing rate, causing GIN cells to exhibit

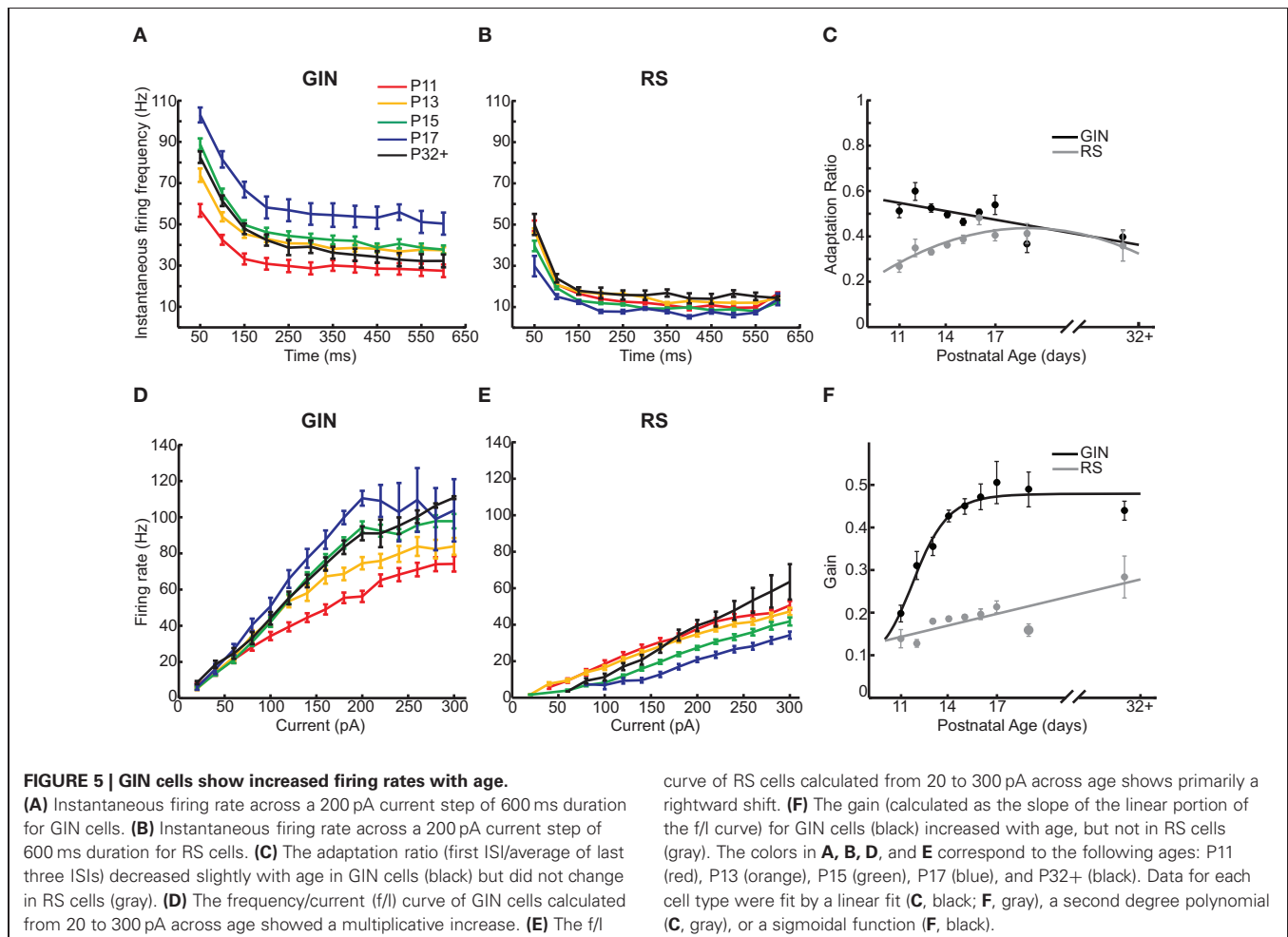
more adaptation in older animals. In comparison, we observed no significant change in the initial firing rate, steady state firing rate, or adaptation ratio across ages in RS cells (Table 2; Figures 5B,C).

In order to fully understand how the firing dynamics of GIN cells and RS cells changed during development, we examined the input–output relationship of both cell types from P11 to P32+. When we compared the input–output relationship across age, GIN cells showed a “multiplicative” increase in firing rate from P11 to P32+ (Figure 5D). This was primarily because whereas the firing rates increased with age, the rheobase current stayed constant from P12 through P32+ (Figures 2C and 5D). To quantify this change, we calculated the slope of the linear portion of the input–output relationship, or the “gain” of the cell. The gain of the GIN cells increased significantly across development (P11:  $0.2 \pm 0.02$  Hz/pA; P32+:  $0.44 \pm 0.02$  Hz/pA,  $p < 0.001$ ; Figure 5F). RS cells also showed a significant increase in gain from P11 ( $0.14 \pm 0.02$  Hz/pA) to P32+ ( $0.28 \pm 0.05$  Hz/pA,  $p < 0.05$ ; Figure 5F). However, the dominant change in the input–output curve of RS cells was a rightward shift with age (Figure 5E). This was due to the increase in rheobase current with age in RS cells (Figure 2C), resulting in more current being required to drive RS cells to fire at a given rate as the neurons matured.

## DISCUSSION

In this study, we sought to determine whether physiological features of GIN inhibitory cells changed as a function of age. Our data demonstrated that multiple intrinsic physiological characteristics of L2/3 GIN and RS cells in somatosensory cortex changed across the developmental time period investigated here (P11–P32+). GIN and RS cells showed decreased intrinsic excitability over the second and third weeks of postnatal development, based on features such as input resistance, membrane time constant, and rheobase current. However, despite these changes, the net change in GIN cell output during this time was an increase in firing rate as a function of age. This was due to an increase in initial and steady state firing rates with age, which resulted in a multiplicative increase in the input–output relationship. In contrast, RS cells did not show substantial changes in gain with age. Together, these results suggest that, for the same level of input current, GIN cells became more active as the animal matured through the age range investigated, whereas RS cells became less readily activated.

The results we reported here for GIN cells are consistent with developmental changes to intrinsic properties reported for pyramidal neurons in the current study and elsewhere (Maravall et al., 2004) and for other types of inhibitory interneurons



(Goldberg et al., 2011) in the upper layers of the somatosensory cortex. Therefore, this may be a general developmental trend across many cell types within this neocortical region. It should be noted that a recent study investigated maturation of SOM and PV cells in the visual cortex during the first few weeks of postnatal development (Lazarus and Huang, 2011). Interestingly, they observed different trends than we reported here, as SOM cells in the visual cortex displayed increases in input resistance and membrane time constant with age. These different developmental trajectories seen by Lazarus and Huang (2011) could reflect differences between cortical areas. Additionally, these authors investigated SOM and PV cells from ages P15–P30, and grouped the data into bins of multiple days, including P15–P17. Because the decreases we observed in SOM cell input resistance and membrane time constant occurred at earlier ages (greatest changes prior to day 14), it is difficult to compare across these studies.

### INTRINSIC CELLULAR PROPERTIES DURING POSTNATAL DEVELOPMENT

Previous studies have examined the properties of pyramidal neurons during the age range studied here and have observed a similar decrease in intrinsic excitability of these cells with age (McCormick and Prince, 1987; Maravall et al., 2004; Oswald and Reyes, 2008). Interestingly, although we observed changes to these properties in GIN and RS cells as a function of age, GIN cells consistently exhibited higher  $R_{in}$ , higher  $\tau_m$ , and lower rheobase current than RS cells of the same age. This illustrates that the relative intrinsic excitability between GIN and RS cells is preserved at all ages. This may be important for maintaining the proper ratio of excitation to inhibition throughout development (Zhang et al., 2011).

In excitatory neurons and non-GIN inhibitory interneurons, such as the FS cells, changes in input resistance are driven primarily by increased expression of potassium leak currents (Goldberg et al., 2011; Guan et al., 2011), which could have caused the change in input resistance we reported for GIN cells. It is currently unknown whether the amount of synaptic input or gap junction coupling changes over development in GIN cells, but either could also contribute to the changes in intrinsic properties observed here.

A prominent feature of GIN cells is the presence of a strong “sag” current and related rebound depolarization during and after negative current pulses, respectively (McGarry et al., 2010; Ma et al., 2012). Both of these properties are mediated by  $I_h$  currents in GIN cells (Ma et al., 2006). We found a pronounced increase in the magnitude of the sag current and rebound depolarization in GIN cells, illustrating that the expression of  $I_h$  is likely increasing in GIN cells over development.  $I_h$  currents often underlie the generation of rhythmic activity (McCormick and Pape, 1990; Maccaferri and McBain, 1996; Pape, 1996; Luthi and McCormick, 1998; Griguoli et al., 2010). GIN cells in both the neocortex and hippocampus show a strong preference for firing at theta frequencies (Chapman and Lacaille, 1999a,b; Klausberger et al., 2003; Lawrence et al., 2006; Fanselow et al., 2008; Griguoli et al., 2010). This could result partially from the expression of  $I_h$  current (Griguoli et al., 2010). A developmental increase in  $I_h$  current could cause the theta preference

found in GIN cells to mature during this period of postnatal development.

### MATURATION OF GIN ACTION POTENTIAL WAVEFORM

A dominant factor that controls the action potential waveform is the expression of voltage-gated potassium channels (Rudy and McBain, 2001). In particular, the potassium channel family, Kv3, has been shown to underlie the narrow action potential waveform in FS inhibitory interneurons (Rudy and McBain, 2001; Lien and Jonas, 2003; Goldberg et al., 2011). The action potential half-width observed in FS cells decreases during the second and third weeks of postnatal development and this property is paralleled by increased expression of Kv3 over development (Goldberg et al., 2011). It is possible that developmental increases in potassium currents could also underlie the decreased action potential half-widths observed in GIN cells in the current study. Although Kv3 channels are observed in deep layer SOM inhibitory interneurons, they are not present in superficial SOM cells (Chow et al., 1999), which were recorded here. The specific types of potassium channels expressed in superficial SOM cells are not known, so it is difficult to know which potassium currents could underlie the developmental changes in action potential waveform we observed in our study.

We demonstrated that the voltage trajectory following the AHP trough depolarized more rapidly in older GIN cells than in younger ones. The ability of mature GIN cells to repolarize more rapidly following an action potential may underlie the increased firing rates observed at older ages.  $I_h$  currents can act on the shape and duration of the AHP and increase the slope of the membrane potential during the interspike interval (McCormick and Pape, 1990; Maccaferri and McBain, 1996; Pape, 1996; Griguoli et al., 2010). The changes observed here in the AHP shape and slope, therefore, further support the idea that  $I_h$  currents increase in GIN cells over the second and third postnatal weeks.

### GIN CELLS DISPLAY MULTIPLICATIVE INCREASES IN THE INPUT–OUTPUT RELATIONSHIP WITH AGE

We assessed the firing output of GIN and RS cells over development by examining changes in firing rates. The initial and steady state firing rates of GIN cells increased with age. Because the rheobase current remained largely constant in GIN cells, the increase in firing rate resulted in a multiplicative increase in the input–output curve of GIN cells as a function of age. Such gain changes can arise from alterations in the rate and balance of excitatory and inhibitory synaptic inputs to a neuron (Chance et al., 2002; Mitchell and Silver, 2003; Larkum et al., 2004). Another way to modulate the gain of a neuron is to affect a process that is spike dependent, such as the action potential AHP (Mehaffey et al., 2005; Diaz-Quesada and Maravall, 2008). This will increase or decrease the firing rate of a neuron without a concomitant change in rheobase current. Our observed changes to the AHP in GIN cells as a function of age could account for the increased gain we observed in these cells.

In contrast, in RS cells the more prominent change in firing rate over development was an increase in rheobase current, accompanied by a rightward shift of the input–output curve (Oswald and Reyes, 2008). Subtractive shifts in input–output



curves can be caused by alterations in intrinsic neuronal excitability (Silver, 2010), which do not cause changes in gain. The changes in intrinsic properties (such as input resistance) of RS cells are likely the cause of the subtractive change in the input–output relationship observed in these cells over development.

### CHARACTERIZING SUBTYPES OF INHIBITORY NEURONS

Establishing relevant distinctions between subtypes of inhibitory neurons, while critical for characterizing their role(s) in the neocortex, has been difficult (Gupta et al., 2000; Markram et al., 2004; Ascoli et al., 2008; Guerra et al., 2011). This has been aided, but not entirely ameliorated, by the use of lines of mice that express fluorescent proteins in inhibitory neurons of interest. It is not clear whether GIN cells form a homogeneous population. These cells have been subdivided into three (McGarry et al., 2010) and four (Halabisky et al., 2006) groups based on physiological and morphological characteristics. In our study, we showed that physiological factors frequently used to characterize inhibitory neurons vary with age. Therefore, in order to use these factors to accurately categorize GIN neurons it is necessary to take age into account. Here, we focused on changes in the physiological features of GIN cells, but other features, such as morphological characteristics, can also be considered when subdividing GIN cells (McGarry et al., 2010). It would be interesting to know whether the morphological features of GIN cells also develop over this time window, and whether they change in concert with the physiological changes we have described. In fact, developmental morphological changes could constitute possible mechanisms for some of the physiological changes we observed. For example, changes in overall dendritic length with age could account for the decreased input resistance in GIN cells we report in this study.

### IMPACT OF GIN CELL MATURATION ON CORTICAL CIRCUITRY

This study represents the first in-depth analysis showing how the physiology of GIN cells in layers 2/3 of somatosensory cortex changes during the second and third postnatal weeks. This encompasses a period of massive reorganization of the neocortex correlated with the onset of sensory input and plasticity. Our results suggest that GIN cells become more active in the cortical network around P14, which corresponds to the approximate time of whisking onset in mice and rats (Mosconi et al., 2010). Although a precise function for GIN cells, and more broadly SOM cells, in sensory processing is still being investigated, there are several implications of our results.

First, changes in intrinsic properties of SOM cells can affect how these cells communicate amongst themselves via electrical connections, or gap junctions. Gap junctions are common between SOM cells (Gibson et al., 1999; Fanselow et al., 2008), and are involved in the generation of synchronous activity between SOM interneurons (Beierlein et al., 2000; Fanselow et al., 2008). The ability of electrically coupled cells to synchronize depends on both the properties of the coupling as well as the intrinsic properties of the individual cells (Amitai et al., 2002; Lewis and Rinzel, 2003; Mancilla et al., 2007; Hayut et al., 2011). Therefore, as the intrinsic properties of SOM cells mature during postnatal development the ability of SOM cells to synchronize their activity through gap junctions may change. Factors such

as input resistance, membrane time constant, and AHP size and shape could all influence gap junction-mediated synchrony between GIN cells. However, determining how the changes we saw in these factors as a function of age would individually or collectively affect GIN–GIN synchrony would require detailed computational and experimental investigations that are beyond the scope of this study.

Second, SOM cells participate in cortical “UP-states” (Fanselow and Connors, 2010), brief periods of depolarization and higher neuronal activity observed in a range of neocortical regions and layers. UP-states have been described *in vitro* (Sanchez-Vives and McCormick, 2000; Shu et al., 2003; Fanselow and Connors, 2010), *in vivo* during anesthesia (Steriade et al., 1993; Sanchez-Vives and McCormick, 2000) and in sleep and waking states (Steriade et al., 2001; Petersen et al., 2003). The function of UP-states is not well understood, but they represent a high-conductance state with neuronal activity that is reminiscent of firing observed in intact animals (Destexhe and Pare, 1999; Destexhe et al., 2003; Rudolph et al., 2005; Fanselow and Connors, 2010). Work by Fanselow and Connors (2010) suggested that GIN cells could be involved in terminating UP-states. If, as our current results suggest, GIN cells play a larger role in cortical activity as an animal progresses through the second and third weeks of postnatal development, then the propensity for GIN cells to terminate UP-states might also increase during the same period. Indeed, Fanselow and Connors (2010) demonstrated a strong inverse correlation between UP-state duration and age, as well as a decrease in UP-state frequency with increasing age from P13 to P17. Such an ability to terminate UP-states could potentially extend to termination of other periods of heightened activity within the cortex, such as seizures. In fact, loss of SOM-expressing, dendrite-targeting inhibitory cells of the hippocampus has been associated with increased seizure activity (Buckmaster and Jongen-Relo, 1999; Cossart et al., 2001).

Third, SOM cells influence the activity of the surrounding neuronal network in multiple ways. These mechanisms would likely be affected by the type of age-driven changes in the input–output relationship (gain) of GIN cells that we observed. For example, because SOM interneurons directly inhibit FS cells (Gibson et al., 1999; Ma et al., 2012), an increase in SOM cell gain could paradoxically increase local excitatory neuron activity through disinhibition. In contrast, it has been shown that activation of even a few pyramidal cells exerts strong disynaptic inhibition onto surrounding pyramidal cells via SOM interneurons (Kapfer et al., 2007; Silberberg and Markram, 2007). In this way, increases in SOM cell gain may serve to increase SOM-mediated disynaptic self-inhibition of pyramidal cells, resulting in a decrease in cortical excitability. Note that these two methods of regulating cortical activity are not mutually exclusive, but may instead work antagonistically to fine tune cortical activity.

It will be useful in future studies to determine how developmental changes to synaptic connections complement development of the intrinsic properties we have described here, and, in turn, affect the influence SOM cells have on neighboring neurons. Excitatory inputs onto SOM cells are initially weak, but display strong short-term facilitation (Gibson et al., 1999;

Fanselow et al., 2008), which suggests these cells are engaged during periods of heightened neuronal activity (Kapfer et al., 2007; Silberberg and Markram, 2007; Fanselow et al., 2008; Hayut et al., 2011). Interestingly, inhibitory output from SOM cells initially exhibits weak depression followed by weak facilitation (Ma et al., 2012). Together, these short-term dynamics suggest that SOM cells would be able to increase and then sustain their inhibitory influence on downstream targets during sensory processing. This is in contrast to FS cells, which receive initially strong but rapidly depressing synapses from upstream neurons and make depressing connections onto target neurons (Beierlein et al., 2003; Ma et al., 2012). This suggests that FS cells, while exerting strong inhibitory output at first, would decrease their influence on surrounding cells as ongoing neuronal activity progressed. These opposing short-term dynamics suggest SOM and FS cells may be specialized for distinct modes of inhibitory processing: FS cells are optimized for fast, transient inhibition, whereas SOM cells can provide somewhat delayed, but sustained, inhibition (Tan et al., 2008). The extent to which each of these functions is fulfilled at a given point during development remains to be determined. Developmental changes in the short-term dynamics of synapses onto and from SOM cells would dictate the degree to which these cells could be activated by high-frequency inputs and the time course over which they could affect their downstream targets. Additionally, inhibitory synapses between SOM cells and FS cells can produce synchronous activity between these different types of inhibitory interneurons (Hu et al., 2011). Therefore, developmental regulation of the inhibitory output from SOM cells may impact the ability of SOM cells to synchronize with FS cells through inhibitory synapses. Further investigation of SOM input and output properties (e.g., synaptic dynamics) as a function of age will be critical to our understanding of the role of SOM cells during this postnatal time period.

Finally, multiple studies *in vitro*, *in vivo*, and using computational models have suggested unique roles for SOM inhibitory interneurons during cortical sensory processing (Tan et al., 2008; Vierling-Claassen et al., 2010; Hayut et al., 2011; Gentet et al.,

2012; Ma et al., 2012). For example, inhibition onto the apical dendrite of pyramidal cells can inhibit pyramidal cell firing by influencing active currents in this dendritic sub-region (Larkum et al., 1999, 2001; Perez-Garci et al., 2006; Gentet et al., 2012; Lovett-Barron et al., 2012). This suggests that SOM cells may be involved in regulating excitatory synapses onto such apical dendrites, including long-range projections from other cortical and subcortical areas (Petreanu et al., 2007, 2009). In contrast, FS cells, which terminate on the perisomatic regions of their targets, would not directly control these inputs. Thus, developmentally mediated increases in SOM cell activity due to increases in gain might more closely regulate long-range inputs to somatosensory cortex at later ages. In addition, SOM cells exhibit different behaviors *in vivo* than other inhibitory interneurons, including FS cells. Specifically, it has been shown that SOM cells are preferentially active during quiet wakefulness, and are hyperpolarized in response to sensory input, whereas FS cells respond robustly to sensory input (Gentet et al., 2012). If, as these findings collectively suggest, increased SOM cell activity gates out excitatory distal dendritic input during sensory processing, then it would appear from our study that such dendritic input (e.g. from motor cortex) becomes increasingly gated as a function of age. Whether and why more excitatory dendritic input might be useful early in the development of the sensorimotor system, or a detriment at older ages, is not yet known. In any case, it is clear that SOM and FS cells possess distinct intrinsic and synaptic properties that may contribute to their different behaviors *in vivo*. The development of these properties in both FS and SOM cells happens concurrently, over the second and third postnatal weeks, and may contribute to the dichotomous roles of these subsets of interneurons in neocortical processing.

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