THE ROLE OF PV NEURONS IN CORTICAL PLASTICITY DURING DEVELOPMENT OF MOUSE VISUAL CORTEX

by

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ABSTRACT: ON THE ROLE OF PV NEURONS IN CORTICAL PLASTICITY DURING DEVELOPMENT OF MOUSE VISUAL CORTEX

Understanding plasticity and learning has been a fundamental question of neuroscience for decades. The visual cortex is a classical system for studying plasticity specifically using the paradigm of ocular dominance plasticity (ODP) in which it is seen that brief periods of monocular deprivation (MD) as short as three days can cause changes in the relative responsiveness of excitatory neurons to stimulation of both eyes. Prior to these shifts in responsiveness of excitatory neurons, monocular deprivation causes a rapid reduction of firing rates in parvalbumin-expressing (PV) inhibitory neurons which is causally linked to the reorganization of excitatory networks following the perturbation. Converging evidence suggests that deprivation, not an imbalance between eye inputs triggers the rapid plasticity in PV neurons. This has not been directly tested in vivo, however. Using two-photon guided cell-attached recording we examined the impact of closing both eyes for 24 hours on PV neuron response properties in primary visual cortex of mouse. We found that binocular deprivation causes a 30% reduction in stimulus-induced mean evoked firing rate, similar to the decrease in PV neuron firing seen following 24 hours of monocular deprivation.

This rapidly induced decrease in stimulus-induced mean evoked firing of PV neurons is specific to the critical period as it is not seen in post critical period aged mice. Whereas stimulus-induced mean evoked firing rate of PV neurons changes under perturbation back towards immature firing rates for PV neurons, other simultaneously developed properties, namely tuning properties do not significantly revert following deprivation. Unlike evoked mean firing rate, measurements of trial-to-trial variability

revealed that stimulus-induced decreases in variability are significantly dampened by deprivation during both the critical period and the post critical period. These data establish that open-eye inputs are not required to drive deprivation-induced weakening of PV neuron evoked activity and that aspects of PV neuron response properties are malleable throughout life.

This paradigm of binocular deprivation, although not useful for eliciting ODP (Frenkel & Bear, 2004), can still elicit the same effects on PV neurons as monocular deprivation, namely the 30% decrease in stimulus-evoked firing rate. Binocular deprivation is therefore a useful paradigm for the identification of molecular mechanisms that couple visual experience to postnatal regulation of PV neuron firing rate without complications such as inputs of callosal projections which can innervate primary visual cortex (Métin, Godement, & Imbert, 1988). We used this paradigm of 24 hours binocular deprivation to test the necessity of ErbB4 for the development and regulation of PV neuron firing. Indeed we found that ErbB4 is necessary for PV neurons to develop their normal stimulus-evoked firing rate. In addition, PV neurons lacking ErbB4 lose their ability to decrease their responses following deprivation. This decreased inhibition from PV neurons lacking ErbB4 is especially interesting since ERBB4 is a schizophrenia risk factor and schizophrenics have been found to have a deficit of inhibition within primary visual cortex (Yoon et al., 2009, 2010).

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Table of Contents

1.0 Introduction......1

1.1 Visual cortex: sensory pathway, architecture, and cortical circuitry......1

1.1.1 Stimuli: from eye to cortex......1

1.1.2 Rodent visual cortex organization......2

1.1.3 Visual cortex circuitry......4

1.1.4 Inhibitory neurons within rodent visual cortex......6

1.2 Maturation of inhibitory PV neurons......8

1.2.1 Origins of PV neurons......8

1.2.2 Development of PV neurons......10

1.2.3 Roles of PV neurons within the circuit......13

1.3 Experience is required for proper maturation of PV neurons......19

1.3.1 The effects of experience on structure......19

1.3.2 The effects of experience on synaptic physiology......22

1.3.3 The effects of experience on intrinsic physiology......25

1.3.4 The effects of experience on *in vivo* response properties......28

1.4 Inhibition beyond the critical period......32

1.4.1 Adaptability of mature PV neurons......32

1.4.2 Types of adult plasticity......34

1.4.3 Clues from outside the cortex......38

1.5 Pathophysiology of improper inhibition......40

1.5.1 Diseases linked to improper inhibition......40

1.5.2 The role of ErbB4 in proper inhibition......45

1.6 Goals of this dissertation and summary of findings......49

2.0 Binocular deprivation induces both age-dependent and age-independent forms of plasticity in parvalbumin inhibitory neuron visual response properties......60

2.1 Abstract......60

2.2 Introduction......61

2.3 Materials and Methods......63

2.3.1 Animal preparation and surgery......64

2.3.2 In vivo cell-attached recording......65
2.3.3 Visual stimulation...... 67
2.3.4 Eye shutterying......67
2.3.5 Data analysis and statistics...... 68
2.4 Results...... 70
2.4.1 Brief binocular deprivation induces a reduction of evoked PV neuron firing rate...... 70
2.4.2 Brief deprivation during the critical period increases percentage of tuned PV neurons...... 72
2.4.3 PV neurons exhibit a stimulus-induced reduction in variability of spike times...... 75
2.4.4 The stimulus-induced reduction in Fano Factor is sensitive to deprivation throughout life...... 76
2.5 Discussion......78
2.5.1 Deprivation-induced rapid plasticity of PV neurons does not require open eye inputs...... 78
2.5.2 Deprivation induces a decrease in spike time variability during spontaneous but not visually evoked epochs, throughout life...... 82
3.0 Binocular deprivation induced plasticity of L2/3 parvalbumin inhibitory neurons is dependent upon ErbB4...... 93
3.1 Abstract......93
3.2 Introduction...... 94
3.3 Materials and Methods......97
3.3.1 Animal preparation and surgery...... 97
3.3.2 In vivo cell-attached recording......99
3.3.3 Visual stimulation...... 100
3.3.4 Data analysis and statistics...... 101
3.4 Results...... 103
3.4.1 ErbB4 necessary for the proper development of PV neuron evoked firing rates......103

3.4.2 Binocular deprivation induced reduction of PV neuron evoked firing rate is dependent upon ErbB4 receptor tyrosine kinase.............. 105
3.4.3 Spontaneous firing rates of PV neurons are still affected by deprivation even in the absence of ErbB4....................................105
3.4.4 Stimulus-induced reduction in Fano factor diminished in PV neurons lacking ErbB4.. 106
3.5 Discussion..107
3.5.1 ErbB4 is necessary for the normal development and plasticity of PV inhibitory neurons...108
3.5.2 ErbB4 affects stimulus induced reduction in Fano factor............. 110
4.0 Discussion..116
4.1 Differentiating role of PV neurons in ODP.. 116
4.2 Dissociation of regulation of firing rate and tuning properties in PV neurons.. 120
4.3 ErbB4 necessary for normal development and plasticity of PV neurons..... 123
4.4 Altered inhibition, ErbB4, and implication for disease..................................127
5.0 References…..134

Index of Tables & Figures

Figure 1.1 NRG1 and ErbB4 strengthen and maintain synapses in an activity dependent manner.......... 57

Table 1.1 Summary of effects of deprivation on rodent visual cortex.......... 58-59

Figure 2.1 Open-eye inputs are not required for rapid plasticity of visually evoked responses in PV neurons.......... 84

Figure 2.2 Deprivation reduces PV neuron evoked responses at all orientations.......... 85

Figure 2.3 PV neuron orientation tuning sharpens slightly yet remains broad in bV1 following brief deprivation.......... 86

Figure 2.4 Deprivation-induced sharpening of PV neuron orientation tuning is age-restricted.......... 87

Figure 2.5 Orientation selectivity indices plotted as a function of evoked firing rates.......... 88

Figure 2.6 PV neurons are more tuned within monocular than binocular zone of primary visual cortex.......... 88

Figure 2.7 Deprivation-induced suppression of PV responsiveness is age-restricted.......... 89

Figure 2.8 Spike time variability during non-stimulus epochs is reduced by deprivation.......... 90-91

Figure 2.9 Two alternative models for the role of PV neurons in ODP.......... 92

Figure 3.1 PV neurons lacking ErbB4 show 25% reduction in evoked firing rates.......... 112

Figure 3.2 ErbB4 is necessary for rapid decrease in PV neuron evoked firing rate following 24 hours of binocular deprivation.......... 113

Figure 3.3 Binocular deprivation still decreases spontaneous firing rate even in the absence of ErbB4.......... 114

Figure 3.4 Post-stimulus reduction in spike time variability reduced in the absence of ErbB4.......... 115

Figure 4.1 Binocular deprivation causes sustained decrease in PV neuron firing.......... 133

1.0 Introduction

1.1 Visual cortex: sensory pathway, architecture, and cortical circuitry

1.1.1 Stimuli: from eye to cortex

Our eyes immediately come to mind when we think of seeing, and certainly they play an essential role in the process, but much of what we think of as seeing is actually accomplished by our brains. The process of seeing involves many steps in order to transmit signals from the eyes to our brains where they can be interpreted. Initially, sight begins with photons entering the eye through the cornea, passing through the aqueous humor and pupil to reach the lens. From the lens these photons are directed through the vitreous humor before finally contacting the first light sensitive neurons in the retina. The retina is where photons are first transformed into an electrical signal which is then transferred to the brain. The retina is composed of five basic classes of neurons: photoreceptors, bipolar cells, ganglion cells, horizontal cells, and amacrine cells (Purves et al., 2012). Although the horizontal and amacrine cells play essential roles in vision, they will not be discussed in detail. The photoreceptor cells are further divided into two classes of cells the rods and cones. Named after their physical shapes, each of these types of photoreceptors has specialized roles in regards to seeing. Rods have extreme sensitivity to light, but low spatial resolution. Cones, on the other hand, have high spatial resolution, but are fairly insensitive to light. Photoreceptors are responsible for transforming the photons received into electrical signal to be carried elsewhere in the central nervous system. The flow of information passes from photoreceptor cell to bipolar cell to ganglion cells, the axons of which form the optic nerve and transport the signals out of the eye.

The optic nerve runs from the back of the eyeball to the optic chiasm where a majority of the axons cross the chiasm to innervate the opposite hemisphere; whereas, some continue on to the same side from which they originated. Once past the optic chiasm, the ganglion cell axons form the optic tract which now contains axons originating from both eyes. The primary target of the optic tract is the dorsal lateral geniculate nucleus (dLGN) of the thalamus. From the dLGN the signals then get relayed on to the primary visual cortex (V1).

Within V1, inputs first arrive to layer 4 before continuing onto layers 2/3. The properties of the inputs from the thalamus to layer 4 are still an active area of research. Initially it was thought that untuned inputs came into layer 4 where they were then transformed such that neurons had preference. This idea has been challenged more in recent years, however. Several studies have found that in mice both orientation and direction tuned inputs come from dLGN to V1 (Niell, 2013; W. Sun, Tan, Mensh, & Ji, 2015). Much is still unknown of exactly how these tuned inputs may contribute to the computations made in visual cortex. The tuned neurons in dLGN do not get their tuning from reciprocal feedback from visual cortex (Lien & Scanziani, 2013; Zhao, Chen, Liu, & Cang, 2013), but there is reciprocal connectivity (Sillito, Cudeiro, & Jones, 2006).

1.1.2 Rodent visual cortex organization

A property shared fairly ubiquitously across mammals is that inputs from the dLGN go to layer 4 of V1 before going to layers 2/3, but other aspects of cortical organization vary more from species to species. In carnivores (some of the more classically studied species being cats, ferrets, and monkeys) V1 is organized into columns where neurons with similar preferred orientations and ocular dominance

properties are located close to each other. This sort of functional architecture does not exist in rodents, however (Espinosa & Stryker, 2012). Instead of being organized into ocular dominance columns, neurons with different orientation preferences and different eye preferences are scattered apparently at random (Ohki, Chung, Ch'ng, Kara, & Reid, 2005). Rodents do still exhibit a precision to the organization of receptive fields which indicates that the connections made in V1 are specific at the level of single cells (Ko et al., 2011). This suggests that the mechanisms of development and plasticity should be similar or identical despite the difference in organization.

Indeed, three stages of development do seem to remain quite similar across species which are the formation of topographic maps, development of orientation selectivity in V1 neurons, and binocular matching (Espinosa & Stryker, 2012). The first stage, the formation of topographic maps, happens prior to eye opening and consists of projections from the dLGN forming high-resolution point-to-point connections with neurons in layer 4 of V1. This is accomplished through a combination of molecular signaling and spontaneous neural activity. The second stage of development in V1, the development of orientation selectivity in neurons, occurs right around the time of eye opening, but surprisingly, spontaneous activity is sufficient to drive it. Vision is not actually required. The third stage, when binocular matching occurs, happens during the critical period, a stage in which experience is highly important and changes in experience can lead to lifelong changes in circuitry.

V1 is the first cortical stop for information coming from the eyes, but it is far from the last stop. In primates and humans, information seems to be broken into ventral and dorsal streams, each of which play differing roles in perception of visual stimuli. Often

times the ventral stream is thought of as the “what” stream whereas the dorsal stream is thought of as the “where” stream. In mice, this process is still not as well understood. It has been suggested that areas LM (lateromedial) and AL (anterolateral) which are the principal targets of V1 (Quanxin Wang & Burkhalter, 2007) may act as the gateways to the ventral and dorsal streams respectively (Q. Wang, Gao, & Burkhalter, 2011). At least nine higher visual areas have been found surrounding V1 which are LM, LI (laterointermediate), AL, RL (rostrolateral), A (anterior), AM (anteromedial), PM (posteromedial), P (posterior), and POR (postrhinal) and seven of these (all except POR and LI) directly share borders with V1 (Marshel, Garrett, Nauhaus, & Callaway, 2011). Higher areas preferentially respond to specific features of stimuli such as spatial frequency, temporal frequency, and speed (Glickfeld, Andermann, Bonin, & Reid, 2013; Marshel et al., 2011).

1.1.3 Visual cortex circuitry

Within the visual cortex exist circuits at various scales. Microcircuits as well as larger circuits, local connections as well as inputs from other layers and brain areas all exist together. Connections to other higher visual areas as well as feedback from those areas also occur. All of these circuits combine to contribute various aspects of vision as we know it.

One series of papers nicely addresses the formation and connectivity of microcircuits in layer 2/3 V1 of mice by pairing calcium imaging *in vivo* with *in vitro* electrophysiology. A main take-home message from the first paper was that neurons with similar responses to stimuli were more than twice as likely to be connected than neurons not sharing stimulus response properties, and bidirectional connectivity was

increased in correlated pairs of neurons (Ko et al., 2011). The second paper looked at the development of these favored connections and found that following eye opening a massive reorganization of synaptic connectivity occurs (Ko et al., 2013). This consists of the elimination of connections between non-responsive neurons, as well as the strengthening of connections between similarly-tuned neurons. The third paper in this series looked at the role played by experience in the development of these microcircuits. The abundance of changes following eye opening might lead one to believe that it was a result of experience, but this is only partly the case. Overall, the strengthening of connectivity between similarly-tuned neurons happens with or without visual experience, it is only the elimination of connections between non-responsive neurons that seems to depend upon experience (Ko, Mrsic-Flogel, & Hofer, 2014).

Within rodent microcircuits although a lack of organization exists in relation to stimulus orientation preferences, retinotopy still occurs. Neurons close to each other in space will have receptive fields that are likewise nearby. This means that a stimulus, depending upon its size, may very well activate more than one neuron within a microcircuit. This can have differing effects. Although this has not been studied much in the mouse, in primate studies a few possible results have been seen. It is possible for a stimulus which is larger than a neuron's classical receptive field to cause either suppression, or, on occasion, facilitation. This surround modulation may or may not be uniform.

Not only are there microcircuits which can influence the responses of individual neurons, but also feedback from higher areas can modulate neuronal responses. For instance, silencing area LM using optogenetics results in an approximately 20 percent

decrease in firing rate of layer 2/3 excitatory neurons in response to their preferred stimuli (Pafundo, Nicholas, Zhang, & Kuhlman, 2016). This effect is not seen in layer 4 or in interneurons in layer 2/3. Furthermore, even this top-down feedback is altered by nucleus basalis activation, indicating that not only can higher visual areas affect V1, but also other areas that connect to the higher areas. Clearly a large number of inputs both bottom-up, top-down, and local all converge to affect neurons located within V1.

1.1.4 Inhibitory neurons within rodent visual cortex

Within the local circuitry composing V1, occur many inputs from both local and projecting excitatory neurons, but inputs from inhibitory GABAergic interneurons also take place. In rodent cortex and hippocampus over 20 inhibitory cell types have been identified (X. Jiang, Lachance, & Rossignol, 2016; Zeisel et al., 2015), but 3 main ones will be the focus of this section. These three types are the parvalbumin expressing (PV) which can be further subdivided into basket and chandelier cells, somatostatin (SOM) expressing neurons, and 5HT3aR a subpopulation of which express vasoactive intestinal peptide (VIP) (van Versendaal & Levelt, 2016). These neurons types compose roughly 40, 30, and 30 percent of the GABAergic neurons in the cortex respectively. Each of these neuron types plays a different role in regulating excitatory neurons and each other.

Basket cells are the predominant class of PV neurons, and they innervate excitatory neurons on their somata and proximal dendrites. They also are heavily connected to other PV neurons and even can form synapses onto themselves, or autapses (Deleuze, Pazienti, & Bacci, 2014; Pfeffer, Xue, He, Huang, & Scanziani, 2013). The way in which PV neurons receive input, both thalamic and local, makes

them well positioned to provide strong feedforward inhibition as well as able to regulate dynamic ranges of responses (van Versendaal & Levelt, 2016). PV neurons play an important role in maintaining the proper level of excitation to inhibition in the brain, helping control plasticity, and orchestrating oscillatory activity. Chandelier neurons are the other class of PV expressing neurons which target the axon initial segments of excitatory neurons quite possibly contributing to either depolarizing or hyperpolarizing the excitatory neurons. Their functions are less well known overall.

SOM expressing neurons, the second most abundant type of GABAergic neurons in the cortex, receive excitatory input from nearby excitatory neurons and primarily output to dendritic tufts in layer 1, but also sometimes on other distal dendrites (van Versendaal & Levelt, 2016). SOM neurons play an important role in surround suppression, but are not the only inhibitory cell type involved in this process (Adesnik, Bruns, Taniguchi, Huang, & Scanziani, 2012). SOM neurons also provide inhibition to PV and VIP neurons, but do not really connect to other SOM neurons (Pfeffer et al., 2013). Also, SOM neurons seem to be more heavily influenced by anesthesia than PV neurons are.

5HT3aR or VIP neurons form the majority of remaining inhibitory neurons in cortex. Most of these VIP neurons are bipolar cells which primarily inhibit other inhibitory neurons especially SOM neurons, but to a lesser extent PV neurons (Pfeffer et al., 2013; Pi et al., 2013; van Versendaal & Levelt, 2016). Also present are some VIP positive bitufted neurons which inhibit excitatory neurons (X. Jiang et al., 2015). In the visual cortex, VIP neurons especially seem to be activated by running, which, in turn, causes increased inhibition of SOM neurons and increased firing of excitatory neurons

(Fu et al., 2014; Wilson & Glickfeld, 2014). The effects on PV neurons are less clear, which makes sense due to there being fewer connections from VIP onto PV neurons.

Overall, although only composing roughly 20 percent of total cortical neurons (Kubota, 2013) inhibitory, GABAergic interneurons are a critical part of cortical circuits. Microcircuits and larger circuits themselves would not function properly if not for the influence of inhibitory neurons. As will be discussed in following sections, many aspects of cortical function, and, as a result, behavior, go awry in the cases of inhibitory malfunction. Despite being only a fraction of total neurons present, and having not been appreciated fully until recent years, these inhibitory neurons are critical for keeping the brain in working order.

1.2 Maturation of inhibitory PV neurons

1.2.1 Origins of PV neurons

All inhibitory neurons originate from a select few brain regions, but 70% of cortical inhibitory neurons, including PV neurons, which are the main focus of this dissertation, originate from the medial ganglionic eminence (MGE) (Jiang et al., 2016). Many factors affect the differentiation and migration of progenitor cells which finally lead them to become cortical PV neurons. Not nearly all of them will be covered in this section, but a basic overview of the process with highlight placed on certain key factors that will be of interest further on will be the main focus.

Progenitor cells within the MGE appear to go on to become either PV or SOM neurons depending upon whether they originate from the ventral or dorsal portion of the MGE respectively (Wonders & Anderson, 2006). A few primary transcription factors early on that play a key role are: the Dlx homeobox genes Dlx1/2 and Dlx5/6, the NK2

homeobox 1 gene (Nkx2-1), the LIM homeobox protein 6 gene (Lhx6), and its downstream effector SRY-box 6 (Sox6) (X. Jiang et al., 2016; Marín, 2012; Wonders & Anderson, 2006). Not surprisingly these factors are very important and some of the genes involved have been linked to diseases such as epilepsy. Once the progenitor cells exit the cell cycle and the fledgling inhibitory neurons are produced, they very soon begin their migration towards the circuits into which they will be integrated.

The initial movement of inhibitory neurons away from the MGE is likely mediated largely by chemorepulsive cues within the MGE (Marín, 2013). First, the new neurons go through a process of tangential migration, before moving onto radial migration. Some of the aforementioned transcription factors continue to influence the neurons as they begin their migration. For neurons that will go onto become PV neurons, these include Lhx6, Dlx1/2, and especially Dlx5/6 (X. Jiang et al., 2016; Wonders & Anderson, 2006), further factors that also come into play for migration include NRP2, ERBB4, and GAD67 (Marín, 2012).

To date, the only chemoattractive molecule shown to affect migration of MGE derived neurons to the cortex is neuregulin (NRG1) (Marín, 2013). This appears to be mediated through ErbB4, the NRG1 receptor expressed by MGE derived neurons (Flames et al., 2004; Marín, 2013). ErbB4 can be detected to be expressed in neurons as early as embryonic day 13, right at the start of tangential migration (Yau, Wang, Lai, & Liu, 2003). Two different isoforms of NRG1 play differing roles in this migration. A membrane bound form CRD-NRG1 is expressed along the route followed by MGE neurons on their way to the cortex, and Ig-NRG1 is a diffusible form of NRG1 that is produced in the pallium (Flames et al., 2004). Evidence suggests that these two forms

of NRG1 act as short and long range attractants, respectively, for the migrating neurons. Disrupting the function of ErbB4 causes a decrease in the number of neurons that tangentially migrate to cortex. Not only do NRG1 and ErbB4 play vital roles in the initial migration of PV neurons, but as will be seen later, they also play roles in their proper development and integration into the circuit.

Once the inhibitory neurons make their way across the pallial-subpallial boundary, one phase of their migration is complete. They then enter into one of the available migratory streams to begin their dispersion, but do not enter the cortical plate during this process (Marín, 2013). It is still not clear exactly what triggers the switch from tangential to radial migration, but once this change is undergone; the inhibitory neurons begin invading the cortical plate. From there they begin to find their appropriate places in the laminar structure. This colonization of the layers is done in a seemingly backwards order in which the early born neurons populate the infragranular layers while the late born neurons populate the supragranular layers. It appears that the change of GABA from being depolarizing to hyperpolarizing likely signals the end to inhibitory neuron migration, and KCC2 a potassium/chloride exchanger seems to play a critical role in this process (Bortone & Polleux, 2009). Once the neurons have reached their proper location, proper integration into their circuits is still essential. For this to happen properly, the neurons must both develop their appropriate morphologies and physiological properties.

1.2.2 Development of PV neurons

Once the PV neurons have made their way into the cortical plate and found their appropriate laminar position, they must next begin forming their appropriate connections

and integrating into the circuit. During this time, approximately 40% of inhibitory neurons undergo apoptosis and are eliminated rather than integrated into the circuit (Southwell et al., 2012). Although different mechanisms have been proposed for controlling this, such as competition for extrinsically derived trophic signals, work by Southwell et al. (2012) suggests that there is either an intrinsic cell autonomous mechanism for eliminating excess inhibitory neurons, or that a competition for survival signals produced by other inhibitory neurons allows some to survive while others undergo apoptosis. Preliminary data suggests that Mef2c transcription factor could in part regulate this selection process for PV neurons (X. Jiang et al., 2016).

Various trophic factors, along with contributing to migration, can influence the branching and growth of axons and dendrites. For instance, the chemoattractive molecule mentioned earlier, NRG1, has the ability to increase the growth and branching of axons of cultured hippocampal neurons (Fazzari et al., 2010). The growth undergone is quite substantial since in PV basket cells the postnatal dendritic and axonal length grows by 354 and 608% respectively, and there is a fivefold increase in the number of axonal braches (Doischer et al., 2008).

Once the basic structure is achieved, synapses must still be formed to allow for more precise GABA transmission. The PV basket cells must form their characteristic targeting of excitatory neuron somas which give them their name, but they also have numerous other connections to make including GABAergic synapses, electrical synapses, and autapses (Deleuze et al., 2014; X. Jiang et al., 2016). Furthermore, the proper wiring of excitatory neurons onto PV neurons must also be accomplished. An example of how wiring from excitatory onto inhibitory neurons or inhibitory onto

excitatory neurons can be affected is found with the NRG1 and ErbB4 signaling pathway which was mentioned in relation to migration of PV neurons. The deletion of ErbB4 from PV-expressing chandelier neurons causes a decrease in the number of axonal boutons formed by the PV neurons (Fazzari et al., 2010). Furthermore, the same study found that in hippocampal neurons the conditional deletion of ErbB4 caused a decrease in the number of excitatory synapses onto the PV neurons. A later study from the same lab showed that ErbB4 was necessary for the normal development of excitatory synapses onto PV neurons, both chandelier and basket cells (Del Pino et al., 2013). Thankfully, this study went on to more closely examine how both types of PV neurons were affected by the deletion of ErbB4. In basket cells, ErbB4 is not necessary for the formation of the normal size or number of boutons, but it does affect the amount of the α1 subunit of $GABA_A$ receptors on the excitatory neurons, which could affect their ability to receive and respond to input from the PV neurons.

As well as having to undergo various morphological changes during development, PV neurons also undergo certain physiological changes. One change that occurs is a reduction in membrane resistance likely caused by an increase in potassium leak channels (Doischer et al., 2008; Okaty, Miller, Sugino, Hempel, & Nelson, 2009). A characteristic of PV neurons is their fast firing phenotype. During the process of maturation, the firing pattern phenotype changes along with the shape of action potentials. The maximal rate of rise and decay along with the maximal amplitude of action potentials increases with age (Doischer et al., 2008). It has been demonstrated that the Kv3 channels can enable the fast spiking phenotype and that their gene transcripts are upregulated during the process of development (Okaty et al., 2009).

One way that changes both in connectivity and physiological changes can be seen is through the responses of neurons to various stimuli *in vivo*. In a mouse's visual cortex, responses of PV neurons to visual stimuli change in a few ways. One, an increased maximum firing rate is seen in mature as opposed to immature PV neurons, and two, the range of stimuli that elicit responses from PV neurons broadens (Kuhlman, Tring, & Trachtenberg, 2011). Another response seen in the visual cortex that signals a state of immaturity is prolonged discharge (Fagiolini et al., 2004). Proper inhibition and PV neurons reaching a more mature state seem to help aid in stopping this excitatory neuron response. Changes in PV neuron maturity can also be seen reflected in changes to overall plasticity and learning ability. With the increasing availability of various strains of transgenic mice, more studies continue to be done in which manipulations can be made to one class of inhibitory neuron, such as PV neurons, and then the responses of either PV neurons themselves, or the surrounding excitatory network can be assayed as a way of better understanding the roles various molecular factors play in the development and maturation of circuits.

1.2.3 Roles of PV neurons within the circuit

Despite only comprising approximately 10% of the circuit, PV neurons serve several vital functions. Susceptibility to hyperactivity or seizures, as well as a spectrum of diseases such as autism, schizophrenia, and intellectual disabilities result from improper inhibition and have been linked to PV neuron malfunction (X. Jiang et al., 2016; Marín, 2012). PV neurons contribute to feedforward and feedback inhibition as well as network oscillations, especially in the gamma (30-80 Hz) frequency range (Hu, Gan, & Jonas, 2014; Marín, 2012). Furthermore, PV neurons play a role in gain control

and act in regulating plasticity and learning (Atallah, Bruns, Carandini, & Scanziani, 2012; Caroni, 2015; Hensch, 2005; van Versendaal & Levelt, 2016). Part of what makes them so well equipped for several of these various roles is how they sample inputs from large numbers of nearby neurons and are set up to give precise, fast, outputs to excitatory, and other inhibitory, neurons to which they connect.

Normal brain function depends upon proper levels of excitation and inhibition. Synchronization and network oscillations of excitatory neurons arise from precise drive from inhibitory neurons regulating when they should fire and when they should not. PV neurons especially contribute to oscillations within the gamma frequency range. Evidence suggests that gamma oscillations are involved in various cognitive processes such as attention, memory, and information processing (Cardin et al., 2009; X. Jiang et al., 2016). It is further possible that disruptions in gamma oscillations may underlie some of the cognitive and behavioral deficits often seen in diseases such as epilepsy and schizophrenia. The precise role played by PV neurons in epilepsy is still not understood as different studies optogenetically manipulating PV neurons in different animal models of epilepsy in vivo and in vitro have reported contrasting findings depending upon neural state at the time of stimulation, the site of the stimulation, and which seizure model was being studied (X. Jiang et al., 2016). Lack of proper inhibition fails to stop the brain from going into a state of hyperactivity, but it has also been suggested that by synchronizing excitatory activity, PV neurons could actually contribute to the onset of seizures.

Feedforward and feedback inhibition both contribute to the regulation and precision of excitatory output (Hu et al., 2014). For the purpose of this section, we will

focus on feedforward and feedback inhibition within the local circuit, and not at the whole network level. Feedforward inhibition occurs when an excitatory input connects both to another excitatory neuron, and an inhibitory neuron which is also connected to the excitatory neuron. Both neurons will receive excitation at the same time, and the inhibitory neuron will very quickly (2ms or less) inhibit the excitatory neuron, providing only a brief window in which it can signal. This form of inhibition can be important for regulating the summation of incoming EPSPs or action potentials. When present, feedforward inhibition provided by PV neurons limits the time scale on which 2 separate events can be summated. For subthreshold events, this can mean the difference between whether the excitatory neuron fires or not. Therefore, feedforward inhibition plays a crucial role in coincidence detection.

Feedback inhibition can be separated into recurrent and lateral inhibition. Recurrent inhibition is when an excitatory neuron both gives and receives feedback from the same inhibitory neuron. Lateral inhibition is when an excitatory neuron receives feedback from an inhibitory neuron that is being stimulated by other, surrounding excitatory neurons. Feedback inhibition allows the circuit to be able to institute a sort of winner-take-all scenario such that when one excitatory neuron fires, it can activate the inhibitory neurons it is connected to and silence competing excitatory neurons from firing (Hu et al., 2014). The fast time scale on which PV neurons can act is especially good for this. Lateral inhibition can also help shape the response properties of excitatory neurons to the stimuli driving them. Although the response of excitatory neurons in the visual cortex is driven primarily by stimuli within their receptive fields, they can be modulated by neurons responding to stimuli surrounding their receptive fields. This has

primarily been looked at in higher species. PV neurons are not the only inhibitory neurons involved in this process, and SOM neurons play an important role as well.

Another way that PV neurons can contribute to the responses of excitatory neurons is by modulating gain. Gain modulation is a response amplitude change of a neuron independent of its selectivity or receptive field characteristics (Salinas & Thier, 2000). Studies which have manipulated PV neurons optogenetically have not always reached the same conclusions as to what affect PV neuron modulation has on excitatory neuron tuning (Atallah et al., 2012; Lee et al., 2012 and trying to reconcile the differences Lee, Kwan, & Dan, 2014), but disinhibition of excitatory neurons allows them to spike more, while increased inhibition reduces their firing. In the auditory cortex, it appears that VIP neurons are largely responsible for controlling the disinhibition of PV neurons in relation to gain modulation (Pi et al., 2013). In relation to the effect PV neurons have on excitatory neuron tuning it is interesting that the refinement of excitatory neuron tuning properties as well as feature-specific connectivity development in the visual cortex occurs independently of PV neuron maturation (Ko et al., 2014; Kuhlman et al., 2011).

An extremely important role that PV neurons further play in circuits is their role in regulating and allowing plasticity and learning. A classical way that plasticity has been studied in the visual cortex dates back over 50 years to the work of Hubel and Wiesel who studied plasticity in cats during various periods of development and found that occluding vision in one eye could cause lifelong changes to the circuit (Espinosa & Stryker, 2012). This form of plasticity, called ocular dominance plasticity (ODP), has been used to learn much about plasticity of the cortex during specific critical periods of

development. Critical periods are strict periods of time during which experience is critical to normal development of the circuit after which the overall performance of the circuit can be permanently altered. It has been demonstrated that there is a GABAergic control to the critical period such that prematurely increasing GABA can lead to an early onset of the critical period, while reducing GABA can lead to a delay in the critical period (Hanover, Huang, Tonegawa, & Stryker, 1999; Hensch, 2005; Hensch et al., 1998). Although classically studied in cats and other high mammals, rodents have proven to be useful due to genetic tractability and the ability to more easily study the roles of specific neuronal cell types. The ability to study specific cell types has been useful since control of the critical period is mediated by inhibition (Hensch, 2005). One reason that PV neurons were of interest was because blocking the Kv3.1 potassium channel which regulates the fast spiking phenotype of PV neurons resulted in a slowing of the rate of ODP (Hensch, 2005).

It is fairly clear that an initial disinhibition of the network, resulting in a change in the inhibitory/excitatory ratio, is critical for plasticity to be able to occur in rodent V1 (Kuhlman et al., 2013). It has remained unclear, however, as to whether changes in inhibition are instructive or permissive for plasticity. Whole cell recordings along with some basic modeling, has suggested that plasticity of PV neurons could directly instruct excitatory neurons by selectively disinhibiting preferred inputs and/or suppressing non-preferred inputs (Yazaki-Sugiyama, Kang, Câteau, Fukai, & Hensch, 2009). Other studies, such as one which used optogenetics to assay the roles of PV, SOM, or VIP neurons in ODP have agreed that PV neurons were the primary players, but did not find them to be preferentially influencing excitatory inputs from either eye (Saiepour et al.,

2015). This study would suggest that PV neurons play a more passive or permissive role in plasticity rather than actively directing excitatory neurons.

Much of what is known about the roles of PV neurons in learning comes from areas outside the visual cortex, although many of the underlying principals are likely the same. Many insights about the roles and development of PV neurons have come from studies in the hippocampus. One hippocampal example of how PV neurons play a role in learning addresses their role in recognition of novel objects as well as their role in learning a Morris water maze (MWM) (Donato, Rompani, & Caroni, 2013). In both situations, for novel object recognition and learning of the MWM, the authors found that the PV neurons went into a state of less inhibition prior to learning, and pharmacogenetic activation of the PV neurons was able to quench learning. Therefore, the disinhibition of PV neurons plays an important role in both plasticity and learning.

Overall PV neurons play many roles within the circuit and are important for several aspects of proper function. The importance of PV neurons seems to be especially emphasized by the span of devastating diseases and disorders caused by their improper functioning. Albeit a small portion of the circuit as a whole that they compose, many aspects of PV neurons, from their intrinsic properties to their connectivity patterns make them especially suited for the roles they fulfill. Better understanding PV neurons, how they adapt, and the molecular mechanisms underlying their physiology will allow for a better understanding of their contribution to the many circuits that compose the brain.

1.3 Experience is required for proper maturation of PV neurons

Experience is necessary in order for the previously discussed proper maturation of PV neurons to occur. Sensory cortex receives input from the outside world and its job is to make sense of the incoming signals. Therefore, when the incoming signals are removed or hindered in some way, especially during the process of development, it affects the neurons themselves and the circuits which they compose. A number of signaling mechanisms exist within the brain on the molecular level, and many of these signaling molecules (trophic factors, homeoproteins, etc.) are produced in an experience dependent manner. These molecular signals can then go on to affect the structure, synaptic physiology, intrinsic physiology, and *in vivo* response properties of the neurons upon which they act. This section will look at a few examples of the experience-regulated molecular signaling and how PV neurons are affected by it in the aforementioned ways.

1.3.1 The effects of experience on structure

As previously mentioned, the maturation of PV neurons and increase in overall cortical inhibition signals the onset of the critical period (Hensch, 2005; Kuhlman et al., 2013). During this period, the circuit is especially susceptible to perturbations or changes in experience (**Table 1.1**). One aspect of the neuron which can be affected is its anatomical structure. Structure is important, as it is one of the hallmarks that is used for distinguishing various classes of neurons, but also affects what role different classes of neurons are well designed to carry out. Fast-spiking PV basket-cells, the main class of neuron discussed in this dissertation, are characterized by primarily targeting the soma and proximal dendrites of their nearby targets which makes them good for being the main source of somatic inhibition (van Versendaal & Levelt, 2016).

The structure of neurons is important, but they are not just free-floating entities. Their structure can be affected by the extracellular matrix (ECM) which holds them in place. One portion of the ECM that is especially relevant for the experience dependent maturation of PV neurons is perineuronal nets (PNNs). PNNs, which are composed of various parts of the ECM such as chrondroitin sulfate proteoglycans, act like a lattice to form a sheath around PV neurons and their proximal neurites (Benninger, 2013). The presence of mature, fully developed PNNs largely signals an end to the critical period. Without the presence of PNNs, PV neurons do not completely mature and their structure remains more plastic. PNN development is delayed in the case of dark rearing, and this is at least in part due to a homeoprotein called Otx2. Otx2 is produced in the retina in an experience-independent manner, but its transport to the visual cortex, where it is primarily taken up by PV expressing neurons, is dependent upon experience (Sugiyama et al., 2008). Exogenous treatment with Otx2 in dark reared mice allowed for normal maturation of PNNs despite the lack of visual experience, and loss of Otx2 delayed the development of PNNs, further pointing to a pivotal role of Otx2 in the development of PNNs (Sugiyama et al., 2008). Another molecule which plays a role in the development of PNNs is MeCP2. MeCP2 (methyl-CpG binding protein 2) null mice showed a precocious development of PNNs as well as other signs of faster-than-usual development of PV neurons (Krishnan et al., 2015).

Not only can the external framework surrounding PV neurons change with experience, but their dendrite and axon morphology can also be affected. One experience-regulated molecule that has been found to affect axonal morphology, at least in culture, is PSA, a long, linear homopolymer of α-2,8-linked sialic acid which in

vertebrates is attached mostly to the neural cell adhesion molecule (NCAM) (Di Cristo et al., 2007). Unlike Otx2 which is produced by activity, PSA is actually downregulated by activity. When enzymatic removal of PSA was carried out in culture, it increased the amount of PV axonal branching and helped PV neurons form their characteristic basket-cell morphology (Di Cristo et al., 2007). In *Xenopus*, dark or short-term visual enhancement (STVE) both were capable of impacting dendritic morphology of GABAergic neurons in one of two ways. One group of neurons retracted dendrites in the dark and grew during STVE; whereas, the other group grew in the dark and retracted following STVE. However, due to the stage of development when these experiments were done, it was not possible to determine what percentage of these groups were composed of PV or SOM neurons (He et al., 2016).

In addition to changes in axon or dendrite growth and morphology, axonal boutons themselves are sensitive to changes in experience. For instance, in layer 4 of the barrel cortex of mice, deprivation in the form of whisker trimming causes about a 25% decrease in the number of PV positive boutons (Jiao, Zhang, Yanagawa, & Sun, 2006). Similarly, in the visual cortex of mice, monocular injection of TTX right at the start of the critical period reduced the number of boutons onto layer 5 excitatory neurons on average by 36%, showing that experience is necessary for the normal maturation of PV basket-cell boutons onto excitatory somas (Chattopadhyaya et al., 2004) Also, the aforementioned enzymatic removal of PSA in cultures caused an increase in the number and size of boutons, and the enzymatic removal of PSA *in vivo* prior to the age at which PSA would normally naturally decrease caused an increase in the number of puncta from basket-cells around the soma of layer 5 excitatory neurons (Di Cristo et al.,

2007). The trend seen overall is that through several possible mechanisms, bouton number and size can be affected by experience, and as will be discussed shortly, synaptic physiology can also be impacted.

As has been seen in the limited review presented in these past few paragraphs, experience has the potential to influence the structure of PV neurons in many ways. This can happen due to changes in the scaffolding surrounding the neurons or due to changes within the neuron itself, but often it is a combination of multiple factors. Experience works to upregulate some molecules and downregulate others, and through the proper balance of various factors, the maturation of PV neurons and the circuit as a whole, is achieved. When something goes awry, such as lack of proper experience, then the PV neurons fail to develop the proper structure, and physiology (as will be discussed in the next sections) and the system as a whole is thrown off.

1.3.2 The effects of experience on synaptic physiology

In the previous section we saw how, among other things, experience can affect the development (number and size) of axonal boutons. Simply the presence or absence of boutons themselves does not prove that physiological changes are occurring, however. In this section, we will look at some of the ways in which experience, often through various molecular substrates, can alter the physiology of the boutons.

Otx2 can affect the development of PNNs, but it can also play a role in the development of synaptic properties of inhibitory neurons. When miniature inhibitory postsynaptic currents (mIPSCs) were recorded from slices in mice lacking Otx2 the frequency of mIPSCs was reduced, but no change to their amplitude was reported (Sugiyama et al., 2008). This suggests that Otx2 could play a role in stabilizing

synapses from PV neurons onto excitatory neurons, which seems sensible given the role it plays in PNN development. MeCP2, another regulator of PNN development, affects synapses in a different way. In MeCP2 null mice, mIPSC amplitudes were strengthened in PV neurons, but not in excitatory neurons (Krishnan et al., 2015). This suggests that MeCP2 plays a role in the proper regulation of PV to PV connectivity.

Cristo et al., (2007) showed that the enzymatic removal of PSA caused an increase in the number of puncta from PV neurons onto excitatory neurons. PSA levels would normally decrease with age in an experience dependent manner, but through enzymatic removal they could speed the development of basket-like structures around excitatory neurons. They went on to record mIPSCs from excitatory neurons in those same mice and found that the removal of PSA also caused an increase in the frequency of mIPSCs, but not the amplitude, consistent with the increased number of puncta they had seen. It is important to note that although these results show that the removal of PSA caused an increase in inhibition (suggesting that the increased number of puncta seen were functional) it does not rule out the possibility that removing PSA could also cause greater inhibition from neurons not expressing PV.

One molecule that can alter the stability and strength of excitatory synapses onto PV neurons is the neurotrophic factor neuregulin (NRG1) which is produced by excitatory neurons in an activity dependent manner (Mei & Xiong, 2008; Ting et al., 2011). The receptor through which NRG1 acts in this role is the receptor tyrosine kinase ErbB4 which in cortex is expressed in inhibitory neurons and is enriched in PV neurons (Bean et al., 2014; Fazzari et al., 2010). NRG1, through ErbB4, can cause an increase in the number and size of puncta as well as an increase in the frequency and amplitude

of mEPSCs in PV neurons (Ting et al., 2011). Taken together, this means that NRG1 has the potential to cause the growth of more synapses onto PV neurons, and to strengthen previously existing synapses. Both NRG1 and ErbB4 are linked to several diseases, and their roles in PV neuron maturation and plasticity will be discussed in more depth in a later section.

Although much of this section has focused on how experience regulated molecules in turn regulate PV neurons, there is an intensive body of literature which addresses how experience affects circuits, without directly addressing what the underlying molecular pathways are. For example, a rather elegant study performed whole cell recordings from layer 4 of rats following 2 days of visual deprivation (Arianna Maffei, Nataraj, Nelson, & Turrigiano, 2006). Looking at the feedback inhibitory circuitry, they found that the excitatory postsynaptic currents (EPSCs) from excitatory neurons onto PV neurons underwent a threefold increase, and the IPSCs from PV neurons onto excitatory neurons were also increased threefold. Since no change occurred either in the strength of inhibition from PV neurons onto other PV neurons, or in excitatory to excitatory connections, it appears that the visual deprivation was causing a shift in the circuit to favor inhibition. They went on to show that this potentiation of feedback inhibition was due to a long-term potentiation of inhibition (LTPi) like mechanism. They hypothesized that this could be due to the decrease in firing of excitatory neurons following eye closure which would increase the chances of PV neurons firing without any excitatory neuron firing due to PV neurons having a higher spontaneous firing rate.

“Neurons that fire together wire together” is a common summary of Hebb’s postulate. The basic idea is that coactivity of neurons strengthens synapses while non-

correlated activity can lead to the weakening of synapses. Long-term potentiation (LTP) and long-term depression (LTD) are phenomena possibly expressed at every excitatory synapse within the mammalian brain (Malenka & Bear, 2004). LTP and LTD correlate with spine enlargement and shrinkage, respectively, and in hippocampal slices it is found that synapse elimination accompanies LTD (Bastrikova, Gardner, Reece, Jeromin, & Dudek, 2008). LTP and LTD are some of the most basic substrates of plasticity, and therefore the growth or removal of functional spines is fundamental to the brain's ability to learn and adapt. Within the primary visual cortex of mice, repeated exposure to visual stimuli induces a form of LTP (Cooke & Bear, 2010). Therefore, visual experience can lead to learning, through the modulation of spines and synapses. Although direct cases of inhibition were not mentioned, it is easy to see how LTP and LTD could affect inhibitory neurons. The strengthening or weakening of inputs onto PV neurons can directly impact their firing and therefore the overall inhibitory state of the circuit. Properly regulating the balance of excitation to inhibition is critical to many functions several of which have been previously discussed.

1.3.3 The effects of experience on intrinsic physiology

There are many factors that go into whether or not a neuron decides to fire an action potential either spontaneously or when it receives input. One of the factors that determines this is the intrinsic physiology of the neuron. The intrinsic physiology is largely regulated by the various ion channels expressed on the cell surface. Different channels can affect the membrane potential of neurons and determine how easy or difficult it is for a neuron to fire.

Mature PV neurons are characterized by several intrinsic properties such as low input resistance, narrow action potentials which have deep after-hyperpolarizations, and the ability to produce high-frequency spike trains with little adaptation of spike-frequency (Miller, Okaty, Kato, & Nelson, 2011). In motor cortex, the abolishment of spontaneous excitatory activity using muscimol over a period of two days resulted in wider action potentials, lowered maximum firing rates, and spike-frequency adaptation when performed on mice that were between P16-P18, right at the stage where the mature properties would usually first be observed. If this same experiment was repeated using mice in the range of P25-P27 instead, it resulted in an increased excitability due to an increased input resistance, reduced maximum firing rate, and slightly more adaptable firing patters; however, the spike width was unchanged (Miller et al., 2011). Minimal changes in gene expression were seen as a result of this deprivation, however, leading the authors to hypothesize that protein localization and trafficking are likely responsible for the changes observed.

An earlier paper (Okaty et al., 2009) from the same lab sheds some potential light on what channels could have been being localized or trafficked differently despite no change being seen in gene expression. Changes in resting membrane potential are likely regulated by the two-pore potassium leak channels TASK1 and TWIK1. Both of these channels are usually more abundant in mature PV neurons than in immature PV neurons. Changes in adaptability are likely, at least in part, due to the small conductance calcium-activated potassium channel *Kcnn2* which in normal development is seen to be downregulated during the process of maturation. The fast-spiking

phenotype and max firing rate tends to be regulated by Kv3 channels which are usually upregulated during the process of maturation.

As briefly discussed in the previous paragraphs, the fast spiking phenotype of PV neurons is made possible due to their expression of the voltage-gated potassium channels Kv3.1/Kv3.2 (Chow et al., 1999). Their fast spiking capabilities make them especially fitted for carrying out their roles in circuit control. Kv3.1/Kv3.2 channels are regulated by visual experience, but not particularly in intuitive ways (Grabert & Wahle, 2009). Although upregulation of Kv3 channels is normal during development, an overexpression of them can actually lead to a decrease in total inhibition. Using a rat model and assaying levels of mRNA and protein either after normal or dark rearing, Grabert & Wahle (2009) found that the initial production levels of Kv3.1 protein were not affected, but by P30 the protein level was significantly increased, although the level of mRNA never changed from control. Kv3.1 mRNA levels could be increased or decreased by 5 days of constant darkness or 5 days of recovery after dark rearing respectively, but only from P25-P30. 5 days of constant exposure to light could cause the downregulation of Kv3.1 mRNA even at a later date of P40-P45, however. For Kv3.2, on the other hand, by P20 the mRNA levels were aberrantly high in dark reared rats, and by P30 the protein expression was also high. The effects of 5 days of constant dark or recovery following dark rearing were similar for Kv3.2 as Kv3.1 with the exception that the 5 days of recovery, although significantly decreasing levels of Kv3.2 mRNA, still left higher levels than in normal reared controls. As a whole, it seems that spontaneous activity of the network plays more of a role in regulating Kv3.1/3.2 levels than active vision does.

The study by Grabert & Wahle does not really address what quality of experience causes the changes in Kv3.1/3.2 expression, although it mentions that BDNF is not a likely candidate for regulating the expression, at least during the later postnatal stages. Other studies have found at least some molecular regulators of Kv3.1. Otx2 has been discussed previously for the role it plays in developing PNNs and strengthening synapses from PV neurons onto excitatory neurons. Exogenous treatment with Otx2 prior to the onset of the critical period causes an increase in the number of Kv3.1 channels expressed in PV neurons (Sugiyama et al., 2008). Since some increase in Kv3.1 expression is normal during the maturation of PV neurons it appears, therefore, that exogenous Otx2 treatment can not only increase the maturation of PNNs, but also speed up the maturation of PV neurons themselves.

Although only a few of the many ion channels which go into giving PV neurons their unique characteristics have been discussed, it should be evident how important they are to the proper maturation and function of PV neurons. Some of these channels have been shown to be regulated either positively or negatively by experience, whereas for others, it is still unknown. The fact of the matter is, many different pieces must all come together at the proper times in order for the neurons and furthermore the circuits to develop and mature properly.

1.3.4 The effects of experience on *in vivo* response properties

Neurons function within the living brain of a living organism, so although much can be learned from examining connectivity within slices of living brain or looking at immunohistochemistry and cell architecture of preserved brains, to really try to make sense of the brain as an entire structure, *in vivo* studies become very useful. Although *in*

vivo studies, like all others, have their own sets of caveats and limitations, they perhaps offer the closest look at the brain in closer to normal conditions. Working with an *in vivo* system allows, among other things, for the recording of responses directly to sensory stimuli. This section will examine some of the changes that experience has on how neurons respond to various stimuli.

Within the visual cortex, the responses of PV neurons vary with age. During normal development both the spontaneous and evoked firing rates of PV neurons increase and also their tuning increases such that they respond to a larger array of stimuli (Kuhlman et al., 2011). Experience is necessary for the development of tuning and normal firing rates in PV neurons, because dark rearing results in the tuning not broadening and the spontaneous and evoked firing rates staying at immature levels. Since the tuning of PV neurons in mice seems to be a reflection of the tuning of local excitatory neurons (Bock et al., 2011; Hofer et al., 2011), it appears that at least certain excitatory to PV connections may fail to form without visual experience.

Not only can deprivation cause a failure of PV neuron responses to mature correctly, but it can also affect the responses of neurons that have already reached a fairly mature state. Monocular deprivation (MD) causes a drop in PV firing rate after 24 hours in either the binocular or monocular zone of the visual cortex (Hengen, Lambo, Van Hooser, Katz, & Turrigiano, 2013; Kuhlman et al., 2013). This drop in PV neuron firing allows excitatory neuron firing to briefly stay at normal levels and also is permissive of excitatory neuron plasticity. Maintaining network homeostasis is a common theme, and different strategies for accomplishing this exist. Perturbations to normal experience can come in many different forms, and often these different forms

may cause the same result, but through differing mechanisms. One study, using fairly young rats, found that although 3 days of visual deprivation (either through monocular eyelid suture or intraocular TTX injection) both caused an overall increase in spontaneous firing of excitatory neurons, the mechanisms by which this was achieved were different (Arianna Maffei & Turrigiano, 2008). Lid suture caused an increase in intrinsic excitability and a decrease in drive from deeper layers as well as a decrease in interconnectivity; whereas, TTX caused an increase in drive from lower layers along with decreased inhibition. Important to note, the results from the Maffei paper are from slice recordings; whereas, the previous two recordings were both done *in vivo*.

What does each of the three studies in the previous paragraph tell us about how the brain adapts, and the role that inhibitory neurons play in this? Some important differences to note prior to comparing these studies are the organism used, the age, the length of deprivation, and the technique. Kuhlman et al. used mice that were ~P28 which were monocularly deprived for 24 hours and used loose cell attached recordings in either awake or anaesthetized mice and also some glutamate uncaging to look at changes in input from various layers. Hengen et al. used P27-P32 rats and implanted multielectrode arrays which allowed them to record for all 6 days of deprivation. Lastly, Maffei and Turrigiano used P18-P21 rats with monocular deprivation for 3 days and did slice physiology to assay the properties of the neurons. The time course we see is a decrease in PV neuron (or putative fast spiking [pFS] neuron) firing following the first 24 hours of deprivation (Hengen et al., 2013; Kuhlman et al., 2013). Kuhlman et al. showed that this was due to a decrease in L4 and L5a excitatory input onto PV neurons. Hengen et al. showed that pFS neurons had returned to their baseline firing rates by 2 days, but

by that point the excitatory neuron firing had dropped. They also showed that mEPSC amplitude, recorded ex vivo, was decreased at this point. By three days, Hengen et al. saw firing rates for both excitatory and inhibitory neurons returned close enough to baseline to not be a differentiable difference. They did not record mEPSCs at this point, but Maffei saw mEPSC amplitude and frequency both decreased at 3 days compared to controls. These changes at 3 days were both for recurrent connections and inputs from deeper layers. Also at this point, Maffei saw an increase in intrinsic excitability. By four days, Hengen et al. no longer detected a difference in mEPSC amplitude and firing rates were a little higher than baseline, although not significantly. By 6 days, Hengen et al. saw mEPSC amplitudes significantly increased compared to controls. It appears that PV neurons are very quickly affected by deprivation as their firing rates are the first to drop due to a decrease in input from lower layers. What causes the rebound in PV neuron firing rate remains unknown, but perhaps it has to do with changes in intrinsic excitability of PV neurons. The decrease in input, both recurrent and from lower layers, happens somewhere between the 24 and 72-hour time point, because at 24 hours there is no detectable change in the excitatory inputs to excitatory neurons. The rebound in inhibition to baseline levels could act to suppress the excitatory firing rate on day 2. By day 3, the increased intrinsic excitability of the excitatory neurons could lead to their firing rate level being back to baseline. It is possible that the increase in excitatory neuron firing seen at day 4 could be a result of excitatory synapses beginning to strengthen again, combined with a still high intrinsic excitability of excitatory neurons. All of this seems to be a back and forth between inhibitory and excitatory neuron connectivity and intrinsic excitability in an effort to maintain homeostasis.

Sometimes changes in the inhibition provided by PV neurons can be assayed by changes in the surrounding excitatory neuron responses. In mice lacking GAD65 and therefore having deficiencies in GABA transmission, a prolonged discharge is seen upon a bar exiting the neuron's receptive field (Hensch et al., 1998). Prolonged discharge is one of the characteristics of immaturity in the visual cortex (Fagiolini et al., 2004). One way that experience may normally help put an end to prolonged discharge is by aiding in the maturation of PV neurons. Along with many of the other previously discussed functions, early treatment with Otx2 shuts down prolonged discharge (Sugiyama et al., 2008). This is one example of a way that changes in PV neuron maturity can affect not only the responses of the PV neurons themselves, but also the excitatory neurons to which they connect.

1.4 Inhibition beyond the critical period

Classical studies of plasticity in the visual cortex and many studies dealing with inhibitory neurons are conducted during the critical period. How much inhibitory neurons change or are able to change following the critical period and once the circuit has matured is an ongoing topic of study. This section will look at the adaptability of PV neurons as well as types of adult plasticity seen both within and outside of the cortex.

1.4.1 Adaptability of mature PV neurons

A decrease in overall changes to neurons, or at least a slower time scale on which changes occur, often comes along with maturation of cortical circuits. This does not mean that PV neurons reach a state at which they suddenly cease undergoing any changes. However, certain factors can limit the ease with which PV neurons undergo plasticity though. Chondroitin sulfate proteoglycans (CSPGs) are an essential

component of PNNs which form sheaths around the somas and dendrites of PV neurons, and appear right around the time of critical period closure. Degrading CSPGs in the visual cortex leads to increased plasticity (Pizzorusso et al., 2002). In the spinal cord and other areas CSPGs have been shown to inhibit axonal growth (Hübener & Bonhoeffer, 2014). It appears that CSPGs and PNNs are a negative regulator of plasticity, but in what way? One way that PNNs could be acting as inhibitors of plasticity in the adult visual cortex is by acting to capture Otx2 which is produced in the retina and transported to visual cortex where it is especially taken up by PV neurons (Sugiyama et al., 2008). By keeping Otx2 close to the PV neurons and readily available for uptake the PNNs could be aiding in keeping PV neurons in their most mature and inflexible state. Indeed, interfering with the interaction between PNNs and Otx2 increases adult plasticity in the visual cortex (Beurdeley et al., 2012).

Despite PNNs and other molecular factors seemingly making plasticity of PV neurons more difficult in the cortex after the critical period, certain changes can still occur. One change that has been observed is changes in PV neurons' axonal boutons. Although the majority of axonal boutons were found to remain fairly stable over the course of a week, an approximately 10% turnover was found where boutons would disappear, and new ones would form at about the same frequency (Kuhlman & Huang, 2008). Changes have also been observed in dendrite dynamics of inhibitory neurons in superficial layers of mouse V1 (W. C. A. Lee et al., 2006). Although this study did not specify what type of inhibitory neurons were involved, they were able to observe extensions, retractions, and sometimes even branching of dendrites in mature visual cortex. A later study by the same group found that up to 16 percent of the branching tips

of inhibitory neurons underwent remodeling as a result of changes to experience (J. L. Chen et al., 2011) suggesting a structural role for inhibition in plasticity later on in life. Clearly, many factors act to balance and maintain the structure of PV neurons and keep them in a less malleable state once the circuits are mostly mature, but as we will see, some amount of plasticity is still possible.

1.4.2 Types of adult plasticity

Despite cortical circuits in general being more plastic during development than in adulthood, at least four forms of plasticity can occur within the primary visual cortex during adulthood: adult ocular dominance plasticity (ODP), retinal-lesion induced plasticity, perceptual learning, and stimulus-selective response potentiation (SRP) (van Versendaal & Levelt, 2016). Disinhibition is important for all of these forms of plasticity, but which inhibitory neurons are involved, and their precise roles, vary.

Disinhibition of the network (seen in the form of decreased PV neuron firing rate) is necessary to permit plasticity during the critical period. Likewise, diminishing PV neuron activity for 24 hours in the adult is sufficient to restore ODP (Kuhlman et al., 2013). The ODP that can be observed in adults without special intervention is less efficient and less permanent, but can still be induced in mice at up to almost six months of age (Lehmann & Löwel, 2008; van Versendaal & Levelt, 2016). Whereas relatively brief deprivation (around three days) is sufficient to induce plasticity in critical period mice, it can take closer to one or two weeks to induce even mild forms of ODP in adult mice.

The exact roles that PV neurons play in adult ODP are still not fully understood, but their activity is not necessary in order to observe ODP (Kaplan et al., 2016). This

recent study found that whereas stimulus-selective response potentiation (SRP) was dependent upon PV neuron activity, ODP could occur even in its absence. This study was just examining the ability to observe shifts in ocular dominance following a week of MD, and did not investigate whether PV neurons played any role along the way. It is still possible that some amount of disinhibition of PV neurons is necessary to permit ODP as in younger mice (Kuhlman et al., 2013), but perhaps this disinhibition occurs over a slower time course in the adult than in the developing cortex. Shifts in the ocular dominance preference can still be observed even if PV neurons are silenced. What roles PV neurons play in the shift along the way and whether they are still permissive of plasticity in the mature cortex remain unclear.

It is possible that much of adult ODP is actually regulated by SOM neurons (van Versendaal & Levelt, 2016). Much of adult ODP, unlike ODP during the critical period, tends to be related more to changes in superficial layers related to horizontal and feedback connections. In young adult mice, GABAergic synapses onto excitatory neurons are rapidly eliminated during ODP, but which classes of inhibitory neurons these synapses originate from is still uncertain (J. L. Chen et al., 2012; van Versendaal et al., 2012). Since SOM form an abundance of inhibitory synapses in the very top layers and they have been found to be involved in adult learning tasks both motor and visual, they are prime candidates for possible mediators of adult plasticity (van Versendaal & Levelt, 2016). In the motor task mentioned (S. X. Chen, Kim, Peters, & Komiyama, 2015), SOM synapses were found to be lost, while PV synapses persisted; however, it is worth considering that only PV boutons forming synapses close to the excitatory neuron somas were examined in this study. One possibility is that an initial

disinhibition by PV neurons is still required for the onset of ODP plasticity in adult mice, and that SOM neurons actually play a subsequent instructive role in the reconfigurations.

Different forms of sensory deprivation cause differing effects on maturing inhibitory neurons, and the same is true for mature inhibitory neurons. Retinal-lesion induced plasticity is observable across many species (van Versendaal & Levelt, 2016). It involves V1 initially becoming unresponsive to the lesioned portion of the retina, followed by that zone eventually becoming active to stimuli from neighboring visual field positions (Keck et al., 2008; van Versendaal & Levelt, 2016). This is a slow process which occurs over the course of weeks or months and results in a massive turnover of spines with approximately 90% of upper layer spines in the involved region being new (Keck et al., 2008). Further work by the same group found that a group of inhibitory neurons did indeed experience a turnover of spines and boutons prior to the excitatory turnover previously described (Keck et al., 2011). Immunostaining found that the majority (91%) expressed neuropeptide Y, a smaller fraction expressed calretinin (20%), almost none (5%) expressed somatostatin, and none were found to express PV. Although PV neurons have in no way been implicated to be involved in retinal-lesion plasticity, it serves as an example of how plasticity and changes in inhibitory neurons can still occur even in the adult visual cortex.

One form of adult plasticity that PV neurons clearly have a key role in is SRP. SRP is observed in rodent primary visual cortex after a specific stimulus has been seen multiple times. This repeated presentation of stimuli leads to an increased response to those stimuli but not to others. Unlike the previously mentioned forms of adult plasticity,

SRP seems to possibly involve thalamacortical changes in connectivity, rather than being isolated to superficial layers of the cortex (van Versendaal & Levelt, 2016). A recent paper looked quite in depth at the roles of PV neurons for SRP (Kaplan et al., 2016). Both ODP and SRP were previously known to require NMDARs and to cause similar increases in visual cortical response; however, since one does not occlude the other, it suggested that different mechanisms were at play (Frenkel & Bear, 2004). To examine the roles of PV neurons on this process, the authors used DREADD (Designer Receptors Exclusively Activated by Designer Drugs) technology to express a G-protein coupled receptor that could be used to selectively silence PV neurons with clozapine-N-oxide (CNO) which is an otherwise inert molecule (Kaplan et al., 2016). After 6 days of exposure to a familiar stimulus, the visually evoked potential (VEP) amplitude will usually be larger to that stimulus than to a novel stimulus; however, this change in VEP amplitude was eliminated following treatment with CNO indicating that the activity of PV neurons does influence SRP. Not only does the elimination of PV neuron activity affect SRP, but the over-activation of PV neurons can similarly disrupt it. Optogenetically activating PV neurons after SRP had been induced in mice also caused an elimination of the strengthening of VEPs. Overall, the findings of this study suggest that SRP is caused by changes in excitatory to excitatory connectivity, but that it is mediated by stimulus-selective modulation of PV neurons. It is also likely that SOM neurons simultaneously play a role in this process, perhaps in helping to modulate the PV neuron activity.

Overall, changes in circuits in the adult cortex, although harder to evoke, can still be seen but typically on a slower time scale. Inhibition often plays an important part in

these changes. Different types of inhibitory neurons are better positioned to affect the circuit at different time points, and sometimes despite the effects appearing the same in adults as in young mice, the underlying mechanisms may vary. How plastic PV neurons themselves are in the adult cortex and what perturbations could still illicit change is yet to be fully explored.

1.4.3 Clues from outside the cortex

A fair bit of what is known about the adaptability of PV neurons comes from work performed outside of the cortex, especially in the hippocampus. One important study examined how PV neurons in the hippocampus of adult mice were affected by environmental enrichment, Pavlovian fear conditioning, or maze learning and found differing effects on the PV network condition (Donato et al., 2013). Environmental enrichment has been shown to increase plasticity and learning in the hippocampus (Caroni, Donato, & Muller, 2012), so seeing what changes occur during environmental enrichment could give hints to what network states promote plasticity and learning. To examine this, Donato et al. examined PV immunoreactivity which they found to closely parallel levels of GAD67 levels. GAD67 links synaptic activity to GABA levels. What they found was that environmental enrichment caused an increase in the number of PV neurons with low PV immunoreactivity suggesting that a lowering of PV neuron activity or a shift into a less inhibited state promotes learning and plasticity. Indeed, when they examined the state of PV neurons in mice that were involved in learning a water maze, they once again found the low PV neuron state similar to the one caused by an enriched environment, further suggesting that a decrease in PV neuron activity in the hippocampus promotes learning and plasticity.

Environmental enrichment and water maze learning caused a decrease in PV immunoreactivity, but what about Pavlovian fear conditioning? Previous work from the same lab found that when fear is restricted specifically to training contexts it depends on enhanced filopodial synapses onto PV neurons by hippocampal mossy fibers (Ruediger et al., 2011). The effects of fear conditioning are opposite that of environmental enrichment in that they cause an increase in the number of PV neurons with high immunoreactivity, and this form of fear learning seems to block or outweigh learning caused by environmental enrichment (Donato et al., 2013). They further found that pharmacogenetic activation of PV neurons in mice which had undergone environmental enrichment to force PV neurons into a high state was enough to counteract the improvement on a foreign object recognition task that normally accompanies environmental enrichment. Taken together these results suggest that in the hippocampus anyways, changes in the state of PV neuron activity correlate strongly with their ability to learn and for plasticity to occur. This supports the idea that PV neurons throughout the brain could play a critical role in regulating plasticity even in adult animals.

Another way that PV neurons in the hippocampus have been seen to modulate learning or plasticity is by the effects that they produce in LTP. For instance, the trophic factor neuregulin (NRG1) which plays critical roles in the initial development of PV neurons also can affect PV neurons in an adult hippocampus to cause increased GABA transmission and as a result the suppression of LTP induction (Y. Chen et al., 2010). Furthermore, the same study found that removing ErbB4, a receptor tyrosine kinase that NRG1 binds to and activates, from PV neurons caused a deficit in contextual fear

learning in mice, and using ecto-ErbB4 to neutralize NRG1 alters LTP. Additionally PNNs could be involved in regulating the availability of NRG1 and other factors and also further play a role in modulating plasticity (Chevaleyre & Piskorowski, 2014).

1.5 Pathophysiology of improper inhibition

Inhibitory neurons develop in a unique way as compared to excitatory neurons and play differing roles during different periods of development. They clearly play many important functions in helping to regulate the brain and maintain balance. This section will examine some of what can go wrong when inhibition is disturbed in the brain with a special emphasis on the roles that neuregulin (NRG1) and its receptor tyrosine kinase ErbB4 play in maintaining the proper levels of inhibition.

1.5.1 Diseases linked to improper inhibition

Several different factors can all lead to improper levels of inhibition. Defects in differentiation or migration can lead to a deficit in the number of inhibitory neurons that are even available to provide inhibition to a circuit. Improper formation of morphologies can lead to the inhibitory neurons not being in the proper places to form inhibitory synapses, or even if the axons and dendrites are all in the correct place, various defects can lead to a lack of proper synapses. Deficits can exist in the inhibitory synapses onto excitatory neurons or onto other inhibitory neurons conversely too few excitatory synapses onto the inhibitory neurons to drive them can also occur. Furthermore, intrinsic properties of inhibitory neurons can be irregular and can also lead to changes in inhibition. Any of these aforementioned changes or combinations of them can result in various diseases.

Improper inhibitory/excitatory balance and other improper levels of inhibition are seen in numerous diseases ranging from epilepsy to autism spectrum disorders, as well as schizophrenia (Marín, 2012). Since many of these diseases often emerge early in life, it is possible that erroneous development could play a role in them. This could happen in a combination of ways with errors early in development potentially affecting the number or location of inhibitory neurons; whereas, later errors could cause changes in their patterns of connectivity. Many of these errors can be the result of genetic disruptions.

Improper inhibition, especially from PV neurons, can lead to epilepsy. PV neurons can be negatively affected at multiple stages including specification, migration, and maturation which can lead to errors in synaptic function and connectivity, resulting in epilepsy (X. Jiang et al., 2016). Errors in specification can often prove embryonic lethal, but in the case that they are not, they can facilitate seizures. For example, losing the transcription factor *Nkx2.1* at embryonic day 10.5 leads to a reduction in the number of PV and SOM neurons, seizures, and early mortality (Butt et al., 2008). Similarly, the *Lhx6* transcription factor also regulates the number of PV and SOM neurons, and mice lacking it or with a hypomorphic allele have increased numbers of seizures (X. Jiang et al., 2016; Neves et al., 2013). These examples demonstrate how affecting specification can lead to seizure, but most likely is embryonic lethal or results in death very shortly after birth. *Lhx6* is also one example of a factor that can affect the migration of PV neurons, further resulting in seizure. An obvious overlap exists, and many aspects of PV neuron development can be negatively regulated by the same gene mutation or deletion.

As well as specification and migration being affected, the maturation of PV neurons can be altered which can cause an increased susceptibility to seizures. The development of PNNs plays a critical role in the proper maturation of PV neurons as they form sheaths around them and help lock them into place. One aspect of PNN development is a switch in the 4-sulfation/6-sulfation ratio of CSPGs making up the PNN. Transgenic mice which overexpressed the 6-sulfation form of CSPG (therefore affecting the overall ratio) were more susceptible to kainic acid induced seizure, most likely due to the improper maturation of PV neurons (Yutsudo & Kitagawa, 2015). Other ways that proper maturation can be impacted is through changes in the expression of various ion channels. Mutations in the Nav1.1 encoding gene *SCN1A* are a genetic cause of various forms of familial generalized epilepsy (X. Jiang et al., 2016). Not only can sodium channels affect seizures, but potassium channels can as well. Mutations in at least eight different voltage-gated potassium channels have been linked with epilepsy in humans (X. Jiang et al., 2016).

As well as intrinsic properties of PV neurons being affected, their synaptic connectivity is also at risk. PV neurons not only form GABAergic synapses, but also electrical synapses with one another, which help in their ability to synchronize and generate gamma oscillations. Knocking out connexin36, which is the primary connexin which forms gap junctions in neurons, makes mice more susceptible to pentylenetetrazol induced seizures (Jacobson et al., 2010) showing that a decrease in gap junction formation increases susceptibility to seizure. Polysialic acid (PSA) and neural cell adhesion molecule (NCAM) which both act to stabilize GABAergic synapses are found to be upregulated in patients with medial temporal lobe epilepsy and in

rodents after seizures have been induced. The ablation of PSA-NCAM increases susceptibility to seizure, suggesting a tie between the stability of GABAergic synapses and the likelihood of seizure.

Whereas some forms of improper inhibition can cause seizures, other maladaptive forms of inhibition can contribute to diseases within the autism spectrum. Rett's syndrome, for instance, which is characterized by impaired language skills, cognitive deficits, stereotypic behaviors, and respiratory problems, is usually caused by loss-of-function mutations in the gene encoding MECP2 (Marín, 2012). Although determining exactly what it is about MECP2 function that is most crucial is challenging based on its ubiquitous expression, the fact that deleting it specifically from GABAergic neurons in mouse forebrain recapitulated a large number of the symptoms such as repetitive behaviors, cognitive deficits, and impaired motor coordination (Chao et al., 2010) suggests that it could be largely GABA related.

Inhibition has further been implicated for involvement with autism and related disorders due to various studies that have linked neuroligin-neurexin complex genes with susceptibility to autism and Asperger's syndrome. One gene within this group that has garnered a fair amount of attention is CNTNAP2 which is part of the neurexin family. After being found to be linked to autism in human studies, it was studied in mice, and *Cntnap2* knockout mice show deficiencies in the number of interneurons, migration deficits, abnormal cortical synchrony, as well as being prone to epilepsy (Peñagarikano et al., 2011).

One other area in which improper inhibition has been implicated in disease, especially as related to the functions of PV neurons, is schizophrenia. Schizophrenia is

classified and thought of as a psychotic disorder due to the positive symptoms that go along with it such as delusions and hallucinations, but prior to the onset of psychosis and potentially just as damaging, are the cognitive deficits that accompany it (Kahn & Keefe, 2013; Lewis, Curley, Glausier, & Volk, 2012; Marín, 2012) many of which have been found to be linked to improper inhibition specifically from PV neurons. The alteration of PV neuron activity leads to abnormal gamma oscillations in patients with schizophrenia (Uhlhaas & Singer, 2010). At least a couple explanations exist for the reduced PV activity seen in patients with schizophrenia, first it could be a result of reduced GABA transmission such as would be seen as a result of fewer synapses (Woo, Whitehead, Melchitzky, & Lewis, 1998), or second it could be the result of decreased activation of PV neurons by excitatory neurons (Marín, 2012).

Three schizophrenia risk factor genes that could in part explain at least portions of the improper inhibition by PV neurons are *ERBB4*, *DISC1*, and *DTNBP1*. ErbB4 is a receptor tyrosine kinase which can bind to NRG1. ErbB4 plays a role in proper migration of PV neurons during which time NRG1 acts to help guide the neurons, although the exact mechanism through which this is achieved is debated (Flames et al., 2004; Li, Chou, Hamasaki, Perez-Garcia, & O'Leary, 2012), and also plays roles in synapse maintenance and strength (Mei & Xiong, 2008). DISC1 (disrupted in schizophrenia 1) is a scaffolding protein which is important for the development and normal functioning of PV neurons (Marín, 2012). Although not implicated by several recent genetic studies since its initial discovery, the study of DISC1 in the past 10 years or so has been insightful and contributed to our understanding of the disease process (Tomoda, Sumitomo, Jaaro-Peled, & Sawa, 2016). The roles DISC1 plays in

development and synaptic regulation have been quite well characterized and it and the protein network it is a part of appear to offer insights into the pathologies of several diseases such as schizophrenia, autism, and other mood disorders. DISC1, as mentioned, is a scaffolding protein, and as such it has many binding partners, one of which may be dysbindin which is encoded by the *DTNBP1* gene. Dysbindin is important for proper intracellular trafficking and is reduced in the prefrontal cortex and hippocampus of patients with schizophrenia (Weickert et al., 2004). Knocking out dysbindin in mice resulted in PV neurons showing a decrease in excitability resulting in a decrease in overall inhibition to excitatory neurons (Ji et al., 2009). Not all the mechanisms are completely clear, but appear remarkably similar to other mouse models of schizophrenia.

1.5.2 The role of ErbB4 in proper inhibition

The receptor tyrosine kinase ErbB4 and its ligand NRG1 which have both been implicated in schizophrenia play roles in many aspects of PV neuron development. This section will focus on the necessity of them for attaining the proper levels of inhibition. Of special interest will be mouse models in which ErbB4 has been removed to one extent or another and the effects this has on the PV neurons themselves, the surrounding network, and the behavior of the mice.

In the cortex and hippocampus, ErbB4 seems to be expressed exclusively in inhibitory neurons, and is quite heavily expressed in PV neurons (Bean et al., 2014; Fazzari et al., 2010; Vullhorst et al., 2009). It plays a critical role in their migration and depending upon what age it is deleted, some knockout mice show deficits in numbers of PV neurons to reach their final destination (Fazzari et al., 2010; Flames et al., 2004; Li

et al., 2012). Beyond affecting initial migration, ErbB4 and NRG1 also appear to be involved in axonal and dendritic branching and growth. In hippocampal cultures, treatment with NRG1 promotes axonal branching and growth of inhibitory neurons (Fazzari et al., 2010). Similarly, NRG1 was found to stimulate dendritic arborization in an ErbB4 dependent way in hippocampal neurons (Krivosheya et al., 2008).

Once the PV neurons make it to their appropriate places and achieve the right morphologies, it is still necessary for them to form the proper connections, and once again, NRG1 and ErbB4 come into play. One form of synapse which seems to be especially dependent upon proper NRG1/ErbB4 signaling is synapses from excitatory neurons onto PV neurons. In the hippocampus, when ErbB4 is knocked out, a decrease occurs in the number of excitatory boutons onto PV neurons, both basket cells and chandelier neurons, and this is accompanied by a decrease in the frequency of mEPSCs seen (Del Pino et al., 2013; Fazzari et al., 2010; Ting et al., 2011). Some of this decrease in the number of boutons present could be due to decreased stability of boutons since NRG1 stimulates PSD-95 stability through ErbB4 (Ting et al., 2011). In the medial prefrontal cortex, however, ErbB4 deletion is not seen to affect the development of these excitatory synapses onto PV neurons, but it does alter their maturation (Yang et al., 2013). Other synapse formation is likewise affected by the NRG1/ErbB4 pathways in potentially a regionally specific manner. In the hippocampus, ErbB4 seems to be necessary for the proper formation of synapses from chandelier neurons onto excitatory neurons, but it did not seem to affect the formation of basket cell boutons (Del Pino et al., 2013). Although in the hippocampus ErbB4 may play a role in the maturation of synapses onto other inhibitory neurons (Krivosheya et al., 2008), it

does not seem to be necessary for the development or maturation of PV to inhibitory synapses in the medial prefrontal cortex (Yang et al., 2013).

One study in the prefrontal cortex showed that NRG1 can regulate excitatory neuron activity by working through ErbB4 to stimulate GABA release from PV neurons (Wen et al., 2010). Not too surprisingly, the decrease in PV neuron maturation in the medial prefrontal cortex following ErbB4 deletion is accompanied by increased activation of excitatory neurons (Yang et al., 2013). Changes in the balance between excitation and inhibition can cause many problems themselves, or it can lead to maladaptive homeostatic compensations (Mei & Nave, 2014). In order to overcome improper inhibition, for instance, excitatory neurons sometimes downregulate the number or function of synapses (Del Pino et al., 2013; Yin et al., 2013).

Interruptions in the proper amount of inhibition or excitation due to changes in the expression of NRG1 or ErbB4 can cause behavioral phenotypes. Ablation of ErbB4 has been seen to cause many phenotypic results in mice some of which are associated with various symptoms observed in human patients with schizophrenia (Mei & Nave, 2014). The results covered here will focus mostly on studies which limited the ablation to inhibitory or PV neurons. One phenotype that has been recapitulated in multiple studies is the tendency of mice lacking ErbB4 to be hyperactive in open field tests, which is used to model some of the positive symptoms of schizophrenia (Del Pino et al., 2013; Shamir et al., 2012; Wen et al., 2010). Knockout mice consistently travel greater distances with more horizontal activity as well more stereotypic behavior. This hyperactivity is something that has to be accounted for when measuring other behavioral functions as well. Another difference seen in mice lacking ErbB4 is the

attenuation of prepulse inhibition (PPI) which is also often seen in patients with schizophrenia. PPI is often used to measure the sensorymotor gating of rodents and is found deficient in mice lacking ErbB4 in their PV neurons, but acoustic startle responses are not affected (Shamir et al., 2012; Wen et al., 2010). Yet another deficit seen in PV specific ErbB4 knockout mice that is highly relevant to schizophrenia is a deficit in working memory. When an automated radial arm maze was used to assess how well PV-Cre;ErbB4$^{-/-}$ mice would do at remembering which arms they had previously gone down or not in order to get a food reward, mice lacking ErbB4 showed an increase in the number of errors they made, even when hyperactivity was taken into account (Wen et al., 2010). When other forms of memory, such as conditioned fear response have been examined, the results have not been quite as clear. One study found that PV-Cre;ErbB4$^{-/-}$ mice showed impaired contextual fear conditioning (Y. Chen et al., 2010); while another study found that whereas full ErbB4 knockout mice showed deficits in contextual fear conditioning, PV-Cre;ErbB4 mice did not (Shamir et al., 2012). Clearly ErbB4 plays a role in fear conditioning, but the role of PV neurons themselves remains less clear.

Not only can ErbB4 cause hyperactivity and impaired PPI and working memory, but other social behaviors can also be affected. Mice conditionally lacking ErbB4 showed less anxiety in an elevated plus maze and reduced marble burying activity (Del Pino et al., 2013). Elevated plus maze and marble burying are considered emotional behaviors, and this study went on to also assay various social behaviors. In the three chambered-social test mutants showed impaired sociability (specifically impaired sociability towards familiar subjects). When nest building was examined, once again the

knockouts showed deficits. They did not form any recognizable nest and tended to scatter the material. The inability to nest tends to be associated with poor planning, organizational ability, and social withdrawal. What is seen overall is that ErbB4 indeed plays an important role in the maturation of proper circuits and some of these deficits manifest themselves as behavioral phenotypes which are reminiscent of traits seen in human patients.

1.6 Goals of this dissertation and summary of findings

PV neurons play multiple roles within the circuit, and are regulated in multiple ways by experience. The first goal of the research within this thesis is to determine the effects of loss of patterned visual stimuli upon PV neurons during the critical period. It aims to clearly distinguish any changes seen as being due to a lack of stimuli and not due to changes or imbalances in inputs from both eyes to the PV neurons. It is expected that a loss of patterned visual stimuli will cause a decrease in PV neuron firing in as brief of time as 24 hours, because recordings of putative fast spiking (pFS) neurons in the monocular zone of the primary visual cortex decreased following 24 hours of monocular deprivation (MD) (Hengen et al., 2013). Similarly, within layers 2 and 3 of the binocular visual cortex, MD causes a decrease in PV neuron firing rate in as little as 24 hours. After which, excitatory neurons begin to undergo ocular dominance plasticity (ODP) (Kuhlman et al., 2013). ODP involves a competitive interaction between inputs from the two eyes which results in strengthening of inputs from the open eye and weakening of inputs from the deprived eye (Hensch, 2005; Kuhlman et al., 2013). Exactly how much this competitive aspect plays a role in the changes in PV neuron firing remains unclear. Due to studies not agreeing as to what the exact effects of short

term deprivation are on PV neuron firing (Kuhlman et al., 2013; Yazaki-Sugiyama et al., 2009), clarity is still needed as to the effects of loss of patterned visual stimuli alone. Within the binocular zone of the visual cortex, the decrease in PV neuron firing can at least be partially explained by a weakening of excitatory inputs from layer 4 and layer 5a (Kuhlman et al., 2013). The decrease seen in pFS neurons in the monocular zone suggests that these changes are not affected by open eye inputs; however, PV neurons do receive and sample numerous inputs from a large area surrounding them (Bock et al., 2011; Hofer et al., 2011), so it is possible that even PV neurons with their somas located in the monocular visual cortex could still be receiving inputs from the binocular visual cortex and be influenced by both eyes.

My research set out to differentiate between the effects of lack of patterned visual stimuli versus imbalance between eye inputs in a couple of ways. First, instead of using the classical MD paradigm, I used binocular deprivation (BD). This should cause the same degradation of normal vision through a deprived eye as is seen in MD, but it also similarly degrades input from the other eye as well (Blais et al., 2008). This ensures that any effects seen on PV neuron firing are a result of decreased patterned visual stimuli, and not any competition or imbalance between inputs from different eyes. It is more similar to MD than dark exposure or intraocular TTX injections which is necessary because these forms of deprivation have been shown to work through differing mechanisms (A Maffei, Lambo, & Turrigiano, 2010; Arianna Maffei & Turrigiano, 2008).

Furthermore, my experiments examined the similarities and differences between PV neurons located within the binocular and the monocular zones of primary visual cortex. Initially, recordings were targeted towards the monocular zone of the primary

visual cortex, so the neurons should be at least primarily innervated by the contralateral eye. During acquisition of PV neuron firing response the ipsilateral eye was shuttered, so all input drive was coming from the contralateral eye. These experiments were then repeated within the binocular zone of primary visual cortex and neurons were stimulated through each eye independently. This allowed for a confirmation that what was seen in monocular zone was also seen in binocular zone regardless of which eye was being stimulated.

In brief, my research found that binocular deprivation did indeed cause a decrease in PV neuron firing rate throughout the primary visual cortex. I used loose cell attached recordings of PV neurons in the primary visual cortex to assay the effects of 24 hours of BD. An approximately 33% decrease in evoked firing rate was observed as well as an approximately 60% decrease in spontaneous firing rate. This shows that a lack of patterned visual stimuli is sufficient to cause a decrease in PV neuron firing rate such as is seen when the network goes into a disinhibited state prior to ODP and that competition from the open eye is not necessary.

The decrease in PV neuron firing rate following MD or BD drops the evoked and spontaneous firing rates down to levels usually seen prior to their maturation. This opens the possibility that the disinhibition is essentially returning the PV neurons to at least a partially immature state. Along with firing rate, the orientation selectivity of PV neurons broadens with maturation over a similar time course and similarly in an experience-dependent manner (Kuhlman et al., 2011). Therefore, if deprivation is causing a drop towards a somewhat immature state of PV neurons, it is possible that changes in PV neuron tuning back towards a more tuned state would also be seen. For

monocular zone, when two different measures of a neuron's tuning, orientation selectivity index (OSI) and bandwidth, were examined, neither showed a significant change following deprivation. Within the binocular zone there was a small, but significant shift in OSI tuning rates following BD. Overall, BD does cause a shift in the percentage of PV neurons found to be more tuned than their own spontaneous activity in both monocular and binocular zones of primary visual cortex, but 2/3 of the population of neurons remains untuned. It should be noted that even following BD, PV neurons are still broadly tuned.

PV neurons in mice seem to gain their broad tuning from receiving input from a majority of spatially close excitatory neurons (Bock et al., 2011; Hofer et al., 2011) which in the mouse (unlike carnivores) display a lack of organization related to direction selectivity (Espinosa & Stryker, 2012). Arch silencing of PV neurons in L2/3 caused a slight reduction in the orientation selectivity index of excitatory neurons, but tuning sharpness remained essentially unchanged (Atallah, et al., 2012). Taken together, these findings allow us to propose a mechanism to explain the change in evoked firing rate we see across orientations in PV neurons following deprivation. We propose that 24 hours of BD causes a decrease in excitatory drive from L4 and L5a onto L2/3 PV neurons without changing the drive onto L2/3 excitatory neurons. The decrease in L2/3 PV neuron inhibition onto the surrounding excitatory neurons could cause a slight broadening of their selectivity index, so they would fire to a broader array of stimuli, but still fire proportionately more to their previous preferred orientation. If L4 or L5a was contributing to the broad tuning of PV neurons in L2/3 this slight increase in orientations

evoking a response in L2/3 excitatory neurons could pick up the slack for the lost input without causing much sharpening of the overall tuning of L2/3 PV neurons.

In understanding how neurons respond to deprivation, two parameters that have been examined so far are tuning properties (both local and global) and firing rates (both spontaneous and evoked). Beyond just examining the mean firing rates of PV neurons, another aspect that can be examined is the overall variability within those firing rates. It has been previously reported that across multiple sensory modalities (both in anaesthetized and awake cortex) stimulus onset reduces neural variability (Churchland et al., 2010). One measure of firing rate variability that can be used is the Fano factor (FF) which is defined as the spike count variance divided by the spike count mean. Most studies dealing with FF focus on the effects of stimulus onset on excitatory neurons, but there is one study in primate V4 shows decreases in FF of inhibitory neurons following stimulus onset, and theoretical modeling work exists to explain how it could function (Litwin-Kumar & Doiron, 2012; Mitchell, Sundberg, & Reynolds, 2007). Employing a technique adapted from the Churchland (2010) paper, we examined how stimulus onset affected the FF of PV neurons both in critical period mice which had undergone deprivation and those which had not. We found that stimulus onset did indeed cause a quenching of neural variability in PV neurons whether the mice had undergone BD or not. The difference in FF following stimulus onset, however, was significantly more in control than in deprived mice. This suggests that perhaps deprivation is causing the neurons to go into a less variable state. The excitatory neurons recorded from in our study had too low of firing rates to examine them directly, but we believe the results seen in the PV neurons reflect a larger network property.

The rapid changes as a result of MD tend to be seen only during the critical period, and in adult cortex longer periods of deprivation are required to see even more moderate changes (van Versendaal & Levelt, 2016). We set out to examine whether the rapid effects of BD were also confined to the critical period. When PV neurons were recorded from adult (P45-P60) visual cortex either with or without 24 hours of BD no difference was observed in the evoked firing rate, although a small yet significant decrease in spontaneous firing rate was observed following 24 hours of BD. The overall tuning of PV neurons following 24 hours of BD was unchanged both in terms of the bandwidth and OSI. When FF analysis was conducted on the PV neurons from adult mice, once again stimulus onset was seen to quench variability of PV neuron responses. Noticeable though, was the fact that the difference in FF following stimulus onset was decreased by deprivation in the adult, similar to how it was decreased during the critical period.

Overall what these results show is that PV neurons in the adult are indeed less plastic than during the critical period (as would be expected), but they still can undergo some changes. Although changes are not observed in evoked firing rate following deprivation, when variability is examined, a change is detectable. The fact that BD causes a decrease in the drop in variability regardless of age shows that aspects of PV neuron functional response properties do indeed stay plastic into adulthood. The changes that have been observed in dendrite and axon dynamics suggest that PV neurons can retain a degree of flexibility into adulthood, but this is to my knowledge one of the first functional responses of PV neurons in the visual cortex shown to maintain plasticity on such a rapid time scale into adulthood.

Many factors can contribute to the regulation and modulation of firing rates in neurons, so how do you determine a good starting place for understanding the molecular underlying of the rapid plasticity seen in PV neurons following 24 hours of BD? A few of the criteria that we proposed were as follows: we wanted something related to synaptic strength and stability, something specific at least to inhibitory neurons, and something known to be activity dependent (**Figure 1.1**). These criteria are met by the NRG1/ErbB4 pathway. NRG1 through the activity of ErbB4 plays a role in stabilizing synapses via PSD-95 as well as helping to maintain synapses (Mei & Xiong, 2008; Ting et al., 2011). Within the cortex, ErbB4 is limited to expression in inhibitory neurons including PV neurons (Bean et al., 2014; Fazzari et al., 2010), and finally NRG1, the ligand for ErbB4, is regulated by activity (X. Liu et al., 2011; Y. Sun et al., 2016).

NRG1/ErbB4 signaling and the effects of its deletion have been studied a fair amount due to the genes for both of these proteins showing up in screens as schizophrenia risk factors. Studies done in the hippocampus suggest that ErbB4 is important for the formation or maturation of synapses, especially excitatory synapses onto PV neurons (Del Pino et al., 2013; Fazzari et al., 2010; Ting et al., 2011). Furthermore, numerous behavioral and social abnormalities have been observed in mice lacking ErbB4. Although a reasonable amount of *in vitro* slice work and behavioral studies have both been done, there is surprisingly little *in vivo* work looking at either PV neurons or their surrounding excitatory networks in ErbB4 knockout mice. My work aims to take a look at what roles ErbB4 expression in PV neurons may have in determining

the dynamic range in which PV neurons can work and how easily they can be modulated in response to perturbations to their normal environment.

Prior to being able to determine what role if any ErbB4 plays in PV neuron plasticity, it is necessary to see what are the effects of ErbB4 deletion from PV neurons. To look at this, a PVcre line was crossed with $ErbB4^{fl/fl}$ mice to conditionally eliminate ErbB4 from PV neurons. Initial tests showed that ErbB4 deletion caused a decrease in PV neuron firing rates, and further tests which were conducted in mice bred such that within the same litter some mice were wildtype and others were knockouts confirmed these results. An approximately thirty percent decrease in PV neuron firing rate was observed due to the removal of ErbB4 from them.

Although these experiments were not repeated in the littermate control mice, 24 hours of binocular deprivation was performed on mice lacking ErbB4 in their PV neurons. No change was detected in the raw evoked firing rate (21.2 Hz compared to 20.4 Hz), and no significant change was seen in the spontaneous firing rate. The spontaneous firing rate seemed to decrease following 24 hours of BD which is in line with the results seen in PV neurons in other experiments regardless of age, further suggesting that the mechanism controlling spontaneous firing rate may be distinct from those controlling evoked firing. The lack of plasticity seen in PV neurons lacking ErbB4 following 24 hours of BD suggests that ErbB4 is required for the normal adaptability of PV neurons. One possibility is that PV neurons lacking ErbB4 just lack dynamic range and are already at the bottom of their firing rate possibility and just cannot lower their rates more even after deprivation. Whether the excitatory neurons surrounding these PV neurons experience increased activation as in the medial prefrontal cortex (Yang et

al., 2013) would be interesting to know. Alternatively, lower PV neuron firing rates could result in the network trying to rebalance the excitation to inhibition ratio resulting in decreased excitatory firing, or excitatory neurons could stay at their normal firing levels while PV neurons were decreased, also resulting in an abnormal excitatory/inhibitory ratio.

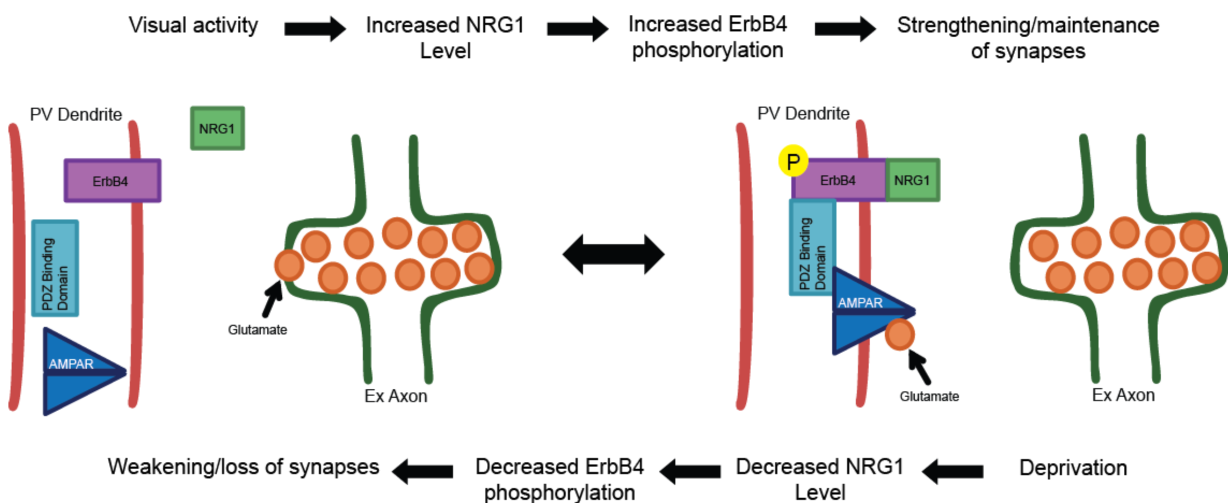


Figure 1.1 NRG1 and ErbB4 strengthen and maintain synapses in an activity dependent manner. Visual activity causes an increase in the production of neuregulin (NRG1) from either excitatory neurons (X. Liu et al., 2011) or inhibitory PV neurons (Y. Sun et al., 2016). NRG1 causes the phosphorylation of the receptor tyrosine kinase ErbB4 which allows it to bind to the PDZ binding domain thereby stabilizing AMPA receptors. Once AMPA receptors are stabilized, there is increased strength of excitatory inputs onto PV neurons. If visual activity is decreased via deprivation, there is a decrease in NRG1 production and therefore a drop in the phosphorylation of ErbB4 leading to a decrease in AMPA receptor stability and a weakening of excitatory synapses onto PV neurons. (Schematic based on Fazzari et al., 2010; X. Liu et al., 2011; Mei & Xiong, 2008; Y. Sun et al., 2016)

Assay	Rodent	Age	Zone	Layer	Deprivation	Result	Reference
in vivo extracellular anesthetized	Mouse	P15 -17	bV1	?	MD (4 day)	Benzodiazepine agonists trigger early ODP via $GABA_A$ α1 containing receptors	(Fagiolini et al., 2004)
in vivo extracellular anesthetized	Mouse	P18 -22	bV1	2/3	MD (4 day)	Otx2 injections caused early onset of ODP	(Sugiyama et al.2008)
in vivo extracellular anesthetized	Mouse	P19 -36	bV1	2 to 6	MD (4 day)	OD shift occurs following contra eye deprivation only. CP peak at P28	(Gordon & Stryker, 1996)
in vivo cell attached anesthetized	Mouse	P23 -30	bV1	2/3	DR (P9-30)	PV evoked and spontaneous firing rate stays at immature levels, broad tuning fails to develop	(Kuhlman et al., 2011)
in vivo cell attached anesthetized & Awake	Mouse	P23 -30	bV1	2/3	MD (1 day)	Pyr ↑ firing both ipsi and contra eye stimulation. PV ↓ firing both ipsi and contra eye stimulation. 3 day MD PV contra remained weak but ipsi returned to normal	(Kuhlman et al., 2013)
in vivo extracellular anesthetized	Mouse	P28	bV1	2 to 6	BD (4 day)	No change detected in putative pyr neurons following BD	(Gordon & Stryker, 1996)
in vivo whole cell anesthetized	Mouse	P24 -32	bV1	2/3	MD (2-3 day) (14 day)	Short and long term MD eliminated spike bias in pyr neurons. Short term MD caused PV spike bias to shift towards contra eye, long term MD caused bias to shift towards ipsi eye	(Yazaki-Sugiyama et al., 2009)
VEPs Awake	Mouse	P28 -35	bV1	4	MD (1,3,5,7 days)	Response depression by 3 days then response potentiation by 7 days	(Frenkel & Bear, 2004)
					BD (1,3,5,7 days)	No change detected	
					MI (3 day) + MD (2 day)	No response depression, but response potentiation by 7 days	
Micro-electrode arrays awake	Rat	P27 -32	mV1	2/3	MD (1-6 days)	pFS firing ↓ after 24 hours MD and ↑ again by 48 hours, ↓ ISI at 24 hours. RSU firing ↓ after 48 hours MD, then ↑ again	(Hengen et al., 2013)
ISOI anesthetized	Mouse	P15 & 26	bV1	5 & 6	MD (5-7 days)	MeCP2 null mice have early onset of ODP and early end to CP due to early maturation of PV neurons	(Krishnan et al., 2015)
ISOI anesthetized	Mouse	P27 - P31	bV1	2/3	MD (4 day)	No ocular dominance shift in the absence of ErbB4 in PV neurons	(Sun et al., 2016)
EPSC following LSPS	Mouse	P27 - P31	bV1	2/3	MD (1-2 day)	↓ pyr input onto PV neurons, but rescued by either bath application of NRG1 or injection of NRG1 during MD (never actually say L2/3 is ↓, but data suggests it)	(Sun et al., 2016)
EPSC following LSPS	Mouse	P28 -29	bV1	2/3	MD (1 day)	Pyr no change in input from any layer. PV ↓ EPSC amplitude from L4 and L5a, ↓ sEPSC frequency.	(Kuhlman et al., 2013)
slice mEPSC & mIPSC	Rat	P15 -17	mV1	4	MD (2 day)	Star pyr neurons ↑ in sEPSC frequency, ↓ in sIPSCs. ↓ amplitude of monosynaptic connections between FS and pyr neurons.	(Maffei, et al., 2004)

Assay	Rodent	Age	Zone	Layer	Deprivation	Result	Reference
slice mEPSC & mIPSC	Rat	P18 -21	mV1	4	MD (2+ day)	↓ spontaneous firing of pyr neurons. Potentiates unitary synapses from FS interneurons to pyr neurons	(Maffei et al., 2006)
slice mEPSC & mIPSC	Rat	P18 -21	mV1	2/3	MD (2 day)	↑ spontaneous firing of pyr neurons, ↓ E/I balance, ↓ mEPSC amplitude. ↓ in threshold voltage for action potential generation	(Maffei & Turrigiano, 2008)
					MI (2 day)	↑ spontaneous firing of pyr neurons, ↑ E/I balance, ↑ mEPSC amplitude	
slice mEPSC & mIPSC	Rat	P18 -21	bV1	4	MD (2+ day)	No change in mEPSCs, ↓ mIPSC amplitude, ↑ mIPSC decay	(Maffei et al., 2010)
		P18 -25			MD (7 day)	↑ in mIPSC amplitude	
		P18 -28			DT	Same time windows as MD, but no changes detected	
		P21 -23			MD (2+ day)	↑ in mIPSC amplitude	
slice mEPSC	Rat	P22 -28	bV1	2/3	MD (1,2,3,6 days)	↓ mEPSC amplitude following 1 or 2 days, and ↑ amplitude by 6 days. No change in frequency, kinetics, or passive neuronal properties. ↑ intrinsic excitability of pyr neurons following 6 days, ↓ current threshold and ↑ input resistance	(Lambo & Turrigiano, 2013)
			mV1		MD 1,2,3,6 days)	↓ mEPSC amplitude following 1 or 2 days, and ↑ amplitude by 6 days.	
			bV1		BD (2,3,6 days)	↑ mEPSC amplitude following 6 days as well as ↓ current threshold and ↑ input resistance	
			mV1		BD (2 day)	↓ mEPSC amplitude	
slice mEPSC & mIPSC	Rat	P25 -28	bV1	4	MD (2+ day)	No change in mEPSCs, ↑ mIPSC amplitude, ↓ mIPSC decay	(Maffei et al., 2010)
mEPSC recordings	Rat	P28, 30, 32	mV1	2/3	MD (2,4,6 days)	Spontaneous mEPSC amplitude ↓ following 2 day MD, was normal at 4 day MD, and ↑ by 6 day MD (Compared to control hemisphere)	(Hengen et al., 2013)
immuno-staining	Mouse	P20 -24	bV1	5	MI (4 day)	TTX injections caused ↓ maturation of perisomatic GABAergic innervation.	(Chattopad hyaya et al., 2004)
fsTRAP & immuno-staining	Mouse	P27 - P31	bV1	2/3	MD (1 day)	↓ NRG1 mRNA & ↓ P-ErbB4	(Sun et al., 2016)

Table 1.1 Summary of effects of deprivation on rodent visual cortex. Abbreviations: P#, postnatal day deprivation was begun; bV1, binocular zone; mV1, monocular zone; MD, monocular deprivation; BD, binocular deprivation; MI, monocular inactivation; DT, dark treatment; DR, dark rearing; VEPs, visually evoked potentials; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; LSPS, laser-scanning photo stimulation; ISOI, Intrinsic signal optical imaging; OD, ocular dominance; ODP, ocular dominance plasticity; pyr, pyramidal neurons; ↑, increased; ↓, decreased; TTX, tetrodotoxin; E/I, excitatory/inhibitory balance, pFS, putative fast-spiking; RSU, regular spiking unit

2.0 Binocular deprivation induces both age-dependent and age-independent forms of plasticity in parvalbumin inhibitory neuron visual response properties

2.1 Abstract

Activity of cortical inhibitory interneurons is rapidly reduced in response to monocular deprivation during the critical period for ocular dominance plasticity and in response to salient events encountered during learning. In the case of primary sensory cortex, a decrease in mean evoked firing rate of parvalbumin-positive (PV) inhibitory neurons is causally linked to a reorganization of excitatory networks following sensory perturbation. Converging evidence indicates that it is deprivation, and not an imbalance between open and closed eye inputs, that triggers rapid plasticity in PV neurons. However, this has not been directly tested in-vivo. Using two-photon guided cell-attached recording we examined the impact of closing both eyes for 24 hours on PV neuron response properties in mouse primary visual cortex. We found that binocular deprivation induces a 30% reduction in stimulus-evoked mean firing rate, and that this reduction is specific to critical period-aged mice. In contrast to evoked mean firing rate, measurements of trial-to-trial variability revealed that stimulus-driven decreases in variability are significantly dampened by deprivation during both the critical period and the post-critical period. These data establish that open-eye inputs are not required to drive the deprivation-induced weakening of PV neuron evoked activity that defines critical period plasticity, and that other aspects of in-vivo PV neuron activity are malleable throughout life.

2.2 Introduction

The development and plasticity of inhibitory circuits plays a central role in determining the timing of critical period plasticity in primary visual cortex (Hensch, 2005; B. Jiang, Huang, Morales, & Kirkwood, 2005). In response to monocular deprivation, a decrease in the evoked firing rate of a specific subclass of inhibitory neurons, referred to parvalbumin-expressing interneurons (PV), initiates critical period plasticity (Kuhlman et al., 2013). The deprivation-induced reduction in mean firing rate of inhibitory neurons is rapid and precedes changes in excitatory neuron evoked firing rate, thereby invoking a transient state of disinhibition that allows ocular dominance plasticity among excitatory neurons to proceed (Aton et al., 2013; Hengen et al., 2013; Kuhlman et al., 2013). Prior to the onset of critical period plasticity, PV neuron response properties are immature, both in terms of mean evoked firing rate, which is approximately 50% lower in the pre-critical period compared to the critical period, and orientation tuning properties (Kuhlman et al., 2011). Visual experience prior to the critical period is required for maturation of these response properties to develop, and in adult mice monocular deprivation does not induce disinhibition. Taken together, these data support a conceptual model in which deprivation-induced disinhibition is permissive, serving to gate the timing of critical period plasticity (Kuhlman et al., 2013), rather than generating an instructive signal during altered sensory experience that suppresses closed eye inputs (Kuhlman, Lu, Lazarus, & Huang, 2010; Yazaki-Sugiyama et al., 2009). A key observable feature of monocular deprivation-induced plasticity is the differential time course of plasticity between inhibitory and excitatory neurons (Hengen et al., 2013). This is important, as it sets a window of opportunity for rewiring of excitatory

connections such that synaptic weights among excitatory neurons can be updated to reflect new sensory experience. Notably, the rapid cell-type specific reduction in firing rate levels following monocular deprivation occurs in both binocular and monocular regions of primary visual cortex (bV1 and mV1, respectively), indicating that this is an ubiquitous feature of primary sensory visual cortex (Hengen et al., 2013; Kuhlman et al., 2013).

A prediction of this conceptual model is that brief deprivation itself is sufficient to induce rapid plasticity of PV neuron responsiveness and that the reduction in firing rate is specific to inhibitory neurons. We tested this prediction in bV1 as well as mV1 by performing binocular deprivation and assaying PV responsiveness using 2-photon guided cell-attached electrophysiological recording. We found that brief binocular deprivation induced a 30-40% reduction in mean evoked firing rate specifically in PV inhibitory neurons and not excitatory neurons in bV1 and in mV1. The impact of binocular deprivation on mean evoked firing was restricted to critical period-aged mice. We also examined the extent to which brief perturbation of vision influences recently developed orientation tuning properties. We found that some orientation tuning properties characteristic of mature PV neurons were resistant to brief deprivation during the critical period, but the percentage of neurons which were tuned increased. Finally, we considered other analyses beyond mean stimulus-evoked firing rate. Although traditionally mean firing rate averaged across repeated stimulus trials has been extremely useful in understanding cortical development and plasticity, recent studies highlight the need for a more thorough analysis of spike times that takes into account variability across trials. For example, Fano factor analysis of response variability across

trials revealed the presence of a cortical state change induced by sensory input. It was observed across a range of brain areas and animals that stimulus onset drives suppression of variable ongoing activity of excitatory neurons. This state change is independent of response magnitude of individual neurons and is therefore likely a property of the local recurrent network (Churchland et al., 2010). The extent to which PV inhibitory neurons also display a stimulus-driven decrease in rate variance in V1 is unknown. Given that PV neurons are highly connected within the local network in terms of the input that they receive, we hypothesized that similar to excitatory neurons, PV neurons exhibit a stimulus-driven decrease in Fano factor. We found that indeed, stimulus onset drives suppression of spike rate variability in PV neurons. Furthermore, we found that the magnitude of the stimulus-driven decrease in Fano factor was reduced following binocular deprivation, and that this reduction occurred both in the critical period as well as the post-critical period.

Our results provide further evidence supporting a conceptual model in which PV inhibitory neurons gate the timing of critical period plasticity by providing a permissive opportunity for reorganization of excitatory neuron connections, rather than generating an instructing signal. Furthermore, in this study we establish that the ability of PV neurons to rapidly modify their average firing rate in response to deprivation is a general property of primary visual cortex and is not restricted to the specialized area of binocular zone. Our examination of spike rate variability revealed a previously unrecognized plasticity in adult PV neurons that may be indicative of a network-level state change inducible throughout life.

2.3 Materials and Methods

2.3.1 Animal preparation and surgery

All experimental procedures were compliant with the guidelines established by the Institutional Animal Care and Use Committee of Carnegie Mellon University and the National Institutes of Health. Monocular zone experiments were performed in mice expressing cre-recombinase (cre) and red fluorescence protein (tdTomato) in parvalbumin (PV)-positive neurons derived from the cross between PV-cre knock-in female mice (Jax: 008069, generated by S. Arbor, FMI) and male tdTomato reporter knock-in mouse (Jax:007908, ′Ai14′, generated by H. Zeng, Allen Brain Institute). Cell-attached mode recordings were made in left hemisphere visual cortex of 28 urethane-anesthetized mice between ages 25-30 days for the critical period experiment, and 15 mice between 45-60 days for the mature group. Both male and female mice were used. Binocular zone experiments were performed in mice expressing cre-recombinase (cre) and red fluorescence protein (tdTomato) in parvalbumin (PV)-positive neurons derived from the cross between PV-cre knock-in female mice (Jax: 008069, generated by S. Arbor, FMI) and male tdTomato reporter knock-in mouse (Jax:007908, ′Ai14′, generated by H. Zeng, Allen Brain Institute), or male tdTomato reporter congenic knock-in mouse (Jax:007914, ′Ai14′, generated by H. Zeng, Allen Brain Institute). Cell-attached mode recordings were made in left hemisphere visual cortex of 16 urethane-anesthetized mice between ages 25-31 days. Both male and female mice were used.

Mice which underwent the binocular deprivation paradigm were anesthetized under isoflurane (3% induction and 1.5-2% maintenance). Silicone oil was applied to both eyes to prevent drying. A single mattress suture (silk 6-0) was made through each eyelid to hold the eye closed. These sutures were made 24 hours prior to the

craniotomy surgery and monitored to ensure maintained closure. Any mice which showed signs of infection or lid separation were removed from the study.

For surgeries mice were anesthetized with isoflurane (3% induction and 1.5-2% maintenance). Their body temperature was kept constant at ~37.5°C using a heating plate. The eyes of any mice not undergoing binocular deprivation were protected with silicone oil at the onset of surgery. For mice with eye sutures, their eyes remained sutured shut until ready for recording at which point the sutures were removed and silicone oil was applied to their eyes. A custom made stainless steel head-bar was affixed to the right side of the skull using ethyl cyanoacrylate glue and dental acrylic and a silver chloride ground electrode was implanted over the cerebellum. A 1.5-2.5 mm craniotomy was made over the left visual cortex. Craniotomies were positioned as described in Kuhlman et al. 2011. A 2.5 mm coverslip was then secured over a portion of the brain using dental acrylic and cortex buffer (125mM NaCL, 5mM KCl, 10mM glucose, 10mM HEPES, 2mM $CaCl_2$, 2mM $MgSO_4$) was used to keep the brain moist as well as facilitating imaging.

2.3.2 In vivo cell-attached recording

Mice were sedated with chlorprothixene hydrochloride(5 mg/kg) and anesthetized with urethane(0.5 g/kg). *In vivo* imaging was performed on a two-photon microscope (Scientifica) imaging system controlled by ScanImage 3 software (Vidrio Technologies, Pologruto, Sabatini, & Svoboda, 2003). The light source was a Chameleon ultra 2 laser (Coherent) running at 930 nm. A 40x water-immersion objective from Olympus was used to pass the laser beam. Surface blood vessels, coverslip, and pipette were viewed in visible-light conditions using a green filtered light.

Pipettes had a resistance of 5-12 MΩ when filled with cortex buffer and 20 μM Alexa Fluor-488 hydrazide (Invitrogen). Labeled neurons were first identified using 2-photon imaging. Their x, y, and z coordinates were recorded and then the pipette was positioned above the neuron's location at low magnification. A Patchstar micromanipulator (Scientifica) was used to back the pipette up an appropriate distance such that moving it in x and z at a 35° angle would result in it hitting the neuron (roughly 1.73 x the depth of the neuron). The pipette was lowered towards the surface of the brain first under low, then high magnification. The pressure of the pipette was raised to approximately 200 mBar positive pressure and a slight increase in resistance marked contact between the pipette tip and the surface of the brain. The pipette was lowered at a 35° angle into the brain and pressure was reduced to 50 mBar as soon as the dura was penetrated. Once through layer 1 the pressure was reduced to 20-30 mBar until the desired neuron was attained. 2-photon imaging was used to guide the pipette towards the desired neuron and minor changes in y were made as needed. Targeting technique was based on Kuhlman, Tring, & Trachtenberg, 2011 and Liu et al., 2009. Once the pipette appeared to be touching the neuron the resistance was lowered and spontaneous spikes could usually be detected. Resistance was decreased to 0 and the pipette was advanced until a 20-200 MΩ loose cell-attached seal was obtained. Occasionally negative pressure was applied up to -50 mBar. Recordings of spontaneous and then evoked spikes were made in current clamp mode. Signal was acquired with a MultiClamp 700B amplifier in current-clamp mode, a National Instruments digitizer, and WinEDR software (J Dempster, Strathclyde University). Signal was sampled at 10.02 kHz. Pipette capacitance was compensated.

2.3.3 Visual stimulation.

Visual stimulation consisted of full field square wave gratings presented at 6 orientations spaced 30° apart moving in two directions (12 total stimuli). A temporal frequency of 1 Hz and spatial frequency of 0.02 cycles per degree (cpd) was used for putative PV neurons while a temporal frequency of 2 Hz and spatial frequency of 0.04 cpd was used for putative excitatory neurons. Stimuli were developed using custom software with PsychToolbox in Matlab (Mathworks). Stimuli were presented one at a time in a random order for 3 seconds at 100% contrast followed by a 3 second blank grey screen with equal mean luminance. Each stimulus was presented 3-12 times. Stimuli were presented on a 40-cm-wide gamma-calibrated LCD monitor. For monocular zone recordings, the monitor was positioned 25 cm in front of the mouse's right (contralateral to site of recording) eye. Mouse was positioned looking straight forward with a 5-15% rightward tilt to accommodate the brain site for recording being relatively flat. The mouse's nose was approximately aimed towards the left of the screen with the right eye looking at the center of the screen ±5 cm right or left. For binocular zone recordings, the monitor was positioned 25 cm in front of the mouse's eyes with the nose pointed towards the center of the screen.

2.3.4 Eye shuttering

Eye shuttering was accomplished by placing an occluding device 5 mm in front of the eye. For each eye, an occluding device was constructed of flexible light-blocking material (1.5 cm x 2 cm) mounted on a flexible linker connected to a vertical post such that either eye could be shuttered or not shuttered in order for each eye to be stimulated.

2.3.5 Data analysis and statistics.

Spike-waveform analysis was conducted using WinEDR and Clampfit software. For putative PV neurons, the first 50-150 spikes exhibiting good peak (P1) to nadir (P2) amplitudes were averaged and the 10-90% rising and falling slopes as well as P1 and P2 were calculated. For putative excitatory neurons, the first 50-150 spikes (if the neuron fired that many times) were averaged and the 10-90% rising and falling slopes as well as P1 and P2 were calculated. The ratios of P2/P1 and falling/rising slope were used to normalize for differences in cell-attached resistance across cells.

WinEDR software along with custom built Matlab software was used to analyze the firing rate of targeted neurons. The spikes elicited from 3 runs of the 12 randomly presented stimuli were first sorted. Then the number of spikes elicited over the 3 runs was averaged for each of the 12 stimuli. The max evoked firing rate was defined as the highest averaged, firing rate over the complete number of runs (usually 9 or 12). Tuning curves were obtained by measuring responses to each of the 12 stimuli. The orientation selectivity index (OSI) was calculated using the circular variance approach where OSI is defined as 1-CV. Bandwidth calculations were based on Ringach, Shapley, & Hawken, 2002, except a von Mises distribution function was used to smooth tuning curves and the concentration parameter 'k' was set to 15. After smoothing, the orientation angles closest to the peak for which the evoked response equaled $1/\sqrt{2}$ height of the peak response on either side of the curve were estimated. Bandwidth is defined as the difference between these two angles. If the tuning curve did not fall below this criterion, the bandwidth was defined as 180°. Neurons assigned a bandwidth value of 180° were not included in the mean bandwidth values. Including these neurons does not change

the interpretation of our results: K-S test p values comparing all neurons in the control and deprived conditions were: p=0.180 and p=0.983 for CP and mature age groups, respectively.

Tuning curves were generated by assigning the orientation with the max firing rate over the whole 3 seconds of stimulus presentation a value of 0 and aligning the rest of the orientations to that. For comparing population tuning curves between control and BD conditions all firing rates were normalized to the max firing rate of the control condition. For percent of sample tuned according to OSI tuned was defined as the OSI value being higher than the OSI calculated based on spontaneous activity of the neuron from the blank grey-screen presentations shown between stimulus presentations (Kuhlman et al., 2011). Even neurons which were tuned according to this definition were still mostly broadly tuned.

Latency to max firing was calculated using 10 ms non-overlapping bins beginning at stimulus onset. For each 10ms bin we calculated the firing rate in Hz for that bin over all the given runs. Latency was defined as the middle of the time bin during the first cycle at which the neuron reached its max firing rate. PSTHs were made by plotting the firing rates of each 10ms bin, where 0 is stim onset. Raster plots were made by plotting individual spikes of a neuron during each run of the preferred stimulus. Once again stimulus onset was defined as 0 and 1 second of grey screen response is shown prior to all 3 seconds of stimulus response. First and third cycle analysis was performed by averaging the response rate of neurons during the first or third cycle (first or third second since the stimuli were being presented at 1Hz) of the preferred stimulus presentation, respectively.

Fano factor was computed in Matlab using code available at http://churchlandlab.neuroscience.columbia.edu/links.html. For more in depth explanation see Churchland et al., 2010. Spike counts were computed using a 200-ms sliding window moving in 25-ms steps. Variance (across trials) and mean of the spike count was then computed. Fano factor is the spike count variance divided by the spike count mean. The raw Fano factor which is the slope of the regression relating the variance to the mean was used. For calculating the difference in Fano factor a single value was obtained for each neuron by taking the average of the Fano factors during the entire 3 seconds of grey screen and subtracting from that the average Fano factors during the 3 seconds of stimulus presentation.

Data are reported as mean ± SEM. Datasets were compared using Mann-Whitney U, Wilcoxon signed rank, binomial probability test, or K-S tests as indicated.

2.4 Results

2.4.1 Brief binocular deprivation induces a reduction of evoked PV neuron firing rate

To understand the effects of binocular deprivation (BD) on PV neurons during the critical period (CP), we performed 2-photon guided cell attached recordings from PV neurons in primary visual cortex in mice at age postnatal day (p) 25-30 (**Fig. 2.1a**). Neural activity was recorded in response to drifting gratings, presented at a temporal frequency of 1 Hz at 12 different orientations. The order of stimulus presentation was randomized and each presentation was interleaved by a grey screen to assess spontaneous activity. The waveform of PV neurons is narrowly shaped (B. Liu et al., 2009); we used this characteristic to confirm that the correct cell type was targeted (**Fig.**

2.1b,c). We found that 24 hours of BD induced a 32% decrease in stimulus-evoked firing rate of PV neurons in bV1, in response to contralateral (contra) eye stimulation, at the preferred orientation (**Fig. 2.1d**; Control: 21.55 ± 2.50 Hz, n = 26 cells from 9 animals; BD: 14.65 ± 1.65 Hz, n = 24 cells from 7 animals; Mann-Whitney U-test p = 0.025). Twenty-four hours of BD also caused a significant decrease in the spontaneous firing rate in these same cells (Control: 5.91 ± 0.88 Hz; BD: 2.18 ± 0.34 Hz; Mann-Whitney U-test p<0.001). Thus, 24 hours of visual deprivation is sufficient to revert both stimulus-evoked and spontaneous firing rates back to immature levels observed during the pre-critical period (Kuhlman et al., 2011), indicating that continued visual experience is required for critical period-aged neurons to maintain their recently developed firing rate levels. Evoked responses to ipsilateral (ipsi) eye stimulation were also recorded in these same neurons. Similar to $bV1_{contra}$, $bV1_{ipsi}$ evoked responses were significantly decreased following 24 hours of BD (Control: 18.88 ± 2.39 Hz, n = 25 cells from 9 animals; BD: 13.84 ± 1.56 Hz, n = 23 cells from 7 animals; Mann-Whitney U test p = 0.0499). To directly confirm that that a rapid decrease in evoked firing rate is a general property of PV neurons in V1 and not restricted to the binocular zone, the experiment was repeated and PV neuron recordings were made in mV1. We found that in mV1, 24 hours of BD induced a 41% decrease in stimulus-evoked firing rate of PV neurons (**Fig. 2.1e,f**; Control: 23.40 ± 1.94 Hz, n = 33 cells from 17 animals; BD: 13.78 ± 2.06 Hz, n = 18 cells from 11 animals; Mann-Whitney U-test p = 0.002). Again similar to bV1, spontaneous firing rate of the same mV1 neurons was significantly decreased in BD animals compared to controls (Control: 6.03 ± 0.65 Hz; BD: 2.24 ± 0.42 Hz; Mann-Whitney U-test p<0.001).

In addition to PV neurons, a total of 21 putative excitatory neurons were recorded in mV1. We define putative excitatory neurons as neurons that do not express red fluorescence and have asymmetric waveforms compared to PV neurons (**Fig. 2.1b,c**). Unlike PV neurons, we did not detect a difference in mean stimulus-evoked firing rate in putative excitatory neurons at their preferred orientation from deprived animals compared to controls (Control: 3.68 ± 0.54 Hz, n = 15 cells from 10 animals; BD: 3.31 ± 1.44 Hz, n = 6 cells from 5 animals; Mann-Whitney U-test p= 0.559), nor did we detect a change in spontaneous firing rate in these same cells (Control: 1.24 ± 0.42 Hz ; BD: 0.79 ± 0.57 Hz; Mann-Whitney U-test p= 0.228). These results establish that in both bV1 and mV1 continued visual experience is required for the recently developed firing rate levels in PV neurons to be maintained. Importantly, the rapid and cell-type specific reduction of firing rate in response to deprivation previously reported in bV1 (Kuhlman et al., 2011) is also present in mV1.

To determine whether mV1 PV neurons in post-critical period animals are sensitive to brief deprivation, recordings were made from mature mice (p45-65). In contrast to critical period aged mice, we found that mean stimulus-evoked rates at the preferred orientation did not change following 24 hours of deprivation (Control: 18.37 ± 1.26 Hz, n = 34 cells from 9 animals; BD: 16.04 ± 1.26 Hz, n = 28 cells from 6 animals; Mann- Whitney U-test p=0.120).

2.4.2 Brief deprivation during the critical period increases percentage of tuned PV neurons.

This rapid reduction in PV neuron firing rate is observed at the preferred orientation, but does it hold true across orientations? We next examined the effect of BD

on evoked firing rate across orientations and found that in both bV1 and mV1 the evoked response to stimulus presentation was reduced for non-preferred stimuli as well (**Fig 2.2**). This would be consistent with an overall decrease in inputs from layers 4 and 5a as was seen following 24 hours MD (Kuhlman et al., 2013).

By the onset of the critical period, PV neuron firing rate increases 2-fold from that of the pre-critical period in a vision-dependent manner (Kuhlman et al., 2011). Along with the change in firing rate, there is also a vision-dependent change in orientation tuning curves. PV neurons are more sharply tuned during the pre-critical period compared to the critical period. In other words, orientation tuning curves become broader with visual experience. We next checked as to whether similar to firing rate levels, orientation tuning curves are reverted back to the immature state following BD initiated during the critical period. We found that the decrease in evoked firing rate following 24 hours BD occurred for all orientations during the critical period, as such, the shape of the tuning curve was not qualitatively altered, as can be observed by comparing normalized tuning curves between control and deprived conditions (**Fig. 2.2**).

The range of tuning values reported within PV neurons from the binocular zone of primary visual cortex do not change between control and BD conditions (**Fig 2.3a**). We examined the percentage of PV neurons that qualify as having some degree of tuning by comparing the mean orientation selectivity index (OSI) calculated as 1 minus circular variance of the PV neurons to the OSI value that would be obtained if OSI was calculated on their spontaneous activity (**Fig 2.3b**). We found that the percentage of neurons which had OSI values which fell above the values calculated from their spontaneous activity was increased following BD for the stimulation of either eye (**Fig**

2.3c,e). There are likewise more PV neurons with narrower bandwidths following BD (**Fig 2.3d,f**).

We examined the monocular zone of primary visual cortex and compared the effects of BD on tuning of PV neurons either during or after the critical period (**Fig 2.4**). We found that once again, BD caused an increase in the percentage of PV neurons which were tuned (**Fig 2.4b-d**) but that this effect is only seen during the critical period and not in recordings made after the critical period (**Fig 2.4e,f**). We also plotted the OSI as a function of firing rate to see whether the results we saw were simply an artifact of changes in firing rates (**Fig 2.5**). Overall, we saw that BD causes an increase in the percentage of tuned neurons only during the critical period, regardless of which zone of visual cortex is examined. We did observe that on average, PV neurons within mV1 were more tuned than PV neurons within bV1 (**Fig 2.6**).

We focused on the larger of the two areas, mV1 for the remainder of the study. In mature animals, V1 PV neurons are characterized as being the first cell type to respond and their response is strongest during the first stimulus cycle (Ma et al., 2010). Next we evaluated the extent to which these properties are developed at the time of the critical period (**Fig. 2.7**). Latency to reach maximum firing rate was similar between critical period and mature age groups (CP: 389.55 ± 48.9 ms; Mature: 315 ± 41.6 ms; Mann-Whitney U-test p=0.415). However the ratio of 1st cycle response/3rd cycle response was not fully developed in critical period aged mice (CP: 1.45 ± 0.05; Mature: 1.76 ± 0.12; Mann-Whitney U-test p=0.014). Despite this incomplete development at the time of the critical period, deprivation induced a decrease in mean evoked firing rate in both the first and last stimulus cycle (**Fig. 2.7c**), indicating that firing rate levels are

sufficiently developed to mediate disinhibition for the full stimulus duration, including the early and late portions of the response.

2.4.3 PV neurons exhibit a stimulus-induced reduction in variability of spike times

It is becoming increasingly clear that in addition to altering the trial-averaged firing rate of individual neurons, stimulus onset alters on-going fluctuations in spontaneous activity of excitatory neurons such that the variability of spike times is dramatically reduced, at time scales of 100-200 ms (Poulet & Petersen, 2008; Shadlen & Newsome, 1998; Sussillo & Abbott, 2009). Notably, the reduction occurs even in response to stimuli that do not elicit a strong mean evoked response, such as occurs in recordings of orientation-tuned neurons presented with a non-preferred orientation. At the population level it is observed that during sensory stimulation spike time patterns occupy a subspace of possible patterns that such that spike patterns during stimulation appear to be constrained by the observed spontaneous activity of the same network (Luczak, Barthó, & Harris, 2009; Shadlen & Newsome, 1998). Together, this is evidence that the population spike patterns that occur during sensory stimulation are drawn from a parameter space of possible patterns observed during spontaneous fluctuations. Sensory responses represent a more narrowly restricted set of patterns and as such, display lower trial-to-trial variability compared to spontaneous activity. Next we estimated across-trial variability of PV spike times to assess the extent to which stimulus onset re-organizes the variability of PV neuron spike times. Given that PV neurons are highly connected within the local network, first we hypothesized that, similar to excitatory neurons, PV neurons exhibit a stimulus-driven decrease in variability of spike times.

We assessed across-trial variability by calculating the Fano Factor of individual neuron spike times prior to the stimulus (grey screen presentation, pre) and after stimulus onset (post). Fano Factor was computed as the spike time variance divided by the mean firing rate. Assuming spike times follow a Poisson process, which would yield a Fano Factor of 1, Fano Factor values greater than 1 can be interpreted as being an indication of cross-trial firing rate variability (Churchland et al., 2010; Mitchell et al., 2007; Nawrot et al., 2008). In both age groups, the mean Fano factor across animals was reduced at stimulus onset and approached a value of 1 (**Fig. 2.8a-d**; CP: pre, 2.75±0.13, post, 1.72±0.10, Wilcoxon signed rank test $p<0.001$; Mature: pre, 2.07±0.10, post,1.27±0.08, Wilcoxon signed rank test $p<0.001$). The magnitude of reduction, defined as the difference between mean pre and mean post-stimulus Fano factor values averaged over 3 seconds was similar for the critical period-aged and mature mice, 31±4.0% and 37±3.0%, respectively. Thus, similar to excitatory neurons, stimulus onset reduces across-trial variability of PV spike times.

2.4.4 The stimulus-induced reduction in Fano Factor is sensitive to deprivation throughout life.

Mechanistically it is unclear what gives rise to the stimulus-induced decline in variability. Decreased variability may be a property of large recurrent networks (Sussillo & Abbott, 2009), on the other hand stimulus-evoked shunting inhibition is well positioned to mediate the decline (Monier, Chavane, Baudot, Graham, & Frégnac, 2003). If stimulus-evoked inhibition is a contributing factor, then manipulations that decrease stimulus-evoked inhibition should prevent stimulus-onset from driving Fano Factor down to a value approaching 1. In other words, decreased inhibition should reduce the

magnitude of the stimulus-induced decline in variability such that Fano Factor is not reduced to 1. It was previously noted that experimentally it is difficult to test this prediction (Churchland et al., 2010). Given we showed that BD in critical period-aged mice creates a network state in which PV inhibitory neuron firing rate is decreased but excitatory neuron firing rate is maintained, we were in an excellent position to directly test the prediction. We found that the ability of stimulus-onset to drive Fano Factor to a value approaching 1 was not disrupted following 24 hours of BD (**Fig. 2.8e**, CP BD: pre, 1.75±0.12, post, 1.36±0.08, Wilcoxon signed rank test $p<0.001$). These results indicate that stimulus-evoked inhibition is not a major contributing factor to the stimulus-induced decrease in spike time variability. Consistent with this interpretation, the stimulus-induced decrease in Fano Factor in adults subjected to 24 hours of BD, which do not have altered PV neuron responsiveness, was similar to that of critical-period aged mice after 24 hours of BD (**Fig. 2.8f**, Mature BD: pre, 1.52±0.08, post, 1.24±0.08, Wilcoxon signed rank test $p=0.003$).

Unexpectedly, in both age groups following BD there was a decline in spike time variability in the non-stimulated epoch preceding stimulus onset (Fano Factor values, critical period: control pre, 2.75±0.13, BD pre, 1.75±0.12, Mann-Whitney U test $p<0.001$; mature: control pre, 2.07±0.10, BD pre, 1.51±0.08, Mann-Whitney U test $p<0.001$). Consistent with this observation, the median magnitude of the stimulus-driven decrease in Fano factor (magnitude of reduction, defined above), was significantly reduced following deprivation by 75% and 38% in critical period-aged and adult mice, respectively (**Fig. 2.8g,h**, K-S test $p<0.001$). These data indicate that brief deprivation

alters the spontaneous spike time patterns of PV neurons and that this deprivation-induced change is not restricted to the critical period.

2.5 Discussion

PV inhibitory neurons are generally thought to be mediators of experience-dependent plasticity. Despite their central role in postnatal development of sensory processing, systematic studies on the development and sensitivity of their response properties to brief deprivation are lacking. Here we identified three previously unrecognized characteristics of PV neuron development and plasticity. First, we established that the ability of PV neurons to rapidly modify their average firing rate in response to deprivation is a general property of primary visual cortex not restricted to the specialized area of binocular zone, and does not require the presence of open-eye inputs. Second, in contrast to evoked firing rate, we found that PV neuron orientation tuning is largely unaltered by brief deprivation during the critical period. Finally, our examination of spike rate variability revealed a previously unrecognized plasticity in adult PV neurons that may be indicative of a network-level state change inducible throughout life.

2.5.1 Deprivation-induced rapid plasticity of PV neurons does not require open eye inputs.

During so-called critical periods of development, cortical connectivity among excitatory neurons is highly malleable. This increased plasticity allows new experiences to shape the neural circuity used to encode behaviorally relevant information available in the animal's environment such that the neural circuitry is matched to local conditions. Classic monocular deprivation (MD) studies establish that the timing of critical period

plasticity is set by the protracted development of cortical PV inhibitory interneurons (Hensch, 2005). Two alternative mechanistic explanations as to how inhibition initiates critical period have been proposed (**Fig 2.9**). The first proposed that in response to MD the *imbalance* of visual input between the two eyes is detected and in response to this imbalance, PV neurons shift their ocular dominance away from the open eye in keeping with the spike-timing dependent plasticity model such that inhibition becomes relatively stronger in the closed-eye pathway and promotes long-term depression and/or suppression of closed eye inputs (Lu, Li, Zhao, Poo, & Zhang, 2007; Yazaki-Sugiyama et al., 2009 **Fig 2.9a**). The discovery that PV neurons, which are equally driven by both eyes in control conditions, shift their responsiveness towards the closed eye with 48 hours of MD is strong evidence in favor of this conceptual model (Yazaki-Sugiyama et al., 2009). Computational models constrained by experimental results provide further support of this proposal (Aton et al., 2013; Kuhlman et al., 2010). Alternatively, rather than an imbalance of input between the two eyes being the initiating factor, it has been proposed that deprivation itself is sufficient to cause an overall reduction in PV neuron responsiveness (**Fig 2.9b**). This proposal is based on the observation that brief MD causes a transient suppression of PV neuron activity in *both* the closed eye and open-eye pathways. Consistent with this observation, putative PV neurons identified by their narrow spike waveform recorded in the monocular zone were shown to rapidly suppress their activity within 24 hours of contralateral MD in freely moving animals in the monocular zone (Hengen et al., 2013). The transient reduction of PV-mediated inhibition is both required and sufficient for ocular dominance plasticity among excitatory neurons to proceed. Thus, it appears that disinhibiting weak, open-eye inputs during

MD creates a temporary permissive environment in which synaptic plasticity can update cortical processing to reflect new sensory conditions (van Versendaal & Levelt, 2016). A key prediction of the former proposal is that deprivation itself is sufficient to suppress PV neuron responsiveness, whereas PV neurons would not be expected to alter their response properties following binocular deprivation if it is an imbalance of input between the two eyes that drives the rapid decrease in PV neuron activity.

Here, we tested this key prediction by recording PV neurons following binocular deprivation and found that deprivation is sufficient to drive a decrease in PV neuron responsiveness. From this we conclude that an imbalance of ocular input is not required for PV neurons to alter their responsiveness, these results support the second proposed model. The distinction between these two models is important; an implication of the first model is that there is a biological circuit capable of computationally detecting closed versus open input pathways. The second model does not require such a pathway-specific detector circuit, rather, in response to deprivation the circuit transiently enters a disinhibited state. In this state despite lower sensory drive, the threshold for induction of LTD is maintained. Without disinhibition, it is expected that the threshold for LTD would be increased, due to BCM metaplasticity (Cooper & Bear, 2012), and closed eye inputs would not undergo LTD. Consistent with this model, blockade of disinhibition via infusion of a GABA receptor use-dependent agonist during monocular deprivation blocks ocular dominance plasticity (Kuhlman et al., 2013).

Our study focused on PV neurons, identified by their molecular expression of parvalbumin and functional narrow spike waveform. Using this targeted approach in both bV1 and mV1, we were able to directly address an open question in the literature.

Previously it was observed that bV1 inhibitory neurons studied as a general class, identified by the molecular expression of the GABA synthesizing enzyme GAD67, exhibit a delayed shift in ocular dominance following MD (Gandhi, Yanagawa, & Stryker, 2008). Computationally it was shown in this same study that a transient mis-match or imbalance between inhibition and excitation can promote LTD of closed eye inputs by suppressing closed eye inputs. In this view, plasticity of inhibition would be instructive rather than permissive, as are the proposed models discussed above (Aton et al., 2013; Kuhlman et al., 2010; Yazaki-Sugiyama et al., 2009). On the other hand, reports in which putative inhibitory neurons are identified by their functional narrow spike waveform, demonstrate that fast-spiking neurons rapidly respond to deprivation in mV1 without delay (Hengen et al., 2013). Our study directly demonstrates that fast-spiking PV neurons rapidly respond to deprivation in both bV1 and mV1; however the question of how the other inhibitory GABAergic neuron subtypes, such as VIP and somatostatin-expressing neurons, shift their eye dominance during deprivation-induced plasticity remains open. Furthermore, the extent to which permissive disinhibition applies to animals with cortical columns and less contralateral bias in excitatory neuron drive, such as the cat, remains unclear. In the case of cats, the data support an instructive model in initial column development and MD (Aton et al., 2013; Hensch & Stryker, 2004).

Our finding that the initial deprivation-induced reduction in evoked inhibitory responses is specific to PV neurons and does not occur in excitatory neurons, and that this cell-type specificity is a general feature of primary visual cortex rather than a specialized property of the binocular zone (see also Hengen et al., 2013), has interesting implications for the engagement of cell autonomous homeostasis and set-

point firing rates of individual cortical excitatory neurons (Hengen et al., 2013). Our results indicate that during the first 24 hours of deprivation, network homeostasis mediated by PV neurons dominates such that excitatory neurons are not induced to alter their firing rate set-point cell autonomously. It will be of interest in future studies to elucidate the interplay between network and cell-autonomous homeostatic mechanisms.

We found that the broad orientation tuning of PV neurons is largely unaltered by brief deprivation, but the percentage of neurons which are tuned increases following deprivation. Even these more tuned neurons do not return all the way back to immature levels, and are likely not tuned enough to truly represent meaningful changes in the network. This differential vulnerability to brief deprivation between firing rates and broad tuning properties indicates that the circuit basis and underlying cellular signaling pathways that underlie the development of these two properties are likely distinct.

2.5.2 Deprivation induces a decrease in spike time variability during spontaneous but not visually evoked epochs, throughout life.

Experimental evidence demonstrates that recent visual experience is reflected in spontaneous cortical activity patterns (Han, Caporale, & Dan, 2008). Considering Hebbian rules of plasticity, it has been proposed that the spontaneous state's statistics observed in the absence of visual drive may reflect past input statistics as experienced during vision, such that the upper limit of the number of spontaneous spike time patterns that are entered is set by how many patterns were recently experienced during vision (Doiron & Litwin-Kumar, 2014). The more spike time patterns present, the higher the variability (Doiron, Litwin-Kumar, Rosenbaum, Ocker, & Josić, 2016; Litwin-Kumar, Rosenbaum, & Doiron, 2016). Thus, an implication of our findings is that 24 hours of

visual deprivation is sufficient to degrade the number of spike time patterns that spontaneously occur in PV neurons. This is consistent with the view that spontaneous activity is structured in space and time, and reflects the underlying network connectivity among neurons (Ringach, 2009). Independent of this implication, we were able to clearly demonstrate that a reduction in sensory evoked inhibition did not impact the ability of stimulus onset to reduce the Fano Factor to values approaching 1.

Notably, the deprivation induced decrease in spike time variability that we observed in the spontaneous state occurred in both critical period-aged and adult mice. Given PV neurons are thought to pool the activity of many neighboring excitatory neurons (Bock et al., 2011), it is possible that our measures of PV neuron spike time variability in the spontaneous state is a readout of the spike time patterns generated by the excitatory network. In this context, our results raise the possibility that the spike time patterns generated by excitatory neurons in the spontaneous are equally sensitive to deprivation in the critical period and the adult.

In summary, the evoked firing rate of PV neurons is dramatically reduced by brief deprivation in a manner that is developmentally restricted to the critical period. The reduction leads to disinhibition, setting the stage for cortical rewiring in response to deprivation. On the other hand in the adult, PV neurons do not initiate cortical rewiring of excitatory network via disinhibition, but are likely to reflect more subtle changes in activity patterns generated by the excitatory network following brief deprivation.

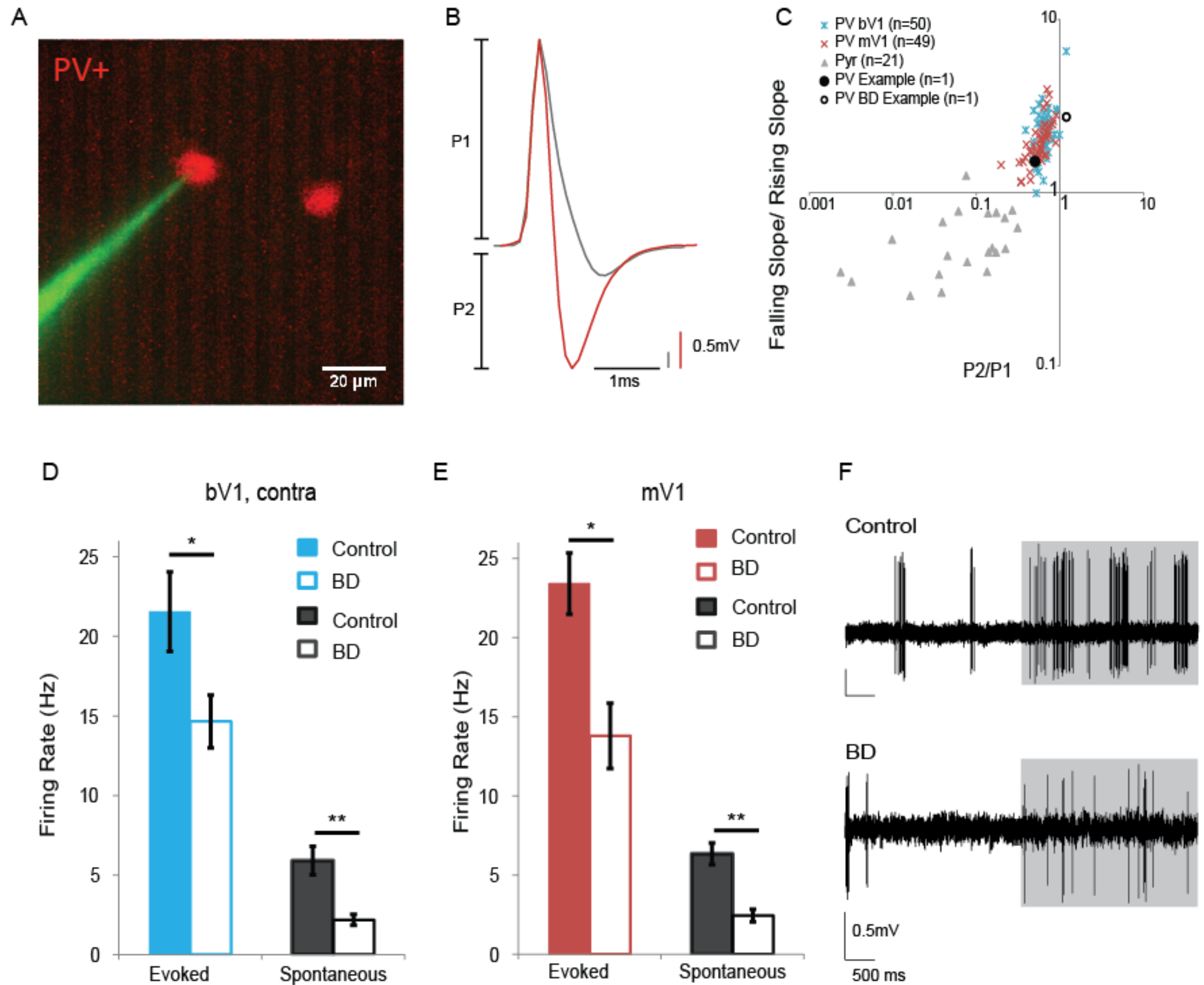


Figure 2.1 Open-eye inputs are not required for rapid plasticity of visually evoked responses in PV neurons. (A) Two-photon image of recording pipette approaching a parvalbumin-positive inhibitory neuron. Scale bar: 20 µm. (B) Average spike waveforms of a parvalbumin-positive inhibitory neuron (red) and a putative excitatory neuron (grey). P1 denotes the amplitude of the spike-wave peak, and P2 denotes the nadir. Scale bars: 1 ms, 0.5 mV. (C) Spike waveforms of inhibitory and excitatory neurons are distinct for all conditions examined. Black circles indicate neurons shown in F. (D) Evoked and spontaneous firing rates for control (filled bars) and BD (open bars) conditions of PV neurons at their preferred orientation for contralateral eye responses in binocular zone (bV1) (Control n=26 cells from 9 animals; BD 24 cells from 7 animals). (E) Similar to D, except for the monocular zone (mV1) (Control n=33 cells from 17 animals; BD 18 cells from 11 animals). (F) Example spike traces, response during a preferred orientation is highlighted by grey. Scale bars: 500 ms, 0.5 mV. * $p<0.05$, ** $p<0.001$ Mann-Whitney U-test.

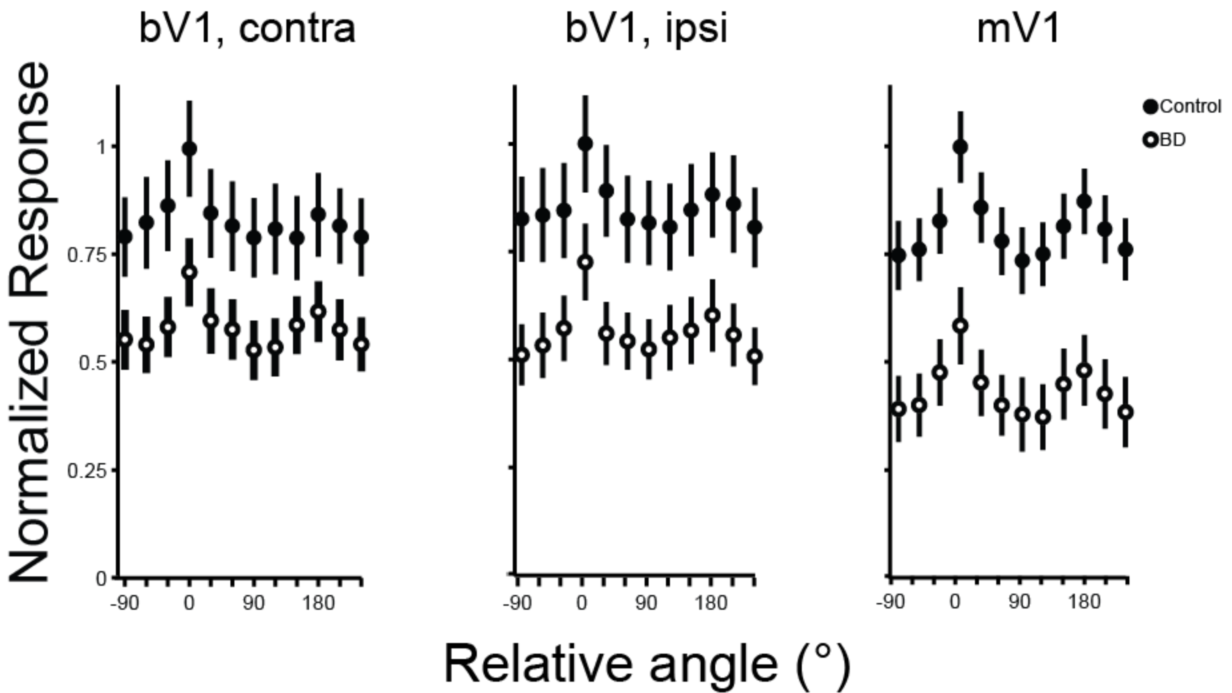


Figure 2.2 Deprivation reduces PV neuron evoked responses at all orientations. Orientation tuning curves averaged across neurons for control (filled circles) and BD (open circles) conditions, brain region (bV1 or mV1) and eye stimulation of recordings is indicated. Data are normalized to the mean preferred firing rate of the control population to qualitatively visualize impact of deprivation on orientation tuning. (bV1 contra: Control n=26 cells from 9 animals; BD 24 cells from 7 animals. bV1 ipsi: Control n=25 cells from 9 animals; BD 23 cells from 7 animals. mV1: Control n=33 cells from 17 animals; BD 18 cells from 11 animals).

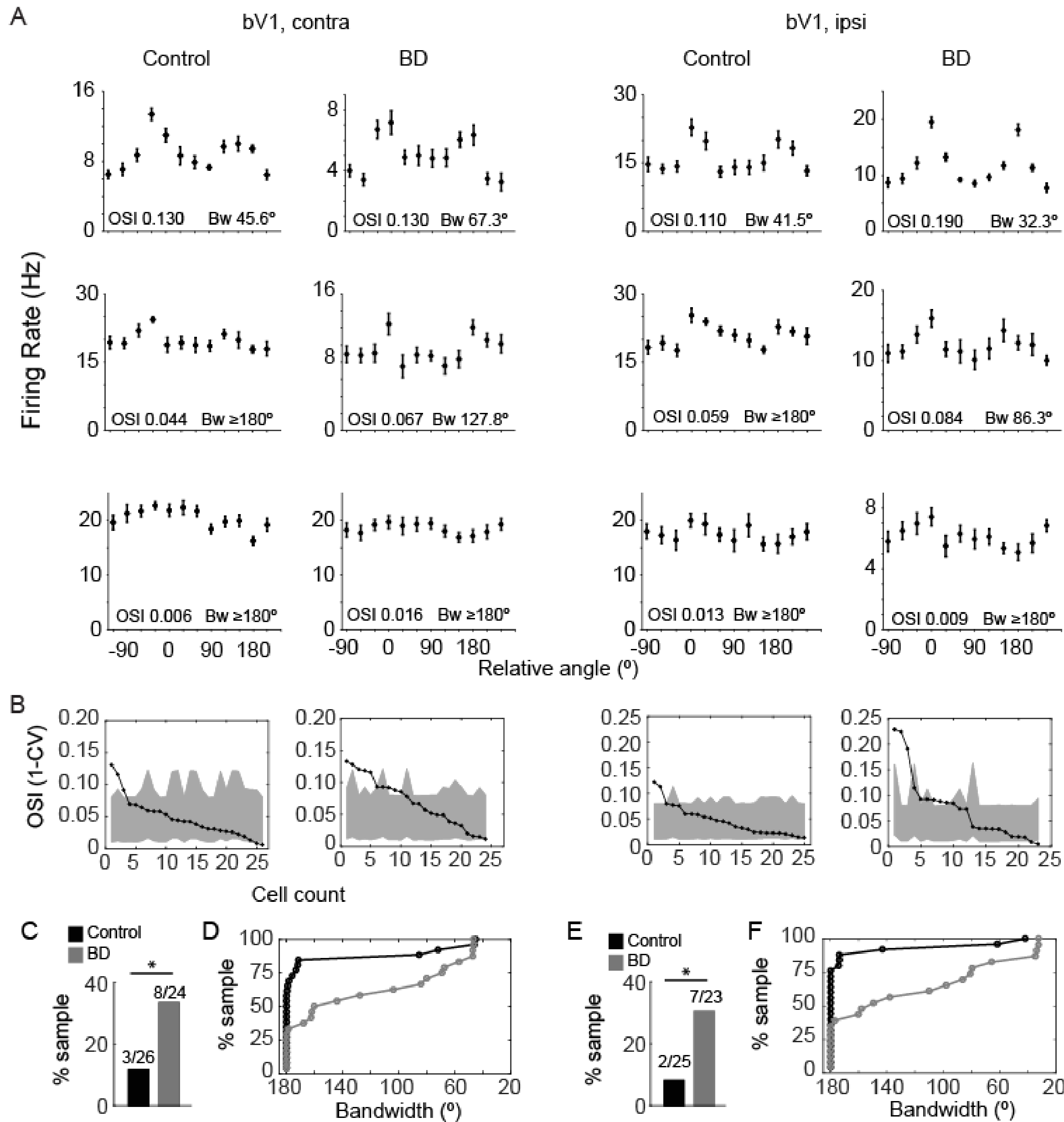


Figure 2.3 PV neuron orientation tuning sharpens slightly yet remains broad in bV1 following brief deprivation. (A) Three example orientation tuning curves of individual neurons recorded in bV1, covering the range of OSI values observed for control and BD conditions. Eye stimulation as indicated by column label (contra or ipsi). Note the majority of neurons have OSI values less than 0.10. (B) Summary plot of OSI values of individual neurons, sorted in descending order, along with their individual confidence interval value (gray shading denotes the 95-5th interval range). Eye stimulation as indicated by column label. Neurons falling above the 95th confidence interval can be considered tuned. (C,E) Fraction of neurons with OSI values greater than the 95th confidence interval in control (black) and deprived (gray). Eye stimulation as indicated by column label. *p<0.05 binomial test. (D,F) Cumulative distribution histogram of bandwidth values of individual neurons. Eye stimulation as indicated by column label. Note that the rightward shift following deprivation can be explained by an increase in the number of neurons having a bandwidth value <180 degrees. (bV1 contra: Control n=26 cells from 9 animals; BD 24 cells from 7 animals. bV1 ipsi: Control n=25 cells from 9 animals; BD 23 cells from 7 animals).

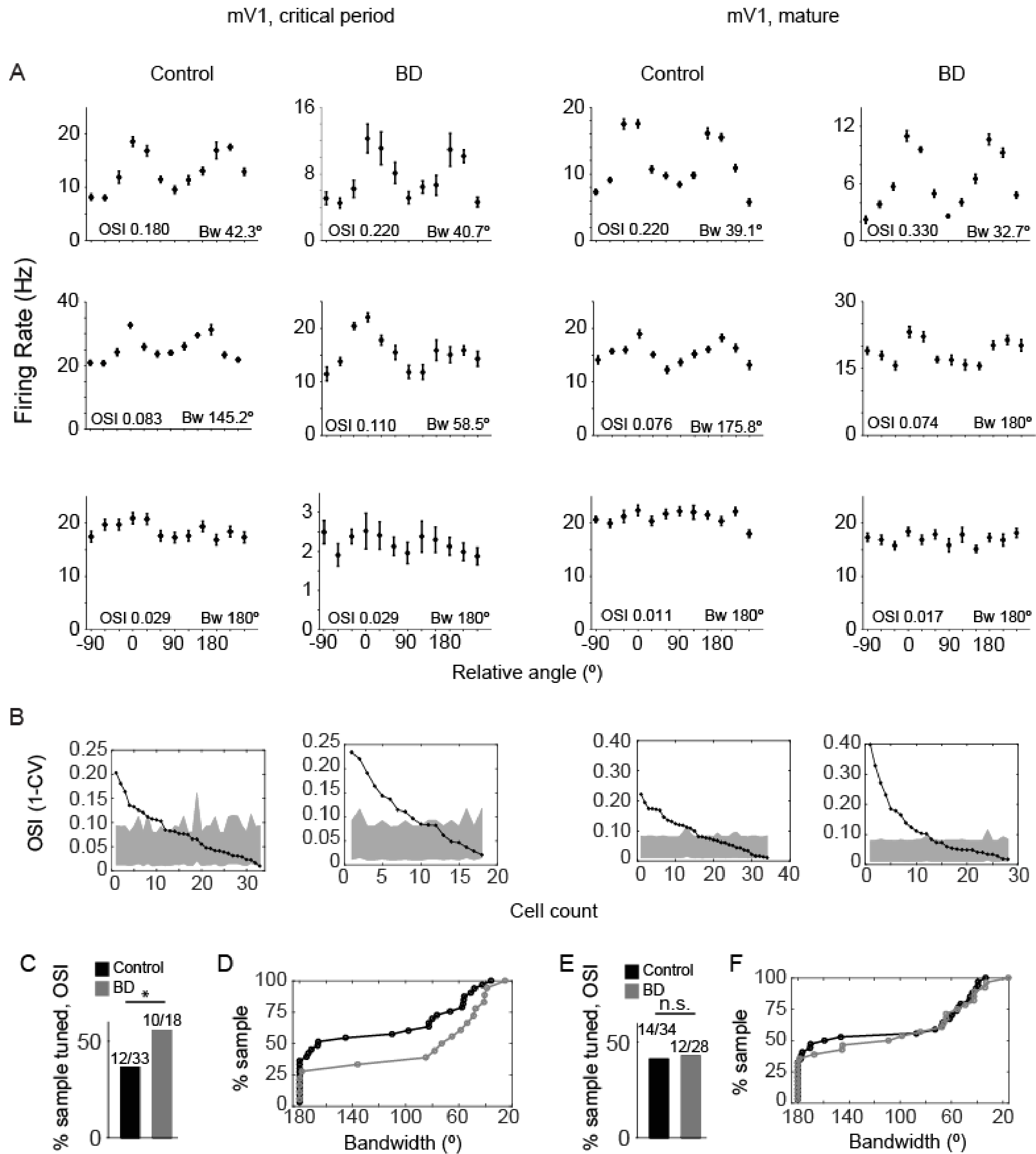


Figure 2.4 Deprivation-induced sharpening of PV neuron orientation tuning is age-restricted. (A) Representative tuning curves of tuned, medium tuned, and untuned PV neurons within monocular zone of primary visual cortex under control and BD conditions. Age of recordings is indicated above. (B) data from mV1 plotted as in Figure 2.3 (mV1 CP: Control n=33 cells from 17 animals; BD 18 cells from 11 animals. mV1 mature: Control n=34 cells from 9 animals; BD n=28 cells from 6 animals). (C,E) Quantification of neurons shown in B which fall above the grey line, percentage of total neurons increases following BD only during the CP. *p<0.05 binomial test (D,F) Comparison of bandwidth between control and BD for CP and Mature. There is only a difference in bandwidth following BD in CP aged mice.

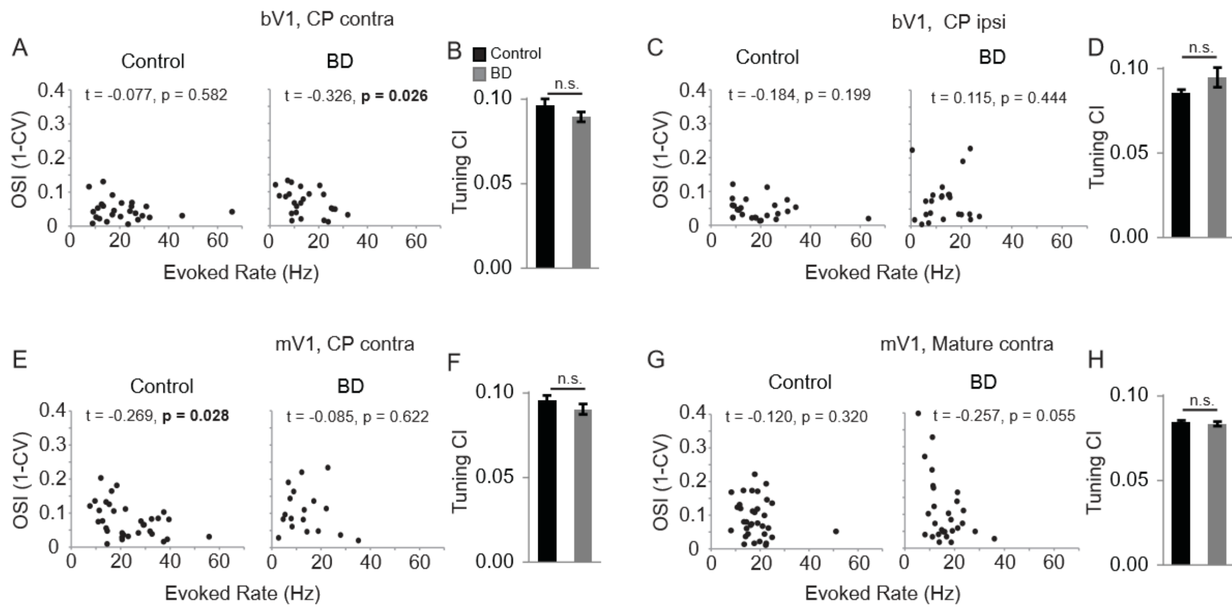


Figure 2.5 Orientation selectivity indices plotted as a function of evoked firing rates. (A,C,E,G) Kendall correlation of evoked spike rate and OSI. Significant p values are in bold. Brain region, age, and eye stimulation as indicated. (B,D,F,H) Mean 95th confidence intervals for OSI tuning, averaged across neurons for a given brain region, eye stimulation, and age as indicated. No differences were detected when comparing control versus BD conditions, indicating that a decrease in signal-to-noise cannot account for the increase in OSI observed following BD in the critical period age group.

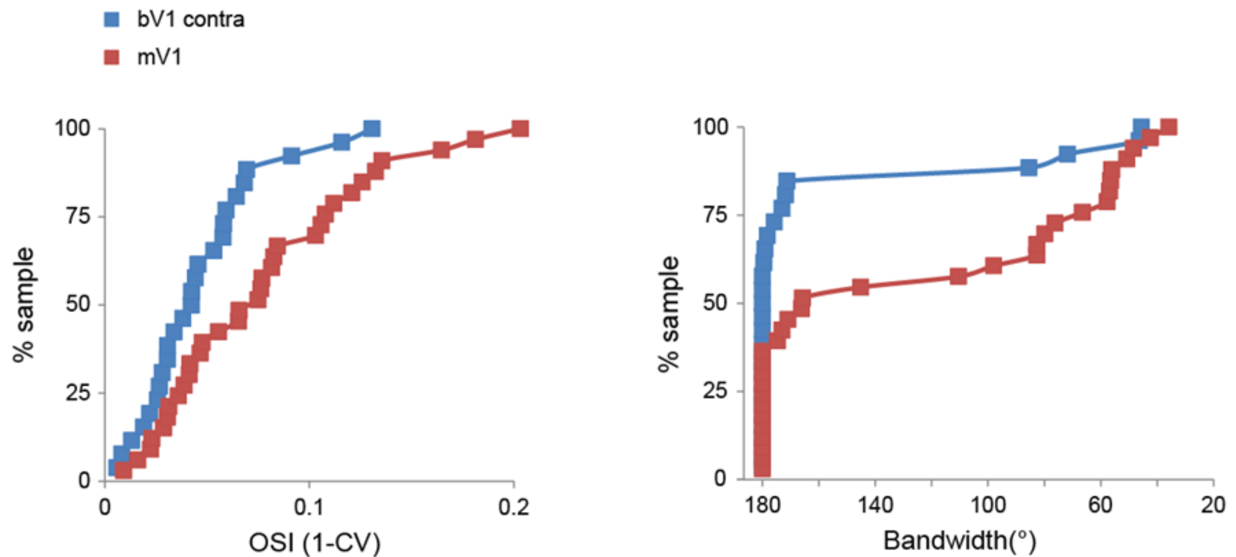


Figure 2.6 PV neurons are more tuned within monocular than binocular zone of primary visual cortex. Cumulative probability plots of OSI and bandwidth of PV neurons in either binocular zone (blue) or monocular zone (red) of primary visual cortex. Both OSI and bandwidth show a higher portion of tuned neurons within the monocular zone.

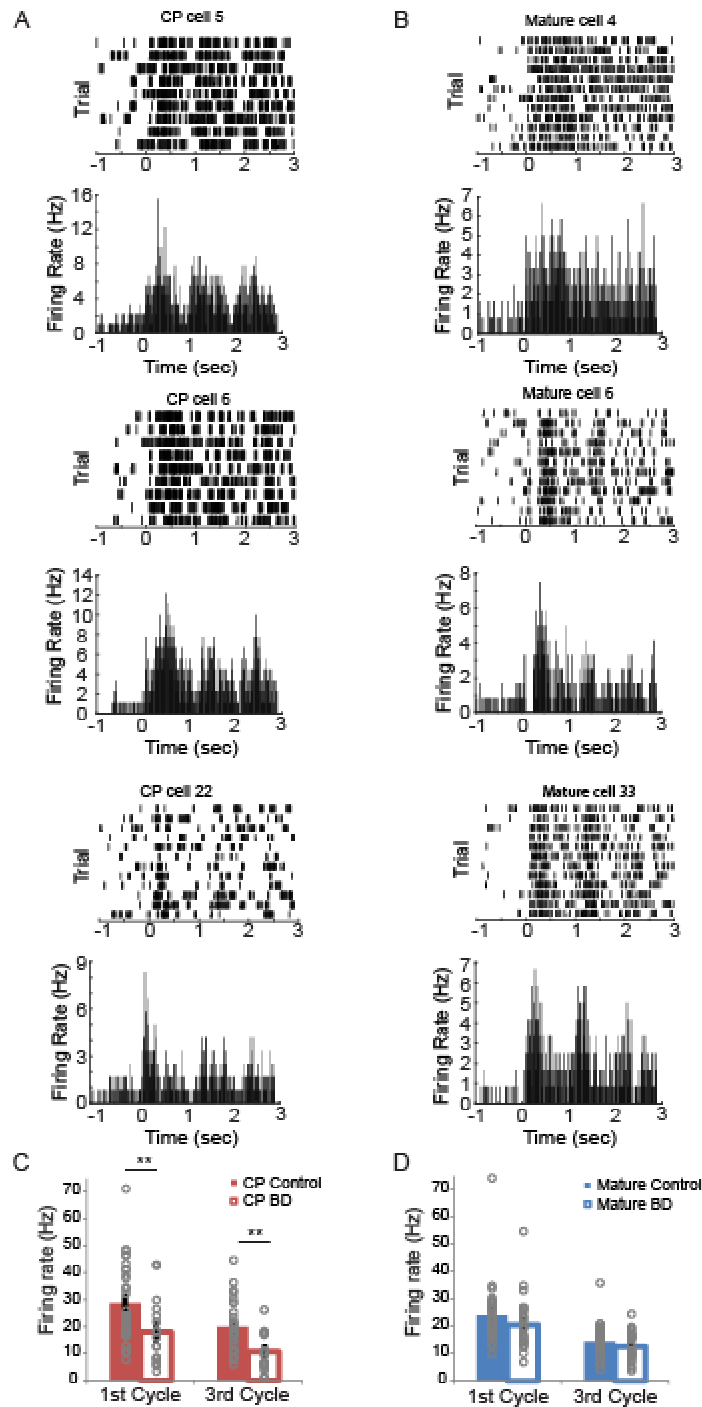
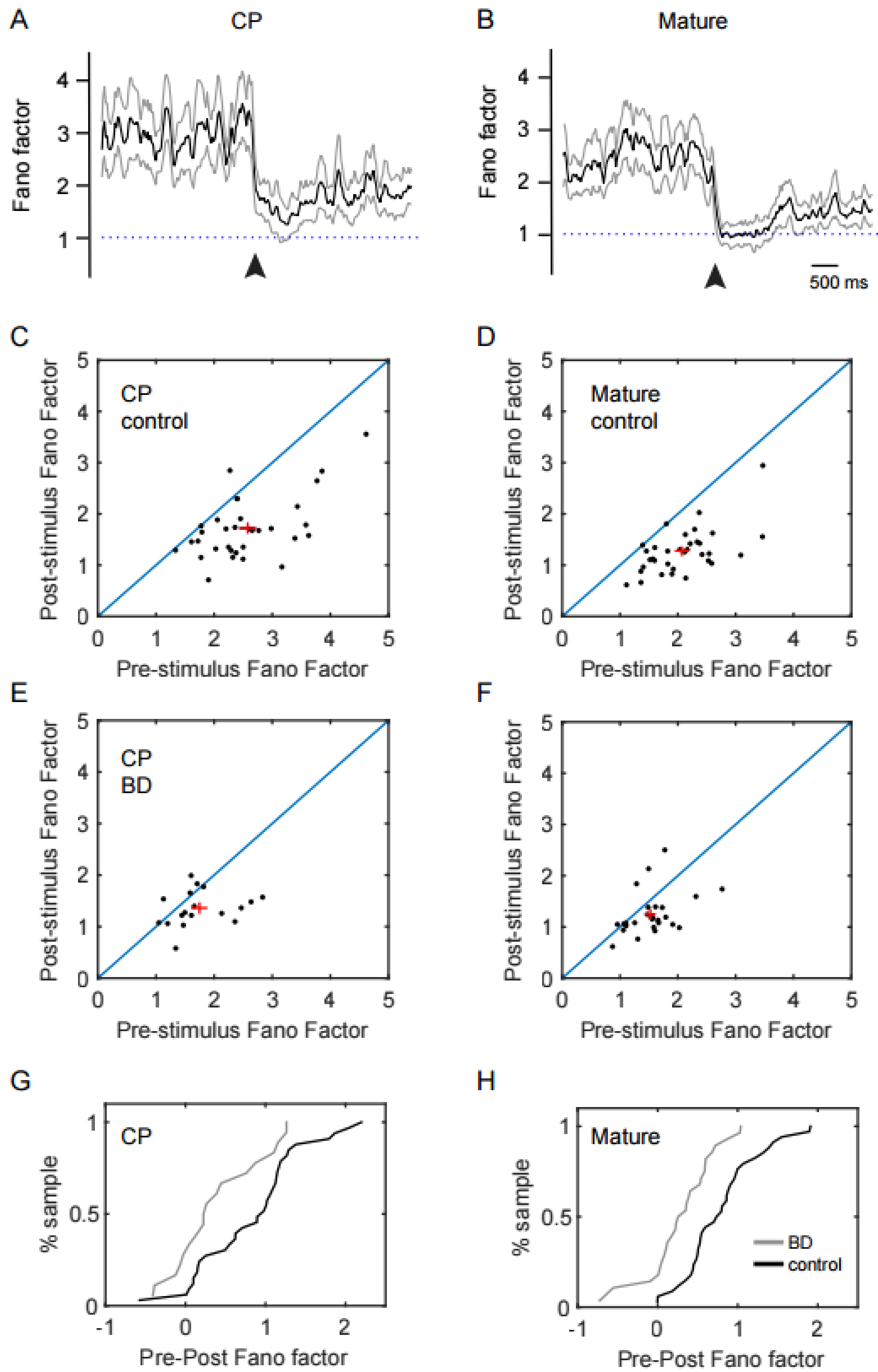


Figure 2.7. Deprivation-induced suppression of PV responsiveness is age-restricted. (A-B) Representative raster plots and peri-stimulus time histograms (10 ms bins) of PV neurons in critical period and mature mice. Stimulus onset is at 0. 1 second of grey-screen is shown and all 3 seconds of stimulus presentation for the preferred stimuli. No difference was detected in the latency to maximum firing rate between the two age groups (p = 0.415 Mann Whitney U). (C-D) First and third cycle evoked firing rates for control (filled bars) and BD (open bars) conditions of PV neurons at their preferred orientation. Animal age group is indicated. * p<0.05, ** p<0.001 Mann-Whitney U-test, Bonferroni corrected for 4 comparisons.


A
CP
Fano factor
4
3
2
1
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Mature
Fano factor
500 ms
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Post-stimulus Fano Factor
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D
Mature
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control

Figure 2.8 Spike time variability during non-stimulus epochs is reduced by deprivation. Fano factor analysis of neural responses to the preferred orientation and the grey screen immediately preceding stimulation. (A,B) Sliding average of Fano factor (bold) across the population (binned by 200 ms) prior to and after stimulus onset (arrowhead). Animal age group is indicated. (C-F) Scatter plots of Fano factor values of individual neurons (binned by 200 ms), averaged across the 3 seconds of grey screen preceding stimulus (pre-stimulus) and 3 seconds of visual stimulation (post-stimulus). Animal age group and treatment condition is indicated. Mean and SEM are indicated by red crosses, note the leftward shift of mean pre-stimulus values in control versus BD in both age groups. (G,H) Cumulative distribution plots of pre-post Fano factor differences for individual neurons. Animal age group and treatment condition is indicated. In both age groups there is a leftward shift following BD.

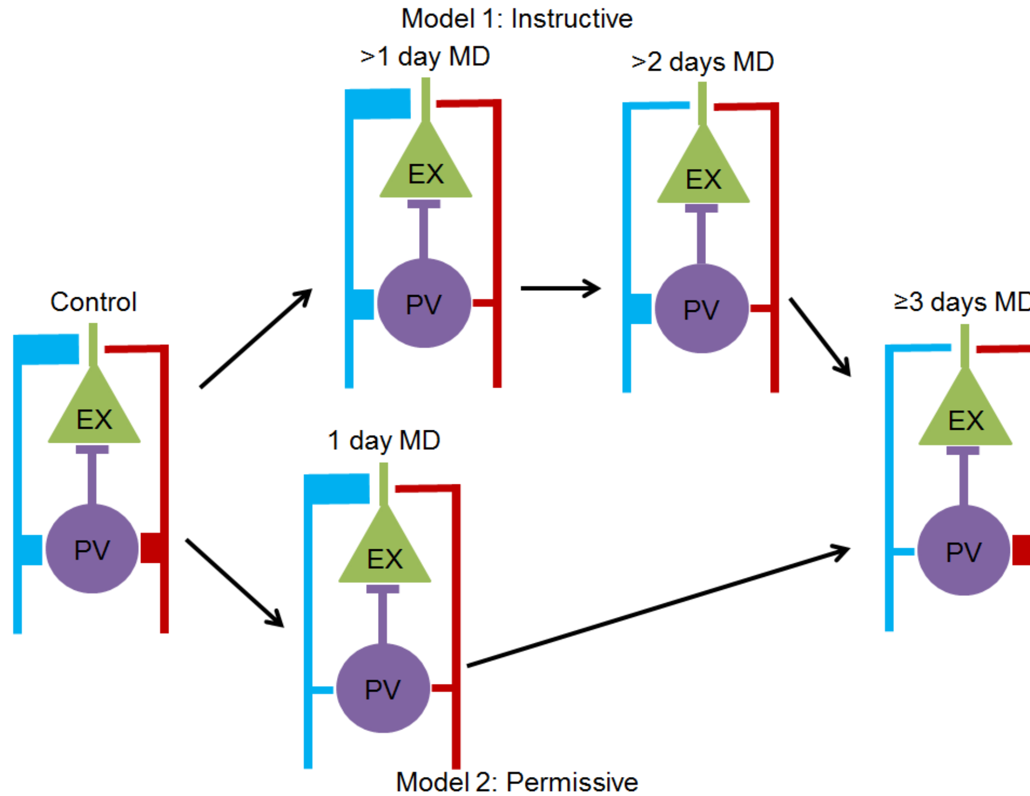

Figure 2.9 Two alternative models for the role of PV neurons in ODP. (A), Within Model 1 PV neurons play an instructive role in excitatory neuron plasticity. Initially excitatory neurons receive stronger inputs from the contralateral eye (illustrated in blue) than from the ipsilateral eye (illustrated in red). PV neurons, however, receive equal input from both eyes and then in turn inhibit the excitatory neurons. Following 2 days of monocular deprivation the input onto PV neurons has shifted such that they are now driven more strongly by the contralateral eye. This allows for the PV neuron to give unequal, instructive inhibition onto the excitatory neuron. By three days, the input from the contralateral eye onto the excitatory neuron has undergone LTD and now the excitatory neuron is driven equally by both eyes. (B), In Model 2 PV neurons play a permissive role in excitatory neuron plasticity. The starting state of the network is the same with excitatory neurons being more strongly driven by contralateral inputs than ipsilateral inputs while the PV neurons are being driven equally well by both. Following 1 day of MD the PV neurons become less driven putting the excitatory neurons into a disinhibited space. This disinhibition allows for plasticity to occur and LTD to suppress contralateral inputs to the excitatory neurons by 3 days.

3.0 Binocular deprivation induced plasticity of L2/3 parvalbumin inhibitory neurons is dependent upon ErbB4

3.1 Abstract

Transient deprivation has been shown to induce a rapid reduction in the activity of parvalbumin expressing (PV) inhibitory interneurons within layer 2/3 of the primary visual cortex. This rapid reduction in PV neuron firing rates results in a disinhibited state of the local circuit and is permissive of excitatory neuron plasticity. This rapid reduction in PV neuron firing rates is proposed to be caused by the weakening of excitatory inputs especially from L4 and L5a onto the PV neurons. Recently, it was shown that this weakening of inputs could be blocked by injecting the ligand of ErbB4, neuregulin-1 (NRG1) during the period of deprivation and that mice lacking ErbB4 in their PV neurons did not undergo ocular dominance plasticity (Y. Sun et al., 2016). This suggests that within PV neurons, ErbB4 may be essential for the regulation of their firing rates, especially following sensory perturbations. A prediction from this observation is that the rapid 30-40% reduction in PV neuron firing rate, which is usually seen following binocular deprivation, is dependent upon ErbB4. Using transgenic mice and two-photon guided cell-attached recordings in vivo and the paradigm of 24 hour binocular deprivation, we found that PV neurons lacking ErbB4 no longer showed this decrease. Therefore, ErbB4 is necessary for the normal plasticity of PV inhibitory neurons. It appears that without ErbB4, PV neurons lose much of their ability to modulate their dynamic range of firing rates. This lack of plasticity could be related to the learning and behavioral deficits seen in mice lacking ErbB4 within PV neurons.

3.2 Introduction

The receptor tyrosine kinase ErbB4 and its ligand neuregulin-1 (NRG1) have been studied largely in relation to the possible roles that they both might play in the neurodevelopmental disease schizophrenia. ERBB4 and NRG1 are both schizophrenia risk factor genes (Mei & Xiong, 2008). In the last few years it has been shown that within the cortex, ErbB4 is expressed exclusively within inhibitory neurons, and it is quite prevalent within parvalbumin-expressing inhibitory interneurons (PV) (Bean et al., 2014; Fazzari et al., 2010). Its ligand, NRG1, is expressed by both excitatory and inhibitory neurons in an activity dependent manner (X. Liu et al., 2011; Y. Sun et al., 2016). Various defects have been found in mice in which ErbB4 is lacking in PV neurons including a decreased number of excitatory synapses onto the PV neurons and increased activation of excitatory neurons (Del Pino et al., 2013; Fazzari et al., 2010; Yang et al., 2013). The mice in which ErbB4 is lacking from the PV neurons also exhibit various behavioral phenotypes including, but not limited to being hyperactive in open field tests, decreased pre-pulse inhibition, and deficits in working memory (Del Pino et al., 2013; Shamir et al., 2012; Wen et al., 2010).

The decreased number of excitatory synapses onto PV neurons which was observed could explain the increased activation of excitatory neurons as well as many of the behavioral phenotypes. It is suggestive that PV neurons just may not be providing as much inhibition to the network. Since PV neurons develop their normal firing properties in an experience dependent manner, and NRG1 is produced in an activity dependent manner, we set out to test the hypothesis that ErbB4 is necessary for PV neurons to develop their normal, high firing rates. Using transgenic mice and two-

photon guided, cell-attached recordings in vivo, we found that PV neurons lacking ErbB4 showed a 25% decrease in firing as compared to their wild-type littermate controls. This decrease in the firing rate of PV neurons lacking ErbB4 raised the question as to whether they had ever developed their normal dynamic range.

Since we first began the study, two papers were published both exploring the role of ErbB4 specifically within the primary visual cortex (Gu et al., 2016; Y. Sun et al., 2016). These papers both addressed aspects of how ErbB4 is involved in ocular dominance plasticity (ODP). ErbB4 is necessary in order for ODP to occur, and this is believed to be due to its function of modulating excitatory synapses onto PV neurons. Excitatory synapses especially from L4 and L5a onto PV neurons were found to be rapidly reduced following 24 hours of monocular deprivation (Kuhlman et al., 2013). Although these synapses can be rapidly lost following deprivation, they are maintained if injections of NRG1 are administered during the course of the deprivation (Y. Sun et al., 2016). NRG1 increases excitation onto PV neurons and increases the excitability of putative PV neurons in vivo (Gu et al., 2016; Y. Sun et al., 2016).

A model based on previously done work, and explained in the Sun paper is as follows. Since NRG1 is regulated in an activity dependent manner, deprivation results in a decrease in its production. Less NRG1 leads to a weakening of excitatory synapses onto PV neurons. The weakening of excitatory synapses and therefore decreased drive onto the PV neurons results in lower firing of the PV neurons and therefore an overall disinhibition of the local circuit. Once disinhibition occurs, ocular dominance plasticity can then be initiated.

A prediction of this model is that the reason ErbB4 is necessary for ODP to occur is because without ErbB4 the PV neurons cannot modulate their firing and allow the circuit to go into a disinhibited state. To test this, we used the paradigm of 24 hour binocular deprivation in mice lacking ErbB4 in their PV neurons. Indeed, we found that whereas binocular deprivation usually would induce a 30-40% decrease in PV neuron evoked mean firing rate, PV neurons lacking ErbB4 no longer showed this decrease. This suggests that ErbB4 is necessary at least for much of the rapid plasticity of PV neurons which is normally seen following sensory perturbations.

We went on to measure the effect of stimulus onset on trial-to-trial variability of PV neurons using Fano factor. Although stimulus onset still decreased the overall variability of PV neurons lacking ErbB4, the magnitude of this decrease was only half as much as in controls. This suggests that not only is ErbB4 necessary for PV neurons to modulate their firing rates in response to deprivation, but even their modulation of firing rates in response to sensory stimuli may be deficient.

Overall, our results provide further evidence supporting the model of ErbB4 and NRG1 being essential for plasticity via the mechanism of decreased activity downregulating NRG1, which in turn destabilizes excitatory synapses on PV neurons via ErbB4, resulting in disinhibition, and allowing plasticity. The inability of PV neurons to modulate their firing rates without ErbB4 could explain the deficits in learning seen in knock-out mice. Furthermore, the direct *in vivo* measurement of firing rates in PV neurons lacking ErbB4 mirrors what would be expected based on behavioral phenotypes with increased hyperactivity.

3.3 Materials and Methods

3.3.1 Animal preparation and surgery

All experimental procedures were compliant with the guidelines established by the Institutional Animal Care and Use Committee of Carnegie Mellon University and the National Institutes of Health. Binocular deprivation experiments were performed in ErbB4PV mice made by crossing ErbB4 floxed mice (MMRRC, B6;129-Erbb4tm1Fej/Mmucd, stock no. 010439-UCD) with PV-cre knock-in mice (Jackson Laboratories, stock no 008069) and then crossing those offspring back to ErbB4fl mice and genotyping for mice homozygous for the ErbB4fl mutation and heterozygous for the PV-cre. These ErbB4fl homozygote/PV-cre heterozygotes were then mated with one another to produce offspring all of which were homozygous for ErbB4fl and approximately ¼ lacking PV-cre, ¼ homozygous for the PV-cre mutation, and ½ heterozygous for the PV-cre mutation. Genotyping was conducted and mice that were either heterozygous or homozygous for the PV-cre mutation were used.

Littermate control mice were generated by crossing PV-cre knock-in mice (Jackson Laboratories, stock no 008069) with the above described ErbB4PV mice. These offspring, designated Eb4PV_het were used as long as they were homozygous for PV-cre. ErbB4 floxed mice (MMRRC, B6;129-Erbb4tm1Fej/Mmucd, stock no. 010439-UCD) were also crossed with with tdTomato reporter knock-in mice from Jackson Laboratory (Jackson Laboratories, stock no. 007908). These offspring were designated Eb4Ai14. Eb4PV_het mice were then crossed with Eb4Ai14 mice to generate Eb4PV_LC mice. Three possible genotypes exist for the Eb4PV_LC mice, all of them are heterozygous for PV-cre, but 25% do not have floxed ErbB4, 50% are

heterozygous for floxed ErbB4, and 25% are homozygous for floxed ErbB4. For our experiments we used the homozygous floxed ErbB4 mice for our knock-out condition and the homozygous for wildtype ErbB4 for our controls. The heterozygous floxed ErbB4 were not used.

Mice which underwent the binocular deprivation paradigm were anesthetized under isoflurane (3% induction and 1.5-2% maintenance). Silicone oil was applied to both eyes to prevent drying. A single mattress suture (silk 6-0) was made through each eyelid to hold the eye closed. These sutures were made 24 hours prior to the craniotomy surgery and monitored to ensure maintained closure. Any mice which showed signs of infection or lid separation were removed from the study.

ErbB4PV mice and Eb4PV_LC mice lacked genetically fluorescent labeled neurons. Between the ages of P12-P14 these mice were given one injection of AAV1.CAG.FLEX.tdTomato.WPRE.bGH (U Penn Vector Core, AV-1-ALL864) into their left primary visual cortex. They were anesthetized under 3% isoflurane induction and maintained under 1.5-2%. Silicone oil was applied to both of their eyes to prevent drying. A 2-3mm incision was made medially of the left ear and skin was retracted to expose the skull. A burr hole was drilled 1 mm anterior of the muscle insertion point. The pipette was lowered to the surface of the dura and then diagonally inserted to a depth of -500 µm. Virus was injected every 50 µm between -500 and -100 µm depth.

For surgeries mice were anesthetized with isoflurane (3% induction and 1.5-2% maintenance). Their body temperature was kept constant at ~37.5°C using a heating plate. The eyes of any mice not undergoing binocular deprivation were protected with silicone oil at the onset of surgery. For mice with eye sutures, their eyes remained

sutured shut until ready for recording at which point the sutures were removed and silicone oil was applied to their eyes. A custom made stainless steel head-bar was affixed to the right side of the skull using ethyl cyanoacrylate glue and dental acrylic and a silver chloride ground electrode was implanted over the cerebellum. A 1.5-2.5 mm craniotomy was made over the left visual cortex. Craniotomies were positioned as described in Kuhlman et al. 2011. A 2.5 mm coverslip was then secured over a portion of the brain using dental acrylic and cortex buffer (125mM NaCL, 5mM KCl, 10mM glucose, 10mM HEPES, 2mM $CaCl_2$, 2mM $MgSO_4$) was used to keep the brain moist as well as to facilitate imaging.

3.3.2 In vivo cell-attached recording

Mice were sedated with chlorprothixene hydrochloride(5 mg/kg) and anesthetized with urethane(0.5 g/kg). *In vivo* imaging was performed on a two-photon microscope (Scientifica) imaging system controlled by ScanImage 3 software (Vidrio Technologies, Pologruto, Sabatini, & Svoboda, 2003). The light source was a Chameleon ultra 2 laser (Coherent) running at 930 nm. A 40x water-immersion objective from Olympus was used to pass the laser beam. Surface blood vessels, coverslip, and pipette were viewed in visible-light conditions using a green filtered light.

Pipettes had a resistance of 5-12 MΩ when filled with cortex buffer and 20 µM Alexa Fluor-488 hydrazide (Invitrogen). Labeled neurons were first identified using 2-photon imaging. Their x, y, and z coordinates were recorded and then the pipette was positioned above the neuron’s location at low magnification. A Patchstar micromanipulator (Scientifica) was used to back the pipette up an appropriate distance such that moving it in x and z at a 35° angle would result in it hitting the neuron (roughly

1.73 x the depth of the neuron). The pipette was lowered towards the surface of the brain first under low, then high magnification. The pressure of the pipette was raised to approximately 200 mBar positive pressure and a slight increase in resistance marked contact between the pipette tip and the surface of the brain. The pipette was lowered at a 35° angle into the brain and pressure was reduced to 50 mBar as soon as the dura was penetrated. Once through layer 1 the pressure was reduced to 20-30 mBar until the desired neuron was attained. 2-photon imaging was used to guide the pipette towards the desired neuron and minor changes in y were made as needed. The targeting technique was based on Kuhlman, Tring, & Trachtenberg, 2011 and Liu et al., 2009. Once the pipette appeared to be touching the neuron, the resistance was lowered and spontaneous spikes could usually be detected. Resistance was decreased to 0 and the pipette was advanced until a 20-200 MΩ loose cell-attached seal was obtained. Occasionally negative pressure was applied up to -50 mBar. Recordings of spontaneous and then evoked spikes were made in current clamp mode. Signal was acquired with a MultiClamp 700B amplifier in current-clamp mode, a National Instruments digitizer, and WinEDR software (J Dempster, Strathclyde University). Signal was sampled at 10.02 kHz. Pipette capacitance was compensated.

3.3.3 Visual stimulation

Visual stimulation consisted of full field square wave gratings presented at 6 orientations spaced 30° apart moving in two directions (12 total stimuli). A temporal frequency of 1 Hz and spatial frequency of 0.02 cycles per degree (cpd) was used for putative PV neurons while a temporal frequency of 2 Hz and spatial frequency of 0.04 cpd was used for putative excitatory neurons. Stimuli were developed using custom

software with PsychToolbox in Matlab (Mathworks). Stimuli were presented one at a time in a random order for 3 seconds at 100% contrast followed by a 3 second blank grey screen with equal mean luminance. Each stimulus was presented 3-12 times. Stimuli were presented on a 40-cm-wide gamma-calibrated LCD monitor. For monocular zone recordings, the monitor was positioned 25 cm in front of the mouse's right (contralateral to site of recording) eye. Mouse was positioned looking straight forward with a 5-15% rightward tilt to accommodate the brain site for recording being relatively flat. The mouse's nose was approximately aimed towards the left of the screen with the right eye looking at the center of the screen (±5 cm right or left).

3.3.4 Data analysis and statistics

Spike-waveform analysis was conducted using WinEDR and Clampfit software. For putative PV neurons, the first 50-150 spikes exhibiting good peak (P1) to nadir (P2) amplitudes were averaged and the 10-90% rising and falling slopes as well as P1 and P2 were calculated. For putative excitatory neurons, the first 50-150 spikes (if the neuron fired that many times) were averaged and the 10-90% rising and falling slopes as well as P1 and P2 were calculated. The ratios of P2/P1 and falling/rising slope were used to normalize for differences in cell-attached resistance across cells.

WinEDR software along with custom built Matlab software was used to analyze the firing rate of targeted neurons. The spikes elicited from 3 runs of the 12 randomly presented stimuli were first sorted. Then the number of spikes elicited over the 3 runs was averaged for each of the 12 stimuli. The max evoked firing rate was defined as the highest averaged, firing rate over the complete number of runs (usually 9 or 12). The orientation selectivity index (OSI) was calculated using the circular variance approach

where OSI is defined as 1-CV. Bandwidth calculations were based on Ringach, Shapley, & Hawken, 2002, except a von Mises distribution function was used to smooth tuning curves and the concentration parameter ‘k’ was set to 15. After smoothing, the orientation angles closest to the peak for which the evoked response equaled $1/\sqrt{2}$ height of the peak response on either side of the curve were estimated. Bandwidth is defined as the difference between these two angles. If the tuning curve did not fall below this criterion, the bandwidth was defined as 180°. Those neurons for which bandwidth was assigned a value of 180° were not included in the mean bandwidth values.

Fano factor was computed in Matlab using code available at http://churchlandlab.neuroscience.columbia.edu/links.html. For a more in-depth explanation see Churchland et al., 2010. Spike counts were computed using a 200-ms sliding window moving in 25-ms steps. Variance (across trials) and the mean of the spike count was then computed. Fano factor is the spike count variance divided by the spike count mean. The raw Fano factor which is the slope of the regression relating the variance to the mean was used. For calculating the difference in Fano factor a single value was obtained for each neuron by taking the average of the Fano factors during the entire 3 seconds of grey screen and subtracting from that the average Fano factors during the 3 seconds of stimulus presentation.

Data are presented as mean ± SEM. Datasets were compared using Mann-Whitney U or Wilcoxon signed rank test.

3.4 Results

3.4.1 ErbB4 necessary for the proper development of PV neuron evoked firing rates

During development, after eye opening, PV neurons within the primary visual cortex begin with lower, immature firing rates which by the time of the opening of the critical period for ocular dominance plasticity have reached their full mature firing rates (Kuhlman et al., 2011). This process occurs in an experience-dependent manner, as PV neurons in mice which are dark reared up until after the critical period fail to develop these same firing rates, but are held at an immature state. We set out to investigate what molecular mechanism could be underlying this experience-dependent development of PV neuron firing rates.

Activity can be a powerful modulator of many molecules, so we decided to look for a molecule that was specific to inhibitory neurons and known to be important for synapse maintenance and strength as well as being activity dependent. All three of these attributes were met by the receptor tyrosine kinase ErbB4 and NRG1. ErbB4 is inhibitory neuron specific and important for synapse maintenance and strength, while NRG1 is regulated in an activity dependent manner (Fazzari et al., 2010; X. Liu et al., 2011; Mei & Xiong, 2008). Based on these roles that ErbB4 plays, we hypothesized that PV neurons lacking ErbB4 would fail to develop normal, mature firing rates.

To investigate the role of ErbB4 in the development of PV neuron firing rates, we bred mice such that we could get from the same litter mice that were lacking ErbB4 in all PV neurons (LC KO) or littermate controls that had normal expression of ErbB4 (LC WT). PV neurons in these mice did not naturally express any fluorescent markers in PV

neurons but did contain cre-recombinase in PV neurons, so between P12-P14 we injected a cre-dependent virus encoding a red fluorescent protein and then recorded from the labeled neuron between the ages of P25-P30 at the height of the critical period (**Fig 3.1a**).

PV neurons recorded from LC WT mice developed as would be expected based upon the studies discussed in chapter 2 of this thesis. We found, however, that PV neurons recorded from LC KO mice had a 25% lower evoked firing rate than their LC WT littermates (**Fig. 3.1b**; LC WT: 23.92 ± 2.05 Hz, n=21 neurons from 6 mice; LC KO: 17.88 ± 2.08 Hz, n=26 neurons from 8 mice; Mann-Whitney U-test p = 0.041). Although LC KO PV neurons still fired at rates higher than has been previously reported in pre-critical period PV neurons (Kuhlman et al., 2011), they did not fire at the full rate the LC WT PV neurons did.

Along with the PV neurons' firing rate, the PV neurons' tuning preferences also mature in an experience-dependent manner. Our results in chapter 2 of this thesis suggested that the mechanisms underlying PV neuron firing rates and tuning properties are primarily distinct, but often simultaneously regulated. This study with ErbB4 supports a different mechanism being involved in these two properties. When we examined the tuning properties of PV neurons lacking ErbB4, we found that neither the mean OSI (**Fig 3.1c**; LC WT: 0.067 ± 0.009; LC KO: 0.048 ± 0.006; Mann-Whitney U-test p = 0.079) nor the mean bandwidth showed a difference in tuning (**Fig 3.1d**; LC WT: 111.38 ± 14.98; LC KO: 131.05 ± 19.33; Mann-Whitney U-test p = 0.547). If anything, the LC KO PV neurons were more broadly tuned than the LC WT which is opposite as to what would be expected if the PV neurons were in an immature state.

3.4.2 Binocular deprivation induced reduction of PV neuron evoked firing rate is dependent upon ErbB4 receptor tyrosine kinase

A second hypothesis that we were testing in mice lacking ErbB4 in their PV neurons was that their PV neurons would no longer undergo the rapid plasticity discussed in Chapter 2 of this thesis following 24 hours of binocular deprivation (BD). A recent study showed that KO mice such as we are using in these studies do not undergo ODP following monocular deprivation (Y. Sun et al., 2016). We theorize that the reason ODP is not observed in these mice may be due to a lack of ability for these PV neurons to modulate their activity and thereby put the network into a less inhibited space.

In this study, we were not using mice with littermate controls (see methods), but they were still mice with ErbB4 specifically knocked-out of PV neurons. We found that 24 hours of BD no longer caused the rapid, 30-40% decrease in PV neuron evoked firing rate that we had previously witnessed (**Fig 3.2** KO: 21.17 ± 2.48 Hz, n=32 neurons from 9 mice; KO BD: 20.39 ± 1.62 Hz, n=18 neurons from 6 mice; Mann-Whitney U-test p=0.69). Therefore, not only is ErbB4 necessary for the normal development of PV neuron evoked firing rates, but also for the rapid plasticity of PV neurons following 24 hours of BD.

3.4.3 Spontaneous firing rates of PV neurons are still affected by deprivation even in the absence of ErbB4

The firing rates, both evoked and spontaneous, of PV neurons mature in an experience-dependent way, and the evoked firing rate is partially dependent upon ErbB4. However, it remains unclear exactly how mutually controlled these two forms of

firing rate are. Therefore, we checked but did not detect a difference in the spontaneous firing rate between the LC WT and LC KO PV neurons (**Fig 3.3A**; LC WT: 8.00 ± 0.72 Hz; LC KO 6.80 ± 0.70 Hz; Mann-Whitney U-test p = 0.24). This suggests that the spontaneous and evoked firing rates of PV neurons may be controlled differently; however, the fact that spontaneous firing rates are lower than evoked firing rates could simply make it more difficult to detect a difference.

In chapter 2 of this thesis, it was shown that 24 hours of BD caused both the evoked and spontaneous firing rates of PV neurons to decrease significantly. BD did not cause any change in the evoked firing rate of KO PV neurons lacking ErbB4, but we did see a significant decrease in the spontaneous firing rate of KO PV neurons (**Fig 3.3B**; KO: 9.53 ± 1.48 Hz; LC 4.49 ± 0.39 Hz; Mann-Whitney U-test p = 0.002). This further suggests that (at least to an extent) the spontaneous and evoked firing rates of PV neurons may be differentially regulated such that the evoked firing rate depends on ErbB4 whereas the spontaneous rate does not.

3.4.4 Stimulus-induced reduction in Fano factor diminished in PV neurons lacking ErbB4

Although the mean firing rates, both evoked and spontaneous, are both interesting and informative, they do not present the entirety of what is going on within the network. Another useful metric to consider is the trial-to-trial variability of the neurons. It has been shown both in the previous thesis chapter and in the literature that stimulus onset normally induces a decrease in variability regardless of whether the preferred stimulus is being shown or not (Churchland et al., 2010). So, we next probed

to see how the elimination ErbB4 would affect the trial-to-trial variability within PV neurons.

We assessed across-trial variability by calculating the Fano Factor of individual neuron spike times prior to the stimulus (grey screen presentation, pre) and after stimulus onset (post). Fano Factor was computed as the spike time variance divided by the mean firing rate. Stimulus onset did cause a reduction in across-trial variability regardless of whether ErbB4 was present or not (**Fig 3.4**: LC WT: Pre, 2.83 ± 0.21, Post, 1.79 ± 0.14, Wilcoxon signed rank test $p<0.001$; LC KO: Pre, 2.56 ± 0.17, Post, 2.05 ± 0.14, Wilcoxon signed rank test $p = 0.004$). The magnitude of reduction, defined as the difference between mean pre- and mean post-stimulus Fano factor values averaged over 3 seconds was significantly different (Mann-Whitney U-test $p = 0.01$) for the LC WT and LC KO, 37% and 20%, respectively.

3.5 Discussion

Generally, PV inhibitory neurons are considered to be the mediators of experience-dependent plasticity. Within the visual cortex, both the development and maintenance of their characteristically high firing rate are dependent upon maintained visual experience. Here we identified three roles that ErbB4 plays in regulating PV neurons. First, we showed that when ErbB4 is knocked out of PV neurons they fail to develop their full firing rate. Second, we showed that PV neurons without ErbB4 fail to undergo rapid plasticity in response to brief, 24 hour, binocular deprivation. Lastly, we showed that when ErbB4 is absent, stimulus onset no longer causes as much reduction in trial-to-trial variability.

3.5.1 ErbB4 is necessary for the normal development and plasticity of PV inhibitory neurons

During development, as the brain is wiring up and neurons are reaching their final mature states, some properties develop just intrinsically; whereas, other properties develop in a manner that is dictated by experience. For instance, excitatory neurons begin with less selectivity to visual stimuli and develop more selectivity in a manner that is independent of experience (Kuhlman et al., 2011). PV neurons, however, begin with more selectivity and develop to have less selectivity in an experience dependent manner. Likewise, certain aspects of circuit wiring develop independent of experience; whereas, other aspects depend upon experience (Ko et al., 2011, 2013, 2014). Neurons with similar tuning properties are preferentially connected within visual cortex, and this connectivity develops with or without experience. The loss of connectivity between non-responsive neurons does depend upon activity, however.

Beyond tuning properties, within PV neurons, their firing rates (both evoked and spontaneous) develop in activity-dependent manners. In this study, we found that the full development of PV neuron evoked firing rates depended upon the receptor tyrosine kinase ErbB4. PV neurons lacking ErbB4 still displayed higher evoked firing rates than has been previously reported in pre-critical period mice (Kuhlman et al., 2011), but they still fired at 25% lower rates than critical period wildtype controls. The spontaneous firing rate did not seem to be significantly affected by the absence of ErbB4, but this could be in part simply more difficult to detect as the spontaneous firing rates are lower.

Although PV neuron firing rates and broad tuning properties seem to develop in parallel and both in experience-dependent manners, ErbB4 does not appear to play an

important role in the development of the broad tuning of PV neurons. If anything, PV neurons lacking ErbB4 were slightly more broadly tuned than PV neurons from their wildtype littermates, which is opposite of what would be expected if the PV neurons were staying in an immature state.

Not only do PV neurons develop in an experience-dependent manner, but brief periods of deprivation can cause a rapid decrease in the firing rate of PV neurons. This allows for the surrounding excitatory network to go into a disinhibited state, which could then be permissive of plasticity (Kuhlman et al., 2013; van Versendaal & Levelt, 2016). Recently, excitatory networks in mice where the PV neurons lacked ErbB4 were shown to not undergo ocular dominance plasticity (Y. Sun et al., 2016). Our work further adds to this finding by showing that PV neurons no longer undergo a rapid decrease in evoked firing rates following brief deprivation. This suggests that ErbB4 may play a vital role in PV neurons being able to regulate their firing rates in response to environmental changes. This could be due to the role that NRG1 plays in strengthening layer 4 and 5 inputs onto L2/3 PV neurons. If ErbB4 is missing then this pathway is no longer present, so the NRG1, which is produced in an activity-dependent manner, can no longer help regulate the strength of the inputs onto PV neurons.

It is possible that PV neurons usually have a dynamic range of firing rates in which they normally function, and that they have the ability to modulate these firing rates in response to the demands of the environment. This modulation of PV neuron firing rates could be important for maintaining proper excitatory/inhibitory balance. Furthermore, it could also be involved in plasticity and learning. The fact that we found both that PV neurons lacking ErbB4 have lower evoked firing rates, and that the evoked

firing rates do not decrease in response to deprivation suggests that without ErbB4 these PV neurons have lost the ability to modulate their firing properly. One possibility is that when ErbB4 is absent the PV neurons are at the floor of their normal dynamic range, and therefore they cannot decrease further following deprivation.

3.5.2 ErbB4 affects stimulus-induced reduction in Fano factor

It has been found that stimulus onset will normally decrease trial-to-trial variability across a wide range of stimuli, even non-preferred ones, and that this represents a decline in the variability of neural state (Churchland et al., 2010). In other words, cortical circuits become more stable when being driven. When using the metric of Fano factor, values larger than one can be interpreted as being indicative of across trial firing rate variability. Therefore, changes in Fano factor such that values are moving towards one means that the overall variability is decreasing.

By examining Fano factor in PV neurons lacking ErbB4, we were able to assess whether stimulus onset still causes the expected decrease in variability. Whereas we did indeed still observe an overall reduction in Fano factor caused by stimulus onset in PV neurons lacking ErbB4, the magnitude of this reduction as compared to the reduction seen in PV neurons with ErbB4 was decreased by half. The average Fano factor following stimulus onset still remained above 2, suggesting that the across trial variability remains rather high. An implication of this is that stimulus onset causes only minimal additional stability of the circuit in mice lacking ErBb4.

In conclusion, our work has shown that the receptor tyrosine kinase ErbB4 is necessary both for the normal full development of PV neurons' characteristically high evoked firing rates, and that it is necessary for these firing rates to rapidly reduce in

response to deprivation. Furthermore, PV neurons lacking ErbB4 no longer demonstrate the same level of decreased variability as is normally seen following stimulus onset. Taken together, these results help explain why mice lacking ErbB4 in their PV neurons may exhibit aberrant behaviors and decreased learning in ways thought to equate to symptoms of the neurodevelopmental disease schizophrenia.

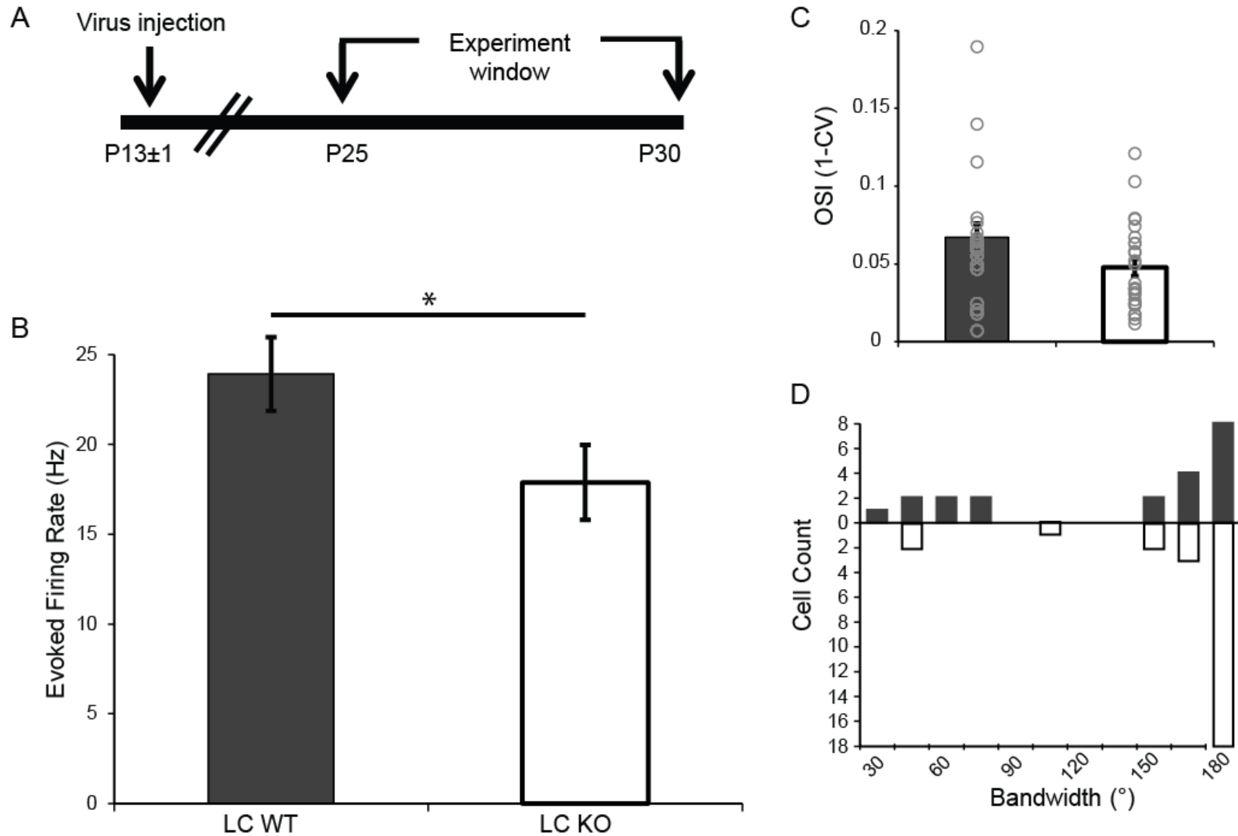

Fig 3.1 PV neurons lacking ErbB4 show 25% reduction in evoked firing rates. A, Experimental protocol, mice were injected with a cre-dependent virus encoding red fluorescent protein at P13±1 days of age. Then, during the critical period, between P25 and P30, the labeled PV neurons were recorded from *in vivo*. B, Mean evoked firing rate is decreased by 25% in PV neurons lacking ErbB4 as compared to their WT littermates (LC WT: 23.92 ± 2.05 Hz, n=21 neurons from 6 mice; LC KO: 17.88 ± 2.08 Hz, n=26 neurons from 8 mice; Mann-Whitney U-test p = 0.041). C, OSI is not significantly changed in PV neurons lacking ErbB4 (LC WT: 0.067 ± 0.009; LC KO: 0.048 ± 0.006; Mann-Whitney U-test p = 0.079) D, The mean bandwidth showed no difference in tuning although it was trending towards being more broadly tuned (LC WT: 137.52 ± 11.78; LC KO: 164.94 ± 7.25; Mann-Whitney U-test p = 0.547)

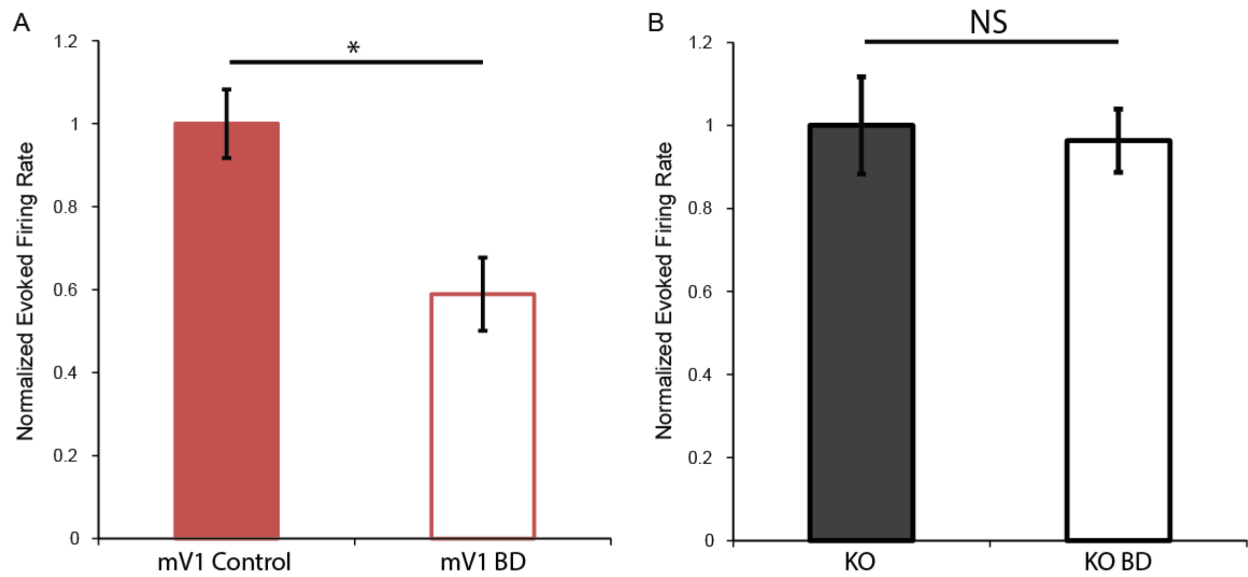

Fig 3.2 ErbB4 is necessary for rapid decrease in PV neuron evoked firing rate following 24 hours of binocular deprivation. A, Replotted data from Chapter 2, PV neurons in monocular zone of V1 normally decrease by ~40% following 24 hours of binocular deprivation * $p<0.05$ Mann-Whitney U-test. (mV1 Control n=33 cells from 17 animals; mV1 BD 18 cells from 11 animals). B, 24 hours of BD no longer causes a decrease in evoked firing in PV neurons lacking ErbB4 $p=0.686$ Mann-Whitney U test. Values are normalized to the evoked firing rates of non-deprived WT or non-deprived KO mice respectively. (KO: n=32 neurons from 9 mice; KO BD: n=18 neurons from 6 mice).

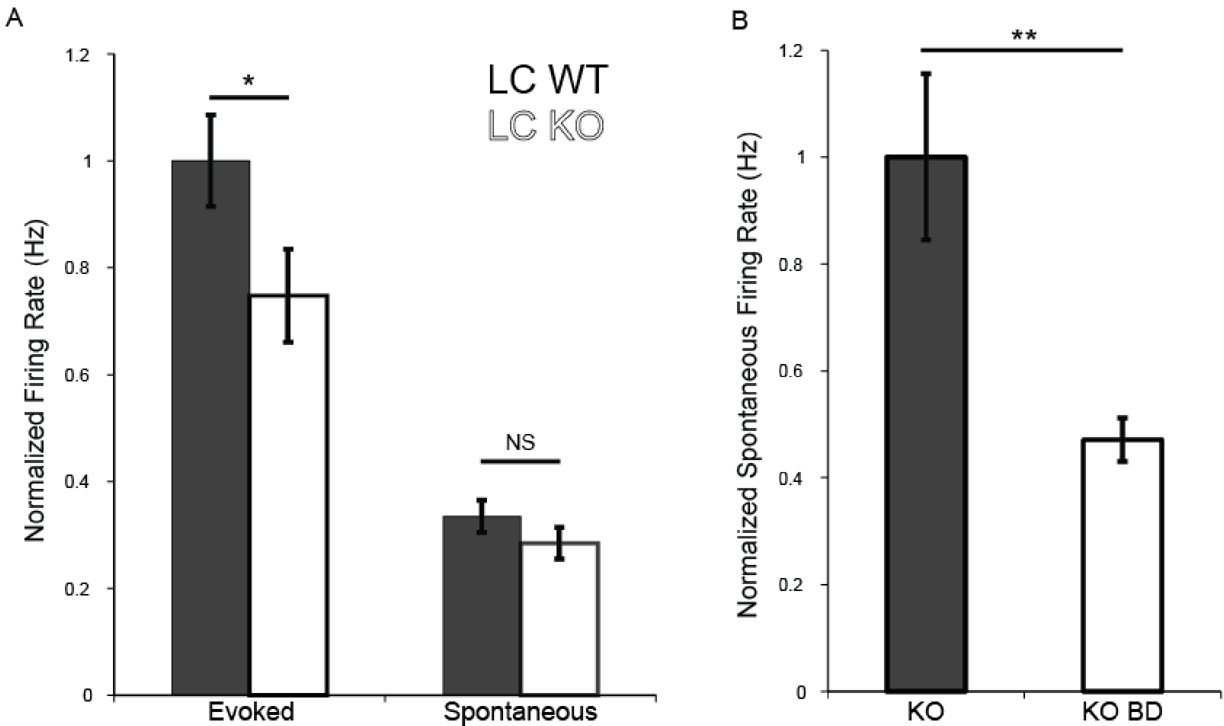

Fig 3.3 Binocular deprivation still decreases spontaneous firing rate even in the absence of ErbB4. A, Whereas ErbB4 is necessary for the normal full development of evoked firing rate in PV neurons; it seems to have minimal effect on the development of spontaneous firing rate. * p = 0.041, NS p = 0.244 Mann-Whitney U-test. (LC WT: n=21 neurons from 6 mice; LC KO: n=26 neurons from 8 mice). B, 24 hours of binocular deprivation still causes a significant decrease in PV neuron firing, even in the absence of ErbB4. ** p = 0.002 Mann-Whitney U-test (KO: n=32 neurons from 9 mice; KO BD: n=18 neurons from 6 mice).

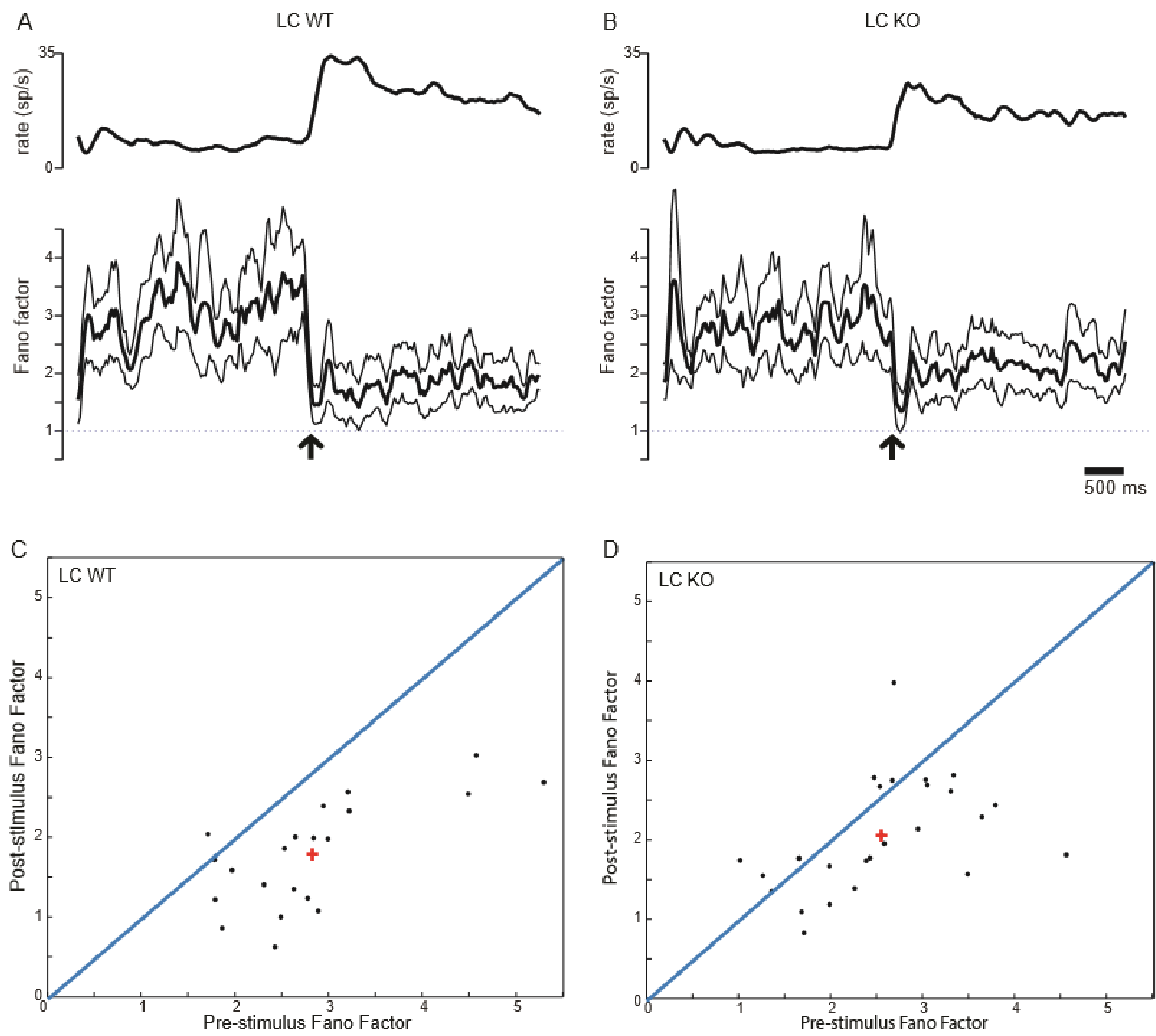

Fig 3.4 Post-stimulus reduction in spike time variability reduced in the absence of ErbB4.Fano factor analysis of neural responses to the preferred orientation and the grey screen immediately preceding stimulation. A-B, Mean firing rate and sliding average of Fano factor (bold) across the population (binned by 200 ms) prior to and after stimulus onset (arrowhead). Animal group is indicated. C-D, Scatter plots of Fano factor values of individual neurons (binned by 200 ms), averaged across the 3 seconds of grey screen preceding stimulus (pre-stimulus) and 3 seconds of visual stimulation (post-stimulus). Animal group is indicated. Mean is indicated by red crosses, note the upward shift of mean post-stimulus values in LC KO versus LC WT.

4.0 Discussion

4.1 Differentiating role of PV neurons in ODP

PV neurons are vital for proper regulation of the critical period, its onset, close, and the plasticity which can occur during it. Although the starting and ending points of the circuit before and after plasticity are fairly ubiquitously agreed upon, the steps taken in the middle are still up for debate. The work presented in this thesis provides further evidence in favor of PV neurons acting as a gate for plasticity rather than being instructive of it.

Within the binocular zone of mouse primary visual cortex, excitatory neurons usually receive drive preferentially from the contralateral eye (Levelt & Hübener, 2012). PV neurons, on the other hand, are fairly ubiquitously driven by both eyes equally (Kuhlman et al., 2013; Yazaki-Sugiyama, Kang, Câteau, Fukai, & Hensch, 2009; for differing opinion see Gandhi, Yanagawa, & Stryker, 2008;). Monocular deprivation for three days can shift this such that PV neurons are preferentially driven by the ipsilateral eye, while excitatory neurons are driven equally by both eyes due to a depression of the inputs from the contralateral eye (Frenkel & Bear, 2004; Kuhlman et al., 2013). If MD is extended up to seven days then a potentiation of the inputs from the ipsilateral eye occurs (Frenkel & Bear, 2004). The importance of GABAergic neurons, specifically PV interneurons, has been known for over a decade; however, some disagreement still exists about the precise role they play in the process of excitatory network reorganization.

One model posits that PV neurons are themselves instructive of excitatory neuron plasticity, and this idea is supported by the findings that following roughly 2 days

of MD there was a shift in PV neuron preference towards the deprived eye (Yazaki-Sugiyama et al., 2009) or that following 2 days of MD inhibitory neurons retained their preference for the contralateral eye (Gandhi et al., 2008). This counterintuitive shift in preference such that PV neurons are driven more strongly by the closed eye could be explained by the spike timing dependent plasticity model (Lu et al., 2007; Yazaki-Sugiyama et al., 2009). This suggests that the PV neuron would therefore provide stronger inhibition to spikes coming from the deprived eye which would already be expected to be less correlated due to the deprivation. This lack of correlation coupled with increased inhibition could lead to the LTD of excitatory inputs onto L2/3 excitatory neurons with the imbalance of inhibition serving to enhance Hebbian plasticity (Gandhi et al., 2008). Alternatively, within this same model, the shift in PV neuron preference towards the deprived eye could lead to a suppression of their impact on excitatory neurons and reflect a cortical manifestation of anti-Hebbian plasticity (Yazaki-Sugiyama et al., 2009). In either case, the PV neurons are themselves actively participating in directing the plasticity of the excitatory neurons via an imbalanced of inhibition.

The other model posits that it is a decrease in firing rate, and therefore a disinhibition of the local network that permits plasticity to occur (Letzkus, Wolff, & Lüthi, 2015). In this model, PV neurons begin with high firing rates, and are equally driven by both eyes, but following 24 hours of monocular deprivation, the PV neurons decrease their firing by almost half. This allows for excitatory responses to the remaining, ipsilateral eye, to increase to levels comparable to binocular viewing prior to deprivation (Kuhlman et al., 2013). This is a very rapid response that occurs prior to the time scale on which LTD or LTP are observed, but could be important in order to allow competitive

plasticity to occur. How this plasticity could occur was predicted by the BCM theory over thirty years ago (Bienenstock, Cooper, & Munro, 1982; Cooper & Bear, 2012). Two essential parts of this theory are that closing the dominant, contralateral eye results in noise instead of patterned visual stimuli coming from those inputs, which in turn, can drive LTD, and that this leads to an adjustment in the overall properties of metaplasticity which facilitates potentiation to occur (Frenkel & Bear, 2004). The portion of this theory relevant to this second model is that the loss of patterned visual stimuli or noise is important for LTD to occur, and that the rapid decrease of PV neuron firing allows the excitatory network to be in a state where competition can occur. Therefore, PV neurons are not themselves directing plasticity to occur, but they are simply permissive of plasticity.

In this dissertation, binocular deprivation was used to differentiate the role of open eye inputs from the loss of patterned visual stimuli in the decrease of PV neuron firing rate which must occur prior to excitatory neuron plasticity being possible. According to the first model presented, it would be expected that the open eye would play an important role in instructing plasticity; whereas, in the second model, the deprivation alone should be sufficient. We found that closing both eyes for 24 hours and therefore creating an environment without patterned visual stimuli, but just noise was sufficient to induce a 30-40% decrease in PV neuron evoked firing rate. This is further support for a lack of stimuli and not an imbalance of inputs being the driving factor in PV neuron plasticity.

It should be noted that clearly binocular deprivation and monocular deprivation are distinct techniques. Monocular deprivation leads to ODP and can result in long term

changes to the overall network; whereas, binocular deprivation even over the same time course does not lead to either LTD or LTP (Frenkel & Bear, 2004). The reason for this could have to do with the role of the ipsilateral, open eye input on excitatory neurons as opposed to inhibitory PV neurons. As was shown in Kuhlman et al. 2013, 24 hours of MD causes a rapid decrease in PV neuron firing rate as well as an increase in excitatory neuron firing. 24 hours of BD similarly causes a rapid decrease in PV neuron firing rate, but does not cause an increase in excitatory neuron firing. This is suggestive that whereas the ipsilateral eye is important for modulating or changing excitatory neuron firing in response to deprivation and ultimately leading to LTD and LTP, it is not necessary for the rapid regulation of PV neuron firing. This is supported by previously published research which found that 24 hours of MD caused putative fast spiking cells (pFS) within mV1 to decrease, and that regular spiking cell rates did not change until 48 hours following MD (Hengen et al., 2013). At the 48-hour time point this study reported the pFS cells to be back to their baseline level. In preliminary experiments we carried out in which BD was performed for 72 hours, we did not see a recovery of PV neuron firing rates to baseline, (**Fig 4.1**; control: 23.40 ± 1.94 Hz, n = 33 cells from 17 mice; 24 hours BD: 13.78 ± 2.06 Hz, n = 18 cells from 11 mice; 72 hours BD: 13.25 ± 1.61 Hz, n = 21 cells from 6 mice; One-Way Anova $p<0.001$, Bonferroni corrected p values are 0.003, 0.001, and 1 respectively), suggesting that open eye inputs might be necessary for that portion of PV neuron plasticity.

Overall, the work presented within chapter 2 of this dissertation shows that PV neuron rapid adaptability which is necessary for ODP to be able to occur is driven by a lack of patterned visual stimuli and not by an imbalance between the two eyes. This

supports a model in which deprivation alone causes disinhibition of the circuit, which then permits plasticity of excitatory neurons to occur rather than a model in which PV neurons are instructive of plasticity.

4.2 Dissociation of regulation of firing rate and tuning properties in PV neurons

During development, various properties of neurons develop in either experience-dependent or experience-independent manners. Two properties of PV neurons that develop in an experience-dependent manner over a similar time course are the characteristic high firing rates and the broad tuning or responding to a large variety of stimuli (Kuhlman et al., 2011). How closely these two properties are linked was unknown, but work presented in this thesis suggests that there are likely two separate mechanisms controlling these two processes, although it is still not completely clear what those mechanism are.

The findings presented in chapters 2 of this thesis illustrate the challenge in trying to differentiate the firing rate and tuning properties of PV neurons. First, the finding that 24 hours of BD caused a decrease in the evoked firing rate, but did not cause a significant change in the tuning (either OSI or bandwidth) within mV1 is suggestive of different mechanisms controlling these two properties. OSI was trending towards being more tuned following BD, so it is possible that sample size simply did not allow us to detect a change that might have been there had a larger amount of data been collected. It is interesting to note that within mV1 PV neurons seemed to be in general more tuned than within bV1. Although no change was detected in OSI or bandwidth, there was a noticeable change in the percentage of PV neurons which were significantly tuned (where significant tuning is defined as being more tuned than the

neuron's own spontaneous activity) following BD. This change in percentage of neurons significantly tuned was seen in both eyes from binocular zone and in monocular zone during the critical period, but not in the monocular zone of adult mice.

Within binocular zone of primary visual cortex, it is a little more complicated, as a small, but significant, change in OSI was detected following BD. Whether this change reflects something that is of functional significance is unclear. The majority of neurons still have tuning that is as broad or broader than the "tuning" of their own spontaneous firing rates, which makes them by definition broad since spontaneous firing cannot actually be tuned. The percentage of PV neurons demonstrating tuning following BD is not to the levels reported in immature PV neurons either prior to the critical period or following dark rearing (Kuhlman et al., 2011), so how much this slight increase in tuning actually functionally means for the circuit is difficult to say.

The finding in chapter 3, that although PV neurons lacking ErbB4 no longer normally develop their full mature firing rates, nor undergo rapid plasticity in response to BD, yet still develop just as broad of tuning rates as control PV neurons further suggests that these two mechanisms arise through different means. If they were intrinsically linked then the decreased firing rate in PV neurons lacking ErbB4 would also be expected to result in a sharpening of tuning. This is not, however, the case.

The decrease in PV neuron firing rates seen following monocular deprivation is due to a loss of inputs primarily from layers 4 and 5a (Kuhlman et al., 2013), and we believe the same mechanism is causing the decrease seen following binocular deprivation. PV neurons seem to primarily gain their broad tuning from sampling the local environment (Bock et al., 2011; Hofer et al., 2011), which in the rodent visual

cortex is a mixed environment due to a lack of organization based on direction selectivity (Espinosa & Stryker, 2012). Therefore, the tuning would not necessarily be expected to vary as much due to a loss of inputs from lower layers. A more recent paper has suggested that local L2/3 inputs are also decreased following 24-48 hours of MD (Y. Sun et al., 2016), so if these local connections are indeed lost, then it might be expected for the tuning of PV neurons to change.

A few possible explanations exist for why we did not detect any change. As previously mentioned, it could simply be a sampling size issue, and if we sampled more neurons, we might be able to detect a change. An alternative is that if the weakening of inputs onto PV neurons is fairly even, then it is possible that the overall sample of the local environment would still be the same, and therefore the tuning could remain unchanged even while the firing rate decreased. A slightly more complicated alternative possibility is that PV neurons may not experience an equal decrease in input from all their innervating excitatory neurons, but that the excitatory neurons actually broaden their tuning a little following the deprivation. Arch silencing of PV neurons in L2/3 has been reported to cause a slight reduction in the orientation selectivity index of excitatory neurons, despite their tuning sharpness remaining essentially unchanged (Atallah et al., 2012). This slight broadening of excitatory neuron responses could make up for an imbalance of losses experienced by the PV neurons, and allow for them to keep their normally broad responses. The drop in spontaneous activity following BD could explain part of the difference in the number of PV neurons more tuned than their own spontaneous activity; however, the fact that mature PV neurons still show a decreased

spontaneous firing rate, but not an increase in the percentage of tuned neurons, suggest that this phenomenon is real.

These possibilities are testable. It is difficult to test whether the result seen is simply due to not a large enough sample, because it seems arbitrary as to how many more samples would be sufficient to not detect a change, and statistical tests are set up to detect changes, not prove that no change occurs. If the lack of change is due to the broadening of excitatory neuron responses then this should be detectible using calcium imaging. If you imaged excitatory neurons, then closed the eyes for 24 hours, and then reimaged the same neurons you could see whether they changed their orientation preference following the BD. This would not directly address the connectivity, but would be suggestive of the second explanation presented above.

4.3 ErbB4 necessary for normal development and plasticity of PV neurons

Recently, it was shown that mice in which ErbB4 is missing from the PV neurons do not undergo ODP (Y. Sun et al., 2016). The reason plasticity is thought to be absent in these mice is due to the fact that the aforementioned weakening of excitatory inputs onto PV neurons following MD seems to be controlled by neuregulin (NRG1), the ligand for ErbB4. The hypothesis is that the decrease in activity following deprivation results in a decreased production of NRG1, resulting in a decrease of excitatory inputs onto PV neurons, therefore resulting in disinhibition and the initiation of LTD in accordance with the BCM theory. However, when ErbB4 is absent, the decrease in NRG1 production following deprivation no longer has an effect, no synapses are weakened onto PV neurons, the level of inhibition in the network does not decrease, and therefore the process that would normally lead to LTD is no longer initiated.

The research presented in this thesis went to further address both the development as well as the plasticity, or lack thereof, of PV neurons in the absence of ErbB4. The first finding is that PV neurons failed to reach their full evoked firing rate potential when ErbB4 is absent. This is in keeping with the finding in Sun et al. 2016 that PV neurons lacking ErbB4 had reduced excitatory synaptic input onto them as compared to PV neurons in littermate controls. It appears that there is normally a subset of inputs, or a strengthening of inputs, one of the two, that are dependent upon ErbB4, and without which PV neurons never reach their normal firing rates. These inputs appear to be responsible for roughly 25% of the evoked firing that PV neurons usually have. ErbB4 does not appear to be necessary for the normal development of the broad tuning properties of PV neurons, as PV neurons lacking ErbB4 were trending towards being even more broadly tuned than normal, so they still undergo the process of broadening, they just do not increase their firing rates to the normal full extent.

The second main finding related to ErbB4 found in this thesis is that PV neurons lacking ErbB4 no longer undergo a rapid decrease in evoked firing rates following 24 hours of BD. Although the spontaneous firing rate of PV neurons lacking ErbB4 still decreases following BD, the evoked rate stays almost identical. This is in keeping with the second model of plasticity that we presented; in which disinhibition of the network is the first step towards plasticity and that PV neurons are permissive of excitatory neuron plasticity. When ErbB4 is absent, PV neurons are no longer able to rapidly regulate their firing rates, and therefore they are not able to be permissive of excitatory neuron plasticity. They seem to have essentially lost their ability to operate within their normal dynamic range, and perhaps are experiencing a bottoming out effect. Whereas

normally, PV neurons can increase or decrease their firing rates in response to external changes in an effort to maintain an overall homeostasis and network balance of inhibition and excitation, in the case where ErbB4 is absent, the PV neurons seem to have lost at least one of the mechanisms by which they can normally quickly adapt.

These recordings were done within the monocular zone of the primary visual cortex with BD, and deprivation was no longer seen to cause a decrease in the firing rate. This suggests that the PV neurons have lost their ability to regulate their firing rate in response to deprivation. This regulation of response and decrease in the overall firing rate is normally the first step of plasticity. The lower firing rate seen in PV neurons lacking ErbB4 could lead to one of two things. Either the system is in a state where it is always essentially disinhibited and plasticity could more easily occur than in controls, or the system has adapted to having less inhibition and still would need even less inhibition for plasticity to be able to occur. Since ODP is not seen in these mice, it suggests that the first of these two scenarios is not correct. Therefore, a change in inhibition would be necessary for plasticity to occur, and these mice seem to have lost this regulatory ability.

The fact that ErbB4 is seen to be necessary for ODP to occur is further support of the model in which PV neurons are permissive, not instructive of plasticity. In the model in which PV neurons are instructive of plasticity, there is first a counterintuitive shift in which the deprived eye initially is the stronger driver of PV neurons. This does not align well with the role that NRG1 is seen to have in synapse stability of excitatory neurons onto PV neurons. For this model to be true, somehow, despite the decreased activity and therefore decreased production of NRG1, this subset of synapses from the

contralateral eye would have to remain preferentially connected as the ipsilateral connections weakened. How NRG1 or ErbB4 would work with this system is unclear. Somehow ErbB4 would have to get phosphorylated for the connections from the contralateral eye, despite its lack of activity, while the ErbB4 was not getting phosphorylated for ipsilateral eye connections despite its maintained activity. It makes more sense if the overall decrease in activity results in an overall decrease in NRG1 and therefore decreased firing of PV neurons and a disinhibited state of the network as a whole.

A couple of experiments remain that could be useful for better understanding the development and plasticity of PV neurons in the absence of ErbB4. As far as understanding the development, it would be interesting to look at pre-critical period firing rates as was done previously in control mice to see whether they still have overall the same developmental timing of firing rates increasing with experience (Kuhlman et al., 2011). It would be interesting to see whether changes in firing rates happen from early on in life, or if it is a failure to mature fully. Since schizophrenia, a disease linked with ErbB4, is a late onset disease; I would hypothesize that the changes seen in PV neurons would not be noticed until around the critical period. To test this, a technique other than the one used in this paper would be necessary due to the time course of when PV-cre turns on and the half-life of the ErbB4 protein. A reliable way to knock-out ErbB4 earlier, but in a way that did not affect initial migration of the PV neurons would be necessary.

An ideal way to test more in depth the effects of ErbB4 deletion would be to do a local knockdown of ErbB4 just in the PV neurons being recorded from, and not

throughout the entirety of the brain. So far, no reliable means of doing this exists. A way that would not directly test this, but would be an interesting follow up would be selectively targeting PSD95 for deletion. Since ErbB4 is thought to regulate AMPARs in a way that is dependent upon PSD95, it would be expected that if PSD95 were selectively knocked down a very similar phenotype would be recapitulated as is seen in neurons lacking ErbB4. This would be an easier experiment to do since shRNA reagents do exist for eliminating PSD95.

Since in Sun et al. 2016, ODP was accessed at the 4 day time point, it is checking the ability of synapses onto the excitatory neurons to undergo LTD. Given further time, these synapses would be expected to undergo LTP of the ipsilateral eye inputs onto the excitatory neurons (Frenkel & Bear, 2004). This LTP is seen to occur even in cases of adult ODP which occurs at a slower time course and through a different mechanism (Espinosa & Stryker, 2012). It would be interesting to see whether mice in which ErbB4 is lacking in PV neurons would still undergo some form of plasticity, just over a longer time course and in a way that more closely mirrors that seen in adult plasticity.

4.4 Altered inhibition, ErbB4, and implication for disease.

Two primary changes seen in PV neurons lacking ErbB4 are described in this thesis; first, that they fail to reach their full level of maturation and secondly, that they have reduced ability to adapt which is likely why these mice show reduced plasticity have interesting implications for models of diseases. One possible disease of interest is schizophrenia for which both ERBB4 and NRG1 are risk factors. Schizophrenia is a neurodevelopmental disease and also a cognitive disorder despite psychosis being the

most striking phenotype associated with it (Fazzari et al., 2010; Lewis et al., 2012). Decreased inhibition, and decreased gamma frequency oscillations have been observed in patients with schizophrenia and various models have been proposed for how this might come about (Lewis et al., 2012).

The failure to develop full, proper levels of inhibition could have multiple effects. One effect is that it could simply cause the whole network to readjust how it has to maintain a physiologically sustainable balance. Mouse models in which NRG1 or ErbB4 are either increased or decreased can cause various behavioral deficits relevant for neuropsychiatric disease suggesting that relatively minor changes in signaling intensity may be very important normally (Mei & Nave, 2014). Although adjustments can be made to keep things as close to normal as possible, effects still are seen.

It has also been reported that eliminating ErbB4 from PV neurons specifically does make mice more prone to seizures and promoted kindling progression (Tan et al., 2012), which would make sense if the excitatory/ inhibitory balance is thrown off in these mice. An ERBB4 mutation has been found in cases of epilepsy in humans (Backx, Ceulemans, Vermeesch, Devriendt, & Van Esch, 2009). Although the mechanism of how ErbB4 relates to epilepsy has not been proven, what is known about its role in synapse stability and strength suggests one possible role. Normally increased neural activity, such as could lead to seizures or would be seen during the kindling process, would increase the levels of NRG1 being produced. This upregulation of NRG1 production would in turn lead to an increased phosphorylation of the receptor tyrosine kinase ErbB4, and an increase in AMPAR stability. This would result in a strengthening of excitatory inputs onto PV neurons, and therefore an overall strengthening of inhibition

from the PV neurons whenever the excitatory neurons were active. This could, in turn, work as a mechanism to keep the excitation from getting out of hand. If ErbB4 is not present, however, then the increased excitation does not result in accompanied increased inhibition and therefore the seizure could more easily occur.

Another potentially interesting effect seen in schizophrenia that could be related to this decrease in PV neuron firing observed when ErbB4 is absent is orientation specific surround suppression (Yoon et al., 2009). It has been seen that people with schizophrenia show a lower ability to detect changes in contrast between a stimulus and a parallel annulus surrounding it than controls. Their ability to differentiate contrast when an orthogonal surround was presented was not seen to be changed however. This deficit has been linked to a reduced GABA concentration in the visual cortex of people with schizophrenia (Yoon et al., 2010). It is feasible that decreased inhibition by the PV neurons in mice lacking ErbB4 could recapitulate a similar phenotype. This could be tested by running a performance-based task modeled after the task used in Yoon et al. and seeing whether mice lacking ErbB4 in their PV neurons did poorer at discriminating a stimulus of differing contrast from its surround than controls do. Additionally, although it would not look at the role of ErbB4, it would also be possible to do an optogenetic study in which Arch-rhodopsin was expressed in PV neurons within the primary visual cortex and see how mice performed at the discrimination task normally or when PV neurons were inhibited. This would be a better test to run first to see whether or not PV neurons are even involved in orientation specific surround suppression or if it is a different class of inhibitory neurons such as SOM neurons that control this.

Whether PV neurons could partially explain the deficits in orientation specific surround suppression or not, the decreased inhibition and altered excitatory/ inhibitory balance is definitely of interest to disease. One of the phenotypes seen in mice lacking ErbB4 in PV neurons is hyperexcitability (Marín, 2012), which suggests that similar effects as were seen in the visual cortex seem to be a general phenomenon which extends to other cortical and possibly subcortical regions. Indeed many of the first studies on the function of ErbB4 were performed in the hippocampus. How broadly applicable the results seen in this thesis are remains to be seen.

The other major effect that we saw in mice lacking ErbB4 in their PV neurons was a lack of the rapid plasticity following 24 hours of BD. This is in keeping with the recently published finding that these mice do not undergo ODP following 3 days of monocular deprivation (Y. Sun et al., 2016). ErbB4 appears to be essential at least in part for the circuit to fairly quickly adapt and adjust. This is in keeping with the finding that there are learning deficits observed in these mice.

Learning deficits and cognitive impairment are observed in cases of schizophrenia prior to the onset of psychosis and are the best predictor of long-term functional outcome (Lewis et al., 2012). Therefore, the learning deficits and lack of plasticity observed in mice lacking ErbB4 (specifically in PV neurons) is of special interest. It suggests one possible mechanism through which a change in expression of a protein can result in rather drastic network level changes which can in turn lead to behavioral and cognitive changes. It does not take that much to throw off the balance of excitation and inhibition in the brain and thereby cause numerous ill effects.

Although interesting to the study of disease and implications that it can have on models and understanding of the brain, it should be noted that we still cannot, nor should we try, to diagnose these or other rodents with schizophrenia (Wong & Josselyn, 2016). There is a recapitulation of many potentially interesting phenotypes or what we think may be the rodent equivalent of symptoms noted in disease, but it still is not completely clear. Also, despite ERBB4 and NRG1 genes being risk factors of schizophrenia, they are not in and of themselves causal. As has been shown by twin studies, genetics alone cannot account for whether or not someone will develop schizophrenia as there are other factors such as environmental exposure that are also important (Insel, 2010). Furthermore, the knockout approach although useful for studying the role of ErbB4 in development and plasticity of PV neurons, is still sort of a sledgehammer approach and not a fine-tuned scalpel for picking up on the intricacies inherent to complex psychiatric diseases. Rarely if ever in human studies do we see the complete elimination of ErbB4 or other risk factors, but more often various point mutations exist which may affect the efficacy of the gene product. Indeed, dysregulated ErbB4 splicing can lead to selective effects on parvalbumin expression by contributing to lower activity of PV neurons (Chung et al., 2015). Overall, diseases such as schizophrenia are much more nuanced than many of the techniques currently available can fully address. That being said, it is still useful to do studies and use model organisms to gain further insight into the construction and maintenance of underlying circuits in the hopes that aspects of it will be applicable and of broader impact to the understanding of mammalian brains.

In conclusion, ErbB4 is necessary for the normal development of PV neuron firing rates within the visual cortex, likely due to its involvement in the formation and maintenance of excitatory inputs onto PV neurons. Furthermore, without the presence of ErbB4, PV neurons no longer undergo the rapid ~30% decrease in evoked firing rate which normally accompanies 24 hours of deprivation. This lack of modulation of PV neurons is a likely cause for the lack of plasticity seen in these mice. The current working hypothesis is that deprivation causes a decrease in activity resulting in the decreased production of NRG1 and through the receptor tyrosine kinase a decrease in PV neuron activity in as little as 24 hours. Then, depending on the type of deprivation, as to whether monocular or binocular, either the excitatory neurons begin to undergo plasticity to balance their ocular dominance preference between the two eyes or they do not undergo further changes, respectively. PV neurons therefore play a critical role in allowing plasticity to initiate, and this process happens in a manner dependent upon ErbB4.

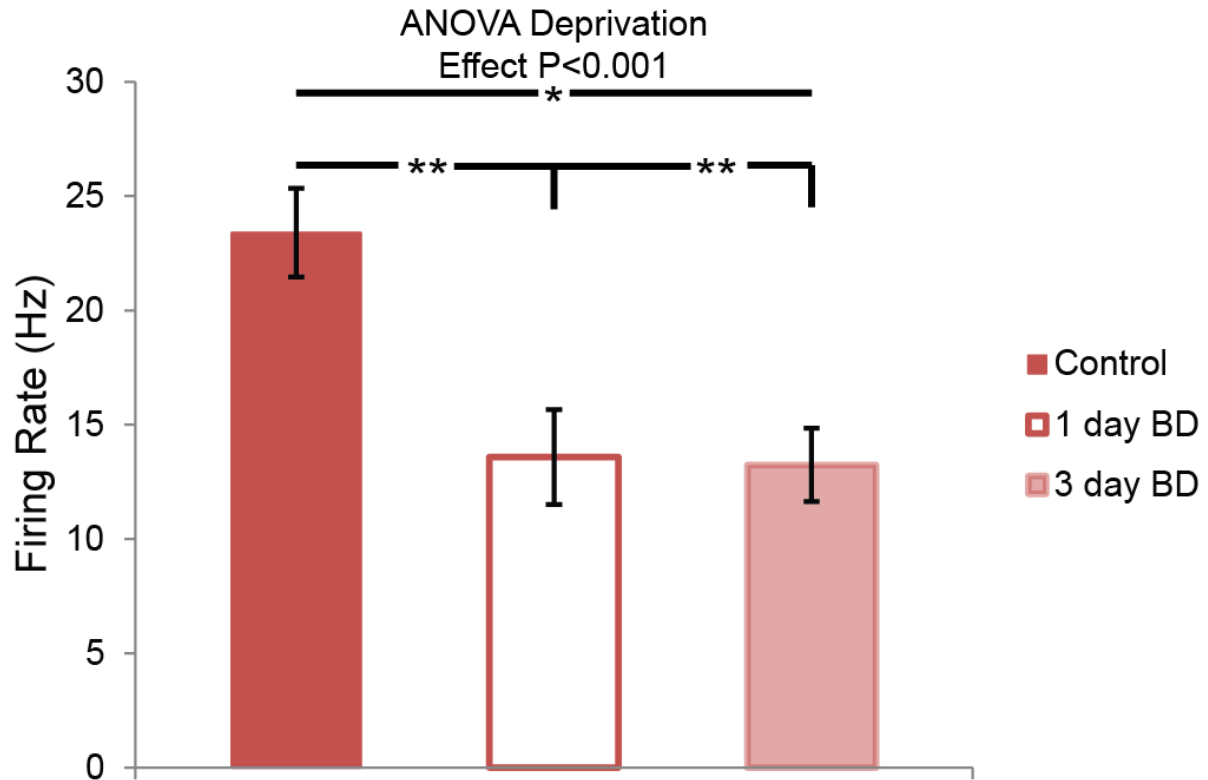

Fig 4.1 Binocular deprivation causes sustained decrease in PV neuron firing. Firing rate of PV neurons is decreased by as little as 24 hours of binocular deprivation, and this decrease in firing rate is sustained for up to 72 hours. Control: 23.40 ± 1.94 Hz, n = 33 cells from 17 mice; 24 hours BD: 13.78 ± 2.06 Hz, n = 18 cells from 11 mice; 72 hours BD: 13.25 ± 1.61 Hz, n = 21 cells from 6 mice; One-Way Anova p<0.001, ** Bonferroni corrected p <0.005

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