Visual Cortical Plasticity: The Role of Parvalbumin Expressing Inhibitory Neurons and Abnormalities in Models of Neurodevelopmental Disorders

By

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B.S. Neurobiology, Physiology, and Behavior
University of California at Davis, 2008

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Abstract

The roles played by cortical inhibitory neurons in experience-dependent plasticity and learning are not well understood. Here we evaluate the participation of parvalbumin-expressing (PV+) GABAergic neurons in two forms of experience-dependent modification of primary visual cortex (V1) in adult mice: ocular dominance (OD) plasticity resulting from monocular deprivation and stimulus-selective response potentiation (SRP) resulting from supplemental visual experience. These two forms of plasticity are triggered by different events but lead to a similar increase in visual cortical response. Both also require the NMDA class of glutamate receptor (NMDAR). However, we find that PV+ inhibitory neurons in V1 play a critical role in the expression of SRP and its behavioral correlate of familiarity recognition, but not in the expression of OD plasticity. Furthermore, NMDARs expressed within PV+ cells play a critical role in SRP, but not in the induction or expression of adult OD plasticity. We also explore the use of visual cortical plasticity paradigms to better understand the function of proteins implicated in autism spectrum disorders (ASDs) and schizophrenia. We find that NMDAR-dependent long-term depression (LTD) and deprived-eye depression in layer 4 of V1 require metabotropic glutamate receptor 5 (mGluR5) signaling during postnatal development. Additionally, schizophrenia-associated protein neurogranin overexpression in V1 disrupts juvenile ocular dominance plasticity. Finally, we evaluate SRP in two models of ASDs associated with excitatory/ inhibitory imbalance: Rett syndrome (RTT) and tuberous sclerosis complex (TSC). Surprisingly, mouse models of RTT and TSC exhibit abnormal SRP phenotypes, but in opposite directions.

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"Contrasting role for parvalbumin-expressing inhibitory neurons in two forms of adult visual cortical plasticity" E.S. Kaplan*, S.F. Cooke*, R.W. Komorowski, A.A. Chubykin, L.A. Khibnik, M.F. Bear, *eLife*, *submitted 2015*

"Metabotropic glutamate receptor 5 is required for NMDA receptor-dependent ocular dominance plasticity and LTD in visual cortex" M.S. Sidorov, E.S. Kaplan, E.K. Osterweil, L. Lindemann, M.F. Bear, *PNAS*, *accepted 9/1/2015*.

"Parvalbumin positive interneurons and the expression of plasticity induced by deprived or enriched visual experience" Picower institute for learning and memory retreat lecture, June 2015. Best lecture award.

"Visual recognition memory, manifested as long-term habituation, requires synaptic plasticity in V1"
S.F. Cooke, R.W. Komorowski, E.S. Kaplan, J.P. Gavornik, M.F. Bear, *Nature Neuroscience*, Volume 18, No 2, Feb. 2015, Pp: 262-271.

"Differential involvement of parvalbumin positive (PV+) interneurons in distinct forms of experience-dependent plasticity in the visual cortex of the adult mouse" E.S. Kaplan, S.F. Cooke, A.A. Chubykin, R.W. Komorowski, J.P. Gavornik, M.F. Bear
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"Differential involvement of inhibition in the expression of experience-dependent cortical plasticity"
Picower institute for learning and memory lecture, Oct., 2013.

"Early mitochondrial abnormalities in hippocampal neurons cultured from *Fmr1* premutation mouse model"
E.S. Kaplan, Z. Cao, S. Hulsizer, F. Tassone, R.F. Berman, P.J. Hagerman, I.N. Pessah,
J. Neurochem., Vol. 123, Issue 4, Nov. 2012, Pp: 613–621.

"mGluR5 expression is required for NMDA receptor-dependent LTD in layer IV of visual cortex"
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"Cytokines upregulated in autism regulate glutamatergic synapse formation between cortical neurons"
P. A. Garay, E. S. Kaplan, A. Goyal, N. Zozulya, S. L. Barrow, S. C. Hulsizer, F. El-sabeawy, A. K. McAllister.
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Chapter 1

Experience-dependent plasticity, learning and memory, and neurodevelopmental disorder pathophysiology

1.1: Introduction

One of the most fundamentally important features of the nervous system is its capacity to guide future behaviors via the ability to store and utilize vast amounts of information gathered from previous experiences. Learning and memory enable a species to adapt to its current environment and optimize its chances of survival and reproduction. *Homo sapiens* in particular have been endowed with an exceptional ability to learn from experiences, as well as a memory system of enormous capacity and functionality. This superior capability has allowed an unprecedented level of species adaptation, communication and cooperation between individuals, and arguably enabled humans unparalleled success as a species, and certainly among Animalia (Pinker 2010, Boyd, Richerson et al. 2011). Experience leaves a lasting physical trace on the brain, which can later be accessed and utilized to inform better decision making. This physical trace is now appreciated as the experience-dependent plasticity of synaptic connections between neurons (Martin, Grimwood et al. 2000). Neurons are dynamic both in structure and physiological function. In particular, the size and strength of synaptic contacts and therefore the communication between cells is known to be modifiable (Kandel 2001, Takeuchi, Duszkiewicz et al. 2014). Importantly, although synaptic plasticity is a life-long capacity, the properties of this plasticity are dynamic and change as a function of age (Hubener and Bonhoeffer 2014). Understanding the mechanisms of experience-dependent synaptic modifications throughout the life-span remains a major goal of contemporary neuroscience.

Over 50 years ago, David Hubel and Torsten Wiesel discovered that manipulating the visual experience of a developing cat could drastically change the responsiveness of neurons in a portion of the brain responsible for decoding visual information, the primary visual cortex (V1) (Wiesel and Hubel 1963). Preceding this discovery, Hubel and Wiesel revealed that neurons in V1 were the first to receive binocular information, which had travelled there from the two eyes via the visual portions of the thalamus (Hubel and Wiesel 1962). This convergence of information from the two eyes at the level of V1 neurons is the basis of binocular vision. Interestingly, they also observed that each neuron in V1 did not necessarily respond with equal strength to input from the two eyes, and they termed this cellular property "ocular dominance" (OD). Most importantly they discovered that depriving the animal of vision through one of the eyes for a short period of time (monocular deprivation, MD), caused a robust change in the ocular dominance properties of V1 cells (Wiesel and Hubel 1963). Whereby, even after the re-opening of the deprived eye, the majority of V1 cells now displayed a significant bias in their

responsiveness towards the non-deprived eye. This temporary loss of experience through one eye caused neurons in V1 to shift their responsiveness to the non-deprived eye, through which they had continued to receive coherent visual input. This phenomenon was referred to as an ocular dominance shift, and this malleability of the visual system referred to more broadly as ocular dominance plasticity. This finding garnered considerable excitement because it was the first example of how changes in the environment can specifically alter the function of the brain at the neuronal level. Furthermore, the discovery set up a paradigm in which the mechanisms of synaptic plasticity could be studied. Importantly, these same mechanisms may be the basis of a wide range of learning types (Hubener and Bonhoeffer 2014, Priebe and McGee 2014), and may also be processes disrupted in neurodevelopmental disorders (Bailey, Phillips et al. 1996, LeBlanc and Fagiolini 2011).

1.2: Ocular dominance plasticity and the critical period

Since the original discovery, the study of ocular dominance plasticity has been a topic of intense investigation in the neuroscience field (Levelt and Hubener 2012, Sur, Nagakura et al. 2013). One path of research has been concerned with the age-dependence of OD plasticity. In some of the original studies by Hubel and Wiesel, they discovered that at relatively young developmental age, the primary visual cortex of a cat was most susceptible to the effect of deprivation (Hubel and Wiesel 1970). This time period in which a juvenile animal is particularly sensitive to experience-dependent synaptic alterations was termed the critical period. The idea of a short window of susceptibility to the effects of experience had its roots in the earlier findings of the ethologist Konrad Lorenz, who had discovered imprinting in certain bird species (Hess 1959). Hubel and Wiesel found that when an adult cat was monocularly deprived, changes in ocular dominance were generally less robust and required a longer period of deprivation to develop. Although there appeared to be a distinction between the plasticity induced in juvenile and adult animals, other researchers began to find evidence of significant plasticity in the adult cortex. In 1984, Merzenich and colleagues discovered robust plasticity of receptive field maps in the somatosensory cortex of the adult monkey after deprivation. Analogous deprivation-induced plasticity was also observed in the auditory cortex of the adult animal (Robertson and Irvine 1989). Shortly after, individuals revisited the idea of plasticity in the adult visual system. Adult plasticity in V1 was observed in monkeys (Gilbert, Hirsch et al. 1990, Kaas, Krubitzer et al. 1990), cats (Gilbert and Wiesel 1992), and mice (Sawtell, Frenkel et al. 2003, Keck, Mrsic-Flogel et al. 2008, Sato and Stryker 2008). Although these studies still agreed with Hubel and

Wiesel's earlier findings that juvenile animals had a greater capacity for change, it was clear that plasticity in the visual system was present in several species in adulthood. Mice, which have the advantages of being relatively inexpensive models and are amenable to new genetic technologies, have in particular been shown to display both robust juvenile and adult plasticity in V1 (Gavornik and Bear 2014, Hubener and Bonhoeffer 2014). Furthermore, the qualities and underlying mechanisms of OD plasticity in juvenile and adult mice appear to be distinct. Great attention has been paid in the field to discover the mechanisms that support shifts in ocular dominance in juvenile as well as adult mice, and to understand what controls the transition between these two plasticity states.

1.3: Measuring ocular dominance plasticity using visually-evoked potentials

Early experiments by Hubel and Wiesel that measured deprivation enabled changes in the responsiveness of the cortex to the two eyes utilized newly developed tungsten microelectrodes. These electrodes allowed for the measurement of action potential responses from individual V1 cells while visual stimulation was presented to the eyes. More recently the study of cortical plasticity has also utilized visually-evoked potential (VEP) responses, which report visually induced changes in voltage integrated from groups of cells. VEPs can be elicited in primary visual cortex (V1) of the rodent by the presentation of a simple visual stimulus, such as a phase-reversing sinusoidal grating of a particular orientation. An implanted VEP electrode in Layer 4, the layer of the cortex receiving input from the thalamus, can enable stable recordings for several days to weeks (Fig. 1.1). If a phase-reversing stimulus of unique orientation is presented to a rodent daily, the resulting VEP will be equivalent in size each day. This VEP magnitude provides a read-out of the strength of the cortical response driven by a visual stimulus (Porciatti, Pizzorusso et al. 1999). The ability to drive VEPs of equal magnitude to any orientation in one recording site is due to the functional cytoarchitecture of rodent V1, which varies considerably from the organization of V1 in carnivores and primates.

Early pioneers who sought to understand the functional cytoarchitecture of the cortex used the monkey and cat as model systems. They resolved that cortical neurons responsive to similar environmental stimuli were organized into columns (Mountcastle 1957, Powell and Mountcastle 1959, Hubel and Wiesel 1962). In the primary visual cortex of the monkey and cat, it was discovered that associated with these columns, were neurons selective for visual stimuli of a particular orientation. These orientation-selective neurons were found clustered together, forming highly ordered "pinwheel" type arrangements (LeVay, Hubel et al. 1975, Blasdel and

Salama 1986, Bonhoeffer and Grinvald 1991). In rodent V1, however; it was revealed that neurons that are preferentially responsive to a particular orientation are not clustered together, but instead are highly distributed (Ohki, Chung et al. 2005). Therefore, in cat or monkey V1, the recordings from an electrode in a single location would result in an evoked response of varying magnitude depending on the orientation of the visual stimulus. This phenomenon is due to the "clustered" arrangement of orientation selective cells in the V1 of these animals. In the rodent, however; the "multi-colored confetti-like" intermingled arrangement of orientation-selective neurons ensures that the visual presentation of stimuli of various orientations will drive VEPs of equal magnitude. This turns out to be a fortunate feature of the rodent visual system, because any change in the magnitude of the VEP observed over time can be ascribed to experience-dependent plasticity and will be independent of the orientation of the stimulus used to evaluate the system.

1.4: Ocular dominance plasticity in the developing mouse

As mentioned, considerable effort has gone into understanding the mechanisms of juvenile and adult OD plasticity in the mouse. In juvenile mice, MD initially results in the weakening of cortical synapses driven by the deprived eye. This is typically measured in the hemisphere contralateral to the deprived eye (Fig 1.1). Synaptic weakening due to MD is known as deprived eye depression (DED). This depression occurs rapidly, is measurable after only 24 hours of eyelid suture, and plateaus following 3 days of MD (Liu, Heynen et al. 2008). This decrease in the magnitude of visual responses in V1 serving the deprived eye has been shown to depend on the mechanisms of homo-synaptic long-term depression (LTD), (Heynen, Yoon et al. 2003, Frenkel and Bear 2004, Chen and Bear 2007, Liu, Heynen et al. 2008, Yoon, Smith et al. 2009, McCurry, Shepherd et al. 2010, Smith and Bear 2010, Espinosa and Stryker 2012) and can be fully expressed as a change in the strength of excitatory connections between the lateral geniculate nucleus of the thalamus (LGN) and V1 (Khibnik, Cho et al. 2010).

Subsequent to the depression of responses downstream of the deprived eye, a strengthening of inputs driven by the ipsilateral (non-deprived) eye begins. Ipsilateral-eye potentiation (also known as open-eye potentiation, OEP) is most commonly observed after more than 3 days of deprivation has occurred (Frenkel and Bear 2004). The mechanisms involved in this distinct phase of juvenile ocular dominance plasticity, characterized by potentiation, are more highly disputed. One hypothesis states that non-deprived eye responses potentiate via homo-synaptic long-term potentiation (LTP) of thalamocortical synapses. There is evidence to

support this idea based on the known dynamic properties of visual cortical neurons. It is believed that the initial phases of OD plasticity lower the overall activity of the cortex, and lead to a metaplastic change, by which the threshold for eliciting LTP at thalamocortical synapses is now lowered (Bear 2003, Cooper and Bear 2012). LTP of these cortical inputs serving the non-deprived eye may then potentiate. The mechanism for this metaplastic change can be explained by increased expression of the NR2B, and decreased expression of the NR2A subunits of the NMDA receptor in V1, which has been shown to occur *in vivo* due to deprivation (Chen and Bear 2007). Furthermore, these NMDA receptor subunit modifications do indeed promote LTP, as well as open-eye potentiation in V1 (Kirkwood, Rioult et al. 1996, Philpot, Cho et al. 2007, Cho, Khibnik et al. 2009).

Another proposed mechanism for non-deprived eye potentiation in the juvenile animal concerns the idea of homeostatic synaptic scaling (Turrigiano and Nelson 2004). This is a phenomenon, first described by Gina Turrigiano in cortical cell culture, where pharmacological blockade of activity with tetrodotoxin (TTX) resulted in a global scaling up of synaptic weights, in an effort to maintain the basal firing levels of neurons (Turrigiano, Leslie et al. 1998). Some evidence in support of synaptic scaling as a feature of juvenile OD plasticity is derived by the discovery that the **cytokine TNFα, which was found to be required for synaptic scaling** (Stellwagen and Malenka 2006), is also required for the occurrence of non-deprived eye potentiation (Kaneko, Stellwagen et al. 2008). There are however, several components of non-deprived eye potentiation, which are inconsistent with a role for homeostatic synaptic scaling. For instance, it has been shown that non-deprived eye potentiation in the juvenile animal can be blocked by NMDA receptor antagonism; however, it is known that NMDA receptors are not required for homeostatic synaptic scaling (Turrigiano, Leslie et al. 1998, Turrigiano and Nelson 2004, Blais, Frenkel et al. 2008, Cho, Khibnik et al. 2009, Smith, Heynen et al. 2009). Lastly, it has been proposed that non-deprived eye potentiation could be accounted for by the relief of gamma-Aminobutyric acid (GABA)-ergic inhibition, though the evidence for this is mixed. Recent studies monitoring the activity of inhibitory GABAergic cells in addition to excitatory glutamatergic cells during MD reported that similarly to excitatory cells, inhibitory cells shift their responsiveness towards the non-deprived eye (Gandhi, Yanagawa et al. 2008, Kameyama, Sohya et al. 2010), suggesting a more passive involvement in the OD shift. In contrast, a different study found that $GABA_A$ receptor blockade after deprivation, caused significant changes in the eye-bias of V1 cells, signifying a GABAergic component to expression of the juvenile OD shift (Yazaki-Sugiyama, Kang et al. 2009). Most recently, there has been evidence

that deprivation longer than 6 days is accompanied by selective disinhibition of non-deprived eye inputs (Ma, Li et al. 2013). The multiple hypotheses that have been suggested to explain non-deprived eye potentiation in the juvenile animal could be accounted for by multiple mechanisms occurring in the cortex simultaneously, or that these processes are dispersed among different cortical cell types or layers. Regardless, this subject continues to be an active area of research.

1.5: Ocular dominance plasticity in the adult mouse

The ocular dominance shift itself is qualitatively different in mature mice. In response to monocular deprivation, the adult mouse displays ipsilateral (non-deprived)-eye potentiation, but not deprived-eye depression (Sawtell, Frenkel et al. 2003, Sato and Stryker 2008). Plasticity in the adult animal is generally weaker than that of the juvenile, and commonly takes a longer period of deprivation to become evident. There are two parallel streams of inquiry concerning adult OD plasticity. One concerns understanding the mechanisms that underlie the distinctive characteristics of the adult OD shift (i.e. the selective potentiation of the non-deprived eye). The other seeks to understand the mechanisms that shift the cortex from displaying juvenile OD plasticity, to exhibiting adult OD plasticity; with emphasis on exploring methods by which it may be possible to return the adult animal to a more juvenile-like state. In terms of understanding the characteristic plasticity featured in the adult mouse, it is known that the potentiation requires the activity of NMDA receptors (Sawtell, Frenkel et al. 2003), and requires αCAMKII, pointing to LTP as a possible mechanism. Furthermore, unlike the juvenile OD shift, the adult shift does not require TNFα (Ranson, Cheetham et al. 2012), which is implicated in homeostatic synaptic scaling.

The transition from juvenile to adult OD plasticity, while not well understood, is correlated with the postnatal maturation of intracortical inhibition. It has been observed that the maturation of cortical GABAergic synapses onto pyramidal neurons correlates with a decline in the magnitude of OD plasticity, which occurs as the animal enters adulthood (Heimel, van Versendaal et al. 2011). Therefore many have investigated the possibility that the strength of cortical inhibition plays a direct role in gating experience-dependent modifications in the cortex. Specifically, the hypothesis reasons that the critical period is dependent on an immature GABAergic network. Hence, a completely matured (adult) inhibitory interneuronal network impedes juvenile-type OD plasticity (Huang, Kirkwood et al. 1999, Chattopadhyaya, Di Cristo et

al. 2004, Maya Vetencourt, Sale et al. 2008, Heimel, van Versendaal et al. 2011). Investigators have shown that manipulations that accelerate the development of inhibition in the cortex cause a premature shortening of the critical period in juvenile animals (Hanover, Huang et al. 1999, Huang, Kirkwood et al. 1999, Fagiolini and Hensch 2000). Conversely, manipulations that slow the maturation of inhibition, such as dark rearing, also appear to be manipulations that delay the closure of the juvenile plasticity state (Fagiolini, Pizzorusso et al. 1994). Other interventions, such as environmental enrichment, also correlate with decreased cortical inhibition (Sale, Maya Vetencourt et al. 2007), and have been shown to "re-activate" juvenile-like plasticity. These experiments have furthered the hypothesis that cortical inhibition determines the quality of plasticity observed in the cortex (juvenile-like vs. adult-like). Because of the strong evidence implicating inhibition in the transition of OD plasticity to the adult state, many have theorized that the mechanisms of adult OD plasticity itself may rely on alterations of the GABAergic system. This however, has not been convincingly demonstrated.

1.6: Inhibitory interneurons of the cerebral cortex

The cerebral cortex is an extremely complex system composed of numerous cell and circuit types. Most cerebral cortical neurons are excitatory glutamatergic cells (~80%), but the remaining minority (~20%) are GABAergic inhibitory interneurons, which display a diversity of morphological, physiological, molecular, and synaptic characteristics (Fig.1.2) (Markram, Toledo-Rodriguez et al. 2004). Interneurons shape the output of local circuits by releasing the inhibitory neurotransmitter GABA, which allows a dynamic balance to be maintained in order for information processing to occur while limiting the possibility of seizure activity. In addition to utilizing different neurotransmitters, excitatory and inhibitory cells undergo vastly different programs of development. While excitatory cortical cells undergo an orderly inside-out migration from the proliferation zone to the overlying cortical plate, inhibitory cortical cells are derived from embryonic subcortical progenitor zones. (Anderson, Eisenstat et al. 1997, Kepecs and Fishell 2014). Almost all inhibitory cortical cells arrive in the cortex via tangential migration, from either the medial ganglionic eminence (MGE) or the caudal ganglionic eminence (CGE). As mentioned, these interneurons display a wide heterogeneity of cellular properties. It is believed that interneurons attain their final properties through a combination of predefined genetic factors, some of which reflect their origin (MGE vs. CGE), and local environmental cues. Although grouping such a diversity of cells into classes remains a challenge, protein expression patterns have recently been used to define and study discrete populations of cortical

interneurons (Fig. 1.2)(Kubota, Shigematsu et al. 2011). In the mouse cortex, it has been found that there are at least three non-overlapping interneuron protein expression markers: parvalbumin, somatostatin, and vasoactive intestinal peptide (Xu, Roby et al. 2010). Calretinin, neuropeptide Y, cholecystokinin, nitric oxide synthase, reelin as well as other protein markers have also been used to define interneuron populations; however, these show overlapping cellular expression. Cell types defined by a particular chemical marker display some characteristic properties, although overlapping features is not uncommon (Fig. 1.2). Parvalbumin positive (PV+) interneurons comprise the most numerous subclass of cortical interneurons, covering ~40% of total interneurons (Xu, Roby et al. 2010). We will focus our attention on this protein marker-defined interneuron subtype.

1.7: Parvalbumin positive GABAergic interneurons

In addition to being the most numerous subtype of cortical GABAergic interneuron, PV+ cells generally display certain characteristic properties, which may provide clues to their function. The parvalbumin protein itself is a calcium binding protein found in cells that exhibit particularly fast changes in membrane potential (Cates, Teodoro et al. 2002). In terms of their intrinsic properties, PV+ cells usually display a "fast-spiking," non-accommodating pattern of activity (Fig 1.2). The expression of several ion channels in PV+ cells allows them to be optimized for fast and consistent action potential generation. These channels include the delayed rectifying potassium channels Kv3.1 and Kv3.2, as well as the hyperpolarization-activated HCN1 and HCN2 pacemaker channels (Rudy and McBain 2001). PV+ interneurons are positioned to strongly control spiking of their synaptic partners, commonly displaying "basket-type" or "chandelier-type" morphology. The more common basket cells tend to form synapses onto the soma and proximal dendrites of other cells, usually forming a basket-like arrangement of synaptic contacts around the cell body. This morphology appears to allow basket cells to adjust gain and exert a strong influence on the integrated synaptic response of their synaptically connected partners. Chandelier cells form contacts on axon initial segments, which enables them to override the final action potential output of other cells (Markram, Toledo-Rodriguez et al. 2004). PV+ interneurons are strongly interconnected, which promotes their synchronous activity (Kepecs and Fishell 2014). Recently, it has been shown that these unique characteristics of PV+ cells allow the generation of cortical network oscillations in the gamma frequency range (30-100Hz). This is significant because of evidence linking gamma oscillations to cognitive functions including: attention and enhanced detection and discrimination of

environmental stimuli (Pritchett, Siegle et al. 2015). The behavioral relevance of gamma oscillations in human visual cortex was strengthened by a study utilizing magnetoencephalography (MEG) recordings, which found that gamma-band activity at the occipital lobe predicted the speed with which subjects were able to report a change in a presented visual stimulus (Hoogenboom, Schoffelen et al. 2010). Evidence linking PV+ interneurons to the generation of behaviorally relevant cortical oscillations suggest that these interneurons may play a significant role in visual function and information processing outside of merely balancing excitation. Indeed, optogenetic activation of PV+ interneurons in mouse V1 has been recently shown to improve visual perception (Lee, Kwan et al. 2012). In what contexts PV+ interneurons contribute to experience-dependent plasticity or learning remains poorly understood.

1.8: Parvalbumin positive interneurons and ocular dominance plasticity

Although it has been known since the early 1980s that interneuron connections can undergo long-term changes in synaptic strength (Buzsaki and Eidelberg 1982), a thorough understanding of their plasticity is still lacking. Similarly to excitatory cells, mechanisms of synaptic plasticity involving inhibitory interneurons are quite diverse (Malenka and Bear 2004, Kullmann and Lamsa 2011). In several brain regions, long-term plasticity has been observed that include modulation of synaptic strength between inhibitory and excitatory cells, as well as between inhibitory cells (Komatsu and Iwakiri 1993, Maffei 2011, Sarihi, Mirnajafi-Zadeh et al. 2012). In the cortex, the modulation of excitatory connections onto PV+ interneurons has been observed (Sarihi, Jiang et al. 2008). Furthermore, it has also been shown that experience can regulate the synaptic strength of excitatory connections onto cortical GABAergic interneurons, and that modifiable thalamocortical inputs onto interneurons can in fact be stronger and more numerous than onto neighboring principal cells (Cruikshank, Lewis et al. 2007, Chittajallu and Isaac 2010). As mentioned, OD plasticity in the developing and adult mouse appear to be distinct and there is support for the involvement of inhibition in the closure of the juvenile critical period and the transition to OD plasticity characteristic of the adult mouse. PV+ interneurons in particular have been hypothesized to be the cell type that regulate this transition (Hensch 2005), because the maturation of the PV+ cell network occurs relatively late in postnatal development, coincident with the critical period. Also it has been shown that rearing mice in the dark affects the expression of parvalbumin, in addition to delaying the timing of the critical period. (Fagiolini, Pizzorusso et al. 1994, Tropea, Kreiman et al. 2006). Furthermore, a recent study revealed that

negatively regulating the function of PV+ cells during MD in an adult mouse, can facilitate a juvenile-like OD shift, similar to what had been previously shown by reducing cortical inhibition more generally (Sale, Maya Vetencourt et al. 2007, Kuhlman, Olivas et al. 2013). Therefore, PV+ interneurons may be an interneuron subtype that gates the timing at which juvenile-type plasticity can be induced. There is, however; very little known about the involvement of PV+ interneurons in experience-dependent plasticity mechanisms which occur in the adult cerebral cortex.

1.9: Stimulus-selective response potentiation

Besides OD plasticity, the adult visual cortex is known to express additional forms of experience-dependent plasticity including stimulus-selective response potentiation (SRP). Unlike OD plasticity, which is triggered by the lack of normal visual input, SRP is elicited by the repeated presentation of supplemental visual experience. As mentioned, in rodent visual cortex presentation of any oriented grating will drive layer 4 VEPs of equivalent magnitude. However, if a stimulus of a specific orientation is re-presented to the animal over days, a potentiation of the VEP magnitude to that specific stimulus is observed (Frenkel, Sawtell et al. 2006). The potentiation of the visually-driven response to this familiar orientation, which plateaus in magnitude over days, is highly selective for the experienced stimulus (*e.g.* orientation) and is long-lasting. Presentation of a novel stimulus (*e.g.* of an alternate orientation), which the animal has never before experienced, reveals a VEP of baseline magnitude. Therefore this form of plasticity may be intimately involved in the discrimination of familiar and novel environmental stimuli. The induction of this form of cortical plasticity depends on *N*-methyl-D-aspartate (NMDA) receptor activity, and is dependent on local insertion of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Frenkel, Sawtell et al. 2006, Cooke, Komorowski et al. 2015). Importantly, SRP does appear to be involved in perceptual learning and stimulus discrimination at the behavioral level. SRP has been demonstrated to be required for a visually guided habituation behavior, known as orientation-selective habituation (OSH) (Cooke, Komorowski et al. 2015). Interestingly, although both adult OD plasticity and SRP are expressed as long-lasting increases in the strength of visual responses, these two forms of plasticity do not occlude one another, which suggests non-overlapping mechanisms. While several of the mechanistic features of SRP induction imply a role for Hebbian long-term potentiation (Frenkel, Sawtell et al. 2006, Cooke and Bear 2010), the known role of gamma oscillations and PV+ cells in stimulus perception and discrimination beg the question of their involvement as well (Pritchett,

Siegle et al. 2015). However, nothing is currently known about the role of PV+ interneurons in the mechanisms underlying SRP.

1.10: Experience-dependent plasticity and neurodevelopmental disorders

Neurodevelopmental disorders such as autism spectrum disorders (ASDs) and schizophrenia are debilitating diseases which cause considerable hardship and have a wide impact on society through the massive economic burden of health care costs (Lewis and Lieberman 2000, Goeree, Farahati et al. 2005, Newschaffer, Croen et al. 2007, Buescher, Cidav et al. 2014). Although the etiology remains a mystery, it is known that increased susceptibility to the development of these disorders can occur via mutations within particular genes. Importantly, a great number of these genes are known to code for proteins, which are critical to synaptic function and plasticity (Bailey, Phillips et al. 1996, Toro, Konyukh et al. 2010). Even though the development of the vast majority of these disorders likely results from a complex interaction of great numbers of genetic insults in addition to environmental factors, we may be able to understand the diseases by studying how the known genetic susceptibilities lead to aberrant neurological function and behavior. Individuals with ASDs and schizophrenia are known to exhibit deficits in cortical plasticity and learning (Braff, Swerdlow et al. 1995, Bailey, Phillips et al. 1996, Newschaffer, Croen et al. 2007, Schretlen, Cascella et al. 2007, Cavus, Reinhart et al. 2012, Park and Gooding 2014). In order to understand how genetic factors may lead to these learning deficits, an important avenue of research entails studying the effects of genetic mutations implicated in disease, on experience-dependent plasticity in animal models. By altering the function of these genes in animal models, and then investigating the impact on plasticity, we may achieve an understanding of the synaptic pathophysiology causing abnormal human phenotypes.

In the case of ASDs, a convergence of data has implicated the involvement of signaling and protein synthesis downstream of group 1 metabotropic glutamate receptors (mGluRs) (Auerbach, Osterweil et al. 2011, Baudouin 2014). Besides metabotropic receptors, evidence of dysfunction of the ionotropic NMDA glutamate receptors, and NMDA receptor-dependent plasticity has been reported in both autism (Lee, Choi et al. 2015), and schizophrenia (Coyle, Tsai et al. 2003). NMDAR-dependent plasticity is known to play an important role in learning in many brain regions, including the cerebral cortex (Martin, Grimwood et al. 2000). It has been

appreciated that mutations in synaptic scaffolding proteins, which are known to link interactions between mGluRs and NMDARs, are also implicated in ASDs (Peca and Feng 2012). Although there appears to be a convergence of pathophysiology in neurodevelopmental disorders concerning these two types of glutamate receptors, there is still much to learn about how their interaction at the synapse can alter plasticity and learning (O'Connor, Bariselli et al. 2014). The study of these interactions may aid in the understanding of neurodevelopmental disorder phenotypes. In addition to the glutamate system, there is considerable evidence linking alterations of the GABAergic inhibitory system to ASDs and schizophrenia (Lewis, Hashimoto et al. 2005, Gogolla, Leblanc et al. 2009, Pizzarelli and Cherubini 2011). Several postmortem studies of patients, as well as the analysis of animal models, has revealed altered expression of GABAergic system-related proteins, including alterations in PV+ interneurons (Hashimoto, Volk et al. 2003, Gogolla, Leblanc et al. 2009, Ramamoorthi and Lin 2011, Durand, Patrizi et al. 2012). How genetic mutations that confer risk to these disorders can alter the excitatory/ inhibitory balance in the brain remains a subject of intense investigation and debate.

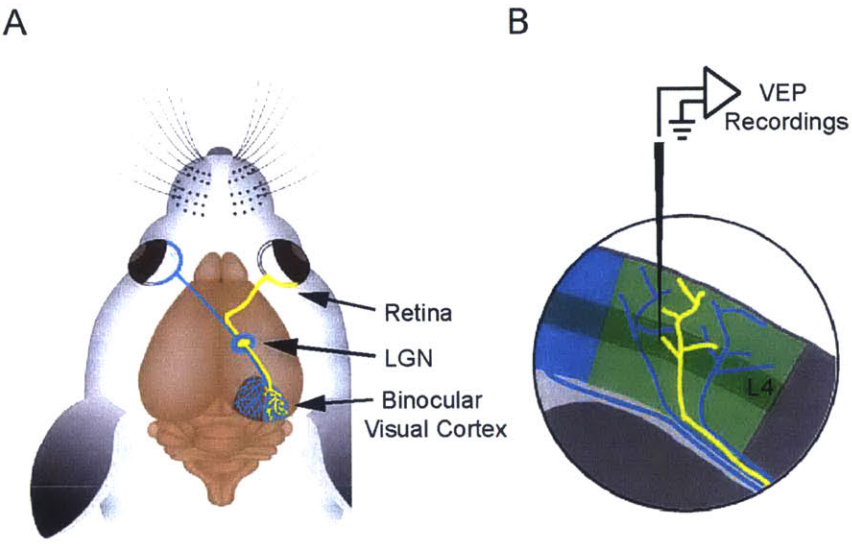

Figure 1.1: Organization of the mouse visual system. (A) Dorsal view of the organization of the visual pathway in a mouse is shown. Visual information is transmitted from both retinas (ipsilateral – yellow; contralateral – blue) to the dorsal lateral geniculate nucleus (LGN) of the thalamus, where inputs remain segregated. Eye-specific thalamocortical axons converge onto neurons of the binocular primary visual cortex. **(B)** Coronal section view of contralateral and ipsilateral thalamocortical input to binocular primary visual cortex. Input downstream of both eyes contact layer 4 (L4) cortical neurons. Visually-evoked potentials (VEPs) can be recorded in layer 4 in response to visual stimulation as a readout of synaptic strength. Figure adapted from Coleman *et al.*, 2010, and Levelt and Hubener 2012.

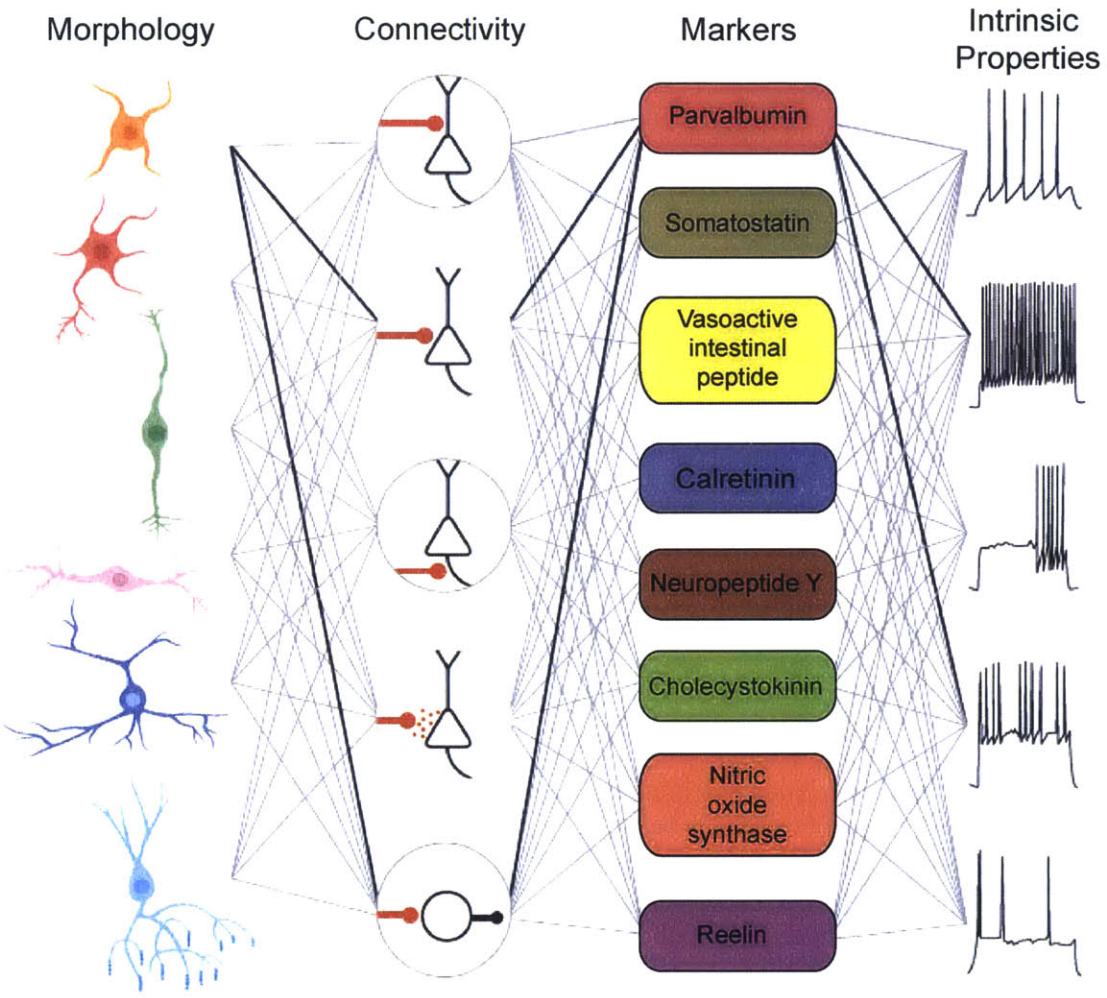

Figure 1.2: Heterogeneity of cortical GABAergic interneurons. The GABAergic interneurons of the cerebral cortex encompass a diversity of characteristics, some distinctive, but many overlapping. Cell types are usually defined by a combination of criteria based on morphology, connectivity pattern, marker expression, and intrinsic firing properties. The thicker connections represent properties of parvalbumin positive fast spiking cortical basket cells. Figure adapted from Kepecs and Fishell 2014.

Chapter 2

The role of parvalbumin expressing inhibitory interneurons in stimulus-selective response potentiation in the adult mouse

Portions of this chapter are under review:

Kaplan ES*, Cooke SF*, Komorowski RW, Chubykin AA, Khibnik LA, Bear MF (in submission). Contrasting Roles for Parvalbumin-Expressing Inhibitory Neurons in Two Forms of Adult Visual Cortical Plasticity

2.1: Abstract

The role played by cortical inhibitory neurons in experience-dependent plasticity is not well understood. Here we evaluate the participation of a major class of GABAergic neurons, the parvalbumin-expressing (PV+) putative fast spiking interneurons, in stimulus-selective response potentiation (SRP), which is a form of perceptual learning that contributes to long-term habituation. We show that PV+ inhibitory interneurons in V1 play a critical role in the expression of SRP. Pharmacogenetic silencing as well as optogenetic activation of PV+ interneurons interferes with SRP expression. Furthermore, we take a genetic knockdown approach to reveal that NMDA receptors expressed within PV+ cells play a critical role in SRP. The psychotomimetic substance ketamine temporarily blocks the expression of SRP, through its action on NMDARs expressed specifically within PV+ interneurons. We also provide evidence for the role of PV+ cells in the expression of SRP, by revealing a stimulus-dependent modulation of gamma frequency oscillations in V1 during SRP expression. Finally, we reveal that inactivation of PV+ interneurons or the removal of NMDA receptors from those cells interferes with behavioral discrimination of familiar and novel stimuli.

2.2: Introduction

Great challenges remain in understanding how sensory events modify the central nervous system (CNS) during development and learning. The effects of sensory experience on the primary sensory cortices serve as highly constrained experimental paradigms to investigate the mechanisms of plasticity that support these processes. The primary visual cortex (V1) has been known as a site of robust experience-dependent plasticity since the pioneering work of Hubel and Wiesel (Wiesel and Hubel 1963). In addition to deprivation, it is now understood that other forms of visual experience modify the properties of V1. A newly discovered form of experience-dependent plasticity that occurs in V1 is known as stimulus-selective response potentiation (SRP). SRP is a phenomenon in which the magnitude of a visually-evoked response to a brief daily presentation of a simple oriented grating stimulus potentiates over days (Frenkel, Sawtell et al. 2006). This potentiation is long lasting and selective, in that the potentiation occurs only in response to the previously experienced stimulus, and not in response to another stimulus, *e.g.* a grating stimulus of a different orientation. Interestingly, it is known that this cortical plasticity is required for a visually-guided habituation behavior. Mice viewing an oriented grating stimulus will physically react to the onset of this oriented grating stimulus. However, over days this behavioral response to the visual stimulus will habituate in a stimulus-specific manner. While the familiar visual stimulus will now often fail to elicit a behavioral response, a reliable behavioral response will re-emerge to a novel stimulus. This phenomenon, termed orientation-selective habituation (OSH) is known to depend on SRP occurring in V1 (Cooke, Komorowski et al. 2015). If the experience-dependent changes in V1 are interfered with, OSH is also disrupted.

While it is known that the activity of *N*-methyl-D-aspartate receptors (NMDARs) is required for SRP (Frenkel, Sawtell et al. 2006), little is understood about what cortical cell types may be involved. Here we investigated the role of the parvalbumin expressing (PV+) inhibitory interneurons, to the expression of SRP in the adult mouse. In addition to the glutamatergic principal cells of V1, PV+ inhibitory interneurons receive substantial feed-forward glutamatergic input from the thalamus (Cruikshank, Lewis et al. 2007), suggesting that these synapses may also serve as a potential site of NMDAR-dependent modification. Roughly 20% of neurons in the cerebral cortex are gamma-Aminobutyric acid (GABA)-ergic inhibitory neurons, which themselves can be subdivided into a number of distinct sub-classes based on gene expression, morphology, and electrophysiological properties (Markram, Toledo-Rodriguez et al. 2004). PV+

fast-spiking inhibitory neurons comprise the most numerous sub-class of GABAergic cortical neurons (Xu, Roby et al. 2010). The mechanistic contribution of this class of neuron to cortical plasticity is of particular importance given the evidence for cortical PV+ interneuron dysfunction in various developmental psychiatric disorders (Lewis, Hashimoto et al. 2005, Gogolla, Leblanc et al. 2009). In the current study, we found that the expression of SRP is dependent on PV+ cell activity, and that perturbations of PV+ neuron function, most notably cell-type specific ablation of NMDARs, disrupt the expression of both SRP and its behavioral correlate, familiarity recognition.

2.3: Results

2.3.1: Inactivation of PV+ interneurons disrupts expression of SRP.

In order to understand the role of PV+ neurons in the expression of experience-dependent visual cortical plasticity, we selectively inactivated these cells using a Dreadd (Designer Receptors Exclusively Activated by Designer Drugs) pharmacogenetic system. Specifically, we expressed a re-engineered G-protein coupled receptor, hM4D(Gi), which is activated exclusively by an otherwise inert small molecule, clozapine-N-oxide (CNO) (Nichols and Roth 2009). The binding of CNO to the hM4D(Gi) receptor activates intracellular G_i-mediated signaling and subsequent hyperpolarization of the cell in which the receptors are expressed. We locally expressed hM4D(Gi) receptors in PV+ cells of binocular V1 using an adeno-associated viral vector containing a construct for Cre-selective expression (AAV9-hSyn-DIO-HA-hM4D(Gi)-IRES-mCitrine) in mice that express Cre recombinase only in PV+ cells (B6;129P2-*Pvalb*$^{tm1(cre)Arbr}$/J, PV-Cre). We confirmed the selective expression of hM4D(Gi) receptors in PV+ neurons in binocular V1 using immunohistochemistry (Fig. 2.1A-E). In order to ensure that PV+ neurons could be inactivated by this method, we took slices of V1 for *ex vivo* intracellular recordings. Bath application of CNO considerably inhibited current evoked action potential firing of PV+ neurons in Layer 4 (2-way repeated measures ANOVA, interaction of CNO x current injection, $F_{(8,72)} = 6.227$, $P < 0.001$, $n = 10$ cells, significant at data points above 100pA: $q(8) = 3.716$, $P = 0.014$) but had no effect on neighboring non-expressing cells (Fig. 2.1F-G). Layer 4 recordings *in vivo* demonstrated that systemic administration of CNO resulted in the elevation of visually-evoked potential (VEP) magnitude, and an increase in the firing rate of excitatory single units (Fig. 2.1H-I), which are expected consequences of inactivating PV+ inhibitory neurons in binocular V1.

With a functioning method of cell inactivation in hand, we assayed the role of PV+ neurons in the expression mechanism underlying stimulus-selective response potentiation (SRP), a form of experience-dependent visual cortical plasticity that manifests as an increase in V1 responses (Frenkel, Sawtell et al. 2006, Cooke and Bear 2010). Binocular VEPs were recorded from awake, head-fixed adult mice viewing phase-reversing gratings of a particular orientation (X° stimulus) on each of 6 consecutive days (Fig. 2.2A and B). On the 7th day mice viewed blocks of the now familiar visual stimulus interleaved with blocks of a novel oriented stimulus (X +/- 60°). To address whether PV+ neuron activity is required for the expression of SRP, familiar and novel oriented grating stimuli were presented before and after mice received CNO (Fig. 2.2A).

As is characteristic of SRP, there was a significant increase in the magnitude of the average VEP evoked by the familiar oriented grating stimulus over days (Friedman 1-way repeated measures ANOVA on ranks, n = 10 mice, $X^2_{(5)} = 40.40$, $P < 0.001$, Fig. 2.2C). This potentiation was evident by day 2 (263.3 ± 19.96 μV) in comparison with day 1 (184 ± 17.13 μV, SNK post hoc test, $q_{(9)} = 4.472$, $P < 0.05$). The stimulus selectivity of VEP magnitude potentiation was apparent on day 7 and significantly affected by inactivating PV+ inhibitory neurons in V1 (2-way repeated measures ANOVA, interaction of treatment x stimulus, $F = 78.927_{(1,9)}$, $P < 0.001$, Fig. 2.2D): Prior to delivery of CNO the average VEP magnitude driven by the familiar stimulus (324.7 ± 22.18 μV) was significantly greater than that driven by a novel oriented stimulus (162.9 ± 16.31 μV, SNK post hoc test, $q_{(9)} = 10.709$, $P < 0.001$). Following intraperitoneal (I.P.) injection of CNO to inactivate hM4D(Gi)-infected neurons in V1, there was no longer a significant difference in the magnitude of VEPs driven by the familiar stimulus (537.2 ± 66.69 μV) compared with a novel stimulus (525.1 ± 68.30 μV, SNK post hoc test, $q_{(9)} = 0.804$, $P = 0.58$). The selectivity of the potentiation to a familiar stimulus can be summarized by plotting the ratio of VEP magnitudes driven by familiar and novel stimuli (Fig. 2.2E). Mice expressed a significantly larger familiar/novel ratio before CNO injection (2.11 ± 0.17), corresponding to a greater response to the familiar stimulus, compared to after CNO injection (1.03 ± 0.04, Mann Whitney rank sum test, $U = 0.00$, $P < 0.001$). CNO injection did not affect the stimulus selectivity of SRP in wild-type animals infected with virus (Fig. 2.S1A-B). These animals underwent SRP (Fig. 2.S1A) and on test day showed significantly larger VEPs to the familiar stimulus, both prior to CNO injection (familiar VEP: 361.06 ± 27.97 μV, novel VEP: 219.25 ± 17.9 μV, 2-way repeated measures ANOVA, n = 8 mice, SNK post hoc test, $q_{(7)} = 13.618$, $P < 0.001$) and following CNO injection (familiar VEP: 369 ± 31.16 μV, novel VEP: 256.75 ± 19.59 μV, SNK

post hoc test, $q_{(7)} = 10.779$, $P < 0.001$, Fig. 2.S1B). These results show that the inactivation of PV+ neurons in binocular V1 disrupts the expression of SRP.

2.3.2: Disruption of SRP by PV+ neuronal inactivation is not due to saturation of responses.

Cortical neurons respond within a dynamic range, and it is possible that discrimination of familiar and novel stimuli would be lost as responses approach saturation. To address the possibility that a "ceiling effect" contributes to the disruption of SRP expression during PV+ cell inactivation, we conducted an additional experiment in which mice viewed sinusoidal grating stimuli across a range of contrast values (5, 10, 25, 50, 100%). VEPs were progressively greater in magnitude the greater the contrast of the viewed stimulus (2-way repeated measures ANOVA, n = 4 mice, effect of contrast, $F_{(4,12)} = 25.908$, $P < 0.001$) both before and during PV+ neuronal inactivation using the hM4D(Gi) system (SNK post hoc test, 5 vs. 100 percent contrast, baseline; $q_{(4)} = 5.178$, $P = 0.013$; CNO; $q_{(4)} = 17.595$, $P < 0.001$, Fig 2.3A). Preservation of the relationship of contrast and response during PV+ neuron inactivation indicates that responses have not exceeded their dynamic range.

We then induced SRP to different orientations in a different set of mice, one stimulus at 50% contrast and the other stimulus at 100% contrast. Stimuli at 50% contrast evoke VEPs of decreased magnitude compared to 100% contrast, which enabled the opportunity to test the requirement for PV+ cell activity in the expression of SRP at a lower absolute VEP magnitude. After 6 days of SRP at 50%, there was a modest but significant difference in VEP magnitude for familiar (188.81 ± 11.80 μV) and novel orientations (145.06 ± 8.11μV, 2-way repeated measures ANOVA, n = 8 mice, SNK post hoc test, $q_{(7)} = 3.608$, $P = 0.023$, Fig 2.3B), just as there was for the familiar (259.81 ± 17.66 μV) and novel orientations (192.81 ± 17.27 μV, SNK post hoc test, $q_{(7)} = 3.793$, $P = 0.018$, Fig 2.3C) at 100% contrast. SRP expression was abolished by PV+ neuronal inactivation using the hM4D(Gi) system at 50% contrast as VEPs elicited by familiar (404.69 ± 59.17 μV) and novel orientations (376.94 ± 56.79 μV) were no longer significantly different (SNK post hoc test, $q_{(7)} = 2.289$, $P = 0.128$, Fig 2.3B). The same was true at 100% contrast as VEPs evoked by the familiar (532.44 ± 63.82 μV) and novel orientations (514.88 ± 40.38 μV) were also no longer significantly different (SNK post hoc test, $q_{(7)} = 0.994$, $P = 0.494$, Fig 2.3C). Again, the blockade of SRP expression was also clearly observed in the familiar/novel ratio, which dropped significantly from (1.31 ± 0.05) to (1.08 ± 0.08) with CNO application at 50% contrast (student's paired two-tailed t-test, $t_{(7)} = 2.983$, $P = 0.02$, Fig. 2.3D)

and from (1.40 ± 0.10) to (1.02 ± 0.05) with CNO application at 100% contrast (student's paired two-tailed t-test, $t_{(7)} = 2.955$, $P = 0.021$, Fig. 2.3E). Thus, SRP could still be abolished through selective loss of PV+ neuron activity even at reduced contrasts eliciting submaximal responses. The disruption of SRP expression by inactivation of PV+ neurons does not appear to be a consequence of a physiological "ceiling effect".

2.3.3: Activation of PV+ interneurons also disrupts expression of SRP.

PV+ neurons have been implicated in the sharpening of orientation selectivity in V1 (Runyan, Schummers et al. 2010, Adesnik, Bruns et al. 2012, Atallah, Bruns et al. 2012, Lee, Kwan et al. 2012, Wilson, Runyan et al. 2012). If orientation selectivity were completely abolished by inactivation of PV+ neurons then the loss of SRP expression could simply be attributed to a failure of orientation discrimination rather than familiarity. A previous experiment has indicated that activation of PV+ neurons in V1 actually mildly enhances orientation-selectivity and visual discrimination (Lee, Kwan et al. 2012). Therefore, we used optogenetics to activate PV+ neurons while mice were presented with familiar and novel stimuli to test whether SRP expression would be enhanced, unaffected or disrupted. PV+ neurons in binocular V1 of PV-Cre mice expressed Channel-rhodopsin 2 (ChR2) as a result of infection with AAV5-EF1α-DIO-hChR2(H134R)-eYFP. During the same surgery VEP recording electrodes were implanted and optic fibers chronically implanted to deliver light to the recording site (Fig. 2.4A). After stable expression 4 weeks after infection, mice were habituated to head-fixation on each of 2 days before undergoing a standard SRP experiment over 6 days (Fig. 2.4B, C).

Mice showed a significant increase in the magnitude of the average VEP evoked by the familiar oriented grating stimulus over days (Friedman repeated measures ANOVA on ranks, n = 11 mice, $X^2_{(5)} = 24.818$, $P < 0.001$, Fig. 2.4C). This potentiation was evident by day 2 (237.55 ± 29.01 μV) in comparison with day 1 (182.59 ± 21.97 μV, SNK post hoc test, $q_{(10)} = 7.675$, $P < 0.05$). On day 7, mice were presented with interleaved blocks of familiar and novel stimuli. On 50% of these blocks, blue light (473 nm) was also continuously delivered via the optic fiber to the recording site in binocular V1. VEPs elicited by the novel X + 90° stimulus were significantly lower in magnitude (221.34 ± 33.55 μV) than those elicited by the familiar stimulus prior to optogenetic activation of PV+ neurons (310.70 ± 36.70 μV, 2-way repeated measures ANOVA, n = 11 mice, SNK post hoc test, $q_{(10)} = 11.799$, $P < 0.001$, Fig. 2.4D). Optogenetic activation of PV+ neurons significantly diminished the selectivity of SRP, as VEPs elicited by the familiar stimulus (181.23 ± 26.43 μV) and novel stimulus (157.80 ± 21.05 μV) were more similar in

magnitude (interaction of stimulus x laser, $F_{(1,10)} = 46.606$, $P < 0.001$; familiar vs. novel SNK post hoc test, $q_{(10)} = 3.094$, $P = 0.045$, Fig. 2.4D). The reduction of SRP selectivity is most clearly observed in the significant difference in the familiar/novel ratio without (1.55 ± 0.14) and with optogenetic stimulation (1.16 ± 0.07, student's paired two-tailed t-test, $t_{(10)} = 4.423$, $P = 0.001$, Fig. 2.4E). Laser stimulation did not affect the stimulus selectivity of SRP in wild-type animals infected with virus (Fig. 2.S1), as there was no significant interaction between stimulus and laser (2-way repeated measures ANOVA, $F_{(1,8)} = 0.677$, $P = 0.434$). These mice underwent SRP (Fig. 2.S1C) and on test day showed significantly larger VEPs to the familiar stimulus, both with the laser off (familiar VEP: 273.69 ± 28.25 μV, novel VEP: 200.19 ± 19.53 μV, 2-way repeated measures ANOVA, n = 9 mice, SNK post hoc test, $q_{(8)} = 5.234$, $P = 0.005$) and the laser on (familiar VEP: 277.08 ± 29.80 μV, novel VEP: 194 ± 18.39 μV, $q_{(8)} = 5.916$, $P = 0.002$, Fig. 2.S1D). Thus, SRP expression was disrupted with activation of PV+ neurons, just as it was with inactivation (Fig. 2.2D-E). This result implies that PV+ neurons play a specific role in SRP expression beyond any role in enhancing orientation tuning.

2.3.4: SRP expression requires NMDA receptors (NMDARs) expressed in PV+ neurons.

Multiple lines of evidence indicate that SRP is dependent upon the NMDA class of glutamate receptors (Frenkel, Sawtell et al. 2006, Cooke, Komorowski et al. 2015). Given the additional clear requirement for normal PV+ inhibitory cell function in SRP (Fig. 2.2D,E) we selectively ablated the NMDARs from just PV+ cells by crossing the PV-Cre line of mice with a line in which the mandatory GluN1 subunit of NMDARs is excised by Cre recombinase activity (B6.129S4-*Grin1*^{tm2Stl}/J, GluN1 fl/fl). The progeny of this cross, in which both alleles of Grin1 (the gene encoding GluN1) were floxed, are henceforth described as either PV-GluN1 KO or Wildtype (WT)-GluN1 fl/fl depending on whether, respectively, Cre was expressed or not. We implanted PV-GluN1 KO (n = 14) and littermate WT-GluN1 fl/fl mice (n = 17) with VEP recording electrodes in layer 4, binocular V1. After recovery and 2 daily sessions of habituation we recorded VEPs elicited by a X° oriented grating stimulus. Immediately apparent was the significantly greater basal magnitude of VEPs recorded in the PV-GluN1 mice (242.45 ± 20.20 μV) relative to their littermate controls (130.88 ± 9.69 μV, student's two-tailed t-test, $t_{(29)} = -5.269$, $P < 0.001$), consistent with the occurrence of disinhibition as a result of reduced glutamatergic drive on cortical PV+ inhibitory cells (Fig. 2.5A). We then presented the same stimulus to these mice over several consecutive days and observed a significant difference in SRP across genotypes (2-way repeated measures ANOVA, interaction of genotype x day,

$F_{(4,116)}$ = 3.835, P = 0.006, Fig. 2.5A). Beyond day 3, VEP magnitudes were no longer significantly different between the PV-GluN1 KO mice (289.15 ± 24.57 μV) and WT-GluN1 fl/fl littermates (239.94 ± 20.72 μV, SNK post hoc test, $q_{(29)}$ = 2.242, P = 0.119), suggesting an occlusion of SRP by the already elevated basal VEP magnitude in the PV-GluN1 KO mice. The significant deficit in SRP is clearly apparent when the data are normalized to day 1 values (2-way repeated measures ANOVA, interaction of genotype x day, $F_{(4,116)}$ = 12.326, P < 0.001, Fig. 2.5B). Again, a significant deficit in SRP emerged by day 3 in the PV-GluN1 KO mice (119.26 ± 10.13% day 1) compared with WT-GluN1 fl/fl littermates (183.33 ± 15.83% day 1, SNK post hoc test, $q_{(29)}$ = 4.727, P = 0.002), demonstrating that SRP is compromised by a loss of NMDARs expressed in PV+ neurons.

We also tested for the stimulus-selectivity of SRP expression by presenting both groups of animals with interleaved blocks of the familiar X° stimulus and a novel X + 90° stimulus (Fig. 2.5C). Significant stimulus selectivity was present in both genotypes (2-way repeated measures ANOVA, stimulus, $F_{(1)}$ = 67.397, P < 0.001, interaction of genotype x stimulus, $F_{(1,29)}$ = 2.359, P = 0.135, Fig. 2.5C): Although PV-GluN1 KO mice showed significant differences in VEP magnitude for familiar (340.24 ± 27.96 μV) and novel orientations (242.54 ± 24.40 μV, SNK post hoc test, $q_{(13)}$ = 6.372, P < 0.001) the difference was more pronounced in the WT-GluN1 fl/fl mice (SNK post hoc test, $q_{(16)}$ = 10.254, P < 0.001), in which the familiar stimulus elicited VEPs 318.74 ± 25.19 μV in magnitude and the novel stimulus elicited VEPs 176.06 ± 11.55 μV in magnitude. The familiar stimulus evoked VEPs that were not significantly different in the WT-GluN1 fl/fl mice (318.74 ± 25.19 μV) and the PV-GluN1 KO mice (340.24 ± 27.96 μV, SNK post hoc test, $q_{(29)}$ = 0.947, P = 0.507) but those evoked in the WT-GluN1 fl/fl by the novel stimulus (176.06 ± 11.55 μV) were significantly lower in magnitude than in the PV-GluN1 KO mice (242.54 ± 24.40 μV, SNK post hoc test, $q_{(29)}$ = 2.926, P = 0.045). The significant deficit in SRP expression in the PV-GluN1 KO mice is most apparent when the familiar/novel ratio of VEP magnitude (1.48 ± 0.13) is compared with the WT-GluN1 fl/fl littermates (1.83 ± 0.13, Mann Whitney rank sum test, U = 60.000, P = 0.020, Fig. 2.5D). Thus, loss of NMDAR function selectively within PV+ neurons impairs SRP expression.

2.3.5: Acute ketamine treatment reversibly eliminates SRP expression.

A group of non-competitive, open-channel NMDAR blockers, including ketamine, PCP and MK801 are known to have the paradoxical impact of increasing net neuronal activity in the brain. It is thought that this apparent disinhibition arises from the preferential impact of these

molecules on fast-spiking neurons, due to the tonic activation and the increased open-time of NMDARs expressed within these cells (Homayoun and Moghaddam 2007, Seamans 2008). Interestingly, these compounds are also psychotomimetic and can reproduce, at high sub-anesthetic doses, most of the symptoms of schizophrenia (Krystal, Karper et al. 1994). Here we tested the possibility that a single acute dose of one of these substances, ketamine, would have an impact on the expression of SRP due to its action on NMDARs expressed in PV+ fast spiking inhibitory neurons.

We implanted VEP recording electrodes in layer 4 of binocular V1 in a group of 10 C57BL/6 mice. After recovery and a standard SRP protocol we recorded VEP magnitudes driven by familiar and novel stimuli before, during, and 2 days after recovery from systemic (I.P.) injection of a high but sub-anesthetic dose of ketamine (50 mg/kg)(Fig. 2.6A). Ketamine had a significant effect on SRP expression (2-way repeated measures ANOVA, interaction of treatment x stimulus, $F_{(2,18)} = 29.479$, $P < 0.001$, Fig. 2.6B). After SRP but prior to ketamine delivery the familiar X° stimulus elicited VEPs of significantly greater magnitude (245.47 ± 21.00 μV) than a novel X + 60° stimulus (138.86 ± 9.32 μV, SNK post hoc test, $q_{(9)} = 9.228$, $P < 0.001$) in these mice. After an hour of respite from head-fixation, mice were injected with ketamine and, 15 minutes later, they were returned to head-fixation and VEP magnitudes were again recorded. Under the influence of ketamine, the familiar X° stimulus elicited VEPs of significantly increased magnitude (381.90 ± 37.25 μV) relative to pre ketamine (245.47 ± 21.00 μV, SNK post hoc test, $q_{(9)} = 7.223$, $P < 0.001$). However, VEPs elicited by a second novel X - 60° stimulus were increased relative to pre-ketamine by an even greater extent (397.53 ± 37.62 μV) relative to pre ketamine (138.86 ± 9.32 μV, SNK post hoc test, $q_{(9)} = 13.695$, $P < 0.001$), such that VEPs were no longer significantly different in magnitude in response to familiar and novel stimuli (SNK post hoc test, $q_{(9)} = 1.353$, $P = 0.349$). After 2 day's rest, allowing for complete recovery from drug effects, the mice were returned to the head-fixation apparatus and exposed to the familiar X° stimulus and a third novel stimulus, X + 90°. Just as observed prior to the ketamine injection, the X° stimulus evoked VEPs of significantly greater magnitude (267.46 ± 26.13 μV) than the novel X + 90° stimulus (149.40 ± 9.85 μV, $q_{(9)} = 10.219$, $P < 0.001$)(Fig. 2.6A-B).

This significant effect of ketamine on the discrimination of familiar and novel stimuli is summarized by the ratio of VEP magnitude driven by the familiar/novel stimulus (1-way repeated measures ANOVA, $F_{(2,18)} = 24.683$, $P < 0.001$, Fig. 2.6C). This familiar/novel ratio dropped significantly from 1.76 ± 0.10 prior to ketamine to 0.96 ± 0.02 after ketamine (SNK post hoc test, $q_{(9)} = 8.443$, $P < 0.001$). Upon recovery, the familiar/novel ratio significantly recovered

(1.79 ± 0.16, SNK post hoc test, $q_{(9)} = 8.759$, $P < 0.001$) and was not significantly different from the first test after SRP but prior to ketamine injection (SNK post hoc test, $q_{(9)} = 0.316$, $P = 0.826$). Thus, the psychotomimetic non-competitive NMDAR antagonist ketamine disrupts SRP. The fact that this is an acute effect on already established SRP that recovers after drug washout indicates that the role for NMDARs in PV+ fast-spiking GABAergic neurons may be in memory retrieval rather than learning.

2.3.6: Ketamine affects V1 responses through NMDARs expressed in PV+ neurons.

Ketamine blocks NMDARs expressed in all cell types throughout the CNS and is also known to have targets other than the NMDAR (Chen, Shu et al. 2009). In order to determine if ketamine has its effect on V1 responses specifically through NMDARs expressed in PV+ cells, we tested if there was a differential effect of ketamine on VEP magnitude in PV-GluN1 KO mice and WT-GluN1 fl/fl mice. After a typical implantation and habituation protocol (Fig. 2.6D) we tested VEP magnitudes elicited by a novel oriented X° stimulus in WT-GluN1 fl/fl mice (178.11 ± 38.41 μV, n = 8) and PV-GluN1 KO mice (260.79 ± 50.75 μV, n = 8)(Fig. 2.6E). We then removed mice from head-fixation and allowed them to recover in their home-cage before delivering 50 mg/kg ketamine (I.P.). After 15 minutes, the mice were returned to head-fixation and we observed the impact of ketamine on VEPs elicited by a novel X + 90° oriented stimulus, which was significantly different in its effect on the two genotypes (2-way repeated measures ANOVA, interaction of genotype x treatment, $F_{(1,14)} = 18.454$, $P < 0.001$). In the control WT-GluN1 fl/fl mice there was a significant potentiation of VEP magnitude as a result of ketamine application (386.65 ± 70.75 μV) in comparison to pre-treatment (178.11 ± 38.41 μV, SNK post hoc test, $q_{(7)} = 8.505$, $P < 0.001$, Fig. 2.6E), replicating our previous finding (Fig. 2.6B). However, in the PV-GluN1 KO mice, ketamine had no significant impact on VEP magnitude (258.67 ± 44.86 μV) in comparison to pre-treatment (260.79 ± 50.75 μV, SNK post hoc test, $q_{(7)} = 0.087$, $P = 0.952$). A ratio of VEP magnitudes pre and post ketamine treatment reveals the significant difference between ketamine's action on WT-GluN1 fl/fl mice (2.40 ± 0.25) and PV-GluN1 KO mice (1.12 ± 0.13, student's two-tailed t-test, $t_{(14)} = 4.610$, $P < 0.001$)(Fig. 2.6F). Thus, while ketamine has a wide range of effects in the CNS, it exerts itself on the response of V1 to visual input selectively through NMDARs expressed in PV+ cells.

2.3.7: Experience-dependent changes in gamma oscillations in V1 are absent in PV-GluN1 KO mice.

We next explored how the SRP deficit observed in the PV-GluN1 KO mice might be related to abnormalities in local field potential (LFP) activity recorded from layer 4 of V1 as these animals view familiar and novel stimuli. LFP spectra were filtered from 1-120 Hz and normalized for presentation by applying a 1/*f* correction (Sirota, Montgomery et al. 2008). Our analysis focused on the high-gamma range (65-100 Hz) of oscillations as PV+ interneurons are known to be instrumental in the generation of these oscillations (Wang and Buzsaki 1996, Bartos, Vida et al. 2007, Cardin, Carlen et al. 2009, Siegle, Pritchett et al. 2014), and our earlier results implicated PV+ interneuron activity and the NMDARs within these cells in the expression of SRP. A comparison between PV-GluN1 KO mice and WT-GluN1 fl/fl littermates revealed significant differences in the average normalized power in the high-gamma range (Fig. 2.7). In wild-type mice there was significantly greater normalized high-gamma power when mice viewed the novel oriented visual stimulus (9.50 ± 1.31), compared to the familiar oriented stimulus (6.75 ± 0.71, 2 way repeated measures ANOVA, SNK post hoc test, n = 10 mice, $q_{(1)} = 7.545$, $P < 0.001$, Fig. 2.7A,C). However, we observed a significant difference in the modulation of high-gamma power across genotypes (2 way repeated measures ANOVA, interaction of genotype x stimulus, $F_{(1,16)} = 6.446$, $P = 0.022$). The PV-GluN1 KO mice did not display a significant modulation of high-gamma power while viewing familiar (5.50 ± 0.31) and novel stimuli (6.29 ± 0.54, 2 way repeated measures ANOVA, SNK post hoc test, n = 8 mice, $q_{(1)} = 1.931$, $P < 0.191$, Fig. 2.7B-C). Most apparent from the analysis was the significantly greater high-gamma power in response to the novel stimulus displayed by the wild-type mice (9.50 ± 1.31), compared to the PV-GluN1 KO mice (6.29 ± 0.54, SNK post hoc test, $q_{(1)} = 3.647$, $P < 0.018$, Fig. 2.7C). These findings indicate that wild-type animals, which exhibit SRP, also show modulation of high-gamma oscillations in V1 in a stimulus specific manner, consistent with the involvement of PV+ interneurons. On the other hand, PV-GluN1 KO mice, which display impaired SRP, lack any significant modulation of gamma frequency power to familiar and novel stimuli.

2.3.8: Visual novelty detection requires PV+ interneuron activity within V1.

Given the clear involvement of PV+ neurons in the expression of SRP we wanted to determine if loss of PV+ neuronal function local to V1 would have any behavioral impact. Head-restrained mice viewing a phase reversing visual grating stimulus are known to exhibit a stereotyped motor response called a visually-induced fidget, or “vidget” (Cooke, Komorowski et

al. 2015). Vidgets can be measured via a piezoelectric sensor located beneath the forepaws of the mouse (Fig 2.8A). Importantly, it has been shown that the magnitude of the vidget response is inversely correlated to the familiarity of the visual stimulus. That is, a visual stimulus that is very familiar to the animal will on average evoke a relatively weak vidget behavioral response. In contrast, the presentation of a novel stimulus will on average evoke a vidget of significantly greater magnitude. Therefore, this behavioral response reflects the ability of the animal to discriminate and respond to a novel visual stimulus in its environment. Importantly, genetic and pharmacological manipulations local to V1 that inhibit SRP also disrupt the behavioral discrimination of familiar and novel stimuli (Cooke, Komorowski et al. 2015), demonstrating that this differential vidget response to familiar and novel stimuli is dependent on the plasticity in visual cortex. Since PV+ neuron inactivation disrupted the expression of SRP, we tested whether this manipulation would likewise disrupt visual novelty detection.

A group of PV-Cre mice expressing hM4D(Gi) receptors in PV+ cells (n = 19), underwent a SRP protocol similar to the previous experiment (Fig. 2.2A) in which mice viewed phase-reversing gratings of a particular orientation (X° stimulus) each day for 6 days. On the 7th day mice viewed blocks of the now familiar visual stimulus interleaved with blocks of a novel oriented stimulus (X + 60°). On day 7, vidget behavioral responses were acquired via a piezoelectric device situated underneath the forepaws of the mice (Fig 2.8A), in order to measure the animal's discrimination of familiar and novel stimuli. On day 8, PV+ cells in V1 were then inactivated by systemic delivery of CNO (i.p.) and vidget responses were acquired to the familiar X° stimulus and a second X - 60° novel stimulus. Inactivation of PV+ neurons in V1 significantly affected stimulus discrimination (2-way repeated measures ANOVA, interaction of treatment x stimulus, $F_{(1,18)} = 13.644$, $P = 0.002$, Fig. 2.8B): Prior to CNO, mice exhibited significantly larger vidget responses to the novel visual stimulus (3.64 ± 0.32 a.u.) than the familiar stimulus (1.95 ± 0.26 a.u., SNK post-hoc test, $q_{(18)} = 9.237$, $P < 0.001$). During PV+ cell inactivation in V1, behavioral responses to the familiar (2.01 ± 0.16 a.u.) and novel visual stimuli (2.46 ± 0.22 a.u.) were no longer significantly different (SNK post-hoc test, $q_{(18)} = 2.503$, $P = 0.086$). The deleterious effect of PV+ cell inactivation in V1 on the animal's ability to discriminate familiar and novel stimuli is most obvious when a ratio of response to familiar/novel visual stimuli is calculated. Prior to CNO delivery this ratio was significantly lower (0.54 ± 0.04) than during CNO treatment (1.00 ± 0.15, Wilcoxon signed rank test, $W = 138.000$, $Z = 2.777$, $P = 0.004$, Fig. 2.8C). These findings indicate that PV+ neuron activity is required not only for the expression of SRP, but also for visual novelty detection.

2.3.9: Discrimination of familiar and novel stimuli requires NMDARs within PV+ neurons.

Finally, given the observation that the discrimination of familiar and novel visual stimuli is disrupted by inactivation of PV+ neurons in V1, we tested whether a similar failure would occur in the PV-GluN1 KO mice (n = 17) compared with WT-GluN1 fl/fl littermates (n = 15, 2-way repeated measures ANOVA, stimulus, $F_{(1)}$ = 14.632, P < 0.001, Fig. 2.8D). In the PV-GluN1 KO mice, the familiar stimulus produced less behavioral response (2.75 ± 0.42 a.u.) than a novel stimulus (3.67 ± 0.79 a.u.). However, the difference was not statistically significant (SNK post hoc test, $q_{(16)}$ = 2.570, P = 0.079). In comparison, the concurrently tested WT-GluN1 fl/fl littermate mice showed a suppression of behavioral response to the familiar stimulus (1.76 ± 0.26 a.u.) relative to the novel stimulus (4.08 ± 0.51 a.u.) that was statistically significant (SNK post hoc test, $q_{(14)}$ = 5.008, P = 0.001). This observation was reinforced by a comparison of the ratio of behavior produced by familiar and novel stimuli (Fig. 2.8E), in which there was a significant difference between the PV-GluN1 KO mice (0.59 ± 0.11) and their WT-GluN1 fl/fl littermates (0.94 ± 0.14, student's one-tailed t-test, $t_{(30)}$ = -1.992, P = 0.028), reflective of the deficit in discrimination of familiar and novel stimuli as a result of lost NMDAR function in PV+ cells. Individual animals' average vidget responses to familiar and novel stimuli (Fig. 2.S2) reveal significantly decreased stimulus selectivity subsequent to CNO administration (Fig 2.S2A-B), and in the PV GluN1 KO mice as compared to WT-GluN1 fl/fl littermate controls (Fig 2.S2C-D). Thus, not only do NMDARs in PV+ cells contribute to SRP expression but they are also involved in its behavioral correlate.

2.4 Discussion

We have investigated the contribution of a class of GABAergic cells, the PV+ inhibitory interneurons, to a form of experience-dependent plasticity known as stimulus-selective response potentiation. We found that the expression of SRP, as well as a behavioral readout of familiarity recognition is dependent on the activity of PV+ interneurons as well as the function of NMDARs expressed on these same cells.

2.4.1: PV+ interneuron function in the cortex

PV+ cells continue to be the subject of concentrated inquiry due their relative abundance, interconnectivity, fast-spiking electrophysiological characteristics, and proclivity to

innervate principal cell soma and axon initial segments (Di Cristo, Wu et al. 2004, Markram, Toledo-Rodriguez et al. 2004, Kubota, Shigematsu et al. 2011). Collectively, these characteristics of PV+ interneuron circuitry suggest an inhibitory system that is temporally precise and highly capable. Much of the investigation into PV+ cells in V1 has focused on their role in several basic features of neural visual processing including orientation selectivity, direction selectivity, and gain control (Runyan, Schummers et al. 2010, Adesnik, Bruns et al. 2012, Atallah, Bruns et al. 2012, Lee, Kwan et al. 2012, Wilson, Runyan et al. 2012). Though PV+ cells themselves appear to lack strong orientation selectivity (Atallah, Bruns et al. 2012), their activity has been shown to modestly enhance principal cell orientation selectivity and thereby affect visual perception (Lee, Kwan et al. 2012). Another interesting feature of cortical PV+ cells is that they are efficiently activated by feedforward excitatory projections from the thalamus (Cruikshank, Lewis et al. 2007), which subsequently leads to intense inhibition of excitatory neurons; thereby suppressing the collective cortical response. In effect, this potent di-synaptic inhibition allows PV+ cells to influence processing by gating the overall responsiveness of the cortex to environmental stimuli. Furthermore, due to their intrinsic properties, PV+ cells have been shown to be instrumental in cortical gamma oscillation generation (Gray and Singer 1989, Wang and Buzsaki 1996, Fukuda and Kosaka 2000, Bartos, Vida et al. 2007, Cardin, Carlen et al. 2009), which is thought to play an important role in attention and sensory perception (Eckhorn, Bauer et al. 1988, Engel and Singer 2001, Moore, Carlen et al. 2010, Siegle, Pritchett et al. 2014, Pritchett, Siegle et al. 2015). Due to the apparent ability of PV+ interneurons to strongly and precisely modulate cortical activity, they are poised to play a direct role in experience-dependent plasticity and memory.

2.4.2: SRP and perceptual learning

SRP is a long lasting form of visual cortical response potentiation that develops over days in response to repeated presentation of a particular visual stimulus (Frenkel, Sawtell et al. 2006). Since its discovery 10 years ago, a great deal has been learned about its underlying mechanisms as well as its behavioral significance. Repeatedly experiencing particular environmental stimuli is known to elicit perceptual learning, where repeated exposure allows for the stimulus to be recognized more quickly, or for the details inherent in that stimulus to be more easily perceived (Poggio, Fahle et al. 1992, Watanabe and Sasaki 2015). These gains in performance can occur extremely selectively to the features of the stimulus. In the visual system perceptual learning is known to occur to very basic visual features such as orientation or spatial

frequency (Fiorentini and Berardi 1980), implicating primary visual cortex as a possible site for the underlying experience-dependent changes. SRP shares the characteristics of perceptual learning such as stimulus-selectivity and input specificity, and therefore may contribute as an underlying mechanism for visual perceptual learning (Cooke and Bear 2014). Perceptual learning allows for increased performance in detection and leads to changes in behavior (Watanabe and Sasaki 2015). Recently, SRP has been shown to be accompanied by a visual stimulus-selective behavioral habituation. As the size of the VEP driven by the familiar stimulus increases over days, the magnitude of the behavioral response to that stimulus decreases in parallel (Cooke, Komorowski et al. 2015). This habituation reflects the ability of the animal to discriminate familiar from novel stimuli in its environment. Importantly, genetic and pharmacological manipulations that inhibit SRP also disrupt this habituation (Cooke, Komorowski et al. 2015). Therefore, it is believed that this differential behavioral response to familiar and novel stimuli is dependent on SRP expression in V1.

The mechanisms underlying SRP appear to be rather complex. Previous studies have shown that the induction of SRP relies on mechanistic features associated with Hebbian long-term potentiation (LTP) (Bliss and Lomo 1973, Collingridge, Kehl et al. 1983, Shi, Hayashi et al. 2001). For instance, the induction of SRP requires NMDAR activity (Sawtell, Frenkel et al. 2003, Frenkel, Sawtell et al. 2006). Additionally, SRP can be disrupted by preventing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor membrane insertion (Frenkel, Sawtell et al. 2006). Furthermore, thalamocortical LTP induced *in vivo* by theta burst stimulation (TBS) of the visual thalamus; and SRP, are mutually occluding (Cooke and Bear 2010).

2.4.3: PV+ cell modulation, orientation tuning, and dynamic range

Distinct from feedforward LTP-like processes shown to be critical to the induction of SRP, here we have revealed an expression mechanism for SRP that relies on PV+ interneuron inhibition. By either inactivating PV+ cells using the Dreadd system, or activating them via channelrhodopsin-2 (ChR2), we were able to disrupt SRP expression (Fig. 2.2, 2.4). We reason that by interfering with the modulation of PV+ cells' activity driven by familiar and novel stimuli, the expression of SRP was lost. One concern is that the lack of discrimination of familiar and novel orientations could be accounted for simply by a failure of orientation selectivity and not by a failure of memory. Throughout the experiments presented here we have used visual stimulus orientations that are at least 60° and often 90° different, meaning that orientation discrimination

should be as unchallenging as possible. Given the rather modest impact on orientation tuning of suppressing PV+ interneuron activity in V1 (Runyan, Schummers et al. 2010, Adesnik, Bruns et al. 2012, Wilson, Runyan et al. 2012) we believe that this explanation is improbable. Moreover, stimulation of PV+ cells via ChR2 similarly disrupted SRP. Stimulation of PV+ inhibition has been shown to actually mildly enhance orientation-selectivity (Lee, Kwan et al. 2012) and yet our experiments reveal that the difference between visual cortical response to familiar and novel orientations is abolished to the same degree by activation of PV+ neurons as it is to inactivation of PV+ neurons. This result casts PV+ neurons in a very specific role in the recognition of familiar stimuli in addition to modest participation in orientation-selectivity.

A second concern is that, after the suppression of PV+ inhibition, V1 may be operating at a 'ceiling' of activity in which differential response to stimuli is not possible. To address this concern of a ceiling effect we conducted an experiment in which SRP is induced to separate stimuli at different contrast levels (Fig. 2.3). Lower contrast stimuli evoke lower magnitude responses than higher contrast stimuli, but SRP expression is present in both cases. Importantly, SRP is abolished through inactivation of PV+ cells in both cases but the differential response to contrast is maintained. This finding demonstrates that, even in a highly disinhibited state, SRP expression is compromised even though cortical response is not saturated. It is also worth noting that pharmacological approaches to suppressing intracortical inhibition, which are less specific, (Khibnik, Cho et al. 2010) result in much greater magnitude of response to visual input than we observe here for PV+ neuron inactivation, PV-GluN1 KO mice or ketamine application, indicating that our manipulations do not represent a physiological ceiling.

2.4.4: SRP modulates gamma frequency oscillations

Changes in cortical gamma oscillations further support the contribution of PV+ cells to SRP expression. We observed significant shifts in the power of neuronal oscillations in the high gamma frequency when an animal views familiar versus novel stimuli (Fig. 2.7). Cortical gamma oscillations have been proposed to mediate cognitive functions such as attention, sensory binding of features into a coherent percept, and increases in ability to detect stimuli (Engel and Singer 2001, Bartos, Vida et al. 2007, Siegle, Pritchett et al. 2014, Pritchett, Siegle et al. 2015). There is also significant evidence that PV+ interneurons are able to drive these rhythms (Wang and Buzsaki 1996, Bartos, Vida et al. 2007, Cardin, Carlen et al. 2009, Siegle, Pritchett et al.

2014). In agreement with these studies, it appears that PV+ cells may mediate a modulation in the power of gamma oscillations in SRP. This modulation of gamma was not apparent in animals lacking NMDARs in PV+ cells, which also show a deficit in SRP (Fig. 2.7). Lastly, we showed that the behavioral readout of familiar and novel stimuli discrimination, which is dependent on SRP (Cooke, Komorowski et al. 2015), is also dependent on the modulation of PV+ interneurons in V1, as disruption of PV+ cells activity disrupted not only the electrophysiological but also the behavioral readout of experience-dependent visual recognition (Fig. 2.8).

2.4.5: PV+ interneurons and familiarity

Other studies have recognized that the function of PV+ interneurons may be of importance in plasticity observed during instances of repeated exposure to sensory stimuli. For instance, mice lacking the synaptic protein neuronal activity regulated pentraxin (NARP), a synaptic protein specifically enriched at excitatory synapses on PV+ cells, displayed a reduction in excitatory synapses onto PV+ cells and a deficit in SRP-like effects (Chang, Park et al. 2010, Gu, Huang et al. 2013). Meyer and colleagues studying cortical area IT in monkeys, showed that image familiarization resulted in a sharpening of cortical responses to an experienced stimulus, which was likely orchestrated by local fast-spiking putative PV+ cells (Meyer, Walker et al. 2014). Additionally, Makino and Komiyama recently showed a stimulus-selective reduction in PV+ interneuron activity in V1 as mice are repeatedly exposed to a specific visual stimulus (Makino and Komiyama 2015).

2.4.6: Role of PV+ interneurons in SRP

These previous findings implicating PV+ cells in visual familiarity are in line with our current results indicating the necessity of PV+ interneuron activity in the expression of SRP. We hypothesize that presentation of a specific visual stimulus over days drives changes to the PV+ inhibitory interneuron network. Thereafter, presentation of the familiar stimulus evokes reduced PV+ interneuron activity in V1. This relaxation of PV+ cell-mediated inhibition results in a potentiation of VEPs to the previously experienced stimulus. This change is simultaneously reflected as a decrease in the power of cortical gamma oscillations. It also appears that stimulus-selective modulation of PV+ interneuron activity relies on NMDARs on PV+ cells, as their genetic deletion or pharmacological disruption via ketamine interferes with SRP. The

disruption of SRP we observed in the PV-GluN1 KO mice was a non-reversible genetic modification; therefore, the experiment did not allow us to pinpoint if NMDARs on PV+ cells were important for the induction, maintenance, or expression mechanism of SRP. Ketamine, a non-competitive NMDAR antagonist, has been shown to preferentially antagonize NMDARs on PV+ cells (Homayoun and Moghaddam 2007, Seamans 2008). By using ketamine to induce a temporary blockade of these receptors, we were able to discriminate that interference with NMDARs on PV+ cells disrupts the expression of SRP. Importantly, temporary pharmacological blockade of these receptors did not affect the stability of the information storage/ maintenance, since a strong bias for the familiar stimulus returned after ketamine washout. Previous inquiries into the mechanisms underlying SRP have implicated excitatory feedforward LTP-like processes (Frenkel, Sawtell et al. 2006, Cooke and Bear 2010, Cooke, Komorowski et al. 2015). We hypothesize that these feedforward excitatory cell synaptic changes underlie the induction and possibly information storage component of this form of experience-dependent plasticity. However, the modulation of PV+ interneuron activity to familiar and novel stimuli is essential for its expression/ retrieval. Interference with the modulation of PV+ cell activity prevents the differential cortical response, which is critical to the selectivity of SRP and the behavioral discrimination of familiar from novel stimuli.

2.4.7: PV+ interneurons, familiarity, and schizophrenia

We have shown here that modulation of PV+ neuron activity in V1 is required for selective recognition of familiarity and consequent novelty detection. Loss of NMDAR function in these same cells also impairs familiarity recognition. Importantly, the cognitive symptoms of schizophrenia, and other psychiatric disorders, are characterized by deficits in habituation and familiarity (Braff, Swerdlow et al. 1995, Ramaswami 2014), and individuals with schizophrenia display impairment in visual cortical plasticity (Cavus, Reinhart et al. 2012). Those with schizophrenia have also been shown to exhibit abnormal cortical gamma oscillations (Gonzalez-Burgos, Cho et al. 2015). Dysfunction of PV+ inhibitory neurons and the NMDARs expressed within PV+ cells have been implicated in these same psychiatric disorders through a range of approaches (Coyle, Tsai et al. 2003, Lewis, Hashimoto et al. 2005, Gogolla, Leblanc et al. 2009, Lewis, Fish et al. 2011). This notably includes observations of the profound psychotomimetic effect in humans of non-competitive NMDAR antagonists such as ketamine (Krystal, Karper et al. 1994, Krystal 2015), a substance that, as we show here, prevents SRP expression. We suggest that understanding the cortical physiology underlying the processes of familiarity

recognition and novelty detection may yield great insight into dysfunction underlying some symptoms of these disorders, and that because these fundamental forms of learning are critical across species, the underlying processes are likely to be conserved.

2.5: Materials and Methods

2.5.1: Mice

All procedures adhered to the guidelines of the National Institutes of Health and were approved by the Committee on Animal Care at MIT, Cambridge, MA, USA. For all experiments mice were male, aged between P60-90 and on a C57BL/6 background (Charles River laboratory international, Wilmington, MA). They were housed in groups of 2-5 with food and water available *ad libitum* and maintained on a 12 hour light-dark cycle. For hM4D(Gi) experiments, mice were Parvalbumin-Cre recombinase knock-in mice (B6;129P2-*Pvalb*$^{tm1(cre)Arbr}$/J, PV-Cre) on a C57BL/6 background. For GluN1 knockdown experiments, these PV-Cre mice were bred with homozygous mice in which the Grin1 gene, which encodes the GluN1 sub-unit was flanked by LoxP sites (B6.129S4-*Grin1*^{tm2Stl}/J, GluN1 fl/fl), enabling Cre-dependent ablation of this mandatory subunit of the NMDA receptor. Multiple generations were required to set up crosses yielding offspring that were homozygous GluN1 fl/fl and Cre-expressing. Of these approximately 50% expressed Cre and 50% served as wild-type littermates on the GluN1 fl/fl background. All experiments were conducted blind to genotype and treatment.

2.5.2: Surgery

Mice were first injected with 0.1 mg/kg Buprenex sub-cutaneously (s.c.) to provide analgesia. They were then anesthetized with an intraperitoneal (i.p.) injection of 50 mg/kg ketamine and 10 mg/kg xylazine. Prior to surgical incision, 1% lidocaine hydrochloride anesthetic was injected under the mouse's scalp. The skull was then cleaned with iodine and 70% ethanol. A steel head post was affixed to the skull anterior to bregma using cyanoacrylate glue. Burr holes (< 0.5 mm) were then drilled in the skull over binocular V1 (3.1 mm lateral of lambda). Tapered tungsten recording electrodes (FHC, Bowdoinham, ME, US), 75 µm in diameter at their widest point, were implanted in each hemisphere, 450 µm below cortical surface. Silver wire (A-M systems, Sequim, WA, US) reference electrodes were placed over prefrontal cortex. Mice were allowed to recover for at least 24 hours prior to head-fixation.

2.5.3: Viral infections

For hM4D(Gi) experiments, we infected V1 of P30-60 PV-Cre mice or wild-type littermates with an AAV9-hSyn-DIO- HA-hM4D(Gi)-IRES-mCitrine virus (UNC viral core – generated by Dr. Brian Roth's laboratory) and for optogenetic experiments we infected mice from the same line with AAV5-EF1α-DIO-hChR2(H134R)-eYFP (UNC viral core – generated by Dr. Karl Deisseroth's laboratory). Using a glass pipette and nanoject system (Drummond scientific, Broomall, PA, US), we delivered 81 nl of virus at each of 3 cortical depths: 600, 450, and 300 μm from the cortical surface, and allowed 5 minutes between re-positioning for depth. Mice were allowed 3-4 weeks recovery for virus expression to peak before experiments were initiated.

2.5.4: Drug delivery

Clozapine-N-oxide (CNO, Enzo Life Sciences) was diluted in saline and injected i.p. at a dosage of 5 mg/kg 30 minutes prior to stimulus delivery. Ketamine hydrochloride (Vedco) was diluted in water and delivered i.p. at 50 mg/kg 15 minutes prior to stimulus delivery.

2.5.5: Stimulus delivery

Visual stimuli consisted of full-field, 100% contrast, sinusoidal gratings that were presented on a computer monitor. Visual stimuli were generated using custom software written in either C++ for interaction with a VSG2/2 card (Cambridge Research systems, Kent, U.K.) or Matlab (MathWorks, Natick, MA, U.S.) using the PsychToolbox extension (http://psychtoolbox.org) to control stimulus drawing and timing. The display was positioned 20 cm in front of the mouse and centered, thereby occupying 92° × 66° of the visual field. Visual stimuli consisted of full-field sinusoidal grating stimuli phase reversing at a frequency of 2 Hz. Mean stimulus luminance was 27 cd/m^2. Grating stimuli spanned the full range of monitor display values between black and white, with gamma-correction to ensure constant total luminance in both gray-screen and patterned stimulus conditions. Throughout, stimulus orientation varied such that a novel orientation was always a minimum of 25° different from any experienced previously by the individual mouse and was never within 20° of horizontal because these orientations are known to elicit VEPs of greater magnitude than vertical or oblique stimuli. If more than 1 orientation was shown within a session, stimuli were pseudo-randomly interleaved such that 3 consecutive presentations of the same stimulus never occurred. For monocular presentation an occluding paddle was positioned in front of one eye to limit stimulus presentation to the opposite eye.

2.5.6: *In vivo* electrophysiology

VEP recordings were conducted in awake, head-restrained mice. Prior to recording, mice were habituated to the restraint apparatus by sitting in front of a gray screen for a 30-minute session on each of two consecutive days. For experiments in which monocular VEPs were subsequently acquired, mice were also habituated to the occluding paddle positioned in front of each eye. On stimulus presentation days, mice were presented with 5 blocks of 200 phase reversals of each oriented stimulus separated by gray screen presentation for ~30 seconds. For monocular presentations, recordings were conducted in sequence for each eye. VEP magnitude was then quantified by measuring trough-peak response magnitude averaged over all phase reversals.

Unless otherwise mentioned, recordings presented were amplified and digitized using the Recorder-64 system (Plexon Inc., Dallas, TX, US). Two recording channels were dedicated to recording EEG/VEPs from V1 in each implanted hemisphere and a third recording channel was reserved for the Piezo-electrical input carrying the behavioral information. Recordings presented in figures 2.5 and 2.6 were amplified using DAM80 amplifiers (World Precision Instruments, Florida, U.S.) and digitized using a custom National Instruments system (National Instruments, Texas, U.S.). Local field potential was recorded from V1 with 1 kHz sampling and a 500 Hz low-pass filter. Data was extracted from the binary storage files and analyzed using custom software written in C++, Matlab and Labview. VEPs were averaged across all phase reversals within a block and trough-peak difference measured during a 200-millisecond period from phase reversal.

2.5.7: LFP power spectrum analysis

Local field potential data was acquired using an Omniplex recording rig (Plexon Inc.), downsampled to a rate of 1000Hz, and filtered from 1-300Hz. The power spectrum was computed using multi-taper estimation in Matlab with the Chronux package (http://chronux.org/) (Bokil, Pesaran et al. 2006), using a 0.5 second sliding window and 3-5 tapers. Spectra were then normalized for 1/f pink noise (Sirota et al., 2008). Both the instantaneous amplitude and the phase time series of a filtered signal were computed from the Hilbert transform, which was obtained by using the hilbert routine from the Signal Processing Toolbox (MATLAB).

2.5.8: *Ex vivo* electrophysiology

Three weeks after infection with AAV9-hSyn-DIO- HA-hM4D(Gi)-IRES-mCitrine virus, visual cortical slices were prepared from the injected mice as previously described (Philpot, Sekhar et

al. 2001). After dissection 350-μm-thick coronal slices recovered for 30 min at 32 °C and then for an additional 2 hours at room temperature, in a holding chamber filled with warmed artificial cerebrospinal fluid (ACSF), which contained: 124 mM NaCl, 3.5 mM KCl, 1.25 mM Na_2PO_4, 26 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM dextrose, saturated with 95% O_2, 5% CO_2. Whole cell patch clamp recordings were performed at 30°C from parvalbumin-positive interneurons in the layer 4, identified by the ECFP fluorescence. Pipette tip resistances were 3–5 MΩ. Internal solution contained: 20 mM KCl, 100 mM Na-gluconate, 10 Hepes, 4 mM MgATP, 0.3 mM Na2GTP, 7 mM phosphocreatine-Tris, 0.2% biocytin with pH adjusted to 7.2 and osmolarity adjusted to 300 mOsm. All recordings were made using Axopatch 200B (Molecular Devices) at 10 kHz sampling rate. Cells with a series resistance <30 MΩ were included for analysis. Data analysis was done using pClamp (Molecular Devices) and custom-written python scripts.

2.5.9: Behavior

All behavioral experiments were performed during the subject's light cycle. A piezo-electrical recording device (C.B. Gitty, Barrington, NH, USA) was placed under the forepaws of head-restrained mice during all sessions. Mice became accustomed to the apparatus by sitting in front of a gray screen for a 30-minute session on each of 2 days. Before stimulus presentation on each day mice also underwent 5 min of gray screen presentation after the experimenter had left the room. A continuous voltage signal was recorded from the piezo device for the entire session. Movements were detected as a shift in the voltage signal. The recording system was automated so that no one was ever present in the closed room for any of the recording period and white noise was played at 67 dB in order to mask outside noise.

For vidget scoring, the continuous voltage signal was down-sampled to 100 Hz. The period of interest in the experiments described here lasted from 2 seconds prior to stimulus onset until 5 seconds after stimulus onset (which was the first 10 phase reversals in a block). Quantification of movement driven by the onset of the stimulus (the vidget) was calculated by taking the Root Mean Square ($SQRT(X^2)$) of the voltage signal. Post-stimulus signal was then normalized to the average magnitude during the 2-second period prior to stimulus onset. The average normalized magnitude across the 5-second period subsequent to stimulus presentation was then used to quantify the degree of stimulus-driven movement and this is described throughout in arbitrary units (a.u.).

2.5.10: Optogenetics

After viral infection mice were also bilaterally implanted with VEP recording electrodes positioned in layer 4. Ready-made optic fibers (200 µm diameter) mounted in stainless steel ferrules (1.25 mm diameter, 2 mm fiber projection, Thor labs, Newton, NJ, US) were then implanted positioned lateral (3.5 mm lateral to lambda) to the recording site and at a 45° angle to the recording electrode, 0.1 mm below surface in each hemisphere. One month later, after the peak of viral expression, mice were habituated to the head-fixation apparatus over 2 days before conducting optogenetic experiments. We delivered 31-second long continuous pulses of blue light (473 nm, 150 mW) into V1 using a laser (Optoengine LLC, Midvale, UT, US). These light pulses were delivered simultaneous to 50% of the 30-second long visual stimulus presentations, commencing 30 ms prior to visual stimulus onset and ending 30 ms after offset. Animals were sacrificed and perfused within a week after this experiment for histological analysis.

2.5.11: Immunohistochemistry

Mice were deeply anaesthetized with fatal plus (pentobarbital) and perfused with saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and post-fixed for 24 hours at room temperature. After fixation, the brain was sectioned into 60 µm coronal slices using a vibratome. Slices were incubated with blocking solution (10% fetal bovine serum in PBS with 0.2% Triton X-100) for 1 hour at room temperature and then with anti-Parvalbumin mouse primary antibody (MAB1572, Millipore; 1:1,000) and anti-GFP antibody (Ab290, Abcam; 1:7000) diluted in blocking solution overnight at 4 degrees Celsius. Slices were then washed three times with PBS and incubated with the secondary antibody for 1 hour at room temperature (Alexa488-conjugated anti-rabbit IgG, Invitrogen, 1:500, Alexa594-conjugated anti-mouse IgG, Invitrogen, 1:500). Slices were washed three times with PBS and mounted with 49,6-diamidino-2 phenylindole (DAPI)-containing Vectashield (Vector Laboratories). Fluorescence images were taken with a confocal fluorescence microscope (Olympus).

2.5.12: Statistics

In the results section, all data is expressed as a mean ± standard error of the mean (S.E.M). Sigmaplot was used for statistical analysis. Normality of distribution and homogeneity of variation was tested and parametric ANOVAs (for multiple groups) or t-tests (for 2 groups) were performed when data passed these tests. Otherwise, non-parametric ANOVAs or t-tests on

ranks were used. If ANOVAs yielded significance, Student-Newman-Keuls post-hoc tests with adjustment for multiple comparisons were applied for individual comparisons. Repeated measures ANOVAs or paired t-tests were applied for all within subject comparisons. For other comparisons unpaired ANOVAs or t-tests were used. Individual tests used are described in the results. $P < 0.05$ is used as a threshold for significance throughout but exact P values are given for all comparisons for which the P value is above 0.001. Technical replicates were only used as N for the *ex vivo* electrophysiological test of hM4D(Gi) efficacy, when 10 cells were recorded from 7 mice. Throughout the remainder of the study the N is an individual animal (biological replicate). Technical replicates (secondary samples within each animal) were not undertaken for *in vivo* electrophysiology. Although both hemispheres were implanted initially, a decision was made as to the best hemisphere, based on response magnitude, morphology and binocularity after the very first recording (prior to any measure of plasticity). Technical replicates were undertaken for the behavior, with 10, 6 (for PV-HM4D(Gi) experiments) or 5 stimulus onsets (for PV-GluN1 KO experiment) being delivered per day, although we only report the daily average (biological replicate). There variations in protocol were due experiments being conducted by different experimenters at different times. However, experimental protocols were completely consistent across treatments/genotypes. The individual technical replicates are available in the uploaded source data files.

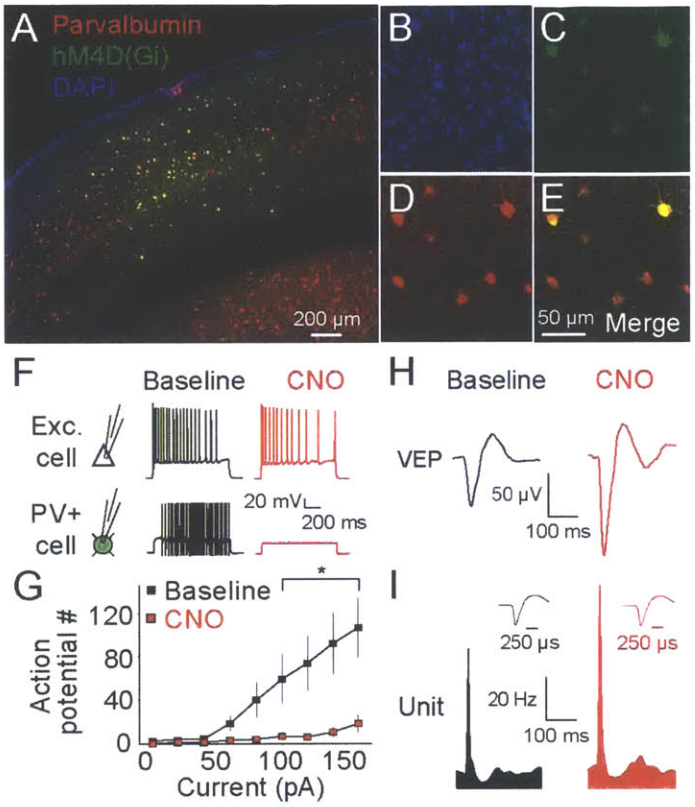

Figure 2.1: The hM4D(Gi) DREADD system locally inactivates parvalbumin+ neurons in binocular primary visual cortex (V1). (A) An example of V1 expression of hM4D(Gi) in a parvalbumin (PV)-Cre recombinase (Cre) mouse infected locally in binocular V1 with AAV9-hSyn-DIO-hM4D(Gi)-mCitrine. **(B)** A DAPI stain for cell nuclei is shown in blue. **(C)** Infected cells expressing hM4D(Gi) are labeled in green. **(D)** Immuno-labeled PV+ cells are shown in red. **(E)** The merged image reveals that hM4D(Gi)-expressing cells are also PV+. **(F)** Intracellular current clamp recordings of hM4D(Gi)-infected PV+ layer 4 neurons in ex vivo slices of V1 reveal that green-labeled infected cells exhibit a non-adapting fast-spiking phenotype typical of fast-spiking inhibitory neurons (black). These cells do not fire action potentials in the presence of CNO (red), the exogenous ligand for hM4D(Gi) receptors, despite depolarizing current injection. In contrast, neighboring cells that are not mCitrine+ show no impact of CNO application. **(G)** HM4D(Gi)-mediated inactivation of putative fast-spiking PV+ inhibitory neurons is here summarized as the number of action potentials resulting from a given current injection before (black) and after CNO application (red). **(H)** The effects of hM4D(Gi)-mediated inactivation of putative PV+ fast-spiking inhibitory neurons in vivo are apparent from electrophysiological recordings from V1 of awake, head-fixed mice viewing phase-reversing sinusoidal grating stimuli. Averaged Visually Evoked Potential (VEP) recordings recorded in layer 4 reveal increased VEP magnitude in the presence of CNO (red), relative to pre-CNO (baseline) recordings (black), indicative of reduced inhibition. (I) Phase reversal-evoked action potentials recorded from neurons in layer 4 also exhibit a similar effect with elevated firing rates in the presence of CNO (red) relative to the baseline recording (black). Labeled scale bars are presented throughout. Error bars are standard error of the mean (S.E.M.).

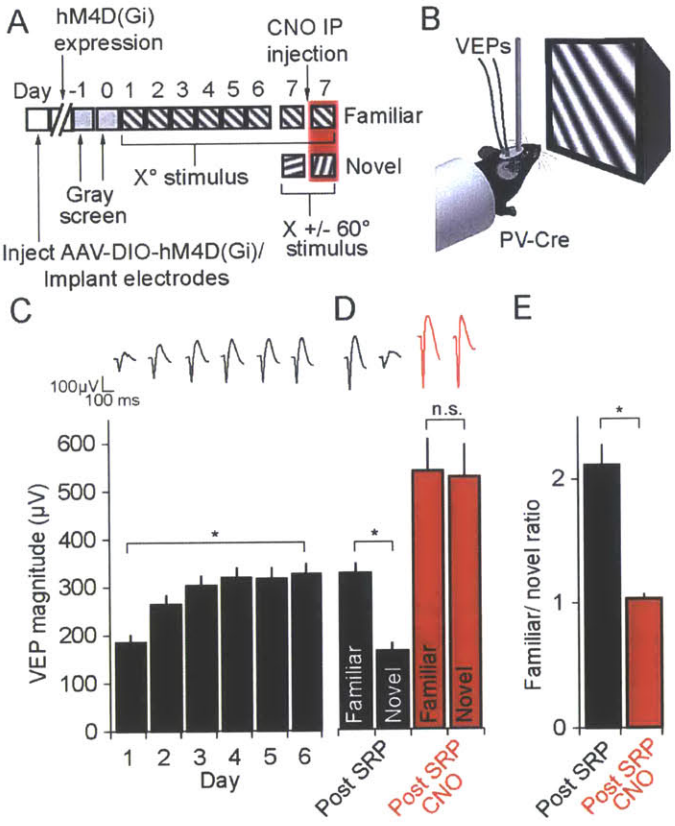

Figure 2.2: The expression of Stimulus-selective Response Potentiation (SRP) requires activity in PV+ neurons in V1. (A) Mice expressing hM4D(Gi) receptors in PV+ cells underwent a standard SRP induction protocol. On day 7, mice viewed a novel oriented stimulus in addition to the familiar stimulus; before and after CNO injection. **(B)** We acquired binocular VEPs from awake, head-fixed mice elicited by the same full-field oriented sinusoidal grating stimulus over several days. **(C)** As a result of multiple days of experience cortical response was dramatically potentiated such that the familiar stimulus evoked VEPs of significantly greater magnitude than the novel stimulus (black bars). **(D)** After application of CNO, VEPs underwent a notable increase in magnitude as a result of disinhibition (red bars). Most notably, CNO rendered response to familiar and novel stimuli equivalent in magnitude. **(E)** This lost discrimination of familiar and novel stimuli is reflected as a drop in the ratio of response to familiar and novel stimuli from approximately 2:1 (black) to approximately 1:1 (red). Significant comparisons are labeled with an asterisk and non-significant comparisons with n.s. throughout. Error bars are standard error of the mean (S.E.M.).

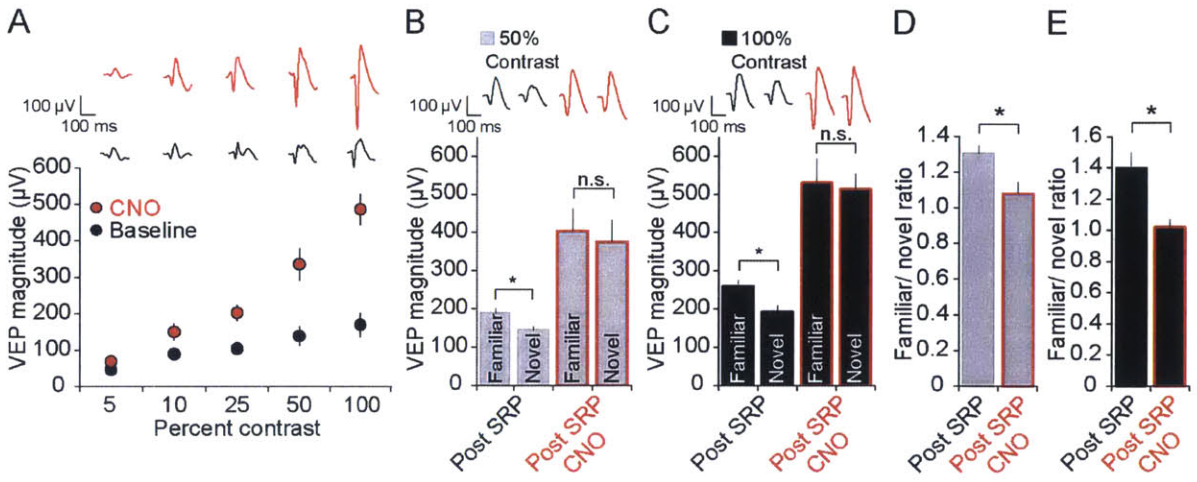

Figure 2.3: Expression of SRP to two separate contrast values is blocked by hM4D(Gi)-mediated PV+ neuron inactivation, but differential response to contrast is maintained. (A) CNO delivery to PV-Cre mice that had been infected with AAV9-hSyn-DIO-hM4D(Gi)-mCitrine impacted VEP magnitude (red) significantly across a range of contrast compared to baseline (black). **(B)** SRP was induced to two differently oriented stimuli, each at different contrast values: 50% (gray) and 100% (black). Modest but significant SRP was expressed at 50% contrast prior to CNO application. After CNO application (red outlines), VEPs increased significantly in magnitude but were no longer significantly different for familiar and a second novel orientation. **(C)** SRP was also expressed for a different orientation at 100% contrast and, again, VEP magnitude was increased and SRP blocked by delivery of CNO. **(D)** The blockade of SRP expression by CNO at 50% contrast was apparent in the reduction in the familiar/novel ratio for VEP magnitude. **(E)** The blockade of SRP expression at 100% contrast was also observed as a significant drop in familiar/novel ratio of VEP magnitude after CNO delivery. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.).

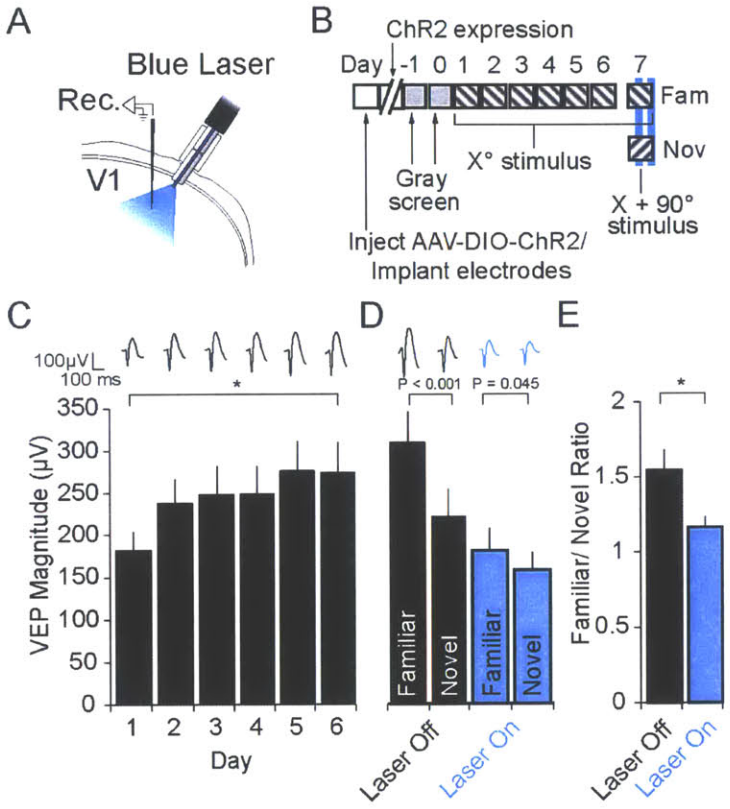

Figure 2.4: Optogenetic stimulation of PV+ inhibitory neurons impairs SRP expression. (A) Blue light was delivered locally into V1 via optic fibers chronically implanted at a 45° angle to target the VEP recording site in layer 4 of binocular V1 of PV-Cre mice infected with AAV5-EF1α-DIO-hChR2(H134R)-eYFP. **(B)** Experimental timeline showing that after viral infection, electrode implantation, and ChR2 expression; mice were accustomed to head-fixation and gray screen viewing. Subsequently, they underwent a standard SRP induction protocol over 6 days. On day 7, mice viewed a novel oriented stimulus in addition to the familiar stimulus and, on 50% of presentations of each stimulus, blue light (473 nm) was delivered to cortex to optogenetically activate PV+ cells. **(C)** Significant SRP was induced over 6 days as VEPs underwent a typical potentiation. **(D)** On day 7, SRP was expressed through significantly larger VEP magnitude in response to the familiar Xo orientation than a novel X + 90° stimulus when blue light was not delivered (Black bars). In the presence of blue light (blue bars), VEPs were suppressed, and there was a significant reduction in the differential magnitude of VEPs driven by familiar and novel stimuli. **(E)** The ratio of VEP magnitude elicited by familiar/novel stimuli was significantly reduced by optogenetic activation of PV+ neurons, reflecting a decrement in SRP expression. Significant comparisons are marked with an asterisk and post hoc test p values are reported in D to emphasize the impact of laser stimulation on SRP selectivity. Error bars are standard error of the mean (S.E.M.).

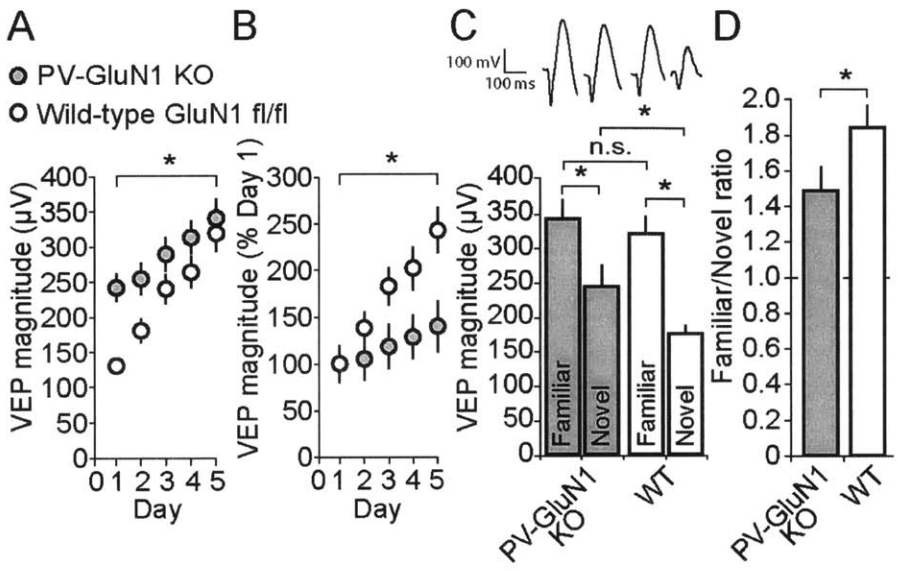

Figure 2.5: Loss of NMDA receptors selectively from parvalbumin+ cells impacts SRP. (A) VEPs recorded from mice in which the mandatory GluN1 subunit of the NMDA receptor was genetically ablated from PV+ cells using Cre recombinase technology (PV GluN1 KO, gray) were significantly greater in magnitude than those recorded from WT littermates (black), suggesting disinhibition of the visual response. In these same PV GluN1 KO mice, SRP was also significantly impacted as there is was significantly less gain in magnitude over days of repeated presentation of an X° stimulus than observed in WT littermates. **(B)** This significant reduction in the magnitude of SRP was most clearly observed if VEP magnitude was normalized to the magnitude on day 1. **(C)** After SRP, both PV GluN1 KO mice and their WT littermates exhibited a significantly greater VEP magnitude elicited by the now familiar stimulus than interleaved presentations of a novel oriented stimulus. However, consistent with the observed difference in magnitude on day 1, VEPs elicited by a novel X + 90° stimulus in PV GluN1 KO mice were significantly greater in magnitude than those in WT littermate mice. No significant difference was observed for VEPs elicited by the familiar stimulus. **(D)** A significant difference in the ratio of VEP magnitude elicited by the familiar and novel stimuli reveals a deficit in SRP expression in PV GluN1 KO mice. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.) (Experiments performed by Samuel F. Cooke).

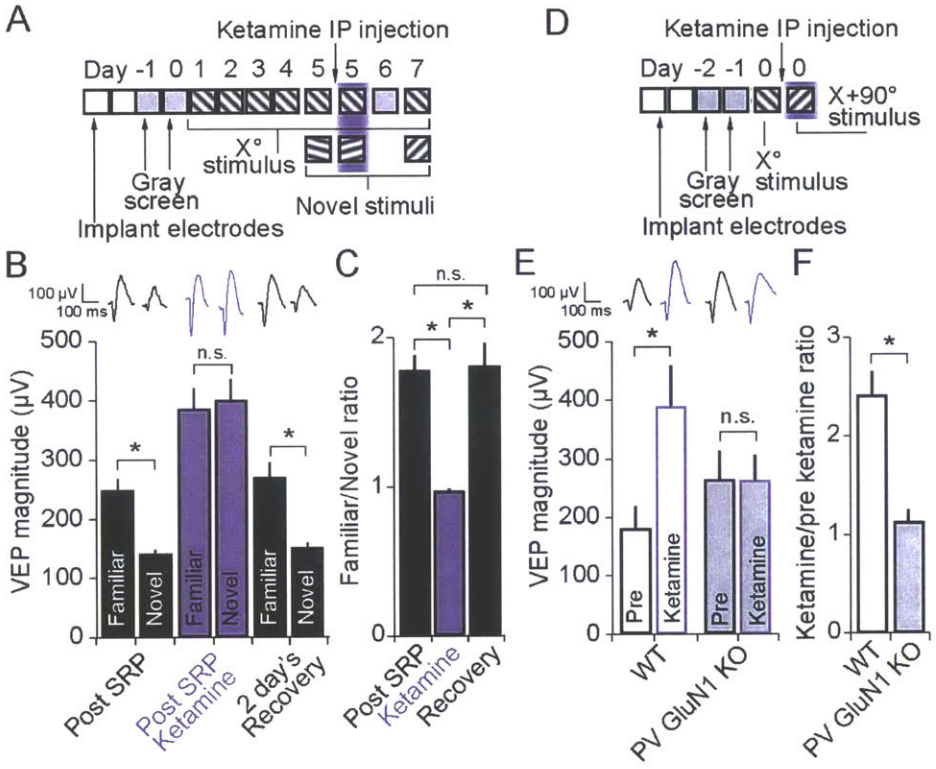

Figure 2.6: Ketamine prevents expression of SRP through blockade of NMDA receptors expressed in PV+ cells. (A) Mice were implanted with electrodes in layer 4 of binocular V1. After habituation to head-fixation and a gray screen for 2 days, SRP was induced over 4 days by repeatedly presenting sessions of an X° stimulus. On day 5, SRP expression was tested by presenting interleaved blocks of the familiar X° stimulus and a novel X + 60° stimulus. In order to test the acute impact of blocking NMDA receptors on SRP expression, 50 mg/kg of ketamine was then delivered systemically before re-acquiring VEPs elicited by the familiar X° stimulus and interleaved presentations of a second novel X - 60° stimulus. Mice were then allowed 2 days recovery and a complete washout of ketamine before re-testing SRP expression by again testing VEP magnitude in response to the familiar Xo stimulus and a third novel X +90° stimulus on day 7. **(B)** Significant SRP was expressed as the familiar X° stimulus elicited VEPs of greater magnitude than the novel stimulus. Delivery of 50 mg/kg ketamine (purple) had two notable impacts on the VEP: First, the overall magnitude of the VEP increased. Second, the significant difference in magnitude of VEPs elicited by familiar and novel stimuli was no longer present. This effect was acute, as SRP expression was again significantly apparent 2 days later. **(C)** The ratio of VEP magnitude elicited by the familiar stimulus over the novel. This ratio was close to 2 and not significantly different prior to or after recovery from ketamine administration but dropped significantly to approximately 1 during ketamine exposure. **(D)** We tested whether ketamine had a differential impact on VEP magnitude in PV GluN1 KO mice and WT littermate mice. (E) In the WT littermate mice (white bars) 50 mg/kg ketamine had a significant potentiating effect on VEP magnitude, consistent with our previous observation. In contrast, ketamine had no significant impact on VEP magnitude in the PV GluN1 KO mice (gray bars). **(F)** The selectivity of ketamine's impact on the WT mice is observed by comparing the ratio of VEP magnitude during ketamine over baseline, which was significantly greater for WT mice than the PV GluN1 KO mice, in which the ratio was approximately 1. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.) (Experiments performed by Samuel F. Cooke).

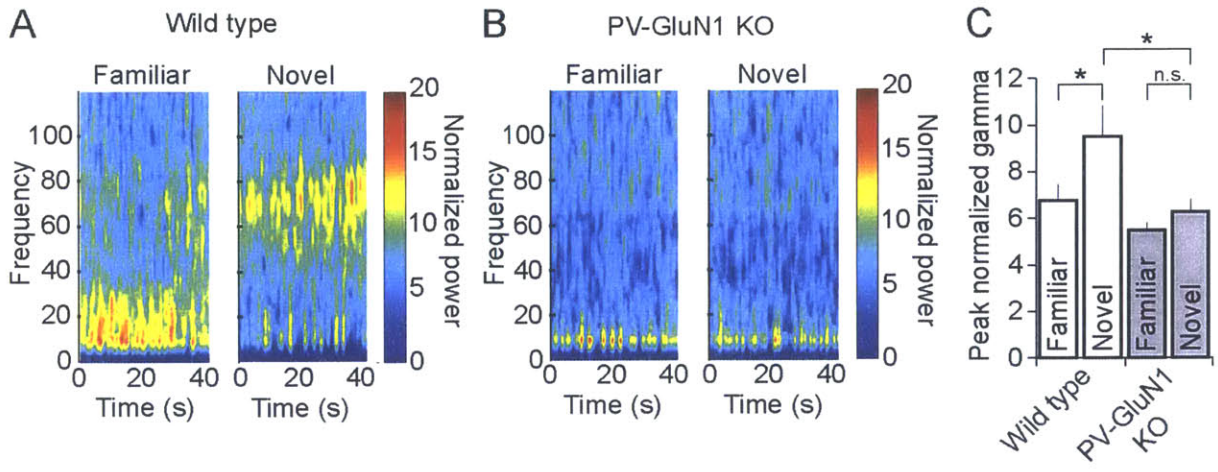

Figure 2.7: Experience-dependent changes in gamma power of V1 LFP are absent in PV GluN1 mice. (A) Using the same protocol as described in figure 3B, mice were progressively familiarized with a specific oriented stimulus. On the test day, mice viewed familiar and novel oriented visual stimuli. Example spectrograms from a wild-type mouse show the normalized power of different LFP frequencies measured in layer 4 of binocular V1, as the animal views familiar and novel stimuli. **(B)** Example spectrograms from a PV-GluN1 KO mouse show the normalized power of different LFP frequencies measured in layer 4 of binocular V1, as the animal views familiar and novel stimuli. **(C)** Significant increases in peak normalized power are visible in the high gamma range (65-90 Hz) as wild-type mice (white bars) view a novel visual stimulus. No modulation of peak normalized power is visible in the high gamma range as PV-GluN1 KO mice (gray bars) view familiar and novel stimuli. Wild-type mice exhibit significantly greater peak normalized power in the high gamma range, compared to PV-GluN1 mice, when viewing a novel stimulus. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.) (Experiments performed by Eitan S. Kaplan, Samuel F. Cooke and Robert W. Komorowski).

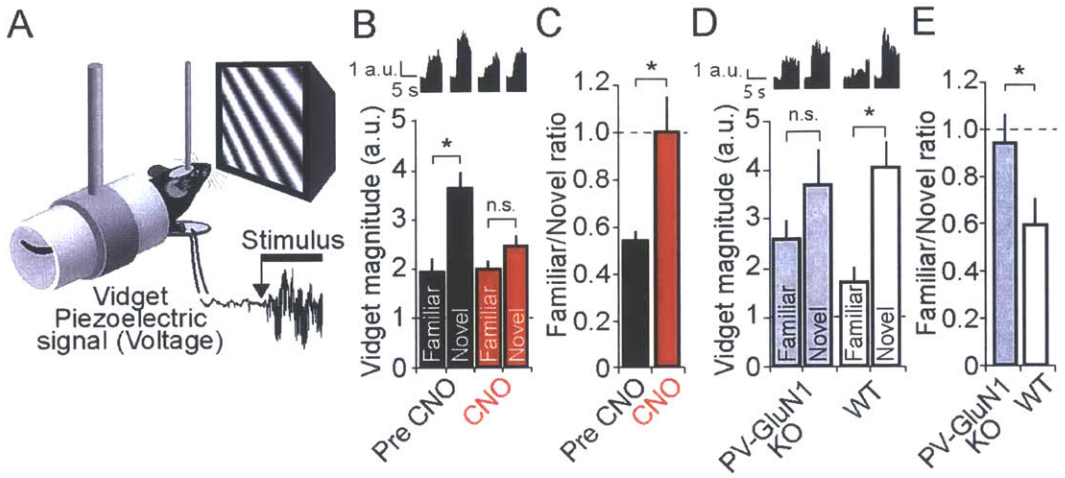

Figure 2.8: Discrimination of familiar and novel oriented stimuli involves PV+ neurons in V1 and NMDARs expressed within PV+ neurons. (A) Using the same protocol as described in figure 3B, mice were progressively familiarized with a specific oriented stimulus. On the test day, as mice viewed familiar and novel stimuli, vidget behavioral responses were measured via a piezoelectric sensor located beneath the forepaws of the head-fixed mouse. **(B)** After a standard SRP protocol, mice expressing hM4D(Gi) receptors selectively within PV+ cells of binocular V1 were exposed to both familiar and novel stimuli. Prior to application of CNO, mice exhibited significant behavioral evidence of discriminating this familiar orientation from interleaved presentations of a novel oriented stimulus (black bars). After application of CNO, there was no longer successful discrimination of familiar and novel stimuli (red bars). Averaged vidget responses are displayed at the top of this panel. **(C)** A significant difference was observed in the ratio of response to familiar and novel stimuli from pre CNO (black) to post CNO (red). **(D)** A deficit in OSH was also apparent in PV GluN1 KO mice as vidget recordings demonstrated a failure to significantly discriminate familiar from novel orientations (gray bars). WT littermates exhibited significantly greater vidget magnitudes for novel than familiar stimuli, indicating unimpaired discrimination of familiarity from novelty (white bars). Averaged behavioral responses are displayed above with accompanying scale bars. **(E)** The significant deficit of PV GluN1 KO mice in discriminating familiar from novel stimuli is apparent in the ratio of behavior elicited by the familiar over the novel stimulus in comparison to WT littermates. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.) (Experiments performed by Eitan S. Kaplan and Samuel F. Cooke).

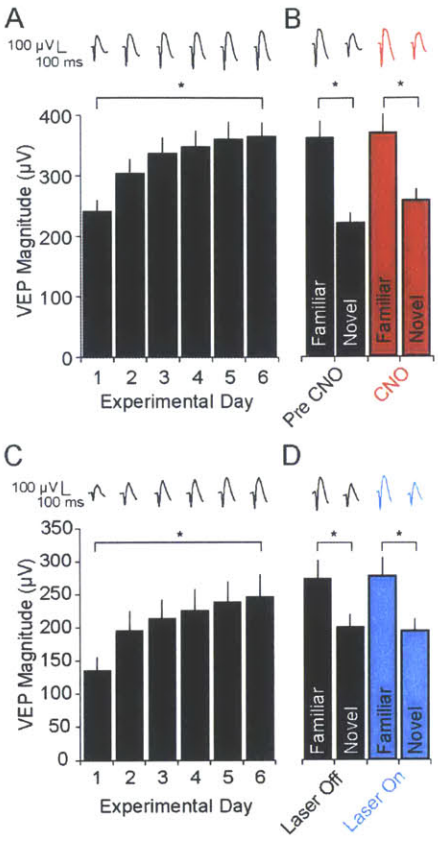

Figure 2.S1: CNO and blue light have no impact on SRP expression. (A) C57BL/6 WT mice were bilaterally infected with AAV9-hSyn-DIO-hM4D(Gi)-mCitrine in binocular V1. Because these mice did not express Cre recombinase there was no subsequent expression of hM4D(Gi) DREADDS receptors. After 4 weeks, during which hM4D(Gi) was expressed at high levels in PV-Cre mice, WT mice were taken through a standard SRP protocol of electrode implantation, habituation and visual experience of a single oriented grating. This progressed as usual, resulting in a significant increase in the magnitude of the VEP. **(B)** Expression of SRP was then tested on day 7 by interleaving presentations of familiar and novel orientations and VEPs elicited by the familiar orientation were of significantly greater magnitude than the novel. After delivery of CNO, VEPs were again tested in response to the familiar orientation and a second novel orientation. The drug had no impact and a similar degree of significant stimulus selectivity was present. **(C)** To check that light itself had no effect on cortical physiology in the optogenetic experiments, WT mice were infected bilaterally with AAV5-EF1α-DIO-hChR2(H134R)-eYFP in binocular V1. After 4 weeks, during which ChR2 was expressed at high levels in PV-Cre mice, WT mice were taken through the standard SRP protocol described above. Again, significant SRP occurred. **(D)** On test day, during interleaved presentations of a familiar and novel oriented stimulus together with either blue light (473 nm) or no light stimulation delivery to V1, VEPs were significantly greater in magnitude for the familiar than the novel stimulus, regardless of the presence of blue light. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.).

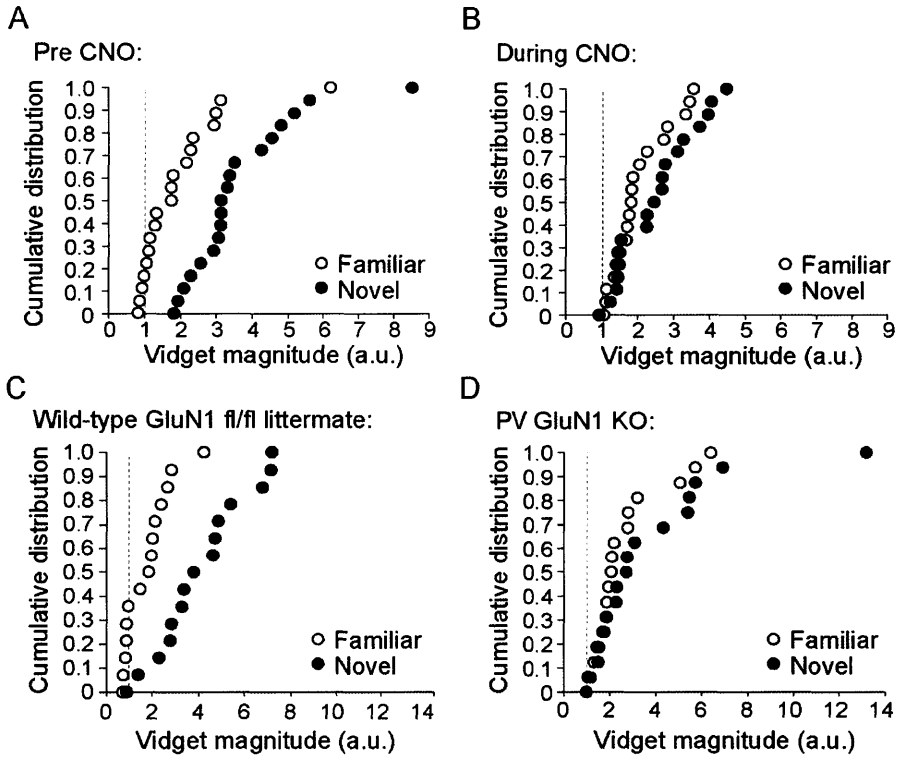

Figure 2.S2: Cumulative distributions of average vidget behavioral response to familiar and novel stimuli for each individual animal. **(A)** Prior to CNO administration, average behavioral response to the familiar stimulus (light gray) and the novel stimulus (black) of each individual animal bilaterally expressing hM4D(Gi) in PV+ neurons of binocular V1. Dotted line represents behavior no greater than pre stimulus baseline. **(B)** Average behavioral response of the same animals to the familiar and a new novel stimulus during PV+ neuron inactivation via CNO delivery, revealing consequent deficit in discrimination of familiar and novel stimuli. **(C)** Cumulative distributions of average vidget behavioral response to the familiar stimulus (light gray) and the novel stimulus (black) of each individual WT GluN1 fl/fl mouse. **(D)** Average vidget behavioral response of each PV-GluN1 KO mouse to the familiar and a new novel stimulus, revealing deficit in discrimination of familiar and novel stimuli. Dotted line represents behavior no greater than pre stimulus baseline (Experiments performed by Eitan S. Kaplan and Samuel F. Cooke).

Chapter 3

The role of parvalbumin expressing inhibitory interneurons in adult ocular dominance (OD) plasticity

Portions of this chapter are under review:

Kaplan ES*, Cooke SF*, Komorowski RW, Chubykin AA, Khibnik LA, Bear MF (in submission). Contrasting Roles for Parvalbumin-Expressing Inhibitory Neurons in Two Forms of Adult Visual Cortical Plasticity

3.1: Abstract

Here we evaluate the participation of a major class of GABAergic neurons, the parvalbumin-expressing (PV+) putative fast spiking neurons to ocular dominance (OD) plasticity resulting from monocular deprivation (MD) in the adult mouse. MD in the adult mouse triggers an increase in visual cortical responses driven through the non-deprived eye. Stimulus selective response potentiation (SRP), though initiated by supplemental visual experience, also results in potentiation of visual responses. Both of these forms of experience-dependent plasticity are known to require the NMDA class of glutamate receptor (NMDAR). However, consistent with observations that the two forms of plasticity do not occlude one another, we show that PV+ inhibitory neurons in V1 do not play a critical role in the expression of OD plasticity, in contrast to what we observed for SRP. Pharmacogenetic inactivation of PV+ interneurons does not affect the inherent contralateral bias of neurons in binocular V1, or the expression of the adult OD shift. Additionally, genetic knockdown and pharmacological blockade of NMDA receptors within PV+ cells does not alter ocular dominance or the OD shift elicited by deprivation. These results contrast with the previous findings regarding a requirement for PV+ interneuron inhibition in SRP.

3.2: Introduction

Mouse visual cortex has proven to be an excellent model system in which to examine experience-dependent neural response modification. One robust type of visual response plasticity is reliably elicited in adult (>P60) mice by simply closing one eyelid. Over the course of 5-7 days of monocular deprivation (MD), the responses in visual cortex evoked by stimulation of the non-deprived eye progressively increase (Sawtell, Frenkel et al. 2003, Sato and Stryker 2008). This deprivation-enabled response potentiation is driven by visual experience through the non-deprived eye, as only the responses through one eye are potentiated (*i.e.*, it is input specific) and it fails to occur if both eyelids are closed or if animals are kept in complete darkness (Blais, Frenkel et al. 2008). There is evidence that the response potentiation is mediated in part by "Hebbian" strengthening of excitatory synaptic transmission in visual cortex, as induction requires cortical NMDA receptor (NMDAR) activation (Sawtell, Frenkel et al. 2003) (Sato and Stryker 2008) and α-calcium/calmodulin-dependent protein kinase II (αCAMKII) expression in principal cells (Ranson, Cheetham et al. 2012). This form of ocular dominance (OD) plasticity is likely responsible for the increase in visual acuity that occurs through the non-deprived eye following adult monocular deprivation (Iny, Heynen et al. 2006), and is of particular interest in the context of recovery of brain function after deprivation, disease, or damage (Cho and Bear 2010).

There has been significant interest in the role of intracortical inhibition in OD plasticity, particularly in the juvenile animal (Hensch 2005, Smith and Bear 2010). Several studies have provided evidence that the postnatal maturation of parvalbumin positive (PV+) inhibitory interneuron circuits guides the opening and closure of the critical period for OD plasticity. In mice, the developmental age in which monocular deprivation causes a juvenile OD shift is around postnatal day 28-35 (P28-35)(Drager 1978, Gordon and Stryker 1996, Frenkel and Bear 2004). As mentioned, it is now well understood that OD plasticity also occurs in the adult mouse (P60+); however, this plasticity is distinct from that which occurs in the juvenile animal and qualitatively unique in that it is characterized by a generalized potentiation of responses driven through the non-deprived eye (Sawtell, Frenkel et al. 2003, Sato and Stryker 2008). Very little is known about the role of inhibition or PV+ interneurons in this adult form of experience-dependent plasticity.

We previously found a requirement for PV+ interneuron activity in the expression of stimulus-selective response potentiation (SRP) induced by selective visual experience in the adult mouse (Chapter 2). We next sought to extend a similar line of investigation to the

mechanisms of adult OD plasticity. Both monocular deprivation and selective visual experience trigger input-specific increases in the short latency VEP measured in layer 4 of mouse visual cortex and, as mentioned above, both OD plasticity and SRP share some molecular requirements (*e.g.* NMDAR activation). Similarities aside, an important finding was that response potentiation after monocular deprivation and SRP do not occlude one another (Frenkel and Bear 2004), suggesting that they employ different mechanisms or are expressed by different synapses. Therefore, we were uncertain as to whether or not the expression of the adult OD shift, would require PV+ cell activity, similarly to their requirement in SRP. In the current study we used a variety of approaches to understand the contribution of PV+ neurons to adult OD plasticity. Here we report that neither the pharmacogenetic silencing of PV+ cells nor the genetic or pharmacological perturbation of the NMDARs within them affect the relative eye dominance or the expression of deprivation-enabled potentiation of VEPs in adult mice.

3.3: Results

3.3.1: Ocular dominance is maintained in the absence of PV+ neuronal activity

Mouse binocular V1 is ~2-3 times more strongly activated by the contralateral (contra) eye than the ipsilateral (ipsi) eye, an inherent bias known as ocular dominance (OD). We probed the involvement of PV+ neuron activity in maintaining baseline OD. PV-Cre mice were infected with AAV virus to deliver hM4D(Gi) Dreadd receptors to PV+ neurons in binocular V1 (Fig. 3.1A). Electrodes were simultaneously implanted into layer 4 of V1 for VEP recordings. VEPs elicited through just contralateral or ipsilateral eyes were acquired consecutively before and after CNO administration (Fig. 3.1A-B). After CNO injection, VEPs driven through each eye increased significantly in magnitude (2-way repeated measures ANOVA, effect of CNO, $F_{(7)} = 75.986$, $P < 0.001$; contralateral eye: 222.18 ± 14.79 μV baseline vs. 679.81 ± 64.12 μV CNO, Student-Newman-Keuls (SNK) post hoc test, $q_{(7)} = 13.925$, n = 8 mice, $P < 0.001$; ipsilateral eye: 105.56 ± 6.78 μV baseline vs. 358.63 ± 35.79 μV CNO, SNK post hoc test, $q_{(7)} = 7.711$, n = 8 mice, $P < 0.001$, (Fig. 3.1C), consistent with the occurrence of cortical disinhibition. However, the OD of visual responses in V1 (contra/ipsi ratio) was not significantly altered by CNO injection (2.13 ± 0.12 baseline vs. 1.96 ± 0.18 CNO, student's paired two-tailed t-test, $t_{(7)} = 0.859$, n = 8 mice, P = 0.42, Fig. 3.1D). This result suggests that the inherent OD of binocular visual cortex is not dependent on PV+ neuron activity.

3.3.2: Expression of adult OD plasticity does not require the activity of PV+ interneurons

The normal OD ratio is altered in the adult mouse by MD of the contralateral eye over 7 days, resulting in an ocular dominance shift that features potentiation of response through the open ipsilateral eye (Sawtell, Frenkel et al. 2003, Sato and Stryker 2008). In a new group of mice we assayed the effect of PV+ neuron inactivation on the expression of the shift in OD caused by MD. We recorded VEPs elicited through each eye from PV-Cre mice expressing hM4D(Gi) receptors in binocular V1 (Fig. 3.1E). After baseline recordings the mice underwent contralateral eyelid suture and 7 days of monocular deprivation. Subsequently, the contralateral (deprived) eye was opened and VEPs were re-recorded in order to reveal the expression of an OD shift. There was a significant potentiation of VEP magnitude driven through the ipsilateral eye following 7 days of MD (69.5 ± 5.81 μV pre MD vs. 139.5 ± 9.33 μV post MD, n = 10 mice, student's paired one-tailed t-test, $t_{(9)} = -10.184$, $P < 0.001$, Fig. 3.1F). This potentiation of response through the ipsilateral (non-deprived) eye resulted in a significant shift in the OD ratio (contra/ipsi ratio, 3.08 ± 0.26 pre MD vs. 1.33 ± 0.10 post MD, 1-way repeated measures ANOVA, SNK post hoc test, $q_{(2)} = 11.77$, $P < 0.001$, Fig. 3.1G). Mice were then injected with CNO and re-recorded to assess whether the OD shift would persist in the absence of PV+ neuron activity. CNO injection resulted in an increase in VEP magnitudes (Fig. 3.1F), but importantly the shift in the contra/ipsi ratio was maintained (1.33 ± 0.10 post MD vs. 1.35 ± 0.18 post MD CNO, SNK post hoc test, $q_{(2)} = 0.127$, $P = 0.93$, Fig 3.1G). Therefore, the expression of the adult OD shift induced by 7 days of MD persists in the absence of PV+ neuron inhibition. This result is in contrast to what we observed for SRP (Fig. 2.2), where PV+ inactivation completely abolished its expression.

3.3.3: Adult OD plasticity does not require NMDARs expressed in PV+ neurons

We also examined if loss of NMDARs from PV+ neurons has an effect on OD plasticity. We implanted VEP recording electrodes in a second cohort of 11 PV–GluN1 KO and 7 WT-GluN1 fl/fl mice. After recovery and habituation over 2 days, monocular VEPs were acquired through each eye. After 7 days of MD, PV-GluN1 KO mice exhibited a significant potentiation of response through the open ipsilateral eye (187.91 ± 25.52 μV) compared with day 0 (90.64 ± 10.69 μV, 2-way repeated measures ANOVA, SNK post hoc test, $q_{(16)} = 8.849$, $P < 0.001$, Fig. 3.2A). Similarly, WT-GluN1 fl/fl mice also showed a significant potentiation of the open ipsilateral eye-response (154.57 ± 7.60 μV) after 7 days of MD compared with responses measured on day 0 (97.57 ± 15.85 μV, SNK post hoc test, $q_{(16)} = 4.137$, $P = 0.01$,Fig. 3.2B).

Comparisons of OD ratios prior to MD in the adult PV-GluN1 KO (3.40 ± 0.29) and WT-GluN1 fl/fl mice (2.57 ± 0.33) did not reveal any significant difference (2-way repeated measures ANOVA, effect of genotype, $F_{(1)} = 3.424$, $P = 0.083$, Fig. 3.2C). Significant shifts in the OD ratio occurred as a result of 7 days of MD in the adult PV-GluN1 KO (1.28 ± 0.33, SNK post hoc test, $q_{(16)} = 10.6$, $P < 0.001$) and WT-GluN1 fl/fl mice (1.19 ± 0.09, SNK post hoc test, $q_{(16)} = 5.517$, $P = 0.001$). The shifted OD ratio also did not differ significantly across genotype (2-way repeated measures ANOVA, interaction of genotype and MD, $F_{(1,16)} = 2.638$, $P = 0.124$, Fig. 3.2C). Thus, the loss of NMDAR function from PV+ neurons did not have a significant impact on either the induction or the expression of adult OD plasticity, in contrast to what we observed for SRP (Fig. 2.5).

3.3.4: Ketamine has no effect on the expression of the adult OD shift

Because we had previously observed ketamine's capacity to perturb the function of NMDARs on PV+ cells in V1, and that this manipulation had blocked the expression of SRP (Fig. 2.6), we tested whether or not ketamine would disrupt either the baseline OD ratio or the shift induced by 7 days of MD. Adult C57BL/6 mice (n = 8) were implanted with VEP electrodes and taken through a standard surgery recovery and habituation protocol before measuring the OD ratio prior to 50 mg/kg ketamine and 15 minutes after ketamine delivery (Fig. 3.3A,B). The normal contra/ipsi OD ratio exhibiting contralateral eye dominance prior to ketamine (2.84 ± 0.18) was not significantly altered by ketamine (2.61 ± 0.19, student's paired two-tailed t-test, $t_{(7)} = 0.871$, $P = 0.413$, Fig. 3.3C). A separate group of adult mice (n = 11) underwent a standard 7 day MD protocol. VEPs elicited by a X° oriented stimulus were recorded at baseline, followed by the deprivation period. After eye opening VEP magnitudes were re-tested with a novel X + 60° stimulus (the standard protocol to avoid contamination of the OD shift by SRP (Frenkel and Bear 2004)). These mice were then tested again with a second novel X - 60° stimulus after 1 hour's rest and an additional 15 minutes after systemic 50 mg/kg ketamine administration (Fig. 3.3D). As expected, VEPs elicited through the open ipsilateral eye (93.73 ± 12.85 μV) were significantly potentiated by 7 days of MD in the adult mouse (155.73 ± 15.86 μV, 2-way repeated measures ANOVA, SNK post hoc test, n = 11 mice, $q_{(10)} = 7.102$, $P < 0.001$, Fig. 3.3E), reflecting the well-documented OD shift. These same ipsilateral VEPs were then further potentiated by ketamine administration (232.03 ± 22.19 μV, SNK post hoc test, $q_{(10)} = 8.741$, $P < 0.001$). However, ketamine had a similar significant potentiating effect on the contralateral VEP (279.07 ± 14.53 μV), relative to pre-ketamine (180.14 ± 11.22 μV, SNK post hoc test, $q_{(10)} =$

11.333, P < 0.001, Fig. 3.3E), suggesting a uniform scaling of response through the two eyes. This observation is confirmed by the fact that the OD ratio, significantly shifted from (2.84 ± 0.29) to (1.23 ± 0.11, Friedman repeated measures ANOVA on ranks, n = 11mice, $X^2_{(2)}$ = 16.545, p < 0.001, SNK post hoc test, $q_{(10)}$ = 5.126, P < 0.05) by 7 days of MD, was not further significantly affected by delivery of ketamine (1.28 ± 0.09, SNK post hoc test, $q_{(10)}$ = 0.426, P > 0.05, Fig. 3.3F). Thus, while ketamine prevents SRP expression through action on NMDARs expressed in PV+ cells (Fig. 2.6), it does not significantly affect the adult OD shift after 7 days of MD, consistent once again with PV+ cells contributing to the expression of SRP but not adult OD plasticity.

3.4: Discussion

3.4.1: The study of ocular dominance plasticity

Experience-dependent synaptic modifications in primary visual cortex (V1) have been prominently studied since the pioneering work of Hubel and Wiesel over 50 years ago (Wiesel and Hubel 1963). These ocular dominance plasticity experiments, originally completed in cats and monkeys, showed that depriving an animal of vision through one eye changed the properties of binocular V1 cells, so that their responsiveness shifted away from the deprived eye and towards the non-deprived eye (Hubel, Wiesel et al. 1977). There work provided a model system as well as insight into the loss of visual function that occurs in the human disorder of sight known as amblyopia (Doshi and Rodriguez 2007). Hubel and Wiesel's discovery also afforded researchers a paradigm in which the mechanisms of synaptic plasticity that occur *in vivo* could be easily studied. Importantly, the mechanisms that underlie ocular dominance plasticity could be shared among some additional forms of experience-dependent plasticity such as those underlying various forms of learning and memory. Hubel and Wiesel also discovered that the deprivation-induced changes they observed in kitten V1, did not occur as reliably when manipulating vision in adult cats (Hubel and Wiesel 1970). This was the first indication that there may be different mechanisms that govern plasticity in young vs. adult animals. As the model organism of choice in the research field has shifted to mice, it is appreciated that indeed visual cortical plasticity occurs in both juvenile and adult animals; however, there is a distinction in the quality of the OD shift that occurs, which may imply different mechanisms prevail at different ages (Sawtell, Frenkel et al. 2003, Sato and Stryker 2008). In juvenile mice (~<P35), a rapid and reliable consequence of MD is the depression of cortical responses mediated by the

deprived eye. This is followed by a progressive compensatory increase in responses through the non-deprived eye (Frenkel and Bear 2004). In adult mice maintained under standard laboratory conditions, depression of deprived-eye responses can be a weak and variable consequence of MD, but potentiation of the non-deprived eye still occurs reliably (Sawtell, Frenkel et al. 2003, Sato and Stryker 2008).

3.4.2: Juvenile and adult OD plasticity

Much of the effort in the field of visual cortical plasticity for the past few decades has been focused on the mechanisms of juvenile OD plasticity. Significant evidence has accumulated that the depression of the responses serving the deprived eye occurs via the category of synaptic plasticity known as long-term depression (LTD). LTD processes have been shown to occur in layers 4 and 2/3 of V1 as a result of derivation (Heynen, Yoon et al. 2003, Frenkel and Bear 2004, Crozier, Wang et al. 2007, Coleman, Law et al. 2009, Yoon, Smith et al. 2009, Khibnik, Cho et al. 2010, Smith and Bear 2010) of the juvenile animal. Importantly, the synaptic depression of thalamocortical synapses has been shown to be sufficient to explain deprived-eye depression in the juvenile mouse (Khibnik, Cho et al. 2010). In addition to these deprivation induced excitatory synaptic changes; there has been great interest in the possible role of PV+ interneurons in juvenile OD plasticity. One theory speculates that the maturation of PV+ interneuron circuity in V1 constrains the sensitivity of the visual cortex to the effects of MD (Fagiolini, Pizzorusso et al. 1994, Hanover, Huang et al. 1999, Huang, Kirkwood et al. 1999, Chattopadhyaya, Di Cristo et al. 2004). There is also some evidence that the functional expression of the OD shift in the juvenile animal could occur via modulation of inhibition (Maffei, Nataraj et al. 2006, Yazaki-Sugiyama, Kang et al. 2009, Smith and Bear 2010).

We have chosen to focus instead on the mechanisms that underlie OD plasticity in the adult. In doing so, we discovered that inactivation of PV+ interneurons, or perturbations of NMDARs on those cells, did not interfere with the expression of the adult OD shift. Interestingly, several groups have shown that manipulations which directly or indirectly reduce the level of cortical inhibition in the adult mouse can promote the induction of juvenile-like plasticity (Sale, Maya Vetencourt et al. 2007, Maya Vetencourt, Sale et al. 2008, Kuhlman, Olivas et al. 2013, Greifzu, Pielecka-Fortuna et al. 2014, Kalogeraki, Greifzu et al. 2014). It is important to recognize that the mechanisms that constrain juvenile OD plasticity may not overlap with the mechanisms that underlie OD plasticity in the adult animal. For instance, tumor necrosis factor alpha (TNFα), a cell signaling protein shown to be important for homeostatic synaptic scaling,

appears to be required for non-deprived eye response potentiation in the juvenile but not in the adult animal (Kaneko, Stellwagen et al. 2008, Ranson, Cheetham et al. 2012). Another study which supports distinctive mechanisms for juvenile vs. adult OD plasticity, conducted by Yazaki-Sugiyama and colleagues, more directly relates to the contribution of intracortical inhibition. These researchers found that $GABA_A$ receptor blockade caused significant changes in the eye-bias of V1 cells after deprivation, signifying a GABAergic component to expression of the OD shift. Interestingly these changes reported for juvenile mice were not observed in adult mice (Yazaki-Sugiyama, Kang et al. 2009). In agreement with this finding, we found that the adult OD shift was unaffected by manipulation of PV+ interneuron activity.

3.4.3: Excitatory synaptic strengthening as a mechanism for adult OD plasticity

We hypothesize that the adult OD shift occurs because deprivation of the dominant contralateral eye causes a metaplastic shift in the LTP threshold, enabling visual experience through the weaker ipsilateral (non-deprived) eye to drive synaptic strengthening (Cooper and Bear 2012). This interpretation is supported by evidence that light deprivation promotes LTP in visual cortex (Kirkwood, Rioult et al. 1996, Philpot, Sekhar et al. 2001, Philpot, Espinosa et al. 2003), and that αCaMKII mutants that lack LTP also lack adult OD plasticity (Ranson, Cheetham et al. 2012). Our hypothesis is further reinforced by a study showing that increasing Rho GTPase activity in V1 during MD, specifically enhances non-deprived eye potentiation in the adult rat, and this potentiation is correlated with increased density of geniculocortical terminals in Layer 4 of V1 (Cerri, Fabbri et al. 2011). These presynaptic changes as a result of MD appear to be mirrored by an increase in the number of dendritic spines of binocular V1 neurons, which may reflect the postsynaptic structural correlate of excitatory synaptic strengthening (Hofer, Mrsic-Flogel et al. 2009). These studies, which suggest feedforward excitatory synaptic strengthening as a mechanism for the adult OD shift, are compatible with our finding that expression of the shift is not dependent on the activity of PV+ inhibitory neurons.

3.4.4: Possible roles for inhibition in adult OD plasticity

Our data are also in agreement with those of Saiepour and colleagues who recently found that optogenetic inactivation of PV+ cells after deprivation did not alter the expression of the adult OD shift (Saiepour, Rajendran et al. 2015). Although the findings that PV+ neurons are

not necessary for the expression of adult OD plasticity do not rule out a modulatory role in the induction mechanisms; similar to what some have proposed for juvenile OD plasticity (Fagiolini and Hensch 2000, Hensch 2005), we note that the adult OD shift still occurred normally in mutant mice in which PV+ neurons lack NMDARs. Still, there may exist disparate mechanisms operating in different layers of V1, some inclusive and some exclusive of changes in inhibition. For instance, there is evidence for the loss of inhibitory synapses in Layer 2/3 following MD in the adult animal (Chen, Lin et al. 2011, Chen, Villa et al. 2012, van Versendaal, Rajendran et al. 2012). Our results also cannot rule out the contribution of other classes of interneurons besides PV+ cells, to the adult OD shift. For instance, is was recently reported that the induction of the adult OD shift can be facilitated by the stimulation of vasoactive intestinal peptide positive (VIP+) interneurons, which in turn inhibit somatostatin positive (SOM+) interneurons (Kaneko and Stryker 2014, Fu, Kaneko et al. 2015). However, these studies have not sufficiently explored the mechanisms of expression, and others have found a lack of evidence for VIP+ and SOM+ cell contribution to the expression mechanisms of adult OD plasticity (Saiepour, Rajendran et al. 2015). Here we have shown that unlike the mechanisms that support the expression of SRP, PV+ interneurons are not required for the expression of the adult OD shift. Future studies will be necessary to confirm the hypothesis of feedforward excitatory synaptic strengthening as the dominant mechanism behind non-deprived eye potentiation in the adult animal.

3.5: Materials and Methods

All materials and methods used for experiments in chapter 3 are described in chapter 2 materials and methods.

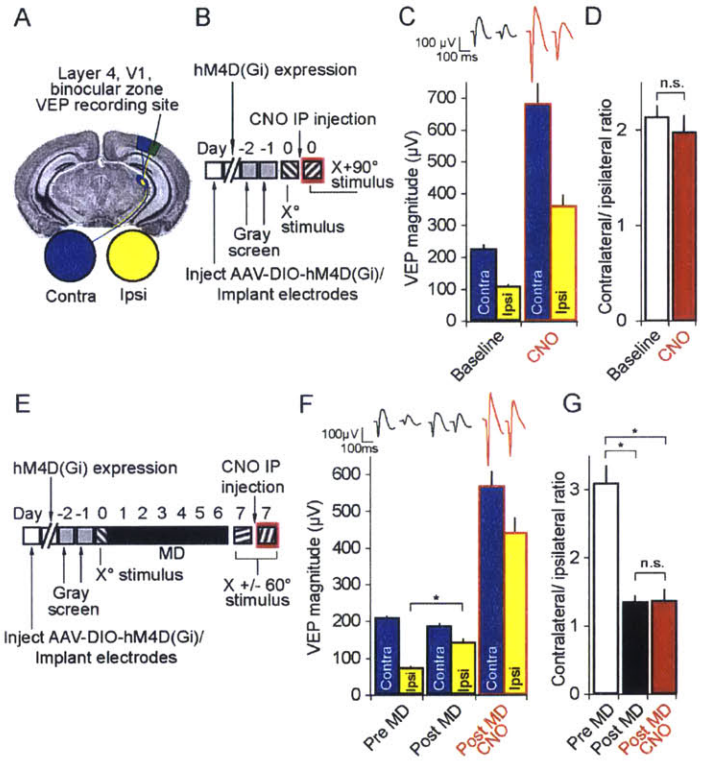

Figure 3.1: Inactivation of parvalbumin+ neurons has no impact on expression of ocular dominance (OD) or the ocular dominance shift as a result of monocular deprivation (MD) in the adult mouse. (A) Mice were infected across all cortical depths bilaterally in binocular V1 (green), and VEP recording electrodes were implanted. **(B)** Mice (P45-60) were infected, implanted with electrodes and then left for 3 weeks of viral expression, after which they were accustomed to head-fixation and a gray screen. Following this, on experimental day 0, mice were presented with a X° phase-reversing sinusoidal grating stimulus separately to each eye. VEPs were recorded from each hemisphere in order to determine ocular dominance in binocular V1. CNO was delivered systemically before re-recording, this time using an orthogonal X + 90° stimulus. **(C)** Responses in V1 to stimuli viewed through the contralateral eye (contra, blue) were greater in magnitude than those elicited through the ipsilateral eye (ipsi, yellow). After application of CNO (red outlines), VEP magnitude dramatically increased. **(D)** This increase was scaled such that the ratio of contra:ipsi VEP magnitude was maintained before (white) and after CNO (red). **(E)** In a second group of mice, a similar experimental protocol was observed prior to measuring ocular dominance. Then the contra eye was sutured closed. After 7 days of monocular deprivation, the eye was opened and VEPs driven through either eye were again recorded, this time elicited by an X + 60° stimulus. Mice were then systemically injected with CNO and subsequently VEPs driven through either eye were recorded, this time elicited by an X - 60° stimulus. **(F)** After MD, there was a significant potentiation of the V1 response to visual input through the ipsi eye (yellow). After CNO (red outlines), VEPs driven through each eye were elevated in magnitude, but again the increase in VEP magnitude was scaled. **(G)** As a result of open eye potentiation after MD, the OD ratio shifted dramatically from the contra bias of pre MD (white) to an almost equal cortical response through the eyes (black). This shifted ratio was unaffected by hM4Di-mediated inactivation of PV+ neurons during CNO application (red), indicating that the expression of OD and its shift as a result of MD in adult mice do not require PV+ cell-mediated inhibition. Significant comparisons are labeled with an asterisk and non-significant comparisons with n.s. throughout. Error bars are standard error of the mean (S.E.M.).

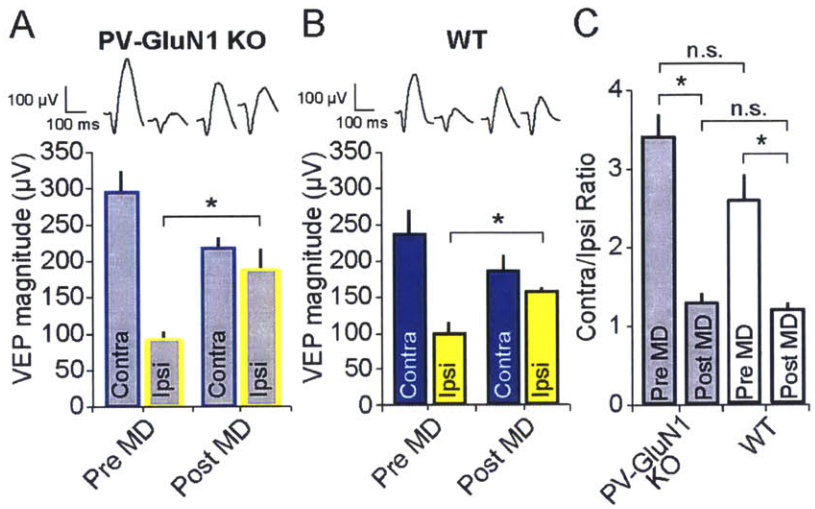

Figure 3.2: Loss of NMDA receptors selectively from parvalbumin+ cells has no effect on adult OD plasticity. (A) Contralateral and ipsilateral VEPs were recorded from mice in which the mandatory GluN1 subunit of the NMDA receptor was genetically ablated from PV+ cells using Cre recombinase technology (PV GluN1 KO, gray). PV GluN1 KO mice exhibited a normal adult OD shift after 7 days of MD, resulting from open eye potentiation (yellow outlines). **(B)** Wild-type (WT) littermates exhibited the same significant open eye potentiation (yellow bars) after 7 days of MD. **(C)** A comparison of the degree of OD shift as a result of 7 days of MD reveals a significant shift in OD ratio in both genotypes but no difference between genotypes, indicating that NMDA receptors in PV+ cells are not required for induction or expression of the OD shift. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.).

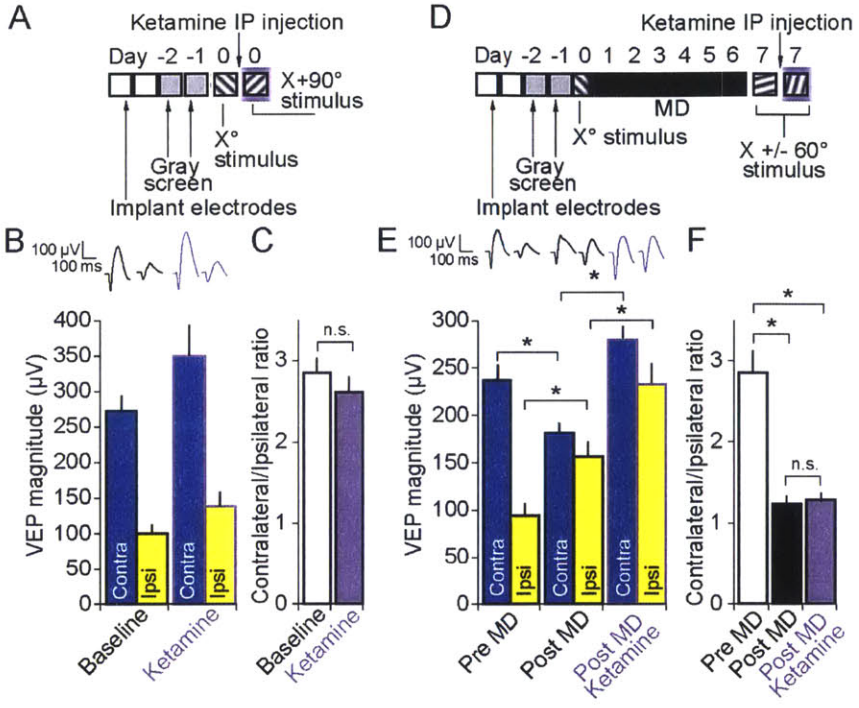

Figure 3.3: Ketamine administration does not impact expression of the adult OD shift. (A) Mice were bilaterally implanted with VEP recording electrodes in layer 4 of binocular V1. After habituation to head-fixation and a gray screen for 2 days, a protocol was used to determine whether the OD ratio is affected by ketamine. **(B)** Ketamine impacted both the VEPs driven through the contralateral eye (blue) and ipsilateral eye (yellow) equally. **(C)** This scaled effect is demonstrated by a lack of significant difference between OD ratios prior to (white) and during 50 mg/kg ketamine (purple). **(D)** We next tested whether ketamine has any impact on the expression of adult OD plasticity by recording VEP magnitudes through either eye in a new group of adult mice before taking them through a standard 7 day MD protocol. **(E)** As anticipated, 7 days of contralateral eye MD induced a significant ipsilateral eye potentiation (yellow) and ketamine then further potentiated VEPs elicited through both contralateral (blue) and ipsilateral eyes. **(F)** The OD ratio shifts significantly from a ratio heavily biased towards the contralateral eye, to less biased ratio. Ketamine administration did not significantly affect the magnitude of the OD shift. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.).

Chapter 4

Visual cortical plasticity and neurodevelopmental disorders

Portions of this chapter were published:

"Metabotropic glutamate receptor 5 is required for NMDA receptor-dependent ocular dominance plasticity and LTD in visual cortex" M.S. Sidorov, E.S. Kaplan, E.K. Osterweil, L. Lindemann, M.F. Bear, *Proc Natl Acad Sci U S A*, Oct. 2015, 112(41): 12852-12857.

4.1: Abstract

A better understanding of the molecules, proteins, and neurotransmitter systems implicated in the etiology of neurodevelopmental disorders is critically important for designing effective treatments. The purpose of this study was to use visual cortical plasticity paradigms as a way to better understand the synaptic function of genetic risk factors for autism spectrum disorders (ASDs) and schizophrenia. We studied the interaction between metabotropic glutamate receptor (mGluR) signaling and NMDA receptor-dependent plasticity. We found that NMDAR-dependent long-term depression (LTD) and deprived-eye depression in layer 4 of V1 require mGluR5 signaling during postnatal development. Additionally, we studied the function of the schizophrenia-implicated protein neurogranin, and report that its overexpression disrupts the juvenile ocular dominance shift. Lastly we studied stimulus-selective response potentiation (SRP) in two models of ASDs associated with excitatory/ inhibitory imbalance: Rett syndrome (RTT) and tuberous sclerosis complex (TSC). MeCP2 KO mice exhibited visually-evoked potentials of increased magnitude and showed deficient SRP. TSC2 Het mice displayed VEPs of decreased magnitude, enhanced SRP, but a deficit in visual recognition memory.

4.2: Introduction

Autism spectrum disorders (ASDs) and schizophrenia are debilitating neurodevelopmental disorders; each of which affect over 1% of the human population (Lewis and Lieberman 2000, Newschaffer, Croen et al. 2007). ASDs are characterized by deficits in social communication and interaction, as well as restricted, repetitive patterns of behavior. In ASDs these deficits either gradually appreciate from birth or begin to be observed within the first 2-3 years of life (Newschaffer, Croen et al. 2007). The onset of schizophrenia occurs at a later developmental stage, commonly during late adolescence or early adulthood. Schizophrenia is expressed in the form of abnormal mental functions and disturbed behavior that includes a diversity of clinical features. Positive symptoms of schizophrenia include hallucinations and delusions, negative symptoms include the loss of motivation and emotion, and disturbances in basic cognitive functions such as executive control, attention, and memory (Lewis and Lieberman 2000). Although the origin of both ASDs and schizophrenia remains elusive, it is clear that both genetic and environmental factors play a role in their development. Fortunately several types of ASDs include fragile-X syndrome, tuberous sclerosis complex, and Rett syndrome have been identified in which the loss of function of a single gene is known to confer a significant risk in developing intellectual disability and autism. These genetically defined syndromes present a unique opportunity to study the downstream effects of these gene mutations, and explore the possibility of common pathophysiology in ASDs. Although specific gene mutations do not appear to be as penetrant in causing schizophrenia, several risk factor gene mutations have been discovered that are highly associated with the disorder (Stefansson, Ophoff et al. 2009). Similarly, the function of these genes' protein products can be explored in order to better understand the disease and eventually devise better therapeutics.

The aim of this study was to use visual cortical plasticity paradigms as a way to better understand genes and neurotransmitter receptors, which are implicated in neurodevelopmental disorders. There is significant evidence for altered metabotropic glutamate receptor (mGluR) signaling and deficient NMDAR-dependent plasticity in several ASD mouse models (Bear, Huber et al. 2004, Bartos, Vida et al. 2007, Dolen, Osterweil et al. 2007, Ehninger, Han et al. 2008, Tropea, Giacometti et al. 2009, Yashiro, Riday et al. 2009, Sato and Stryker 2010, Auerbach, Osterweil et al. 2011, LeBlanc and Fagiolini 2011, Bhakar, Dolen et al. 2012, He, Liu et al. 2014). However, very little is known about how the interaction between these two glutamate receptor types may affect cortical plasticity. We found that NMDAR-dependent long-

term depression (LTD) and deprived-eye depression in layer 4 of V1 require mGluR5 signaling during postnatal development. Additionally, we studied the role of the synaptic protein neurogranin, which is implicated as a risk factor for schizophrenia. Our results showed that neurogranin overexpression disrupts the juvenile ocular dominance shift, and support the role of neurogranin as a synaptic protein, which functions in opposition to the processes of LTD. Lastly, we studied stimulus-selective response potentiation (SRP), a form of cortical plasticity involving parvalbumin expressing (PV+) interneurons, in two models of ASDs associated with excitatory/ inhibitory imbalance; Rett syndrome (RTT) and tuberous sclerosis complex (TSC). Interestingly, the mouse model of RTT exhibited visually-evoked potentials (VEPs) of increased magnitude and showed deficient SRP. Conversely, a mouse model of TSC displayed VEPs of decreased magnitude, and showed enhanced SRP. Interestingly, the mouse model of TSC also exhibited a deficit in visual recognition memory.

4.3: Results

4.3.1: Chronic inhibition of mGluR5 signaling impairs ocular dominance plasticity.

As discussed, significant evidence has implicated mGluRs (metabotropic glutamate receptors) and protein synthesis downstream of these receptors in the pathophysiology of several forms of ASDs (Bear, Huber et al. 2004, Dolen, Osterweil et al. 2007, Dolen and Bear 2008, Osterweil, Krueger et al. 2010, Auerbach, Osterweil et al. 2011, Santoro, Bray et al. 2012, Berry-Kravis 2014). We set out to further understand the links between mGluR signaling and NMDAR-dependent forms of plasticity. Experiments were initially motivated by the finding that ocular dominance plasticity is impaired in *Grm5*$^{+/-}$ mice (Fig. 4.1.1 A-C). This observation was surprising on two counts. First, other than ocular dominance plasticity, broad phenotypic screens had shown little consequence of knocking down mGluR5 by 50% compared to WT (Dolen, Osterweil et al. 2007, She, Quairiaux et al. 2009). Second, the deficit in deprived-eye depression in layer 4 in juvenile animals after 3 days of monocular deprivation was reminiscent of the effects of inhibiting NMDA receptor-dependent LTD (Yoon, Smith et al. 2009, Yang, Xiong et al. 2011), which was believed to be unaffected by mGluR5 blockade (Sawtell, Huber et al. 1999). Therefore we set out to re-examine the role of metabotropic glutamate receptors in ocular dominance plasticity using a different method of mGluR5 inhibition.

CTEP is a highly selective mGluR5 negative allosteric modulator (NAM) that can achieve a steady-state ~75% receptor occupancy in mice by dosing 2 mg/kg subcutaneous *(s.c).* every

second day (Lindemann, Jaeschke et al. 2011, Michalon, Sidorov et al. 2012). Mice were administered CTEP, beginning at P21 and throughout the duration of 3-day monocular deprivation (Fig. 4.1.1D). There was a significant effect of CTEP on the magnitude of deprived-(contralateral-) eye depression (Fig. 4.1.1E-H; two-way repeated measures ANOVA, MD x treatment interaction, $F_{(1,21)} = 6.403$, $P = 0.02$). Both vehicle and CTEP-treated WT mice showed depression of the VEP evoked by the contralateral eye (SNK post-hoc test effect of MD within vehicle, $q_{(1)} = 7.443$, $P < 0.001$; effect of MD within CTEP, $q_{(1)} = 3.562$, $P = 0.02$), but the magnitude of this depression was markedly reduced by CTEP treatment. For VEPs evoked by the ipsilateral eye, there was no interaction between drug treatment and MD ($P = 0.264$). The fractional change in responses through the ipsilateral and contralateral eyes after MD are plotted in Fig. 4.1.1G, and reveals a significant difference in the ocular dominance shift in treated and control mice (MANOVA, $P = 0.008$). The magnitude of baseline VEPs evoked prior to MD by the contralateral eye and ipsilateral eye were not significantly different between vehicle and CTEP treatment (Fig. 4.1.1H; Student's unpaired t-tests, $P = 0.255$ for contralateral VEPs and $P = 0.964$ for ipsilateral VEPs). These findings, considered together with the previous findings in the *Grm5*$^{+/-}$ mice, indicate that a threshold level of mGluR5 signaling during postnatal development is necessary for ocular dominance plasticity in visual cortex.

4.3.2: LTD in layer 4 of visual cortex is impaired in *Grm5* mutant mice.

Low-frequency stimulation (LFS; 900 pulses at 1 Hz) induces NMDA receptor-dependent LTD in visual cortex (Kirkwood and Bear 1994). In layer 4, this LTD is mediated by AMPA receptor internalization (Crozier, Wang et al. 2007), as is deprived-eye depression after MD (Yoon, Smith et al. 2009, McCurry, Shepherd et al. 2010, Yang, Xiong et al. 2011). The finding that ocular dominance plasticity is impaired in the *Grm5*$^{+/-}$ mice led us to ask if LTD was similarly affected. To address this question, we electrically stimulated white matter of visual cortical slices using a standard LFS LTD induction protocol and recorded extracellular field potentials from layer 4. We observed that LTD is deficient in *Grm5*$^{-/-}$ and *Grm5*$^{+/-}$ slices compared to WT littermate controls (Fig. 4.1.2A; one-way ANOVA: $P = 0.012$; SNK post-hoc tests: WT versus *Grm5*$^{-/-}$, $P = 0.012$; WT versus *Grm5*$^{+/-}$, $P = 0.033$). There was not a statistically significant difference between LTD magnitude in *Grm5*$^{-/-}$ and *Grm5*$^{+/-}$ mice ($P = 0.450$). We also examined LFS LTD in layer 3 and confirmed the findings of a previous study (Sawtell, Huber et al. 1999) that there is no deficit in *Grm5*$^{-/-}$ or *Grm5*$^{+/-}$ slices compared to WT slices (Fig. 4.1.2B; one-way ANOVA: $P = 0.936$).

4.3.3: LTD in layer 4 is disrupted by chronic but not acute mGluR5 inhibition.

The reduction in layer 4 LTD in the *Grm5* mutant correlates with the impairment in deprived-eye depression observed *in vivo*. To investigate if this LTD phenotype, like disrupted ocular dominance plasticity, also arises from reduced mGluR5 signaling during postnatal life, we treated mice with CTEP (2 mg/kg, *s.c.*) every other day for 7-11 days from P14 until slice recording at P21-25. We found that chronic inhibition of mGluR5 significantly reduced the magnitude of LTD in layer 4 of visual cortex in wild-type mice (Fig. 4.1.2C; student's unpaired t-test, P = 0.047).

Previous work had shown that synaptic depression in layer 4 is mediated by NMDA receptor-dependent modification of postsynaptic AMPA receptors. In hippocampus, mGluR5- and NMDA receptor-dependent forms of LTD are distinct and non-occluding. We therefore examined the effects of acute pharmacological manipulations on layer 4 LTD. We found that the LTD was indeed blocked by 50 μM D-APV, an NMDA receptor antagonist (Fig. 4.1.2D; paired Student's t-test, pre and post LFS, P = 0.956), but not by 60 μM cycloheximide, a protein synthesis inhibitor that interferes with expression of mGluR5-dependent LTD in the hippocampus (Fig. 4.1.2D; paired Student's t-test, pre and post LFS, P = 0.014). Acute inhibition of mGluR5 with the selective NAM 2-methyl-6-(phenylethynyl)pyridine (MPEP, 10 μM) also had no effect on LTD. Under some experimental conditions, blockade of mGluR-dependent LTD in the hippocampus requires inhibition of both mGluR5 and mGluR1 (Volk, Daly et al. 2006). Therefore we also tested whether simultaneous inhibition of both group 1 mGluRs, using MPEP and LY367385 (100 μM), would inhibit LFS-LTD in layer 4. However, there was no effect of acute pharmacological group 1 mGluR inhibition on LTD magnitude (Fig. 4.1.2E; one-way ANOVA, P = 0.939). The effects of chronic and acute inhibition of mGluR5 on LTD are compared in Fig. 4.1.2F. These findings indicate that mGluR5 activation is not a trigger for LTD induction in layer 4 of visual cortex, but that mGluR5 signaling during postnatal development is necessary to establish the conditions that make LTD in visual cortex possible.

4.3.4: NMDA receptor function and inhibition are unaffected by chronic inhibition of mGluR5.

Genetic knockdown and chronic pharmacological inhibition of mGluR5 resulted in impaired NMDAR-dependent plasticity *in vivo* and *in vitro*. Therefore we tested whether NMDA receptors were functionally impaired in *Grm5* mutants. First, we confirmed that basal synaptic

transmission, driven mainly by AMPA receptor-mediated currents, was normal in *Grm5*$^{+/-}$ and *Grm5*$^{-/-}$ mice, as measured by input/output functions (Fig. 4.1.3A; two-way repeated measures ANOVA, no interactions between stimulation intensity and genotype, P = 0.985 for extracellular recordings and P = 0.628 for intracellular recordings). Given that basal transmission was normal, we used AMPA/NMDA ratio as a way to assay NMDAR function. AMPA and mixed AMPA/NMDA-mediated currents were isolated in layer 4 neurons (Fig. 4.1.3B), and showed no difference in *Grm5*$^{+/-}$ or *Grm5*$^{-/-}$ mice compared to WT controls (Fig. 4.1.3B; one-way ANOVA, P = 0.990). Western blotting of the obligatory NMDA receptor subunit NR1 also showed no significant differences between WT, *Grm5*$^{+/-}$, and *Grm5*$^{-/-}$ visual cortical slices (Fig. 4.1.3C; one-way ANOVA, P = 0.766). As expected, mGluR5 protein expression was decreased as a function of genotype (Fig. 4.1.3C; one-way ANOVA, $p < 0.001$).

In both hippocampus and layer 2/3 of visual cortex, there is evidence that mGluR5 is involved in the developmental shift in the NMDA receptor NR2 subunit from predominantly NR2B to predominantly NR2A (Matta, Ashby et al. 2011). Specifically, *Grm5*$^{-/-}$ mice show enhanced synaptic expression of NR2B during development. The nature of the NR2 subunits regulates the conductance of NMDA receptors and intracellular protein interactions, and therefore, their functional consequences when activated (Monyer, Sprengel et al. 1992, Vicini, Wang et al. 1998). The relative levels of NR2A and NR2B in visual cortex are known to have important consequences for the induction of NMDAR-dependent plasticity. NR2A knockout mice display impaired LFS-LTD induced by 1 Hz stimulation and impaired ocular dominance plasticity (Fagiolini, Katagiri et al. 2003, Philpot, Cho et al. 2007, Cho, Khibnik et al. 2009). Therefore we hypothesized that mGluR5 regulates plasticity in visual cortex via regulation of the developmental NR2B-to-NR2A shift. We tested this hypothesis by measuring the decay kinetics of NMDA receptor-mediated EPSCs in layer 4 neurons in slices from animals treated chronically with either CTEP or vehicle. NR2A currents have faster kinetics than NR2B currents (Vicini, Wang et al. 1998, Townsend, Liu et al. 2004). However, chronic CTEP treatment did not affect the decay kinetics of layer 4 neurons at P21-P25 (Fig. 4.1.3D; student's t-test, P = 0.940). There was also no difference in either the decay kinetics of layer 4 neurons (Fig. 4.1.3D; one-way ANOVA, P = 0.729) or the protein expression of NR2A and 2B subunits in visual cortical slices from *Grm5*$^{+/-}$ or *Grm5*$^{-/-}$ mice (Fig. 4.1.3C; one-way ANOVAs, P = 0.168 for NR2A, P = 0.434 for NR2B). Furthermore, neither *Grm5* gene dosage nor CTEP treatment affected the intrinsic membrane resistance of layer 4 neurons (R_m (MΩ): WT 94.6 ± 11.1, *Grm5*$^{+/-}$ 91.2 ± 21.8, *Grm5*$^{-/-}$ 108.9 ± 16.9, Vehicle 108.8 ± 24.7, CTEP 91.1 ± 13.5).

Because of the voltage-dependence of NMDA receptor conductance, NMDA receptor-dependent forms of synaptic plasticity are particularly sensitive to levels of inhibition. For example, a genetic reduction in GABAergic inhibition impairs LTD (Choi, Morales et al. 2002) and ocular dominance plasticity (Hensch, Fagiolini et al. 1998) in mouse visual cortex. Therefore we asked whether inhibition was functionally altered in visual cortex by mGluR5 knockdown. We measured evoked EPSCs and IPSCs within individual layer 4 neurons in response to varying intensities of white matter stimulation (Dong, Wang et al. 2004). However, there was no significant change in EPSC or IPSC magnitude as a function of *Grm5* genotype (Fig. 4.1.3E; two-way repeated measures ANOVA, main effect of genotype, P = 0.546 for EPSCs, P = 0.464 for IPSCs).

4.3.5: NMDAR-dependent synaptic strengthening persists after partial but not complete inhibition of mGluR5.

We next assessed whether the requirement for mGluR5 signaling was limited to specific forms of plasticity such as synaptic weakening in layer 4, or if they generalized to all forms of NMDAR-dependent plasticity. Stimulus-specific response potentiation (SRP) is an experience-dependent form of synaptic strengthening in visual cortex that requires NMDA receptor activation. The induction mechanisms of SRP also appear to share features of canonical long-term potentiation (LTP) (Frenkel, Sawtell et al. 2006, Cooke and Bear 2010). During SRP, repeated exposure to a visual stimulus potentiates VEPs that are evoked by this familiar stimulus but not by a stimulus of novel orientation (Fig. 4.1.4A). We discovered that there was a significant effect of *Grm5* genotype on SRP, measured by growth of VEP magnitude over days (Fig. 4.1.4B-C; two-way repeated measures ANOVA, genotype x day interaction, P = 0.011). There was also a significant effect of *Grm5* genotype on the ability to distinguish between familiar and novel stimuli on day 6 of testing (Fig. 4.1.4D; one-way ANOVA, P = 0.001). Post-hoc tests revealed a significantly impaired ratio of familiar to novel VEP magnitudes in $Grm5^{-/-}$ mice compared to WT mice (P = 0.005) and compared to $Grm5^{+/-}$ mice (P = 0.001). However, there was no significant difference between WT and $Grm5^{+/-}$ mice (P = 0.864). Baseline day 1 raw VEP magnitude was increased in $Grm5^{-/-}$ mice (WT: 88 ± 7 μV, $Grm5^{+/-}$: 71 ± 4 μV, $Grm5^{-/-}$: 136 ± 25 μV; one-way ANOVA, P = 0.013; post-hoc $Grm5^{-/-}$ versus WT, P = 0.020; post-hoc $Grm5^{-/-}$ versus $Grm5^{+/-}$, P = 0.005). In sum, SRP was impaired in $Grm5^{-/-}$ but not $Grm5^{+/-}$ mice, measured both by the ability to distinguish familiar from novel stimulus, and by growth of VEPs over days.

The finding that *Grm5* null but not *Grm5*$^{+/-}$ mice showed deficient SRP prompted us to study the effect of CTEP treatment on SRP induction in wild-type mice. Mice were treated chronically every 48h with CTEP or vehicle, beginning at P21 and continuing throughout the duration of six-day SRP protocol from P30-P35 (Fig. 4.1.4E), the same treatment regimen that impaired ocular dominance plasticity. There was no difference in the magnitude of SRP between vehicle and CTEP-treated mice (Fig. 4.1.4F-G; repeated measures two-way ANOVA, treatment x day interaction, P = 0.329) and no difference in the ability to discriminate novel from familiar stimulus on test day (Fig. 4.1.4H; student's t-test, P = 0.570). Baseline day 1 raw VEP magnitude was not affected by CTEP treatment (vehicle: 159 ± 10 μV, CTEP: 142 ± 17 μV; student's t-test, P = 0.402). Together, the data indicate that partial inhibition of mGluR5 signaling during development selectively impairs the mechanism of NMDA receptor-dependent synaptic weakening.

Abnormal signaling through mGluRs during development appears capable of altering the conditions required for normal NMDAR-dependent plasticity. This is of particular importance given the evidence for altered mGluR function in some models of autism spectrum disorders (Bear, Huber et al. 2004, Auerbach, Osterweil et al. 2011). Based on the findings reported here, individuals with certain forms of ASDs associated with altered mGluR signaling, could develop abnormal NMDAR-dependent plasticity and learning deficits. In fact, it is known that some models of neurodevelopmental disorders including fragile-X syndrome and Angelman syndrome, display abnormal ocular dominance plasticity (Dolen, Osterweil et al. 2007, Tropea, Giacometti et al. 2009, Yashiro, Riday et al. 2009, Sato and Stryker 2010, LeBlanc and Fagiolini 2011, He, Liu et al. 2014, Krishnan, Wang et al. 2015). Here we have shown that OD plasticity may be an especially useful paradigm for uncovering cortical circuit deficits based on either mGluR or NMDAR dysfunction. This is noteworthy that OD plasticity deficits in models of neurological disorders may be useful for uncovering molecular level impairments, and provide a phenotype to attempt correction via pharmacological or other interventions.

4.3.6: Neurogranin overexpression in binocular visual cortex blocks juvenile OD plasticity.

Given that several models of ASDs display deficits in experience-dependent plasticity and learning, including in ocular dominance plasticity (LeBlanc and Fagiolini 2011); we extended our investigation to the role of a protein recently implicated in the etiology of schizophrenia, called neurogranin. Genome-wide association studies (GWAS) of patients with

schizophrenia found that a single nucleotide polymorphism (SNP) adjacent to the neurogranin gene was commonly observed in those with the disorder (Stefansson, Ophoff et al. 2009). Additionally, a post-mortem study of the brains of individuals with schizophrenia, found decreased neurogranin immunoreactivity in areas of the prefrontal cortex (Broadbelt, Ramprasaud et al. 2006). Neurogranin is a post synaptic protein that binds and regulates the calmodulin protein, a calcium binding protein, that in turn interacts with CAMKII and calcineurin proteins, all of which are known to be critically involved in synaptic plasticity (Bliss and Collingridge 1993, Li, Pak et al. 1999, Huang, Huang et al. 2007, Zhong and Gerges 2010). The neurogranin protein is highly expressed in the cerebral cortex, including in V1 (Represa, Deloulme et al. 1990). Due to the known interaction between neurogranin and proteins involved in long-lasting changes in synaptic efficacy, we hypothesized that the manipulation of neurogranin may alter NMDA receptor-dependent forms of cortical plasticity such as ocular dominance plasticity (Kirkwood and Bear 1994, Gordon and Stryker 1996, Frenkel and Bear 2004, Philpot, Cho et al. 2007, Cho, Khibnik et al. 2009, Yoon, Smith et al. 2009). Studies in the hippocampus have shown that neurogranin facilitates increases in synaptic strength in an activity-dependent and NMDAR-dependent manner (Zhong, Cherry et al. 2009, Zhong, Kaleka et al. 2011, Zhong and Gerges 2012). In order to understand the role of neurogranin in V1, we overexpressed neurogranin during short-term monocular deprivation in the juvenile mouse. Because short-term deprivation in the juvenile mouse is known to cause NMDAR-dependent synaptic weakening of inputs serving the deprived eye (Gordon and Stryker 1996, Frenkel and Bear 2004, Yoon, Smith et al. 2009), we hypothesized that neurogranin overexpression may interfere with the juvenile ocular dominance shift.

At postnatal day 21 (P21), C57Bl6 Wild-type (WT) mice were locally injected in binocular V1 with an AAV viral vector containing a construct for either neurogranin overexpression (AAV1/2-CAG-Neurogranin-GFP), or merely a fluorescent reporter (AAV1/2-CAG-GFP) as a control (Fig. 4.2 A-C). Simultaneously the mice were implanted with an electrode in layer 4 of binocular V1 for future VEP recordings. Mice were allowed to recover from surgery for 1 week, during which time they were made accustomed to being head-fixed, and the viral-mediated neurogranin overexpression (or merely GFP expression) occurred. The timeline of the OD plasticity experiment was designed to allow for the deprivation period to occur during the developmental age in which it is known that monocular deprivation causes a depression of the responses serving the deprived (contralateral) eye (P28-31)(Drager 1978, Gordon and Stryker 1996, Frenkel and Bear 2004). Contralateral (contra) eye and ipsilateral (ipsi) eye driven VEPs

were recorded from both neurogranin overexpression and control mice. Before monocular deprivation, neurogranin overexpression and control mice did not significantly differ in the magnitudes of their VEPs evoked via the contralateral eye (Pre MD, Contra VEP, GFP control: 175.67 ± 21.51 μV, n = 6 mice, Neurogranin overexpression: 167.88 ± 9.32 μV, 2-way repeated measures ANOVA, SNK post hoc test, $q_{(1)} = 0.530$, P = 0.711, n = 8 mice) or ipsilateral eye (Pre MD, Ipsi VEP, GFP control: 60.25 ± 14.63 μV, Neurogranin overexpression: 45.00 ± 4.08 μV, 2-way repeated measures ANOVA, SNK post hoc test, $q_{(1)} = 1.496$, P = 0.304, Fig. 4.2D-E). After the initial recording of VEPs (Pre MD), the dominant contralateral eye was deprived of vision for 3 days, and subsequently reopened. At this point, VEPs were alternately recorded from either eye to look for the presence of an ocular dominance shift (Post MD). In the GFP control mice, deprivation induced significant depression in VEP responses driven by the deprived contralateral eye (Post MD, 128.33 ± 5.38 μV, SNK post hoc test, $q_{(1)} = 3.832$, P = 0.019), and significant potentiation of responses driven by the non-deprived ipsilateral eye (Post MD, 127.83 ± 13.27 μV, SNK post hoc test, $q_{(1)} = 9.413$, P < 0.001, Fig. 4.2D). In contrast to the control mice, the neurogranin overexpression mice did not display significant changes in the magnitude of the average contralateral eye VEP (Post MD, 144.63 ± 16.85 μV, SNK post hoc test, $q_{(1)} = 2.173$, P = 0.150) or ipsilateral eye VEP (Post MD, 54.69 ± 8.93 μV, SNK post hoc test, $q_{(1)} = 1.558$, P = 0.292, Fig. 4.2E). A shift in ocular dominance can be easily expressed by plotting the ratio of the magnitudes of contralateral to ipsilateral VEPs (contra/ ipsi ratio). Control mice displayed a significant decrease in the contra/ ipsi ratio following MD (Pre MD: 3.36 ± 0.58, Post MD: 1.07 ± 0.15, student's paired one-tailed t-test, $t_{(5)} = 3.606$, P = 0.007, Fig. 4.2F). Neurogranin overexpression mice did not exhibit a significant shift in contra/ ipsi ratio (Pre MD: 3.96 ± 0.43, Post MD: 3.96 ± 1.41, student's paired one-tailed t-test, $t_{(7)} = -0.00053$, P = 0.50, Fig. 4.2G). Thus, neurogranin overexpression in primary visual cortex disrupts the juvenile ocular dominance shift.

4.3.7: MeCP2 deletion mice display enlarged baseline VEP magnitudes and impaired SRP.

In addition to utilizing ocular dominance plasticity as a paradigm for understanding neural dysfunction in mouse models of disease, we also investigated if deficits would be apparent in the distinct form of experience-dependent plasticity known as stimulus-selective response potentiation (SRP). We were particularly interested in this question because the expression of SRP is known to depend on GABAergic inhibitory circuitry (Chapter 2), and

dysfunction of GABAergic cells has been implicated in many forms of neurodevelopmental disorders (Lewis, Hashimoto et al. 2005, Gogolla, Leblanc et al. 2009, Han, Tai et al. 2012). Rett syndrome, which is caused by mutations in the MeCP2 gene (Amir, Van den Veyver et al. 1999), is a disorder particularly associated with GABAergic circuit dysfunction (Glaze 2005, Gogolla, Leblanc et al. 2009, Chao, Chen et al. 2010, He, Liu et al. 2014, Zhang, Peterson et al. 2014, Krishnan, Wang et al. 2015). Therefore, we were interested to scrutinize SRP, a form of experience-dependent plasticity, in a mouse model of Rett syndrome, the MeCP2 knock out mouse (MeCP2 KO, B6.129P2(C)-Mecp2$^{tm1.1Bird}$/J).

We implanted MeCP2 KO (n = 9) and littermate wild-type (WT) mice (n = 7) with VEP recording electrodes in layer 4, binocular V1. After recovery and 2 daily sessions of head fixation, we recorded binocular VEPs elicited by a X° oriented grating stimulus. Immediately apparent was the significantly greater basal magnitude of VEPs recorded in the MeCP2 KO mice (213.26 ± 14.69 μV) relative to their littermate WT controls (156.43 ± 8.91 μV, student's two-tailed t-test, $t_{(14)}$ = 3.592, P = 0.0029, Fig. 4.3A). We then presented the same stimulus to these mice over several consecutive days (Fig. 4.3B). A significant deficit in SRP is clearly apparent when the data is normalized to day 1 values (2-way repeated measures ANOVA, interaction of genotype x day, $F_{(5,70)}$ = 8.257, P < 0.001, Fig. 4.3C). Again, a significant deficit in SRP was displayed by day 2 in the MeCP2 KO mice (126.62 ± 5.13% day 1) compared with WT littermates (181.94 ± 10.52% day 1, SNK post hoc test, $q_{(5)}$ = 5.432, P = 0.002), demonstrating that SRP is compromised by a loss of MeCP2. This deficit extended until the final day of the experiment on day 7, in which the MeCP2 KO mice displayed relatively less potentiation to the now familiar visual stimulus (173.68 ± 10.77% day 1) compared with WT littermates (230.61 ± 14.95% day 1 SNK post hoc test, $q_{(1)}$ = 5.099, P = 0.002, Fig. 4.3D). We also tested for the stimulus-selectivity of SRP expression by presenting both groups of animals with interleaved blocks of the familiar X° stimulus and a novel X + 90° stimulus on day 7 (Fig. 4.3E). Interestingly, significant stimulus selectivity was present in both genotypes (2-way repeated measures ANOVA, stimulus, stimulus, $F_{(1)}$ = 153.686, P < 0.001, interaction of genotype x stimulus, $F_{(1,14)}$ = 1.583, P = 0.229, Fig. 4.3E). As is typical, WT mice displayed larger VEP magnitudes for the familiar stimulus (357.5 ± 22.29 μV), compared to a novel stimulus (213.07 ± 21.59 μV, SNK post hoc test, $q_{(1)}$ = 10.502, P < 0.001, Fig. 4.3E). This difference was also significant in the MeCP2 KO mice (familiar: 385.94 ± 33.23 μV, novel: 208.89 ± 18.97 μV, SNK post hoc test, $q_{(1)}$ = 14.598, P < 0.001, Fig. 4.3E). Therefore, although the MeCP2 KO mice

showed less potentiation over days to the familiar stimulus, these mice did show significant stimulus selectivity on the test day.

4.3.8: TSC2 heterozygous mice display reduced baseline VEP magnitudes and enhanced SRP.

We were curious if the same abnormalities we found in the MeCP2 KO mice would be recapitulated in another mouse model of autism. We investigated SRP in a mouse model of tuberous sclerosis complex (TSC, B6;129S4-*Tsc2*^{tm1Djk}/J). TSC is caused by heterozygous mutations in the genes encoding the TSC1 or TSC2 proteins (Ehninger, de Vries et al. 2009). Notably, cognitive deficits in this disorder are accompanied by severe and often treatment-resistant epilepsy (Chu-Shore, Major et al. 2010), suggesting the possibility of GABAergic system dysfunction. TSC2 heterozygous rodents have been shown to recapitulate abnormalities seen in the human condition such as increased cell size and learning deficits (Cheadle, Reeve et al. 2000, de Vries and Watson 2008, Ehninger, Han et al. 2008). In addition, these models have shown deficits in synaptic function and plasticity (von der Brelie, Waltereit et al. 2006, Auerbach, Osterweil et al. 2011). Therefore, we hypothesized that TSC2 heterozygous mice may show deficits in SRP, as we observed in the MeCP2 KO mice.

We implanted TSC2 heterozygous mice (TSC2 Het, n = 16) and littermate wild-type (WT) mice (n = 12) with VEP recording electrodes in layer 4, binocular V1. Again, after recovery and 2 daily sessions of head restraint, we recorded VEPs elicited by an X° oriented grating stimulus. To our surprise, the TSC2 Het mice displayed significantly smaller basal VEP magnitudes on day 1 (136.44 ± 9.15 μV) relative to their littermate WT controls (168.38 ± 10.74 μV, student's two-tailed t-test, $t_{(26)} = 2.619$, P = 0.0145, Fig. 4.3F). We then presented the same stimulus to these mice over several consecutive days (Fig. 4.3G). Although both TSC2 Het and WT mice showed potentiation over days, TSC2 Het mice displayed enhanced SRP. This increased magnitude of SRP gradually became apparent over days and was significant by day 6 (2-way repeated measures ANOVA, SNK post hoc test, TSC2 Het : 281.96 ± 21.42% day 1, WT: 227.33 ± 16.18% day 1, SNK post hoc test, $q_{(5)} = 3.853$, P = 0.008, Fig. 4.3H). This phenotype was apparent on the final experimental day 7, in which the TSC2 Het mice displayed relatively greater potentiation to the now familiar visual stimulus (281.22 ± 21.69% day 1) compared with WT littermates (236.98 ± 12.92% day 1, SNK post hoc test, $q_{(1)} = 3.069$, P = 0.035, Fig. 4.3I). We also tested for the stimulus-selectivity of SRP expression by presenting genotypes with interleaved blocks of the familiar X° stimulus and a novel X + 90° stimulus on

day 7 (Fig. 4.3J). Interestingly, significant stimulus selectivity was present in both genotypes (2-way repeated measures ANOVA, stimulus, stimulus, $F_{(1)} = 134.178$, $P < 0.001$, interaction of genotype x stimulus, $F_{(1,26)} = 1.466$, $P = 0.237$, Fig. 4.3J). WT mice displayed larger VEP magnitudes for the familiar stimulus (392.29 ± 22.78 μV), compared to a novel stimulus (187.92 ± 13.00 μV, SNK post hoc test, $q_{(1)} = 19.507$, $P < 0.001$, Fig. 4.3J). This was also apparent in the TSC Het mice (familiar: 358.47 ± 14.59 μV, novel: 171.38 ± 12.43 μV, SNK post hoc test, $q_{(1)} = 20.620$, $P < 0.001$, Fig. 4.3J). Therefore, although the TSC2 Het mice showed more potentiation over days to the familiar stimulus compared to wild-type mice, TSC2 Het mice and WT mice displayed similar stimulus selectivity for the familiar stimulus on day 7. Interestingly, MeCP2 KO mice and TSC2 Het mice displayed opposite VEP and SRP phenotypes. MeCP2 KO mice displayed enlarged baseline VEPs and relatively less SRP compared to WT, while TSC2 Het mice displayed decreased baseline VEPs and relatively greater SRP compared to WT. Notably, these abnormalities did not affect SRP selectivity as both mutant strains showed significantly increased VEP magnitude to the familiar oriented stimulus compared to that evoked by a novel stimulus.

4.3.9: TSC2 heterozygous mice do not display behavioral discrimination of familiar and novel stimuli.

Although TSC2 Het mice showed significant discrimination of familiar and novel stimuli at the electrophysiological level (Fig. 4.3J), we wanted to investigate if this would carry over to behavioral discrimination as described previously (Chapter 2, Fig. 2.8). TSC2 Het mice and wild-type littermates (WT) underwent a standard SRP protocol, being exposed to a specific oriented visual stimulus over days. On the final day (test day) of the experiment, mice were exposed to the familiar stimulus as well as a novel stimulus while behavioral responses were recorded via a piezoelectric device placed underneath the forepaws (Fig. 4.4A). As expected WT mice showed a reduced behavioral response to the familiar oriented visual stimulus (1.73 ± 0.32 a.u.) compared to the response to a novel stimulus (3.05 ± 0.45 a.u., 2 way repeated measure ANOVA, SNK post hoc test, $q_{(1)} = 3.402$, $P = 0.024$, Fig. 4.4B), characteristic of habituation to an experienced stimulus. Surprisingly, TSC2 Het mice did not show significant behavioral discrimination between novel (2.94 ± 0.34 a.u.) and familiar visual stimuli (2.53 ± 0.27 a.u., $q_{(1)} = 1.217$, $P = 0.397$, Fig. 4.4B). The inability of TSC2 Het mice to discriminate familiar and novel stimuli is apparent when calculating the familiar/ novel ratio (Fig. 4.4C). There was a significantly lower ratio in the WT mice (0.75 ± 0.18) compared to the TSC2 mice (1.07 ±

0.19, student's one-tailed t-test, $t_{(24)} = -1.91$, $P = 0.034$), reflective of a deficit in discrimination of visual stimuli in the mice heterozygous for TSC2. Thus, TSC2 mice were unable to discriminate familiar and novel visual stimuli at the level of behavior, whereas this distinction appeared to be represented in primary visual cortex at the electrophysiological level.

4.4: Discussion

Neurodevelopmental disorders such as autism spectrum disorders (ASDs) and schizophrenia are extremely diverse in their phenotypes, however; there may exist common genetic susceptibilities and synaptic pathologies (de Lacy and King 2013). In both ASDs and schizophrenia, synaptic abnormalities likely represent the basis of cognitive and behavioral dysfunction seen in patients (Bailey, Phillips et al. 1996, Bakhshi and Chance 2015). Therefore, it is imperative that we better understand the role of the specific genes/ proteins implicated in the etiology of these disorders to synaptic function and plasticity. Several different neurotransmitter systems and receptors have been implicated in the underlying synaptic pathology of ASDs including the ionotropic and metabotropic glutamate receptors as well as GABAergic-related proteins (Bailey, Phillips et al. 1996, Bear, Huber et al. 2004, Gogolla, Leblanc et al. 2009, Chao, Chen et al. 2010). Thus, it is also important to understand how these neurotransmitter systems interact, and how these interactions may affect synaptic plasticity and cognitive function.

4.4.1: mGluR signaling establishes conditions permissive for NMDAR-dependent synaptic weakening.

One interesting feature of early postnatal neocortical development is an increase in group 1 mGluR signaling that, in visual cortex, coincides with increased sensitivity to MD (Dudek and Bear 1989). Based on this correlation and a theory of synaptic plasticity, it was proposed that postsynaptic mGluR signaling might serve as a trigger for homosynaptic depression at glutamatergic synapses (Bear and Dudek 1991). Although subsequent research showed that LTD can indeed be a consequence of group 1 mGluR activation (Linden and Connor 1995, Bear and Abraham 1996, Kemp and Bashir 2001, Malenka and Bear 2004), surprisingly little progress has been made in establishing a role for postsynaptic mGluRs in visual cortical plasticity *in vivo*. Here we confirm pharmacologically what was previously shown in the *Grm5* mutant (Dolen, Osterweil et al. 2007), that a partial reduction of signaling via mGluR5 interferes with the effects of MD in layer 4 of visual cortex (Fig. 4.1.1). CTEP treatment

during the period of heightened sensitivity to MD, beginning at ~P21 (Gordon and Stryker 1996), was sufficient to reproduce the phenotype observed in the *Grm5*$^{+/-}$ mouse, an impairment in deprived-eye depression. The likely basis for this deficit *in vivo* was revealed by the study of LTD in mutant and treated WT mice (Fig. 4.1.2). In layer 4, both approaches to chronically inhibit mGluR5 produced a clear deficit in NMDA receptor-dependent LTD, a synaptic modification which employs the same mechanisms of postsynaptic AMPA receptor modification as does deprived-eye depression (Cooke and Bear 2014). Interestingly, layer 3 LTD, which has different signaling requirements (Daw, Rao et al. 2004) and is expressed via a presynaptic endocannabinoid-dependent mechanism (Crozier, Wang et al. 2007), was unaffected by mGluR5 inhibition (Fig. 4.1.2), consistent with previous findings (Sawtell, Huber et al. 1999).

In layer 4, LTD is unaffected by acute pharmacological inhibition of mGluR5, mGluR5-dependent signaling pathways, or protein synthesis (Fig. 4.1.2) (Daw, Rao et al. 2004, Ueta, Yamamoto et al. 2008). One appealing hypothesis is that chronic inhibition of mGluR5 affects the activity-dependent NMDA receptor NR2B-to-NR2A subunit switch that occurs postnatally in visual cortex (Carmignoto and Vicini 1992, Sheng, Cummings et al. 1994, Quinlan, Olstein et al. 1999, Quinlan, Philpot et al. 1999). Similar to what we observe after chronic inhibition of mGluR5, both NMDA receptor-dependent LTD and deprived-eye depression are impaired in layer 4 of *Grin2A* null and heterozygous mice (Cho, Khibnik et al. 2009). Normal sensory experience during early life drives the change in NMDA receptor subunit composition, and there is evidence from hippocampus and layer 2/3 of visual cortex that the functional expression of NR2A-containing receptors is triggered by activation of mGluR5 (Matta, Ashby et al. 2011). However, our failure to observe a difference in the NMDA EPSC decay kinetics in layer 4 neurons after chronic CTEP suggests that this subunit switch likely occurred normally in treated animals (Fig. 4.1.3). We also note that another phenotype caused by reduced NR2A expression, enhanced non-deprived-eye potentiation during 3 days of MD (Cho, Khibnik et al. 2009), was not observed following chronic inhibition of mGluR5 (Fig. 4.1.1). Together, these findings argue against the hypothesis that impairment in the NR2B-to-NR2A subunit switch is the basis for the impairment in deprived-eye depression in layer 4.

Other overt changes in NMDA receptors appear to be ruled out by the findings of a normal AMPA/NMDA receptor ratio (Fig. 4.1.3), and normal SRP in the *Grm5*$^{+/-}$ and CTEP-treated WT mice (Fig. 4.1.4). However, we did observe a striking impairment in SRP in the full mGluR5 knockout. SRP shares many mechanisms with canonical LTP (Cooke and Bear 2010),

and NMDA receptor-dependent LTP is severely impaired in the *Grm5*$^{-/-}$ hippocampus (Lu, Jia et al. 1997) and layer 4 of sensory neocortex (She, Quairiaux et al. 2009), so this finding is not totally surprising.

We speculate that the cause of altered synaptic depression in layer 4 following chronic mGluR5 inhibition is related to adjustments in intracellular signaling that occur with a slow time-course. Although acute inhibition of mGluR5 by itself has little effect, modulatory *augmentation* of phospholipase C signaling has been shown to promote LTD *in vitro* and synaptic depression *in vivo* at layer 2/3 synapses in visual cortex (Choi, Chang et al. 2005, Huang, Trevino et al. 2012). Chronic down-regulation of PLC-dependent signaling might have the opposite effect, for example, by altering intracellular Ca^{2+} stores. Another possibility, not involving the canonical Gq11 signaling pathway, relates to regulation by mGluR5 of local synaptic protein synthesis (Weiler and Greenough 1993) via activation of a Ras-ERK-MAP kinase pathway (Gallagher, Daly et al. 2004, Osterweil, Krueger et al. 2010). Chronic inhibition of ERK (Di Cristo, Berardi et al. 2001) and mRNA translation (Taha and Stryker 2002) also interfere with ocular dominance plasticity. Consistent with this hypothesis, genetic deletion of the mRNA translation repressor FMRP, which boosts basal protein synthesis, is sufficient to restore deprived-eye depression and normal ocular dominance plasticity in the *Grm5*$^{+/-}$ mice (Dolen, Osterweil et al. 2007).

Loss of the protein FMRP is the cause of fragile X syndrome, the most common inherited form of human intellectual disability and autism. A core pathophysiological mechanism of the loss of FMRP is excessive protein synthesis downstream of an mGluR5-dependent signaling pathway. Therefore it is important to recognize the interactions between mGluR signaling and NMDAR signaling when considering phenotypes of, and treatments for, neurodevelopmental disorders. The data suggest that ongoing signaling at mGluR5 during a critical period establishes biochemical conditions that are permissive for activity-dependent sculpting of excitatory synapses via the mechanism of NMDA receptor-dependent LTD.

4.4.2: Neurogranin overexpression disrupts juvenile OD plasticity.

NMDA receptors and NMDAR-plasticity is not only affected by mGluR signaling, but also by the activity of postsynaptic proteins such as neurogranin (Zhong and Gerges 2010). Neurogranin, which is implicated in schizophrenia (Broadbelt, Ramprasaud et al. 2006, Stefansson, Ophoff et al. 2009), is one of the most abundant binding proteins of calmodulin (CaM (Gerendasy, Herron et al. 1994)). When calcium ions enter the cell via NMDARs, CaM

binds these ions and transduces this signal to other protein partners. The level of calcium and CaM in the post synaptic compartment affects the activation of the enzymes calcium/CaM-dependent protein phosphatase calcineurin and calcium/CaM dependent protein kinase II (CaMKII) (Lisman, Schulman et al. 2002). In turn, the activation of calcineurin and CaMKII affect the induction of synaptic weakening and synaptic strengthening, respectively. Therefore, the availability of calmodulin, which is regulated by neurogranin, can greatly influence whether LTP or LTD mechanism will be set in motion at the synapse. Thus, through its interaction with other synaptic proteins, neurogranin may be able to alter the balance between LTP and LTD induction processes at the synapse (Zhong, Cherry et al. 2009, Zhong and Gerges 2010).

Studies in the hippocampus have shown that neurogranin overexpression enhances CAMKII activity and promotes LTP. Conversely reducing the expression of neurogranin in the hippocampus blocks LTP induction (Zhong, Cherry et al. 2009, Zhong and Gerges 2010). Based on the known function of neurogranin, we hypothesized that its role in visual cortex would be similar to that observed in the hippocampus; promoting LTP mechanisms. Therefore, by overexpressing neurogranin in the visual cortex during monocular deprivation, we expected to block the weakening of contralateral eye inputs characteristic of short-term MD in juvenile mice. We also hypothesized that neurogranin overexpression may promote the potentiation of responses serving the non-deprived ipsilateral eye. As expected, increasing the amount of neurogranin in visual cortex did block the weakening of inputs downstream of the deprived eye (Fig. 4.2). This finding supports the proposed function of neurogranin as a protein that opposes LTD processes.

Interestingly; we did not observe any ipsilateral eye potentiation in the mice overexpressing neurogranin as predicted. In fact, we observed significant ipsilateral eye potentiation in the control animals (Fig. 4.3D), but none in the neurogranin overexpression animals (Fig. 4.3E). One possibility for the lack of open eye potentiation in the neurogranin overexpression mice concerns metaplasticity. It is widely accepted that the properties of synaptic plasticity can change as a function of the recent history of synaptic or cellular activity (Bear 2003). In the case of the juvenile ocular dominance shift, it is appreciated that deprived eye depression precedes ipsilateral (non-deprived) eye potentiation (Frenkel and Bear 2004). It is believed that deprivation produces uncorrelated neural activity, which leads to LTD processes from inputs serving the deprived eye (Crozier, Wang et al. 2007, Yoon, Smith et al. 2009). This decreases the overall activity of the cortex, and leads to a metaplastic change, in which the threshold for eliciting LTP is now lowered (Bear 2003). The mechanism for this metaplastic

change is at least partly dependent on NMDAR subunit composition modifications (Chen and Bear 2007, Cho, Khibnik et al. 2009). Normally, metaplasticity allows for the subsequent potentiation of non-deprived eye inputs. It is possible that in the neurogranin overexpression mice, neurogranin opposes the synaptic weakening of inputs from the deprived eye, and this lack of depression limits any metaplastic changes, and thereby also blocks the subsequent synaptic potentiation of non-deprived eye inputs. This could be tested by measuring changes in NMDAR subunit composition as a result of deprivation (Chen and Bear 2007) in the neurogranin overexpression mice. It is also possible that non-deprived eye potentiation in the neurogranin overexpression mice may have been observed with a longer deprivation protocol.

Recently, Zhong and colleagues found that overexpression of neurogranin in the prefrontal cortex enhances LTP and increases the rate of extinction learning (Zhong, Brown et al. 2015). This recent article and our findings are in agreement that neurogranin overexpression in the cortex promotes LTP and opposes LTD, similar to neurogranin's reported function in the hippocampus. Zhong and colleagues argue for neurogranin overexpression in the cortex as a universal method for facilitating plasticity and learning in general. Interestingly, our results do not agree with this, as neurogranin overexpression caused a deficit in experience-dependent plasticity in V1. Indeed, other forms of plasticity/ learning in the cortex may rely on synaptic weakening, just as in juvenile OD plasticity. Based on our findings, these forms of plasticity, which rely on synaptic weakening, would be hindered by increasing the activity or expression of neurogranin. In order to confirm the role of cortical neurogranin in experience-dependent synaptic strengthening in the future; it will be useful to examine the effect of neurogranin manipulation on visual cortical plasticity that is characterized by response potentiation, such as adult OD plasticity, or SRP (Sawtell, Frenkel et al. 2003, Frenkel, Sawtell et al. 2006, Cooke and Bear 2010).

Investigating the role of neurogranin in the cortex is greatly motivated by the association of this protein with schizophrenia (Broadbelt, Ramprasaud et al. 2006, Stefansson, Ophoff et al. 2009). Abnormal synaptic plasticity could play a major role in the behavioral abnormalities and learning deficits observed for those with this disorder (Park and Holzman 1992, Braff, Swerdlow et al. 1995, Park, Puschel et al. 2003, McGrath, Saha et al. 2008, Park and Gooding 2014, Bakhshi and Chance 2015). Unusual cortical activity and plasticity has been reported in those with schizophrenia in the prefrontal as well as visual cortices (Andreasen, O'Leary et al. 1997, Cavus, Reinhart et al. 2012). Additionally, an influential theory on the synaptic pathophysiology of schizophrenia, supported by clinical data, is related to hypofunction of the NMDAR (Coyle,

Tsai et al. 2003, Coyle 2006). Therefore understanding the role of synaptic proteins downstream of NMDAR signaling, such as neurogranin, is of utmost importance to unraveling the basis of the debilitating positive and negative symptoms of schizophrenia. Our results support the idea that disruption of neurogranin function in those with schizophrenia (Stefansson, Ophoff et al. 2009) may significantly alter the balance between experience-dependent synaptic strengthening and weakening, and therefore greatly alter cortical function and learning.

4.4.3: SRP is altered in MeCP2 KO mice.

Rett syndrome, caused by mutations in the X-linked MeCP2 gene, occurs in about 1 in 10,000 live female births (hemizygous mutations in males are usually lethal). The disorder is characterized by a regressive phenotype that is common in ASDs, where the first 6 months of life proceed relatively normally, but are followed by deceleration of head growth, gait abnormalities, loss of speech, breathing disturbances, epilepsy, and the replacement of purposeful hand motions with repetitive stereotypies (Neul, Kaufmann et al. 2010, Lyst and Bird 2015, Pohodich and Zoghbi 2015). Although ASDs are a heterogeneous group of diseases, several have been associated with alterations in inhibitory GABAergic circuitry (Pizzarelli and Cherubini 2011, Ramamoorthi and Lin 2011). Rett syndrome, which has been modeled using MeCP2 KO mice, is associated with epilepsy, excitatory/ inhibitory imbalance and dysfunction of GABAergic interneurons (Glaze 2005, Chao, Chen et al. 2010, Zhang, Peterson et al. 2014). In fact, many of the overt phenotypes present in MeCP2 KO mice can be recapitulated by selectively eliminating MeCP2 in PV+ GABAergic interneurons (He, Liu et al. 2014). Interestingly, although there is evidence for excitatory/ inhibitory imbalance with the removal of the MeCP2 gene, the direction of the imbalance is a matter of controversy. Several studies have found an increase in excitation (Chao, Chen et al. 2010, He, Liu et al. 2014), and others have found an increase in inhibition (Durand, Patrizi et al. 2012, Krishnan, Wang et al. 2015). Conflicting results may be attributed to the experimental conditions, including the use of anesthesia, the differences in mouse models, or the age of the mice tested. Regardless, the evidence for alterations in the GABAergic system in Rett syndrome as well as in the MeCP2 KO mouse presented ample reason to suspect that these mice may display a deficit in stimulus-selective response potentiation, which is dependent on PV+ interneurons (Chapter 2).

We found the baseline magnitude of VEPs to be increased and a deficit in SRP in MeCP2 KO mice relative to littermate controls (Fig. 4.3 A-D). This phenotype is suggestive of decreased GABAergic function and is reminiscent of the phenotype of PV-GluN1 KO mice (Fig.

2.5). Similarly to the PV-GluN1 KO mice, MeCP2 KO mice displayed relatively less potentiation over days, but still showed significant stimulus selectivity in the form of larger VEP magnitude to the familiar stimulus, relative to the novel stimulus (Fig. 4.3 E). Although it is known that disruption of the MeCP2 gene causes Rett syndrome, the function of MeCP2 is still unclear (Lyst and Bird 2015). There is substantial evidence that MeCP2 acts as a transcriptional regulator, either acting as an activator, a repressor, or both in different circumstances. As mentioned, there is evidence that the deletion of MeCP2 causes a disruption of the GABAergic system (Chao, Chen et al. 2010, He, Liu et al. 2014). Therefore, there may be a subset of genes, critical to the function of the GABAergic system, that is regulated by MeCP2. There is in fact evidence for such genes being regulated by MeCP2, which could explain a dysfunctional inhibitory system in MeCP2 KO mice. Durand and colleagues found that GAD65 expression is decreased in V1 neurons of MeCP2 KO mice (Durand, Patrizi et al. 2012). Also reported in the same study was a decrease in expression of the voltage-gated potassium channel Kv3.1, a channel that enables fast repolarization following action potential firing and is known to be critical to the function of fast spiking PV+ inhibitory interneurons (Rudy and McBain 2001, Lien and Jonas 2003). Notably, it has also been shown that deficits in juvenile ocular dominance plasticity in the MeCP2 KO mouse can be recapitulated by specifically knocking out the gene only within PV+ interneurons (He, Liu et al. 2014). A related study, specifically deleted MeCP2 from GABAergic interneurons and found evidence of decreased GABA protein expression as well as decreased *GAD1* and *GAD2* mRNA, which code for proteins GAD65 and GAD67 respectively (Chao, Chen et al. 2010). On the whole these studies suggest that mutation or deletion of MeCP2 can disrupt the balance of excitation and inhibition in the brain, possibly with an exorbitant effect on fast spiking PV+ interneurons. Therefore, it is not surprising that we observed increased baseline VEP magnitude and a deficit in SRP in MeCP2 KO mice. The results presented here add further insights into the synaptic pathophysiology of Rett syndrome and provide motivation for future pharmacological treatments that target the GABAergic system.

4.4.4: TSC2 mice display enhanced SRP but deficient habituation behavior.

We extended out investigation to another type of ASD, the tuberous sclerosis complex (TSC), due to the human condition's severe epilepsy phenotype. TSC is a disorder caused by heterozygous mutations in either the TSC1 or the TSC2 gene, and is often associated with intellectual disability and autism (Ehninger, de Vries et al. 2009). Tuberous sclerosis is also accompanied by cognitive, behavioral, and learning deficits (de Vries and Watson 2008,

Ehninger, de Vries et al. 2009, Curatolo, Moavero et al. 2015). TSC, which occurs in about 1 in 6000 newborns, represents one of the most common genetic causes of epilepsy (Chu-Shore, Major et al. 2010, Curatolo, Moavero et al. 2015). An incredible 90% of those with TSC have epilepsy and greater than half of those patients display drug-resistant forms of epilepsy (Curatolo, Moavero et al. 2015, Wong and Roper 2015). This phenotype represents a severe alteration in excitatory/ inhibitory balance, and may involve alterations in the GABAergic system, including in the PV+ expressing interneurons. Therefore we hypothesized that SRP may be dysfunction in a mouse model of TSC, the TSC2 heterozygous (TSC2 Het) mouse.

Intriguingly, we found that baseline VEPs were reduced in size in TSC2 Het mice (Fig. 4.3F). This was a surprising result given that we expected an increase in excitatory/ inhibitory balance in these mice, due to the high prevalence of seizure activity in the human condition (Chu-Shore, Major et al. 2010). We also were surprised since the opposite phenotype had been observed in MeCP2 KO mice (Fig. 4.3A). Interestingly, although plasticity and learning deficits have been observed in TSC1 and TSC2 heterozygous mice (Cheadle, Reeve et al. 2000, de Vries and Watson 2008, Ehninger, Han et al. 2008, Auerbach, Osterweil et al. 2011), there is little evidence of seizures or overt brain pathological abnormalities in TSC heterozygous mice (Wong and Roper 2015). It is possible that given the hardiness of mice in comparison to humans, the loss of a single copy of TSC1 or TSC2 is not sufficient to induce an epilepsy phenotype. In humans as well as mice, the homozygous loss of TSC1 or TSC2 is embryonic lethal and therefore a conventional homozygous TSC deficient mouse cannot be studied (Wong and Roper 2015). However, several groups have investigated conditional knock outs utilizing homozygous inactivation of either the TSC1 or TSC2 genes in subsets of brain cells. Of interest to what has been studied here, the homozygous deletion of TSC1 from GABAergic cells, generates a mouse line with reduced survival, physically enlarged but reduced in number cortical GABAergic cells, and reduced seizure threshold (Fu, Cawthon et al. 2012). This study suggests that in mice, a more pronounced or targeted insult to the TSC genes may be necessary to display some of the human phenotypes, including epilepsy It is possible that the TSC2 Het mice may show more modest changes in synaptic function than would be observed in the human, or that network level compensation in these mice obscures changes in excitatory/ inhibitory balance.

In our investigation of SRP, we found that TSC2 Het mice displayed enhanced potentiation to a familiar stimulus over days (Fig. 4.3G-I). Again this finding was unexpected

because we had seen diminished SRP in another ASD model, the MeCP2 KO mouse (Fig 4.3B-D). In the case of TSC2 heterozygous rodents, synaptic plasticity deficits have been previously reported in the hippocampus, including reduced mGluR-dependent LTD (Auerbach, Osterweil et al. 2011), reduced NMDAR-dependent LTD (von der Brelie, Waltereit et al. 2006), and enhanced hippocampal NMDAR-dependent LTP (Ehninger, Han et al. 2008). An interesting interpretation of these results stems from the known role of the protein products of the TSC genes. The proteins TSC1 and TSC2 form a TSC1/2 complex. This TSC1/2 complex interacts with proteins Rheb and mTOR to inhibit the mTOR complex-mediated translation of mRNAs (Tee, Fingar et al. 2002, Kwiatkowski and Manning 2005). The heterozygous loss of TSC1 or TSC2 is believed to lead to unchecked mRNA translation downstream of the mTOR complex, which is known to be pathogenic in TSC (Ehninger, de Vries et al. 2009). Based on studies of TSC heterozygous mouse lines it is hypothesized that the mRNAs upregulated in the disorder are those which promote activity-dependent potentiation of synapses and oppose synaptic depression (Auerbach, Osterweil et al. 2011). This hypothesis suggests an explanation for the reported enhancement of LTP and disruption of LTD in the hippocampus of TSC heterozygous rodents. These studies also may inform the enhanced stimulus-selective response potentiation that we observed in the visual cortex of TSC2 Het mice. Similarly to what has been observed in the hippocampus, the loss of one copy of the TSC2 gene may enhance LTP processes in the cortex (Ehninger, Han et al. 2008). We know that in addition to the requirement for PV+ mediated inhibition in the expression of SRP, NMDAR-dependent LTP mechanism are intimately involved in the induction and/ or maintenance of SRP (Cooke and Bear 2010, Cooke, Komorowski et al. 2015). Therefore, the loss of one TSC2 allele may promote the mTOR complex-mediated expression of LTP related proteins, and thereby act to enhance SRP.

Lastly, we observed a deficit in orientation-selective habituation (OSH) behavior in the TSC2 Het mice (Fig. 4.4). This represents an exciting finding as humans with TSC display learning deficits including in attention-related tasks (de Vries and Watson 2008, Ehninger, de Vries et al. 2009, Curatolo, Moavero et al. 2015). It is peculiar though that TSC2 Het mice showed normal VEP stimulus selectivity (Fig. 4.3J), but were unable to show behavioral discrimination for familiar and novel stimuli (Fig. 4.4). While it was recently shown that SRP in V1 is necessary for orientation-selective habituation behavior (Cooke, Komorowski et al. 2015), SRP has not been shown to be sufficient. Therefore, there may be other experience-dependent synaptic modification occurring elsewhere in the brain, which work in conjunction with SRP to

elicit OSH. These additional mechanisms downstream of the primary visual cortex may be altered in TSC2 Het mice and lead to deficiencies in visual recognition memory.

4.5: Materials and Methods

4.5.1: Animals and drug treatment. All procedures adhered to the guidelines of the National Institutes of Health and were approved by the Committee on Animal Care at MIT, Cambridge, MA, USA. For all experiments mice were male and on a C57BL/6 background (Charles River laboratory international, Wilmington, MA). They were housed in groups of 2-5 with food and water available ad libitum and maintained on a 12 hour light-dark cycle. Male and female $Grm5^{+/-}$ mice (Jackson Labs) were bred on a C57BL/6 background, yielding $Grm5^{-/-}$, $Grm5^{+/-}$, and $Grm5^{+/+}$ (wild-type, WT) littermates. TSC2 Het male and female mutant mice (Jackson labs: B6;129S4-$Tsc2^{tm1Djk}$/J) on the C57Bl/6J clonal background were bred with C57Bl/6J WT partners to produce the WT and TSC2 Het offspring used in this study. MeCP2 KO male mice used in this study were derived from breeding MeCP2 heterozygous female mice (Jackson labs: B6.129P2(C)-$Mecp2^{tm1.1Bird}$/J) to C57Bl/6J WT male partners. All experiments were performed on male littermate controls. All experiments were performed by an experimenter blind to genotype and/ or to CTEP treatment. CTEP (Roche) was formulated as a microsuspension in vehicle (0.9% NaCl, 0.3% Tween-80). Chronic treatment consisted of once per 48 h dosing at 2 mg/kg (s.c.), as described previously (Lindemann, Jaeschke et al. 2011).

4.5.2: Electrophysiological recordings and Western blotting. *In vivo* VEP recordings were described previously in chapter 2. Extracellular field potential recordings for LTD experiments from layer 4 or layer 2/3, as noted, were obtained using an interface chamber using standard methods (Philpot, Cho et al. 2007). P21-P30 mice were used for comparisons of genotype, and this age was restricted to P21-P25 in CTEP experiments. Intracellular recordings were obtained from layer 4 pyramidal neurons using P21-25 mice using a submersion chamber. AMPA/NMDA ratio (Myme, Sugino et al. 2003), E/I balance (Dong, Shao et al. 2004), and NMDA decay (Philpot, Cho et al. 2007) experiments were performed essentially as described. Visually-evoked potential (VEP) electrode implantation, electrophysiological recordings and analysis were performed as previously described (Frenkel and Bear 2004, Cooke, Komorowski et al. 2015). Briefly, for ocular dominance (OD) plasticity experiments, novel oriented visual stimuli were used for recordings both before and after the three day deprivation period. For stimulus-selective response potentiation (SRP) experiments, a visual stimulus of specific orientation was

presented each day; except on the final experimental day, where blocks of a novel oriented stimulus were interleaved. Detailed information regarding slice preparation, extracellular LTD recordings and analysis, intracellular recordings and analysis, in vivo recordings and analysis, and Western blotting are described in SI Materials and Methods.

4.5.3: Statistics. To determine if there were significant differences between groups, one-way ANOVA was used, followed by post-hoc Student-Newman-Keuls tests. For experiments comparing two conditions (*e.g.* CTEP vs. vehicle), Student's t-test was used. To test whether significant depression occurred within an experimental group, paired Student's t-test was used on raw (non-normalized) field potential magnitudes. For MD experiments, two-way repeated measures ANOVA was used with treatment and time as factors to determine if there was significant depression of the contralateral VEP with MD. MANOVA was used to test whether CTEP treatment affected contralateral eye depression and ipsilateral eye potentiation. For all LTD and in vivo experiments, n represents number of animals. 1-3 slice recordings (LTD) or 1-2 hemispheres (SRP) were averaged together per animal. For intracellular current recordings, n represents number of cells. For all figures, * indicates $p<.05$ and error bars indicate SEM. Outliers more than two standard deviations from the mean were excluded.

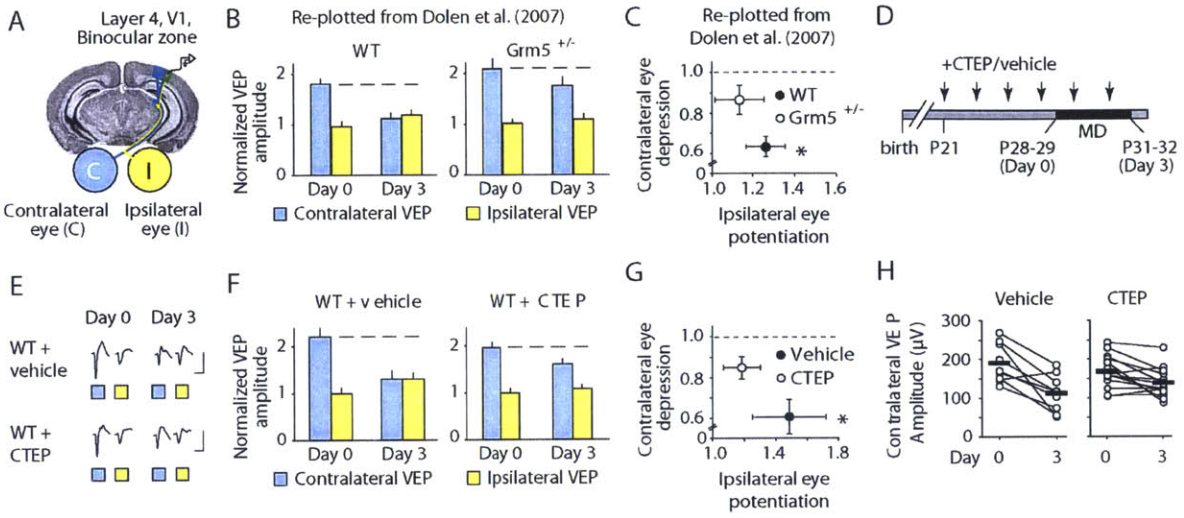

Figure 4.1.1: Chronic inhibition of mGluR5 impairs deprived-eye depression in wild-type mice. (A) Schematic of contralateral and ipsilateral-eye inputs to mouse binocular visual cortex. **(B-C)** In wild-type mice, three day monocular deprivation (MD) induces an ocular dominance shift which is expressed primarily as depression of VEP responses driven by the deprived- (contralateral-) eye. *Grm5*$^{+/-}$ mice display deficient deprived-eye depression. Data re-plotted from Dolen, et al. **(Dolen, Osterweil et al. 2007)**. **(D)** CTEP or vehicle treatment beginning at P21 and lasting throughout the duration of 3-day MD. **(E)** Averaged waveforms across all experiments, pre- and post-MD. Scale bars: 100 μV, 100 ms. **(F)** MD induced depression of the contralateral-eye driven VEP is impaired with CTEP compared to vehicle treatment. Data are normalized to day 0 ipsilateral response. **(G)** Average fractional changes in the contralateral and ipsilateral-eye driven VEP responses after MD. CTEP treatment had a significant effect on the magnitude of the ocular dominance shift. **(H)** Raw VEP amplitudes pre and post-MD plotted by animal. Error bars indicate SEM.

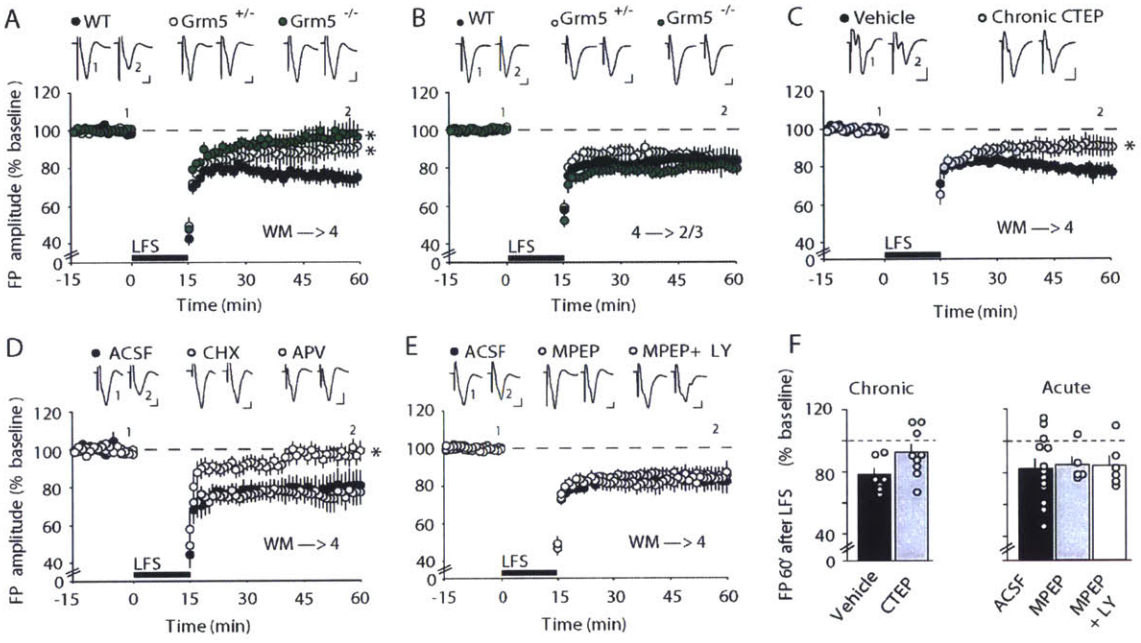

Figure 4.1.2: NMDAR-dependent LFS-LTD is impaired in layer 4 with genetic reduction and pharmacological inhibition of mGluR5. (A) LTD induced by stimulation of white matter and recording in layer 4 is significantly reduced in *Grm5*$^{-/-}$ *and Grm5*$^{+/-}$ mice. **(B)** The magnitude of LTD is similar in layer 2/3 across genotypes. **(C)** Chronic mGluR5 inhibition reduced the magnitude of LFS-induced LTD in layer 4 in wild-type mice. **(D)** LFS-LTD in layer 4 is NMDAR-dependent and not protein synthesis-dependent in WT animals. **(E)** Acute inhibition of group 1 mGluRs did not affect LFS-LTD. **(F)** Summary of LTD experiments. For all figures, displayed traces were averaged across all experiments and scale bars: 0.2 mV, 50 ms. Error bars indicate SEM (Experiments performed by Michael S. Sidorov).

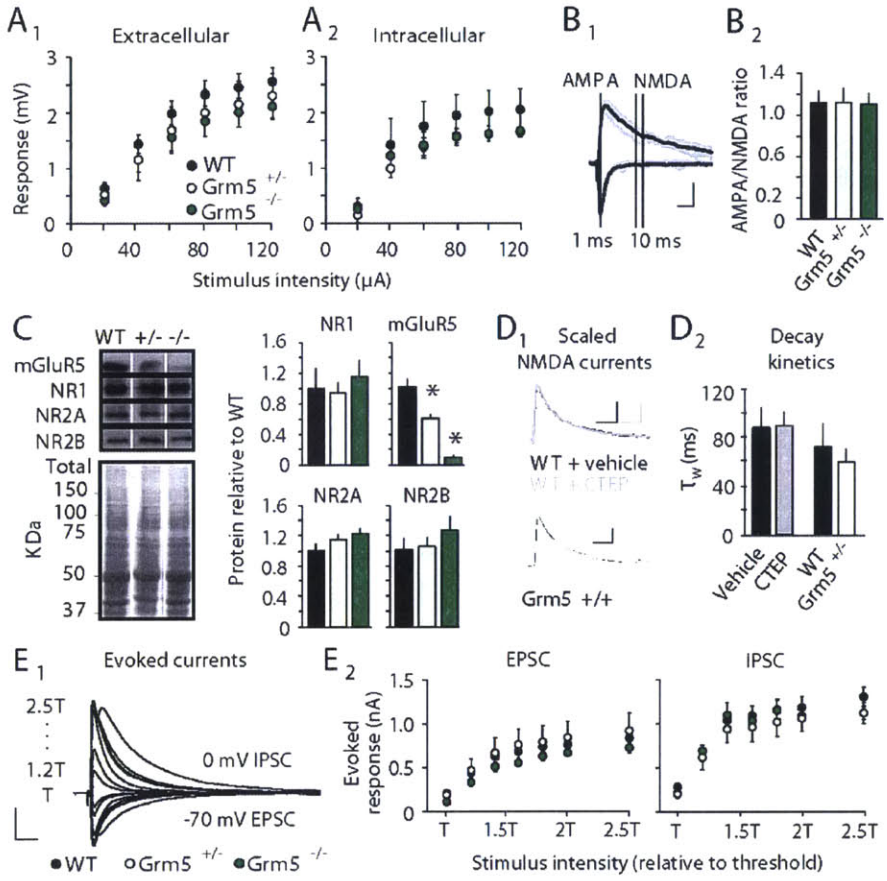

Figure 4.1.3: NMDA receptor function is normal in layer 4 with chronic mGluR5 downregulation. Input/output functions from **(A_1)** extracellular field potential LTD experiments and **(A_2)** intracellular voltage clamp recordings showed no change in basal synaptic transmission in *Grm5*$^{+/-}$ or *Grm5*$^{-/-}$ mice compared to wild-type. **(B_1)** AMPA/NMDA ratio in layer 4 was calculated by comparing AMPA-only responses to NMDA-only responses. The AMPA-only component of the response at +40 mV was taken from a 1 ms window corresponding to the peak at -70 mV, and the NMDA-only response was taken from a 10 ms window at +40 mV where no AMPA response was present. Scale bar: 25 ms, 50 pA. **(B_2)** AMPA/NMDA ratio was normal in *Grm5*$^{+/-}$ and *Grm5*$^{-/-}$ neurons. **(C)** Levels of NR1 protein were normal in *Grm5*$^{+/-}$ and *Grm5*$^{-/-}$ visual cortical slices. Levels of mGluR5 protein were reduced. Levels of NR2A protein and NR2B protein were normal. **(D_1)** NMDA currents were isolated at +40 mV in the presence of NBQX. Scale bar: 50 ms, 50 pA. **(D_2)** The weighted decay constant of NMDA currents was similar between WT mice treated chronically with vehicle and CTEP, and between WT mice and *Grm5* mutants. **(E_1)** Evoked IPSCs and EPSCs were isolated in layer 4 neurons by holding cells at 0 mV and -70 mV, respectively. White matter stimulation yielded a threshold stimulation intensity required to evoke responses in layer 4 ("T"). The amplitude of evoked IPSCs and EPSCs were recorded as a function of stimulation intensity relative to threshold. Scale bar: 200 ms, 50 pA. There was no effect of *Grm5* genotype on **(E_2)** evoked EPSC amplitude or evoked IPSC amplitude. Error bars indicate SEM (Experiments performed by Michael S. Sidorov and Emily K. Osterweil).

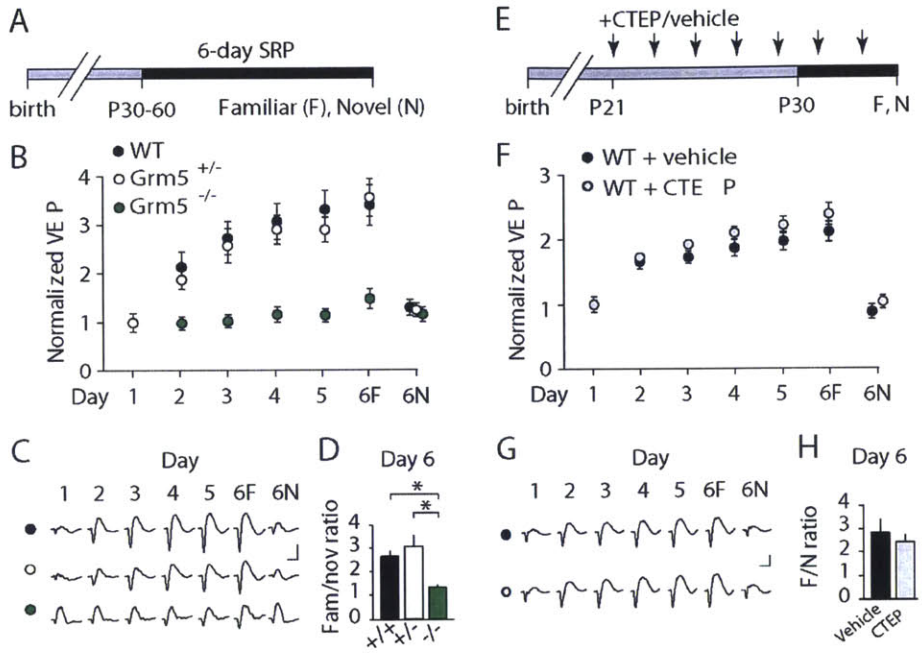

Figure 4.1.4: SRP persists in *Grm5*$^{+/-}$ and CTEP-treated mice but is impaired in *Grm5*$^{-/-}$ mice. (A) SRP was induced by presentation of a familiar stimulus (45° - "F") on six consecutive days, followed by interleaved presentation of a novel stimulus (135° - "N") on test day 6. **(B)** SRP (normalized to day 1 VEP within group) was significantly impaired in *Grm5*$^{-/-}$ mice. **(C)** Averaged VEPs across test days. **(D)** *Grm5*$^{-/-}$ mice showed significant impairments in distinguishing familiar from novel stimuli. **(E)** SRP was induced beginning on P30 following chronic CTEP or vehicle treatment, which began at P21. **(F)** SRP magnitude was not significantly different between CTEP and vehicle-treated wild-type mice. **(G)** Averaged VEPs across test days. **(H)** Chronic CTEP did not affect the ability to distinguish familiar from novel stimuli (n = 7 mice). Scale bars: 100 ms, 100 μV. Error bars indicate SEM (Experiments performed by Michael S. Sidorov).

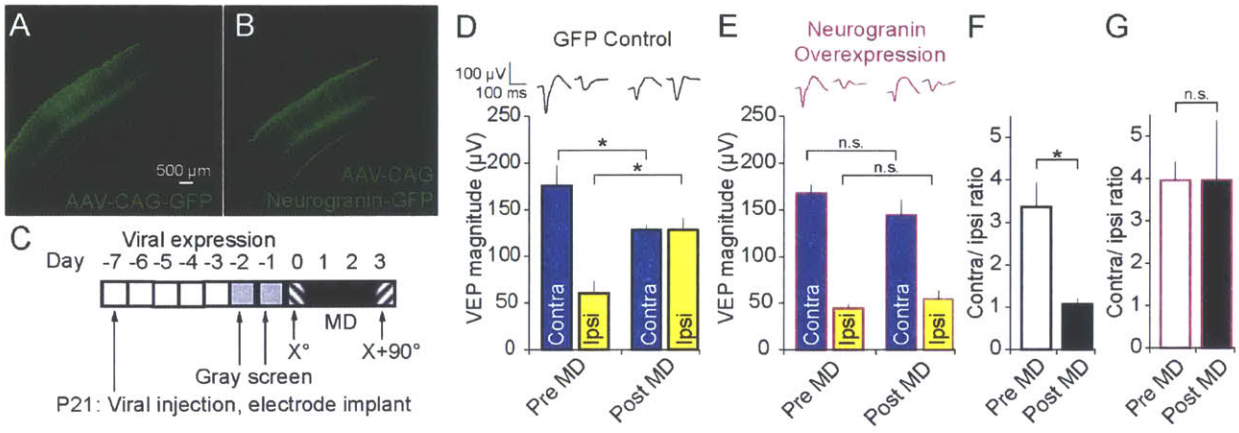

Figure 4.2: Neurogranin overexpression in binocular visual cortex disrupts juvenile ocular dominance plasticity. (A) Viral-mediated expression of AAV GFP control virus in binocular visual cortex. **(B)** Viral-mediated expression of neurogranin overexpression virus in binocular visual cortex. **(C)** Experimental timeline illustrating P21 wild-type (WT) mice were locally infected with AAV virus and implanted with electrodes in binocular visual cortex. One week elapsed to allow for viral-mediated protein expression, during which the mice recovered from surgery and experienced head fixation while viewing a gray screen. On day 0, VEPs were acquired as mice viewed an X^{o} oriented visual stimulus alternately through the contralateral and ipsilateral eyes. Monocular deprivation of the contralateral eye occurred for 3 days, at which point the eye was reopened and VEPs were recorded to an $X + 90^{o}$ stimulus. **(D)** Mice receiving the AAV GFP control virus showed a robust ocular dominance (OD) shift, characterized by both deprived-eye depression and ipsilateral-eye potentiation. Average VEP waveforms are displayed above. **(E)** Mice receiving the AAV-mediated neurogranin overexpression did not display a significant OD shift. **(F)** A shift in the contralateral/ ipsilateral-eye driven VEP ratio (contra/ ipsi ratio) is apparent in the GFP control mice. **(G)** No shift in the contra/ ipsi ratio is observed in the mice overexpressing neurogranin. Error bars indicate SEM (Experiments performed by Eitan S. Kaplan and Kyung Seok Han).

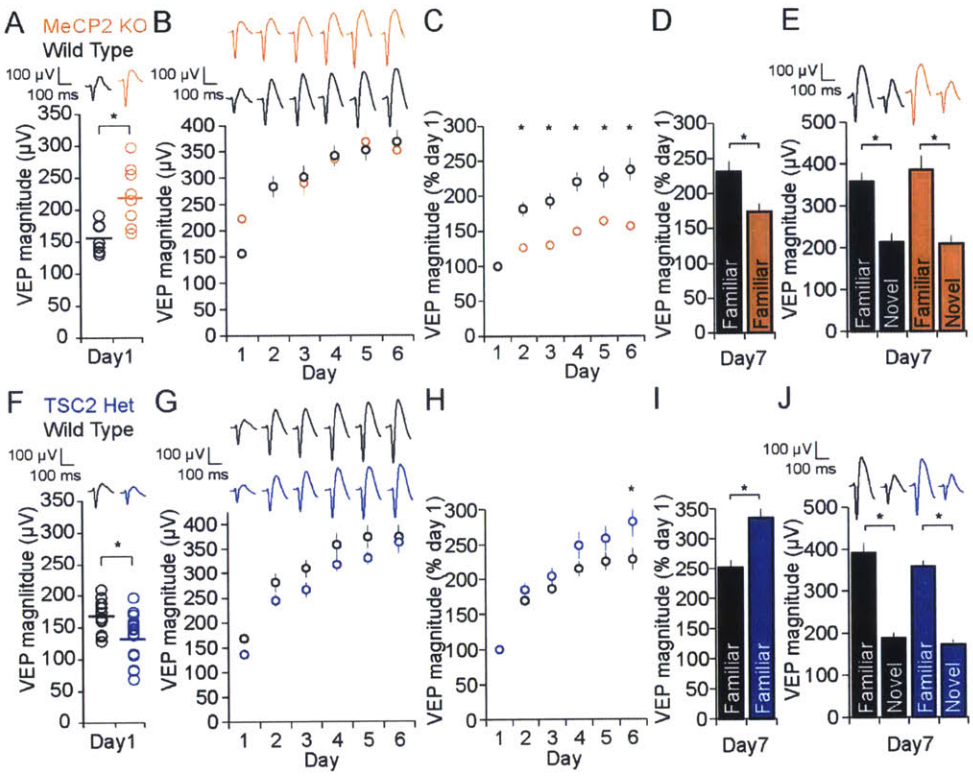

Figure 4.3: MeCP2 KO mice and TSC2 Het mice display contrasting baseline VEP magnitude and SRP phenotypes. (A) Baseline average VEP magnitudes were significantly elevated in MeCP2 KO mice compared to wild-type (WT) littermate controls. VEPs were recorded from awake, head-fixed mice binocularly viewing a phase-reversing sinusoidal grating of a particular orientation. Individual circles represent each individual animal's average VEP on day 1. Averaged VEPs waveforms for MeCP2 KO and wild-type littermates are shown above. **(B)** VEPs were recorded daily as mice repeatedly viewed the phase-reversing X° oriented visual stimulus. Averaged VEP waveforms for MeCP2 KO and wild-type littermates are shown above. **(C)** MeCP2 KO mice display significantly less gain in VEP magnitude over days compared to their littermates, when viewing the X° stimulus. This is evident when VEP magnitude is normalized to day 1. **(D)** On day 7, MeCP2 KO mice show significantly less VEP magnitude potentiation to the familiar stimulus than wild-type littermates. **(E)** On day7, mice viewed the familiar (X°) oriented stimulus, as well as interleaved presentations of a novel (X+90°) oriented visual stimulus. Both MeCP2 KO mice and wild-type littermates exhibited significantly larger VEPs elicited by the now familiar stimulus compared to a novel stimulus. **(F)** In contrast to the MeCP2 KO mice, baseline average VEPs were significantly reduced in TSC2 Het mice compared to wild-type littermate controls. Averaged VEP waveforms for MeCP2 KO and wild-type littermates are shown above. **(G)** VEPs were recorded daily as mice repeatedly viewed the phase-reversing X° oriented visual stimulus. Averaged VEPs for TSC2 Het and wild-type littermates are shown above. **(H)** TSC2 mice display significantly greater gain in VEP magnitude over days compared to their littermates, when viewing the X° stimulus. This is evident when VEP magnitude is normalized to day 1. **(I)** On day 7, TSC2 Het mice show significantly greater VEP magnitude potentiation to the familiar stimulus than wild-type littermates. **(J)** On day7, mice viewed the familiar (X°) oriented stimulus, as well as interleaved presentations of a novel (X+90°) oriented visual stimulus. Both TSC2 Het mice and wild-type littermates exhibited significantly larger VEPs elicited by the now familiar stimulus compared to a novel stimulus. Scale bars: 100 ms, 100 µV. Error bars indicate SEM.

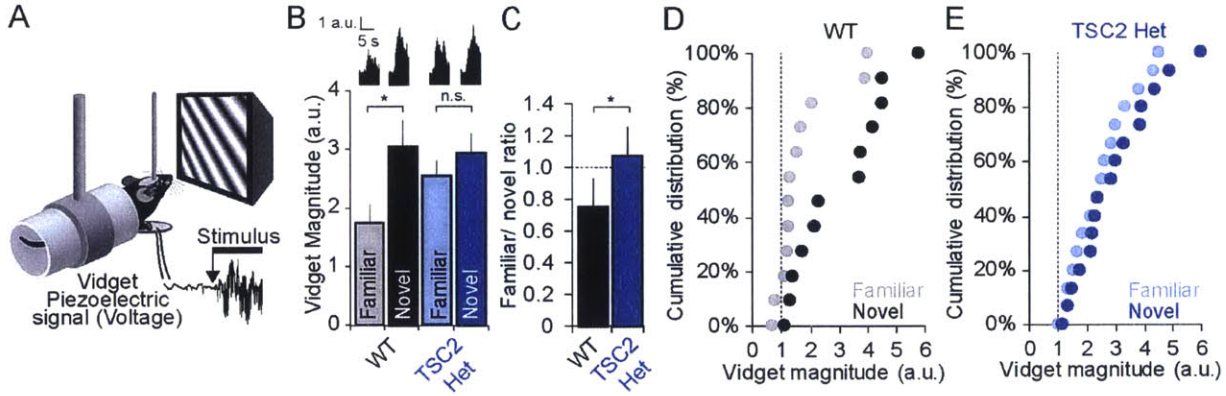

Figure 4.4: TSC2 Het mice display deficit in discrimination of familiar and novel stimuli. (A) Using a standard protocol for stimulus-selective response potentiation (SRP), mice were progressively familiarized with a specific oriented stimulus over a week. On the test day, as mice viewed familiar and novel stimuli, vidget behavioral responses were measured via a piezoelectric sensor located beneath the forepaws of the head-fixed mouse. **(B)** A deficit in OSH was apparent in TSC2 Het mice as vidget recordings demonstrated a failure to significantly discriminate familiar (light blue bar) from novel orientations (dark blue bar). WT littermates exhibited significantly greater vidget magnitudes for novel (black bar) compared to familiar stimuli (gray bar), indicating unimpaired discrimination of familiarity from novelty. Averaged behavioral responses are displayed above with accompanying scale bars. **(C)** The significant deficit of TSC2 Het mice in discriminating familiar from novel stimuli is apparent in the ratio of behavior elicited by the familiar over the novel stimulus in comparison to WT littermates. **(D)** Cumulative distributions of average vidget behavioral response to the familiar stimulus (gray) and the novel stimulus (black) of each individual WT mouse. **(E)** Average vidget behavioral response of each TSC2 Het mouse to the familiar stimulus (light blue) and the novel stimulus (dark blue), revealing deficit in discrimination of familiar and novel stimuli. Dotted line represents behavior no greater than pre stimulus baseline. Significant comparisons are marked with an asterisk throughout while non-significant comparisons are marked with n.s. Error bars are standard error of the mean (S.E.M.).

Chapter 5

Implications and future directions

5.1: Introduction

The study of experience-dependent plasticity paradigms *in vivo* can enable the mechanistic dissection of learning and memory processes in the brain (Martin, Grimwood et al. 2000). Furthermore, these synaptic modifications can be altered in models of neurodevelopmental disorders, and thereby provide a means to understand synaptic pathophysiology which lead to abnormal behavioral phenotypes in humans (Newschaffer, Croen et al. 2007, Peca and Feng 2012). Here we studied the contribution of PV+ inhibitory GABAergic interneurons to the expression of two forms of experience-dependent plasticity in adult V1. The intact adult brain is capable of multiple forms of cortical plasticity, which are enabled by distinct processes at the cellular and molecular levels. The expression of stimulus-selective response potentiation (SRP) requires the involvement of PV+ interneuron activity. Additionally, NMDA receptors in these cells appear to play a necessary role in the function of the PV+ interneuron circuit. These finding implicate a specific cell type, which likely comprises ~8% of total cerebral cortical neurons (Markram, Toledo-Rodriguez et al. 2004), in the cognitive process of distinguishing previously experienced environmental stimuli from those which are unfamiliar. Further understanding of this process at the synaptic, cellular, and behavioral level may greatly inform the understanding of atypical circuit function in schizophrenia. We also established that the expression of the adult ocular dominance (OD) shift does not require the contribution of PV+ interneurons in V1. This finding paints an interesting picture of a dynamic landscape of activity-dependent plasticity in the brain throughout development. The mechanistic underpinnings of OD plasticity in the juvenile animal appear to be rather distinct from that which occurs in the adult. Furthermore, there may be unique roles for inhibitory and excitatory circuits embedded in the induction and expression phases of plasticity and learning.

We have found here several interesting experience-dependent plasticity phenotypes that illuminate the function of specific genes implicated in neurodevelopmental disorders. Metabotropic glutamate receptor (mGluR) signaling, which has been associated with autism spectrum disorders (ASDs) (Bear, Huber et al. 2004), was shown here to be required for NMDAR-dependent synaptic weakening *in vivo* and *in vitro*. A deficit in juvenile OD plasticity was also observed by overexpressing the synaptic protein neurogranin in V1. Because mutations in neurogranin have been shown to occur in some individuals with schizophrenia, this finding may inform the learning deficits and prefrontal cortex abnormalities observed in this disorder (Braff, Swerdlow et al. 1995, Stefansson, Ophoff et al. 2009, Bakhshi and Chance

2015, Gonzalez-Burgos, Cho et al. 2015). Lastly, we probed two models of ASDs, which have been associated with excitatory/ inhibitory imbalance, Rett syndrome and tuberous sclerosis complex (Chao, Chen et al. 2010, Curatolo, Moavero et al. 2015, Krishnan, Wang et al. 2015, Wong, Denkers et al. 2015). We found abnormal VEP magnitude and SRP phenotypes in both of these mutant mouse lines, but surprisingly their abnormal phenotypes were in opposite directions. MeCP2 KO mice, which are a model of Rett syndrome, displayed baseline visually evoked potentials of enhanced magnitude, and reduced potentiation to a familiar stimulus over days. Based on our findings as well as other evidence, these mice may show enhanced excitation due to a dysfunctional PV+ interneuron circuit (Durand, Patrizi et al. 2012). TSC2 Het mice displayed the opposite phenotype in V1, including enhanced SRP. This may be related to increased intracortical inhibition or the known LTP-facilitating action of deleting a copy of TSC2 (Cooke and Bear 2010, Auerbach, Osterweil et al. 2011). Interestingly, these mice also displayed a profound behavioral deficit in distinguishing familiar and novel stimuli. By further investigating the mechanisms that give rise to abnormal cortical plasticity found in these mouse models of disease, we will be better equipped to understand these debilitating developmental disorders and design targeted therapeutic treatments.

5.2: SRP and PV+ inhibitory interneurons

The ability to discern familiar and novel environmental stimuli is a cognitive ability of great significance to all animals. Two forms of implicit (non-declarative) memory, known as perceptual learning and habitation are involved in an animal's ability to discriminate between familiar and novel, and subsequently guide behavior. Repeated exposure to environmental stimuli can alter how those stimuli are perceived. Generally this exposure results in improved perception, whereby a stimulus becomes easier to recognize and subtle differences in the stimulus become more apparent. This process is known as perceptual learning (Watanabe and Sasaki 2015). As an example, consider a scenario in which an individual purchases a litter of young golden retriever puppies. At first, the puppies all appear rather identical to the buyer; however, after days of repeated exposure the buyer begins to distinguish each of the puppies upon quick visual inspection. It is known that supplemental sensory experience can induce reorganization of receptive field maps in various primary sensory cortical areas (Recanzone, Schreiner et al. 1993, Elbert, Pantev et al. 1995). Indeed, this primary sensory plasticity is believed to underlie some forms of perceptual learning. Perceptual learning allows animals to quickly detect and discriminate stimuli in the environment, which can alter attention and guide

behavior. Another form of implicit learning related to the discrimination of stimuli, is habituation. Habituation is the decrease in the strength or occurrence of a behavior after repeated exposure to an environmental stimulus. It is a vital form of learning for distinguishing dynamic/ meaningful environmental stimuli from irrelevant ones, and is advantageous because it reduces the amount of attention and energy needed to address familiar stimuli known to lack danger or significance (Thompson 2009). Like perceptual learning, habituation can occur unconsciously and without any explicit training. A common example of habituation learning, involves the rapid unconscious disregard of a repetitive sound, such as the ticking of a clock. Perceptual learning is believed to inform habituation, in that once an organism can rapidly recognize a familiar stimulus, it can be hastily deemed unimportant, and consequently ignored by the animal.

Stimulus-selective response potentiation occurs in primary visual cortex as a result of repeated exposure to a particular visual stimulus. This form of experience-dependent plasticity bears all the hallmarks of perceptual learning (Cooke and Bear 2010, Cooke and Bear 2014). Additionally, SRP is known to be required for habituation of a visually-induced behavioral response in a head-fixed mouse (Cooke, Komorowski et al. 2015). It was previously found that manipulations that inhibited SRP induction, were shared with those that blocked NMDAR-dependent LTP, such as NMDA receptor antagonism or the perturbation of AMPA receptor membrane insertion (Frenkel, Sawtell et al. 2006). Although these findings implied a straightforward explanation of SRP as LTP of thalamocortical synapses driven by a particular stimulus, we decided to investigate a possible role for PV+ interneuron inhibition. PV+ interneurons are known to be involved in the generation of gamma frequency oscillations in the cortex, which are associated with cognitive functions such as attention, and increased ability to detect environmental stimuli (Engel and Singer 2001, Bartos, Vida et al. 2007, Siegle, Pritchett et al. 2014, Pritchett, Siegle et al. 2015). The activity of PV+ interneurons themselves has also been reported to enhance perception and discrimination of stimuli (Lee, Kwan et al. 2012, Pritchett, Siegle et al. 2015). Therefore, we hypothesized that these cells may be required for the expression of SRP and the retrieval of information necessary for its behavioral correlate.

We discovered that PV+ interneuron activity, and the NMDA receptors expressed within these cells are required for the expression of SRP. In the case of SRP, the PV+ interneuron circuit appears to undergo plasticity as a result of repeated exposure to a specific oriented stimulus. It is possible that this exposure eventually results in the familiar stimulus inducing less recruitment of PV+ cells, causing an increased VEP response and decreased power in the

gamma band of the local field potential. A behavioral habituation to this stimulus is likely engaged via mechanisms of SRP and other processes downstream of V1. It is vital to consider that PV+ interneuron activity may be necessary for the expression of SRP, but that the mechanisms of induction may be dissimilar. There are many known forms of learning and synaptic plasticity in which the mechanisms underlying the expression of that plasticity differ from those required for its induction. A classic example is the discovery that NMDA receptor activity is required for LTP of the hippocampal Schaffer collateral pathway during the induction protocol, but is unnecessary for the expression or maintenance of subsequent potentiation (Collingridge, Kehl et al. 1983). Indeed this is also true of NMDA receptor activity for the induction of some forms of learning (Morris 1989). In the case of SRP, it is known that LTP-like mechanisms are involved, and that these alterations occur in excitatory cells (Frenkel, Sawtell et al. 2006). SRP may be quite complex in that synaptic alterations between excitatory cells may underlie the induction and information storage components of SRP. However, the expression/ retrieval of this plasticity may require cortical activity to be precisely coordinated via the modulation of PV+ cell circuit activity and gamma frequency oscillations. PV+ interneurons' connectivity and intrinsic properties allow them to influence the flow of information in the cortex precisely (Kepecs and Fishell 2014). The modulation of this PV+ interneuron network may therefore play a key role in allowing the information held in the excitatory synapses, to be expressed in the appropriate circumstances.

Additional experiments will be needed to gain a confident understanding of the role of PV+ interneurons in the processes underlying SRP. We have taken an interventionist approach by altering the activity of PV+ interneurons via pharmacological, genetic, pharmacogenetic and optogenetic techniques. By utilizing these approaches we have provided substantial evidence for the importance of PV+ cells in the expression of this form of experience-dependent plasticity. However, it will be important to monitor the activity of these cells *in vivo* over days as an animal becomes familiar with a visual stimulus. It will also be useful to understand the activity of PV+ cells as an animal views familiar and novel stimuli. Based on our experiments, we have a simple hypothesis that PV+ interneurons will be less active while the animal is viewing a familiar stimulus, which contributes to the observed increase in VEP magnitude. However, the role of these cells may be more nuanced and complex. Techniques such as *in vivo* calcium imaging and *in vivo* patch clamp recordings in awake mice could provide useful information to test our hypothesis concerning PV+ cell involvement.

While the mechanisms underlying SRP are interesting in that these are processes mediating familiarity discrimination and habituation, they are also significant based on the abnormalities reported in schizophrenia. Individuals with schizophrenia have been reported to have learning deficits in familiarity, working memory, and habituation (Park and Holzman 1992, Braff, Swerdlow et al. 1995, Park and Gooding 2014, Bakhshi and Chance 2015). Patients also exhibit abnormalities in cortical plasticity as well as gamma frequency oscillations (Cavus, Reinhart et al. 2012, Gonzalez-Burgos, Cho et al. 2015). Furthermore, studies have found alterations in PV+ interneurons and evidence of the hypofunction of NMDA receptors within PV+ cells (Krystal, Karper et al. 1994, Coyle, Tsai et al. 2003, Hashimoto, Volk et al. 2003, Lewis, Hashimoto et al. 2005, Coyle 2006). Based on the findings reported here concerning SRP and its related habituation behavior, we can hypothesize that these stated abnormalities in schizophrenia are functionally related, as we have implicated PV+ cells in the expression of a form of cortical plasticity, which is necessary for familiarity recognition/ novelty detection. It may be possible that individuals with schizophrenia have deficits in the human correlate of SRP, similar to what was observed in the PV-GluN1 KO mice. Although it is likely that the cognitive dysfunction observed in those with schizophrenia is independent of any processes in V1, the same mechanisms of experience-dependent plasticity we are studying in the visual system may be shared with other sensory cortices, the prefrontal cortex, or in subcortical structures. Therefore, by comprehending the relationship between PV+ interneurons and SRP, we may be better equipped to understand the synaptic pathophysiology in schizophrenia, which leads to abnormal cognitive and behavioral phenotypes. The results reported here reinforce that familiarity discrimination, perceptual learning, and habituation depend on the functions of NMDA receptors within PV+ interneurons in the cortex. These receptors may constitute a target for treatment of the cognitive symptoms of schizophrenia.

5.3: Adult OD plasticity

Ocular dominance plasticity, which has been studied for over 50 years, remains an important model of how experience can alter the brain. Importantly, enough progress has been made in understanding its mechanisms, that OD plasticity is now understood to occur via distinct mechanism depending on the developmental age of the animal (Hubener and Bonhoeffer 2014). This is particularly interesting because it implies that completely distinct mechanisms underlying learning and memory may operate depending on an animal's age. In terms of ocular dominance plasticity in the adult mouse, only potentiation of the non-deprived

eye is observed, and there is evidence that LTP-like mechanisms may be involved (Sawtell, Frenkel et al. 2003, Ranson, Cheetham et al. 2012). Indeed it is known that that NMDAR-dependent LTP occurs in layer 4 of visual cortex of the adult animal (Kirkwood and Bear 1994, Heynen and Bear 2001). There is also evidence that the expression of this potentiation does not rely on PV+ interneuron inhibition (Saiepour, Rajendran et al. 2015). Experiments conducted here support this conclusion, as we did not observe an effect on the OD shift by any of the PV+ interneuron manipulations we conducted. Further experiments will be necessary to confirm that non-deprived eye potentiation in the adult animal occurs via LTP-like processes, and to assay whether these processes are present at the level of the thalamocortical synapse. In order to better understand the site of plasticity, the thalamocortical VEP could be isolated after adult deprivation, as was previously accomplished when studying deprived eye depression in the juvenile mouse (Khibnik, Cho et al. 2010). If the plasticity needed for the expression of the OD shift does indeed occur at the thalamocortical synapse, silencing intracortical activity should leave the post-deprivation OD ratio intact. Additionally, it would be helpful to assay whether LTP induced *in vivo* could occlude non-deprived eye potentiation and vice versa. This could be accomplished either by electrical or optogenetic high frequency stimulation of the thalamus. These experiments and others, in which the known mechanisms of LTP are interfered with, could bolster the hypothesis that LTP mechanisms underlie deprivation-induced potentiation in adult V1. It will also be important to assay the contribution of interneurons including PV+ cells, to the induction mechanisms involved in adult OD plasticity. Based on previous studies which have observed changes in inhibitory synapses during adult deprivation (Chen, Lin et al. 2011, Chen, Villa et al. 2012), it is not unrealistic to imagine a scenario in which changes in inhibitory networks are needed in the induction phase of the adult OD shift. Additional experiments aimed at monitoring the activity of interneuron subtypes during the OD shift could shed light on this aspect of deprivation induced plasticity.

An abundance of studies have focused on the mechanisms by which juvenile-like plasticity can be "re-instated" in an adult animal (Hensch 2005, Sale, Maya Vetencourt et al. 2007, Maya Vetencourt, Sale et al. 2008, Harauzov, Spolidoro et al. 2010, Kuhlman, Olivas et al. 2013). This work has implicated inhibition in gating the plasticity characteristic of the juvenile animal, i.e. the depression of deprived eye responses, and a generally greater level of plasticity. These studies may be of interest in terms of promoting recovery subsequent to injury of the adult human brain (Greifzu, Pielecka-Fortuna et al. 2014). Indeed, increasing plasticity in the adult brain following physical trauma may allow for enhanced recovery of function. Studies

aimed at re-opening the critical period have even envisioned the possibility of enhancing learning in human adults via a reduction in cortical inhibition. It is important to reflect, however, on the purpose of developmental critical periods to begin with, and why manipulating the rules and constraints of this type of plasticity in the adult brain may be inadvisable or undesirable. At the time of birth, neural connections throughout brain systems are commonly immature. Experience is necessary to modify synaptic connections and the strength of those connections. The influence of sensory experience on neural wiring is potent during phases of an animal's early life, after which the same environmental input has less effect. These time periods are referred to as critical periods (Levelt and Hubener 2012). This heightened sensitivity of neural circuits makes the system adaptable, and are useful in the initial construction of a functional brain. As an animal enters adulthood, the nervous system is well adapted to its contemporary environment based on sensory input and feedback it has acquired over time. By adulthood, considerable information has been gained and it is vital that it be properly stored and utilized. If neural circuits are made too malleable in the adult, vital information may be lost or corrupted. Therefore, besides the dangers inherent in reducing inhibition in the brain, which concern promoting epileptic activity, the "re-opening" of the critical period in adult humans could potentially be damaging to previously stored information. Alternatively, it may be more useful to understand the processes that serve learning in the adult brain. There may be other interventions that could promote the mechanisms of synaptic plasticity and learning already at work in the adult, which may be more desirable. Therefore it is critical to better understand the mechanisms of synaptic plasticity and learning in the adult animal.

5.4: Cortical plasticity paradigms and the study of neurodevelopmental disorders

We have taken the approach of studying how alterations in genes which are implicated in ASDs and schizophrenia may affect experience-dependent cortical plasticity. Although the mechanisms that contribute to both OD plasticity and SRP are not fully understood, we do at least know some of the particular synaptic proteins and cell types involved. Therefore, we can examine whether the loss or gain of function of a gene implicated in a neurodevelopmental disorder interferes with these mechanisms. This approach enables an understanding of the downstream effects of these specific genetic insults, and may illuminate synaptic pathophysiology that is shared between neurodevelopmental disorders (Auerbach, Osterweil et al. 2011, de Lacy and King 2013, Baudouin 2014). In the case of ASDs, several genetic factors have been associated with the disease. Interestingly, the majority of these are directly related to

the structure and function of the synapse (Bailey, Phillips et al. 1996, Peca and Feng 2012). These include alterations in mGluR signaling (Bear, Huber et al. 2004, Baudouin 2014), NMDARs (Lee, Choi et al. 2015), GABAergic system proteins (Pizzarelli and Cherubini 2011), scaffolding molecules (Peca and Feng 2012, Baudouin 2014), and regulators of synaptic protein synthesis (Auerbach, Osterweil et al. 2011, Bhakar, Dolen et al. 2012).

Results presented here linked the developmental signaling downstream of mGluR5 to NMDAR-dependent synaptic plasticity. Although we do not yet fully understand how these distinct glutamate receptors affect one another, there are several possibilities. For instance, it known that mGluRs activate phospholipase C, which can increase calcium levels and lead to alterations in the trafficking of ionotropic glutamate receptors (O'Connor, Bariselli et al. 2014). It is also been shown that mGluRs and NMDARs interact through scaffolding proteins such as homer, shank, SAPAP, and PSD95. Importantly, genetic mutations in many of these scaffolding proteins are themselves associated with ASDs (Peca, Feliciano et al. 2011, Peca, Ting et al. 2011). There is also a high prevalence of excitatory/ inhibitory imbalance in neurodevelopmental disorders, which may include alterations in excitatory or inhibitory synaptic function (Gogolla, Leblanc et al. 2009). Because of the highly interweaved nature of the signaling pathways at the synapse, it will important to recognize which proteins are most crucial to the development of functionally normal synapses, and which protein perturbations contribute to the most debilitating symptoms of these disorders. This information will be extremely useful for the development of pharmacological or other therapeutic interventions.

Our studies and many others point to the idea that perturbations of mGluR signaling or the misregulation of synaptic protein synthesis can lead to abnormalities in NMDAR-dependent plasticity and possibility GABAergic system dysfunction. In line with results reported here, other researchers have found evidence of NMDAR dysfunction in some cases of autism, including those involving *de novo* mutations in either the *GRIN2A* or *GRIN2B* genes, which code for the NR2A and NR2B subunits of the NMDAR (Tarabeux, Kebir et al. 2011, O'Roak, Vives et al. 2012). Furthermore, studies of mouse models of ASDs have found that drug treatments targeting NMDARs can be therapeutic (Lee, Kwan et al. 2012). Interestingly, these studies have found that either positive or negative modulation of NMDAR function is efficacious, depending on the type of ASD being modeled. This observation is similar to what has been reported for the therapeutic effects of modulating mGluR signaling, where some ASD models react positively to the enhancement of mGluR signaling (*e.g.* tuberous sclerosis complex), whereas others react

positively to a reduction in mGluR signaling (*e.g.* fragile-X syndrome) (Auerbach, Osterweil et al. 2011). The exploration of treatments targeting both NMDARs and mGluRs reaffirm the idea that ASDs represent a diverse collection of diseases in which bidirectional changes may result in similar behavioral phenotypes.

There is increasing evidence that the neurobiological underpinnings of ASDs and schizophrenia are related. Although historically the diagnoses of the disorders have been formulated based on psychiatric criteria; many of the same genetic insults including disruptions in NMDAR function and PV+ interneuron function, have now been associated with both disorder types (de Lacy and King 2013). Genome wide association studies have found candidate genes that are associated with both ASDs and schizophrenia including but not limited to: DISC1, MeCP2, GRIN2B, and GAD. If there are major similarities in the genetic alterations associated with these disorders, why are the onsets of these disorders set apart to different developmental stages, and why do individuals present with different behavioral phenotypes? One idea is that in humans, neural development is characterized by several critical periods, and that these disorders occur during two distinct critical periods of neurological development. Autism may arise during an earlier critical period in the first few years of life that is characterized by sensory system refinement, motor coordination, social awareness, as well as language development (LeBlanc and Fagiolini 2011). Schizophrenia on the other hand presents at a later age, during puberty or the transition into adulthood, which is characterized by its own neural rearrangements that include the development of executive function, response inhibition, and maturation of the prefrontal cortex. Why some individuals with the same genetic mutations present with neurological problems at different ages remains unclear. One hypothesis contends that those that develop schizophrenia instead of autism had greater "neural resources" to counteract or compensate for the effects of the genetic insult, and were therefore able to "hold off" the presentation of a neurological break for longer. What is likely, is that other genetic characteristics that vary between individuals as well as environmental factors contribute to the distinct presentation of one or the other of these disorders (de Lacy and King 2013). Understanding changes in how the disorder-implicated proteins function at the synapse through development and in different brain regions will advance our knowledge on both disorders in the future.

5.5: Concluding remarks

Experience-dependent plasticity paradigms allow researchers to investigate the mechanisms by which the brain is altered by environmental experience. By understanding how the mouse brain changes in response to experience, we can gain vital insight into humans' remarkable capacity to learn and utilize information. Here we have focused on two forms of experience-dependent plasticity that occur in the primary visual cortex of the adult mouse. Ocular dominance plasticity is enabled by depriving V1 of normal visual exposure through one of its main inputs, the contralateral eye. This form of plasticity occurs as the visual cortex adapts to dynamic changes in the quality of input it is receiving, and adjusts its responsiveness accordingly. Stimulus selective response potentiation on the other hand, is triggered by supplemental visual experience. This form of plasticity is intriguing as a basis for familiarity recognition/ novelty detection, which is a fundamental cognitive process. A central theme in this thesis was to understand the contribution of PV+ interneurons to the expression mechanisms underlying OD plasticity and SRP in the adult animal. The results suggest that the modulation of PV+ interneuron activity is required for the differential cortical responses, which are driven by familiar and novel visual stimuli. NMDA receptors in these PV+ interneurons are necessary for the expression of this form of plasticity. Conversely, PV+ interneuron activity is not required for the inherent contralateral-eye bias of evoked cortical responses in V1, or the shift in the responses evoked by the two eyes that can occur as a result of deprivation. Although we cannot rule out the possibility that PV+ interneurons play a role in the induction process, their activity is not needed for the expression of adult OD plasticity.

Another major goal of this thesis was to use experience-dependent plasticity paradigms to better understand the functions of synaptic proteins which have been implicated in autism spectrum disorders and schizophrenia. The contribution of PV+ interneurons to familiarity and habituation represents a link of particular significance to individuals with schizophrenia. Additionally, we found that the schizophrenia-associated protein neurogranin is capable of interfering with the experience-dependent plasticity in juvenile V1, and further supports its role as an LTD-opposing synaptic protein. We also revealed that signaling downstream of mGluR5 during development is required for NMDAR-dependent synaptic weakening in V1. This finding, which is of particular importance to ASDs, is an interesting window into the interconnectedness of neurotransmitter receptor signaling pathways at the synapse. Results described here also revealed cortical plasticity deficits in mouse models of Rett syndrome and tuberous sclerosis complex. Both of these disorders are associated with excitatory/ inhibitory imbalance and

epilepsy. For this reason we assayed SRP, which was previously shown to depend on PV+ interneuron activity. MeCP2 KO mice displayed increased magnitude VEPs and a deficit in SRP, which is consistent with a compromised PV+ interneuron circuit. Surprisingly, the TSC2 Het mice showed the opposite phenotype, characterized by decreased VEP magnitudes, and enhanced SRP. Intriguingly, although TSC2 mice showed selectivity for familiar and novel stimuli at the level of the VEP, they did not display a distinction at the behavioral level.

This thesis has touched on several interesting aspects of neural plasticity, learning, and memory. It is clear that the mechanisms of plasticity in the brain are not simple or uniform, but rather diverse and complex. The rules that govern plasticity in the cerebral cortex are dynamic and change as function of the age of the animal. These mechanisms can be quite varied in cases of both synaptic weakening and strengthening. Additionally, a seemingly simple and distinctive form of plasticity such as OD plasticity or SRP may include multiple sets of mechanisms that come into play during the stages of induction, maintenance, and expression of plasticity. These mechanisms also may vary between different cortical layers and cell types. The study of plasticity deficits in models of neurodevelopmental disorders will be aided in the future by the use of new technologies, including those which will allow for the investigation of plasticity processes in multiple brain areas simultaneously. This will enable a more cohesive approach to recognizing the circuits which give rise to behavioral abnormalities in these disorders. A major aspect of the research discussed in this thesis concerned the mechanisms involved in the *expression* of experience-dependent plasticity, which is directly related to the retrieval of information. Memory retrieval is an especially intriguing topic, which deserved considerable attention, as recent research into cases of extraordinary memory in humans, has singled out retrieval as the key factor in these individuals' superior memory abilities (LePort, Mattfeld et al. 2012). Future research in the field should continue to investigate the mechanisms which are vital to the expression of experience-dependent plasticity and the retrieval of stored information across various developmental ages.

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