Distinct maturation profiles of perisomatic and dendritic targeting GABAergic interneurons in the mouse primary visual cortex during the critical period of ocular dominance plasticity

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Lazarus MS, Huang ZJ. Distinct maturation profiles of perisomatic and dendritic targeting GABAergic interneurons in the mouse primary visual cortex during the critical period of ocular dominance plasticity. J Neurophysiol 106: 775–787, 2011. First published May 25, 2011; doi:10.1152/jn.00729.2010.—In the rodent primary visual cortex, maturation of GABA inhibitory circuitry is regulated by visual input and contributes to the onset and progression of ocular dominance (OD) plasticity. Cortical inhibitory circuitry consists of diverse groups of GABAergic interneurons, which display distinct physiological properties and connectivity patterns. Whether different classes of interneurons mature with similar or distinct trajectories and how their maturation profiles relate to experience dependent development are not well understood. We used green fluorescent protein reporter lines to study the maturation of two broad classes of cortical interneurons: parvalbumin-expressing (PV) cells, which are fast spiking and innervate the soma and proximal dendrites, and somatostatin-expressing (SOM) cells, which are regular spiking and target more distal dendrites. Both cell types demonstrate extensive physiological maturation, but with distinct trajectories, from eye opening to the peak of OD plasticity. Typical fast-spiking characteristics of PV cells became enhanced, and synaptic signaling from PV to pyramidal neurons became faster. SOM cells demonstrated a large increase in input resistance and a depolarization of resting membrane potential, resulting in increased excitability. While the substantial maturation of PV cells is consistent with the importance of this source of inhibition in triggering OD plasticity, the significant increase in SOM cell excitability suggests that dendrite-targeted inhibition may also play a role in OD plasticity. More generally, these results underscore the necessity of cell type-based analysis and demonstrate that distinct classes of cortical interneurons have markedly different developmental profiles, which may contribute to the progressive emergence of distinct functional properties of cortical circuits.

γ-aminobutyric acid; development

DURING A BRIEF POSTNATAL PERIOD, the closure of one eye can permanently shift the response property of neurons in the primary visual cortex (V1) to favor inputs from the open eye, i.e., ocular dominance (OD) shift (Hubel and Wiesel 1970). OD plasticity has been a premier model to study how sensory experience shapes the development of cortical circuits during a critical period. To shift eye preference during monocular deprivation, visual cortical neurons must first be able to detect the imbalance of converging visual inputs, relayed to the cortex as altered spiking patterns in thalamic axons, before they can engage a cascade of molecular, cellular, and circuitry mechanisms to weaken the deprived eye-associated inputs and strengthen the open eye-associated inputs (Hensch 2005). GABAergic interneurons are crucial in shaping and detecting the precise spatiotemporal patterns of electrical signaling in cortical circuits and in regulating synaptic plasticity (Markram et al. 2004). Accumulating evidence has indicated that proper functioning of GABAergic inhibitory neurons within V1 are critical to establish the necessary physiological milieu that enables OD plasticity. Mice lacking the synaptic isofrom of the GABA-synthetic enzyme GAD65 show no OD plasticity, a deficit that can be rescued by cortical infusion of a GABA$_A$ receptor agonist (Hensch et al. 1998). In addition, genetic (Huang et al. 1999) and pharmacological (Fagioliini and Hensch 2000) enhancement of the maturation and function of GABA inhibition in V1 induces a precocious critical period. However, the cellular and circuitry mechanisms by which the maturation of cortical inhibition promotes OD plasticity are not well understood.

Synaptic inhibition in the neocortex is achieved by diverse groups of interneurons, which mediate GABA transmission at discrete spatial and temporal niches during circuit operation and demonstrate distinct physiological properties and connectivity patterns (Markram et al. 2004; Burkhhalter 2008). Although our understanding of this diversity is far from complete, previous studies have established a major dichotomy in the inhibitory control of pyramidal neurons. Interneurons that innervate pyramidal cell dendrites are responsible for controlling the efficacy and plasticity of glutamatergic inputs that terminate in the same dendritic domain (Miles et al. 1996; Tamas et al. 1997). On the other hand, interneurons targeting the perisomatic region control action potential (AP) generation, timing, and synchrony in pyramidal cell populations (Cobb et al. 1995; Miles et al. 1996). Whether or how these two major sources of inhibition differentially engage molecular and cellular plasticity mechanisms and contribute to OD plasticity is unclear. Previous studies have focused on parvalbumin-expressing (PV) interneurons, which are fast spiking and innervate the perisomatic region of pyramidal neurons (Miles et al. 1996; Tamas et al. 1997). For example, the morphological maturation of perisomatic innervation from PV cells correlates with the timing of the critical period (Chattopadhyaya et al. 2004). In addition, PV interneurons signal through $\alpha_1$-subunit-containing GABA$_A$ receptors, and inhibition through these receptors appears critical for OD plasticity (Fagioliini et al. 2004). Furthermore, the homeoprotein orthodenticle homolog 2, which is able to trigger plasticity
when transported from the retina to V1, is prominently taken up by PV cells (Sugiyama et al. 2008). These results indicate an important role of PV interneurons and perisomatic inhibition in the onset of OD plasticity. On the other hand, the role of dendrite-targeted inhibition through somatostatin-expressing (SOM) interneurons has not been well studied. OD plasticity ultimately involves structural rewiring of excitatory synapses onto the dendritic spines of pyramidal neurons (Oray et al. 2004; Mataga et al. 2004). Given the powerful role of dendrite-targeted inhibition in controlling synaptic integration (Miles et al. 1996; Perez-Garcì et al. 2006), dendritic Ca\(^{2+}\) spikes (Murayama et al. 2009), plasticity (Ballard et al. 2009), and learning (Collinson et al. 2002; Maubach 2003), it is likely that this source of inhibition also contributes to aspects of OD plasticity.

A necessary step toward further understanding the role of these two major classes of GABAergic interneurons in OD plasticity is a characterization of their functional maturation during the critical period. It has been known for decades that maturation of the GABAergic system in the rodent visual cortex follows a postnatal time course (Luhmann and Prince 1991). However, previous studies have primarily used methods such as spontaneous, miniature, or field-evoked inhibitory postsynaptic current (IPSC) recordings (Bosman et al. 2002, 2005; Heinen et al. 2004; Morales et al. 2002), which cannot distinguish the synaptic sources of inhibition. To date, the physiological maturation of PV interneurons during the critical period has not been fully characterized, and the maturation of SOM interneurons has not been explored. Using two green fluorescent protein (GFP) reporter mouse lines, here, we present the first comparison of the maturation of inhibition provided by PV and SOM interneurons before and during the critical period for OD plasticity. We found that both classes of interneurons demonstrate substantial physiological maturation from eye opening to the peak of OD plasticity, but with distinct trajectories. While PV cells demonstrated significant maturation in their characteristic fast signaling properties, which plateaued after the onset of critical period, SOM cells demonstrated a profound and steady increase in their excitability, which continued to the peak of OD plasticity.

**METHODS**

**Animals**

To identify PV and SOM cells, we used two transgenic mouse lines, B13 and GIN, respectively, that have been previously used to identify these classes of neurons. The B13 line (Dumitriu et al. 2007; Goldberg et al. 2008; Ango et al. 2008; Daw et al. 2010) expresses enhanced GFP (EGFP) driven by the \(\text{Pv}\) gene. EGFP in the B13 line is expressed selectively in ~50% of PV cells in the neocortex (Dumitriu et al. 2007). The GIN line expresses EGFP driven by the \(\text{Gad}1\) promoter (Oliva et al. 2000), and EGFP is restricted to a subclass of SOM neurons (Oliva et al. 2000; Ma et al. 2006; Halabisky et al. 2006). EGFP in the GIN line is expressed in SOM neurons in both superficial and deep layers of the neocortex (Oliva et al. 2000; Ma et al. 2006), labeling approximately one-third of SOM cells in layer II/III (Ma et al. 2006). Mice were treated in accordance with Cold Spring Harbor Laboratory guidelines on animal husbandry and care/welfare. Experiments were performed on animals between 15 and 30 days after birth [postnatal day (P)15 and P30], as indicated.

**Slice Preparation**

Acute brain slices were prepared at the appropriate ages. Animals were deeply anesthetized with avertin (tribromoethanol in amyl hydrate, intraperitoneal injection, 0.2 ml/kg), and decapitated. Brains were rapidly removed and placed into ice-cold, oxygenated cutting solution, containing (in mM) 110 chlorides, 2.5 KC1, 25 NaHCO\(_3\), 1.25 NaH\(_2\)PO\(_4\), 0.5 CaCl\(_2\), 7 MgCl\(_2\), 25 glucose, 11.6 ascorbic acid, and 3.1 pyruvic acid bubbled with 95% O\(_2\)-5% CO\(_2\). The anterior one-third of the brain and the posterior section containing the cerebellum were removed with coronal cuts. The brains were then glued to the slicing block, anterior face down. Slices were prepared in the choline-based cutting solution on a Microm HM650V (Walldorf, Germany). Coronal slices contained V1 and were 350 \(\mu\)m thick. Slices were transferred to artificial cerebrospinal fluid (aCSF) and incubated at 32–34°C for at least 30 min. aCSF contained (in mM) 126 NaCl, 2.5 KCl, 25 NaHCO\(_3\), 14 glucose, 1.25 NaH\(_2\)PO\(_4\), 1 MgSO\(_4\), and 2 CaCl\(_2\) bubbled with 95% O\(_2\)-5% CO\(_2\) to pH 7.4. For recording, slices were transferred to a recording chamber continuously perfused with oxygenated aCSF and maintained at 28–30°C.

**Biocytin Filling**

GFP-positive cells were identified in layer II/III of the visual cortex and patched with a recording pipette containing 0.2% biocytin. These slices were then incubated overnight at 4°C in 4% paraformaldehyde in PBS (pH 7.4). After fixation, slices were rinsed in PBS (3 times for 5 min) and then incubated overnight in Alexa fluor 568-conjugated streptavidin (1:1,000, Invitrogen) with 0.3% Triton X-100 in PBS. Slices were then rinsed in PBS (3 times for 5 min) and mounted in Vectashield mounting medium (Vector Labs). Fluorescently labeled neurons were imaged using a Zeiss LSM 510 confocal microscope and reconstructed using NeuroLucida (MicroBrightField).

**Electrophysiology**

All recordings were performed in layer II/III in coronal cut slices and were restricted to V1. Dual whole cell recordings were performed on a two-channel Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). For paired and single whole cell recordings, interneurons were identified by GFP expression under a narrow-band GFP filter set (Chroma Technology, Brattleboro, VT) in an Axioskop FS2 upright microscope (Zeiss, Thornwood, NY) with an ORCA-ER camera (Hamamatsu, Hamamatsu City, Japan). The GFP-positive cell was subsequently visualized with differential interference contrast (Zeiss). For paired recordings, a nearby pyramidal neuron (~50 \(\mu\)m) was visually identified by a triangular soma with a distinct apical dendrite oriented toward the pial surface. Patch pipettes were pulled from borosilicate glass on a Flaming-Brown micropipette puller (Sutter Instruments, Novato, CA) and had a resistance of 2–4 M\(\Omega\). In both paired and single cell recordings, the intracellular solution for the interneuron contained (in mM) 130 K-glucitone, 6 KCl, 2 MgCl\(_2\), 2 EGTA, 2 HEPES, 2.5 Na-ATP, 0.5 Na-GTP, and 10 Na-phosphocreatin \((\text{pH} 7.25, 285–295\text{ mosM})\). The interneuron recording was performed in current clamp for all experiments. For paired recordings, a high internal Cl\(^-\) concentration was used to magnify IPSC responses in pyramidal neurons to aid with analysis. The pyramidal neuron internal solution contained (in mM) 65 K-glucitone, 65 KCl, 2 MgCl\(_2\), 2 EGTA, 2 HEPES, 2.5 Na-ATP, 0.5 Na-GTP, and 10 Na-phosphocreatin \((\text{pH} 7.25, 285–295\text{ mosM})\). The postsynaptic pyramidal neuron was held in voltage clamp at \(-75\text{ mV}\). Zolpidem was used to assess GABA\(_{A}\) receptor \(\alpha\)-subunit composition and was purchased from Tocris (Ellisville, MO).

To assess intrinsic properties, interneurons were stimulated with increasing 1-s-long current steps. PV cells were assessed with 50-pA steps starting from \(-200\text{ pA}\) increasing up to \(+700\text{ pA}\), and SOM cells were assessed with 20-pA steps starting from \(-100\text{ pA}\) increases...
ing up to +200 pA. Maximum current injections of +700 and +200 pA for PV and SOM cells, respectively, were chosen because these were the highest levels that would reliably not produce spike inactivation. For paired recordings, the presynaptic interneuron was stimulated with a brief suprathereshold current injection (0.8–1.2 nA, 3–5 ms). With the exception of short-term plasticity experiments, 1 spike was initiated per trial, with an intertrial interval (ITI) of 5 s. For analysis, 25–50 trials were averaged. For short-term plasticity experiments, presynaptic interneurons were stimulated at 20 Hz, with an ITI of 10 s.

Analysis and Electrophysiological Parameters

All analysis was performed offline with the ClampFit 9.0 (Molecular Devices) program.

Only stable recordings were included for analysis. Data were discarded if series resistance was >25 MΩ or varied by >25%, if resting membrane potential (V_mem) was greater than −50 mV several minutes after break in, or if membrane resistance (R_mem) was <50 MΩ. Pairs of neurons were considered synaptically connected if the averaged IPSC was >2 pA.

Membrane properties. The membrane time constant (τ_m; in ms) was determined by a monoexponential fit of the hyperpolarizing voltage response (∼10 mV) to a suitable current injection, from resting level to minimum point of sag, if present.

R_mem (in MΩ) was the slope of the linear portion of voltage responses to a series of negative current steps (current-voltage response curve).

Membrane capacitance (C_M; in pF) was determined from the following equation: C_M = τ_m/R_mem.

V_mem (in mV) was the stable membrane potential, as determined with no current injection a few minutes after the seal was broken.

AP threshold (V_T; in mV) was membrane potential when the rate of rise equaled 5 V/s, as measured in response to the smallest current step able to evoke a spike.

The excitability index (EI; in pA) incorporated multiple properties of a cell to estimate intrinsic excitability, as determined from the following equation: EI = (V_T - V_mem)/R_mem.

Spiking properties. Spike half-width (in ms) was the width measured at half-amplitude, between V_T and the peak of the AP, measured in response to the smallest current step able to evoke a spike.

Afterhyperpolarization (AHP) amplitude (in mV) was the voltage difference between V_T and the most negative point reached after an AP, measured in response to the smallest current step able to evoke a spike.

Spike frequency (in Hz) was the inverse of the first interspike interval (ISI).

Frequency-current slope (in Hz/pA) was the slope of the linear portion of the frequency-current response curve using the initial spike frequency.

Spike frequency adaptation (dimensionless) was the ratio of the last ISI to the fourth ISI. It was assessed at +700 pA for PV cells and +200 pA for SOM cells.

Synaptic properties. Amplitude (in pA) was measured from baseline (average of 5 ms) to the peak of the averaged IPSC.

The rise time of averaged IPSCs (RT_Avg; in ms) was the time from 10–90% of the rising phase of the averaged IPSC.

The rise time of individual IPSCs (RT_ind; in ms) was the average of the 10–90% rise time determined from at least 10 individual IPSC responses that could be resolved from noise. This measure was used to avoid potential error from jitter in presynaptic spiking or IPSC delay but could only be measured for PV→pyramidal (PV→Py) IPSCs due to the very small amplitude of SOM→pyramidal (SOM→Py) IPSCs.

Decay time (in ms) was determined by a monoexponential fit of the decaying phase of averaged IPSCs.

The paired-pulse ratio (PPR; dimensionless) was the ratio of the second IPSC amplitude to the first IPSC amplitude, stimulated at 20 Hz.

Statistics

All data are reported as means ± SD. Significance was determined by one-way ANOVA with a post hoc Tukey’s test for all data with greater than two comparison groups. For comparison of two groups, two-tailed unpaired Student’s t-test was used. For comparison of distributions, the Kolmogorov-Smirnov test was used. The significance level was set at P < 0.05.

RESULTS

Targeted whole cell current-clamp recordings were made from PV and SOM neurons to analyze membrane and spiking properties. Dual whole cell recordings were made from PV→Py and SOM→Py pairs to analyze synaptic properties. Three age groups were chosen to study the maturation of inhibition in relation to the onset of OD plasticity: the precritical period after eye opening (P15–P17), early in the critical period (P22–P24), and the peak of the critical period (P28–P30) (Gordon and Stryker 1996). All statistical comparisons were across these three age groups unless otherwise noted.

Morphology of GFP-Labeled Cells

Morphological reconstructions were performed to determine which types of cells were labeled in the B13 and GIN lines and to confirm previous reports (see Methods). Three cells each in animals from the young age group (P15–P17) and mature age group (P28–P30) in the B13 and GIN lines (total of 12 cells) were reconstructed (Fig. 1). Reconstructed cells in the B13 line (Fig. 1A) generally had dense local axonal arborization restricted to layer II/III, with occasional horizontal or vertical collaterals. This is consistent with identity as nest or small basket cells but not with large basket cells (Wang et al. 2002; Markram et al. 2004). In the GIN line (Fig. 1B), all reconstructed cells had axonal projections up toward the pial surface and extensive arborization within layer I. This supports previous work identifying GIN line cells as Martinotti (MN) cells (Ma et al. 2006).

Cell Type-Specific Maturation of Membrane Properties

PV and SOM neuron membrane properties demonstrated distinct developmental profiles over the period studied (Fig. 2). PV cells showed a decrease in τ_m from eye opening (P15–P17, 12.2 ± 5.3 ms) to the early critical period (P22–P24, 8.2 ± 3.2 ms, P < 0.05), when it reached a steady state (Fig. 2A). On the other hand, in SOM cells, τ_m increased between eye opening (P15–P17, 29.3 ± 13.0 ms) and the peak of the critical period (P28–P30, 43.2 ± 12.3 ms, P < 0.05; Fig. 2B).

The mechanisms of the changes in τ_m were different in PV and SOM cells (Fig. 3). In PV cells, R_mem did not change significantly over the period studied (Fig. 3A); therefore, the reduction in τ_m of PV cells may be due to a decrease in C_M between P15–P17 (87.7 ± 22.5 pF) and P22–P24 (67.7 ± 14.2 pF, P < 0.01; Fig. 3A, 2). In contrast to PV cells, SOM cells showed a substantial R_mem increase across all three ages studied (P15–P17: 299 ± 99 MΩ and P28–P30: 445 ± 79 MΩ, P < 0.01; Fig. 3B), but C_M did not change significantly over the developmental time period tested (Fig. 3B, 2). This increase in
The VM of PV cells was stable over the period studied (Fig. 4A, 1). The VM of SOM cells was stable between P15–P17 and P22–P24 but became significantly more depolarized before the peak of the critical period at P28–P30 (P22–P24: −61.3 ± 7.7 mV and P28–P30: −55.9 ± 5.1 mV, P < 0.05; Fig. 4B, 1). Since VM was measured after the experimental internal solution would have replaced the natural cellular contents, this observed depolarization may reflect a developmental reduction of K permeability at rest rather than a change in ion concentrations. A reduction of open K channels in SOM cells may explain both the developmental depolarization of VM as well as the developmental increase in RM (Cameron et al. 2000).

Two approaches were used to quantify cell excitability: 1) cumulative distribution of the minimum current injection required for at least one spike to occur (100-pA intervals for PV cells and 20 pA intervals for SOM cells) and 2) EI [expressed in pA and determined by the equation EI = (VT - VM)/RM, as described above]. A lower EI would reflect a more excitable cell, and vice versa.

No changes in cell excitability were observed in PV cells; however, SOM cells demonstrated a substantial increase in excitability with maturation. The current required to evoke spikes in PV cells did not change with development (Kolmogorov-Smirnov test, P = 0.89; Fig. 4A, 2), nor did EI change significantly over the period studied (Fig. 4A, 2). In SOM cells, likely due to increased RM and more depolarized VM, a significant shift to the left in the distribution of current required to evoke spikes was observed with maturation (Kolmogorov-Smirnov test, P < 0.01; Fig. 4B, 2), along with a twofold reduction of EI (P15–P17: 53.6 ± 25.3 pA and P28–P30: 25.9 ± 7.5 pA, P < 0.01; Fig. 4B, 2).

It should be noted that with the EI measure in mature mice (P28–P30), SOM cells were over 10-fold more excitable than PV cells (Fig. 4A, 2 and B, 2). At all ages, SOM cells, compared with PV cells, demonstrated more hyperpolarized VT (no developmental change; data not shown), larger RM, and more depolarized VM. In other words, all three factors used to determine EI would make SOM cells more excitable than PV cells, suggesting that the SOM source of inhibition may be more frequently or more readily engaged. Additionally, these results are consistent with the description of SOM cells as “low threshold spiking” interneurons (Gibson et al. 1999).

Cell Type-Specific Maturation of Spiking Properties

PV cells showed the fast-spiking properties (Kawaguchi and Kubota 1997) described by McCormick et al. (1985). These include low spike frequency adaptation, large fast
AHP, narrow AP half-width, and high spiking frequency. These last three features showed significant maturational changes, leading to more typical fast-spiking characteristics, in PV cells.

AP morphology substantially changed with maturation in PV cells but not in SOM cells (Fig. 5). The AP half-width of PV cells decreased between P15–P17 (0.80 ± 0.14 ms) and P22–P24 (0.60 ± 0.31 ms, *P < 0.05; Fig. 5A), whereas AHP
However, in PV cells, the frequency response to a given frequency-current curve shifted upward during development. Both PV and SOM cells (Fig. 6). In both cell types, the frequency-current curves demonstrated maturational changes for over the time period studied (Fig. 5).

Plots of initial spike frequency versus current injected (frequency-current curves) demonstrated maturational changes for both PV and SOM cells (Fig. 6). In both cell types, the frequency-current curve shifted upward during development. However, in PV cells, the frequency response to a given current input increased with age only for larger current steps ($P < 0.05$ for $500 \text{ pA}$ and $P < 0.01$ for $600$ and $700 \text{ pA}$; Fig. 6A, 2). In SOM cells, higher spike frequencies in older animals were observed across the range of current inputs ($P < 0.01$ for $40, 80, 120, 160,$ and $200 \text{ pA}$; Fig. 6B, 2). To determine if changes in $R_M$ may contribute to the shift of frequency-current curves, we adjusted these data for developmental differences in $R_M$ by multiplying the current input value by the average $R_M$ for the specific age group and cell type. This gave an estimation of the predicted membrane potential response (spike activation prevents the full membrane voltage response from occurring) and was expressed in millivolts. Since $R_M$ of PV cells did not change with development, adjusting for $R_M$ in this cell type had a minimal impact on the relative position of frequency-current curves (Fig. 6A, 3). SOM cells, however, showed a substantial change in $R_M$ with development. Adjusting the frequency-current curves of SOM cells for $R_M$ elimi-
nated the differences in the frequency response and led to overlapping curves for the different age groups (Fig. 6B, 3). This result suggests that increased $R_m$ in SOM cells can largely account for developmental changes in frequency-current response features. Quantification of the frequency-current slope in PV cells showed the increased frequency response occurred between P15–P17 (0.240 ± 0.039 Hz/pA) and P22–P24 (0.429 ± 0.166 Hz/pA, $P < 0.01$; Fig. 6A, 4); however, in SOM cells, the frequency-current slope increased gradually between P15–P17 (0.457 ± 0.093 Hz/pA) and P28–P30 (0.558 ± 0.092 Hz/pA, $P < 0.05$; Fig. 6B, 4). Spike adaptation, which is typically low in fast-spiking cells (McCormick et al. 1985), was stable in PV cells over the period studied (Fig. 6A, 4) but increased gradually in SOM cells (P15–P17: 1.94 ± 0.75 and P28–P30: 3.70 ± 2.19, $P < 0.05$; Fig. 6B, 4).

The increased excitability of SOM cells, and the resultant impact on spiking characteristics, appears unique to this cell type, as it is not observed in pyramidal cells (Oswald and Reyes 2008; Desai et al. 2002) or PV cells (Doischer et al. 2008; Okaty et al. 2009). This may reflect increased involvement of this source of inhibition in more mature cortical circuits. The developmental profile of PV cells was consistent with previous studies (Doischer et al. 2008; Okaty et al. 2009; Kuhlman et al. 2010) and demonstrated the postnatal acquisition of fast-spiking characteristics (McCormick et al. 1985).

### Source-Specific Maturation of Inhibitory Transmission

The maturational changes of intrinsic properties in PV and SOM cells determine when, if, and how AP firing occurs. But, the impact of cell spiking is, of course, dependent on communication with postsynaptic cells. Therefore, we decided to study the maturation of synaptic connections in PV→Py and SOM→Py pairs during the same developmental time period (Figs. 7 and 8).

Similar to PV cell intrinsic properties, PV→Py synaptic transmission showed significant maturation in numerous characteristics after eye opening (Fig. 7A). These developmental changes largely stabilized by the early critical period. In contrast, SOM→Py synaptic transmission did not demonstrate any significant changes over the time period studied (Fig. 7B). Synaptic features at both PV→Py and SOM→Py connections are quantified and compared in Fig. 8.

The kinetic properties of IPSCs became faster in PV→Py pairs. IPSC rise times decreased, mostly between P15–P17 ($RT_{\text{avg}}$: 0.77 ± 0.14 ms and $RT_{\text{indiv}}$: 0.71 ± 0.14 ms) and P22–P24 ($RT_{\text{avg}}$: 0.65 ± 0.11 ms, $P < 0.05$; $RT_{\text{indiv}}$: 0.57 ± 0.14 ms, $P < 0.05$). IPSC decay times also decreased and followed a similar time course (P15–P17: 12.68 ± 3.85 ms and P22–P24: 8.94 ± 1.41 ms, $P < 0.01$). IPSC amplitude showed

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**Fig. 6. Maturation of spiking properties.** A, 1: example current-step responses to −100, 0, and +700 pA in PV cells from a young animal (P16) and a mature animal (P28) along with an overlay of the first 30 ms from young (gray trace) and mature (black trace) animals. B, 1: example current-step responses to −40, 0, and +200 pA in SOM cells from a young animal (P15) and a mature animal (P29) along with an overlay of the first 30 ms. A, 2: initial spike frequency response to current injections in PV cells at ages P15–P17 (gray squares), P22–P24 (gray triangles), and P28–P30 (solid circles). Initial spike frequency in response to large current injections increased with age in PV cells between P15–P17 and P22–P24. The solid horizontal bars indicate the current levels at which a significant difference was observed. B, 2: initial spike frequency response to current injections in SOM cells. Initial spike frequency in SOM cells in response to all current injection levels increased with age. A, 3: data from A, 2 adjusted to the mean $R_m$ for each age group. Adjusting for $R_m$ did not account for the age-related changes in spike frequency in PV cells. B, 3: data from B, 2 adjusted to the mean $R_m$ for each age group. Overlapping data points indicate that adjusting for $R_m$ largely accounted for the age-related changes in spike frequency in SOM cells. A, 4: quantification of frequency-current ($F/I$) slope and spike adaptation across three ages in PV cells. A steepened frequency-current response in PV cells was apparent between P15–P17 and P22–P24, whereas spike adaptation remained stable. B, 4: quantification of frequency-current slope and spike adaptation across three ages in SOM cells. A steepened frequency-current response occurred gradually between P15–P17 and P28–P30. Scale bars = 500 ms and 50 mV in A, I and B, I for full traces and 5 ms and 50 mV in A, I and B, I for overlays. *$P < 0.05$; **$P < 0.01$.**

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Fig. 7. Maturation of inhibitory postsynaptic current (IPSC) properties. A, left: example averaged IPSC responses from PV cell (gray traces) to pyramidal cell (black traces) paired recordings in young (P16; top) and mature (P30; bottom) animals. Right, amplitude-scaled overlay of IPSC responses at PV→pyramidal cell (PV→Py) connections in young (gray trace) and mature (black trace) animals. Inset, first 3 ms of overlaid IPSC responses. At PV→Py connections, both IPSC rise time and decay time became faster with development. B, left: example averaged IPSC responses from SOM cell (gray traces) to pyramidal cell (black traces) paired recordings in young (P17; top) and mature (P28; bottom) animals. Right, amplitude-scaled overlay of IPSC responses at SOM→pyramidal cell (SOM→Py) connections in young (gray trace) and mature (black trace) animals. Inset, first 7 ms of overlaid IPSC responses. No developmental changes of IPSCs were detected at SOM→Py connections. C, left: example IPSC responses from PV→Py connections in young (P16) and mature (P24) animals before (gray traces) and during (black traces) the application of zolpidem. In the young animal example, the pre-zolpidem trace is mostly obscured by the zolpidem trace. Right, IPSC kinetics at PV→Py connections were more sensitive to zolpidem in mature mice, suggesting a developmental increase of α1-subunit-containing GABA_A receptors. D, left: amplitude-scaled overlay of paired-pulse ratio (PPR)-20 responses at PV→Py connections from young (P16; gray trace) and mature (P30; black trace) animals, slightly offset for clarity. PPR-20 increased with age at PV→Py connections. Right, amplitude-scaled overlay of PPR-20 responses at SOM→Py connections from young (P17; gray trace) and mature (P28; black trace) animals, slightly offset for clarity. No change in PPR-20 was observed at SOM→Py connections. Scale bars = 10 ms, 100 pA, and 190 mV in A, left; 10 ms in A, right; 1 ms in A, inset; 10 ms, 5 pA, and 210 mV in B, left; 10 ms in B, right; 1 ms in B, inset; and 10 ms in C. *P < 0.05 by Student’s t-test.

A nonsignificant increase (amplitude varied substantially). All of these changes stabilized by P22–P24, with little change apparent between P22–P24 and P28–P30.

To determine if a shift in GABA_A receptor subunit composition may underlie the maturational changes in IPSC kinetics, the α1-subunit-specific agonist zolpidem (Munakata et al. 1998) was applied (0.4 μM) (Ali and Thomson 2008) to some PV→Py paired recordings in young (P15–P17) and mature (P22) slices. Numerous studies have demonstrated a shift in α-subunits from α2/3- to α1-subunits during postnatal maturation (Laurie et al. 1992; Fritschy et al. 1994; Heinen et al. 2004; Hashimoto et al. 2009). α1-Subunit-containing GABA_A receptors have faster deactivation properties (Lavoie et al. 1997) and contribute to faster IPSC decay kinetics (Vicini et al. 2001; Bosman et al. 2005). In paired recordings, the application of zolpidem led to a significantly larger increase in IPSC half-width in mature (>P22: 29.3% ± 20.1) compared with young animals (P15–P17: 2.5% ± 6.1, P < 0.05 by Student’s t-test; Fig. 7C). This suggests that a relative increase in α1-subunit-containing GABA_A receptors contributes to the faster IPSC kinetics observed in older animals. The application of zolpidem did not consistently potentiate IPSC amplitude in any age group (data not shown), likely due to the saturation of GABA_A receptors on layer II/III pyramidal neurons (Hajos et al. 2000).

In contrast to PV→Py pairs, SOM→Py connections did not show any maturational changes in IPSC properties. The lack of developmental changes in IPSC kinetics is likely due to the use...
of GABA<sub>A</sub> receptor subtypes different from those of perisomatic targeting interneurons; dendritic targeting interneurons signal primarily through /H9251 5-subunit-containing GABA<sub>A</sub> receptors (Ali and Thomson 2008). Although expression of /H9251 5-subunits decreases with age (Heinen et al. 2004; Yu et al. 2006), these do not appear to be replaced with a different /H9251 -subunit, and therefore kinetic properties would not be expected to change.

In addition to the features of individual IPSCs, short-term synaptic plasticity can also be a key determinant in the function of different types of synaptic connections (Reyes et al. 1998; Markram et al. 1998). For example, excitatory input to PV cells shows short-term depression, whereas excitatory input to SOM cells shows short-term facilitation, possibly resulting in differential engagement of these two sources of inhibition depending on network activity (Reyes et al. 1998). To assess the maturation of short-term plasticity at the output synapses of PV and SOM cells, we measured the PPR at 20 Hz in both PV<sub>»</sub>PY and SOM<sub>»</sub>PY pairs (Fig. 7D). Nearly every single paired recording, at both types of synapses and at all ages, demonstrated synaptic depression (one PV<sub>»</sub>PY pair in the P28–P30 age group was slightly facilitating, PPR = 1.02, but this was the only exception). A gradual increase in PPR occurred in PV<sub>»</sub>PY pairs from P15–P17 (0.65 ± 0.09) to P28–P30 (0.81 ± 0.16, P < 0.05). This is the only PV synaptic feature that did not appear to stabilize by the early critical period and may reflect an increased ability to sustain activity in the PV inhibitory network. The mechanism of developmental reduction in synaptic depression at these synapses is unclear and would not be explained by increased PV expression (Caillard et al. 2000; Muller et al. 2007). SOM<sub>»</sub>PY pairs did not demonstrate any changes in PPR.

The distinct developmental profiles of PV and SOM cells contribute to the physiological differences in these two sources of inhibition in the mature V1 (Fig. 8). The mature PV network, by inhibiting proximal cell regions via fast /H9251 1-subunit-containing GABA<sub>A</sub> receptors, is generally expected to provide fast synaptic inhibition. However, in young animals (P15–P17), the decay times of PV<sub>»</sub>PY and SOM<sub>»</sub>PY IPSCs were indistinguishable (Fig. 8C). Additionally, mature (P28–P30) PV<sub>»</sub>PY connections demonstrated less synaptic depression than SOM<sub>»</sub>PY connections, but this also was a feature that only appeared later in development as a result of maturation of PV synapses (Fig. 8C). Throughout the time period studied, PV<sub>»</sub>PY IPSCs, compared with SOM<sub>»</sub>PY IPSCs, had larger amplitudes and faster rise times.

**DISCUSSION**

Cortical inhibitory circuits consist of diverse classes of interneurons with distinct physiological properties and connec-
tivity patterns (Miles et al. 1996; Kawaguchi and Kubota 1997; Markram et al. 2004; Burkhalter 2008). The maturation profiles of different cell classes and their differential regulation by experience may contribute to the progressive sharpening of functional properties of pyramidal neurons, yet most previous studies on the maturation of cortical inhibition (Bosman et al. 2002, 2005; Heinen et al. 2004; Morales et al. 2002) have not distinguished among different sources of synaptic inhibition. This is the first study, to our knowledge, that directly compares the maturation of two major classes of interneurons during the critical period in V1.

**Maturation of Perisomatic Inhibition From PV Interneurons**

During the onset phase of the critical period, both the intrinsic and synaptic properties of PV cells become significantly faster and more robust at multiple stages of signal propagation and transmission (Fig. 9). Indeed, the PV τ_M and AP waveform become faster, and the kinetics of unitary IPSCs become more rapid. The faster membrane properties (lower τ_M) result from a reduction in C_M, which would allow for more rapid integration with enhanced precision in response to synaptic inputs, effectively creating a highly stringent coincidence detector. The rapid AP, along with fast and strong AHP, in PV cells might result from the regulation of ion channel expression or spontaneous IPSC recordings, which do not differentiate between presynaptic sources, demonstrate the development of faster inhibitory signaling (Bosman et al. 2002, 2005; Heinen et al. 2004; Vicini et al. 2001; Kotak et al. 2008) have not distinguished among different sources of synaptic inhibition. This is the first study, to our knowledge, that directly compares the maturation of two major classes of interneurons during the critical period in V1.

![Fig. 9. Summary of changes in SOM and PV cells and synaptic output. Percentages given indicate changes between P15–P17 and P28–P30. CP, critical period. †Measured at 700 pA.](image-url)
enable V1 neurons to engage the plasticity mechanisms involved in OD plasticity.

**Maturation of Dendrite-Targeted Inhibition From Somatostatin-Expressing Interneurons**

SOM interneurons likely include several subgroups (Kawaguchi and Shindou 1998; Wang et al. 2004; Ma et al. 2006). The GIN line used in this study (Oliva et al. 2000) labels subsets of SOM cells, especially MN cells (Fig. 1B) (Ma et al. 2006), which are characterized by their ascending axonal projections, with extensive branching in layer I, and slowly accommodating firing pattern with spikes triggered at low threshold. Importantly, MN cells mediate frequency-dependent disynaptic inhibition (FDDI) among nearby pyramidal cells (Silberberg and Markram 2007), and this is a generic circuit motif prevalent across cortical areas and layers (Berger et al. 2009). FDDI has been postulated to gate synaptic plasticity in distal dendrites (Buchanan and Sjostrom 2009). Furthermore, the dendritic encoding of sensory stimuli in pyramidal neurons is highly sensitive to inhibitory control from MN cells (Murayama et al. 2009). However, the maturation of MN cells and other SOM cells has not been examined, especially during the critical period in V1.

We found that the most apparent developmental changes in SOM interneurons are in cellular biophysical properties: a substantial increase in $R_M$ underlies a slower $V_M$ in mature cells as well as a steepened frequency-current response curve (Fig. 9). Since $C_M$ remained constant, the increase of $R_M$ likely results from decreased density of leak channels (Camero et al. 2000). Increased $R_M$ along with depolarized $V_M$ leads to substantially higher excitability of these cells. Additionally, slower membrane properties would increase the time period over which SOM cells can integrate and respond to synaptic inputs.

These maturation profiles of SOM cells are in sharp contrast to those of pyramidal neurons and PV cells. Postnatal development of layer II/III pyramidal cells leads to lower input resistance (Luhmann and Prince 1991; Desai et al. 2002; Kuhlman et al. 2010) and hyperpolarized $V_M$ (Luhmann and Prince 1991; Desai et al. 2002; M. S. Lazarus and Z. J. Huang, unpublished observations), which should reduce cell excitability. The substantial increase of excitability of SOM cells may reflect a stronger engagement of this type of inhibition in V1 circuits during the critical period. Compared with PV cells, we noted that while the maturation of fast signaling in PV cells plateaus early in critical period, the increase of SOM excitability continues to the peak of OD plasticity.

Previous studies on the function of inhibition in OD plasticity have mainly focused on PV cells and their role in triggering the onset of the critical period. Whether and how GABAergic inhibition influences the execution of plasticity mechanisms and promotes the progression of OD shift after the critical period onset is poorly understood. In particular, the functional role of dendrite-targeted inhibition and SOM cells is unexplored. OD plasticity ultimately involves physical rewiring of excitatory synapses onto the dendritic spines of pyramidal neurons (Hofer et al. 2009). A major question remains as to how inputs representing the closed and open eyes compete along the apical dendrites for synaptic connections; in particular, it is unclear whether and how dendrite-targeted inhibition contributes to such competition. The intrinsic and membrane properties of mature SOM cells seem ideally suited to represent the strength of excitatory input and to convert it proportionally into inhibitory outputs, which could act to suppress competing inputs along pyramidal cell dendrites. Our results indicate that the maturational increase in the excitability of SOM cells correlates with the progression of OD plasticity, implying a stronger engagement of dendritic inhibition after the onset of the critical period. Genetic manipulation of SOM cells (e.g., using a SOM cell-specific Cre mouse line) offers the opportunity to directly test their function in OD plasticity.

**Conclusions**

By studying two distinct classes of visual cortical interneurons during the critical period of OD plasticity, we discovered their distinct maturation profiles. The most prominent feature in fast-spiking, perisomatic targeting PV interneurons is the maturation of fast signaling at the onset of the critical period. The most prominent feature in regular-spiking, dendritic targeting SOM interneurons is the maturation of their excitability and therefore the strength of inhibition, which continues to the peak of OD plasticity. In addition, the maturation of PV and SOM cells seem to exhibit a developmentally enhanced dichotomy: PV cells appear increasingly tuned to detect precisely timed inputs, whereas SOM cells develop stronger ability to detect and thus represent an overall level of input activity. Although our present results are descriptive and correlative by nature, they demonstrate that distinct classes of cortical interneurons have different developmental trajectories, which may progressively sharpen functional and plasticity properties in cortical circuits. This finding underscores the necessity of cell type-specific analysis when studying the development of cortical microcircuitry and has general implications in other cortical areas and plasticity paradigms.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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