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

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Running Title: Maturation of two classes of cortical GABAergic interneurons in V1

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Abstract

In 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 is not well understood. We used GFP 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 become enhanced, and synaptic signaling from PV to pyramidal neurons becomes faster. SOM cells demonstrate 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.
Keywords: GABA, Interneurons, Development, Ocular Dominance Plasticity, Critical Period
Introduction

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 shift (Hubel and Wiesel 1970). Ocular dominance (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 (MD), 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 indicates 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 isoform of the GABA-synthetic enzyme GAD65 show no OD plasticity, a deficit that can be rescued by cortical infusion of a GABA<sub>A</sub> receptor agonist (Hensch et. al. 1998). In addition, genetic (Huang et. al. 1999) and pharmacologic (Fagiolini 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 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; Burkhalter 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 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 the 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 α1 subunit containing GABA_A receptors, and inhibition through these receptors appears critical for OD plasticity (Fagiolini et al. 2004). Furthermore, the homeoprotein OTX2, which is able to trigger plasticity when it is 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 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-Garcia et al. 2006), dendritic calcium 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 towards 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 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 post-synaptic 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 critical period has not been fully characterized, and the maturation of SOM interneurons has not been explored. Using two GFP reporter mouse lines, here we present the first comparison of the maturation of inhibition provided by PV and SOM interneurons prior to and during the critical period for ocular dominance plasticity. We found that both classes of interneurons demonstrate substantial physiological maturation from eye opening to the peak of ODP, but with distinct trajectories. While the PV cells demonstrate significant maturation in their characteristic fast signaling properties, which plateaus after the onset of critical period, SOM cells
demonstrate a profound and steady increase in their excitability, which continues 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 used previously 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 green fluorescent protein (EGFP) driven by the \( P_v \) gene. EGFP in B13 line is expressed selectively in approximately 50% of PV cells in neocortex (Dumitriu et. al. 2007). The GIN line expresses EGFP driven by the \( Gad1 \) 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 GIN line is expressed in SOM neurons in both superficial and deep layers of neocortex (Oliva et. al. 2000; Ma et. al. 2006), labeling approximately 1/3 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, 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/g), and decapitated. Brains were rapidly removed and placed into ice-cold, oxygenated cutting solution, containing in millimolar: 110 choline chloride, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 0.5 CaCl₂, 7 MgCl₂, 25 glucose, 11.6 ascorbic acid, 3.1 pyruvic acid, bubbled with 95%O₂-5%CO₂. The anterior 1/3 of the brains 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 the primary visual cortex, and were 350 μm thick. Slices were transferred to artificial cerebrospinal fluid (ACSF), and incubated at 32-34°C for at least 30 minutes. ACSF contained in millimolar: 126 NaCl, 2.5 KCl, 25 NaHCO₃, 14 glucose, 1.25 NaH₂PO₄, 1 MgSO₄, 2 CaCl₂, bubbled with 95%O₂-5%CO₂ to pH 7.4. For recording, slices were transferred to a recording chamber continuously perfused with oxygenated ACSF, maintained at 28-30°C.

**Biocytin Filling**

GFP+ cells were identified in layer II/III of 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 minutes), and then incubated overnight in AlexaFluor 568 conjugated streptavidin (1:1000; Invitrogen), with 0.3% triton X-1000 in PBS. Slices were then rinsed in PBS (3 times for 5 minutes) 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 primary visual cortex. 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+ cell was subsequently visualized with differential interference contrast (Zeiss). For paired recordings, a nearby pyramidal neuron (<50 μm) 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 MΩ. In both paired and single cell recordings, the intracellular solution for the interneuron contained in millimolar: 130 K-gluconate, 6 KCl, 2 MgCl₂, 2 EGTA, 2 HEPES, 2.5 Na-ATP, 0.5 Na-GTP, 10 Na-phosphocreatine, pH 7.25, 285-295 mOsm. The interneuron recording was performed in current clamp for all experiments. For paired recordings, high internal [Cl⁻] was used to magnify IPSC responses in pyramidal neurons to aid with analysis. The pyramidal neuron internal solution contained in millimolar: 65 K-gluconate, 65 KCl, 2 MgCl₂, 2 EGTA, 2 HEPES, 2.5 Na-ATP, 0.5 Na-GTP, 10 Na-phosphocreatine, pH 7.25, 285-295 mOsm. The post-synaptic pyramidal
neuron was held in voltage clamp at -75 mV. Zolpidem was used to assess GABA\textsubscript{A} receptor α-subunit composition, and was purchased from Tocris (Ellisville, MO).

To assess intrinsic properties, interneurons were stimulated with increasing 1 second long current steps. PV cells were assessed with 50 pA steps starting from -200 pA increasing up to +700 pA, and SOM cells were assessed with 20 pA steps starting from -100 pA increasing up to +200 pA. Maximum current injections of +700 pA 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 suprathreshold 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 inter-trial interval of 5 seconds. For analysis, 25-50 trials were averaged. For short-term plasticity experiments, presynaptic interneurons were stimulated at 20 Hz, with an inter-trial interval of 10 seconds.

**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 was > -50 mV several minutes after break-in, or if membrane resistance was < 50 MΩ. Pairs of neurons were considered synaptically connected if the averaged IPSC was >2 pA.

**Membrane Properties**
Membrane Time Constant ($\tau_M$, ms): Determined from monoexponential fit of hyperpolarizing voltage response (~10 mV) to a suitable current injection, from resting level to minimum point of sag, if present.

Input Resistance ($R_M$, M$\Omega$): Slope of linear portion of voltage responses to a series of negative current steps (I-V response curve).

Membrane Capacitance ($C_M$, pF): Determined from the equation, $C_M = \frac{\tau_M}{R_M}$.

Resting Membrane Potential ($V_M$, mV): Stable membrane potential, determined with no current injection a few minutes after breaking seal.

Action Potential Threshold ($V_T$, mV): Membrane potential when rate of rise ($dV/dt$) equals 5 V/s, measured in response to smallest current step able to evoke a spike.

Excitability Index (EI, pA): Incorporates multiple properties of a cell to estimate intrinsic excitability, determined from the equation $EI = \frac{(V_T - V_M)}{R_M}$.

Spiking Properties

Spike Half-Width (HW$_{AP}$, ms): Width measured at half-amplitude, between $V_T$ and peak of action potential, measured in response to smallest current step able to evoke a spike.

Afterhyperpolarization Amplitude (AHP, mV): Voltage difference between $V_T$ and most negative point reached following action potential, measured in response to smallest current step able to evoke a spike.

Spike Frequency (Hz): Inverse of first interspike-interval (ISI).

F-I Slope (Hz/pA): Slope of linear portion of the frequency-current response curve, using initial spike frequency.
Spike Frequency Adaptation (dimensionless): Ratio of last ISI to fourth ISI. Assessed at +700 pA for PV cells, +200 pA for SOM cells.

Synaptic Properties

Amplitude (pA): Measured from baseline (average of 5 ms) to peak of averaged IPSC.
Rise Time of Averaged IPSC (RT_{Avg}, ms): Time from 10% -90% of rising phase of averaged IPSC.
Rise Time of Individual IPSC (RT_{Indv}, ms): Average of 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→Py IPSC’s due to very small amplitude of SOM→Py IPSC’s.

Decay Time (τ_{IPSC}, ms): Determined from monoexponential fit of decaying phase of averaged IPSC.
Paired-Pulse Ratio (PPR, dimensionless): Ratio of second IPSC amplitude to first IPSC amplitude, stimulated at 20 Hz.

Statistics

All data are reported as mean ± standard deviation (SD). Significance was determined by one-way ANOVA with post-hoc Tukey’s test for all data with >2 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. Significance level was set at P<0.05.
Results

Targeted whole cell current-clamp recordings were made from PV and SOM expressing neurons to analyze membrane and spiking properties. Dual whole-cell recordings were made from PV to pyramidal neuron pairs (PV→Py) and SOM to pyramidal neuron pairs (SOM→Py) to analyze synaptic properties. Three age groups were chosen to study maturation of inhibition in relation to the onset of ocular dominance plasticity: Pre-critical period after eye opening (P15-17); early in critical period (P22-24); and peak of critical period (P28-30; Gordon and Stryker 1996). All statistical comparisons are across these three age groups, unless otherwise noted.

Morphology of GFP labeled cells

Morphological reconstructions were performed to determine which types of cells are labeled in the B13 and GIN lines, and to confirm previous reports (see Methods). Three cells each in animals from young age group, P15-17, and mature age group, P28-30, in 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 2/3, 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 1. This supports previous
description identifying GIN line cells as Martinotti 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 $\tau_M$ from eye opening
(P15-17, 12.2 ms ±5.3) to the early critical period (P22-24, 8.2 ms ±3.2, $P<0.05$), when it
reached a steady state (Fig. 2A). On the other hand, in SOM cells $\tau_M$ increased between
eye opening (P15-17, 29.3 ms ±13.0) and the peak of critical period (P28-30, 43.2ms
±12.3, $P<0.05$; Fig. 2B).

The mechanisms of the changes in $\tau_M$ were different in PV and SOM cells (Fig. 3). In PV
cells, $R_M$ did not change significantly over the period studied (Fig. 3A); therefore, the
reduction in $\tau_M$ of PV cells may be due to a decrease in membrane capacitance ($C_M$)
between P15-17 (87.7 pF ±22.5) and P22-24 (67.7 pF ±14.2, $P<0.01$; Fig. 3A2). In
contrast to PV cells, SOM cells showed substantial $R_M$ increase across all three ages
studied (P15-17, 299 MΩ ±99; P28-30, 445 MΩ ±79; $P<0.01$; Fig. 3B), but $C_M$ did not
change significantly over the developmental time period tested (Fig. 3B2). The increase
in $R_M$ likely underlies the developmental increase in $\tau_M$ observed in SOM cells.

Resting membrane potential ($V_M$) of PV cells was stable over the period studied (Fig.
4A1). The $V_M$ of SOM cells was stable between P15-17 and P22-24, but became
significantly more depolarized prior to the peak of critical period at P28-30 (P22-24, -
61.3 mV ±7.7; P28-30, -55.9 mV ±5.1; P<0.05; Fig 4B1). Since $V_M$ 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 $V_M$, as well as the developmental increase in $R_M$ (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, 20 pA intervals for SOM cells); 2) excitability index (EI, expressed in pA and determined by the equation $EI = (V_T - V_M) / R_M$, 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. Current required to evoke spikes in PV cells did not change with development (Kolmogorov-Smirnov Test, $P=0.89$; Fig. 4A2), nor did EI change significantly over the period studied (Fig. 4A2). In SOM cells, likely due to increased $R_M$ and more depolarized $V_M$, 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. 4B2), along with a two-fold reduction of EI ($P15-17, 53.6$ pA ±25.3; P28-30, 25.9 pA ±7.5; $P<0.01$; Fig. 4B2).
It should be noted that with the EI measure in mature mice (P28-30), SOM cells are over ten-fold more excitable than PV cells (Fig. 4A2 and 4B2). At all ages, SOM cells, compared to PV cells, demonstrate more hyperpolarized $V_T$ (no developmental change, data not shown), larger $R_M$, and more depolarized $V_M$. In other words, all three factors used to determine EI would make SOM cells more excitable than PV cells, suggesting the SOM-expressing 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 show fast-spiking properties (Kawaguchi and Kubota 1997) described by McCormick et. al. (1985). These include low spike frequency adaptation, large fast after-hyperpolarization (AHP), narrow action potential half-width (HW$_{AP}$), and high spiking frequency. These last three features showed significant maturational changes, leading to more typical fast-spiking characteristics, in PV cells.

Action potential morphology substantially changed with maturation in PV, but not in SOM cells (Fig. 5). HW$_{AP}$ of PV cells decreased between P15-17 (0.80 ms ±0.14) and P22-24 (0.60 ms ±0.31; p<0.05; Fig. 5A), while AHP became stronger between P22-24 (17.1 mV ±4.4) and P28-30 (21.0 mV ±2.7; p<0.05; Fig. 5A). The development of larger AHP was the only maturational feature of PV cells that did not show changes between P15-17 and P22-24. Both HW$_{AP}$ and AHP of SOM cells were stable over the time period studied (Fig. 5B).
Plots of initial spike frequency versus current injected (F-I curves) demonstrated maturational changes for both PV and SOM cells (Fig. 6). In both cell types, the F-I 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 pA, P<0.01 for 600 pA and 700 pA; Fig 6A2). In SOM cells, higher spike frequencies in older animals were observed across the range of current inputs (P<0.01 for 40 pA, 80, 120, 160, and 200; Fig. 6B2). To determine if changes in membrane resistance may contribute to the shift of F-I 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 gives an estimation of the predicted membrane potential response (spike activation prevents the full membrane voltage response from occurring), and can be represented in millivolts (mV). 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 F-I curves (Fig. 6A3). SOM cells, however, showed a substantial change in R_M with development. Adjusting the F-I curves of SOM cells for R_M eliminated the differences in frequency response, and led to overlapping curves for the different age groups (Fig. 6B3). This result suggests that increased R_M in SOM cells can largely account for developmental changes in frequency-current response features.

Quantification of F/I slope in PV cells shows the increased frequency response occurs between P15-17 (0.240 Hz/pA ±0.039) and P22-24 (0.429 Hz/pA ±0.166; P<0.01; Fig 6A4); however, in SOM cells, F/I slope increased gradually between P15-17 (0.457 Hz/pA ±0.093) and P28-30 (0.558 Hz/pA ±0.092; P<0.05; Fig 6B4). Spike adaptation,
typically low in fast-spiking cells (McCormick et. al. 1985), was stable in PV cells over
the period studied (Fig 6A4), but increased gradually in SOM cells (P15-17, 1.94 ±0.75;
P28-30, 3.70 ±2.19; P<0.05; Fig 6B4)

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 is consistent with
previous studies (Doischer et. al. 2008; Okaty et. al. 2009; Kuhlman et. al. 2010), and
demonstrates 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 will determine
when, if, and how action potential firing occurs. But the impact of cell spiking is, of
course, dependent on communication with post-synaptic 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 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.

Kinetic properties of inhibitory post-synaptic currents (IPSC’s) became faster in PV→Py pairs. IPSC rise-time decreased, mostly between P15-17 (RT\_Avg, 0.77 ms ±0.14; RT\_Indv 0.71 ms ±0.14) and P22-24 (RT\_Avg, 0.65 ms ±0.11, P<0.05; RT\_Indv, 0.57 ms ±0.14; P<0.05). IPSC decay time also decreased, and followed a similar time-course (P15-17, 12.68 ms ±3.85; P22-24, 8.94 ms ±1.41; P<0.01). IPSC amplitude showed a non-significant increase (amplitude varied substantially). All of these changes stabilized by P22-24, with little change apparent between P22-24 and P28-30.

To determine if a shift in GABA\(_A\) receptor subunit composition may underlie the maturational changes in IPSC kinetics, the \(\alpha_1\)-subunit specific agonist zolpidem (Munakata et. al. 1998) was applied (0.4 \(\mu\)M; Ali and Thomson 2008) to some PV→Py paired recordings in young (P15-17) and mature (>P22) slices. Numerous studies have demonstrated a shift in \(\alpha\) subunits from \(\alpha_{2/3}\) to \(\alpha_1\) during postnatal maturation (Laurie et. al. 1992; Fritschy et. al. 1994; Heinen et. al. 2004; Hashimoto et. al. 2009). \(\alpha_1\)-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, zolpidem application led to a significantly larger increase in IPSC half-width in mature (>P22, 29.3\% ±20.1) compared to young animals (P15-17, 2.5\% ±6.1, P<0.05, Student’s T-test; Fig. 7C). This suggests that a relative increase in \(\alpha_1\)-subunit
containing GABA<sub>A</sub> receptors contributes to the faster IPSC kinetics observed in older animals. Zolpidem application did not consistently potentiate IPSC amplitude in any age group (data not shown), likely due to saturation of GABA<sub>A</sub> 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 though α<sub>5</sub>-subunit containing GABA<sub>A</sub> receptors (Ali and Thomson 2008). Although expression of α<sub>5</sub> subunits decreases with age (Heinen et. al. 2004; Yu et. al., 2006), these do not appear to be replaced with a different α subunit, and therefore kinetic properties would not be expected to change.

In addition to the features of individual IPSC’s, 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, while 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 maturation of short-term plasticity at the output synapses of PV and SOM cells, we measured paired-pulse ratio (PPR) at 20 Hz in both PV→Py and SOM→Py pairs (Fig. 7D). Nearly every single paired recording, at both types of synapses and at all ages, demonstrated synaptic depression (one PV→Py
pair in the P28-30 age group was slightly facilitating, PPR=1.02, but this was the only exception. A gradual increase in PPR occurred in PV→Py pairs from P15-17 (0.65 ±0.09) to P28-30 (0.81 ±0.16; P<0.05). This is the only PV synaptic feature that did not appear to stabilize by early critical period, and may reflect 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→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 $\alpha_1$-subunit containing GABA$_A$ receptors, is generally expected to provide fast synaptic inhibition. However, in young animals (P15-17), the decay times of PV→Py and SOM→Py IPSC’s are indistinguishable (Fig. 8C). Additionally, mature (P28-30) PV→Py connections demonstrate less synaptic depression than SOM→Py connections, but this also is a feature that only appears later in development as a result of maturation of PV synapses (Fig. 8C). Throughout the time period studied, PV→Py IPSC’s, compared to SOM→Py IPSC’s, had larger amplitude and faster rise-time.

Discussion
Cortical inhibitory circuits consist of diverse classes of interneurons with distinct physiological properties and connectivity 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, which directly compares the maturation of two major classes of interneurons during the critical period in primary visual cortex.

**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, PV cell membrane time constant and action potential wave-form become faster, and the kinetics of unitary IPSCs become more rapid. The faster membrane properties (lower $\tau_M$) result from a reduction in membrane capacitance ($C_M$), which would allow for more rapid integration with enhanced precision in response to synaptic inputs, effectively creating a highly stringent coincidence detector. The rapid action potential, along with fast and strong afterhyperpolarization, in PV cells might result from the regulation of ion channel expression, and allows PV cells to fire at high frequencies with minimal spike frequency
adaptation (Erisir et al. 1999). Additionally, PV cells have the unique property of electrical coupling with one another (Galarreta and Hestrin 1999; Gibson et al. 1999; Beierlein et al. 2000), which could also contribute to biophysical features of these cells (Veruki et al. 2010).

The more rapid IPSC kinetics (i.e. faster decay) at PV→Py synapses seem to result from increased α1-subunit content of postsynaptic GABA_A receptors, as evidenced by enhanced sensitivity to a low concentration of zolpidem. This is consistent with results from multiple brain regions and species showing high expression of α_2- or α_3-subunits at early stages, with up-regulation of α_1-subunits at later times (Fritschy et al. 1994; Bosman et al. 2002; Heinen et al. 2004; Vicini et al. 2001; Hashimoto et al. 2009). Consequently, mIPSC or sIPSC 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; Hashimoto et al. 2009). This developmental switch in subunit composition and IPSC properties appears to be a highly conserved, cell-type specific mechanism to sharpen inhibitory transmission during GABA circuit maturation in different cortical areas and species. The concerted changes in synaptic and intrinsic properties, similar to the maturation of basket cells in the dentate gyrus (Doischer et al. 2008), essentially convert V1 PV cells into fast signaling devices during the onset of OD plasticity.

Previous developmental studies on PV cells in dentate gyrus (Doischer et al. 2008) and somatosensory cortex (Okaty et al. 2009) included much younger, neonatal ages, and
showed, not surprisingly, more profound maturation of some characteristics, including the strength and reliability of synaptic transmission. Our study focuses around the critical period and revealed that faster signaling appears to be the main, if not the only, property of PV cells which shows significant maturation, possibly contributing to the onset of OD plasticity.

During ocular dominance plasticity, the rearrangement of synaptic connections in V1 is guided by altered visual inputs from the open and closed eyes, which are encoded in the temporal spiking patterns of thalamic axons. The firing of PV cells can precisely reflect the spiking patterns of excitatory inputs, and groups of synaptic and electrically connected PV cells (Galarreta and Hestrin 1999; Gibson et. al. 1999; Beierlein et. al. 2000) can discriminate synchronous vs. less synchronous inputs (e.g. those from the open or closed eye) with millisecond precision (Galarreta and Hestrin 2001). Furthermore, mature PV cells are characterized by their “fast-in and fast-out” signal transmission (Jonas et. al. 2004) and thus are highly effective in converting the pattern of excitatory input into well-timed inhibitory output to the perisomatic region, thereby controlling the spiking pattern of innervated pyramidal neurons. PV-expressing fast-spiking interneurons therefore appear ideally suited to detect and transmit precise input spiking patterns in V1, a necessary step in the engagement of plasticity mechanisms (Huang and Di Cristo 2008; Hensch 2005), e.g. spike-timing dependent plasticity, which may already be in place at that time (Kuhlman et. al. 2010). Here we have shown that the intrinsic and synaptic properties of V1 PV cells remain immature after eye opening, but are significantly sharpened during the onset of OD plasticity. These results suggest that PV
cells may develop improved ability to detect and/or transmit changes in input spiking patterns, e.g. from an open vs. closed eye. Therefore, the maturation of the PV interneuron network could potentially enable V1 neurons to engage the plasticity mechanisms involved in OD plasticity.

Maturation of dendrite-targeted inhibition from somatostatin-expressing interneurons

SOM-expressing 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 Martinotti cells (Figure 1B; Ma et. al. 2006) characterized by their ascending axonal projections, with extensive branching in layer I, and slowly accommodating firing pattern with spikes triggered at low threshold. Importantly, Martinotti (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 Martinotti 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 membrane resistance underlies a slower $\tau_M$ 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 (Cameron et. al. 2000). Increased membrane resistance, along with depolarized resting membrane potential, 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 resting membrane potential (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. When compared to 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 critical period. Whether and how GABAergic inhibition influences the execution of plasticity mechanisms and promotes the progression of OD shift after 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 following 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 ocular dominance 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 critical period. The most prominent feature in regular-spiking, dendrite-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, while SOM cells develop stronger ability to detect and thus represent an overall level of input activity. Although our current 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 microcircuits and has general implications in other cortical areas and plasticity paradigms.
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Disclosures

None.
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Figure 1. Morphological reconstruction of GFP labeled cells. A1, Reconstructed cells from young mice in B13 line. A2, Mature mice in B13 line. B1, Young mice in GIN line. B2, Mature mice in GIN line. Axons, red; soma and dendrites, blue. Specific ages are indicated. Scale bar, 100 μm.

Figure 2. Cell-type specific maturation of membrane properties. A1, Example current step responses (100 pA steps) in PV cells from a young (P16) and a mature (P28) animal. A2, Amplitude-scaled overlay (i.e., -200 pA step response set to 1) of the first 100 ms of current-step responses from A1. Gray trace, young animal; black trace, mature animal. τM Of PV cells decreased during development. B1, Example current-step responses (20 pA steps) in SOM cells from a young (P15) and a mature (P29) animal. The mature animal trace shows some truncated spikes. B2, Amplitude-scaled overlay of the first 150 ms of current-step responses from B1. In contrast to PV cells, τM in SOM cells increased during development. Scale bars in A1 and B1, 300 ms, 20 mV; in A2 and B2, 20 ms; P<0.05. Sample sizes are indicated in parentheses, and apply to figures 2-6.

Figure 3. Distinct causes of maturational changes of τM in PV and SOM cells. A1, Overlayed current-step responses from figure 2 and current-voltage (I-V) relationships of PV cells in young (P16, gray traces; and P15-17, gray square data points) and mature (P28, black traces; and P28-30, black circle data points) animals. A2, Quantification in three age groups of membrane resistance (Rm), determined from the slope of the current-voltage relationship, and membrane capacitance (Cm), determined from the equation: Cm=(τM)/(Rm). Rm did not change significantly with age in PV cells. Cm decreased with
Age, and stabilized by early critical period. B1, Overlayed current-step responses from figure 2 and I-V relationships of SOM cells in young (P15, gray traces; and P15-17, gray square data points) and mature (P29, black traces; and P28-30, black circle data points) animals. The mature trace includes some truncated spikes. B2, $R_M$ of SOM cells increased steadily over the period studied. $C_M$ did not change. Scale bars in A1 and B1, 300 ms, 20 mV. * P<0.05, ** P<0.01.

Figure 4. Cell-type specific maturational changes of neuron excitability. A1, Left, membrane potential ($V_M$) with no injected current and in response to a 400 pA current step in example PV cells from a young (P16) and a mature (P28) animal, and quantification of $V_M$ in three age groups. $V_M$ of PV cells did not change with age. Dashed line represents 0 mV. A2, Left, the distribution of minimum current injection, in 100 pA intervals, that elicited at least 1 spike in PV cells from young (gray line) and mature (black line) animals did not change with development. Right, the calculated excitability index did not change significantly with age in PV cells. B1, Left, $V_M$ with no injected current and in response to a 60 pA current step in example SOM cells in a young (P15) and a mature (P29) animal, and quantification of $V_M$ in three age groups. $V_M$ of SOM cells became more depolarized with development between P22-24 and P28-30. B2, Left, the distribution of minimum current injection, in 20 pA intervals, that elicited at least 1 spike in SOM cells from young (gray line) and mature (black line) animals shifted significantly to the left with age (Kolmogorov-Smirnov, P<0.01). Right, the calculated excitability index decreased with age in SOM cells. Scale bars in A1 and B1, 1000 ms, 30 mV. * P<0.05, ** P<0.01.
Figure 5. Cell-type specific maturation of action potential morphology. A1, Left, example action potentials in PV cells from a young (P16) and a mature (P30) animal. Right, amplitude-scaled overlay (i.e., threshold to peak is set to 1) of young (gray trace) and mature (black trace) action potentials in PV cells demonstrates the reduction in AP half-width and increase of AHP with development. A2, AP half-width and AHP in PV cells are quantified across three ages. B1, Left, example action potentials in SOM cells from a young (P15) and a mature (P29) animal. Right, amplitude-scaled overlay of young (gray trace) and mature (black trace) action potentials. The gray trace is mostly obscured by the black trace, exemplifying the lack of developmental change in AP morphology. B2, AP half-width and AHP in SOM cells are quantified across three ages. Scale bars in A1 and B1 for example spikes, 20 mV, 2 ms; for scaled overlays, 1 ms. * P<0.05.

Figure 6. Maturation of spiking properties. A1, Example current-step responses to -100 pA, 0 pA, and +700 pA in PV cells from a young (P16) and a mature (P28) animal, and an overlay of the first 30 ms from young (gray trace) and mature (black trace) animals. B1, Example current-step responses to -40 pA, 0 pA, and +200 pA in SOM cells from a young (P15) and a mature (P29) animal, along with overlay of the first 30 ms. A2, Initial spike frequency response to current injections in PV cells at ages P15-17 (gray squares), P22-24 (light gray triangles), and P28-30 (black circles). Initial spike frequency in response to large current injections increased with age in PV cells between P15-17 and P22-24. Solid black bars indicate current levels at which a significant difference was
observed. B2, 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. A3, Data from A2, adjusted to the mean R_m for each age group. Adjusting for R_m does not account for the age-related changes in spike frequency in PV cells. B3, Data from B2, adjusted to the mean R_m for each age group. Overlapping data points indicate that adjusting for R_m largely accounts for the age-related changes in spike frequency in SOM cells. A4, Quantification of F/I slope and spike adaptation across three ages in PV cells. Steepened frequency-current response in PV cells was apparent between P15-17 and P22-24, while spike adaptation remained stable. B4, Quantification of F/I slope and spike adaptation across three ages in SOM cells. Steepened frequency-current response and increased spike adaptation occurred gradually between P15-17 and P28-30. Scale bars in A1 and B1 for full traces, 500 ms, 50 mV; for overlays, 5 ms, 50 mV. * P<0.05, ** P<0.01.

Figure 7. Maturation of IPSC properties. A, Left, example averaged IPSC_{PV} responses from PV cell (gray traces) to pyramidal cell (black traces) paired recordings in young (top, P16) and mature (bottom, P30) animals. Right, amplitude-scaled overlay of IPSC_{PV} responses in young (gray trace) and mature (black trace) animals. Inset: First 3 ms of overlaid IPSC_{PV} responses. At PV→Py connections, both IPSC rise time and decay became faster with development. B, Left, example averaged IPSC_{SOM} responses from SOM cell (gray traces) to pyramidal cell (black traces) paired recordings in young (top, P17) and mature (bottom, P28) animals. Right, amplitude-scaled overlay of IPSC_{SOM} responses in young (gray trace) and mature (black trace) animals. Inset: First 7 ms of
overlayed IPSC\textsubscript{SOM} responses. No developmental changes of IPSC’s were detected at SOM→Py connections. C, Left, example IPSC\textsubscript{PV} responses in young (P16) and mature (P24) animals prior to zolpidem application (gray traces) and during zolpidem application (black traces). In the young animal example, the pre-zolpidem trace is mostly obscured by the zolpidem trace. Right, IPSC\textsubscript{PV} kinetics were more sensitive to zolpidem in mature mice, suggesting a developmental increase of $\alpha_1$-subunit containing GABA\textsubscript{A} receptors.

D, Left, amplitude-scaled overlay of 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 in A, 10 ms, 100 pA, 190 mV, Overlay, 10 ms, Inset, 1 ms; in B, 10 ms, 5 pA, 210 mV, Overlay, 10 ms, Inset, 1 ms; in C, 10 ms; * P<0.05, Student’s T-test.

Figure 8. Comparison of maturation of IPSC\textsubscript{PV} and IPSC\textsubscript{SOM} properties. A, Example average traces of IPSC\textsubscript{PV} (left) and IPSC\textsubscript{SOM} (right) from mature animals (P28). Gray trace, presynaptic cell; black trace, pyramidal cell. B, Amplitude-scaled overlay of IPSC\textsubscript{PV} (black trace) and IPSC\textsubscript{SOM} (gray trace). C, Quantification of IPSC properties. Rise-time indicated is RT\textsubscript{Avg}. At all ages studied, IPSC\textsubscript{PV} had larger amplitude and faster rise time than IPSC\textsubscript{SOM}. Only at mature ages, IPSC\textsubscript{PV} had faster decay time and larger PPR-20 than IPSC\textsubscript{SOM}. Scale bars in A, 10 ms, 100 pA, 160 mV; in B, 10 ms. * P<0.05,
** P<0.01, one-way Anova; # P<0.05, ## P<0.01, ### P<0.001, Student’s T-test; sample sizes indicated in parentheses.

Figure 9. Summary of changes in SOM and PV cells and synaptic output. Percentages given indicate change between P15-17 and P28-30. CP, critical period. ‡‡ Measured at 700 pA.
A

B

C

D

0.4 µM Zolpidem

Change of IPSC Half-Width

P15-17 > P22

50 ms

20Hz
**A**

PV+ Inputs

SOM+ Inputs

P28

**B**

**C**

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Note: * indicates a significant difference between PV+ and SOM+ inputs.
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<th>Time-Course</th>
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<td>Steady from eye opening to CP peak.</td>
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