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Plasticity of Cortical Circuit Activation within the Monocular Region of Primary Visual Cortex of the Rat

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Trevor Charles Griffen

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Abstract of the Dissertation

Plasticity of Cortical Circuit Activation within the Monocular Region of Primary Visual Cortex of the Rat

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After eye opening, rodent visual cortex undergoes a period of rapid changes in cellular and synaptic properties that coincides with the maturation of visual receptive fields. During this developmental window, cortex is particularly sensitive to manipulations in sensory experience. Here, I used voltage sensitive dye imaging in acute cortical slices to show that the maturation of visual cortex is accompanied by changes in the spatio-temporal propagation of activity through the cortical circuit. The developmental alteration in the voltage signal is mediated in part by changes in the components of the signal mediated by NMDA, AMPA and $GABA_A$ receptors. Next, I used *in vivo* whole-cell recordings to examine the responsiveness of visual cortical neurons to flash stimuli and the sensitivity of these responses to manipulation of visual drive. I found that in addition to responding to stimulation of the contralateral eye, the monocular region of visual cortex responded to brief flash stimulation of the ipsilateral eye as well. However, responses to ipsilateral eye stimulation were delayed relative to contralateral eye stimulation, which suggests that these responses have distinct anatomical origins. After a single day of

monocular visual deprivation, ipsilateral responses to the open eye were potentiated, leading to a novel form of ocular dominance plasticity. Together, these experiments show plasticity of cortical circuit activation during both healthy and pathological postnatal development.

Dedication

This dissertation is dedicated to Brittney Wolf and Diana, Charles, Jessica and Emily Griffen. Thank you for providing unfailing love, patience and support!

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Chapter III: Rapid Ocular Dominance Plasticity in Monocular Visual Cortex

List of Abbreviations

A1	Primary auditory cortex
ACSF	Artificial cerebrospinal fluid
auROC	Area under the receiver operator curve
L	Layer
LGN	Lateral geniculate nucleus of the thalamus
LTD	Long term depression
LTP	Long term potentiation
ODI	Ocular dominance index
P	Postnatal day
ROI	Region of interest
S1	Primary somatosensory cortex
SEM	Standard error of the mean
TFS	Time from stimulus
V1	Primary visual cortex
V1b	Primary visual cortex, binocular region
V1m	Primary visual cortex, monocular region
VSD	Voltage sensitive dye

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Chapter I

General Introduction

Sensory Cortical Plasticity

Faced with limited resources, organisms must have the capacity to adapt to their environment in order to ensure survival. Sensory systems provide the means for receiving information about the environment and the primary sensory regions of the mammalian neocortex provide the capacity for complex processing of environmental inputs. The neocortex is comprised of neurons that can be broadly classified by their primary neurotransmitters as either glutamatergic/excitatory, GABAergic/inhibitory or neuromodulatory, and glia. The primary sensory cortices are layered structures, with unique distributions of subclasses of cell types and connectivity schemes comprising each layer. Between different cell types and cortical layers, a multitude of mechanisms for plasticity have been identified (Malenka and Bear, 2004; Maffei, 2011; Turrigiano, 2011). This diversity of mechanisms is likely essential for circuit rewiring and facilitating adaptation to the environment.

Primary visual (V1), somatosensory (S1) and auditory (A1) cortices are classically divided into six laminae. The majority of sensory input is thought to arrive at these sensory cortices from the thalamus. The thalamus projects its largest output to layer 4 (L4), which is generally considered the first stage of cortical sensory processing. Here, I will examine plasticity within the visual cortical circuit surrounding the primary input layer, with a special focus on a window in early postnatal development during which there is a heightened capacity for plasticity in response to changes in environmental input.

Ocular Dominance Plasticity

In 1921, Juler observed that the restoration of vision following surgery on a cataract secondary to trauma was more complete if the cataract had formed after the age five or six (Juler, 1921). This paramount observation provided evidence for the existence of time windows during development in which there is a heightened sensitivity to manipulation of visual drive. To understand how temporary loss of vision in one eye might lead to a permanent loss of visual function, Hubel and Wiesel pioneered recordings from neurons in cat V1. They observed that most neurons in cat V1 respond to visual stimuli presented in the same portion of visual space to either eye; however, some neurons responded preferentially to stimuli presented to one eye (Hubel and Wiesel, 1962). They termed the ratio of responsiveness to similar stimuli presented separately to the two eyes "ocular dominance." Following the prolonged unilateral disruption of visual experience by suturing shut a single eyelid, kittens became unable to see out of the deprived eye after removal of the sutures. Additionally, the distribution of ocular dominance for V1 neurons shifted dramatically to favor the eye that was left open (Wiesel and Hubel, 1963b). This shift in responsiveness was termed "ocular dominance plasticity" and was assumed to provide the mechanism for the observed blindness. However, if monocular lid suture was performed on adult cats, ocular dominance plasticity did not occur (Hubel and Wiesel, 1970). This experimental evidence suggested that the capacity to induce ocular dominance plasticity by monocular lid suture is limited to a sensitive period during early postnatal development (Wiesel and Hubel, 1963b; Hubel and Wiesel, 1970).

The existence of a sensitive period for ocular dominance plasticity in V1 has been observed in many species, including rodents, ferrets and non-human primates (LeVay et al., 1980; Fagiolini et al., 1994; Gordon and Stryker, 1996; Horton and Hocking, 1997; Issa et al.,

1999). To understand how deprivation during a sensitive period leads to loss of visual acuity, several fundamental questions must be answered. What are the specific changes within the cortical circuit that permit plasticity to occur maximally within a given developmental window? What changes in network activity during sensory deprivation drive plasticity? What mechanisms of plasticity underlie changes in responses to sensory stimuli?

Mechanisms of the Expression of Ocular Dominance Plasticity

Competitive versus non-competitive mechanisms of plasticity

After observing that prolonged lid suture of a single eye during the sensitive period leads to a loss of responsiveness to stimuli shown to that eye, Wiesel and Hubel hypothesized that simultaneous lid suture of both eyes would cause V1 neurons to stop responding to all visual stimuli (Wiesel and Hubel, 1965). They were surprised to find that most V1 neurons remained responsive to visual stimuli after a long period of binocular lid suture. They interpreted this result as evidence that the loss of responsiveness to the deprived eye seen after monocular deprivation was driven by competition between inputs to cortical neurons that served both the open eye and the closed eye (Wiesel and Hubel, 1965). In this model, the competitive loss of responsiveness to the closed eye reduces the allocation of computational power away from inputs providing little relevant visual information, but only when a better source of visual information exists.

One prediction that followed from the competitive theory of ocular dominance plasticity was that in the portion of V1 that only receives direct thalamic input from the contralateral eye, monocular V1 (V1m), monocular lid suture should not suppress visual responses as strongly as in the binocular portion of V1 (V1b; **Figure 1.1**). In V1m of cats and rats and in V1 of rabbits, where most neurons are driven entirely by the contralateral eye (Rose and Malis, 1965), prolonged periods of visual deprivation greatly reduced, but did not eliminate, visual responsiveness to stimuli shown to the contralateral, deprived eye (Wilson and Sherman, 1977; Crabtree et al., 1981; Iurilli et al., 2012; see also Mrsic-Flogel et al., 2007). However, while very brief periods of monocular lid suture (~2 days) were sufficient to induce ocular dominance plasticity in V1b, a significant loss of responsiveness has not been observed following brief

monocular lid suture in V1m (Mrsic-Flogel et al., 2007; Blais et al., 2008; Smith et al., 2009; Iurilli et al., 2012). Together, these findings have led to the conclusion that competition between inputs from both eyes can accelerate and enhance, but is not required for, loss of responsiveness to the deprived eye (Iurilli et al., 2012).

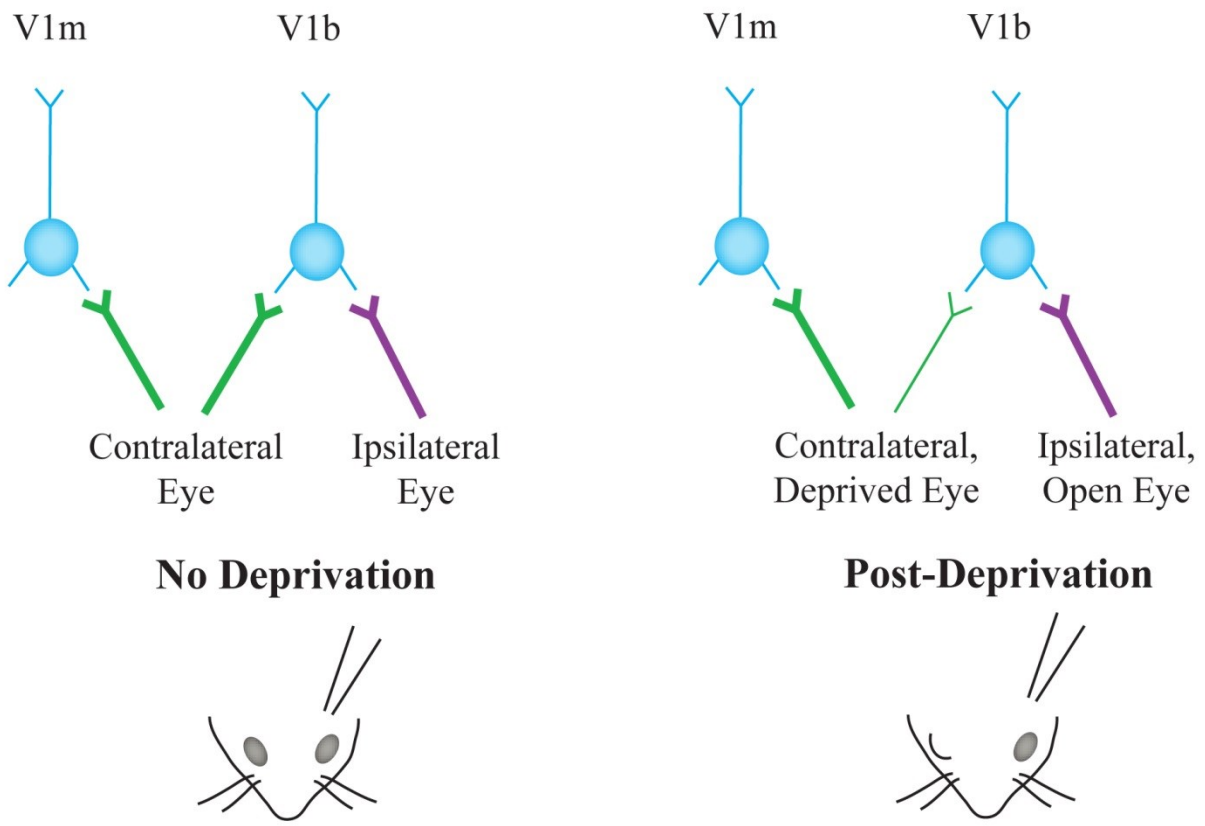


Figure 1.1. Competitive model of ocular dominance plasticity following lid suture. Schematics showing example neurons in V1m and V1b receiving inputs from the contralateral (green) and ipsilateral (plum) eyes. Schematics represent an un-manipulated V1 (left) and a V1 in the hemisphere contralateral to a deprived eye (right). According to the competitive model of ocular dominance plasticity: Following monocular deprivation by lid suture (right), responses to the deprived eye are reduced in V1b (thin green line), which also receives open eye input (plum line), while responses to the deprived eye remain unchanged in V1m (thick green line), which does not receive open eye input.

Depression and potentiation of inputs following monocular deprivation

Monocular deprivation led to a shift in the ratio of responsiveness to the closed eye versus the open eye (Wiesel and Hubel, 1963b). This ocular dominance plasticity could be expressed either by a weakening of drive to V1 neurons from the deprived eye, a strengthening of drive to V1 neurons from the open eye or a combination of these changes (**Figure 1.2**). Additionally, several loci of plasticity are possible: Weakening of thalamic drive, weakening of intracortical excitatory drive or an increase in intracortical inhibitory drive could lead to a decreased responsiveness to the deprived eye. Two sets of early studies suggested that the loss of responsiveness to the deprived eye following monocular lid suture was caused by a weakening of thalamic inputs to cortex from the deprived eye. Prolonged monocular lid suture led to the atrophy of some neurons in the lateral geniculate nucleus of the thalamus (LGN) driven by the deprived eye (Wiesel and Hubel, 1963a) and to the retraction of LGN afferents serving the region of cortex driven by the deprived eye. These changes were coupled with an expansion of LGN afferents responsive to the open eye (Antonini and Stryker, 1996; Antonini et al., 1999).

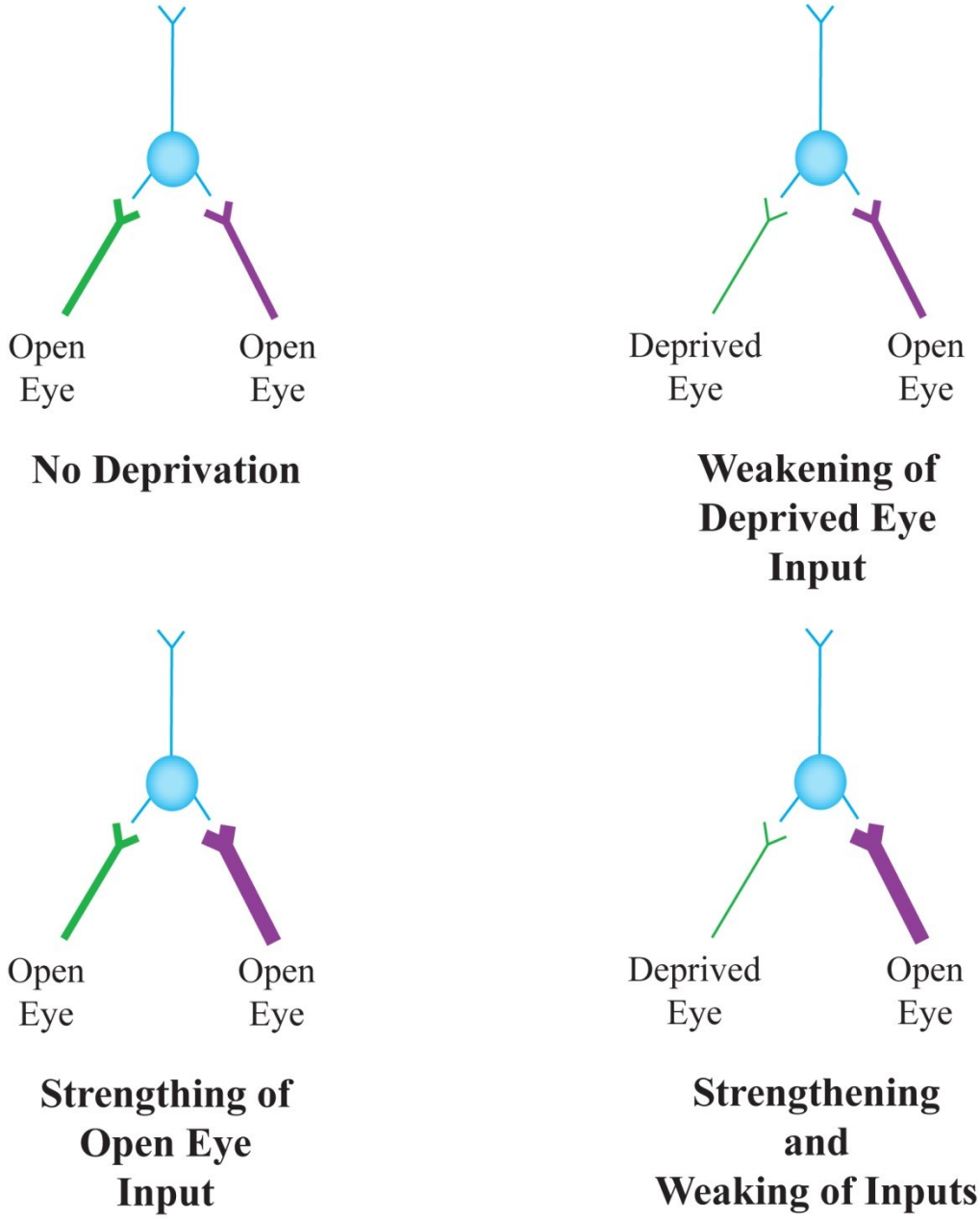


Figure 1.2. Possible mechanisms of ocular dominance plasticity. Schematics showing example neurons in V1b receiving inputs from both eyes. Following monocular deprivation by lid suture, ocular dominance plasticity could occur if inputs to cortical neurons from the deprived eye are weakened (top right), if inputs to cortical neurons from the open eye are strengthened or if deprived eye inputs are weakened and open eye inputs are strengthened.

To determine the time course of ocular dominance plasticity and differentiate between strengthening of open eye responses and weakening of deprived eye responses directly, a chronic recording system was developed. Local field potentials were recorded from chronically implanted electrodes in awake, behaving mice. When mice viewed high contrast sine wave gratings that abruptly reversed direction, a large deflection time locked to the direction change was observed in the local field potential recorded in V1b (Huang et al., 1999). These local field potential changes were called "visually evoked potentials." The ratio of the amplitudes of visually evoked potentials from stimuli presented separately to each eye was used to calculate an ocular dominance index (ODI). At the most binocularly responsive recording position in V1b, the ODI was approximately 2.4: 1 (contralateral: ipsilateral) in mice. Ocular dominance measured with visually evoked potentials was stable over time during the sensitive period and was similar to the ODI measured using single unit recordings (Hanover et al., 1999; Huang et al., 1999). The stability of individual visually evoked potential recordings over time was a critical technical development for allowing pre- and post-manipulation comparisons to be made not just between relative ratios of responses to each eye, but between responses to each eye alone.

The most seminal finding of these experiments was that monocular lid suture of juvenile mice led to a switch in ocular dominance through two sequential steps. First, shortly after lid suture, the amplitude of the visually evoked potential driven by the closed eye was reduced while the visually evoked potential driven by the open eye remained unchanged (Frenkel and Bear, 2004). Second, after more than three days, the amplitude of responses driven by the open eye was enhanced, while the deprived eye responses returned towards baseline but remained significantly depressed (Frenkel and Bear, 2004). This result was later confirmed using calcium dye imaging of large populations of superficial neurons (Mrsic-Flogel et al., 2007). Differently,

no significant changes were observed in either ocular dominance ratios or absolute responses following binocular lid suture or monocular lid suture if recordings were obtained in V1m (Frenkel and Bear, 2004; Smith et al., 2009). Together, these results were interpreted as providing evidence that ocular dominance plasticity during the sensitive period involves two sequential steps and strengthened the hypothesis that competition between inputs from an open and closed eye enhances plasticity (**Figure 1.3**; Frenkel and Bear, 2004; Smith et al., 2009).

An additional pair of experiments provided evidence for the independence of the changes observed during juvenile ocular dominance plasticity. Using chronic visually evoked potential recordings, it was found that ocular dominance plasticity could be induced in adult mice. Unlike in juveniles, however, adult ocular dominance plasticity was expressed solely through a potentiation of open eye inputs (Sawtell et al., 2003). Instead of lid suture, monocular visual deprivation can be performed using chronic intravitreal tetrodotoxin injections to block voltage gated sodium channels in the retina. Deprivation by retinal silencing also induced an ocular dominance shift; like adult ocular dominance plasticity, it was expressed only through potentiation of open eye responses (Frenkel and Bear, 2004). Therefore, the weakening of closed eye responses in visual cortex is restricted to a sensitive period and requires that one eye remains open while the other eye retains activity but only receives blurred input through the closed eyelid (Sawtell et al., 2003; Frenkel and Bear, 2004; Rittenhouse et al., 2006).

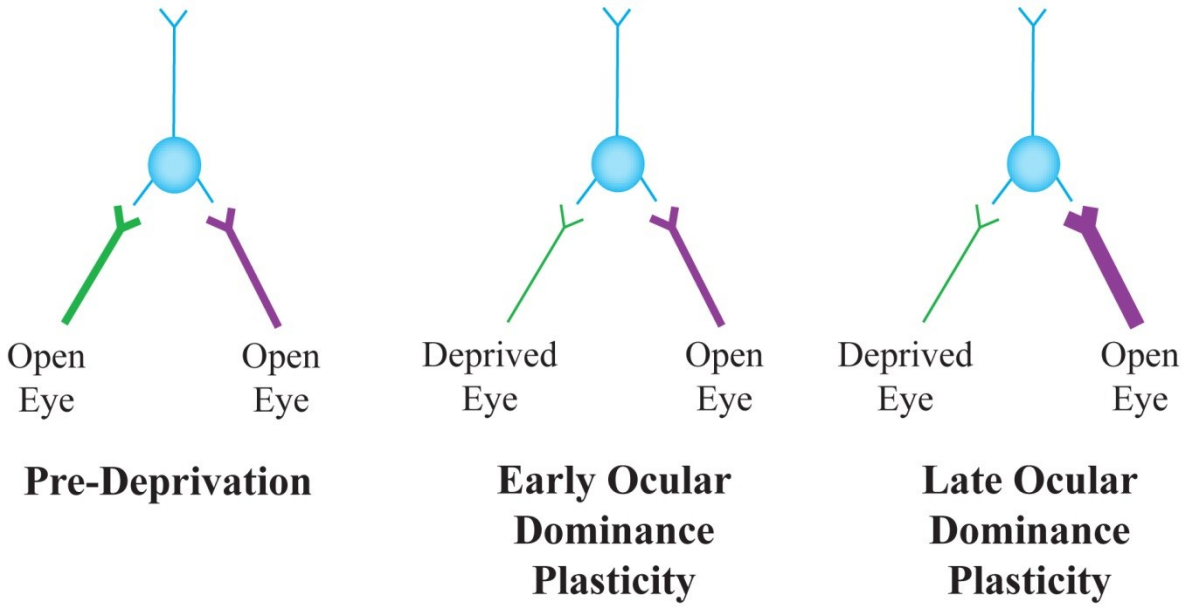


Figure 1.3. Sequential sensitive period ocular dominance plasticity in binocular visual cortex. Schematics showing example neurons in V1b receiving inputs from both eyes prior to monocular deprivation by lid suture (left), shortly after lid suture (~3 days, middle), and after a longer period of lid suture (~7 days, right). The early phase of juvenile ocular dominance plasticity is expressed through a reduction in input from the deprived eye (middle, thin green line). During the late phase of juvenile ocular dominance plasticity, there is a strengthening of open eye inputs (left, thick plum line). This figure is based on the theory of Smith and colleagues (Smith et al., 2009).

The observed rapid weakening of responses to deprived eye stimulation led to the formulation of the hypothesis that monocular deprivation induced long term depression at thalamocortical synapses and that this eye specific depression in synaptic strength resulted in the loss of deprived eye responses (Smith et al., 2009; Cooke and Bear, 2014). Eyelid suture was shown to decorrelate the firing of LGN neurons, potentially making it more difficult for them to drive action potentials in visual cortex (Linden et al., 2009). According to Hebbian rules of plasticity, if LGN afferents continue to fire but become less associated with post synaptic action potentials, thalamocortical synapses should undergo depression (Caporale and Dan, 2008; Smith et al., 2009). Several observations have confirmed that thalamocortical depression occurs following the loss of visual inputs. A direct demonstration of this phenomenon was made by selectively activating thalamocortical afferents and recording responses in L4 pyramidal neurons using photoactivation of LGN terminal fields (Wang et al., 2013b). This finding has been corroborated by a substantial body of work that showed monocular deprivation led to changes in the level of expression of receptors and genes associated with long term depression, that monocular deprivation occluded acute induction of long term depression and that manipulations that block the induction of certain forms of long term depression could prevent the induction of ocular dominance plasticity (Cooke and Bear, 2014).

To determine whether changes in the feedforward LGN input to cortex could fully account for the expression of the first stage of ocular dominance plasticity, weakening of responsiveness to the deprived eye, Khibnik and colleagues attempted to isolate thalamocortical inputs to V1 in an intact animal and record visual responses in V1 (Khibnik et al., 2010). Their approach was to apply a cocktail of $GABA_A$ and $GABA_B$ receptor agonists to hyperpolarize V1 neurons and block all action potential firing. After preventing action potential firing of V1

neurons, they were still able to record visually evoked potentials and assumed that these were carried solely by mostly unaltered inputs from the LGN (Khibnik et al., 2010). This assumption, however, is tenuous. The authors showed that application of their drug cocktail weakened white matter (putative LGN) stimulation induced monosynaptic inputs to V1 (Khibnik et al., 2010). Whether this weakening in non-deprived tissue was due to a selective effect on inputs driven by one eye was not tested. An additional factor complicating the interpretation of these experiments is that there is no evidence regarding whether the drug cocktail used might affect thalamocortical transmission directly. The ocular dominance ratio following monocular deprivation was observed to be similar before and after cortical silencing, and the authors concluded that weakening of feedforward inputs accounted for the entirety of the ocular dominance shift (Khibnik et al., 2010). In addition to possible changes to thalamocortical transmission induced by application of the drug cocktail, the authors failed to investigate whether cortical silencing might alter the activity of LGN neurons themselves or lead to rapid, selective plasticity at thalamocortical synapses. Therefore, the possibility exists that when V1 is active, the relative strength of ipsilateral and contralateral LGN afferents is different than when cortex is silenced pharmacologically.

Several observations have strongly challenged the hypothesis that long term depression at LGN to V1 synapses is the primary mechanism of early ocular dominance plasticity: 1) Ocular dominance plasticity was expressed later in cat L4, which receives the majority of direct LGN drive, compared to other cortical layers (Trachtenberg et al., 2000). 2) Silencing the contralateral visual cortex unmasked responses to the deprived eye, putatively via callosal projections (Restani et al., 2009). 3) Fast spiking inhibitory neurons in V1b initially shifted their ocular dominance to favor the deprived eye before eventually favoring the open eye (Yazaki-Sugiyama

et al., 2009). 4) Just one day after monocular deprivation visually evoked contralateral and ipsilateral eye responses of individual L2/3 neurons were potentiated simultaneously (Kuhlman et al., 2013). 5) During the first day of monocular lid suture, the firing rates of fast spiking neurons in V1m were reduced before returning to baseline on the second day of deprivation. On the second day of deprivation, the firing rates of regular spiking neurons were reduced and returned to baseline on day three (Hengen et al., 2013). Together, these results suggest that multiple mechanisms may act synergistically to alter visual responses after monocular deprivation while maintaining action potential firing within a dynamic range.

Additional consequences of sensitive period lid suture

Beyond ocular dominance plasticity, many complex changes in visual cortical function have been observed following lid suture during the sensitive period. After binocular lid suture, many cat V1 neurons remained visually responsive, but most had abnormal receptive field properties and only limited, superior colliculus-mediated visually guided behaviors remained intact (Wiesel and Hubel, 1965; Sherman, 1977; Watkins et al., 1978). Long term monocular deprivation led to a near complete loss of cells with complex receptive fields in cat V1m (Wilson and Sherman, 1977). In young rabbits, monocular lid suture rapidly altered the distribution of receptive field types among V1 neurons (Chow et al., 1971; Grobstein et al., 1973; Chow and Spear, 1974; Mathers et al., 1974; Grobstein et al., 1975; Murphy, 1985); paradoxically, as the duration of the deprivation increased, the number of non-visually responsive neurons decreased over a period of 14 months before increasing again after 20 months (Crabtree et al., 1981). Finally, monocular deprivation decreased the behaviorally measured visual acuity of the

deprived eye and increased the acuity of the open eye (Iny et al., 2006). Thus, it has long been known that lid suture causes changes in visual response properties that go beyond the magnitude of peak responses and ocular dominance (Wilson and Sherman, 1977).

Circuit Level Plasticity following Monocular Deprivation

Changes in visual response properties following monocular deprivation are thought to be driven by changes in the brain circuits responsible for these visual processes. Several studies have directly uncovered plasticity in neural circuits that is induced by monocular lid suture; however, there is still considerable debate as to how visual responses are derived within neural circuits (Griffen and Maffei, 2014). Therefore, it is difficult to infer the effects these cellular and synaptic changes on visual processing.

Within visual cortex, plasticity of both glutamatergic and GABAergic neurons and synapses following juvenile lid suture has been observed. Within L4, brief monocular lid suture started before eye opening decreased drive from fast spiking neurons onto pyramidal neurons in V1m (Maffei et al., 2004), whereas lid suture of the same duration started during the sensitive period potentiated fast spiking to pyramidal neuron synapses in L4, both pre- and postsynaptically (Maffei et al., 2006; Nahmani and Turrigiano, 2014). A similar transition occurred around the start of the sensitive period in V1b. Early in development, a brief period of lid suture weakened spontaneous inhibitory drive onto pyramidal neurons; however, the same manipulation strengthened inhibitory drive during the peak of the sensitive period (Maffei et al., 2010). Changes at recurrent excitatory synapses within L4 have only been observed prior to the sensitive period in V1m, when they were potentiated by monocular deprivation (Maffei et al., 2004; Maffei et al., 2006; Maffei et al., 2010).

Induction of long term potentiation at L4 fast spiking to pyramidal neuron synapses, either through lid suture or acutely, switched the sign of plasticity induced at pyramidal neuron recurrent glutamatergic synapses from long term potentiation to long term depression (Wang et

al., 2012; Wang and Maffei, 2014). Acute depotentiation of the fast spiking to pyramidal neuron synapses after lid suture restored the capacity for long term potentiation of glutamatergic recurrent inputs, demonstrating that the control of inhibitory plasticity over the capacity for plasticity at excitatory synapses can be modulated by experience (Wang and Maffei, 2014). While synapse specific, experience-dependent inhibitory control over excitatory plasticity has only been shown to exist at one locus within one cortical microcircuit, this finding raises the intriguing possibility that plasticity at inhibitory synapses may control the expression of other forms of plasticity throughout cortex.

Differently from L4, circuit changes in L2/3 and L5 were shown to involve plasticity of the intrinsic firing properties of pyramidal neurons. Early in the sensitive period, brief monocular lid suture reduced the intrinsic excitability of V1m L5 pyramidal neurons and increased the excitability of V1m L2/3 pyramidal neurons (Maffei and Turrigiano, 2008b; Nataraj et al., 2010). Increased intrinsic excitability was also seen in V1b with longer periods of deprivation (Lambo and Turrigiano, 2013). In L2/3 of V1m and V1b, lid suture also modulated excitatory drive onto pyramidal neurons in a biphasic manner, with brief deprivation weakening excitatory drive and longer deprivation enhancing excitatory drive (Maffei and Turrigiano, 2008b; Lambo and Turrigiano, 2013). While brief monocular deprivation did not alter inhibitory drive onto L2/3 excitatory neurons, interlaminar excitatory drive onto L2/3 parvalbumin positive inhibitory neurons was reduced after only one day (Maffei and Turrigiano, 2008b; Kuhlman et al., 2013).

To examine how these complex changes in circuit connectivity might alter the activation of V1m, Wang and colleagues used voltage sensitive dye (VSD) imaging to measure the spread of activity following extracellular stimulation of L4 or L2/3 (Wang et al., 2011). They found that

following brief monocular deprivation circuit activation in response to stimulation of L4 was substantially reduced, while direct activation of L2/3 remained unchanged (Wang et al., 2011).

Circuit level plasticity has also been observed at non-cortical locations in the visual circuit following monocular lid suture. In the LGN, visual deprivation lasting less than one week increased the frequency and amplitude of miniature excitatory postsynaptic potentials from corticothalamic afferents onto neurons serving the deprived eye (Krahe and Guido, 2011). Prolonged lid suture led to the atrophy of some neurons in the LGN and prevented developmental synaptogenesis from occurring in the superior colliculus (Wiesel and Hubel, 1963a; Lund and Lund, 1972). Very little is known about how corticothalamic pathways or tectothalamocortical pathways might modulate responses in rodent V1.

With the possible exception of examining the contribution of thalamic inputs to ocular dominance plasticity, no experiments have succeeded in directly tying a specific change in circuitry to visual responses following monocular deprivation. Perhaps the closest connection between circuit plasticity and a change in visual responses was observed by Kuhlman and colleagues. Following one day of monocular deprivation, intracortical excitatory inputs to V1b L2/3 parvalbumin positive cells were weakened and their visually evoked firing rates were reduced. In turn, visually evoked excitatory neuron responses were enhanced (Kuhlman et al., 2013). While these changes were all internally consistent with a reduction in inhibition leading to enhanced excitation, whether the visually driven inputs to L2/3 parvalbumin positive neurons were also reduced was not tested. Further, the specific mechanism driving increased visual responses of L2/3 pyramidal neurons was assumed to be disinhibition, but this hypothesis was not directly tested. Therefore, considerable work remains to determine how changes in cortical circuits may alter visual function.

Possible Roles for GABAergic Neurons in Ocular Dominance Plasticity

While evidence for depression of thalamic inputs (Wang et al., 2013b) playing a role in ocular dominance plasticity is strong (Cooke and Bear, 2014), the function of changes at intracortical excitatory and inhibitory synapses is less clear. Thus far, no study has elucidated a role for plasticity of intracortical excitatory connections in monocular lid suture induced changes in visual responses. The possible roles of plasticity at inhibitory synapses, however, have been hotly debated (Smith and Bear, 2010; Griffen and Maffei, 2014).

Brief unilateral lid suture was shown to strongly modulate the binocularity of fast spiking inhibitory neurons. While fast spiking inhibitory neurons are normally more binocular than pyramidal neurons (Swadlow, 1988; Yazaki-Sugiyama et al., 2009; Aton et al., 2013), soon after the onset of monocular lid suture or eye patching, L2/3 fast spiking neurons became strongly driven by the closed eye (Yazaki-Sugiyama et al., 2009; Aton et al., 2013; but see Kuhlman et al., 2013), although their spontaneous firing frequency was shown to be reduced (Yazaki-Sugiyama et al., 2009; Aton et al., 2013; Hengen et al., 2013). This increase in fast spiking neuron responsiveness to the deprived eye could contribute to reducing excitatory neuron responsiveness to that eye (Yazaki-Sugiyama et al., 2009). Interestingly, if the monocular deprivation was maintained for several days, the changes in the ocular dominance of L2/3 fast spiking neurons (Yazaki-Sugiyama et al., 2009; Aton et al., 2013; Kuhlman et al., 2011) and their spontaneous firing frequency were reversed (Aton et al., 2013; Hengen et al., 2013; but see Yazaki-Sugiyama et al., 2009).

In L4 pyramidal neurons of mice, brief monocular lid suture similarly decreased both peak evoked excitatory and inhibitory conductances, and longer deprivation selectively reduced

inhibition driven by the open eye (Ma et al., 2013). Differently, in rats longer monocular lid suture reduced excitation and inhibition driven by the deprived eye equally (Iurilli et al., 2013). Together, these results suggest that other mechanisms, such as changes in thalamocortical drive (Shatz and Stryker, 1978; LeVay et al., 1980; Khibnik et al., 2010; Wang et al., 2013b) may cooperate with altered intracortical inhibition to mediate the functional effects of sensory deprivation.

At first glance, studies showing that monocular lid suture does not affect the ratio of excitatory and inhibitory inputs to L4 pyramidal neurons (Iurilli et al., 2013; Ma et al., 2013) appear contradictory to the hypothesis that potentiation of inhibition directly suppresses visual responses (Maffei et al., 2006). However, many questions still exist. Visual stimuli used in these experiments were either moving bars or drifting gratings presented over seconds or noise stimuli presented for several hundred milliseconds and were presented to one eye at a time. Additionally, only responses to stimuli presented at peak orientation were examined (Iurilli et al., 2013; Ma et al., 2013). These stimuli may not be ideal for investigating the contribution of inhibitory neurons to visual responses: Fast spiking neurons in visual cortex have response properties that make them ideally suited to modulate extremely rapid visual responses to stimuli presented binocularly (Swadlow and Weyand, 1987; Swadlow, 1988; Yazaki-Sugiyama et al., 2009; Kameyama et al., 2010; Cardin, 2012; Aton et al., 2013; Zhuang et al., 2013). The broad tuning of fast spiking neurons raises the possibility that they could contribute to changes in orientation tuning or response suppression following lid suture. Alternatively, changes in patterns of fast spiking neuron activity and synaptic strength could gate activity at other neurons' synapses (Levelt and Hubener, 2012; Wang and Maffei, 2014). Finally, recordings of somatic inhibitory conductances are unable to resolve differential contributions from interneuron subgroups: Changes in input

from one interneuron group may be offset by opposite changes in input from other groups. A recent study examining changes in excitatory and inhibitory conductances following whisker removal underscored the difficulty in interpreting results using this technique (Li et al., 2014). Short and long periods of whisker trimming reduced evoked excitatory and inhibitory drive, and both decreased inhibition more than excitation. Interestingly, brief whisker trimming increased evoked spiking responses, while longer removal decreased responses (Li et al., 2014). Thus, additional unidentified mechanisms must have contributed to this change in responsiveness.

A recent, particularly exciting study shed light on a possible role of inhibitory plasticity in modulating visual responses using surgical strabismus to modulate visual experience (Scholl et al., 2013). Like lid suture, long term strabismus has been shown to disrupt ocular dominance (Hubel and Wiesel, 1965). Strabismus has also been shown to lead to binocular suppression, a condition in which monocular stimuli elicit stronger responses than binocular stimuli (Sengpiel and Blakemore, 1994; Sengpiel et al., 1994). Scholl and colleagues demonstrated that strabismus induced binocular suppression through a selective increase in inhibitory drive during binocular stimulation (Scholl et al., 2013). This result suggests that changes in cortical inhibition induced by sensory experience may alter sensory processing in more subtle and complex ways than simply repressing unilateral deprived eye responses to optimal stimuli.

Cortical Circuit Development during the Sensitive Period

The expression of ocular dominance plasticity through a reduction in deprived eye responsiveness is restricted to a sensitive period in development. During this time period, visual response properties mature rapidly under normal rearing conditions (**Figure 1.4**; Espinosa and Stryker, 2012). Extensive developmental changes in inhibitory neuron function occur during the sensitive period and are thought to regulate the capacity for ocular dominance plasticity (Hensch, 2004; Levelt and Hubener, 2012). Developmental changes in circuit function likely contribute to both the refinement of visual response properties and the capacity to undergo plasticity.

In rodent V1, parvalbumin positive fast spiking neurons showed a progressive decrease in membrane time constant, cell capacitance and action potential width and an increase in intrinsic excitability during the first two weeks of postnatal development after eye opening (Goldberg et al., 2011; Lazarus and Huang, 2011). Conversely, somatostatin positive neurons showed a progressive developmental increase in membrane time constant, input resistance and spike frequency adaptation (Lazarus and Huang, 2011). The properties of inhibitory synapses also changed during this developmental time window (**Figure 1.4**; Morales et al., 2002; Heinen et al., 2004; Maffei and Turrigiano, 2008a; Pinto et al., 2010). During the postnatal weeks following eye opening, progressive changes in the proportions of subunits of the $GABA_A$ receptor were reported (Heinen et al., 2004). Changes in receptor subunit composition underlie changes in conductance, clustering and susceptibility to allosteric modulators, all factors that play fundamental roles in determining the amplitude and time course of postsynaptic events (Bosman et al., 2002; Heinen et al., 2004; Mohler, 2006; Eyre et al., 2012). In mature brains $GABA_A$ receptors containing specific α subunits were shown to have preferential locations in specific subcellular compartments (Nusser et al., 1996; Klausberger et al., 2002; Wisden et al., 2002;

Chandra et al., 2006; Ali and Thomson, 2008; Wu et al., 2012). Whether $GABA_A$ receptor subunits are similarly localized during development is not known; however, the observed changes in levels of expression suggest that changes in the subcellular targeting of GABAergic synapses may occur.

Developmental changes in inhibitory circuit function go beyond subunit composition, strength and kinetic properties. Inhibitory drive tends to increase during postnatal development (**Figure 1.4**; Blue and Parnavelas, 1983; Morales et al., 2002; Chattopadhyaya et al., 2004; Maffei and Turrigiano, 2008a; Kuhlman et al., 2011; Li et al., 2012; Kuhlman et al., 2013). Changes in the strength of inhibitory inputs have been shown to decrease the ability of excitatory neurons to fire action potentials (Saraga et al., 2008; Pouille et al., 2013). Thus, developmental regulation of inhibitory synaptic strength may have significant effects on the activity of excitatory neurons. Precise patterns of neural activity are required for the induction of different forms of plasticity; therefore, changes in circuit activity may alter how stimuli drive changes at synapses. Patterns of activity or sensory experiences that facilitate the induction of long term potentiation or depression at inhibitory synapses could gate the induction of specific forms of plasticity at glutamatergic synapses in cortical circuits (Levelt and Hubener, 2012). Gating of excitatory plasticity could occur by altering activity patterns of glutamatergic neurons (Aton et al., 2013), by activating neuromodulatory signals (Huang et al., 2013) and/or through the activation of intracellular signaling cascades (Hayama et al., 2013; Wang and Maffei, 2014).

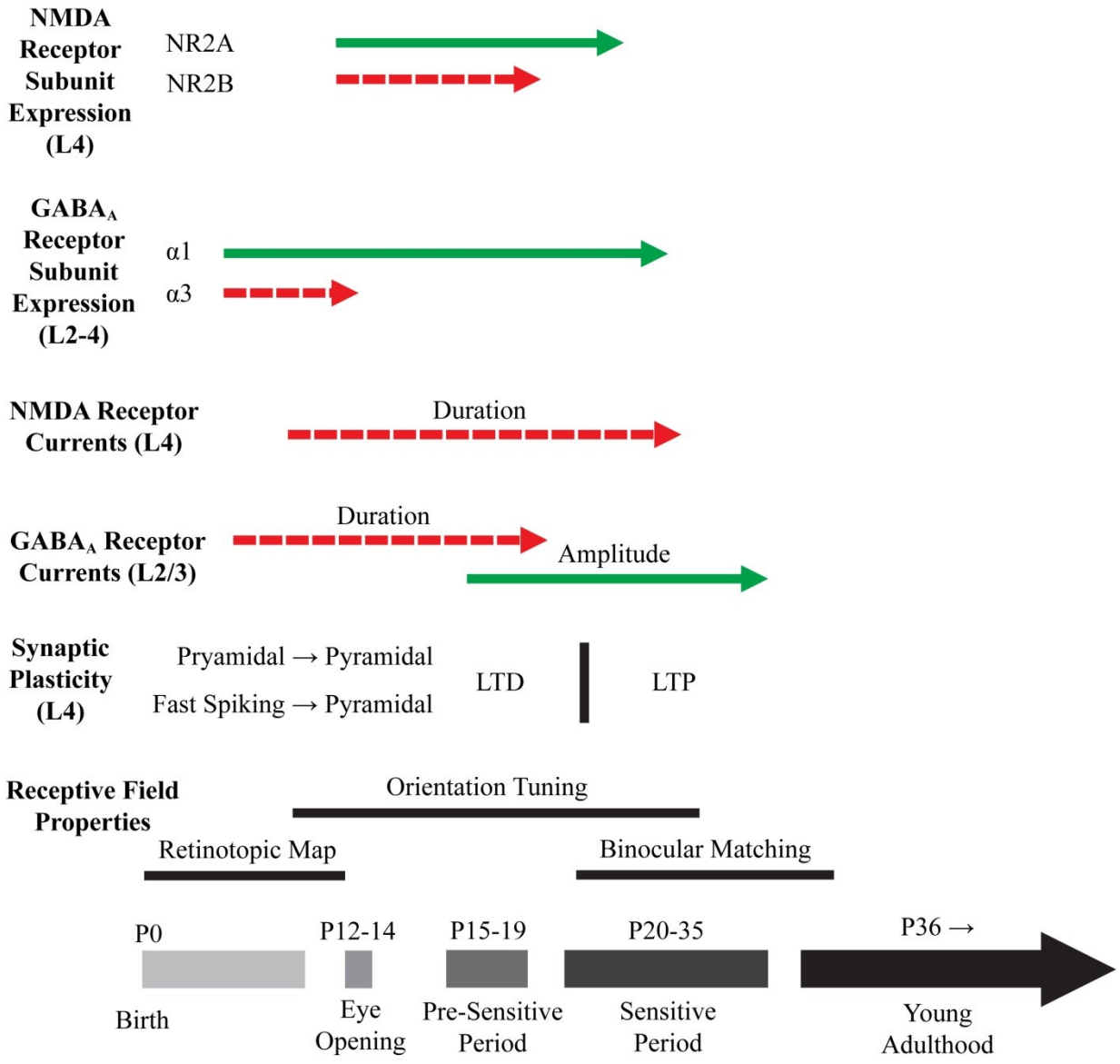


Figure 1.4. Selected developmental changes in primary visual cortex function. (Bottom) Timeline of early postnatal development. (Top) Selected properties of V1 neurons that change during early postnatal development. Horizontal black bars indicate the time periods in development when specific receptive field properties mature. The vertical black bar delineates a developmental switch in the sign of plasticity induced by an induction protocol. Arrows indicate the time period during which there is a developmental increase (green) or decrease (red dash) in a specific property. L: Layer. LTD: Long term depression. LTP: Long term potentiation. P: Postnatal day. This figure is based on Carmignoto and Vicini, 1992, Nase et al., 1999, Morales et

al., 2002, Heinen et al., 2004, Espinosa and Stryker, 2012, Wang et al., 2012 and Lefort et al., 2013.

The capacity for plasticity at inhibitory synapses in V1 changes sharply during postnatal development at a time point corresponding to the transition between the pre-sensitive and the sensitive periods for visual cortical plasticity (**Figure 1.4**; Lefort et al., 2013). In response to the same pattern of activity, monosynaptic connections from fast spiking neurons onto L4 pyramidal neurons showed long term depression in the pre-sensitive period and long term potentiation in the sensitive period (Lefort et al., 2013). This shift in capacity for plasticity paralleled that reported for L4 recurrent excitatory connections (**Figure 1.4**; Wang et al., 2012). Surprisingly, the switch in sign of plasticity at excitatory synapses was reversed by inducing inhibitory plasticity in a connection specific fashion, indicating that inhibitory inputs may contribute to selective rewiring of local circuits despite their widespread connectivity (Wang and Maffei, 2014). This interaction between inhibitory and excitatory plasticity is mediated by G protein signaling; therefore, it may affect the pyramidal neuron long after its induction (Wang and Maffei, 2014). Such signaling crosstalk between mechanisms for excitatory and inhibitory plasticity could provide a memory trace that promotes experience-dependent rewiring of local microcircuits with a high degree of specificity and contributes to developmental circuit refinement.

The maturation of inhibitory synapses has been proposed to be a critical step for the onset of and a modulator of the duration of sensitive periods (Hensch et al., 1998). Manipulations of inhibition *in vivo* during early postnatal development shifted the onset of the sensitive period for visual cortical plasticity in V1. In GAD65 knockout mice, which were shown to have low levels of GABA throughout life, the sensitive period never opened (Fagiolini and Hensch, 2000). Increasing intracortical inhibitory drive with benzodiazepines or zolpidem in early development,

or at any time in GAD65 knockouts, led to the onset of a window for rapid ocular dominance plasticity (Fagiolini and Hensch, 2000; Fagiolini et al., 2004; Wang et al., 2013a; Chen et al., 2014). This effect was mediated by $GABA_A$ receptors containing α1 subunits, which are preferentially located at fast spiking to pyramidal neuron somatic synapses (Fagiolini and Hensch, 2000; Klausberger et al., 2002; Iwai et al., 2003; Fagiolini et al., 2004; Katagiri et al., 2007). Either overexpressing BDNF or removing polysialic acid accelerated the development of inhibitory synapses and caused a precocious sensitive period (Hanover et al., 1999; Huang et al., 1999; Di Cristo et al., 2007).

Similarly, sensory deprivation by dark rearing prolonged the onset of sensitivity to ocular dominance plasticity until the animal was introduced to light (Cynader and Mitchell, 1980; Mower and Christen, 1985; Mower, 1991; Fagiolini et al., 1994). Dark rearing from shortly after birth delayed the formation of mature innervation onto excitatory neurons; whereas dark rearing started after eye opening did not affect inhibitory drive (Morales et al., 2002; Maffei et al., 2010). Dark rearing also decreased the level of expression of GAD65 in L2/3 inhibitory neurons (Kreczko et al., 2009) and prevented postnatal increases in inhibitory post synaptic current amplitude onto pyramidal neurons from occurring, consistent with a delay in GABAergic input maturation (Morales et al., 2002).

Conversely, pharmacologically decreasing inhibition in adult animals reopened a window for ocular dominance plasticity (Harauzov et al., 2010; Maya-Vetencourt et al., 2012). This was accomplished by reducing cortical GABA with GAD inhibitors, with the peptide hormone IGF-1, or by blocking $GABA_A$ receptors (Harauzov et al., 2010; Maya-Vetencourt et al., 2012). Environmental enrichment, which also decreased intracortical inhibition, could restore the capacity for ocular dominance plasticity in adult rodents in an IGF-1 dependent manner (Sale et

al., 2007; Baroncelli et al., 2010; Maya-Vetencourt et al., 2012). Direct evidence that the age of inhibitory neurons, rather than overall inhibitory tone, plays a major role in sensitive period regulation came from experiments in which implants of GABAergic neural progenitor cells from embryonic mice were made into V1 of older mice. These implants later opened a window for ocular dominance plasticity that matched the age of the implanted neurons (Southwell et al., 2010). Substantial evidence exists that the maturation of inhibition regulates the timing of sensitive periods: Uniformly, manipulations that prevent that maturation of inhibition prevent the onset of sensitive periods, while those that enhance inhibition early in development cause a precocious sensitive period. Once inhibition has matured, reducing inhibition acutely can restore the capacity for ocular dominance plasticity.

During the developmental window in which inhibitory synapses mature, there are also substantial changes in glutamatergic receptor subunit composition, subcellular localization and function (**Figure 1.4**; Carmignoto and Vicini, 1992; Nase et al., 1999; Hensch, 2004; Corlew et al., 2007; Yashiro and Philpot, 2008), voltage signal propagation through the cortical circuit (Barkat et al., 2011) and receptive field properties (Fagiolini et al., 1994; Katz and Shatz, 1996; Huang et al., 1999; Zhang et al., 2001; Inan and Crair, 2007; Wang et al., 2010; Espinosa and Stryker, 2012; Chen et al., 2014; Li et al., 2014). Whether and how GABAergic neurotransmission contributes to these developmental processes is not fully understood. Evidence is beginning to emerge that changes in the receptive fields of the inhibitory drive onto excitatory neurons may at least partially underlie developmental receptive field sharpening (Dorrn et al., 2010; Sun et al., 2010; Li et al., 2012). However, the underpinning synaptic and circuit mechanisms of this process remain elusive.

The sensitive period for ocular dominance plasticity is a time of substantial changes within the visual cortical circuit affecting both glutamatergic and GABAergic neurons (**Figure 1.4**). The heightened capacity for plasticity during this window allows the visual cortex to develop mature visual processing in an experience-dependent fashion. This heightened capacity for plasticity and experience-dependent adaptation leaves the cortical circuit vulnerable to pathological perturbations in visual drive. Responses to perturbations in visual drive can have both positive and negative adaptive consequences: The loss of responsiveness to a deprived organ may allow reallocation of the cortical circuitry responsive to that organ to respond to a non-deprived organ; however, this repurposing of cortical circuitry comes at the expense of responsiveness to the deprived organ should normal sensory input be restored.

Chapter II

Developmental Regulation of Spatio-temporal Patterns of Cortical Circuit Activation

Preface

The following study investigated changes in cortical circuit activation that occur during normal sensory development. These experiments were performed simultaneously with those reported by Wang and colleagues to provide a template to contrast changes in cortical circuit activation that occur during normal development with those that occur during pathological development (Wang et al., 2011).

Abstract

Neural circuits are refined in an experience-dependent manner during early postnatal development. How development modulates the spatio-temporal propagation of activity through cortical circuits is poorly understood. Here we used VSD imaging to show that there are significant changes in the spatio-temporal patterns of intracortical signals in primary visual cortex from postnatal day 13 (P13), eye opening, to P28, the peak of the sensitive period for rodent visual cortical plasticity. Upon direct stimulation of L4, activity spread to L2/3 and to L5 at all ages. However, while from eye opening to the peak of the sensitive period, the amplitude and persistence of the voltage signal decreased, the peak of activation was reached more quickly and the interlaminar gain increased with age. The lateral spread of activation within layers remained unchanged throughout the time window under analysis. These developmental changes in spatio-temporal patterns of intracortical circuit activation were mediated by differences in the contributions of excitatory and inhibitory synaptic components. Our results demonstrate that after eye opening the circuit in primary visual cortex is refined through a progression of changes that shape the spatio-temporal patterns of circuit activation. Signals became more efficiently propagated across layers through developmentally regulated changes in interlaminar gain.

Introduction

During postnatal development, neural circuits in sensory cortices are extensively refined in an experience-dependent fashion (Carmignoto and Vicini, 1992; Katz and Shatz, 1996; Finnerty et al., 1999; Morales et al., 2002; Hensch, 2004; Espinosa and Stryker, 2012). The onset of sensory experience is known to promote the maturation of sensory circuits. In rodent V1, the time between eye opening and the fourth postnatal week has been characterized by changes in receptor expression (Carmignoto and Vicini, 1992; Nase et al., 1999; Corlew et al., 2007; Yashiro and Philpot, 2008), gene expression (Lyckman et al., 2008), neurotransmitter release (Morales et al., 2002), connectivity (Blue and Parnavelas, 1983), mechanisms for plasticity (Ramoa and Sur, 1996; Huang et al., 1999; Rozas et al., 2001; Wang et al., 2012; Lefort et al., 2013) and cell intrinsic properties (Etherington and Williams, 2011; Lazarus and Huang, 2011; Wang et al., 2012). The developmental regulation of synaptic and intrinsic properties in different cell types has been shown to contribute to establishing mature connectivity and is thought to be the underpinning mechanism for the acquisition of mature visual processing (Hensch, 2004; Li et al., 2012). Specifically, the acquisition of a balance between excitation and inhibition is considered crucial to healthy circuit wiring (Hensch and Fagiolini, 2005). Shifts in the excitatory/inhibitory balance may affect not only the excitability of single neurons but also how stimuli are propagated in the cortical circuit (Rozas et al., 2001; Wang et al., 2011).

Previous findings from a number of cortical regions have shown that synaptic components that, in V1, are strongly affected by early postnatal experience, such as synaptic transmission mediated by GABA and NMDA receptors, could significantly affect the spatio-temporal patterns of signal propagation (Laaris et al., 2000; Sato et al., 2008). These studies suggest the importance of excitatory and inhibitory synaptic transmission for the spread of circuit

activation in neocortex, but did not assess whether these patterns of activation were modulated during development. In A1, passive tone rearing was shown to modify tonotopic maps of cortical activation in an experience-dependent fashion (Barkat et al., 2011). In addition, during postnatal development the dynamics of V1 responsiveness to repeated white matter stimulation were shown to be altered, and the experience-dependent maturation of cortical inhibition was thought to underlie this change (Rozas et al., 2001).Consistent with these findings, it has been shown that in V1 the spatio-temporal patterns of circuit activation are extensively modulated by changes in visual drive during the sensitive period and depend on changes in the cortical level of inhibition (Wang et al., 2011). These data suggest that changes in synaptic properties during development may contribute to sculpting the patterns of activation of the circuit. How spatio-temporal patterns of visual cortical activation change during postnatal development under conditions of normal rearing is unknown.

Here we used VSD imaging in acute rat brain slices containing V1 to determine how the spatio-temporal propagation of activity within and between cortical layers was modulated during early postnatal development. VSD imaging provided high spatial and temporal resolution of cortical circuit activity and allowed us to quantify patterns of intracortical circuit activation in response to electrical stimulation (Laaris et al., 2000; Tominaga et al., 2000; Petersen and Sakmann, 2001; Sato et al., 2008; Barkat et al., 2011; Wang et al., 2011; Wester and Contreras, 2012). To bypass thalamocortical projections, which undergo significant postnatal refinement (Kato et al., 1983; Carmignoto and Vicini, 1992; Antonini and Stryker, 1993) and measure the spatio-temporal patterns of intracortical signal propagation, we directly stimulated L4 and measured the voltage changes in V1. We found that the spatio-temporal propagation of stimuli was significantly affected by the maturation of the circuit. During the time window between eye

opening and the peak of the sensitive period for visual cortical plasticity (P13 to P28) the activation of V1 was characterized by a progressive decrease in the peak and duration of the optical voltage signal that was mediated by changes in synaptic transmission. As the circuit in V1 matured, the signal leaving L4 became amplified upon reaching L2/3, suggesting an increase in the gain of the vertical signal propagation. Our results indicate that the maturation of healthy cortical circuits occurs through a series of events leading to temporally and spatially refined propagation of signals across layers. These developmentally regulated changes in circuit activation likely contribute to a progressive acquisition of mature visual processing (Fagiolini et al., 1994; Huang et al., 1999; Prevost et al., 2010; Li et al., 2012).

Materials and methods

Animals and acute slice preparation

All experimental procedures were approved by the Stony Brook University Animal Use Committee and followed National Institute of Health guidelines. Long Evans rats obtained from Charles River aged P13 – P28 were used for recordings and were grouped as follows: P14 ± 2, P20 ± 1, and P27 ±1. Acute slices containing V1 were prepared (Maffei et al., 2004; Wang et al., 2011). Slices were mounted on Omnipore filters and placed in a container filled with artificial cerebrospinal fluid (ACSF) continually perfused with a gas mixture of 95% CO_2 and 5% O_2 to maintain oxygenation and humidity (Tominaga et al., 2000; Wang et al., 2011). Slices were maintained at 37° C for approximately 20 min and then at room temperature.

Voltage sensitive dye staining and imaging

VSD staining and imaging were performed as previously described (Tominaga et al., 2000; Wang et al., 2011). Di-4-ANEPPS (Invitrogen; absorption: 496 nm, emission: 705 nm) dissolved in a 2:1 mixture of ethanol and 10% Cremophor-EL solution (v/v in ddH_2O, Sigma) was prepared as stock solution (final concentration of 3.3 mg/ml) and stored at 4° C for no longer than 3 months. On the day of recording, a small volume of the stock solution was dissolved in a 1:1 mixture of fetal bovine serum (Sigma) and oxygenated ACSF (final di-4-ANEPPS concentration: 0.2 mM). Prior to recording, the slice was covered with 100 µl of VSD solution and allowed to incubate for 40 min at room temperature. The excess dye was washed off and the slice was placed in a 1 ml recording chamber mounted on an upright microscope (Olympus, BX51WI) and held in place with a flattened platinum ring.

For recording, a constant flow of oxygenated, 35° C ACSF was perfused into the chamber at a rate of 1.5 ml/min and the slice was allowed to sit for 10 min to wash out excess dye prior to the start of recording (Wang et al., 2011). A halogen lamp (150 W, TH 4-100, Olympus) with an electronically controlled shutter (Smart Shutter with Lambda 10B controller, Sutter Instruments) was used to activate the dye and detect voltage signals. The excitation light was first passed through an excitation filter (λ 530 ± 10 nm), then projected onto a dichroic mirror (λ = 565 nm) and finally projected through the objective lens to illuminate the slice. The fluorescent signal generated by the tissue was passed through an absorption filter (λ = 590 nm) to a CCD camera connected to a PC via an I/O interface (MiCAM 02, SciMedia, Brainvision). A high numerical aperture 4× objective (NA 0.28, Olympus), reduced with a 0.5× lens on the c-mount of the CCD camera, was used to visually identify the region for imaging. The image resolution was 60 × 88 active pixels, with single pixel size of 20 μm, for a total imaged area of 1.2 mm × 1.8 mm. For each stimulus, 256 frames were acquired at 400 Hz, for a total of 640 ms. Stimuli were repeated 16 times with 15 s intervals, and the signal was averaged across the 16 repetitions. A peristaltic pump (Watson-Marlow Sci 400) was used to maintain a constant volume of ACSF in the recording chamber and prevent changes in the depth of focus. The shutter controller and the stimulus isolation unit were driven by the I/O interface of the CCD camera and the duration of the stimuli was controlled though the MiCam data acquisition software (Brainvision).

Extracellular stimulation was delivered with a tungsten unipolar electrode covered with a glass pipette (0.1 MΩ, Harvard Apparatus) and inserted below the surface of the slice to allow for stable recordings. The electrode was positioned at the center of the region identified as V1m in the lower portion of L4, as visually identified by laminar distinctions. The reference electrode

was in the bath. 0.2 ms unimodal pulses were delivered at a stimulation intensity of 50 μA, which allowed for comparison of VSD signals between treatment groups (Wang et al., 2011).

Analysis and statistics

Analysis of the VSD signal was performed with procedures developed in Image J, SigmaPlot (Systat Software, Inc.) and Igor Pro (WaveMetrics). To allow for comparisons between different conditions, voltage signals were measured as the transition in fluorescence from the baseline (ΔF/F), and signals were normalized to the initial background measured over the 25 ms immediately preceding each stimulus. VSD signals measured as ΔF/F have been shown to correlate with voltage changes measured intracellularly and with local field potentials (Laaris et al., 2000; Tominaga et al., 2000; Petersen and Sakmann, 2001; Wang et al., 2011).

Interlaminar spread of the VSD signal was analyzed with line scans 3 pixels wide (60 μm) from the pial surface to a depth of 1000 μm (50 pixels) positioned next to the stimulating electrode and perpendicular to the pia (**Figure 2.1A**). Regions of interest (ROIs) 2 x 2 pixels (40 μm x 40 μm) were selected to analyze the time course of activation in L4, L2/3 and L5 over 50 ms as follows: The L2/3 ROI was placed at the point of maximal activation in L2/3. The L4 ROI was placed as close to the stimulating electrode as possible while avoiding the stimulation artifact from the electrode. The L5 ROI was placed 600 μm below the pial surface (**Figure 2.1A**). All three ROIs were aligned vertically, perpendicular to the pial surface. Threshold for signal detection was set at ±2 standard deviations from the baseline. Signals not reaching threshold were set to 0 ΔF/F for analysis.

Horizontal spread of the VSD optical signal was analyzed by fitting line scans 60 μm wide through L2/3, at the peak of horizontal spread, and L4, above the stimulating electrode (**Figure 2.1A**), with the following one-dimensional Gaussian equation using Igor Pro (WaveMetrics):

$$Y(x) = a \exp -[(x - b)^2 / c^2]$$

where a is the amplitude of the curve, b is the offset of the peak and c is the width (Wang et al., 2011).

Data are presented as mean ± the standard error of the mean (SEM). n = 13 slices from 4 rats for the P14 group, n = 10 slices from 3 rats for the P20 group, except for the drug application experiments, where n = 9, and n = 11 slices from 3 rats for the P27 group. Statistical analysis was performed using SigmaPlot (Systat Software, Inc.). To determine significance, the Kruskal-Wallis one-way ANOVA on Ranks was performed, and if significant, was followed by Dunn's Test. P values < 0.05 were considered significant.

Solutions and drugs

ACSF contained (mM): NaCl 126, KCl 3, $MgSO_4$ 2, $NaHPO_4$ 1, $NaHCO_3$ 25, $CaCl_2$ 2 and dextrose 25. The pH was adjusted to 7.2 by bubbling with a gas mixture of 95% CO_2 and 5% O_2. To dissect the synaptic receptor components of the signal, the slices were then perfused cumulatively with blockers of NMDA, AMPA and $GABA_A$ receptors (Wang et al., 2011). The following drugs were delivered in additive sequence in ACSF (μM): APV 50 (Tocris), DNQX 20 (Tocris) and picrotoxin 20 (Tocris).

Results

We studied the effects of development on the spatio-temporal propagation of activity through the cortical circuit. We used optical imaging of VSD coupled with electrical stimulation in L4 of V1 acute slice preparations from three age groups: P14, P20 and P27.

Reduced circuit activation across layers during development

To begin investigating the effects of neurodevelopment during normal rearing on the spatio-temporal pattern of circuit activation, we first examined how activity elicited by direct stimulation of L4 spreads to other cortical layers in each age group (**Figure 2.1A**). Across all ages, circuit activation was centered on the site of stimulation in L4 at time from stimulus (TFS) 2.5 ms (**Figure 2.1B**). By TFS 5 ms the signal spread beyond L4 and peaked in L2/3. Activation of L5 also became apparent by TFS 5 ms (**Figure 2.1B**). Following the fast activating component of the optical signal, there was a persisting component with slow decay that became apparent after the fast component had died out by TFS 30 ms in all layers (**Figure 2.1C**).

To quantitatively assess circuit activation, we measured the time course of the VSD signal intensity for ROIs in L4, L2/3 and L5 (**Figure 2.1C**). In L4 at P14 the time to peak was 8.7 ± 0.5 ms, significantly slower than in the later developmental windows (**Figure 2.1C**; P20: 6.0 ± 0.6 ms; P27: 6.1 ± 0.5 ms; Dunn's Test: P14 vs. P20, $p < 0.05$; P14 vs. P27, $p < 0.05$). As development progressed, the amplitude of the peak of L4 activation was significantly reduced, from a $\Delta F/F$ of 0.89 ± 0.034 at P14 and 0.73 ± 0.044 at P20 to 0.42 ± 0.020 at P27 (**Figure 2.1C**; Dunn's Test: P14 vs. P27, $p < 0.05$; P20 vs. P27, $p < 0.05$).

From L4, activity propagated to L2/3. At P14, the time to peak of the optical signal in L2/3 was 10.2 ± 0.3 ms, significantly slower than 7.0 ± 0.3 ms at P20 and 8.2 ± 0.4 ms at P27 (**Figure 2.1C**; Dunn's Test: P14 vs. P20, $p < 0.05$; P14 vs. P27, $p < 0.05$). In L2/3, the peak of the VSD signal was not significantly different between P14 and P20; however, by P27 there was a significant reduction in the peak amplitude (**Figure 2.1C**; P14: 0.83 ± 0.019; P20: 0.82 ± 0.056; P27: 0.52 ± 0.029; Dunn's Test: P14 vs. P27, $p < 0.05$; P20 vs. P27, $p < 0.05$).

Activity induced by L4 stimulation propagated to deep layers at all ages. The time to peak in L5 was 8.8 ± 0.6 ms at P14, 7.5 ± 0.8 ms at P20, and 8.0 ± 0.7 ms at P27, indicating that there was no significant difference in the time to peak activation of deep layers between age groups (**Figure 2.1C**; ANOVA on ranks: $p = 0.34$). As in L4 and L2/3, peak activation in L5 did not change significantly from P14 to P20, but was significantly reduced by P27 (**Figure 2.1C**; P14: 0.57 ± 0.019; P20: 0.55 ± 0.026; P27: 0.33 ± 0.017; Dunn's Test: P14 vs. P27, $p < 0.05$; P20 vs. P27, $p < 0.05$).

These data show that from eye opening through the fourth postnatal week, the magnitude of activation of the cortical circuit is reduced. As development progresses, the circuit becomes less strongly activated; however, temporal propagation of the signal becomes faster as the peak activation is reached more quickly in L4 and L2/3 in the older age groups.

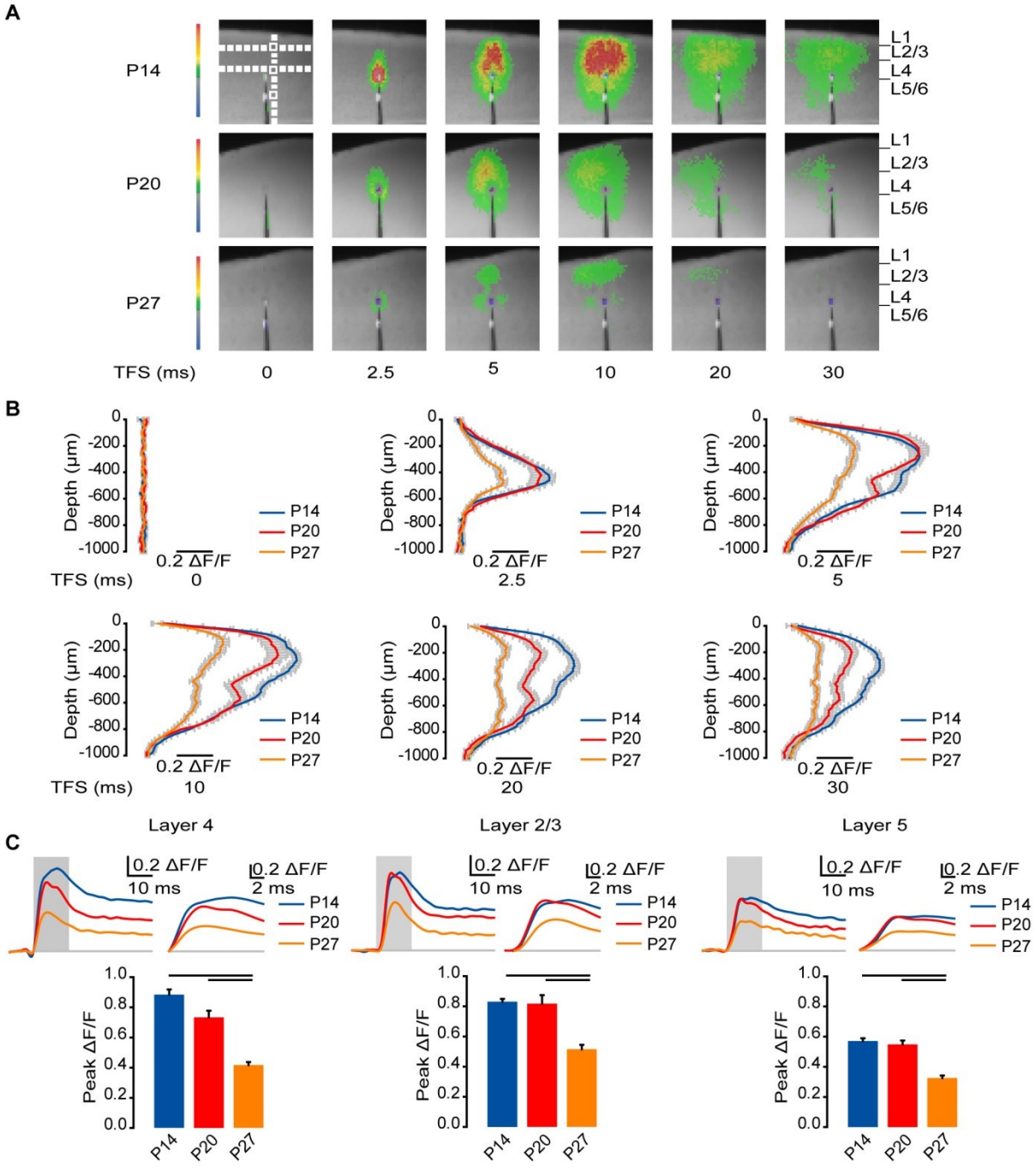

A
P14
P20
P27
L1
L2/3
L4
L5/6
TFS (ms)
0
2.5
5
10
20
30
B
Depth (µm)
0
-200
-400
-600
-800
-1000
0.2 ΔF/F
P14
P20
P27
TFS (ms)
0
2.5
5
10
20
30
Layer 4
Layer 2/3
Layer 5
C
0.2 ΔF/F
10 ms
2 ms
Peak ΔF/F
1.0
0.8
0.6
0.4
0.2
0.0

Figure 2.1. Developmental reduction in cortical activation. (A) Representative sample VSD images at 0, 2.5, 5, 10, 20 and 30 ms from L4 stimulation for each age group. Images were cropped to better visualize the activated region (from 60 x 88 to 45 x 50 pixels, 20 μm per pixel). Top left panel: White boxes: ROIs quantified in **Figures 2.1C**, **2.2**, **2.3**, **2.5**, **2.6** and **2.7**. Vertical white dashed line: ROI quantified in **Figure 2.1B**. Horizontal white dashed lines: ROIs quantified in **Figure 2.4**. **(B)** Time course of the ΔF/F measured by line scans perpendicular to the pial surface. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. **(C)** Top: Time course of optical signals measured from ROIs in L4, L2/3 and L5 from 10 ms before stimulation to 50 ms after stimulation on the left. The grey box indicates TFS 0 to 15 ms, which is shown amplified in the traces on the right to highlight changes in the time to peak. Blue: P14. Red: P20. Orange: P27. Light grey line: 0.0 ΔF/F. Bottom: Peak ΔF/F measured from ROIs in L4, L2/3 and L5. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. Dark bars indicate significant changes, $p < 0.05$.

Signal persistence decreases across development

In all layers and at all ages, the VSD signal had fast and slow components, corresponding to the fast peak of activation followed by a slow phase of signal persistence. It has previously been shown that this slow phase is present in both local field potential and VSD recordings in our preparation and can be almost entirely eliminated by blocking AMPA and NMDA receptors (Wang et al., 2011). To determine the time course of cortical activation and quantify possible developmental differences in the persistence of the voltage signal within each layer following stimulation, we calculated the ratio of the VSD signal at each time point to the peak signal (**Figure 2.2A**). We compared the fractions of the peak signal that remained at 30 ms, a time point after the fast component of the VSD response had died out.

Within L4, a larger portion of the signal persisted at 30 ms at P14 compared to P20 and P27 (**Figure 2.2A**; P14: 0.64 ± 0.017; P20: 0.50 ± 0.024; P27: 0.48 ± 0.029; Dunn's Test: P14 vs. P20, $p < 0.05$; P14 vs. P27, $p < 0.05$). Likewise, in L2/3, the VSD signal persisted most in the P14 group, and significantly less in the P20 and P27 groups (**Figure 2.2A**; P14: 0.53 ± 0.013; P20: 0.43 ± 0.028; P27: 0.35 ± 0.017; Dunn's Test: P14 vs. P20, $p < 0.05$; P14 vs. P27, $p < 0.05$). In L5, there was a significant decrease in the persistence of the signal from P14 to P20 and a trend towards a reduction at P27 (**Figure 2.2A**; P14: 0.69 ± 0.015; P20: 0.49 ± 0.035; P27: 0.57 ± 0.039; Dunn's Test: P14 vs. P20, $p < 0.05$). While we did not directly compare the signal persistence between layers, the slope of the slow phase in L2/3 appeared less steep than in L4 or L5, which might be a result of the relatively greater intralaminar connectivity within L2/3 (Burkhalter, 1989). These data show that as the animal gets older, the activation of V1 becomes temporally restricted, suggesting a more time constrained propagation of activity in the cortical circuit.

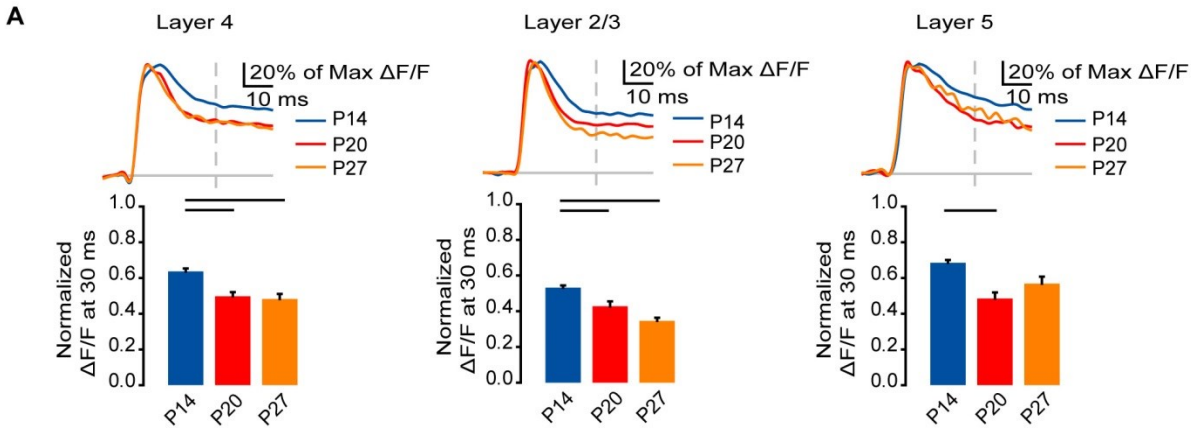


Figure 2.2. Developmental reduction in signal persistence. (A) Top: Time course of optical signals from ROIs in L4, L2/3 and L5 from 10 ms before stimulation to 50 ms after stimulation normalized to the peak ΔF/F measured in each ROI. Blue: P14. Red: P20. Orange: P27. Light grey line: 0.0 ΔF/F. Light grey dash: TFS 30 ms. Bottom: ΔF/F at 30 ms normalized to the peak ΔF/F measured from ROIs in L4, L2/3 and L5. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. Dark bars indicate significant changes, $p < 0.05$.

Interlaminar signal gain increases during development

The decrease in intensity of the VSD signal in the later stages of development that we examined could imply L4 stimulation is not as effective at activating V1. To address this, we examined the ratio of VSD signals from ROIs in L2/3 and L5 to ROIs in L4 in each age group as a measure of signal gain (**Figure 2.3**). From P14 to P27, signal gain from L4 to L2/3 increased significantly (**Figure 2.3B**; P14: 0.95 ± 0.029; P20: 1.13 ± 0.069; P27: 1.24 ± 0.059; Dunn's Test: P14 vs. P27, $p < 0.05$). The signal gain from L4 to L5 was also increased in the older age groups (P20 and P27) compared to P14 (**Figure 2.3B**; P14: 0.63 ± 0.022; P20: 0.76 ± 0.035; P27: 0.78 ± 0.024; Dunn's Test: P14 vs. P20, $p < 0.05$; P14 vs. P27, $p < 0.05$). These results suggest that while L4 stimulation elicited a smaller activation of all layers in older animals, feedforward activation actually became amplified. Thus, during development the cortical circuit acquires the capacity to propagate a temporally more restricted signal with increased gain.

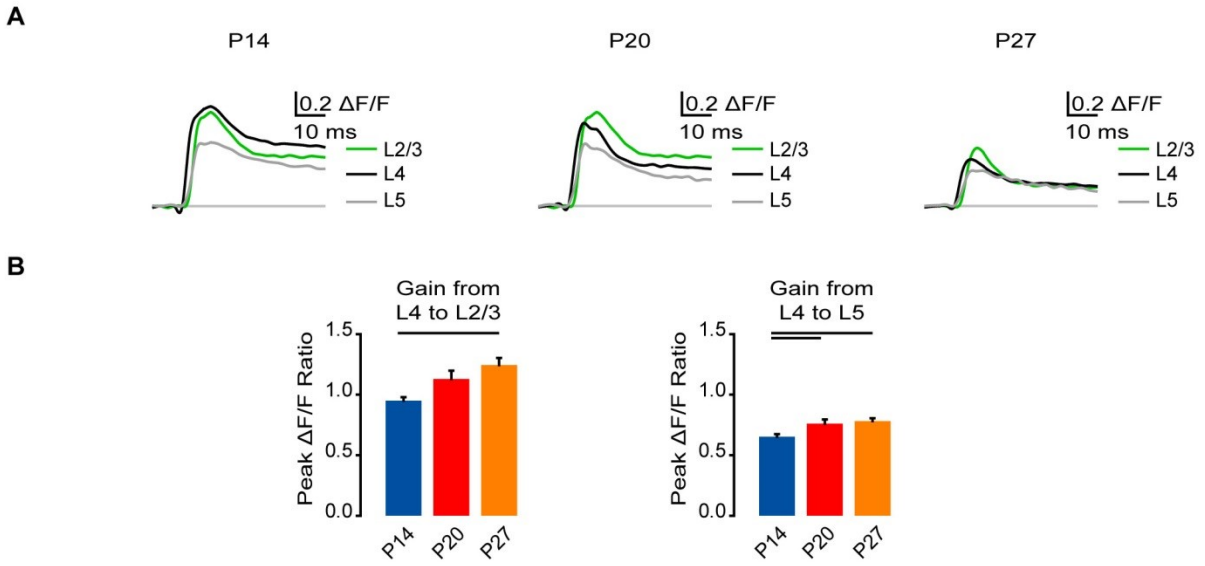


Figure 2.3. Developmental increase in interlaminar gain. (A) Time course of optical signals measured from ROIs at P14, P20 and P27 from 10 ms before stimulation to 50 ms after stimulation. Green: L2/3. Black: L4. Grey: L5. Light grey line: 0.0 ΔF/F. The unique color scheme of this panel reflects data organized by cortical layer, whereas in other panels, the color scheme reflects data organized by developmental age group. **(B)** Gain from L4 to L2/3 and L5 measured as the ratio of the peak ΔF/F from a ROI in L2/3 or L5 to the peak ΔF/F in a ROI in L4. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. Dark bars indicate significant changes, $p < 0.05$.

Intralaminar horizontal circuit activation is unchanged throughout development

Besides being propagated in the feedforward direction, stimuli elicited in L4 evoked voltage signals that were also propagated horizontally within each layer. To address whether the horizontal spread of activation was modulated by development, we analyzed line scans through L4 (**Figure 2.4A**) and L2/3 (**Figure 2.4B**) at TFS 5 ms, 10 ms, 20 ms and 30 ms. Each line scan was fit with a Gaussian curve to assess the width of signal spread within each layer (**Figure 2.4C**). In L4, the maximum width of activation was not significantly different between age groups (P14: 510 ± 117 μm; P20: 470 ± 99 μm; P27: 520 ± 118 μm; ANOVA on ranks: p = 0.88). Within L2/3, the maximal spread of activation was also not significantly different between groups (P14: 729 ± 133 μm; P20: 785 ± 198 μm; P27: 678 ± 108 μm; ANOVA on ranks p = 0.99). We conservatively chose not to analyze the horizontal spread of activation within L5 because the stimulating electrode obscured a portion of L5 from the camera. For all age groups, horizontal propagation of voltage signals within each layer remained constant, and the ratio of the lateral spread of activation in L2/3 to L4 was greater than 1 by 20 ms following stimulation, suggesting that the recurrent circuit in L2/3 is activated more broadly than that in L4 throughout the developmental window examined (**Figure 2.4D**).

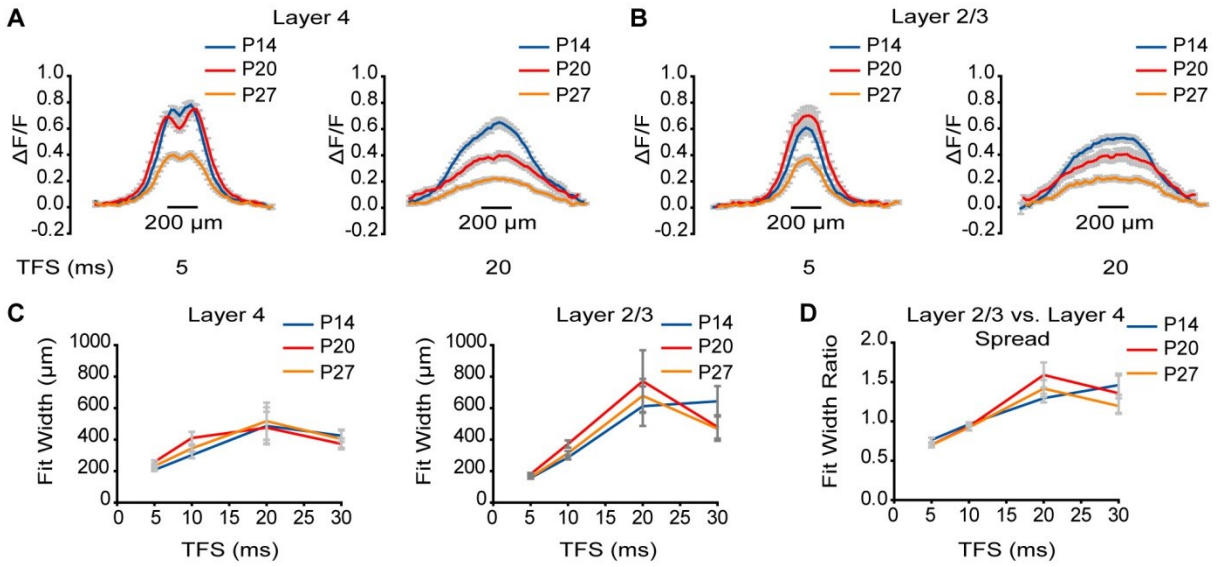


Figure 2.4. No developmental change in horizontal spread of voltage sensitive dye signal. **(A)** ΔF/F measured by line scans through L4 at TFS 5 and 20 ms. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. **(B)** ΔF/F measured by line scans through L2/3 at TFS 5 and 20 ms. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. **(C)** Plot of the width of activation in L4 and L2/3 at different TFS. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. **(D)** Plot of the ratios of the width of activation in L2/3 to L4 at different TFS. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM.

NMDA receptors account for signal duration

Excitatory synaptic transmission is significantly changed during development (Carmignoto and Vicini, 1992; Nase et al., 1999; Corlew et al., 2007; Yashiro and Philpot, 2008; Wang et al., 2012) in a way that may affect the spatio-temporal propagation of the VSD signal. To dissect the contribution of synaptic receptors to the VSD signal, we sequentially applied the NMDA receptor antagonist APV, the AMPA receptor antagonist DNQX and the $GABA_A$ receptor antagonist picrotoxin (Wang et al., 2011). Receptor mediated components of the VSD signal were calculated by subtracting the signal recorded after application of a specific antagonist from the signal recorded prior to application of that antagonist.

NMDA receptors can mediate the spatio-temporal spread of cortical circuit activation (Laaris et al., 2000; Petersen and Sakmann, 2001). Their expression and subunit composition is developmentally regulated and confers NMDA mediated responses with different properties (Carmignoto and Vicini, 1992; Nase et al., 1999; Corlew et al., 2007; Yashiro and Philpot, 2008). When isolated pharmacologically, the NMDA component of the VSD signal showed fast and slow components, with the fast component representing the peak activation followed by a slow component of signal persistence. We quantified the fast component of NMDA receptor mediated activation as the sum of the ΔF/F measured at each time point during the first 20 ms following stimulation. There was a decrease in the NMDA receptor mediated component of the VSD signal in L4 across age groups, which was significant between P14 and P27 (**Figure 2.5A**; P14: 0.96 ± 0.19; P20: 0.51 ± 0.20; P27: 0.023 ± 0.046; Dunn's Test: P14 vs. P27, $p < 0.05$). Similarly, in L2/3 the NMDA receptor mediated component of the VSD signal was significantly smaller at P27 compared to P14 (**Figure 2.5A**; P14: 0.59 ± 0.087; P20: 0.45 ± 0.30; P27: 0.10 ± 0.056; Dunn's Test: P14 vs. P27, $p < 0.05$). In L5, there was no significant change in the NMDA

receptor mediated component of the VSD signal across development (**Figure 2.5A**; P14: 0.48 ± 0.22; P20: 0.31 ± 0.13; P27: 0.12 ± 0.074; ANOVA on ranks: $p = 0.21$).

We next asked whether changes in the NMDA receptor mediated signaling across development could account for changes in the persistence of the slow component of the VSD signal. The NMDA receptor component was normalized to the peak signal before application of synaptic blockers. We compared the normalized signal that remained at 30 ms, after the fast phase of the NMDA receptor mediated signal had subsided. In both L4 and L2/3, there was a significant reduction in the persistence of NMDA receptor mediated activation from P14 to P27 (**Figure 2.5B**; L4: P14: 0.17 ± 0.022; P20: 0.10 ± 0.037; P27: 0.014 ± 0.014; Dunn's Test: P14 vs. P27, $p < 0.05$; L2/3: P14: 0.12 ± 0.020; P20: 0.091 ± 0.045; P27: 0.0055 ± 0.014; Dunn's Test: P14 vs. P27, $p < 0.05$). In L5, there was no significant difference in the persistence of the NMDA receptor mediated signal between age groups (**Figure 2.5B**; P14: 0.15 ± 0.048; P20: 0.051 ± 0.045; P27: 0.093 ± 0.039; ANOVA on ranks $p = 0.38$). These results suggest that the reduction in VSD signal persistence in L4 and L2/3 across age groups is likely related to the developmentally regulated decrease in the NMDA receptor mediated portion of the VSD signal.

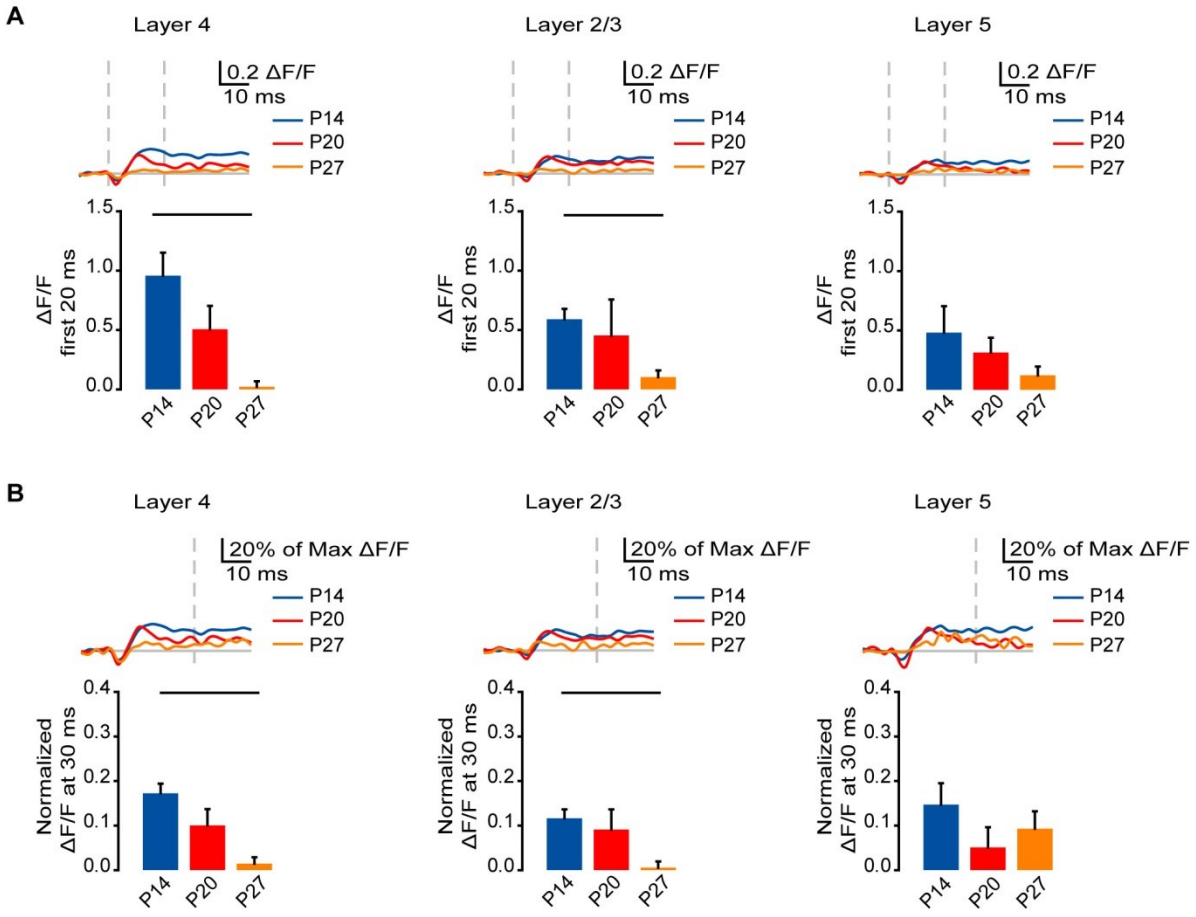


Figure 2.5. Developmental decrease in the fast and slow NMDA receptor components of circuit activation. (A) Top: Time course of the NMDA receptor component of the optical signals measured from ROIs in L4, L2/3 and L5 from 10 ms before stimulation to 50 ms after stimulation. The NMDA receptor mediated component of the signal was obtained by subtracting the VSD signal remaining after perfusion of APV from the one recorded in ACSF. Blue: P14. Red: P20. Orange: P27. Light grey line: 0.0 ΔF/F. Light grey dashes: TFS 0 ms and 20 ms. Bottom: Total ΔF/F of the NMDA receptor component of the optical signal for 20 ms from stimulation measured from ROIs in L4, L2/3 and L5. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. Dark bars indicate significant changes, $p < 0.05$. **(B)** Top: Time course of the NMDA component of the optical signals from ROIs in L4, L2/3 and L5 from 10 ms before stimulation to 50 ms after stimulation normalized to the peak ΔF/F measured in each ROI before application of synaptic blockers. Blue: P14. Red: P20. Orange: P27. Light grey line: 0.0 ΔF/F. Light grey dash: TFS 30 ms. Bottom: ΔF/F of the NMDA receptor component of the optical signal at 30 ms normalized to the peak ΔF/F before application of synaptic blockers measured from ROIs in L4, L2/3 and L5. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. Dark bars indicate significant changes, $p < 0.05$.

AMPA receptor mediated activation decreases across development

After blocking NMDA receptor mediated transmission, we measured the AMPA receptor mediated component of the VSD signal. Similar to the NMDA receptor mediated signal, the time course of the AMPA receptor mediated signal also had fast and slow components; we quantified the fast AMPA receptor mediated component of the VSD signal as the sum of the ΔF/F measured at each time point during the first 20 ms after stimulation. The AMPA receptor mediated component of the optical signal in L4 decreased significantly from P14 and P20 to P27 (**Figure 2.6A**; P14: 2.92 ± 0.17; P20: 2.35 ± 0.19; P27: 1.32 ± 0.10; Dunn's Test: P14 vs. P27, $p < 0.05$; P20 vs. P27, $p < 0.05$). In L2/3, the AMPA receptor mediated component of the signal was significantly smaller at P27 compared to P14 and P20 (**Figure 2.6A**; P14: 2.66 ± 0.073; P20: 2.41 ± 0.21; P27: 1.51 ± 0.14; Dunn's Test: P14 vs. P27, $p < 0.05$; P20 vs. P27, $p < 0.05$). In L5, there was a significant reduction of the AMPA receptor mediated VSD signal from P14 to P27 (**Figure 2.6A**; P14: 1.93 ± 0.20; P20: 1.74 ± 0.17; P27: 1.19 ± 0.095; Dunn's Test: P14 vs. P27, $p < 0.05$). Therefore, the AMPA receptor mediated component of the VSD signal decreases across development coincident with the decrease in the amplitude of the full signal.

To determine whether changes in AMPA receptor mediated synaptic transmission contribute to changes in signal persistence, we normalized the AMPA receptor mediated portion of the VSD signal to the peak signal measured before the application of drugs in each layer for each age group (**Figure 2.6B**). We compared the normalized signals that remained in each layer at 30 ms, after the fast phase of the VSD signal had subsided. In L4, L2/3 and L5 there were no significant differences between the normalized VSD signal remaining at each age (**Figure 2.6B**; L4: P14: 0.27 ± 0.015; P20: 0.21 ± 0.030; P27: 0.20 ± 0.023; ANOVA on ranks: $p = 0.06$; L2/3: P14: 0.23 ± 0.011; P20: 0.18 ± 0.025; 0.15 ± 0.031; ANOVA on ranks: $p = 0.08$; L5: P14: 0.33 ±

0.044; P20: 0.20 ± 0.034; P27: 0.21 ± 0.044; ANOVA on ranks: p = 0.06). These data are consistent with the interpretation that changes in AMPA receptor mediated circuit activation contribute primarily to the changes in peak VSD signal; however, changes in the peak circuit activation mediated by AMPA receptors may influence the signal persistence by supporting the depolarization that leads to NMDA receptor activation.

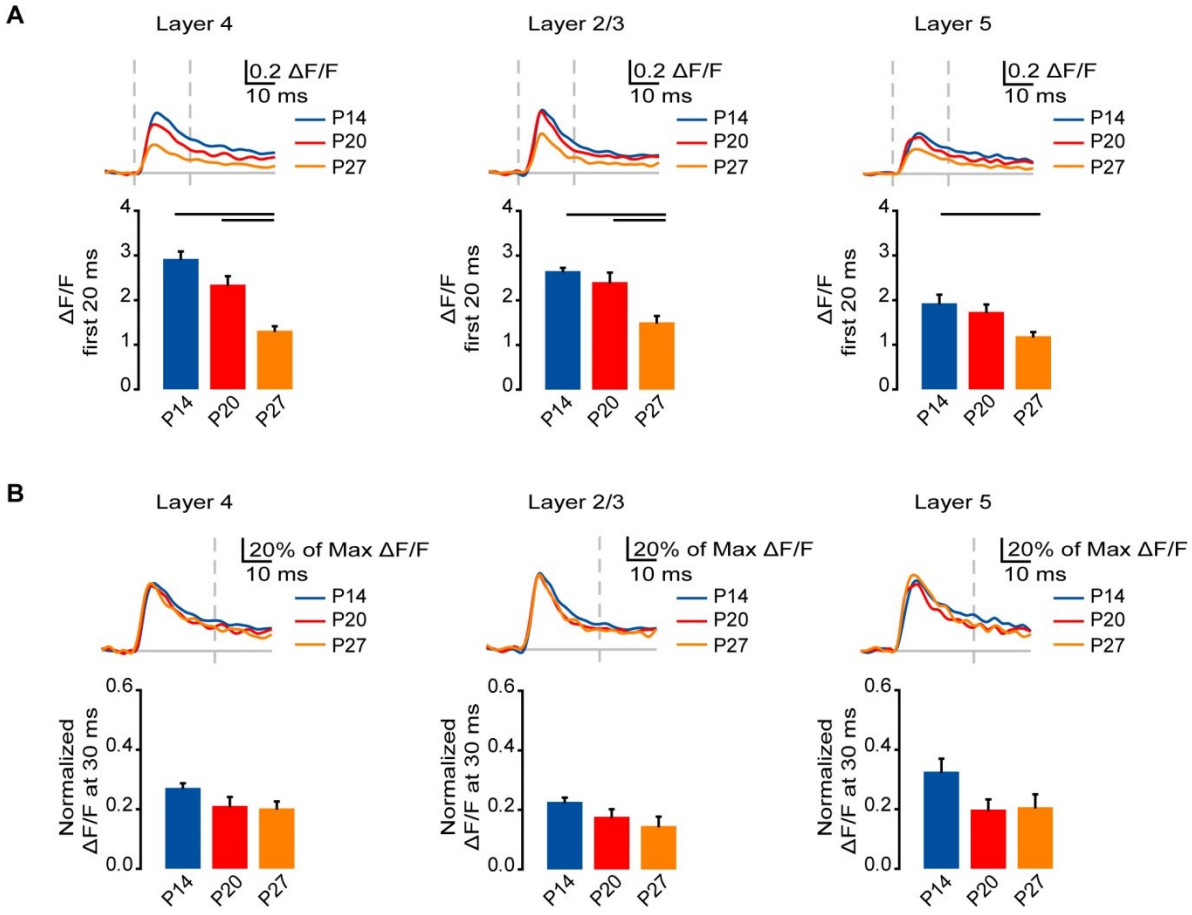


Figure 2.6. Developmental decrease in the fast AMPA receptor component of circuit activation. (A) Top: Time course of the AMPA receptor component of the optical signals measured from ROIs in L4, L2/3 and L5 from 10 ms before stimulation to 50 ms after stimulation. The AMPA receptor component of the signal was obtained by subtracting the VSD signal remaining after DNQX from the signal measured in ACSF with APV. Blue: P14. Red: P20. Orange: P27. Light grey line: 0.0 ΔF/F. Light grey dashes: TFS 0 ms and 20 ms. Bottom: Total ΔF/F of the AMPA receptor component of the optical signal for 20 ms from stimulation measured from ROIs in L4, L2/3 and L5. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. Dark bars indicate significant changes, $p < 0.05$. **(B)** Top: Time course of the AMPA component of the optical signals from ROIs in L4, L2/3 and L5 from 10 ms before stimulation to 50 ms after stimulation normalized to the peak ΔF/F measured in each ROI before application of synaptic blockers. Blue: P14. Red: P20. Orange: P27. Light grey line: 0.0 ΔF/F. Light grey dash: TFS 30 ms. Bottom: ΔF/F of the AMPA receptor component of the optical signal at 30 ms normalized to the peak ΔF/F before application of synaptic blockers measured from ROIs in L4, L2/3 and L5. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM.

$GABA_A$ receptor mediated inhibition increases in layer 4 across development

The maturation of GABAergic inhibition is critical for the functional development of visual cortex (Huang et al., 1999; Li et al., 2012; Griffen and Maffei, 2014) and has been shown to regulate the spatio-temporal propagation of voltage signals (Laaris et al., 2000; Petersen and Sakmann, 2001; Sato et al., 2008; Wang et al., 2011). To determine possible developmental changes in the contribution of $GABA_A$ receptor mediated activity to the changes in VSD signal we analyzed the portion of the optical signal remaining after additive blockade of AMPA and NMDA receptors. As the $GABA_A$ receptor blocker was applied only following complete blockade of glutamatergic synaptic transmission, our experimental design allowed us to isolate the inhibitory component of the VSD signal due to direct stimulation of local GABA releasing neurons and axons in the vicinity of the stimulating electrode (Wang et al., 2011). We quantified the $GABA_A$ receptor mediated signal as the sum of the $\Delta F/F$ measured at each time point during the first 10 ms following stimulation.

In L4, the $GABA_A$ receptor mediated component of the VSD signal increased in magnitude from P14 to P20 and P27 (**Figure 2.7A**; P14: 0.041 ± 0.020; P20: -0.10 ± 0.042; P27: -0.11 ± 0.041; Dunn's Test: P14 vs. P20, $p < 0.05$; P14 vs. P27, $p < 0.05$). There was no significant change in the $GABA_A$ receptor mediated component of the VSD signal in L2/3 and L5, away from the site of stimulation (**Figure 2.7A**; L2/3: P14: 0.012 ± 0.016; P20: -0.076 ± 0.034; P27: -0.11 ± 0.049; ANOVA on ranks: $p = 0.06$; L5: P14: -0.0029 ± 0.039; P20: -0.014 ± 0.049; P27: -0.063 ± 0.024; ANOVA on ranks: $p = 0.27$). Our data show that direct stimulation of L4 activates a larger $GABA_A$ receptor mediated VSD signal within L4 during the peak of the sensitive period than it does just after eye opening.

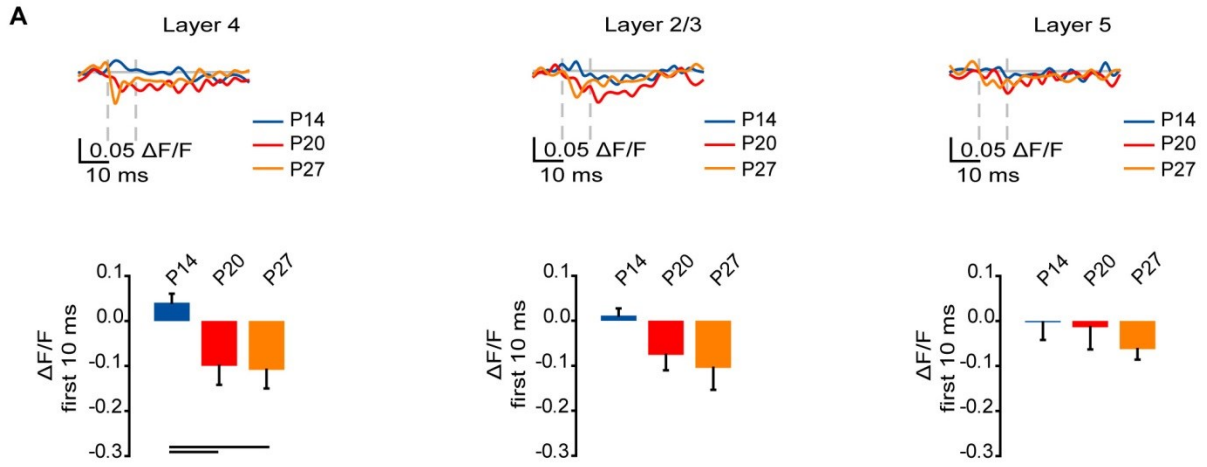


Figure 2.7. Developmental increase in the $GABA_A$ receptor component of circuit activation. **(A)** Top: Time course of the $GABA_A$ receptor component of the optical signals measured from ROIs in L4, L2/3 and L5 from 10 ms before stimulation to 50 ms after stimulation. The $GABA_A$ receptor mediated component of the signal was obtained by subtracting the VSD signal remaining after perfusion of picrotoxin from the one recorded in ACSF with APV and DNXQ. Blue: P14. Red: P20. Orange: P27. Light grey line: 0.0 ΔF/F. Light grey dashes: TFS 0 ms and 10 ms. Bottom: Total ΔF/F of the $GABA_A$ receptor component of the optical signal for 10 ms from stimulation measured from ROIs in L4, L2/3 and L5. Blue: P14. Red: P20. Orange: P27. Error bars: ± SEM. Dark bars indicate significant changes, $p < 0.05$.

Discussion

We have shown that during postnatal development there are significant changes in the spatio-temporal activation of the visual cortical circuit. Our data demonstrate that from eye opening through the fourth postnatal week, stimuli of comparable amplitude elicited smaller signals that decayed more rapidly and were propagated with greater gain to other cortical layers. While the interlaminar dynamics of activation were altered, the width of activation remained unchanged. Changes in the synaptic components of the VSD signals correlated well with the modulation of spatio-temporal patterns of circuit activation.

At all ages, the sequence of circuit activation following L4 stimulation was similar. The optical signal was initially confined almost entirely to L4, before spreading primarily to L2/3 and also to L5. Upon reaching L2/3, the voltage signal spread laterally beyond the borders of the region activated in L4. While field stimulation in L4 could depolarize axons from other layers, we did not observe robust activation outside of L4 until 5 ms after stimulation. Therefore, the majority of the optical signal in our preparation was likely due to direct stimulation of L4 and recurrent circuit activity secondary to this initial stimulation. In rat V1, L4 pyramidal neurons have been shown to send axons vertically, primarily to L2/3, but also to L5 and L6, while L2/3 axons project horizontally (Burkhalter, 1989). Our optical signal followed the architectural organization of the V1 circuit.

Neural circuits must consume energy in order to propagate signals and circuits that require more voltage changes consume more energy (Ames, 2000). In an efficient neural circuit, activity is thought to become spatio-temporally constrained to maximize the ratio of information to total activation (Laughlin and Sejnowski, 2003). We showed that over the course of

development, the overall responsiveness to electrical stimulation of L4 decreased: We observed a reduction of both fast and persistent circuit activation. The decrease in overall activation of the circuit was partly due to an increase in $GABA_A$ receptor mediated inhibition in L4 and a decrease in AMPA and NMDA receptor mediated excitation. Similarly, developmental increases in paired pulse depression between white matter stimulation and L2/3 recording were shown to depend on increasing intracortical inhibition (Rozas et al., 2001). Together, these results are consistent with synaptic development in rodent visual cortex: from the second through fourth postnatal weeks, the number of inhibitory synapses increases substantially, increasing total cortical inhibition (Blue and Parnavelas, 1983; Morales et al., 2002; Chattopadhyaya et al., 2004) and there is an approximate halving of the number of presynaptic NMDA receptors in visual cortex, which act to increase glutamatergic neurotransmitter release (Corlew et al., 2007). Therefore, changes in $GABA_A$, AMPA and NMDA mediated currents contribute to the reduction in the fast component of the VSD signal. While neurotransmitter gated ion channels contributed substantially to the reduction in circuit excitability, they did not account for the entire reduction is the VSD signal observed from P13 to P28. Other developmental changes, including changes in intrinsic excitability and myelination, could account for the remainder of the change in excitability (Tanaka et al., 2003; Etherington and Williams, 2011; Lazarus and Huang, 2011; Wang et al., 2012).

Following sequential application of NMDA and then AMPA receptor antagonists, we showed that the decrease in the signal persistence in L2/3 and L4 from P13 to P28 was mediated predominantly by NMDA receptors in L2/3 and L4, although the decrease in total circuit depolarization (e.g. through AMPA receptors) may have contributed to the reduced NMDA receptor activation. The timing of this NMDA receptor dependent change in circuit activation

kinetics coincides with developmental decreases in NMDA receptor decay time and deactivation time that have been observed previously (Carmignoto and Vicini, 1992; Nase et al., 1999; Yashiro and Philpot, 2008). NMDA receptor mediated signaling did not account for changes in L5 signal persistence; consistent with our results, APV did not differentially affect synaptic transmission between connected L5 pyramidal neurons from P11 to P29 (Etherington and Williams, 2011). Therefore, by the fourth postnatal week, V1 reaches peak activation more quickly and the voltage signal becomes less persistent. These changes suggest that the efficiency of signal propagation within the V1 circuit improves during postnatal development.

While the circuit in V1 became less excitable and its activation less persistent during postnatal development, there was an increase in the gain of signals leaving L4. By the end of the third postnatal week, activation of L4 resulted in an amplified signal reaching L2/3. This circuit refinement occurred with a similar time course as the refinement of visual cortex receptive field properties (Fagiolini et al., 1994; Huang et al., 1999; Prevost et al., 2010; Espinosa and Stryker, 2012; Li et al., 2012). Intracortical amplification has been proposed as a mechanism for the development of mature receptive fields (Chance et al., 1999; Antolik and Bednar, 2011). We speculate that an increase in the gain of interlaminar circuit activation may play a role in the maturation of receptive fields. Thus, during the maturation process occurring in normal development the visual cortical circuit becomes more efficient while simultaneously developing receptive field properties via an increase in interlaminar gain.

How does the propagation of voltage signals through visual cortex compare to those through circuits in other primary sensory cortices? In insular and barrel cortices, direct stimulation of L4 was shown to elicit distinct patterns of interlaminar propagation of VSD signals (Sato et al., 2008). In P15 – P22 insular cortex, stimulation at the border of the

dysgranular and agranular regions in "L4" resulted in spread to L2/3 and L5 confined to the columnar width around the stimulation site (Sato et al., 2008). While stimulation of L4 in V1 led to the activation of L2/3 and L5, the VSD signal was greatest in L2/3, where it spread horizontally more widely than in L4. In barrel cortex at P13 – P15 and P18 – P22, stimulation of L4 resulted in a spread of depolarization similar to what we observed in V1; however, signal spread to L5 was minimal (Petersen and Sakmann, 2001; Sato et al., 2008). As V1 and barrel cortex both have a well-defined L4 that receives most of the thalamic input (Bolz, 1994; Miller et al., 2001), it is not surprising that they show a similar responsiveness to stimulation, which is distinct from that at the agranular/dysgranular junction in insular cortex (Sato et al., 2008) where thalamic fibers distribute diffusely (Maffei et al., 2012). The differences in spread of VSD signal to L5 between visual and barrel cortex could be due to the later maturation of visual cortex relative to barrel cortex (Cheetham and Fox, 2010) or could represent differences in signal propagation between the cortices. Our findings indicate that from the onset of visual experience, the spatio-temporal propagation of cortical activation is refined dependent upon changes in receptor mediated synaptic transmission. The propagation of activity in the cortical circuit is modulated not only by development but also by changes in visual experience (Palagina et al., 2009; Wang et al., 2011), a manipulation that is known to alter synaptic transmission (Morales et al., 2002; Maffei et al., 2006; Maffei and Turrigiano, 2008a). Similarly, neurodevelopmental disorders that alter synaptic transmission (Harrison and Weinberger, 2005; Paluszkiewicz et al., 2011; Werner and Covenas, 2011; Zoghbi and Bear, 2012) may disrupt the normal development of spatio-temporal circuit activation, resulting in dysfunctional propagation of stimuli through cortical circuits.

Chapter III

Rapid Ocular Dominance Plasticity in Monocular Visual Cortex

Preface

These experiments were initially conceived under a working hypothesis based on the observation that a single day of visual deprivation was sufficient to potentiate fast spiking neuron to pyramidal neuron synapses in V1m (Maffei et al., 2006; Maffei et al., unpublished data). As brief stimuli such as sounds and whisker touch rapidly and strongly co-activate inhibition in their primary sensory cortices (Griffen and Maffei, 2014), I reasoned that a brief visual stimulus might be better suited to exploring the early effects of visual deprivation than a patterned visual stimulus presented over several seconds or a noise stimulus presented for 50 ms (ten times longer than the duration of my stimuli). Ipsilateral eye responses were recorded primarily as a control to differentiate V1m from V1b. However, flash stimuli have been shown to evoke propagating cortical activity beyond V1 (Stroh et al., 2013). Therefore, the possibility existed that ipsilateral eye flash stimuli would activate both V1b and V1m. It has been shown that monocular deprivation can alter not only the vertical, interlaminar spread of cortical circuit activation, but also the horizontal spread of activation (Wang et al., 2011) and a long period of monocular deprivation was shown to lead to an expansion of the area served by the open eye from the ipsilateral V1b into the ipsilateral V1m (Tagawa et al., 2005). Therefore, a secondary hypothesis arose that deprivation might alter the ipsilateral eye responses within V1m.

Abstract

Monocular visual deprivation shifts the responsiveness of binocular neurons in primary visual cortex to favor the open eye over the deprived eye. This ocular dominance shift is thought to occur through a two-step process: a loss of responsiveness to the deprived eye followed by a potentiation of open eye responses. In V1m, a loss of responsiveness to the deprived eye was shown to occur. The loss of responsiveness observed in V1m did not occur following a brief deprivation, it required a longer of period of deprivation than reported for ocular dominance plasticity in V1b. Using *in vivo* whole-cell recordings, I show that neurons in V1m can respond to flash stimuli presented independently to both the contralateral and ipsilateral eyes. After just one day of monocular deprivation, ipsilateral, open eye responses were potentiated in V1m, while closed eye responses remained unchanged. Thus, in V1m brief alteration of visual drive shifted the interocular bias toward the open eye more rapidly than previously expected. This early form of ocular dominance plasticity may provide a competitive mechanism for the expansion of ipsilateral eye responses beyond the borders of V1b.

Introduction

Following the loss of a sensory input, cortical circuits may be rewired to respond more robustly to other sensory inputs that remain intact (Bence and Levelt, 2005; Tagawa et al., 2005; Margolis et al., 2014; Schreiner and Polley, 2014). For example, monocular deprivation during a sensitive period in development was shown to lead to a change in the ocular dominance of neurons in V1b (Wiesel and Hubel, 1963b; LeVay et al., 1980; Fagiolini et al., 1994; Gordon and Stryker, 1996; Horton and Hocking, 1997; Issa et al., 1999). This change is thought to occur through two temporally distinct processes: Shortly after the onset of visual deprivation, neurons became less responsive to the deprived eye. After a delay, there was an increase in responsiveness to the non-deprived eye (Frenkel and Bear, 2004). These sequential changes have been proposed to be driven by a competitive interaction between inputs to cortical neurons driven independently by the two eyes (Smith et al., 2009). V1m has been shown to be driven monosynaptically by thalamic inputs from the contralateral eye (Tagawa et al., 2005; Coleman et al., 2009). As in V1b, monocular deprivation led to a decrease in responsiveness in V1m L4 to the stimuli presented to the contralateral, deprived eye and to a weakening of thalamic input to V1m (Iurilli et al., 2012; Wang et al., 2013b); however, a longer period of deprivation was required before loss of responsiveness could be observed in V1m (Iurilli et al., 2012). This slower loss of responsiveness was attributed to a lack of direct, open eye inputs to drive competitive, Hebbian plasticity (Iurilli et al., 2012).

Additional lines of evidence suggest that the classical model proposed to explain ocular dominance plasticity may be an oversimplification. First, in cat visual cortex, ocular dominance shifts were observed in extra-granular layers that do not receive strong thalamic input, but not in L4, after a single day of monocular deprivation (Trachtenberg et al., 2000). Second, prior to the

expression of an ocular dominance shift in mouse V1b, there was an increase in responsiveness to stimuli delivered to both eyes in the hemisphere contralateral to the deprived eye (Kuhlman et al., 2013). The increased responsiveness to the open, ipsilateral eye was thought to maintain visually evoked spiking in V1 at a rate comparable to that driven by binocular stimulation prior to the onset of the deprivation (Kuhlman et al., 2013). Third, during the early phase of ocular dominance plasticity, fast spiking GABAergic neurons became more responsive to stimuli presented to the deprived eye than to the open eye (Yazaki-Sugiyama et al., 2009). However, extended periods of monocular deprivation led to the loss of inhibitory neurons' responsiveness to the deprived eye and did not alter the ratio of excitatory and inhibitory inputs to pyramidal neurons (Gandhi et al., 2008; Yazaki-Sugiyama et al., 2009; Iurilli et al., 2013). Finally, in V1m, population firing rates were suppressed soon after the onset of monocular deprivation but returned to baseline after only two days (Hengen et al., 2013). These observations suggest that, in addition to Hebbian competition between thalamic inputs serving the two eyes, other mechanism of plasticity, possibly induced at intracortical connections, likely contribute to alter visual responses and spontaneous firing rates in visual cortex in response to manipulations of visual drive.

It is well known that the topographic representations of sensory stimuli in cortex have some degree of flexibility and can be altered by experience (Buonomano and Merzenich, 1998). Following sensory deprivation, regions of cortex that were once less responsive to a stimulus may become more responsive to that stimulus (Wiesel and Hubel, 1963b; Bence and Levelt, 2005; Tagawa et al., 2005; Margolis et al., 2014; Schreiner and Polley, 2014). For example, after two weeks of monocular deprivation, L4 of V1m contralateral to the deprived eye can become activated by stimulation of the ipsilateral, open eye (Tagawa et al., 2005). To investigate

possible mechanisms underlying this plasticity in V1m, I characterized the response of visual cortical neurons to brief (5 ms) flash stimulation delivered to each eye of an acutely anesthetized rat. Using whole-cell patch-clamp, I was able to monitor membrane potential and spiking neuronal responses. I chose brief flash stimuli because they have been shown to activate cortical regions beyond V1 through spreading calcium waves (Stroh et al., 2013). I showed that flash stimuli were capable of activating neurons in ipsilateral V1m. I then asked whether a single day of monocular deprivation could alter the balance of contralateral and ipsilateral responses to flash stimuli in V1m. Consistent with previous reports, I found no change in visual responsiveness to the deprived eye at this time point (Iurilli et al., 2012); however, I observed a shift in responsiveness in the hemisphere contralateral to the deprived eye: Excitatory neurons responded more strongly to the ipsilateral, open eye. This increase in open eye responsiveness could enhance competition between inputs responding to the open and closed eyes within V1m and provide a mechanism for post-deprivation widening of the region of V1 responsive to the ipsilateral eye.

Materials and Methods

Subjects

All experimental procedures were approved by the Stony Brook University Institutional Animal Care and Use Committee and followed National Institute of Health guidelines. Long-Evans rats were obtained from Charles River as full litters on P5. After weaning on P20-21, juvenile rats were housed in groups of one to four. Access to chow and water was provided *ad libitum* and a 12 hour light/12 hour dark cycle was maintained with lights turned on at 7:00 AM local time. Forty-three rats of both sexes, aged P26-33 on the day of recording, were used for these experiments.

Monocular deprivation

Monocular lid suture was performed similar to previous experiments (Maffei et al., 2004; Maffei et al., 2006). Animals selected for visual deprivation were randomly chosen to have their left or right eye sutured shut. During the late morning or early afternoon, subjects were briefly anesthetized with a mixture of 70 mg/kg ketamine, 3.5 mg/kg xylazine hydrochloride and 0.7 mg/kg acepromazine maleate, intraperitoneally. Antibiotic ointment and artificial tears were applied to both eyes to prevent infection and drying. One eye was sutured shut with four or five mattress sutures and animals were allowed to recover. To prevent littermates from removing the sutures, animals were housed alone during and after recovery.

Surgery

For monocularly deprived animals, induction of surgical anesthesia was performed 24 ± 2 hours following the induction of anesthesia for lid suture. Animals were anesthetized with 1.2 g/kg urethane and 40 mg/kg pentobarbital. Recordings were performed at least 3 h after anesthesia induction, after the pentobarbital was expected to have worn off (Stone et al., 2011). Adequate anesthesia was assessed by repeatedly testing for the absence of a hind limb withdrawal reflex throughout the experiment and administering additional doses of urethane (10-20% of induction) as needed. Bupivacaine hydrochloride was injected at all incision points to further ensure analgesia. Body temperature was maintained at 37° C with a heating pad (FHC Inc.). The lid sutures were examined and, if still intact without any sign of infection or eye opening, the experiment proceeded. The lid sutures were replaced, under low light, with one or two sutures, which was sufficient to keep the eye closed yet allow for easy reopening prior to recording. Throughout the experiment, the subject's eyes were kept moist with artificial tears as necessary.

The animal was next placed on a stereotactic apparatus (Narishige) and a small craniotomy (~1 mm in diameter) was opened up over a portion of primary visual cortex 89% of the distance between bregma and lambda. V1m was targeted 2.8 mm lateral to the midline and V1b was targeted 4.2 mm lateral to the midline. For most animals, a single craniotomy was performed over the left hemisphere. Prior to recording, a small hole in the meninges was created with a fine needle and lid sutures were removed. After removal of lid sutures, care was taken to keep the experimental room as dark as possible.

In vivo whole-cell recordings

Whole-cell recordings were performed similar to standard procedures (Ferster, 1988; Margrie et al., 2002). 4-8 MΩ borisillicate patch pipettes were fashioned using a Flaming–Brown puller (Sutter Instruments) and filled with intracellular solution (in mM: 116 K-Gluconate, 10 KCl, 1 HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-Phosphocreatine; ~7.35 pH; ~295 mOsm; biocytin 0.1%). Pipettes were slowly lowered into cortex perpendicular to the surface of the brain at the midline with a hydraulic micromanipulator (Narishige) under ~200-250 mbar of outward pressure for ~400 μm. Pressure was then reduced to ~30 mbar and cells were searched for in voltage clamp mode. After encountering a cell, pressure was released and, if necessary, gentle suction was applied to obtain a > 1.0 GΩ seal. A ramp of suction was applied to obtain whole-cell configuration. Cells were allowed to stabilize for several minutes. Recordings proceeded in current clamp mode. Capacitance was manually compensated online and series resistance was partly (10-20 MΩ) compensated online. Correction of parameters for the liquid junction potential was not applied either online or offline. No holding currents were applied during recordings. Cells were considered for analysis only if the resting membrane potential was more negative than -50 mV and did not change more than 5 mV during the course of the recording and if series resistance was below 100 MΩ with less than 20% change during recording. The distance that the pipette traveled prior to encountering a cell was noted. The depth was then corrected based on the angle between the pipette and the surface of the brain as estimated from recovered pipette tracks: 15° for V1m and 28° for V1b. Recordings were obtained between 380 and 1060 μm below the cortical surface.

Visual stimulation and data collection

Membrane potential data were acquired at 20 kHz with a Digidata 1440A board (Molecular Devices) connected to a computer running Clampex 10 acquisition software (Axon Instruments) in current clamp mode. Different stimulus presentations and recording protocols were tested in different subpopulations of cells. Visual stimuli were delivered in sets of ten (or five for two of the recorded neurons) 5 ms flashes spaced 6.0 s apart and delivered to either the right or the left eye. All analysis was performed on twenty (or for a small subset of cells, ten) stimuli delivered to each eye. Flash stimuli were delivered with a custom made stimulation apparatus: a 5V TTL pulse (Master-8, A.M.P.I.) was passed through a 7000 mcd, 25 mA forward current, 3.6V maximum supply, 30° viewing angle, white-light LED (Radio Shack, model #276-017) in series with a 100Ω resistor. The LEDs could deliver ~10 mW of power at steady state. LEDs were positioned within ~2mm of the eyes at an angle of ~20° from the midline. In preliminary experiments with local field potential recordings, small deviations in LED position did not affect the visually evoked potential (data not shown). **Figure 3.1** is a schematic of the recording setup.

To ensure that recordings remained stable, the resting, or baseline, membrane potential was measured as the lowest value of the membrane potential, after application of a 10 ms mean filter, during a 1.5 s window within the inter-stimulus interval. Action potential threshold was measured as the point at which the derivative of the membrane potential reached 20 V/s and was averaged over at least three action potentials per cell. Spike width was measured as the full width at half maximum spike height from threshold. To allow for maximal unambiguous *post hoc* identification of neurons, no more than two neurons were recorded from a single craniotomy.

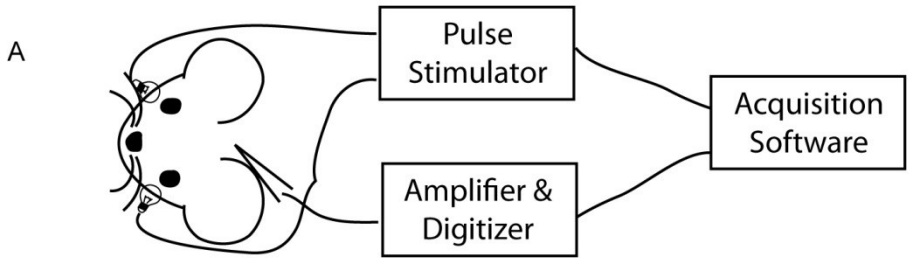


Figure 3.1. Schematic of experimental setup. (A) Schematic drawing of rat with LEDs (bulbs) positioned in front of both eyes, connected to Master-8 stimulus generator. Patch pipette is connected to Molecular Devices amplifier and board. A computer running Clampex 10 software controls the Master-8 and records data from the digitizing board.

Histology

Following each experiment, deep anesthesia was ensured with an overdose of surgical anesthetics. Rats were then transcardially perfused with chilled PBS followed by chilled 4% paraformaldehyde in PBS. Brains were post-fixed in 4% paraformaldehyde in PBS for 1 to 3 days, and then transferred to PBS until slicing. Brains were then sectioned 100 μm thick on a vibratome and stained using standard peroxidase histochemical procedures.

Cell classification and inclusion

Cells were classified as fast spiking interneurons if their spike width was < 0.5 ms, they responded to current injection with spiking typical of an interneuron and they could be unambiguously identified as having interneuron morphology without dendritic spines *post hoc*. Five neurons from five rats fit these inclusion criteria and were located within L3 and L4. Cells were classified as putative excitatory neurons if they had spike widths > 0.5 ms, spike height > 30 mV and responded to current injection with spiking patterns typical of cortical pyramidal neurons: bursting and regular spiking patterns (Zhu and Connors, 1999). Of cells fitting these criteria, twenty-five were recovered and had morphologies typical of pyramidal neurons from L3, L4 and L5, including star pyramidal neurons and pyramidal neurons. One neuron was morphologically identified as a spiny stellate cell, a L4 excitatory interneuron. Nineteen neurons could not be unambiguously identified *post hoc* but fit the inclusion criteria for putative excitatory neurons.

Analysis and statistics

Analysis was performed with procedures developed in SigmaPlot (Systat Software, Inc.), Igor Pro (WaveMetrics), Excel (Microsoft) and Matlab (Mathworks). Membrane potential traces were passed through a 5 ms median filter to remove action potentials prior to analysis of membrane potential responses. To determine the peak of the post stimulus response velocity, the absolute value of the first derivative of the membrane potential was passed through a 5 ms median filter. The time of the peak during the visually identified post stimulus response was determined. This method for identifying response latency was used because for many neurons the direction of change of the membrane potential following stimulus was highly dependent upon the membrane potential prior to stimulus onset (see results for further discussion).

Action potential responses to visual stimuli were analyzed using the area under the receiver operator curve (auROC) to normalize data (Parker and Newsome, 1998). An auROC value was obtained by comparing ten 100 ms baseline bins immediately preceding the stimulus to a single 100 ms post-response bin beginning at the peak rate of change of the membrane potential during stimulus response. The auROC normalization returns values between 0 and 1, with a response value of 1 indicating that all response bins contained more action potentials than all baseline bins, and a value of 0 indicating that all response bins contained fewer action potentials than all baseline bins. A value of 0.5 is returned if there is no change from response to baseline. The auROC-ODI was computed as the auROC response to ipsilateral eye stimulation subtracted from the auROC response to the contralateral eye. Possible auROC-ODI values range from 1.0 to -1.0.

Unless otherwise stated, data are presented as mean ± SEM for the number of neurons recorded (in the number of animals indicated). To determine significance, a two-tailed Student's *t* test was used to compare between groups. For distributions that deviated significantly from normality (as per the Shapiro-Wilk test), the nonparametric Mann-Whitney rank sum test was used. Paired tests were used only to compare ipsilateral and contralateral response latencies within groups of neurons. To determine whether the distribution of neuron depths was different between groups, a two-sample Kolmogorov-Smirnov test was applied. Significance was set at $p \leq 0.05$.

Results

Data were obtained from 50 neurons recorded from V1 of 45 rats. Prior to recording, a subset of animals were subjected to monocular deprivation by lid suture for 24 hours. Neurons were recorded from both V1m and V1b, and were classified as either excitatory neurons or fast spiking neurons based on spiking responses to depolarizing current steps and action potential widths. This classification was subsequently verified with *post hoc* morphological analysis (**Table 3.1**, **Figures 3.2A**, **3.2B**, **3.2E**, **3.2F**). Excitatory neurons that were successfully recovered all had morphologies consistent with excitatory neurons (**Figure 3.2A**). Five neurons with fast spiking firing patterns were also recovered and had typical interneuron morphologies (**Figure 3.2E**).

	No Visual Deprivation	Ipsilateral to Deprived Eye	Contralateral to Deprived Eye
V1m Excitatory	11 (10)	17 (14)	13 (13)
V1b Excitatory	4 (4)	0	0
V1m Fast Spiking	3 (3)	1 (1)	1 (1)

Table 3.1. **Classification of neurons by cell type and location.** Number of neurons (in number of rats).

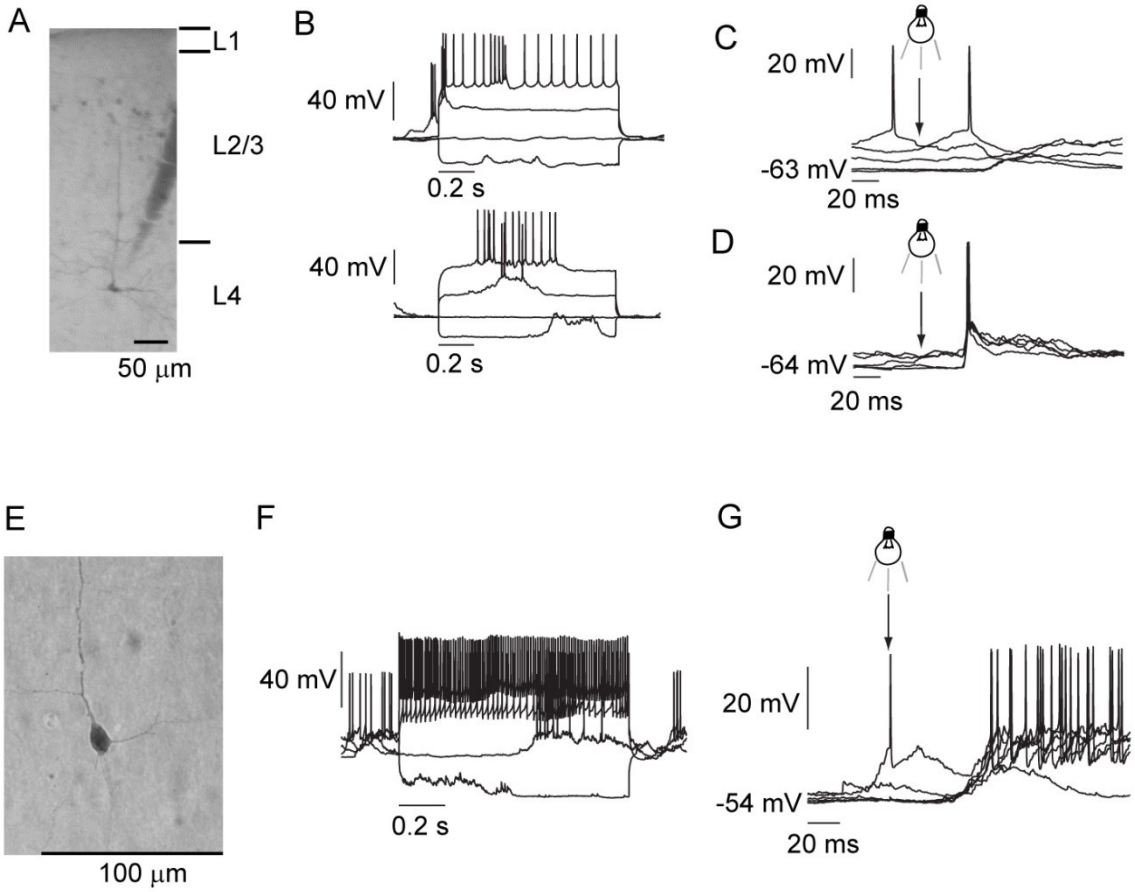


Figure 3.2. Whole-cell recordings of primary visual cortex excitatory and fast spiking neurons and flash responses. (A) Recovered L4 V1m pyramidal neuron with morphology representative of the majority of recovered neurons. The electrode track is visible next to the recovered cell. **(B)** Two representative current injection/firing relationships from V1 excitatory neurons in response to sequential 1 s current injections (-250 pA, 0 pA, 250 pA, 500 pA). **(C-D)** Representative examples of five overlapped responses of two excitatory V1 neurons to flash stimuli presented to the contralateral eye. Stimulus onset at arrow. **(E)** Recovered L4 V1m fast spiking neuron with typical interneuron morphology. **(F)** Representative current injection/firing relationships from V1 excitatory neurons in response to sequential 1 s current injections (-250 pA, 0 pA, 250 pA, 500 pA). **(G)** Five overlapped responses of a V1 fast spiking neuron to flash stimuli presented to the contralateral eye. Stimulus onset at arrow.

Flash evoked membrane potential responses in visual cortical neurons

Brief flashes of light can robustly activate V1; however, responses to brief flashes have only been analyzed using extracellular and calcium sensitive dye recording techniques (Uhlrich et al., 2005; Stroh et al., 2013). To determine how individual neurons in visual cortex respond to flash stimuli, I recorded the membrane potential and action potential responses of V1 neurons to brief (5 ms) light flashes delivered to either eye. Following stimulation, all neurons had clear, visually identifiable changes in membrane potential that were time locked to the stimulus and occurred within 200 ms (**Figures 3.2C**, **3.2D**, **3.2G**). Reliable responses could be evoked in both V1m and V1b neurons in response to stimulation of either eye. Ipsilateral eye responses in V1m are not entirely surprising, given previous reports of flash evoked responses recorded from both somatosensory and frontal cortices (Manning and Uhlrich, 2009; Stroh et al., 2013). Similar to previous reports of superficial neurons in the barrel cortex of behaving mice (Crochet et al., 2011), the magnitude and sign of individual post-stimulus membrane potential changes was dependent on the pre-stimulus membrane potential. For several neurons, if the membrane potential was near threshold at the time of stimulus, the response was a negative deflection of membrane potential; however, if the membrane potential was near baseline at the time of stimulus, the response depolarized the membrane potential to a point below action potential threshold (**Figure 3.2C**). Regardless of the membrane potential at stimulus onset, neurons with this membrane potential response profile exhibited a reduction in firing rate immediate following the membrane potential response. For other neurons, the membrane potential moved consistently towards threshold following stimulation, rapidly increasing action potential frequency (**Figure 3.2D**). Both excitatory neurons and fast spiking interneurons responded to flash stimuli (**Figure 3.2G**).

Delayed evoked ipsilateral responses in monocular visual cortex

Flash stimuli evoked membrane potential and spiking responses that showed considerable variability. However, some response properties appeared uniform within the population of recorded neurons. Importantly, for all neurons, there appeared to be a relatively consistent latency to the onset of the membrane potential response. Previously, response latency had been quantified in V1 as the time from stimulus at which the mean membrane potential across trials crossed a threshold (Ma et al., 2013). This method is incompatible with many of the responses that I recorded: Because post-stimulus membrane potential changes were highly dependent upon pre-stimulus membrane potential, the amplitudes of the average membrane potential changes were highly variable. For example, in some neurons there was a time locked change in membrane potential (either positive or negative depending on the state of the neuron) on every trial following stimulation but, because of opposite sign evoked responses over multiple trials, no significant changes in the mean membrane potential could be detected (**Figure 3.2C**). Therefore, I chose to measure the latency from stimulus onset to the peak rate of change of the membrane potential during the initial post stimulus response (**Figures 3.3A**, **3.3B**). This method allowed me to determine when the membrane potential changed in concert across trials relative to the stimulus, without relying on the amplitude of the mean response or the sign of individual responses.

Membrane potential responses to stimuli delivered to both ipsilateral and contralateral eyes were visually identifiable in all recorded neurons in V1m and V1b. Ipsilateral visually evoked responses in V1m had not been previously observed, as V1m was thought to respond directly only to stimuli presented to the contralateral eye. To better characterize these ipsilateral responses, I began by comparing the response onset latencies of stimuli presented to the two eyes

in V1m. Responses to ipsilateral eye stimulation were significantly delayed relative to contralateral eye stimulation, consistent with the interpretation that these responses were not evoked directly by feedforward ipsilateral thalamic afferents (**Figure 3.3A**). This result was observed across the entire populations of V1m neurons (Ipsilateral - Contralateral, 60.1 ± 4.2 ms; $n = 27$ (24); $p < 0.0001$) and when analysis was restricted to the population of excitatory neurons recorded from non-deprived animals (Ipsilateral - Contralateral, 60.3 ± 8.5 ms; $n = 5$ (6); $p < 0.001$).

To determine if the delayed response to ipsilateral flash stimuli was a general property of visual cortex or whether this delay was specific to V1m, I recorded from four excitatory neurons in V1b: The response onset latencies did not differ for responses evoked in V1b by contralateral and ipsilateral eye stimulation, consistent with the interpretation that both responses were driven by feedforward thalamic inputs (**Figure 3.3B**; Ipsilateral - Contralateral, 6.9 ± 6.1 ms; $n = 4$ (4); $p > 0.30$). Together these data indicate that responses to ipsilateral eye stimulation could be recorded in V1m neurons and that the delayed onset of ipsilateral responses was specific to V1m.

In order to ensure that contralateral eye responses in V1m were similar to those observed in V1b, I compared the response latencies between neurons recorded from V1m and V1b (**Figure 3.3C**). There was a trend towards slightly earlier responses to the contralateral eye in V1b when only excitatory neurons from non-deprived rats were considered (V1b, 42.5 ± 4.5 ms, $n = 4$ (4); V1m 55.9 ± 3.6 ms, $n = 11$ (10); $p = 0.06$); however, this trend disappeared when the whole population of V1m neurons was considered (V1b, 42.5 ± 4.5 ms, $n = 4$ (4); V1m 49.3 ± 2.1 ms, $n = 45$ (41); $p > 0.30$). Response latencies to ipsilateral eye stimulation were significantly shorter in V1b compared to V1m, when compared between non-deprived putative excitatory neurons only (V1b, 49.4 ± 3.9 ms, $n = 4$ (4); V1m 113.4 ± 7.0 ms, $n = 6$ (5); $p < 0.0005$) and the

entire population of V1m neurons (V1b, 49.4 ± 3.9 ms, n = 4 (4); V1m 108.4 ± 4.0 ms, n = 28 (25); $p < 0.005$).

The latencies to response onset that I observed were consistent between regions of visual cortex thought to be directly activated by visual stimuli presented to a given eye: On average, there was only a modest, non-significant difference in latencies between contralateral eye responses recorded in V1m and both contralateral and ipsilateral eye responses recorded in V1b. These response latencies were similar to, but slightly shorter than, the latencies previously observed in V1b in response to a noise stimulus (Ma et al., 2013). The mean latency to ipsilateral eye responses recorded in V1m, however, was more than twice as long as the other observed latencies. This result suggests that the ipsilateral flash responses observed in V1m have a different anatomical origin within the visual circuit than either the ipsilateral flash responses observed in V1b or contralateral flash responses.

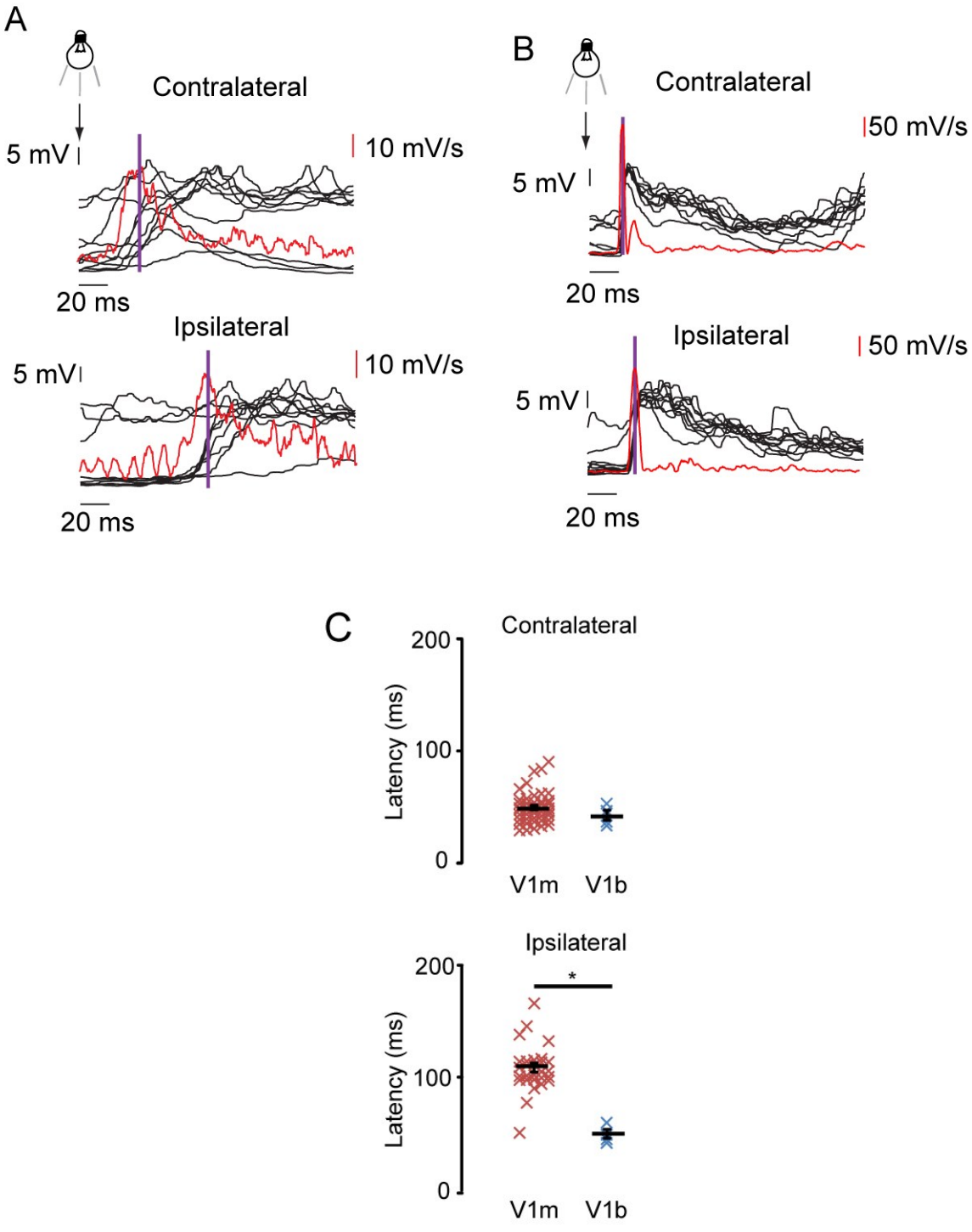

A
Contralateral
5 mV
10 mV/s
20 ms
Ipsilateral
5 mV
10 mV/s
20 ms
B
Contralateral
50 mV/s
5 mV
20 ms
Ipsilateral
50 mV/s
5 mV
20 ms
C
Contralateral
200
100
0
Latency (ms)
V1m
V1b
Ipsilateral
*
200
100
0
Latency (ms)
V1m
V1b

Figure 3.3. Whole-cell recordings from primary visual cortex excitatory and fast spiking neurons and their responses to flash stimuli. (A) Ten overlapped membrane potential responses (black) to flash stimuli presented to the contralateral (top) and ipsilateral (bottom) eyes from a V1m excitatory neuron. Action potentials were removed with a 5 ms median filter. The mean of the absolute value of the derivative of the action potentials is shown in red. The purple line indicates the time of the peak rate of change of the membrane potential during the stimulus response. The arrow indicates stimulus onset. **(B)** Same as in **A**, except traces are from a V1b excitatory neuron. **(C)** Latency to the peak rate of change of the membrane potential from the entire population neurons in response to stimulation of the contralateral (top; V1m: $n = 45$ (41); V1b: $n = 4$ (4); $p > 0.30$) and ipsilateral (bottom; V1m: $n = 28$ (25); V1b: $n = 4$ (4); $p < 0.005$) eye. Each × denotes the latency measured from an individual neuron (red: V1m; blue: V1b).

Action potential responses to flash stimuli

Immediately following the onset of the membrane potential response to flash stimuli, some neurons increased their action potential frequency, some were inhibited and some experienced no change in action potential frequency. Across experimental conditions and cell types, there was a considerable range of auROC response values, ranging from 0.37 to 1.00 for contralateral eye stimulation and 0.41 to 0.98 for ipsilateral eye stimulation. The auROC value for two of the five fast spiking neurons was in the top 15% of all contralateral responses recorded. The auROC value of one of the fast spiking neurons was in the top 20% of all ipsilateral responses recorded. One fast spiking interneuron was only observed to fire action potentials during current injection. This neuron did not fire any action potentials in response to either contralateral or ipsilateral stimulation, but did have a clear membrane potential response. Thus, fast spiking neurons had response properties that fell within the range of parameters of excitatory neurons. While, from my limited sample size, the response properties of fast spiking neurons fell within the range of responses observed for excitatory neurons, a larger sample is necessary to make comparisons. Because fast spiking neurons have been observed to have markedly different sensory response properties and exhibit distinct patterns of response plasticity compared to excitatory neurons (Griffen and Maffei, 2014), they have been excluded from further analysis.

Early ocular dominance plasticity of monocular visual cortex excitatory neurons

Previous work has shown that after two days of monocular deprivation, there is no change in the responsiveness of V1m L4 putative excitatory neurons to contralateral, patterned

visual stimuli (Iurilli et al., 2012). However, it is currently unknown whether monocular deprivation alters visual responses to brief flash stimuli, which activate the circuit more broadly (Stroh et al., 2013). Furthermore, the observation that neurons in V1m respond to flash stimulation of the ipsilateral eye, albeit with delayed onset, raises the possibility that brief visual deprivation may affect these newly identified responses.

To assess the effects of a brief manipulation of visual drive on V1 visual response properties, I first compared the effects of a 1 day monocular lid suture on neurons recorded from non-deprived animals and from the hemisphere contralateral to the open eye. There were no qualitative or statistically significant differences in auROC-ODI, contralateral eye responses or ipsilateral eye responses between V1m neurons recorded in untreated animals and neurons recorded from the hemisphere contralateral to the open eye (data not shown, p values ≥ 0.15). Therefore, these neurons were pooled into a Control group (n = 28 (24)). Next, I compared Control neurons with those recorded from the hemisphere contralateral to the deprived eye (the Deprived group; n = 13 (13)). The distribution of recording depths did not differ significantly between Control and Deprived groups (25-75%, median: Control, 460 – 680 μm, 520 μm; Deprived, 490 – 610 μm, 490 μm; $p > 0.75$). There was no difference between these groups in series resistance, action potential half-width, action potential threshold, or resting membrane potential (**Table 3.2**).

	Control	Deprived	*p* >
n **=**	28 (24)	13 (13)	
Series Resistance	56.1 ± 2.5 MΩ	54.7 ± 2.7 MΩ	0.70
Action Potential Threshold	-36.4 ± 0.8 mV	-34.8 ± 1.0 mV	0.25
Action Potential ½ Width	1.04 ± 0.04 ms	0.94 ± 0.04 ms	0.10
Resting Membrane Potential	-65.1 ± 1.3 mV	-64.6 ± 2.3 mV	0.85

Table 3.2. Biophysical properties of putative excitatory neurons.

Visual inspection of recordings obtained from V1m of the hemisphere contralateral to the deprived eye (Deprived) revealed an increase in the relative responsiveness of neurons to stimuli presented to the open eye relative to the closed eye. To determine whether monocular deprivation changed relative responsiveness of V1m excitatory neurons to contralateral versus ipsilateral flash stimuli, I analyzed the auROC-ODI, which can return values between 1.0 (maximal contralateral response, maximal ipsilateral inhibition) and -1.0 (maximal contralateral inhibition, maximal ipsilateral response). I found that just one day of monocular deprivation was sufficient to shift the responsiveness of Deprived neurons towards the open, ipsilateral, eye (**Figures 3.4A**, **3.4B**, **3.4C**; Control, 0.14 ± .03, n = 16 (13); Deprived, -0.11 ± 0.08, n = 8 (8); $p < 0.05$).

A shift in the ODI could result from an increase in responsiveness to one eye, a decrease in responsiveness to the other eye or differential changes in responsiveness to both eyes. To investigate which of these possibilities might explain the shift that I observed, I compared contralateral and ipsilateral eye responses between the Control and Deprived groups. Consistent with previous reports (Iurilli et al., 2012), a brief period of monocular deprivation had no effect on the responses of V1m excitatory neurons to stimuli delivered to the contralateral (closed) eye (**Figure 3.4D**; Control, 0.58 ± 0.03, n = 28 (24); Deprived, 0.60 ± 0.05, n = 12 (12); $p > 0.75$). For responses to ipsilateral eye stimulation, there was a trend toward an increase in responsiveness in neurons in the hemisphere contralateral to the closed eye (**Figure 3.4E**; Control, 0.58 ± 0.04, n = 16 (13); Deprived, 0.71 ± 0.06, n = 9 (9); p = 0.07). These results suggest that the observed switch in ocular dominance is driven by changes in responsiveness to the open eye, rather than to the closed eye.

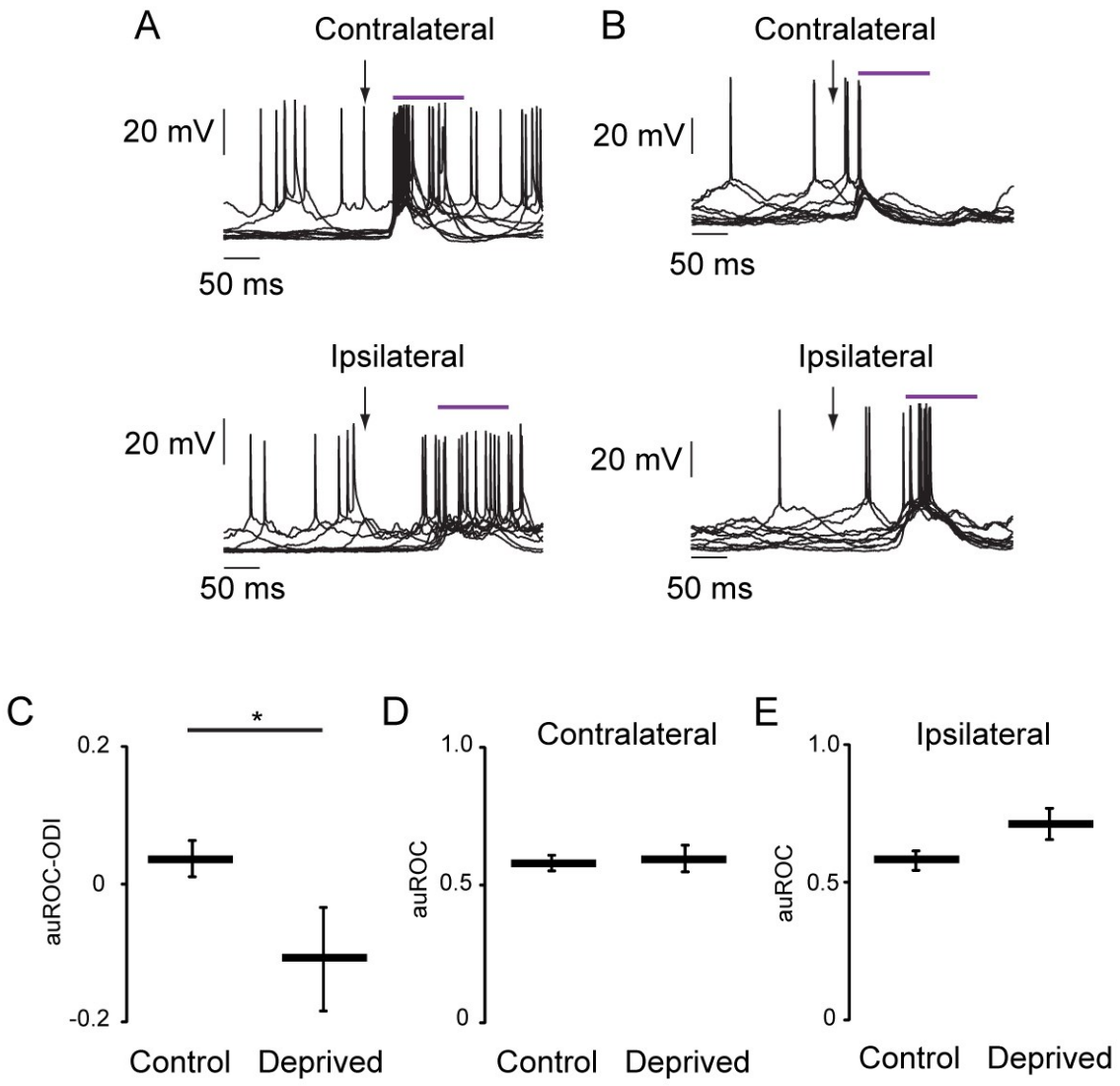


Figure 3.4. Rapid ocular dominance plasticity in monocular visual cortex. (A) Ten overlapped responses (black) to flash stimuli presented to the contralateral (top) and ipsilateral (bottom) eye from a representative Control V1m excitatory neuron. Arrow indicates stimulus onset. Purple bar indicates the 100 ms time window during which spiking responses were recorded. **(B)** Same as in **A**, except traces are from a Deprived neuron with more ipsilateral dominant responses than was observed in any Control neuron. **(C)** auROC-ODI for Control and Deprived V1m excitatory neurons (Control: n = 16 (13); Deprived: n = 8 (8); *$p < 0.05$). **(D)** Responses to contralateral eye stimuli for Control and Deprived V1m excitatory neurons (Control: n = 28 (24); Deprived: n = 12 (12); $p > 0.75$). **(E)** Responses to ipsilateral eye stimuli for Control and Deprived V1m excitatory neurons (Control: n = 16 (13); Deprived: n = 9 (9); p = 0.07).

Discussion

I characterized the membrane potential and spiking responses of neurons in primary visual cortex to brief flashes of light presented independently to either eye. I report here, for the first time, electrophysiological responses of V1m neurons to stimuli presented to the ipsilateral eye. Finally, I show plasticity of visual responses in V1m that occurs shortly after the onset of visual deprivation when ocular dominance was thought to remain unchanged.

Previously, responses to brief light flashes had only been observed using recording methods that sampled the membrane potential or intracellular calcium concentration over a large, heterogeneous population of visual cortical neurons (Uhlrich et al., 2005; Stroh et al., 2013). In the experiments reported here I found that at the level of individual neurons, there is considerable response heterogeneity to flash stimuli. This was especially true for spiking responses, with some neurons being excited by flash stimuli, some inhibited and some unaffected. Despite the heterogeneity, all recorded neurons responded to visual stimulation with membrane potential changes at consistent latencies over multiple trials.

What visual pathway is responsible for driving the flash stimuli responses that I have observed? One explanation for these results is that contralateral flash responses seen throughout visual cortex and ipsilateral flash responses within V1b may be driven by direct thalamic input. However, flash stimuli led to population responses in V1 before single unit responses were observed in the LGN (Stroh et al., 2013). This result was interpreted to mean that very few thalamic relay neurons are responsible for carrying flash responses to cortex (Stroh et al., 2013). It is also possible that flash responses are carried to V1 via pathways that do not involve the LGN. Following flash stimuli, multi-unit responses have been recorded in the ferret superior

colliculus with mean onset latencies faster than any single neuron membrane potential responses that I observed in rat V1 (Stitt et al., 2013). The differences in latency may depend on the fast activation of a tectothalamocortical pathway by flash stimuli (Zagorul'ko and Lukanidina, 1978; Stitt et al., 2013; Stroh et al., 2013).

V1m is not thought to be driven by ipsilateral visual stimuli via direct monosynaptic input from the thalamus (Tagawa et al., 2005; Coleman et al., 2009). However, my data show that stimuli to the ipsilateral eye consistently evoke responses in V1m neurons. These responses had a longer latency than responses evoked by flashes to the contralateral eye. Within V1b, responses to stimuli delivered to the contralateral and ipsilateral eye had similar latencies. Based on these response properties, I propose that within V1m contralateral and ipsilateral eye inputs have different anatomical origins. One attractive hypothesis to explain the delayed ipsilateral flash responses in V1m is that cortico-cortical projections from V1b to V1m drive the delayed response. This hypothesis is supported by the observation that flash stimuli could evoke calcium waves that spread across the cortex (Stroh et al., 2013). It is also possible that delayed ipsilateral responses may be generated through inter-hemispheric, cortico-thalamocortical or even cortico-tectal interactions.

Unexpectedly, I found that spiking responses of the population of Control V1m neurons and V1b neurons did not exhibit a strong ocular dominance bias towards the contralateral eye. Visually evoked potentials have an approximately 1.6:1 ratio in the strength of responses to contralateral versus ipsilateral stimuli in urethane anesthetized juvenile rats (Pietrasanta et al., 2014). This discrepancy may be due to the differences in the pathways involved in the generating flash responses compared to those responsible for responses to patterned visual stimuli.

Interestingly, I found that following one day of visual deprivation, neurons in the deprived hemisphere exhibited a shift in their ocular dominance to favor the ipsilateral, open eye in V1m. Ocular dominance shifts have been observed following one day of deprivation outside of L4 in cat V1b (Trachtenberg et al., 2000) and after two days in L4 of rat V1b (Iurilli et al., 2012). The early phase of ocular dominance shifts is currently thought to be driven by a decrease in responsiveness to deprived eye stimulation (Smith et al., 2009); however, the early ocular dominance shift that I have observed is expressed primarily as a potentiation of responsiveness to the open eye. What could underlie a potentiation of open eye, ipsilateral inputs to V1m? One reported mechanism could explain my findings. In L2/3 of mouse V1b, one day of monocular lid suture leads to a potentiation of visual responses in the hemisphere contralateral to the deprived eye (Kuhlman et al., 2013). If the ipsilateral activation of V1m by flash stimuli is secondary to activation of V1b, then an increased responsiveness of V1b could lead to increased ipsilateral responses in V1m. The previously observed rapid increase in responsiveness within V1b following one day of monocular deprivation was attributed to a weakening of inhibitory drive onto pyramidal neurons (Kuhlman et al., 2013). Disinhibition has been proposed as a mechanism for increased responsiveness soon after the onset of sensory deprivation in both visual and barrel cortices (Kuhlman et al., 2013; Li et al., 2014). My data show that some fast spiking interneurons respond robustly to flash stimuli. The membrane potential response latencies of the recorded fast spiking neurons were similar to those of pyramidal neurons, and fast spiking neurons responded to both ipsilateral and contralateral stimuli. From my small sample of fast spiking neurons, it is not possible to determine whether their responses are altered by deprivation or if they play a role in the ocular dominance plasticity of excitatory neurons.

A second possible explanation for ocular dominance plasticity in V1m is that the inputs to V1m that drive the ipsilateral response are rapidly potentiated. Monocular deprivation can change the horizontal spread of circuit activation within V1m (Wang et al., 2011). Similarly, strabismus, which also causes changes in ocular dominance, can rapidly alter the morphology of the horizontal projections of L2/3 neurons (Trachtenberg and Stryker, 2001). Whether horizontal projections from V1m to V1b are altered following a single day of monocular deprivation remains untested.

If flash stimuli activate V1 through tectal pathways, the visual pathway involving the superior colliculus could play a role in the plasticity that I have observed. Very little is known about the possible role of the superior colliculus in monocular lid suture induced plasticity in cortex; however, lid suture can modulate activity in the superior colliculus. Metabolic activity in the superior colliculus is reduced by diffuse light stimulation compared to either patterned visual stimulation or darkness (Rooney and Cooper, 1988). Monocular lid suture disrupts normal developmental patterns of synaptogenesis (Lund and Lund, 1972), alters receptive field properties and leads to changes in ocular dominance within the super colliculus (Wickelgren-Gordon, 1972; Hoffmann and Sherman, 1974; Berman and Sterling, 1976; Fox et al., 1978).

The function of rapid ocular dominance plasticity within V1m is not clear. Competitive interactions between LGN inputs to cortex are thought to drive the weakening of deprived eye LGN afferents following monocular deprivation in V1b: Binocular lid suture, which affects both eyes similarly, does not significantly weaken visually evoked potentials after a week (Frenkel and Bear, 2004). Differently, monocular deprivation leads to a weakening of thalamic inputs to V1m (Wang et al., 2013b) and a decrease in responsiveness to contralateral visual stimulation (Iurilli et al., 2012). These changes have not been reported to occur until after the onset of ocular

dominance plasticity in V1b and have been attributed to a non-competitive mechanism (Iurilli et al., 2012). My data show that following monocular deprivation, the inputs driving ipsilateral responses in V1m are strengthened. This strengthening could increase competition between inputs from the open and deprived eyes. Instead of competition between LGN inputs, competition between the inputs driving ipsilateral eye responses in V1m and contralateral LGN afferents could drive plasticity at V1m thalamocortical synapses. This competitive mechanism could promote the expansion of the portion of V1 driven by the open eye over time (Tagawa et al., 2005).

I have shown that in addition to responding to contralateral eye stimuli, neurons in V1m respond consistently to stimulation of the ipsilateral eye. The delay to contralateral and ipsilateral eye responses is significantly different within V1m, suggesting that ipsilateral and contralateral stimuli engage different circuits. This finding is consistent with previous reports that flash stimuli evoke propagating cortical activity (Stroh et al., 2013). In addition, I found that brief monocular deprivation induces rapid ocular dominance plasticity in V1m. Differently from what has been proposed in V1b, my data suggest that ocular dominance plasticity in V1m is expressed as a rapid increase in responsiveness to the ipsilateral eye. This shift to favor the open eye may foster the expansion of the circuit driven by the open eye and favor competition between inputs even in a region of V1 predominantly driven by the contralateral eye (Tagawa et al., 2005).

Chapter IV

Conclusions and Future Directions

General Conclusions

I have provided evidence for the existence of complex changes in circuit activation of the monocular region of rat primary visual cortex during early postnatal development. With the first set of data, I have shown that visual cortex proceeds through a set of sequential changes that alter the spatio-temporal patterns of cortical circuit activation. These changes occur over the course of normal development, during the time when visual response properties are maturing. With the second set of experiments, I found that an alteration of visual drive during a defined window in development very rapidly changes the response properties of cortical neurons. Taken together, these experiments provide evidence that patterns of cortical circuit activation in V1m mature during healthy development, can be influenced by visual input to both eyes and are susceptible to a shorter period of visual deprivation than was previously thought.

Outstanding Questions Following Chapter II

Perhaps the most interesting and pertinent question following from the experiments described in **Chapter II** was addressed in a manuscript published just prior to Griffen et al., 2013 (adapted in **Chapter II**): How does monocular deprivation affect the spatio-temporal spread of cortical circuit activation? Brief periods of monocular deprivation (two and four days) reduce both the vertical (interlaminar) and horizontal spread of circuit activation following monocular deprivation (Wang et al., 2011). This reduction in cortical excitability is largely accounted for by a strengthening of inhibition.

The experiments described in **Chapter II** were specifically designed to bypass thalamocortical inputs to visual cortex; however, these projections likely change over development as well. It would be interesting to repeat the experiments described in **Chapter II**, substituting direct thalamic stimulation for the L4 electrical stimulation. Several technical limitations make this experiment difficult. Due to the complex three dimensional anatomy of the thalamocortical pathway between the LGN and V1, preserving thalamic neurons, including their cell bodies and complete axons, and visual cortex in a single acute slice is extremely difficult. Stimulation of white matter tracts has been used to approximate direct thalamic stimulation; however, there is likely considerable contamination with other inputs when using this technique. In theory, expression of light gated ion channels specifically in the LGN could be used to allow for the selective stimulation of thalamic afferents in visual cortex. This could be combined with a class of VSDs sensitive to the infrared portion of the electromagnetic spectrum. The temporal relationship between photoactivation and image acquisition would need to be carefully synchronized to allow for effective signal detection. Perhaps more importantly, the length of the incubation time between injection of the viral vectors used to express light gated channels and

their robust expression makes performing experiments on pre-sensitive period rats extremely difficult at this time.

Across development, I observed a substantial decrease in the contribution of NMDA receptors to cortical signal propagation. However, several studies have identified NMDA receptor activation as acutely required for the induction of ocular dominance plasticity. While, the presumed locus for this plasticity is thalamocortical terminals and the developmental reduction in NMDA receptor medicated circuit spread was observed in a preparation that contains only a minor contribution from the activation of thalamic terminals. The possibility that there is a developmental regulation of thalamocortical NMDA receptors and that this contributes to sensitive period regulation cannot be excluded. To address this, the acute visual cortical slice preparation could be combined with optogenetic stimulation of thalamic afferents (Wang et al., 2013b), pharmacological isolation of the feedforward NMDA receptor mediated component of thalamocortical drive and whole-cell recordings from L4 pyramidal neurons during and after the sensitive period.

A fourth question is whether and how cortical signal propagation changes during the development of other sensory cortices. Sato and colleagues performed similar VSD imaging experiments to those reported here with acute slices of insular and barrel cortices prepared from P15-22 and P18-22 rats, respectively (Sato et al., 2008). They reported several differences in signal propagation between these two regions. This result is not surprising, given that the region of insular cortex studied lacks a granular layer and is thus structurally very different from barrel cortex. However, the development of different cortical regions does not necessarily occur in parallel. It would be interesting to know whether and how specific features of the development of signal propagation through cortical circuits are conserved across regions and what features may

be truly region specific. It is possible that some of the interregional differences reported by Sato and colleagues are absent in the mature cortex but observed in juveniles because the age range used samples two cortical regions at different stages of development (Sato et al., 2008). As discussed in **Chapter II**, signal propagation through visual cortex at the ages studied shares some features with those reported in insular cortex and some features with those reported in barrel cortex (Sato et al., 2008).

Outstanding Questions Following Chapter III

To directly determine whether a single day of monocular visual deprivation increases the spread of cortical activation in V1b, VSD imagining experiments similar to those described in **Chapter II** and by Wang and colleagues could be undertaken in V1b following a single day of visual deprivation (Wang et al., 2011). Based on my results and those of Kuhlman and colleagues, I hypothesize that after only one day of visual deprivation, the hemisphere contralateral to the deprived eye would be more sensitive to electrical stimulation (Kuhlman et al., 2013). Further, I hypothesize that the horizontal spread of activation following stimulation would increase. This would provide evidence supporting increased horizontal spread of activity as mechanism for V1m ocular dominance plasticity.

The question of whether the rapid potentiation of L4 fast spiking inhibitory synapses following monocular visual deprivation plays a direct role in altering visual responses to environmentally relevant stimuli remains unanswered. This is likely in large part due to the fact that the role of these neurons in shaping visual responses is stimulus dependent, largely unknown and hotly debated (see Griffen and Maffei, 2014). An attractive possibility exists that a brief period of visual deprivation could narrow orientation tuning within visual cortex via an inhibitory mechanism, a possibility that has never been tested. To date, experiments examining inhibition following monocular lid suture have only examined visual responses to optimal patterned stimuli, white noise or brief flashes of light (in the present work).

Some inhibition dependent effects of monocular deprivation may not be measurable in anesthetized preparations. Recent work has shown that, at least in L2/3, visual responses in awake mice actively sensing visual stimuli are dominated by inhibitory conductances, while responses in the anesthetized animal are comprised of approximately balanced excitatory and

inhibitory conductances. Thus, the ideal experiment would be to determine whether brief visual deprivation alters visual responses to a broad range of stimuli in an awake animal using voltage clamp to identify the excitatory and inhibitory components of the response. However, even this extremely technically demanding approach is limited by its ability to only resolve only somatic excitation and inhibition. Thus, even the most technically advanced experiments presently available may fail to draw definitive conclusions.

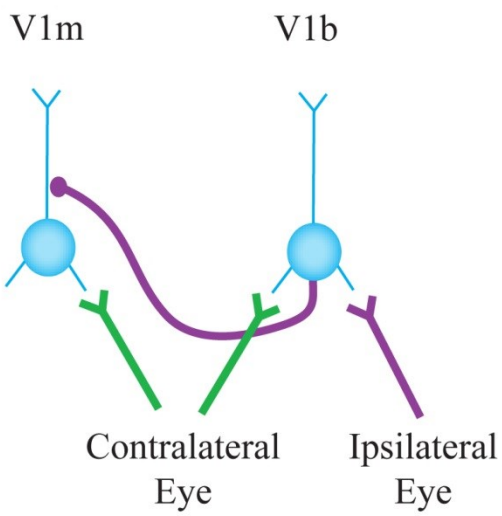
Other possibilities exist to explain the observed potentiation of inhibition following monocular deprivation. The potentiation of inhibition can, through intracellular signaling mechanisms, alter the outcome of long term plasticity induced at excitatory synapses (Wang and Maffei, 2014). Therefore, the primary purpose of inhibitory plasticity may be to alter how cortical activity is translated into plastic changes. This could explain why the maturation of inhibition regulates sensitive period timing and why disruption of inhibitory function disrupts ocular dominance plasticity (Heimel et al., 2011; Griffen and Maffei, 2014). Alternatively, the plasticity at fast spiking to pyramidal neuron synapses might be reversed so quickly by visual experience that even the minimal exposure to light during experiments between removing lid sutures and recording prevents its observation.

Revised Model of Rapid Ocular Dominance Plasticity

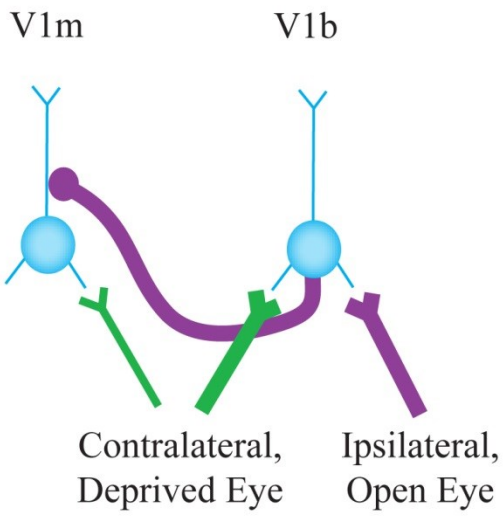
The results that I have presented in **Chapter III**, when combined with those of Tagawa and colleagues present an important and critical challenged to the interpretation of experiments examining the effects of lid suture on V1m (Tagawa et al., 2005). Tagawa and colleagues developed a method to induce expression of the immediate early gene Arc within mouse visual cortex following selective stimulation of one eye (Tagawa et al., 2005). In older animals, they found that visual stimulation primarily led to Arc expression in the entire contralateral V1 and ipsilateral V1b, although some Arc positive neurons were seen beyond ipsilateral V1b. Interestingly, they found that as late as P22, ipsilateral eye stimulation induced a strong Arc translational response in V1m (Tagawa et al., 2005). These results, coupled with my findings, suggest that the "monocular" region of visual cortex may be influenced by certain types of visual input to both eyes, especially early in development. Most studies of visual function have used stimuli that had equal net luminance between pre- and post-stimulus conditions, while the experiments presented here used a bright, transient stimulus presented during near darkness. Tagawa and colleagues stimulated Arc expression by exposing an animal that had been in complete darkness to a lighted environment (Tagawa et al., 2005). Therefore, large changes in luminance may affect V1 differently than patterned stimuli.

The activation of V1m by the ipsilateral eye likely occurs through a distinct pathway compared to the activation of V1m by the contralateral eye; however, it can still modulate activity in V1m neurons. Therefore, even within V1m, inputs to cortical neurons carrying information from both the contralateral and ipsilateral eyes may be able to compete. Based on my results and those of others (Tagawa et al., 2005; Iurilli et al., 2012; Kuhlman et al., 2013; Stroh et al., 2013), I propose a new model of plasticity within V1m following monocular lid

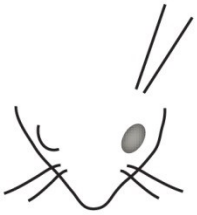
suture (**Figure 4.1**): Shortly after monocular lid suture, V1b becomes more responsive to stimuli presented to both eyes independently (Kuhlman et al., 2013). This results in an increase in responsiveness to ipsilateral eye stimuli that activate V1m secondary to V1b activation (**Chapter III**, Stroh et al., 2013). The increased responsiveness to ipsilateral eye stimulation within V1m enhances competition between open eye and deprived eye inputs within V1m, ultimately leading to a reduction in deprived eye responses (Iurilli et al., 2012) and an expansion of the cortical area responsive to the open, ipsilateral eye.

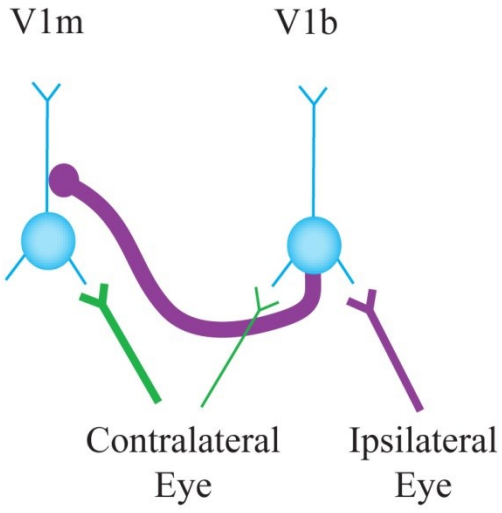


No Deprivation

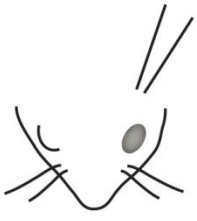


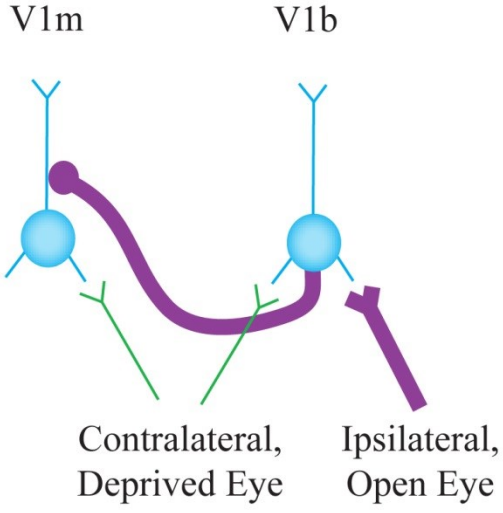
One Day Deprivation





Two Day Deprivation





Long Term Deprivation

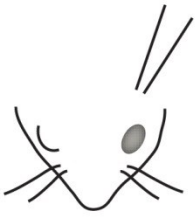


Figure 4.1. Revised model of ocular dominance plasticity following lid suture. Schematics showing example neurons in V1m and V1b receiving inputs from the contralateral (green) and ipsilateral (plum) eyes. Schematics represent an un-manipulated V1 (top left) and a V1 in the hemisphere contralateral to a deprived eye (top right, bottom left, bottom right). According to my revised model of ocular dominance plasticity: One day after monocular deprivation by lid suture (top right), responses to both eyes are enhanced in V1b, which leads to enhanced ipsilateral, open eye responses in V1m through V1b. Next, contralateral, deprived eye responses are selectively reduced in V1b (bottom left). Finally, after a long period of visual deprivation ipsilateral, open eye responses are potentiated and contralateral, deprived eye responses are depressed throughout V1.

Final Remarks

Sensitive period monocular lid suture leads to many complex changes in cortical circuits and visual response properties that are not limited to ocular dominance shifts. The mechanisms of ocular dominance plasticity have been studied extensively; however, much remains to be uncovered. With the experiments described here, I showed two changes in the activation of the visual cortical circuit, one that occurs during normal, healthy development and one that occurs in response to a pathological disruption of visual experience.

Manipulations that change the timing of the sensitive period for ocular dominance plasticity alter receptive field properties (Griffen and Maffei, 2014). Pharmacologically enhancing inhibition with benzodiazepines early in development causes a precocious sensitive period and disrupts binocular matching of orientation preference in complex cells (Wang et al., 2010; Wang et al., 2013a), widens the spacing of orientation columns and disrupts direction selectivity (Hensch and Stryker, 2004). These results suggest that the timing of the sensitive period with respect to the development of the visual circuit may be important for the development of receptive field properties. Perhaps the coordination of non-pathological changes in cortical circuit function, such as those that I observed using VSD imaging, and the availability of mechanisms for plasticity during the sensitive period must simultaneously occur for the proper development of the visual system.

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