ABSTRACT

Title of dissertation: REACTIVATION OF PLASTICITY BY DARK EXPOSURE PROMOTES ANATOMICAL AND PHYSIOLOGICAL RECOVERY FROM CHRONIC MONOCULAR DEPRIVATION IN ADULTS

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Chronic monocular deprivation, initiated early in postnatal life and maintained until adulthood, causes severe amblyopia, characterized by a significant decrease in strength and selectivity of visual cortical responses evoked by stimulation of the deprived eye. Amblyopia is highly resistant to reversal in adulthood, but binocular visual deprivation through dark exposure can be used to promote recovery from chronic monocular deprivation. To identify the locus of the changes in excitatory synaptic transmission that accompany the response to, and recovery from chronic monocular deprivation, I quantified the density of dendritic spines throughout the depth of the primary visual cortex. I demonstrate that chronic monocular deprivation induces a significant loss of dendritic spine density in all cortical laminae. Importantly, recovery of visual responses induced by dark exposure followed by reverse deprivation is accompanied by a significant recovery of dendritic spine density. As the majority of excitatory synaptic transmission is mediated by spine synapses, this suggests significant loss and recovery of excitatory synaptic density during loss and

recovery of vision. The observation that mid cortical laminae, which are enriched for thalamocortical synapses, participates in the recovery from chronic monocular deprivation in adulthood was unexpected, given that plasticity at thalamo-recipient synapses has been demonstrated to be constrained very early in postnatal life. Isolation of the thalamocortical component of the visually evoked potential via cortical silencing confirmed an experience-dependent strengthening during the recovery from amblyopia. This work further supports the hypothesis that dark exposure in adulthood returns the visual cortex to a “juvenile” state, capable of expressing plasticity at thalamocortical synapses.

Severe amblyopia is characterized by a loss of the strength and selectivity of visually evoked activity in primary visual cortex. The reduction in visually evoked responses recovers completely when dark exposure is followed by reverse deprivation (open deprived eye, close nondeprived eye). However, the recovery of spatial acuity, measured by performance in a spatial frequency discrimination task, is incomplete. Therefore, I designed a strategy to promote the strengthening of synapses serving the deprived eye that utilizes tetanic visual stimulation. Dark exposure followed by visual tetanus induced a significant strengthening of synapses serving the deprived eye. Importantly, the potentiation of visual responses generalized to novel stimuli without modifying stimulus selectivity. Subsequent repetitive performance of a two-choice spatial frequency discrimination task, promoted a recovery of orientation selectivity and spatial acuity. The combination of dark exposure (to reactivate plasticity), visual tetanus (to promote synaptic strength) and perceptual learning (to promote neuronal

stimulus selectivity) may accelerate and enhance recovery of visual functions, thereby optimizing the recovery from severe amblyopia.

REACTIVATION OF PLASTICITY BY DARK EXPOSURE PROMOTES ANATOMICAL AND PHYSIOLOGICAL RECOVERY FROM CHRONIC MONOCULAR DEPRIVATION IN ADULTS

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PREFACE

All physiology and anatomy experiments were conducted by Karen Montey. Behavior data was acquired in collaboration with Nicolette Eaton. Publications resulting from this work are listed below:

Montey KL, Quinlan EM.
Recovery from chronic monocular deprivation following reactivation of thalamocortical plasticity by dark exposure. Nat Comm. 2011;2(317): doi:10.1038/ncomms1312.

Chronic monocular deprivation induces severe amblyopia that is resistant to spontaneous reversal. However, dark exposure initiated in adulthood reactivates synaptic plasticity in the visual cortex and promotes recovery from chronic monocular deprivation in Long Evans rats. Here we show that chronic monocular deprivation induces a significant decrease in the density of dendritic spines on principal neurons throughout the deprived visual cortex. Nevertheless, dark exposure followed by reverse deprivation promotes the recovery of dendritic spine density of neurons in all laminae. Importantly, the ocular dominance of neurons in thalamo-recipient laminae of the cortex, and the amplitude of the thalamocortical visually evoked potential recover following dark exposure and reverse deprivation. Thus, dark exposure reactivates widespread synaptic plasticity in the adult visual cortex, including thalamocortical synapses, during the recovery from chronic monocular deprivation.

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List of Abbreviations

ACSF	artificial cerebrospinal fluid
AMPAR	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANOVA	analysis of variance
BDNF	brain-derived neurotrophic factor
CBI	contralateral bias index
cMD	chronic monocular deprivation
DE	dark exposure
EEG	Electroencephalography
fMRI	functional magnetic resonance imaging
GABA	γ-Aminobutyric acid
HSD	honestly significant difference
IPSC	inhibitory postsynaptic current
KS Test	Kolmogorov-Smirnov Test
LFS	low frequency stimulation
LGN	lateral geniculate nucleus
LTP	long-term potentiation
LTD	long-term depression
mEPSC	mini excitatory postsynaptic current
MD	monocular deprivation
NgR	nogo receptor
NMDAR	N-methyl-D-aspartate receptor
Non-REM	non-rapid-eye-movement
OD scores	ocular dominance scores
P	postnatal
PNNs	perineuronal nets
PSD	post-synaptic density
PSTH	post-stimulus time histogram
RD	reverse deprivation
rTMS	repetitive transcranial magnetic stimulation
SEM	standard error of the mean
SRP	stimulus-selective response potentiation
TBS	theta burst stimulation
tDCS	transcranial direct current stimulation
TTX	tetrodotoxin
VEP	visually evoked potential
VPL	visual perceptual learning
WGA	wheat germ agglutinin

CHAPTER 1: BACKGROUND/INTRODUCTION

1.1 The use of the rodent visual system as a model for visual impairments in higher mammals

1.1.1 Comparison of visual system features of the rodent and higher mammals

The spatial acuity of rodents is low (1.0 cycle/degree in adult rats; 0.5 cycles/degree in adult mice; Prusky et al., 2000) relative to higher mammals (6 cycles/degree in cats; 40 cycles/degree in macaques; Mitchell et al., 1976; Van Hooser, 2007; Patel et al., 2010). However, the short generation time, experimental accessibility and molecular/genetic tools make the rodent visual system a popular model to study visual cortical plasticity. Visual cortical neurons from rodents to humans share common properties, including the preferential activation by specific features of a visual stimulus (Braddick et al., 1986, 2005; Hubel and Wiesel, 1963; Dräger, 1975; Niell and Stryker, 2008; Wang et al., 2010a; Zariwala et al., 2011; Rochefort et al., 2011). Neuronal selectivity, such as preference for a specific orientation, emerges over postnatal development. In the rat, neuronal stimulus selectivity and spatial acuity reach adult levels by the seventh postnatal week (Fagiolini et al., 1994). A similar correlation between the establishment of neuronal stimulus selectivity and spatial acuity is apparent across mammals, including humans (Braddick and Atkinson, 2011; Ellemberg et al., 1999). However, as spatial acuity increases, visual cortical synaptic plasticity becomes constrained (Mitchell and MacKinnon, 2002; Fagiolini et al., 1994).

In contrast, neurons in the visual cortex of higher mammals including humans are organized into anatomical columns (Burkhalter et al., 1993; Hubel and Wiesel, 1968; LeVay et al., 1980). Neurons clustered within these columns share functional properties, such as ocular dominance or orientation selectivity. Indeed, ~95% of neurons in the mature cat visual cortex are orientation selective (Chapman and Stryker, 1993). Although neurons in the rodent visual cortex display stimulus selectivity, rodents lack columnar organization of functionally-matched neurons (Ohki et al., 2005; Van Hooser et al., 2005). However, the majority (~74%) of visual cortical neurons in binocular mice (> postnatal day 60; P60) are orientation selective (Niell and Stryker, 2008). While the criteria to classify neurons as orientation selective varies across studies, the criterion commonly used in rodent studies is typically less stringent than that applied to feline studies. For example, in the cat visual cortex, neurons were classified as orientation selective by applying a Fourier transform to the orientation selectivity of post stimulus time histograms and by examining the normalized amplitude of the second harmonic component (Chapman and Stryker, 1993). This measure of orientation selectivity is influenced by bandwidth as well as depth of modulation. In contrast, neuronal responses from the mouse visual cortex were classified as orientation selective if a ≥3:1 spike rate is observed in response to visual stimuli of one orientation relative to the orthogonal orientation (Niell and Stryker, 2008). However, a recent report highlights the fundamental processing of visual cortical neurons and the degree of orientation selectivity from the mouse to the monkey is relatively invariant (Huberman and Niell, 2011). In all species, neuronal

stimulus selectivity is robustly decreased following monocular deprivation (Rentschler and Hilz, 1979; Fagiolini et al., 1994).

1.2 Synaptic plasticity is robust in the visual cortex during an early postnatal critical period

1.2.1 Physiological response to monocular deprivation during the critical period

During a critical period in early postnatal development, the potential to modify synaptic structure and function is high, but this synaptic plasticity significantly declines with age. Synaptic plasticity in the binocular region of the primary visual cortex is revealed by the response to manipulations in visual input, such as monocular deprivation (MD). During the critical period, brief monocular deprivation induces a rapid shift in the ocular dominance of binocular neurons away from the deprived eye. The shift in ocularity is accompanied by widespread changes in synaptic input, neuronal output, and neuronal anatomy (Sawtell et al., 2003; Wiesel and Hubel, 1963; Gordon and Stryker, 1996; Hofer et al., 2009; Coleman et al., 2010).

Visually evoked potentials (VEPs) are one of the methods I used to study ocular dominance plasticity. VEPs are local field potentials recorded from the visual cortex *in vivo* that reflect a summation of excitatory synaptic currents evoked synchronously by visual stimulation. It is approximated that an electrode (0.5-1.0 MΩ impedance) records from a volume of tissue with a diameter of ~130 microns (Gray et al., 1995). The shape of the VEP waveform is determined by current sinks/sources generated in response to visual stimulation and is dominated by the large current sink in deep layer III/layer IV. This large current

sink reflects feedforward excitatory synaptic transmission from the thalamic afferents to the mid cortical laminae (Sawtell et al., 2003; Heynen and Bear, 2001). Accordingly, the shape of the VEP depends on the placement of the recording electrode. In superficial laminae of the visual cortex (layer II and superficial III), the VEP has a large positive-peak waveform. The VEP reverses to a negative-peak waveform as the recording electrode approaches the thalamo-recipient laminae (deep layer III, IV and V; Porciatti et al., 1999; Figure 5a; page 41).

The normal contralateral bias of the rodent visual system is revealed as a two-fold larger VEP amplitude evoked in response to stimulation of the contralateral eye versus the ipsilateral eye (Porciatti et al., 1999). This bias reflects the large number (~90%) of retinal ganglion cell axons that decussate at the optic chiasm (Hübener, 2003). Importantly, the contralateral bias is dependent on normal binocular visual experience. Brief monocular deprivation (3 days) during the critical period decreases the amplitude of VEP evoked by stimulation of the deprived eye, while prolonged monocular deprivation (5 days) increases the amplitude of VEP evoked by stimulation of the nondeprived eye (Frenkel and Bear, 2004). These changes decrease the VEP contralateral bias in the binocular visual cortex contralateral to the deprived eye (Frenkel and Bear, 2004; McCurry et al., 2010; Huang et al., 1999).

The normal contralateral eye preference in rodents is also reflected in the ocular dominance distribution of evoked single unit activity (Dräger, 1978). Single unit spiking represents action potentials generated by a single proximal neuron,

and can be used to characterize ocular dominance and stimulus selectivity of single neurons. Evoked single unit activity reveals a strong preference for the contralateral eye in binocular rodents (Liu et al., 2008; McGee et al., 2005; Pizzorusso et al., 2006; Montey and Quinlan, 2011). Brief (4 days) monocular deprivation during the mouse critical period (P28) induces a shift in the ocular dominance of single unit activity away from the deprived eye (Gordon and Stryker, 1996). This shift in ocularity of single unit responses is similar to that described in other mammals, including monkeys (Liao et al., 2004; Wiesel and Hubel, 1963; Blakemore et al., 1978).

Other types of stimulus selectivity of visual cortical neurons are also regulated by monocular or binocular visual deprivation during the critical period (Kim and Bonhoeffer, 1994; Issa et al., 1999; Liao et al., 2004; Faulkner et al., 2006; Beaver et al., 2002; White et al., 2001; Heimel et al., 2010). For example, monocular deprivation of kittens during the critical period reduces orientation selectivity of neurons in the visual cortex contralateral to the occlusion (Hubel and Wiesel, 1970). The deprivation-induced loss of stimulus selectivity correlates with severe visual impairments, including the loss of spatial acuity and decreased visuo-motor coordination (Wiesel and Hubel, 1963). Similarly, prolonged binocular deprivation by dark exposure in rats (birth to postnatal day 60+) reduces orientation and direction selectivity of neurons in the binocular visual cortex (Benevento et al., 1992; Fagiolini et al., 1994). Together this suggests that normal visual experience during the critical period is necessary to maintain neuronal stimulus selectivity in the visual cortex.

1.2.2 Anatomical response to monocular deprivation in the critical period

Significant changes in neuronal synaptic anatomy are also induced by monocular deprivation in young subjects. Indeed, monocular deprivation of kittens during the critical period induces a retraction of the thalamic afferent arbors serving the deprived eye (Shatz and Stryker, 1978; Antonini and Stryker, 1993; 1996). A similar decrease in the density of thalamic afferent terminals is observed in thalamo-recipient cortex following brief monocular deprivation (3 days) in juvenile (P21) mice (Coleman et al., 2010).

Many anatomical studies track changes in the number and/or shape of postsynaptic dendritic spines, as the majority of the excitatory synaptic transmission in the mammalian cortex occurs on dendritic spine heads. Indeed, spine head volume is positively correlated with the area of the postsynaptic density in the visual cortex (Arellano et al., 2007), and serial section transmission electron microscopy combined with immunogold labeling of AMPA receptors reveals that spine head area is positively correlated with the number of AMPA receptors (Nusser et al., 1998; Takumi et al., 1999).

In the visual cortex, spine density/morphology is regulated by visual experience. The majority of the feedforward excitation occurs along the proximal segment of the basolateral dendrites of pyramidal neurons, while intracortical excitation occurs along the distal length of basolateral dendrites and apical dendrites. Brief (4 days) monocular deprivation in juvenile mice decreases spine density along apical dendrites of pyramidal neurons located in the superficial laminae of the visual cortex. The time course of monocular deprivation-induced spine loss (Mataga et al., 2004) is similar to the depression of deprived eye

physiological responses (Gordon and Stryker, 1996). Interestingly, brief (6 hours) monocular deprivation during the ferret critical period (P42) induces a decrease in dendritic spine density that can be rapidly reversed to control levels within 24 hours of reopening the eye (Yu et al., 2011). Binocular deprivation by dark rearing mice from birth induces a decrease in dendritic spine density along apical dendrites in the visual cortex (Valverde, 1967). While dark rearing rats to ~P30 increases the diameter of dendritic spine heads on basolateral dendrites of deep layer III visual cortical pyramidal neurons, which can be reversed by subsequent (~10 days) light exposure (Wallace and Bear, 2004). Since approximately 95% of all dendritic spines form synapses in the adult (P56-84) mouse visual cortex (Arellano et al., 2007), these anatomical changes predict excitatory synaptic strength and may reflect the potential for subsequent plasticity.

1.2.3 Anatomical and physiological plasticity are constrained early at thalamocortical synapses

It is well established that synaptic plasticity is constrained over the course of postnatal development. This developmental decrease in synaptic plasticity may lend to the stability of cortical circuits, yet it also severely constrains the ability of the cortex to recover from long-term sensory deprivation or injury. Interestingly, different populations of cortical synapses appear to lose plasticity at different times over development. For example, anatomical and physiological plasticity is constrained early in the thalamo-recipient laminae relative to extragranular laminae of several cortical regions including visual, somatosensory (barrel), and auditory (Crair and Malenka, 1995; Feldman et al., 1998; Hogsden and Dringenberg, 2009). Indeed, early monocular deprivation (eye opening

through ~8 months of age) in kittens shifts ocularity away from the deprived eye in all cortical layers (Shatz and Stryker, 1978). However, the shift in ocularity in response to monocular deprivation initiated in older kittens (8-9 months of age) is constrained in layer IV but persists in extragranular layers (II/III and V/VI; Daw et al., 1992).

The decrease in plasticity in layer IV is thought to be due to decreased plasticity at thalamocortical synapses. However, it is predicted that thalamic input is a relatively small percentage (5-20%) of input to layer IV neurons in the rodent, as previously demonstrated in the mouse somatosensory cortex (Benshalom and White, 1986), cat visual cortex (Binzegger et al., 2004; Ahmed et al., 1994; Peters and Payne, 1993) and monkey visual cortex (Peters et al., 1994). Despite the relatively small number of thalamic inputs to layer IV neurons, these synapses exert significant control of layer IV neuronal excitability. The synchronous pattern of the convergent thalamic inputs can reliably drive cortical neurons in the rat somatosensory cortex (Bruno and Sakmann, 2006) and the cat visual cortex (Wang et al., 2010b; Stratford et al., 1996). Thalamic afferents also have a significantly higher probability of release than cortical afferents (Gil et al., 1999). To focus specifically on the contribution of thalamocortical synaptic transmission to ocular dominance plasticity, a pharmacological method (described in more detail in Chapter 2) has recently been developed which isolates thalamocortical synaptic transmission by silencing intracortical spiking (Liu et al., 2007). This technique revealed a thalamocortical contribution to the ocular dominance shift induced by brief (3 days) monocular deprivation in

juvenile mice (Khibnik et al., 2010). Plasticity at thalamocortical synapses is thought to only be present early in postnatal development.

1.3 Synaptic plasticity is constrained over development

1.3.1 Physiological and anatomical responses to monocular deprivation are constrained in the post-critical period

The hallmark of reduced plasticity in the visual cortex is the loss of an ocular dominance shift in response to brief monocular deprivation (Wiesel and Hubel, 1963; Gordon and Stryker, 1996; McGee et al., 2005; He et al., 2006; Hubel and Wiesel, 1970; Blakemore et al., 1978). Indeed, prolonged (7 days) monocular deprivation in adult (P90+) rats does not reduce the VEP contralateral bias (Maya Vetencourt et al., 2008; Spolidoro et al., 2011). Likewise, no shift in the distribution of single units is induced by monocular deprivation (7 days) in adult (>P100) rats (Pizzorusso et al., 2002). The developmental decrease in the level of synaptic plasticity has been previously described in many other species, including cats and monkeys (Hubel and Wiesel, 1970; Blakemore et al., 1978). Even prolonged (3 months) monocular deprivation of post-critical period cats did not result in a physiological response, including no change in the ocular dominance distribution of single units (Wiesel and Hubel, 1963). One exception to this rule may be mice, which retain more plasticity in later life than other mammals, including rats (Fischer et al., 2007; Sato and Stryker, 2008; Tagawa et al., 2005). For this reason, rats are the preferred experimental rodent for my studies that propose to enhance synaptic plasticity during the post-critical period.

Similar to the stability of ocularity during the post-critical period, other forms of neuronal stimulus selectivity are also unaffected by monocular deprivation in the post-critical period. Monocular deprivation in rats (≥P45) after the post-critical period does not alter spatial acuity estimated by VEP amplitudes (Fagiolini et al., 1994). Anatomical plasticity is also constrained over cortical development. Accordingly, large mushroom-shaped spines (Harris et al., 1992), which are more stable than small spines (Trachtenberg et al., 2002) are more prevalent in the adult visual cortex. Similarly, brief (4 days) monocular deprivation in adult mice (P60) does not induce a decrease in dendritic spine density along the apical dendrites of layer II/III pyramidal neurons (Mataga et al., 2004). A decrease in dendritic spine motility is also observed over development in all sensory cortices, including the auditory, somatosensory, and visual cortex (Holtmaat et al., 2005; Grutzendler et al., 2002; Zuo et al., 2005, Majewska et al., 2006). Accordingly, monocular deprivation enhances spine motility in juveniles, but does not change the motility of dendritic spines of apical dendrites in adult mice (Oray et al., 2004).

1.3.2 Consequence of decreased plasticity in the post-critical period

While the developmental decrease in the level of synaptic plasticity may lend to stability of cortical circuits, it severely limits the ability of the cortex to recover from long-term sensory deprivation or injury. For example, monocular deprivation in humans, due to the presence of a unilateral congenital cataract at birth, reduces cortical responsiveness to stimulation of the occluded eye and

induces severe amblyopia (cortical blindness). If amblyopia is not treated early in postnatal life, severe visual impairments develop, including decreased spatial acuity (Levi and Li, 2009), contrast sensitivity (Polat et al., 2004), and positional discrimination (Li and Levi, 2004). One form of therapy commonly used to treat amblyopia involves occlusion or patching of the good eye. This therapy is thought to promote strengthening of the pathway serving the amblyopic eye. While patching initiated early in the postnatal life is thought to provide the best opportunity for restoring vision in amblyopes, this therapy has had limited success. For example, ~75% of patients (mean age 5.1±1.4 years) with anisometropic (unequal refractive power between eyes) or strabismic amblyopia (misalignment of the eyes) that underwent 12 weeks of occlusion (2 to 6 hours/day) recovered only partial visual acuity (Stewart et al., 2004). Furthermore, a study of 429 strabismic amblyopes at age 16 revealed that ~76% had persistent visual impairments, despite therapeutic attempts such as reverse occlusion, surgery, and/or optical correction (Rahi et al., 2006). This suggests that the chronic asymmetry in visual input that induces amblyopia results in significant degradation in the feedforward excitatory pathways serving the affected eye. Furthermore, the developmental loss of ocular dominance plasticity may impose significant limitations on the ability of the visual cortex to recover from amblyopia after the critical period. Clearly, more effective therapies are needed for the treatment of amblyopia.

1.3.3 Recovery from deprivation-induced visual impairments during the critical period versus post-critical period

Severe amblyopia induced by chronic monocular deprivation in experimental animals is also increasingly difficult to reverse with age. For example, it has been demonstrated that the loss of neuronal responses, including orientation selectivity, induced by monocular deprivation in monkeys can be reversed by reverse deprivation (open deprived eye, suture nondeprived eye) during the critical period (~5 weeks). However, the strength of the recovery declines significantly with age (Blakemore et al., 1978). Similarly, kittens that received monocular deprivation followed by reverse deprivation during the critical period (4 to 5 weeks of age) recovered normal neuronal selectivity. However, if the reverse deprivation was delayed until after the critical period (14 weeks of age), no recovery of orientation selectivity is observed (Blakemore and Van Sluyters, 1974). The limited temporal window for the recovery of stimulus selectivity has been described in many species, including monkeys (Movshon, 1976; Blakemore et al., 1978; Kim and Bonhoeffer, 1994). Likewise, chronic monocular deprivation in rats (initiated before eye opening and maintained into adulthood) significantly decreases visual functions, including decreased VEP amplitudes, compromised spiking output, reduced stimulus selectivity (Montey and Quinlan, 2011), and reduction in spatial acuity that do not recover spontaneously following reverse deprivation in adulthood (He et al., 2007).

Arguably, chronic form deprivation, via monocular deprivation or cataract, initiated at eye opening and maintained into adulthood induces the most severe form of amblyopia. For example, chronic monocular deprivation initiated early in

postnatal development in monkeys induced deep amblyopia (Harwerth et al., 1986), however the amblyopia is less severe if the deprivation is interrupted by brief periods of intermittent binocular vision (Wensveen et al., 2006; Zhang et al., 2003). This is also reflected in clinical studies, in which the depth of amblyopia is influenced by the age of onset (Ellemberg et al., 2002), the duration of the deficit (Maurer et al., 1999), and the age and type of therapeutic interventions (Birch et al., 1998; Lewis et al., 1995).

The reduction in physiological plasticity over development is also reflected in the stability of neuronal structure. For example, the decrease in dendritic spine density induced by chronic monocular deprivation (initiated at P21) does not recover following reverse deprivation (7 days) in post-critical period rats (~P160). In this case, the spines of interest were on the proximal basal dendrites (~26 microns from soma) of layer II/III pyramidal neurons in the visual cortex (Pizzorusso et al., 2006). Thus, the ability to recover from a deprivation-induced loss (anatomical and physiological) initiated during the critical period becomes increasingly difficult to reverse in adulthood.

1.4 Reactivation of synaptic plasticity in the post-critical period

1.4.1 Reactivation of ocular dominance plasticity in binocular adults

We have recently shown that ocular dominance plasticity can be reactivated in adulthood by binocular visual deprivation through dark exposure (10 days). Ocular dominance plasticity of VEPs in dark exposed adults resembles a juvenile response to monocular deprivation, including a similar time course and changes in response amplitude (He et al., 2006). These observations

suggest that dark exposure returns the adult visual cortex to a juvenile-like state (tested in Chapter 2). However, the ocular dominance plasticity reactivated by dark exposure is transient (decreases in 3-5 days; He et al., 2006) suggesting that subsequent light exposure/visual experience constrains synaptic plasticity back down to normal adult levels.

Several pharmacological and transgenic manipulations have unmasked synaptic plasticity in the post-critical period visual cortex. The nogo-66 receptor (NgR) is thought to impair axonal regeneration by binding to several myelin associated ligands. Accordingly, transgenic deletion of the nogo-66 receptor (NgR-/-) restores ocular dominance plasticity in response to brief (4 days) MD in post-critical period (P120) mice (McGee et al., 2005). Similarly, removal of structural constraints by enzymatic degradation of chondroitin sulphate proteoglycans, a class of glycoproteins that contribute to the extracellular matrix, reactivates ocular dominance plasticity in post-critical period (>P100) rats (Pizzorusso et al., 2002). Other genetic manipulations, such as deletion of Lynx1, which binds to nicotinic acetylcholine receptors (nAChR) to reduce sensitivity to acetylcholine, have also been shown to promote a shift in ocular dominance plasticity in mice (P60). Interestingly, antagonism of nAChRs in post-critical period Lynx1 knockout mice blocks the ocular dominance shift in response to monocular deprivation (Morishita et al., 2010). While these studies make valuable contributions to the identification of various mechanisms that regulate ocular dominance plasticity, they are not readily transferable to the treatment of amblyopia in a clinical population.

Dark exposure is a less invasive mechanism for the reactivation of ocular dominance plasticity in adulthood. Indeed, the reactivation of ocular dominance plasticity by dark exposure is sufficient to promote a partial recovery from chronic monocular deprivation. Dark exposure (10 days) followed by reverse deprivation (open the chronically deprived eye and suture the nondeprived eye) results in the return to a normal contralateral bias (VEP and single units) and normal spatial acuity (estimated with VEPs). Interestingly, dark exposure followed by reverse deprivation also promotes the recovery of spatial acuity determined behaviorally, but the recovery is slow and incomplete (He et al., 2007). Therefore, in chapter 3 I optimize a protocol to accelerate both the rate, and the magnitude of recovery of spatial acuity from chronic monocular deprivation.

1.5 Synaptic plasticity mechanisms

1.5.1 Ocular dominance plasticity employs LTP and LTD mechanisms

My work on improving recovery of function in our animal model of severe amblyopia was informed by studies of LTP/LTD, which I will briefly address here. The decrease in the response of deprived eye stimulation induced by brief MD during the critical period shares many characteristics with homosynaptic long-term depression (LTD) of excitatory synapses induced by low frequency stimulation (LFS), including activity-dependence (Rittenhouse et al., 1999). Similarly, the increase in the response of nondeprived eye stimulation induced by prolonged monocular deprivation shares characteristics with homosynaptic long-term potentiation (LTP) of excitatory synapses induced by theta burst stimulation

(TBS), including NMDA receptor dependence (Kirkwood et al., 1993; Sawtell et al., 2003).

1.5.2 Developmental constraints on LTP/LTD

It has recently been appreciated that mechanisms of LTD induction can vary across different cortical layers. For example, antagonists of type 1 cannabinoid receptors (CB1R) block LTD in the superficial laminae (II/III) of the visual cortex, but not layer IV (Crozier et al., 2007). Accordingly, CB1 receptor antagonism in juvenile mice *in vivo* blocks the response to monocular deprivation in layer II/III, but not in layer IV (Liu et al., 2008). The difference in LTD induction mechanisms mirrors the laminar distribution of CB1 receptors (Deshmukh et al., 2007). In contrast, blockade of AMPAR endocytosis in juvenile mice prevents a response to monocular deprivation in layer IV, but not in layer II/III (Yoon et al., 2009).

Laminar differences in the mechanism for induction of LTD may contribute to the observation that different synaptic populations have different critical periods. Experiments performed in slices of visual cortex reveal that long-term potentiation and long-term depression at excitatory synapses onto neurons in layer IV are lost early in development (Dudek and Friedlander 1996; Kato et al., 1991; Jiang et al., 2007). In addition, the ability to induce synaptic plasticity by a paired conditioning paradigm (white matter stimulation paired with postsynaptic depolarization) in rodent visual cortical slices is constrained earlier in layer IV than layer II/III (superficial) synapses. In layer IV, LTP is constrained by ~P20

and LTD is constrained by ~P28; however, both of these mechanisms persist in layer II/III through ~P60 (Jiang et al., 2007). The loss of LTP/LTD in layer IV is thought to underlie the early loss of ocular dominance plasticity at thalamocortical synapses.

1.5.3 Metaplastic regulation of ocular dominance plasticity and LTP/LTD

All forms of synaptic plasticity are regulated by the history of synaptic activation (Abraham and Bear, 1996). Metaplasticity, induced by dark rearing from birth, promotes LTP across a range of stimulation frequencies in visual cortical slices (Kirkwood et al., 1996). Dark rearing also prolongs the critical period for the expression of LTP (Philpot et al., 2007) and ocular dominance plasticity (Cynader and Mitchell, 1980; Mower et al., 1981; Cynader, 1983). Even very brief (2 h) dark exposure can promote the potentiation of layer IV VEP amplitudes by repetitive (50 Hz) photic stimulation in adult rats (Kuo and Dringenberg, 2009). One mechanism by which dark exposure (2 days) can increase excitability is via a homeostatic scaling up of mEPSC amplitudes on excitatory neurons. The scaling of mEPSCs by dark exposure persists in superficial laminae of adult mouse cortex (~P95), and can be reversed by light exposure (1 day) (Goel and Lee, 2007). Increased cortical excitability can also be achieved by transcranial magnetic stimulation (TMS). TMS, delivered to humans over the visual cortex evokes the perception of flashes or phosphenes (Pascual-Leone et al., 1998). Interestingly, light deprivation (1 hour) in normal-sighted

humans (41±10.5 years of age) decreased the threshold for TMS evoked phosphenes (Fierro et al., 2005).

1.5.4 Visual tetanus induces synaptic plasticity

Synaptic strength in the visual cortex is also regulated by repetitive exposure to patterned visual stimulation. For example, repetitive presentation of high contrast, low spatial frequency visual stimuli (reversing at 0.5 Hz) induces a slow, stimulus-selective potentiation in VEP amplitude that plateaus after ~3-4 days in binocular mice (~P28-P32; Frenkel et al., 2006; Cooke and Bear, 2010). The strengthening of the VEP amplitude shares many characteristics of Hebbian plasticity, including NMDAR dependence, and involves a similar expression mechanism including synaptic delivery of AMPA receptors. This “photic” LTP also occludes subsequent LTP induction by LGN stimulation and is rigidly input specific (Frenkel et al., 2006). For example, presentation of a visual stimulus rotated 5 degrees from that used during the tetanus does not reveal enhanced VEP amplitudes (Cooke and Bear, 2010). Visual responses in humans can also be potentiated by visual tetanus. A repetitive (9 Hz temporal frequency) checkerboard stimuli presented to normal-sighted humans (23-38 years of age) promoted a synaptic strengthening of VEP amplitudes that persisted at least one hour (Teyler et al., 2005). Tetanic visual stimulation has also been shown to modulate subsequent vision, and can improve performance in a luminance detection task (Beste et al., 2011).

1.5.5 Stimulus-selectivity is modulated by visual experience

The selectivity of visual cortical neurons underlies visual feature detection and subsequent visual acuity (Hubel and Wiesel, 1963; Rentschler and Hilz, 1979). This stimulus selectivity is also likely to be the physiological basis for input specificity of many types of response potentiation in the visual system. Similar to homosynaptic LTP, it is predicted that only the subset of neurons activated by the visual stimulus could be strengthened by repeated presentation (Aberg and Herzog, 2012). Indeed, the enhancements of visual responses seen following visual perceptual learning in binocular subjects are revealed only by the stimulus used for the training. A similar input specificity is observed in visual perceptual learning paradigms in experimental animals. For example, improvements in orientation discrimination thresholds are observed following months of orientation detection training in adult monkeys, however these improvements do not transfer to visual stimuli of novel orientations (Schoups et al., 2001). Similarly, improvements in contrast sensitivity are observed following months of contrast detection training in adult cats, that do not transfer to novel spatial frequencies (Hua et al., 2010). Likewise, the improvements in spatial acuity following a spatial frequency discrimination task in rats (P60-90) do not transfer to novel orientations (Sale et al., 2011).

Repetitive exposure to patterned visual stimuli also regulates neuronal stimulus selectivity. Repetitive exposure to moving visual stimuli accelerates the emergence of direction selectivity in visually naïve (<24 hours of visual experience) juvenile ferrets (P30-35; Li et al., 2006; 2008b). This implies that it may be possible to use visual experience to promote a refinement of neuronal

response properties, especially when neuronal selectivity is relatively low. Additionally, repetitive presentation of high contrast, low spatial frequency gratings to juvenile mice that have already acquired orientation selectivity, sharpens the orientation selectivity of neurons tuned to that orientation, but does not increase the number of neurons tuned to that orientation (Frenkel et al., 2006).

Repetitive visual experience or performance in a visual discrimination task also lowers detection thresholds and improves discrimination capabilities. Repetitions of binocular texture- and face-identification tasks in adult humans with unimpaired vision revealed that as few as 40 trials were necessary to increase accuracy and lower detection thresholds. Importantly, improvements in facial discrimination were orientation-specific and did not transfer to novel or inverted facial discrimination tasks (Hussain et al., 2009). Interestingly, this type of activity-dependent synaptic plasticity is not limited to the critical period (Polat et al., 2009; Li et al., 2008a; Hussain et al., 2012). Therefore, this type of visual perceptual learning has been proposed as a therapy to promote recovery of vision in adult human amblyopes. However, the gains in discrimination detection thresholds in human amblyopes following visual perceptual learning are modest. While repeated (~50 hours of training) performance of a positional discrimination task improves positional acuity in humans (18-40 years) with severe anisometropic and/or strabismic amblyopia, discrimination thresholds remain significantly elevated relative to normal-sighted observers (Li et al., 2008a). This

suggests that there may be opportunities to improve visual perceptual learning paradigms to treat human amblyopia with optimization of current protocols.

The repetitive nature of the visual discrimination tasks commonly used to assess spatial acuity in animal models may inadvertently induce visual perceptual learning. For example, in the Prusky water maze task, rats learn to discriminate between a positive stimulus (high contrast, low spatial frequency gratings) and a luminance-matched grey screen (Prusky et al., 2000). Subjects start the task at a low spatial frequency (0.117 cycles/degree) and advance to higher spatial frequencies based on performance. The spatial frequency at which the subject can no longer discriminate between the high contrast gratings and the grey screen with ≥60% accuracy is used as an estimation of spatial acuity. However, estimation of spatial acuity with this method typically requires hundreds of spatial discrimination trials, which may improve spatial acuity.

1.6 Specific aims

1.6.1 Brief overview

Chronic monocular deprivation induces severe amblyopia that can be partially reversed if dark exposure precedes the reinstatement of visual experience. My goal was to understand the anatomical locus of the loss and recovery of vision during the induction and recovery from deep amblyopia, and to design an approach to maximize recovery.

In chapter 2, I used a combination of anatomical and physiological techniques to test the hypothesis that plasticity at thalamocortical synapses is reactivated by dark exposure in adult amblyopes. Severe amblyopia was induced

with chronic monocular deprivation initiated early and maintained until adulthood. It had been predicted that chronic monocular deprivation results in a degradation of the feedforward excitatory pathway that may limit the anatomical substrate for recovery. Indeed, decreased spines were detected in all visual cortical laminae. Additionally, it had been predicted that synaptic plasticity in cortical layer IV is significantly constrained in adults. However, dark exposure of amblyopic rats followed by reverse deprivation promoted an increase in spine density in all cortical laminae, including layer IV. I used cortical silencing to confirm that dark exposure reactivates plasticity in the thalamocortical synapses and propose that strengthening of these synapses contributes to the recovery from chronic monocular deprivation.

In chapter 3, I characterized several responses to chronic monocular deprivation, including a decrease in the strength of neuronal responses and a loss of neuronal stimulus selectivity. To promote recovery from the deprivation-induced losses, I used a visual tetanus presented to the chronically deprived eye of dark exposed amblyopes to strengthen synapses serving the deprived eye. Interestingly, the potentiation of VEP amplitudes in dark exposed amblyopes was not stimulus selective, and transferred to visual stimuli with novel orientations. Subsequent performance in a visual task, shown to promote the recovery of neuronal orientation selectivity, was accelerated in subjects that received the visual tetanus. Together this suggests that visual tetanus combined with visual perceptual learning may optimize the recovery from deep amblyopia.

CHAPTER 2: DARK EXPOSURE REACTIVATES THALAMOCORTICAL PLASTICITY IN ADULT AMBLYOPES

2.1 Abstract

Chronic monocular deprivation in Long Evans rats induces severe amblyopia that is resistant to reversal. However, dark exposure initiated in adulthood reactivates synaptic plasticity in the visual cortex and promotes recovery from chronic monocular deprivation. Here we show that chronic monocular deprivation induces a significant decrease in the density of dendritic spines on principal neurons in all laminae (II-VI) of the deprived visual cortex. Nevertheless, dark exposure followed by reverse deprivation promotes the recovery of dendritic spine density of neurons in all laminae. Importantly, the ocular dominance of neurons in thalamo-recipient laminae of the cortex, and the amplitude of the thalamocortical visually evoked potential recover following dark exposure and reverse deprivation. Thus, dark exposure reactivates widespread synaptic plasticity in the adult visual cortex, including thalamocortical synapses, during the recovery from chronic monocular deprivation.

2.2 Introduction

2.2.1 Monocular deprivation induces Hebbian LTD in juveniles

The ocular dominance of binocular neurons in the primary visual cortex is maintained postnatally by a competition between synapses serving the two eyes. Brief monocular deprivation in juveniles induces a rapid shift in ocular dominance, mediated by a rapid decrease in the strength of excitatory synapses serving the deprived eye (Frenkel and Bear, 2004). Deprived eye depression

shares many characteristics with the homosynaptic long-term depression (LTD) of excitatory synapses that can be induced by low frequency stimulation (Heynen et al., 2003; Rittenhouse et al., 1999; Yoon et al., 2009), including a decline over the course of postnatal development (Jiang et al., 2007; Gordon and Stryker, 1996). Both deprived eye depression and LTD are induced by de-correlation of pre- and postsynaptic activity (Rittenhouse et al., 1999; Linden et al., 2009). Importantly, the deprived eye depression induced by monocular deprivation occludes subsequent LTD of excitatory synaptic transmission in slices of rat visual cortex (Heynen et al., 2003). Extending the duration of monocular deprivation in juvenile mice reveals a delayed potentiation of the response to stimulation of the nondeprived eye (Frenkel and Bear, 2004; Sawtell et al., 2003).

2.2.2 Anatomical correlates to Hebbian LTD

The majority of excitatory synaptic transmission in the mammalian cortex occurs on the heads of dendritic spines. In the visual cortex, approximately 95% of all dendritic spines form synapses, and spine head volume is strongly correlated with the area of the postsynaptic density (Arellano et al., 2007). Manipulations that regulate synaptic strength, such as sensory input and changes in arousal state, induce changes in dendritic spine morphology and spine density (Wallace and Bear, 2004; Wilbrecht et al., 2010; Popov et al., 2007). Indeed, LTD in hippocampal slice cultures is accompanied by a retraction in dendritic spines (Nägerl et al., 2004) and a decrease in the number of contacts between presynaptic boutons and dendritic spines (Bastrikova et al., 2008;

Becker et al., 2008). In addition, low-frequency stimulation of excitatory synapses in the rat hippocampus induces an NMDAR-dependent shrinkage of spine heads (Medvedev et al., 2010) and depolymerization of actin (Zhou et al., 2004; Okamoto et al., 2004).

2.2.3 Developmental decrease in ocular dominance plasticity

Robust anatomical changes are induced by monocular deprivation during the critical period. For example, in juvenile mice, monocular deprivation induces an initial decrease in the density of dendritic spines on the apical dendrites of supra-granular neurons, which recovers following prolongation of the monocular deprivation (Mataga et al., 2004). Monocular deprivation in juvenile mice also increases the motility of dendritic spines on apical dendrites of layer V principal neurons passing through layers II/III and V (Oray et al., 2004). In contrast, experience-dependent anatomical plasticity is extremely limited in adult rodent visual cortex (Mataga et al., 2004; Oray et al., 2004; Majewska and Sur, 2003), but can be encouraged with repeated episodes of monocular deprivation (Hofer et al., 2009) or enzymatic degradation of the extracellular matrix (Pizzorusso et al., 2006).

The developmental constraint on cortical plasticity also limits the ability to recover from monocular deprivation. For example, the ocular dominance shift induced by brief monocular deprivation in juvenile kittens and ferrets is rapidly reversed if the occlusion is removed during the critical period (Giffin and Mitchell, 1978; Mitchell and Gringas, 1998; Liao et al., 2002). In contrast, chronic

monocular deprivation in rats, from eye opening to adulthood, induces a significant shift in ocular dominance that does not reverse following removal of the occlusion during the post-critical period (Pizzorusso et al., 2006; He et al., 2007; Sale et al., 2007).

Chronic monocular deprivation would be expected to induce considerable depression and subsequent elimination of deprived eye synapses, imposing a formidable obstacle to reversal. Indeed, a significant decrease in thalamocortical afferents serving the deprived eye is observed following prolonged monocular deprivation in monkeys, cats and mice, due to a retraction or an inhibition of further growth of pre-existing afferents (LeVay et al., 1980; Tieman, 1984; Shatz and Stryker, 1978; Antonini et al., 1993, 1999). However, we have recently reported that robust ocular dominance plasticity can be reactivated in adult Long Evans rats by binocular visual deprivation through dark exposure. Dark exposure in adulthood promotes the recovery of visual functions following chronic monocular deprivation (He et al., 2007). This complements a growing list of experimental interventions shown to reactivate synaptic plasticity in the adult, post-critical period, visual cortex (Pizzorusso et al., 2006; Sale et al., 2007; Maya Vetencourt et al., 2008). The ability to recover from severe deprivation amblyopia suggests that some feedforward excitation must persist following chronic monocular deprivation; however, this prediction is contrary with the expectation that chronic monocular deprivation would significantly degrade anatomical connectivity in the pathway serving the chronically deprived eye.

Therefore, I used an unbiased labeling method (DiOlistic staining; Gan et al., 2000) to track the density and morphology of dendritic spines along the basolateral dendrites of pyramidal neurons, which receive the majority of feedforward excitation. Following chronic monocular deprivation, from eye opening to adulthood, we observed a significant, decrease in dendritic spine density throughout the depth of the visual cortex, including the thalamo-recipient zone. Nonetheless, presentation of visual stimuli to the chronically deprived eye continued to evoke neuronal spiking and thalamocortical synaptic potentials. Dark exposure followed by reverse deprivation promoted the recovery of dendritic spine densities throughout the depth of the visual cortex and the recovery of visually evoked activity, including thalamocortical synaptic potentials.

2.3 Methods

Subjects: Long Evans rats (equal numbers of males and females) received chronic monocular deprivation via lid suture, from eye opening (~ postnatal day 14) to adulthood (average ± SEM age at experiment postnatal day 148±11.65). In some cases chronic monocular deprivation was followed by 10 days of dark exposure +/- 7 days of reverse deprivation (opening chronically deprived eye and suturing nondeprived eye). Controls subjects (equal numbers of males and females) were raised in a normal visual environment (12 hours light:12 hours dark per day), and all subjects had food and water available *ad libitum*. All procedures conformed to the guidelines of the U.S. Department of Health and

Human Services and the University of Maryland Institutional Animal Care and Use Committee.

Anatomy: A modification of the DiOlistic staining method of Gan et al., 2000, was used. Briefly, transcardial perfusion (100 mls, 2% paraformaldehyde in 0.1M phosphate buffer, pH 7.3) was followed by post fixation of the brain (1 hr in 2% paraformaldehyde at RT). Coronal sections of visual cortex (200 μm thickness) were prepared in ice-cold phosphate buffered saline (PBS, pH 7.3) on a vibratome. The lipophilic dye, DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate), was coated onto tungsten particles (1.3 μm) and delivered into slices with a Helios gene gun (Bio-Rad; 60-80 psi helium pressure). Cortical slices were incubated in PBS (2.5 hrs at RT) to allow the dye to diffuse followed by a second post-fix (4% paraformaldehyde for 30 min at RT). Slices were mounted in ProLong gold antifade reagent (Invitrogen). Neuronal selection within the binocular region of the visual cortex was based on characteristic pyramidal soma, distinct basolateral and apical dendrites at low magnification (10x), as dendritic spines are not detectable at this magnification. Only one basolateral dendrite was scored per neuron. Neurons were sorted into categories (superficial, mid and deep) based on depth of the parent cell body from the dura and other identifying landmarks. Confocal images were acquired using a Zeiss 710 confocal microscope. A typical z-stack consisted of ~30 optical image planes each of 436x436 pixels. We used a 63x/1.4 Oil DIC Plan Apo objective with 3x zoom with 0.42 μm z-steps. Images were acquired with a 63x/1.4 Oil DIC Plan Apo objective with 3x zoom with 0.42 μm z-steps. Maximum

intensity projections were exported and photomontages were constructed in Photoshop (Adobe), and images were calibrated and quantified using an image processing toolkit (Reindeer graphics). All protrusions with a neck and head were manually marked and quantified using “Count” function. Lines tracing the head diameters and neck length (as demonstrated in figure 2) were drawn on each spine and lengths calculated with “Measure all features” function. An observer blind to the experimental condition analyzed a subset of the morphological data.

Physiology: Visually evoked potentials (VEPs) were recorded from the thalamo-recipient laminae (~500 microns from the dural surface), of the binocular visual cortex (7 mm posterior of bregma, 4 mm lateral of midline) with tungsten microelectrodes (1 MΩ) following urethane anesthesia (1.4 mg/kg, i.p.). VEPs evoked by visual stimulation to each eye separately confirmed electrode placement in the binocular portion of the visual cortex. Visual stimuli were 1 Hz full screen square wave gratings with a spatial frequency of 0.02-0.04 cycles/ degree, 100% contrast and 25 cd/m^2 luminance. The VEP waveform has a characteristic shape based on the cortical depth of the electrode placement. The large negative-going waveform of the VEP reflects the currents sinks in thalamocortical laminae and is characteristic of layer IV (see Figure 5a, page 41). The amplitude of the primary negative (thalamo-recipient) component of the evoked potential was quantified to assess the cortical response to visual stimulation. VEP waveforms were averaged from 100 visual stimuli presentations. Subdural electrode placement allowed for the simultaneous collection of thalamo-recipient VEPs (50 Hz low pass filter) and neuronal spiking

(300 Hz high pass and a 3 kHz low pass) in response to visual stimulation. Multi-unit activity was sorted into single units based on waveform shape and principal component analysis using OpenEx software (TDT). Ocular dominance scores were calculated based on individual eye peak activity responses in PSTHs (contra eye - ipsi eye)/(contra eye + ipsi eye) and were binned in a modified version of the classic scale introduced by Hubel and Wiesel: 1= 1.00 – 0.60, 2= 0.59 – 0.20, 3= 0.19 – -0.19, 4= -0.20 – -0.59, 5= -0.60 – -1.00; where 1 represents units primarily driven by the contralateral eye and 5 represents units primarily driven by the ipsilateral eye. Contralateral bias index was calculated by: [(N1-N5)+(N2-N4)+(Ntot/2Ntot)] where N(tot) is the total number of cells and N1 – 5 is the number of neurons in bins 1 – 5 respectively. The thalamocortical location of the electrode was confirmed anatomically by a post-hoc reconstruction of the electrode track. Prior to transcardial perfusion (4% paraformaldehyde) the electrode was cemented to the skull with dental acrylic to maintain electrode placement during fixation. Following overnight post-fix (4% paraformaldehyde), coronal sections (50 microns) were collected on a vibratome in PBS. A cresyl violet nissl stain was used to reveal the laminar organization of the visual cortex, and the placement of the recording electrode in layer IV. Brightfield images (Figure 1b) were collected on a Zeiss 710 confocal microscope in transmitted light mode using a 10x air objective. A photomontage was constructed in Photoshop to reveal the laminar location of the electrode track. Only contrast or brightness was adjusted as needed for the photomontage.

Pharmacology: The thalamocortical component of the VEP was isolated

using a combination of the $GABA_A$ receptor agonist muscimol (4mM, to silence cortical spiking) and the $GABA_B$ receptor antagonist SCH50911 (6mM to prevent activation of presynaptic $GABA_B$ receptors) as described (Liu et al., 2007; Khibnik et al., 2010). A small craniotomy was made ~500 microns posterior to the recording craniotomy (7 mm posterior of bregma, 4 mm lateral of midline). The drug or vehicle (ACSF) was delivered intracranially (~500nL) via a guide cannula (26 gauge, Plastics One; attached to surrounding skull with dental acrylic) allowed to infuse for ~60 minutes.

Statistics: One-way ANOVAs were used to determine statistical significance between three or more independent experimental groups ($p<0.05$). A subsequent Tukey-Kramer HSD post-hoc analysis was used to make pair-wise comparisons (JMP). A Kolmogorov-Smirnov Test was used to determine statistical significance in the distribution of two independent data sets ($p<0.05$). A paired or unpaired t-test was used to probe the statistical significance for two experimental groups ($p<0.05$). In electrophysiology experiments, n=the number of animals for statistical analysis, in anatomical experiments, n=number of cells.

2.4 Results

2.4.1 Recovery of dendritic spine density from amblyopia

We used visually evoked potentials (VEPs) acquired from layer IV of the binocular visual cortex in response to high contrast vertical gratings (0.02-0.04 cycles/degree, 100% contrast, reversing at 1 Hz) to track the physiological response to manipulations of visual input in Long Evans rats (Figure 1). The contralateral bias of the rodent visual system results in a two-fold larger VEP

amplitude in response to stimulation of the contralateral eye relative to the ipsilateral eye, reflecting the complement of thalamocortical afferents (Coleman et al., 2009). Chronic monocular deprivation of the dominant contralateral eye, from eye opening to adulthood (postnatal day P14 to postnatal day 148±11.65), induced a significant decrease in the VEP contralateral bias.

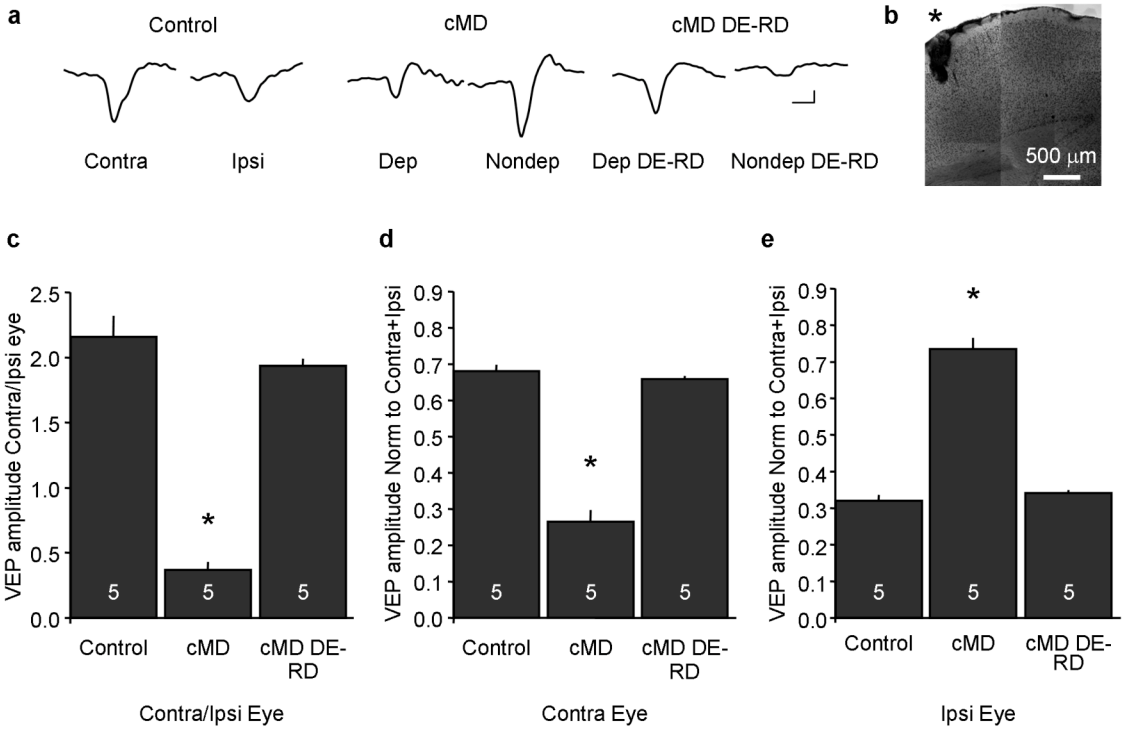

Figure 1: Reversible regulation of VEP contralateral bias in adults. A. Representative layer IV VEP waveforms evoked by visual stimulation of the contralateral (Contra) and ipsilateral (Ipsi) eye in adults with binocular vision (control) chronic monocular deprivation (cMD), cMD followed by dark exposure and reverse deprivation (DE-RD; average of 100 presentations of 0.02-0.04 cycles/degree vertical gratings, 100% contrast, reversing at 1 Hz). Scale bars= 50 ms, 50 μV. B. Photomontage of nissl stain confirms placement of recording electrode (*) in layer IV of visual cortex. Scale bar= 500 μm. C. Chronic monocular deprivation induces a significant reduction in VEP contralateral bias (VEP amplitude in response to stimulation of contralateral eye/ipsilateral eye), which recovers following dark exposure and reverse deprivation (one-way ANOVA, $F_{(2,14)}$=94.5117, p<0.0001). D. Reversible regulation of the deprived eye VEP amplitude (one-way ANOVA, $F_{(2,14)}$=155.2638, p<0.0001). E. Reversible regulation of the nondeprived eye VEP amplitude (one-way ANOVA, $F_{(2,14)}$=114.2875, p<0.0001). *p<0.05 versus control, Tukey-Kramer HSD post-hoc.

Binocular visual deprivation, through dark exposure, followed by reverse deprivation in adulthood (open the chronically deprived eye and close the chronically nondeprived eye) reversed the decrease in the VEP contralateral bias (VEP amplitude in response to stimulation of contralateral/ipsilateral eye; average ± SEM: control = 2.16±0.16; chronic monocular deprivation (cMD) = 0.37±0.06; chronic monocular deprivation + dark exposure + reverse deprivation (cMD DE-RD) = 1.93±0.05; one-way ANOVA, $F_{(2,14)}$=94.5117, p<0.0001, n = 5 each group, Figure 1c). The reversible regulation of the VEP contralateral bias was reflected in the VEP evoked by stimulation of the deprived eye (contralateral eye normalized to amplitude of contralateral + ipsilateral, average ± SEM: control = 0.68 ±0.02; cMD = 0.27 ±0.03; cMD DE-RD = 0.66 ±0.01; one-way ANOVA, $F_{(2,14)}$=155.2638, p<0.0001; Figure 1d) and nondeprived (ipsilateral) eye (control = 0.32 ±0.02; cMD = 0.73 ±0.03; cMD DE-RD = 0.34 ±0.01; one-way ANOVA, $F_{(2,14)}$=114.2875, p<0.0001, Figure 1e).

To identify changes in the anatomy of excitatory circuitry that may underlie the recovery from chronic monocular deprivation, we tracked the density and morphology of dendritic spines in the adult visual cortex (Figure 2). We focused on basolateral dendrites of principal neurons, which receive significant feedforward excitatory input and limited our analysis to dendrites that could be traced back to pyramidal neuron cell bodies in the binocular primary visual cortex. Dendritic spines were binned in 25 micron segments, starting at the cell body, and analyzed across approximately 100 microns of the basolateral dendrite. Chronic monocular deprivation significantly decreased the density of

dendritic spines in the deprived visual cortex. Surprisingly, dendritic spine densities were unchanged following dark exposure; however, dark exposure followed by reverse deprivation in adulthood promoted an increase of dendritic

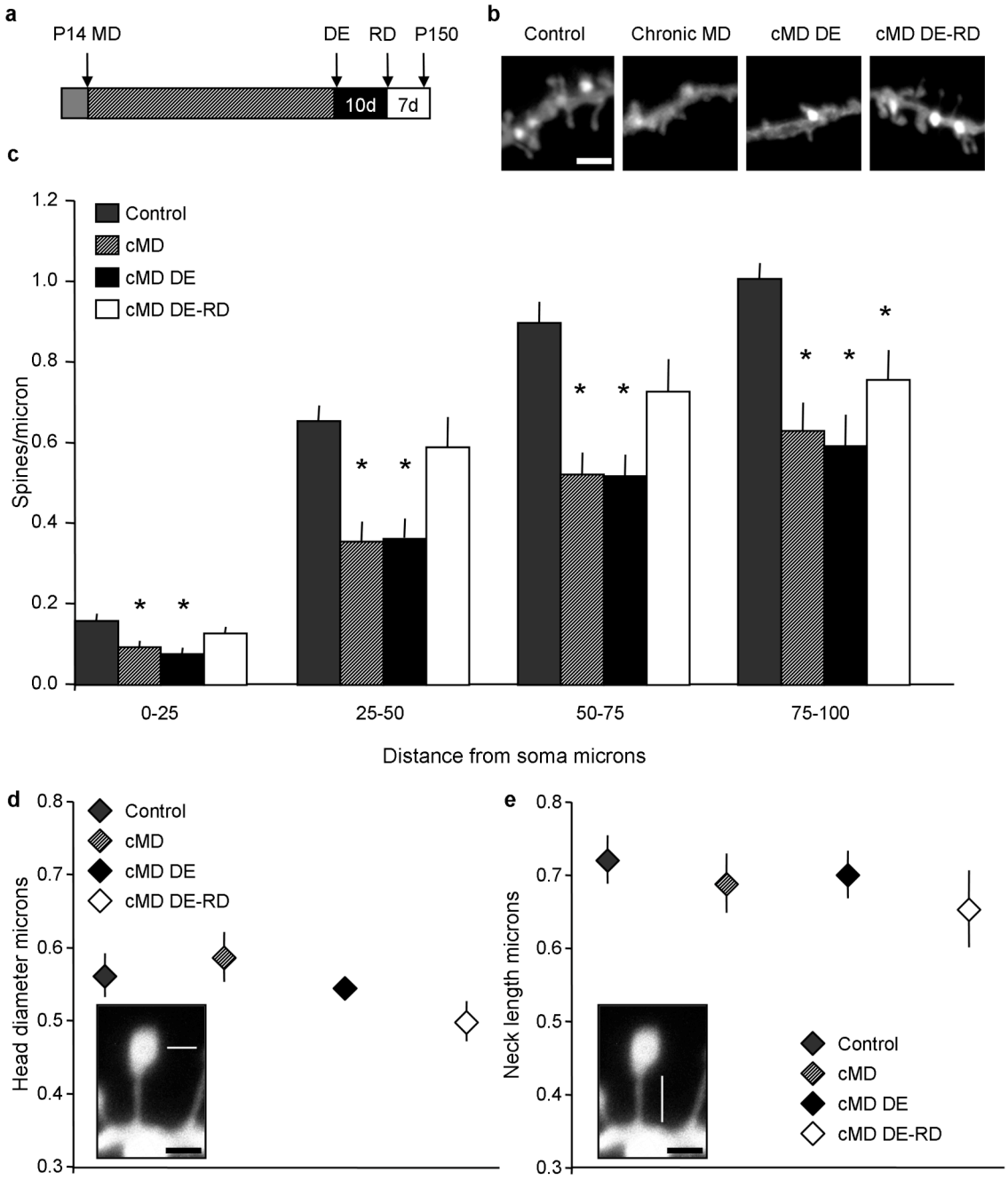

Figure 2: Reversible regulation of dendritic spine density in adult visual cortex. A. Experimental timeline. Chronic monocular deprivation (cMD) from postnatal day 14 – 150 (P14-P150) followed by 10 days of dark exposure (DE) and 7 days of reverse deprivation (RD). B. Representative DiI labeled basolateral dendrites from pyramidal neurons in binocular visual cortex in adult with binocular vision (Con), cMD, cMD plus dark exposure (cMD DE) and cMD DE and reverse deprivation (cMD DE-RD). The dendritic segment shown is 75–100 µm from the cell body. Scale bar= 2.5µm. C. cMD induces a significant reduction in spine density in all dendritic segments, which is unchanged following dark exposure. DE followed by RD increased dendritic spine densities in proximal (0-75 µm) segments (one-way ANOVAs: 0-25 µm $F_{(3,78)}$=5.7840, p=0.0013; 25-50 µm $F_{(3,78)}$=7.1713, p<0.0001; 50-75 µm $F_{(3,75)}$=10.0410, p<0.0001; 75-100 µm $F_{(3,63)}$=8.2569, p=0.0001, *p<0.05 versus within-segment control, Tukey-Kramer HSD post-hoc). D. No significant difference in spine head diameters across experimental conditions. E. No change of spine neck lengths across experimental conditions. Insets: White lines depict area used for measurements of spine head diameter and neck length (white lines), scale bar= 1 µm.

spine densities (average spines/micron across 25 micron segments ± SEM: 0-25 µm control=0.16±0.02 (19,8); chronic MD=0.09±0.01 (24,9); cMD DE=0.08±0.01 (20,8); cMD DE-RD=0.13±0.02 (16,6); n=neurons, subjects; 25-50 µm control=0.65±0.04; chronic MD=0.36±0.05; cMD DE=0.36±0.05; cMD DE-RD=0.59±0.07; 50-75 µm control=0.90±0.05; chronic MD=0.52±0.05; cMD DE=0.52±0.05; cMD DE-RD=0.73±0.08; 75-100 µm: control=1.01±0.04; chronic MD=0.63±0.07; cMD DE=0.59±0.08; cMD DE-RD=0.76±0.07; Figure 2c).

Approximately 50% of dendritic spines on principal neurons of the deprived visual cortex persisted following chronic monocular deprivation. To ask if the morphology of the spared dendritic spines revealed evidence of neuronal compensation for the spine loss, we measured the head diameter and neck length of residual spines across all experimental conditions. No change in the average diameter of spine heads following chronic monocular deprivation, dark exposure or dark exposure followed by reverse deprivation (average spine head diameter ± SEM: control= 0.56±0.03 (19,8); chronic MD=0.59±0.03 (24,9); cMD

DE=0.54±0.01 (20,8); cMD DE-RD=0.50±0.03 (16,6); n=neurons, subjects; Figure 2d). Similarly, no change in dendritic spine neck lengths was observed across experimental conditions (average spine neck length ± SEM control= 0.72±0.03; chronic MD= 0.69±0.04; cMD DE=0.70±0.03; cMD DE-RD=0.65±0.05; Figure 2e).

Complementary changes in dendritic spine density were not observed in the nondeprived visual cortex (ipsilateral to the occluded eye), suggesting that chronic deprivation of the non-dominant input was insufficient to trigger elimination of excitatory synapses (Figure 3). Nonetheless, dark exposure and reverse deprivation induced a significant decrease in dendritic spine density in the nondeprived visual cortex, suggesting that the reactivation of synaptic plasticity by dark exposure promotes synapse elimination following deprivation of the dominant input (average spines/micron across 25 micron segments ± SEM: 0-25 μm control=0.16±0.02 (16,7); Nondep=0.16±0.02 (19,8); Nondep DE=0.13±0.01 (26,8); Nondep DE-RD=0.08±0.02 (9,4); 25-50 μm control=0.67±0.04, Nondep=0.64±0.06, Nondep DE=0.52±0.04, Nondep DE-RD=0.44±0.08; 50-75 μm control=0.93±0.06, Nondep=0.82±0.04, Nondep DE=0.74±0.05, Nondep DE-RD=0.54±0.09 spines/μm; 75-100 μm: control=1.04±0.03, Nondep=0.92±0.04, Nondep DE=0.86±0.05, Nondep DE-RD=0.57±0.09 spines/μm; n=neurons, subjects).

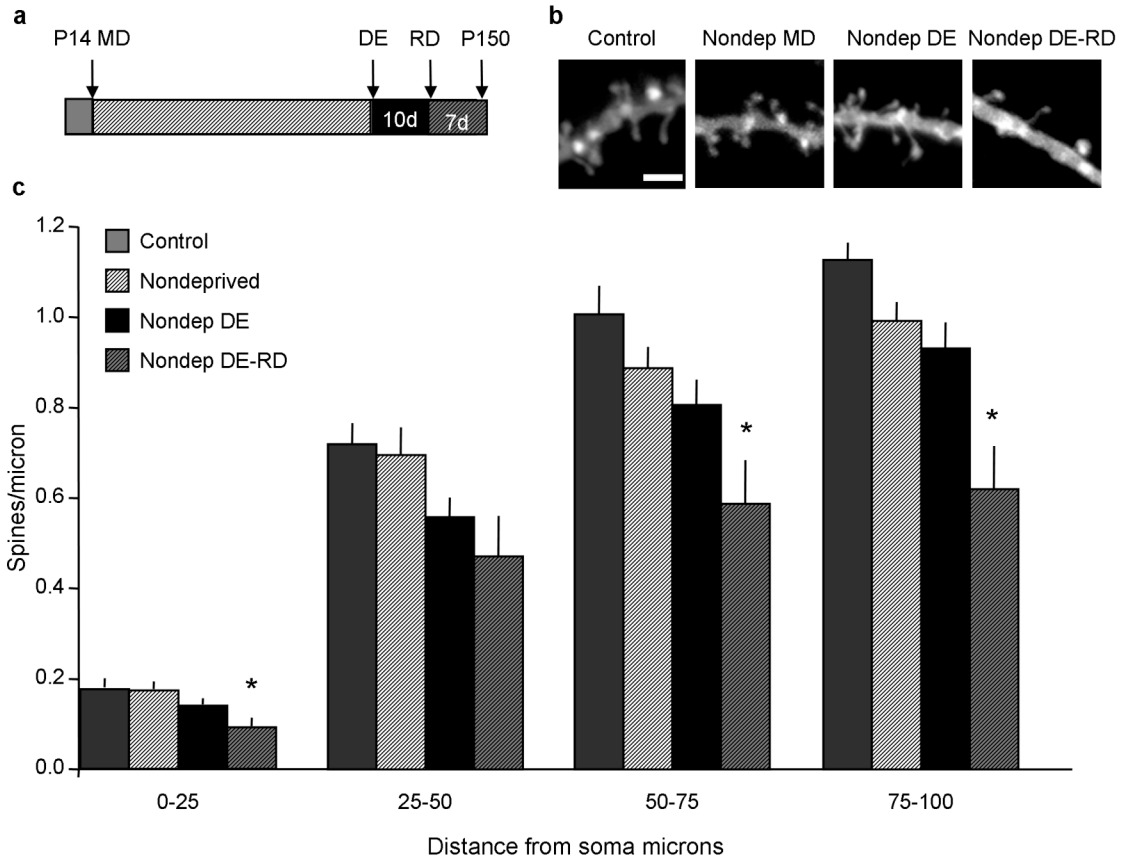

Figure 3: Relative stability of dendritic spine density in the nondeprived adult visual cortex. A. Experimental timeline. B. Representative DiI labeled basolateral dendrites from pyramidal neuron in nondeprived binocular visual cortex from adult with binocular vision, chronic monocular deprivation (Nondep cMD), chronic monocular deprivation plus dark exposure (Nondep cMD DE) and chronic monocular deprivation plus dark exposure and reverse deprivation (Nondep cMD DE-RD). In each case the dendritic segment shown is 75–100 µm from cell body. Scale bar= 2.5 µm. C. No significant differences in spine density in any dendritic segments following chronic monocular deprivation or dark exposure. Dark exposure and reverse deprivation induce a reduction in dendritic spine density in all dendritic segments (one way ANOVAs: 0-25 µm $F_{(3,69)}$=3.4272, p=0.0220; 25-50 µm $F_{(3,69)}$=3.5132, p=0.0199; 50-75 µm $F_{(3,68)}$=5.6344, p=0.0017; 75-100 µm: $F_{(3,61)}$=9.7476, p<0.0001, *p<0.05 versus control, Tukey-Kramer HSD post-hoc).

2.4.2 Parallel regulation of spine density in all cortical laminae

One advantage of the DiOlistic method is that it allowed tracking of dendritic spine density from neurons at all cortical depths. Therefore, we used

DiOlistic labeling to ask if chronic monocular deprivation and subsequent recovery regulated the density of neuronal spines differently in neurons of different cortical laminae. Dendrites were sorted into three groups based on the distance of the parent soma from the dura: 200 – 400 microns = superficial, 400 – 600 microns = middle and 650-850 microns = deep. Chronic monocular deprivation induced a significant, parallel decrease in spine density in all cortical laminae. No change was observed in the density of dendritic spines on neurons at any cortical depth following dark exposure alone. However, dark exposure followed by reverse deprivation significantly increased the density of dendritic spines at all cortical depths, including the middle lamina, which receives rich thalamic innervation (average normalized spine density ± SEM: superficial control=1.00±0.06 (7,4); chronic MD=0.55±0.11 (7,5); cMD DE=0.56±0.11(8,5); cMD DE-RD=0.81±0.19 (5,4); mid control=1.00±0.08 (5,4); chronic MD=0.59±0.10 (7,4); cMD DE=0.57±0.07 (6,4); cMD DE-RD=0.97±0.10 (5,3); deep control=1.00±0.06 (7,6); chronic MD=0.58±0.11 (10,5); cMD DE=0.45±0.14 (6,4); cMD DE-RD=0.83±0.11 (6,5); n=neurons, subjects; Figure 4). Thus, the recovery from chronic monocular deprivation was accompanied by significant spinogenesis throughout the visual cortex, including the thalamo-recipient cortex.

2.4.3 Dark exposure reactivates plasticity at thalamocortical synapses

The involvement of the strengthening of thalamocortical synapses in the recovery from chronic monocular deprivation in adulthood would be unexpected. Although plasticity at thalamocortical synapses can be revealed by direct

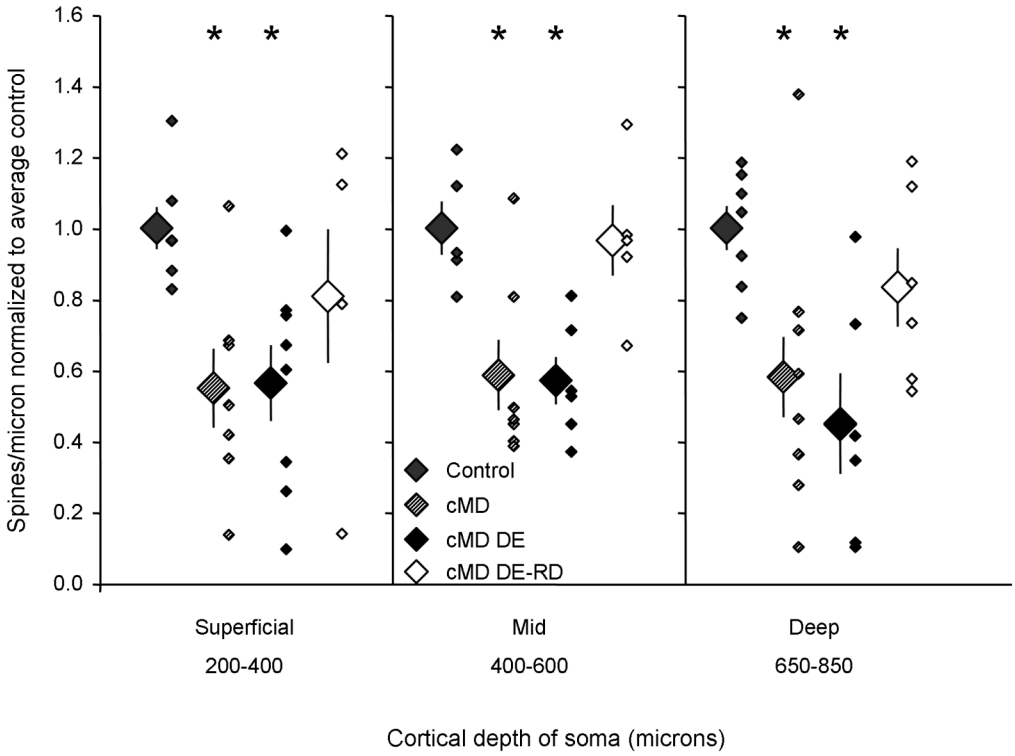

Figure 4: Parallel regulation of dendritic spine density in all laminae of the deprived visual cortex. Chronic monocular deprivation induces a significant decrease in dendritic spine density in all cortical lamina that is unchanged following dark exposure. Dark exposure and reverse deprivation induces a significant increase in dendritic spine density in all cortical laminae (one way ANOVAs: superficial $F_{(3,26)}$=3.7793, p=0.0243; mid $F_{(3,22)}$=6.7756, p=0.0027; deep $F_{(3,28)}$=4.5740, p=0.0110, *p<0.05 versus control, Tukey-Kramer HSD post-hoc. Large symbols represent group average, and small symbols represent individual neurons.

thalamic stimulation *in vivo* (Heynen and Bear, 2001; Mainardi et al., 2010; Hager and Dringenberg, 2010), there are many reports that synaptic plasticity at thalamocortical synapses in many species is constrained very early in postnatal development (Jiang et al., 2007; Crair and Malenka, 1995; Dudek and Friedlander, 1996; Wang and Daw, 2003; Beaver et al., 2001; Daw et al., 1992; Glazewski and Fox, 1996). Therefore, we used two complementary strategies to examine the contribution of plasticity at thalamocortical synapses to the recovery

from chronic monocular deprivation in adulthood. First we examined the regulation of ocular dominance of single units isolated from layer IV of the primary visual cortex. We used the shape of the VEP waveform to guide electrode placement in the thalamo-recipient cortex (Mitzdorf, 1985; Heynen and Bear, 2001) and simultaneously recorded VEPs (50 Hz low pass filter) and neuronal spiking (300 Hz high pass and a 3 kHz low pass, Figure 5a). We observed the expected contralateral bias in the ocular dominance of single units acquired from the thalamo-recipient zone of control binocular adults (Gordon and Stryker, 1996; Daw et al., 1992) reflecting the complement of thalamocortical projections (Coleman et al., 2009). Chronic monocular deprivation shifted the ocular dominance of single units away from the deprived (contralateral) eye, and dark exposure followed by reverse deprivation in adulthood shifted ocular dominance back to control levels (Figure 5b). The reversible change in the response properties of layer IV output was reflected in the contralateral bias index (CBI = [(N1-N5)+(N2-N4)+(Ntot/2Ntot)] where N(tot) is the total number of neurons, and N1-N5 represent the number of neurons in classes 1-5 using a modified version of the scale introduced by Hubel and Wiesel), and the average ocular dominance score (spike rate in response to stimulation of contra – ipsi eye/contra + ipsi eye ± SEM: control=0.25±0.08 (32, 5); cMD= -0.05±0.09 (41,6); cMD DE-RD=0.32±0.08 (40,6); one-way ANOVA, $F_{(2,112)}$=6.1897, p=0.0028, n= units, subjects). The cumulative distribution of ocular dominance scores revealed a significant rightward shift in the ocular preference of layer IV neurons away from the chronically deprived eye (Figure $5c_1$, p=0.018, KS test). Surprisingly,

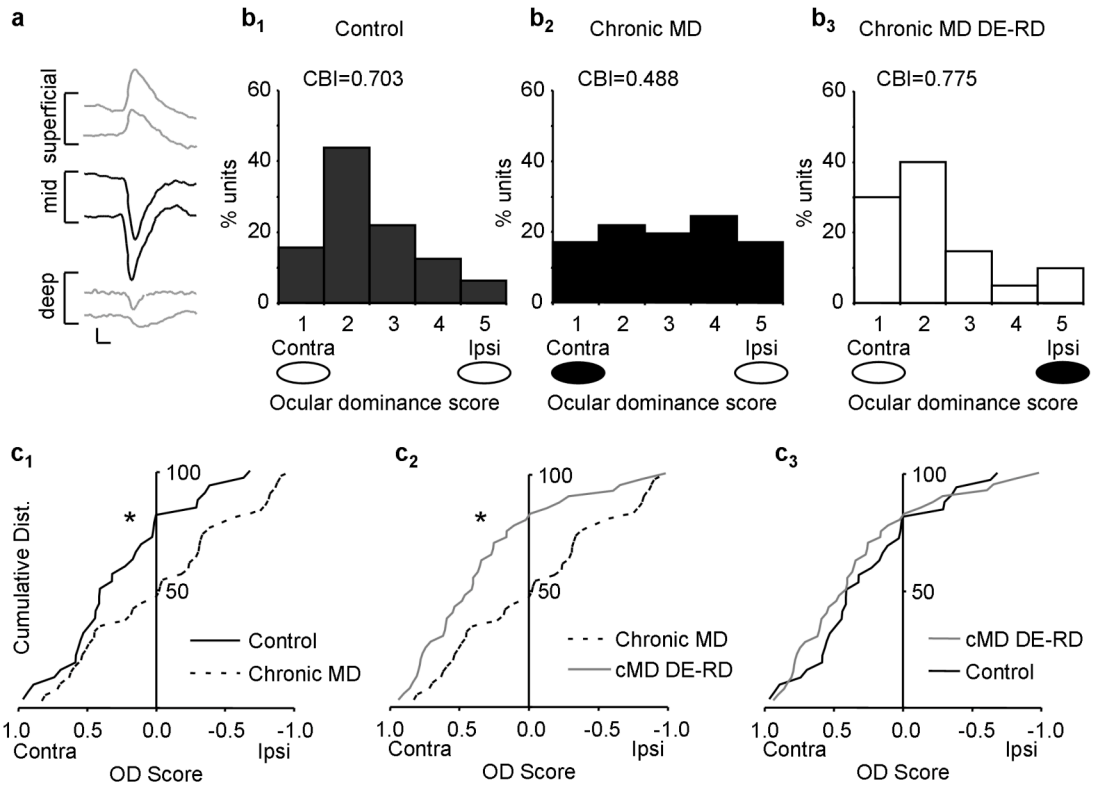

Figure 5: Restoration of ocular dominance of single units isolated from thalamo-recipient cortex during the recovery from chronic monocular deprivation. A. The shape of the VEP waveform changes as the recording electrode moves vertically through the cortex. VEPs are acquired with 50 Hz low pass filter of evoked activity, and each waveform is an average of 100 presentations of 0.02-0.04 cycles/degree, 100% contrast vertical gratings reversing at 1 Hz. Scale bars= 50 ms, 100 μV. A VEP with a large negative component confirms placement of the recording electrode in the thalamo-recipient cortex (mid). Unit activity from thalamo-recipient cortex was acquired with 300 Hz high pass and a 3 kHz low pass filter of evoked activity. Chronic monocular deprivation induces a shift in the ocular preference of single units away from the deprived eye (black oval) and towards the nondeprived eye (white oval). Dark exposure and reverse deprivation induces a shift back to normal contralateral preference. Reversible change in contralateral bias index (CBI) reflects the response to chronic monocular deprivation, and recovery following dark exposure and reverse deprivation. C1. Cumulative distribution of OD scores of single units obtained from thalamo-recipient cortex reveals a significant shift away from deprived (contralateral) eye following chronic monocular deprivation (dashed line) relative to binocular controls (solid black), *p=0.018; KS test. C2. Significant reverse shift in OD scores toward the recovering (contralateral) eye following dark exposure and reverse deprivation (solid gray line) relative to chronic monocular deprivation (dashed line), *p=0.007; KS test. C3. No difference in OD scores in binocular controls (black line) and subjects that received chronic monocular deprivation followed by dark exposure and reverse deprivation solid gray line, p=0.322; KS test.

however, ~34% of neurons retained ocular dominance scores > 0.20 following chronic monocular deprivation, demonstrating the persistence of sensitivity to visual stimulation of the chronically deprived eye. Dark exposure and reverse deprivation induced a significant leftward shift in the ocular dominance scores, indicating increased sensitivity to stimulation of the previously deprived, contralateral eye (Figure $5c_2$, p=0.007, KS test). Ocular dominance scores of chronically deprived subjects with dark exposure followed by reverse deprivation were indistinguishable from normal binocular controls (Figure $5c_3$, p=0.322, KS test).

The recovery of normal ocular dominance of the spiking output of neurons from the thalamo-recipient cortex suggests that enhancement of thalamocortical synaptic transmission may participate in the recovery from chronic monocular deprivation. However, changes in the spiking output of neurons in thalamo-recipient laminae may also reflect changes in the strength of cortico-cortical synaptic inputs. To distinguish between these possibilities, we used cortical silencing to pharmacologically isolate activity at thalamocortical synapses. A combination of the $GABA_A$ receptor agonist muscimol (4mM, to hyperpolarize cortical neurons) and the $GABA_B$ receptor antagonist SCH50911 (6mM, to block activation of presynaptic $GABA_B$ receptors by muscimol) delivered intracranially has recently been used to isolate the thalamocortical component of auditory and visually evoked potentials (Liu et al., 2007; Khibnik et al., 2010). Musc+SCH (500 nL delivered intracranially, infused over ~60 minutes) silenced cortical spiking and induced a significant reduction in the VEP recorded from thalamo-recipient

cortex (average deprived eye VEP ± SEM, pre drug: 129.8±3.6 μvolts; post drug: 39.8±11.8, n =4, p=0.004, paired t-test, Figure 6b, c). Visual stimulation of the chronically deprived eye continued to evoke a small thalamocortical VEP. Cortical spiking and VEP amplitude recovered following washout of Musc+SCH. Cortical silencing was also used to isolate the thalamocortical VEP in subjects that received dark exposure followed by reverse deprivation to promote the recovery from chronic monocular deprivation (average ± SEM: pre drug VEP 323.3±48.5; post drug VEP: 172.5±22.4, n = 4, p=0.005, paired t-test).

Dark exposure followed by reverse deprivation induced a significant increase in the amplitude of the thalamocortical VEP (average ± SEM cMD: 0.31±0.10; cMD DE-RD: 0.54±0.01; p=0.027, unpaired t-test, Figure 6d). The amplitude of the thalamocortical VEP recovered to the level observed in normal binocular controls (VEP amplitude post drug/pre drug, average ± SEM: 0.55±0.10, n=2, unpaired t-test versus cMD DE-RD, p=0.866, not shown). No non-specific reduction in VEP amplitude or contralateral bias was observed in response to intracranial delivery of vehicle alone (post vehicle VEP amplitude normalized to pre vehicle amplitude, average ± SEM: 0.93±0.05, n=2, paired t-test, p=0.13, not shown). Importantly, silencing cortical spiking did not reduce the VEP contralateral bias in adult rats, as previously reported in juvenile mice (Khibnik et al., 2010; average VEP contralateral bias ± SEM cMD pre drug: 0.38±0.070; post drug: 0.38±0.12; cMD DE-RD pre drug: 1.97±0.05; post drug: 2.07±0.1; Figure 6e).

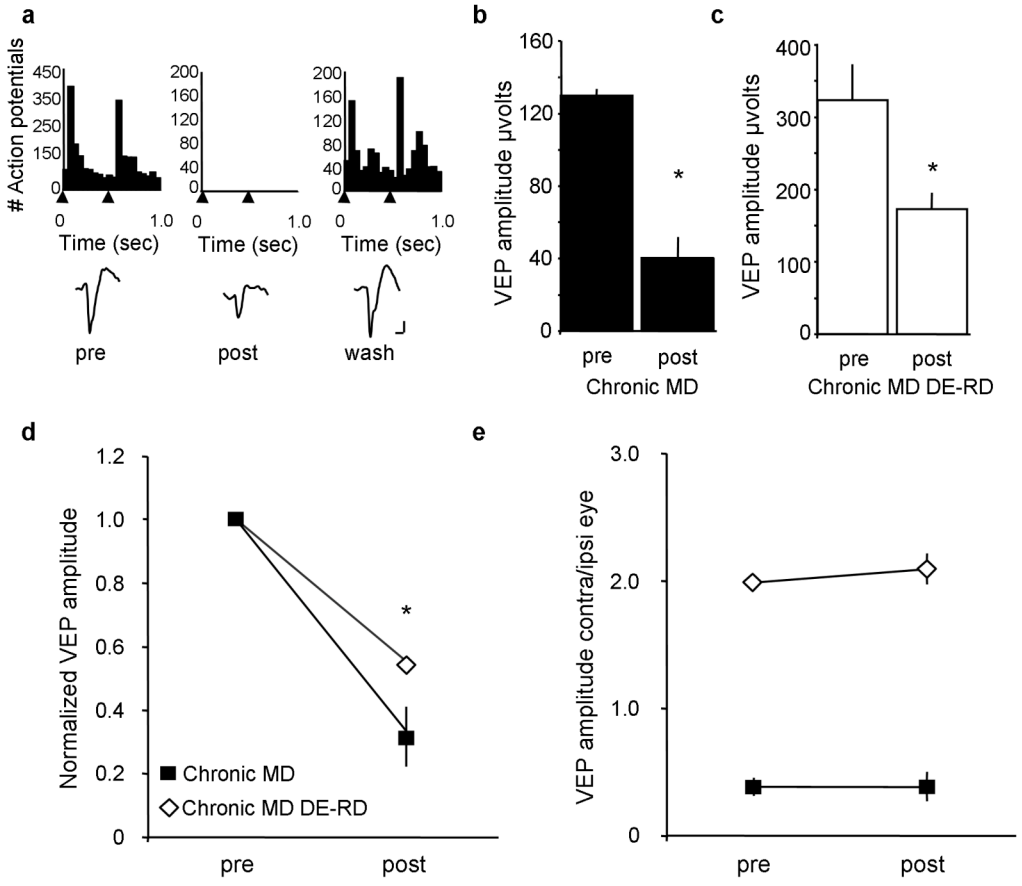

Figure 6: Restoration of thalamocortical VEP amplitude during recovery from chronic monocular deprivation. A. VEPs and unit activity (in 50 ms bins) evoked by visual stimulation before cortical silencing (pre; 0.02-0.04 cycle per degree. 100% contrast gratings at preferred orientation, reversing at 1 Hz, arrows). Intracortical infusion of muscimol+SCH50911 silences cortical spiking and reduces VEP amplitude (post). Cortical spiking and VEP amplitude recover following washout (~ 10 hours; wash). Scale bars= 50 ms, 50 µV. B. Significant reduction in VEP amplitude following cortical silencing in subjects that received chronic monocular deprivation (*p=0.004, paired t-test). C. Significant reduction in VEP amplitude following cortical silencing in subjects that received chronic monocular deprivation followed by dark exposure and reverse deprivation (*p=0.005, paired t-test). D. Increase in the thalamocortical VEP amplitude (% of total VEP) following recovery from chronic monocular deprivation (*p=0.027, unpaired t-test). E. Inhibition of cortical spiking does not modify the VEP contralateral bias in subjects that received chronic monocular deprivation, or chronic monocular deprivation followed by dark exposure and reverse deprivation.

2.5 Discussion

2.5.1 Chronic MD induces a significant decrease in dendritic spine density

Chronic monocular deprivation induces severe amblyopia that is resistant to reversal (Mitchell and MacKinnon, 2002). The obstacles to recovery include the developmental reduction in physiological and anatomical plasticity in the visual cortex, and the considerable degradation of feedforward excitation predicted to result from chronic monocular deprivation. Indeed, we show that chronic monocular deprivation induced a significant reduction in the density of basolateral dendritic spines of principal neurons throughout all laminae of the deprived visual cortex. However, dark exposure followed by reverse deprivation in adulthood promotes the recovery from chronic monocular deprivation (He et al., 2007). Indeed, dark exposure and reverse deprivation in adulthood induced a recovery of dendritic spine density in neurons in all laminae of the visual cortex. Importantly, dark exposure followed by reverse deprivation promotes a recovery of the ocular dominance of neurons from the thalamo-recipient cortex and the amplitude of the thalamocortical VEP. Thus dark exposure in adulthood induces widespread anatomical and physiological plasticity at synapses throughout the visual cortex, including thalamocortical synapses, which promotes the recovery from chronic monocular deprivation.

The DiOlistic labeling technique allowed analysis of dendritic spine density throughout the depth of the visual cortex (850+ microns from dural surface). DiOlistic labeling revealed that the anatomical response to recovery from chronic monocular deprivation differed along the length of the basolateral dendrite. The recovery of dendritic spine density following dark exposure and reverse

deprivation was limited to the proximal (0-75 μm from the soma) segment of the basolateral dendrite. This suggests that synapses in the proximal portion of the dendrite may have a lower threshold for experience-induced spinogenesis following dark exposure and reverse deprivation. Indeed, proximal synapses are more likely than distal to exhibit activity-dependent potentiation in response to the same stimulus parameters (Gordon et al., 2006). Enhancement of correlation-based plasticity and spinogenesis may be due to the higher concentration of voltage gated sodium channels in proximal dendritic segments (Lorincz and Nusser, 2010).

We show that chronic monocular deprivation in Long Evans rats induces a net reduction in dendritic spine density, suggesting that the depression and elimination of synapses serving the deprived eye dominates the response to prolonged monocular deprivation initiated during the critical period. Similarly, monocular deprivation (6-7 days) in kittens induces a decrease in the length of thalamocortical afferents serving the deprived eye (Antonini and Stryker, 1993). However, the anatomical plasticity recruited by monocular deprivation in juvenile mice is biphasic. A rapid decrease in the density of spines on the apical dendrites of layer II/III pyramidal neurons is induced by brief monocular deprivation (Mataga et al., 2004). Extending the duration of the monocular deprivation reveals a recovery of dendritic spines densities. The strengthening of nondeprived eye responses (Frenkel and Bear, 2004) may promote the generation of new excitatory synapses. Monocular deprivation in juvenile mice also induces a biphasic change in thalamocortical afferent number, in which an

initial decrease in afferents serving the deprived eye is supplemented by a delayed increase in afferents serving the nondeprived eye (Coleman et al., 2010).

Chronic monocular deprivation induced a decrease in dendritic spine density on principal neurons throughout the depth of the visual cortex. The parallel anatomical response in all cortical laminae is consistent with a final common pathway for synaptic depression induced by monocular deprivation, despite laminar differences in induction mechanisms (Yoon et al., 2009; Liu et al., 2008; Crozier et al., 2007). However, it is estimated that only ~10 % of excitatory synapses onto principal neurons in layer IV originate from the thalamus as demonstrated in the mouse somatosensory cortex (Benshalom and While, 1986) in the cat visual cortex (Binzegger et al., 2004). Therefore, the ~50% decrease in dendritic spine density we observed in response to chronic monocular deprivation suggests that chronic monocular deprivation induces long-term depression and subsequent elimination of excitatory cortico-cortical synapses as well as thalamocortical synapses. We did not observe a decrease in the spine density on principal neurons in the visual cortex ipsilateral to the occlusion, demonstrating that chronic deprivation of the non-dominant ipsilateral input was insufficient to trigger spine loss.

2.5.2 Dark exposure in adulthood does not regulate dendritic spine density

Contrary to our predictions, dark exposure alone did not induce a change in dendritic spine density or morphology in the visual cortex of chronically deprived adults. We also observed no compensatory changes in the morphology

of residual spines that persisted following chronic monocular deprivation or following dark exposure. A compensatory scaling of dendritic spine density may require a larger reduction in synaptic input (Wallace and Bear, 2004; Petrak et al., 2005). Alternatively, high levels of spontaneous synaptic input may suppress homeostatic scaling of spine density and morphology by dark exposure in chronically deprived adults. Instead, dark exposure in adulthood may reactivate synaptic plasticity by enhancing the motility of residual spines. Indeed, dark-rearing mice until the peak of the critical period induce a persistent enhancement of dendritic spine dynamics on layer V pyramidal neurons (Tropea et al., 2010). Alternatively, regulation of dendritic spine density may require a competitive process, as observed following partial deprivation of neuronal input to multi-whisker septal neurons in the barrel cortex (Wilbrecht et al., 2010) and binocular neurons in the visual cortex (Mataga et al., 2004; Oray et al., 2004; Hofer et al., 2009).

Previous examinations of dendritic spine density by manipulation of visual experience in rodents have been limited to tracking anatomical changes in single cortical lamina (Wallace and Bear, 2004; Mataga et al., 2004; Pizzorusso et al., 2006) or dendritic compartments that do not receive substantial thalamocortical excitatory input (Oray et al., 2004). By tracking dendritic spines on pyramidal neurons throughout the depth of the visual cortex, we demonstrated a significant loss and recovery of dendritic spine density in all cortical laminae, including the thalamo-recipient zone. Although brief monocular deprivation in juveniles shifts the response properties of thalamo-recipient neurons (Khibnik et al., 2010;

Gordon and Stryker, 1996; Beaver et al., 2001), plasticity at thalamocortical synapses is constrained very early in postnatal development (Daw et al., 1992; Glazewski and Fox, 1996). Surprisingly, visual stimuli presented to the chronically deprived eye continued to evoke residual single unit activity in neurons from the thalamo-recipient cortex and a residual thalamocortical VEP, demonstrating the persistence of thalamocortical synaptic transmission following chronic monocular deprivation. The residual feedforward excitation likely provides the anatomical scaffold on which anatomical plasticity, once reactivated in the adult visual cortex by dark exposure, can promote the recovery from chronic monocular deprivation.

2.5.3 Reactivation of juvenile-like anatomical plasticity by dark exposure in adulthood

In the absence of dark exposure, reverse deprivation does not promote the spontaneous recovery of dendritic spine density or spatial acuity in rats (Pizzorusso et al., 2006; He et al., 2007) consistent with the absence of rapid anatomical and physiological plasticity in the adult rodent visual cortex (Holtmaat et al., 2005). A significant increase in dendritic spine density was observed during the recovery from chronic monocular deprivation by dark exposure and reverse deprivation in adulthood. The new spines that emerge following dark exposure and reverse deprivation are likely to form functional synapses and contribute to the increase in the amplitude of the deprived eye VEP observed during the recovery from chronic monocular deprivation. The ocular dominance of neurons in thalamo-recipient laminae of the cortex and the amplitude of the thalamocortical VEP recover when dark exposure is followed by reverse

deprivation, indicating the strengthening of thalamocortical synapses. Thus, dark exposure in adulthood reactivates widespread anatomical and physiological plasticity in the visual cortex, including thalamocortical synapses, to promote the recovery from chronic monocular deprivation.

CHAPTER 3: OPTIMIZATION OF RECOVERY FROM SEVERE AMBLYOPIA

3.1 Abstract

Chronic monocular deprivation induces severe amblyopia, which is characterized by a significant decrease in the strength and the selectivity of cortical neurons serving the deprived eye. Dark exposure reactivates synaptic plasticity in adulthood, and promotes the recovery from chronic monocular deprivation. However, the recovery of spatial acuity assessed behaviorally is slow and incomplete. Here we employ a visual tetanus, presented to the chronically deprived eye of dark exposed adult amblyopic rats, to strengthen deprived eye responses. Visual tetanus induces a rapid potentiation in visually evoked potential amplitudes that transfers to novel attributes of a visual stimulus. Subsequent performance in a visual discrimination task, and the recovery of orientation selectivity in visual cortical neurons is accelerated in subjects that received visual tetanus. Thus a combination of approaches (passive and active) may promote an optimal recovery from deep amblyopia

3.2 Introduction

3.2.1 Chronic monocular deprivation induces a loss in stimulus selectivity

It is well established that neurons in the primary visual cortex are selective for specific features of a visual stimulus, such as spatial frequency or orientation (Dräger, 1975; Niell and Stryker, 2008; Wang et al., 2010a). These neuronal selective features reach mature, adult levels by ~P45 in the rat, with a similar time course to the developmental constraint in ocular dominance plasticity. Long-term monocular deprivation induces severe amblyopia that is partly characterized

by a significant reduction in spatial acuity. The deprivation-induced loss of visual acuity is mediated by a decrease in the strength and selectivity of visually evoked responses in primary visual cortical neurons (Hubel and Wiesel, 1970). Prolonged MD (7 days) in kittens (P21-35) decreases ocularity and orientation selectivity (Bear et al., 1990). The shift in ocularity and decrease in orientation selectivity induced by monocular deprivation (3 weeks) in young ferrets (initiated at eye opening, ~P32) does not recover spontaneously following eye reopening. In contrast, ocularity and orientation selectivity recover spontaneously if the same duration of MD is initiated later (~P45) in the postnatal period (Liao et al., 2004). Importantly, however, ocularity and orientation selectivity can be independently regulated. In transgenic mice, in which the NR2a subunit of the NMDAR has been deleted, ocularity developed normally, but orientation selectivity did not (Fagiolini et al., 2003). In addition, early monocular deprivation (5 days) in kittens (P28) decreased ocularity, but orientation selectivity was maintained in the visual cortex contralateral to the deprived eye (Beaver et al., 2002). Ocularity and stimulus selectivity both become resistant to manipulation after the termination of the critical period (Fagiolini et al., 1994).

3.2.2 Recovery of spatial acuity from severe amblyopia is slow and incomplete

The severe amblyopia that is induced by chronic monocular deprivation is resistant to spontaneous recovery in the post-critical period. Some modest recovery of spatial acuity is seen in kittens after opening a chronically-deprived eye (MD from eye opening to postnatal day 90). The recovery, assessed by

repetitive testing in a spatial frequency discrimination task, was incremental. Spatial acuity was detectable at ~1 cycle/degree at 7-12 days after eye opening, and reached ~50% (~3 cycles/degree) of nondeprived eye spatial acuity by ~120 days (Giffin and Mitchell, 1978). Similarly, the severe amblyopia induced by chronic monocular deprivation in rats (P21 to P180) degrades spatial acuity such that following eye opening, subjects have no measurable vision with the previously deprived eye (Iny et al., 2006). Indeed, subjects are unable to perform a spatial frequency discrimination task with the previously deprived eye at the lowest spatial frequency tested (0.12 cycles/degree). However, a modest spontaneous improvement in spatial acuity is detectable ~35 days after eye opening and reached an asymptote of ~36% of control at ~75 days. In our hands, the loss of spatial acuity in the deprived eye induced by chronic monocular deprivation initiated at eye opening (P14 to ~P90) does not spontaneously improve following reverse deprivation, despite 7 weeks of repeated trials in a visual discrimination task (He et al., 2007).

3.2.3 Ocular dominance plasticity is reactivated by dark exposure in the post-critical period

The loss of ocular dominance plasticity over development is thought to constrain the ability to recover from chronic monocular deprivation. Therefore, in most cases reopening the chronically deprived eye in adulthood does not reveal spontaneous recovery of visual function (He et al., 2007; Spolidoro et al., 2011; but see Iny et al., 2006). However, we have previously shown that ocular dominance plasticity can be reactivated in the adult visual cortex by dark

exposure (He et al., 2006). The reactivation of synaptic plasticity is sufficient to promote enhancement of the amplitude of the deprived eye visually evoked potential across a range of spatial frequencies (He et al., 2007). However, the recovery of spatial acuity, assessed behaviorally with a two-choice spatial frequency discrimination task, is slow and incomplete. Other experimental interventions have been developed that promote the recovery of synaptic strength from milder forms of experimental amblyopia (Maya Vetencourt et al., 2008; Sale et al., 2007; Pizzorusso et al., 2006; Spolidoro et al., 2011). However, in these cases, the effect of monocular deprivation (initiated P21) on neuronal stimulus selectivity was not demonstrated.

3.2.4 Visual perceptual learning

While many forms of activity-dependent synaptic plasticity peak during a postnatal critical period, a subset of plasticity mechanisms appears to persist throughout life. The forms of plasticity that persist throughout development may offer opportunities to enhance synaptic strength that is decreased in amblyopia. One form of synaptic plasticity that remains robust in adults is the improvement in visual perception observed after repetitive visual discriminations (Karni and Sagi, 1991). Visual perceptual learning is typically rigidly stimulus specific. Humans with normal vision that repeatedly performed a face-identification task improved the ability to recognize facial orientation. The visual perceptual learning was limited to stimuli with the orientation that had been presented repeatedly during

the training trials (Hussain et al., 2009). In addition, perceptual gains observed in a trained eye rarely transfer to the untrained eye (Zhou et al., 2006).

Stimulus-selective gains in visual discrimination promoted by repeated visual perceptual learning tasks are observed across mammals, from humans to rats (Sale et al., 2011; Schoups et al., 2001; Hua et al., 2010; Ball and Sekuler, 1987; Fiorentini and Berardi, 1980). Binocular rats (P60-90) repeatedly trained at an orientation-specific two-choice spatial frequency discrimination task do not transfer improvements to novel orientations (Sale et al., 2011).

Visual perceptual learning has also been used to stimulate perceptions in the affected eye in human amblyopes. Visual acuity in human amblyopes improved following performance in a contrast-detection task, and these improvements were not dependent on the age of the subject (ranged 9-55 years of age; Polat et al., 2004). However, current protocols reveal modest success at recovering vision from amblyopia-induced loss. For example, extensive training at a positional-discrimination task resulted in a modest, ~36% improvement, in patients with severe amblyopia (Li et al., 2005). Although, opportunities may exist to use visual perceptual learning paradigms to promote recovery from amblyopia, to date, visual perceptual learning has not been shown to promote recovery across a range of visual functions in any study (Levi and Li, 2009). Importantly however, the gains acquired by visual perceptual learning are often less stimulus selective in amblyopes relative to normal binocular subjects (Huang et al., 2008; Astle et al., 2010; Hou et al., 2011), which may be a consequence of the severity and/or type of amblyopia (Li and Levi, 2004). Increased transfer of perceptual

learning may be more prevalent in severe cases of amblyopia, which is accompanied by a more severe reduction in neuronal stimulus selectivity.

3.2.5 Visual tetanus modulates synaptic strength

Repetitive presentation of simple visual stimuli can also modulate visual responses. A slow, stimulus-selective response potentiation (SRP) in VEP amplitude has been described in mice in response to repetitive presentation of high contrast, low spatial frequency gratings. SRP develops slowly, plateaus in 3-4 days, and appears to be equally robust in ~P28 and P60 mice. Stimulus-selective response potentiation shares many characteristics with Hebbian plasticity including dependence on NMDAR activation, incorporation of AMPA receptors to activated synapses, and is occluded by potentiation of thalamocortical synapses. Similar to visual perceptual learning, SRP is rigidly stimulus selective, and the potentiation of the VEP amplitudes does not transfer to oriented gratings rotated 5 degrees from that used in tetanus (Frenkel et al., 2006; Cooke and Bear, 2010). The visual tetanus used to induce SRP also increases orientation selectivity in cortical neurons tuned to that orientation (Frenkel et al., 2006). Repetitive visual stimulation can also induce the precocious development of direction selectivity in visually naïve ferrets (Li et al., 2006; 2008b). Here, I asked if a combination of passive (visual tetanus) and active (visual perceptual learning) visual stimulation can optimize the recovery from severe amblyopia in rats.

3.3 Methods

Subjects: Long Evans rats (males and females) were raised in a standard visual environment (12 hours light/12 hours dark). A cohort received chronic monocular deprivation (cMD) via eyelid suture from eye opening at postnatal day 14 until adulthood (postnatal day P120 ± 9.78). A subset of binocular (postnatal day 107 ± 8.22) and cMD subjects received complete visual deprivation through 10 days of dark exposure. All subjects had food and water available *ad libitum*. All procedures conformed to the guidelines of the US Department of Health and Human Services and the University of Maryland Institutional Animal Care and Use Committee.

Physiology: Visually evoked potentials (VEPs) were recorded from layer IV (confirmed by maximal amplitude of short latency negative peak; ~500 microns from the dural surface) of the binocular visual cortex (7 mm posterior of bregma, 4 mm lateral of midline) with tungsten microelectrodes (1 MΩ) following isoflurane anesthesia (~2% in 100% O_2). VEPs (50 Hz low pass filter) were recorded in response to full screen square wave gratings with a spatial frequencies ranging from 0.05 – 1.2 cycles per degree, 100% contrast and 25 cd/m^2 luminance reversing at 0.5 Hz. The amplitude of the short latency primary negative component of the visually evoked potential was used to assess the cortical response to visual stimulation. VEP waveforms were evoked by high contrast gratings at 8 orientations (0, 22.5, 45, 67.5, 90, 112.5, 135, and 157.5 degrees) averaged from 100-200 reversals. To determine the spatial frequency detection threshold, a linear regression of VEP amplitude versus spatial frequency (0.04 to 1.2 cycles/degree) was extrapolated to zero VEP amplitude.

Multi-unit activity (300 Hz high pass and a 3 kHz low pass filters) was evoked in response to 25 presentations of a high contrast gratings reversing at 1 Hz at 8 orientations. Multi-unit activity was sorted into single units based on waveform shape and principal component analysis using OpenEx software (TDT). Ocular dominance scores were calculated based on individual eye peak activity in post-stimulus time histograms (PSTHs; contra eye - ipsi eye/contra eye + ipsi eye) where 1 represents units primarily driven by the contralateral eye. Orientation selectivity of single units was determined by calculating (pref-ortho)/(pref+ortho), where pref is the peak response of the post stimulus time histogram (PSTHs; 10 ms bin) at the preferred (pref) orientation relative to the peak response at the orthogonal (ortho) orientation. Spontaneous activity was quantified by calculating the mean number of spikes/second during exposure to blank screen. Evoked activity for the preferred orientation is reported after subtraction of the mean spontaneous activity (spikes/second). All visually responsive neurons (evoked activity >spontaneous activity) were included in the analyses.

Behavior: Monocular adults were trained to perform a water-based two-choice visual discrimination task by learning to discriminate between a visual stimulus (100% contrast, 75 cd/m^2 luminance, 1.8 gamma value and 5000K white balance sinusoidal vertical gratings) and a grey screen of equal luminance (Prusky et al., 2000). Subjects were placed in a shallow tank of water, and trained to associate the monitor displaying the visual stimulus with a submerged escape platform. Introduction of a lane divider (53.3 cm in length) creates a decision point at a fixed distance from the monitors and allows for the calculation

of the spatial frequency. Training of amblyopic adults is initiated with the nondeprived eye, takes ~2-3 weeks and starts at a spatial frequency of 0.208 cycles/degree. Once each subject can successfully detect the grating stimulus with a ≥90% success rate for 3 consecutive days, testing of the spatial acuity of the nondeprived eye commences by increasing spatial frequencies in small increments from 0.208 to 1.2 cycles/degree. Successful advancement to an increased spatial frequency is dependent on the individual performance of each subject. The spatial frequency at which the subject can no longer discriminate between the displays with ≥60% accuracy is reported as estimated spatial acuity.

Transfer. Once the spatial acuity of the nondeprived eye has been obtained at the trained orientation (90°), spatial acuity at novel orientations were tested (45 and 0°). All subjects then received 10 days of dark exposure followed by reverse deprivation (open chronically deprived eye and suture nondeprived eye). We have previously shown that 10 days reprieve from the task does not compromise the ability of subjects to perform the task (He et al., 2007). Following reverse deprivation, subjects were randomly split into two cohorts: one group received low frequency visual tetanus to the previously chronically deprived eye (full field, high contrast vertical gratings, 0.05 cycles/degree, 100% contrast, reversing at 0.5 Hz) under light isoflurane anesthesia, and the sham group received isoflurane anesthesia but no visual tetanus. Spatial acuity was assessed behaviorally in both cohorts using the spatial frequency discrimination task, beginning at 0.173 cycles/degree. Advancement to next higher spatial frequency is performance-based, and the spatial frequency at which the subject can no

longer discriminate between the displays with ≥60% accuracy is the estimated spatial acuity. Acuity assessments are performed 3X/week, before and after visual tetanus.

Statistics: To determine statistical significance between three or more independent experimental groups, a one-way ANOVA ($p<0.05$) was applied to the data, followed by a subsequent Tukey-Kramer HSD post hoc analysis for pair-wise comparisons, when appropriate. A Kolmogorov-Smirnov Test was used to determine statistical significance in the distribution of two independent data sets ($p<0.05$). A paired or unpaired t-test was used to determine statistical significance between two experimental groups ($p<0.05$).

3.4 Results

3.4.1 Chronic monocular deprivation reduced the strength and selectivity of visually evoked responses

Chronic monocular deprivation (cMD) initiated at eye opening (~postnatal day 14; P14) and maintained into adulthood (~P120) induces severe amblyopia that is characterized by significantly compromised vision in the deprived eye. As expected, chronic monocular deprivation induced a significant decrease in the VEP contralateral bias (VEP amplitude of contralateral eye/VEP amplitude of ipsilateral eye (C/I); average ± SEM binocular: 2.02±0.12, n=5; cMD: 0.59±0.13, n=5; unpaired t-test, p=0.002, Figure 1b), due to a significant decrease in the VEP amplitude evoked by stimulation of the deprived, contralateral eye (average μV ± SEM binocular: 225.2±36.91, n=5; cMD: 122.20±13.56, n=5; unpaired t-test, p=0.031, Figure 1c). The decrease in the amplitude of the VEP was observed

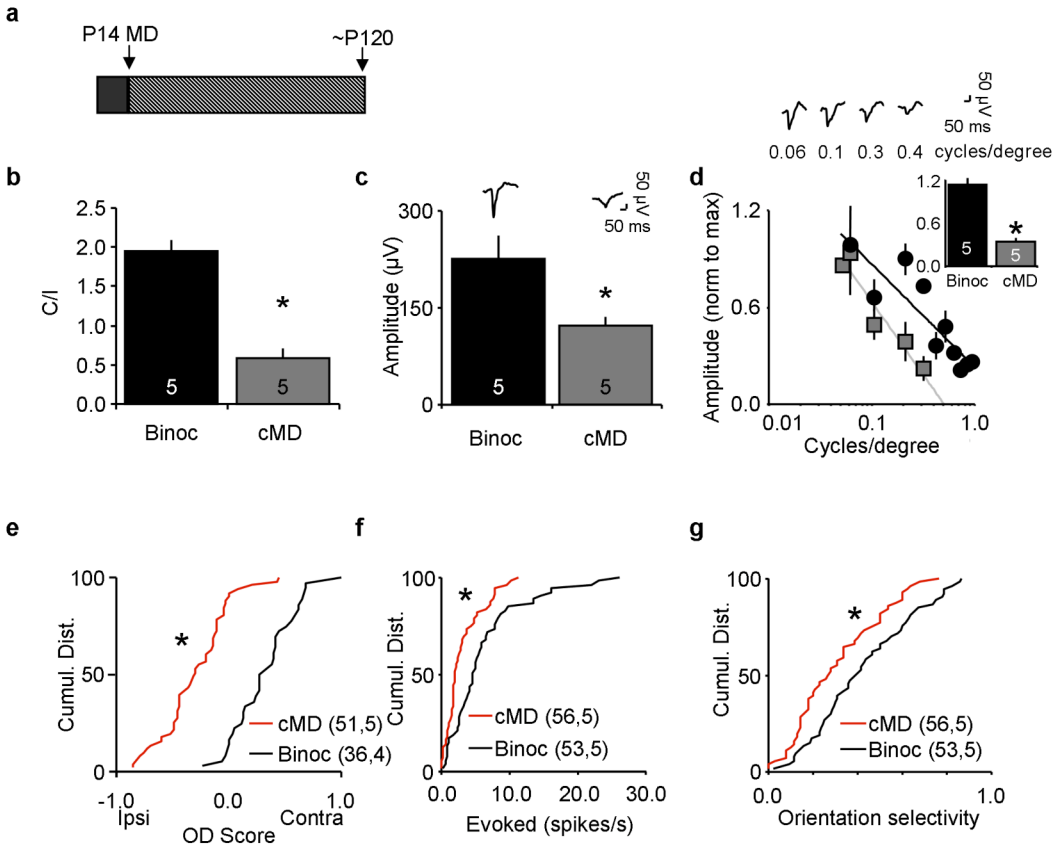

Figure 1: Chronic monocular deprivation induced a decrease in the strength and selectivity of visual cortical neurons. A. Experimental timeline: Chronic monocular deprivation (cMD) initiated at eye opening (postnatal day 14; P14) was maintained into adulthood (~P120). B. cMD significantly decreased the VEP contralateral bias (VEP amplitude of contralateral eye /VEP amplitude of ipsilateral eye; C/I; average ± SEM) relative to binocular (Binoc) controls, *p=0.002, unpaired t-test. C. cMD significantly decreased the amplitude of the deprived eye VEP (average μV ± SEM, *p=0.031, unpaired t-test). Inset: Representative VEPs in response to presentation of visual stimulus to contralateral dominant (binocular) or deprived eye (chronic MD). D. cMD decreased amplitudes of VEPs evoked by visual stimuli with a range of spatial frequencies. Inset: Extrapolation of linear regression to 0 VEP amplitude (average amplitude (norm to max) ± SEM) *p=0.031, unpaired t-test. Representative VEPs at 0.06, 0.1, 0.3 and 0.4 cycles/degree. E. cMD induced a significant shift of the ocular dominance of single unit activity away from the deprived contralateral eye (cMD, red line; n= units, subjects; binoc, black line, *p<0.001, KS test). F. cMD decreased the strength of evoked single unit activity (binoc, black line; cMD, red line, *p=0.001, KS test). G. cMD significantly decreased the orientation selectivity of single unit activity (*p=0.043, KS test). Scale bars= 50μV, 50 ms.

across a range of spatial frequencies (extrapolation of linear regression to 0 VEP amplitude average ± SEM binocular: 1.03±0.07, n=5; cMD: 0.35±0.05, n=5; unpaired t-test, $p<0.001$, Figure 1d). The ocular dominance shift induced by chronic monocular deprivation was also evident in single unit recordings, which revealed a shift in the ocular preference away from the contralateral, deprived eye (cumulative distribution of ocular dominance scores*;* red line, chronic monocular deprivation; 51,5; n=units, subjects; black line, binocular controls; 36,4; KS test, $p<0.001$, Figure 1e). Chronic monocular deprivation also induced a significant decrease in the strength of the single unit responses (cumulative distribution of evoked activity (spikes/second); red line, chronic monocular deprivation; 56,5; black line, binocular controls; 53,5; KS test, $p=0.001$, Figure 1f) and a significant decrease in orientation selectivity (cumulative distribution of neuronal orientation selectivity; red line, chronic monocular deprivation; 56,5; black line, binocular controls; 53,5; KS test, $p=0.043$; (pref-ortho)/(pref+ortho), Figure 1g). Thus chronic monocular deprivation induced a significant decrease in both the strength and the selectivity of visual cortical responses.

3.4.2 Dark exposure enabled activity-dependent potentiation of visual response strength in amblyopic adults

There is little evidence for spontaneous recovery of the severe visual deficits induced by chronic monocular deprivation in rats (Spolidoro et al., 2011; Sale et al., 2007; He et al., 2007; Maya Vetencourt et al., 2008; Pizzorusso et al., 2006). However, we have previously shown that binocular visual deprivation, through dark exposure, promotes experience-dependent recovery of the VEP

contralateral bias and ocularity of evoked single unit responses (He et al., 2007; Montey and Quinlan, 2011). We hypothesized that dark exposure reactivated synaptic plasticity in the visual cortex of post-critical period amblyopes, which promoted activity-dependent strengthening of the pathway serving the chronically deprived eye. To test this hypothesis, I examined the sensitivity to visual stimulation in dark exposed and control adult amblyopes. We adopted a visual stimulation paradigm recently shown to induce robust, slow, stimulus-selective response enhancement of VEP amplitudes in rodents (Frenkel et al., 2006; Cooke and Bear, 2010).

Visual tetanus (full field, 100% contrast vertical gratings, 0.05 cycles/degree, reversing at 0.5Hz) presented to the chronically deprived eye of dark exposed subjects induced a rapid potentiation of deprived eye VEP amplitude (deprived eye VEP amplitude normalized to pre-tetanus baseline: average ± SEM; cMD DE: 120 minutes post-tetanus=1.47±0.13; 240 minutes post-tetanus=1.84±0.13, n=7, one-way ANOVA, $F_{(2,18)}$=18.3033, $p<0.001$; $p<0.05$ versus pre-tetanus baseline; Tukey-Kramer honestly significant difference (HSD) post hoc, Figure 2b). However, the same visual tetanus did not induce a rapid potentiation of the deprived eye VEP in amblyopes that were not dark exposed (deprived eye VEP amplitude normalized to pre-tetanus baseline: average ± SEM; cMD: 120 minutes post-tetanus=0.88±0.08; 240 minutes post-tetanus=0.93±0.08, n=5, one-way ANOVA, $F_{(2,12)}$=0.4602, p=0.4602 versus pre-tetanus baseline, Figure 2e).

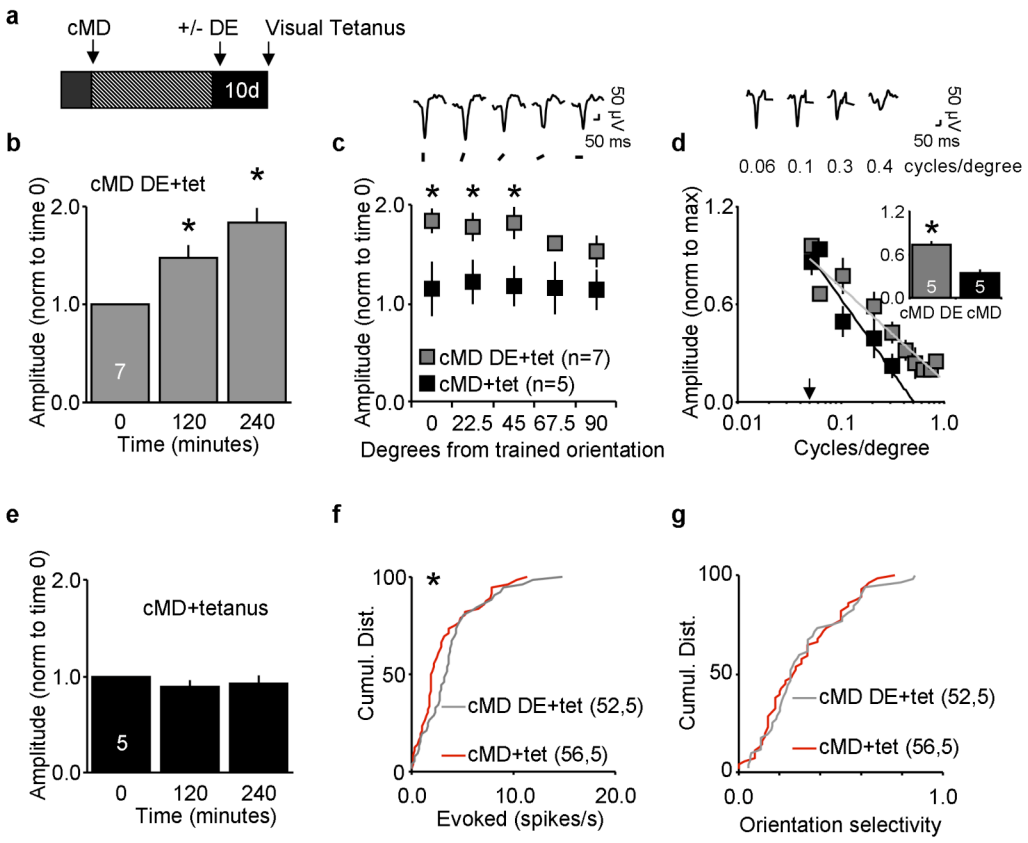

Figure 2: Visual tetanus promoted synaptic strengthening in dark exposed amblyopes. A. Experimental timeline: All subjects received chronic monocular deprivation (cMD; P14-P120); half of the subjects received visual deprivation through dark exposure (DE), all subjects received tetanic visual stimulation (tet) delivered to the chronically deprived eye (100% contrast, 0.05 cycle per degree, vertical gratings reversing at 0.5 Hz). B. Visual tetanus induced a rapid increase in the amplitude of deprived eye VEPs in subjects that received dark exposure (average ± SEM; one-way ANOVA, $F_{(2,18)}$=18.3033, p<0.0001, *p<0.05 versus pre-tetanus baseline, Tukey Kramer HSD *post hoc*). C. The potentiation of deprived eye VEP was observed in response to visual stimuli rotated up to 45 degrees from vertical (average ± SEM; *p<0.05 for matched orientations, unpaired t-test). Inset: Representative VEPs evoked by stimuli 0, 22.5, 45, 67.5 and 90 degrees from the trained orientation in cMD DE+tetanus subjects. D. The potentiation of deprived eye VEP was observed in response to visual stimuli across a range of spatial frequencies (average amplitude (norm to max) ± SEM). Arrow indicates spatial frequency of visual tetanus. Inset: Extrapolation of linear regression to 0 VEP amplitude (average amplitude (norm to max) ± SEM), *p=0.001, unpaired t-test. Representative VEPs at 0.06, 0.1, 0.3 and 0.4 cycles/degree. E. In the absence of DE, visual tetanus did not induce a rapid potentiation of deprived eye VEPs (average ± SEM). F. Visual tetanus promoted a modest increase in the strength of single unit evoked activity (spikes/second) at the peak orientation following dark exposure of cMD (grey line) relative to cMD (red line) no dark exposure (n=units, subjects, *p=0.020, KS test). G. Visual tetanus did not increase the orientation selectivity of single unit activity. Scale bars= 50μV, 50 ms.

The response to repeated visual stimulation is typically input specific. Surprisingly, however, in dark exposed amblyopes, the VEP potentiation induced by this protocol was not stimulus selective. Presentation of low spatial frequency vertical gratings induced rapid potentiation of VEP amplitudes that transferred to gratings with orientations rotated up to 45 degrees from vertical (VEP amplitude 240 minutes post-tetanus normalized to pre-tetanus baseline, average ± SEM cMD DE: 0 degrees=1.84±0.12; 22.5 degrees=1.77±0.14; 45 degrees=1.81±0.16; 67.5 degrees=1.60±0.07; 90 degrees=1.52±0.16, n=7; cMD: 0 degrees=1.15±0.28; 22.5 degrees=1.21±0.22; 45 degrees=1.18±0.21; 67.5 degrees=1.15±0.26; 90 degrees=1.13±0.21, n=5; *$p<0.05$ for matched degrees, unpaired t-test; Figure 2c). In addition, the tetanic stimulation of low spatial frequency vertical gratings induced rapid potentiation of VEP amplitudes that transferred to VEPs evoked by a range of spatial frequencies (extrapolation of linear regression to 0 VEP amplitude average ± SEM cMD DE: 0.73±0.06, n=5; cMD: 0.35±0.05, n=5; unpaired t-test, $p=0.001$; Figure 2d). The visual tetanus also induced a modest increase in the strength of evoked single unit responses in dark exposed amblyopes (grey line; cMD no DE; red line; cMD DE 52,5; KS test, $p=0.020$; Figure 2f), but did not increase orientation selectivity (KS test, $p=0.908$; Figure 2g). Thus, the response to visual tetanus was regulated by the history of visual experience. Importantly, the potentiation of VEP amplitudes induced in dark exposed amblyopes was not stimulus selective, and did not stimulate the recovery of neuronal orientation selectivity.

The rapid potentiation of VEPs appears to be enabled by dark exposure in chronically deprived subjects, as no rapid VEP potentiation was observed in non-DE amblyope controls. To ask if dark exposure accelerated the response to visual tetanus in adult binocular controls, a cohort of normal binocular adults received 10 days of dark exposure (Figure 3a). As we have previously reported, dark exposure alone did not modulate VEP amplitudes or time to peak (He et al., 2006); VEP amplitude (μV) average ± SEM binoc: 225.2±36.91, n=5; binoc DE: 217.8±37.67, n=5; unpaired t-test, p=0.854). Surprisingly, visual tetanus did not induce a rapid potentiation of VEP amplitudes in control or dark-exposed binocular adults (VEP amplitude normalized to pre-tetanus baseline: average ± SEM: binocular DE: 120 minutes post-tetanus=1.24±0.27; 240 minutes post-tetanus=1.30±0.17, n=5; binocular: 120 minutes post-tetanus=1.02±0.13; 240 minutes post-tetanus=1.14 ±0.10, n=5; Figure 3b, d). As expected, no rapid potentiation of VEP amplitudes was observed in binocular subjects in response to stimuli of any other orientation (VEP amplitude 240 minutes post-tetanus normalized to pre-tetanus baseline, average ± SEM binocular DE: 0 degrees=1.28±0.18; 22.5 degrees=1.26±0.23; 45 degrees=1.34±0.37; 67.5 degrees=1.16±0.19; 90 degrees=1.28±0.26, n=5; binocular: 0 degrees=1.14±0.10; 22.5 degrees=1.06±0.10; 45 degrees=1.10±0.11; 67.5 degrees=1.04±0.07; 90 degrees=1.07±0.06, n=5; Figure 3c) or spatial frequency (Figure 3e). This suggests that both chronic monocular deprivation and dark exposure are required to enable the rapid, non-stimulus selective potentiation in VEP amplitude by visual tetanus.

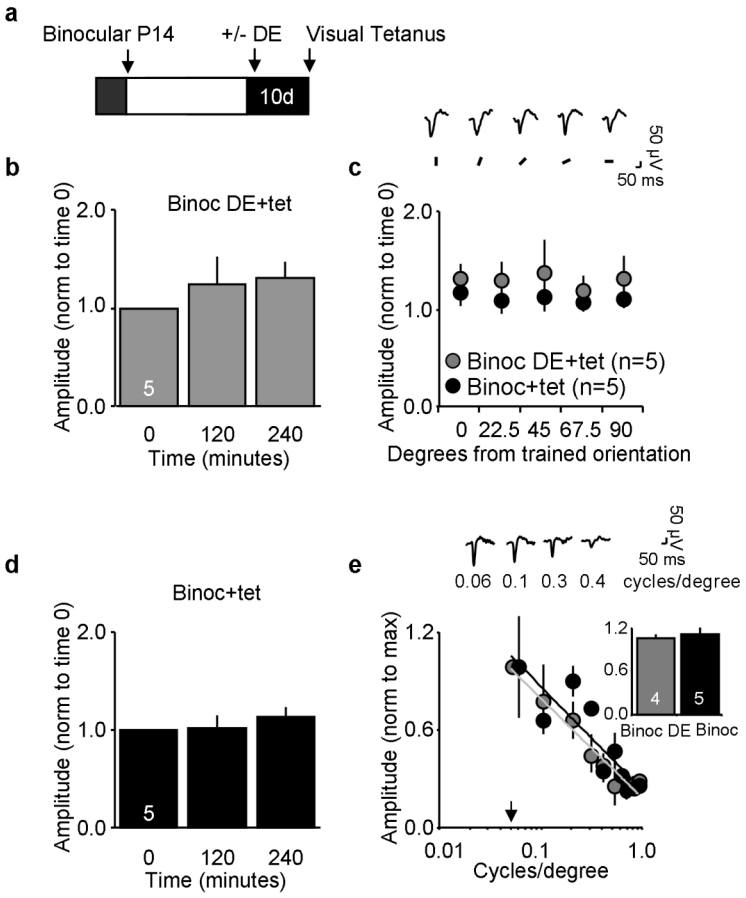

Figure 3: Visual tetanus was ineffective in dark exposed binocular adult controls. A. Experimental timeline: All subjects had normal visual experience (12 hours light/12 hours dark/day) from eye opening to adulthood (P14-P100); half of the subjects received visual deprivation through dark exposure (DE); all of the subjects received tetanic visual stimulation to the dominant, contralateral eye. B. Visual tetanus did not induce a rapid increase in the amplitude of the contralateral eye VEP in subjects that received dark exposure (average ± SEM). C. No potentiation of contralateral eye VEP was observed in response to visual stimuli of any orientation (average ± SEM). Inset: Representative VEPs evoked post-tetanus at 0, 22.5, 45, 67.5 and 90 degrees from the trained orientation. D. Visual tetanus did not induce a rapid potentiation of contralateral eye VEP in binocular subjects that did not receive dark exposure (average ± SEM). E. No potentiation of contralateral eye VEP was observed in response to visual stimuli of any spatial frequency (average amplitude (norm to max) ± SEM). Arrow indicates spatial frequency of visual tetanus. Inset: Extrapolation of linear regression to 0 VEP amplitude (average amplitude (norm to max) ± SEM). Representative VEPs at 0.06, 0.1, 0.3 and 0.4 cycles/degree. Scale bars= 50μV, 50 ms.

3.4.3 Dark exposure enabled experience-dependent recovery of neuronal stimulus selectivity in amblyopic adults

In an effort to identify conditions to promote a maximal recovery from chronic deprivation, we combined visual tetanus (to promote synaptic strengthening) with spatial frequency discrimination (to promote recovery of stimulus selectivity). Following chronic monocular deprivation, subjects received 10 days of dark exposure followed by reverse deprivation and began a routine of performance (3 days/ week for ~5 weeks) in the two-choice spatial frequency discrimination task. Half of the subjects received tetanic visual stimulation to the recovering eye approximately two hours before performance of the visual task. The combination of visual tetanus and spatial frequency discrimination task induced an increase in VEP amplitude (μV average ± SEM cMD: 122.20±13.56, n=5; tetanus + visual perceptual learning: 442.0±61.3, n=3; unpaired t-test, $p<0.001$, Figure 4b). Importantly, the activity and experience-dependent potentiation of VEP amplitudes was observed across spatial frequencies (extrapolation of linear regression to 0 VEP amplitude average ± SEM cMD: 0.35±0.05, n=5; VPL + tetanus: 1.27±0.05, n=3; unpaired t-test, $p<0.001$; Figure 4c). Single unit recordings revealed an increase in the strength of evoked responses to preferred orientations (green line, VPL + tet; 43,3; red line, cMD; 56,5; KS test, p=0.007, Figure 4d). Importantly, subjects that received tetanus + spatial frequency discrimination training also recovered orientation selectivity (KS test, p=0.001; Figure 4e).

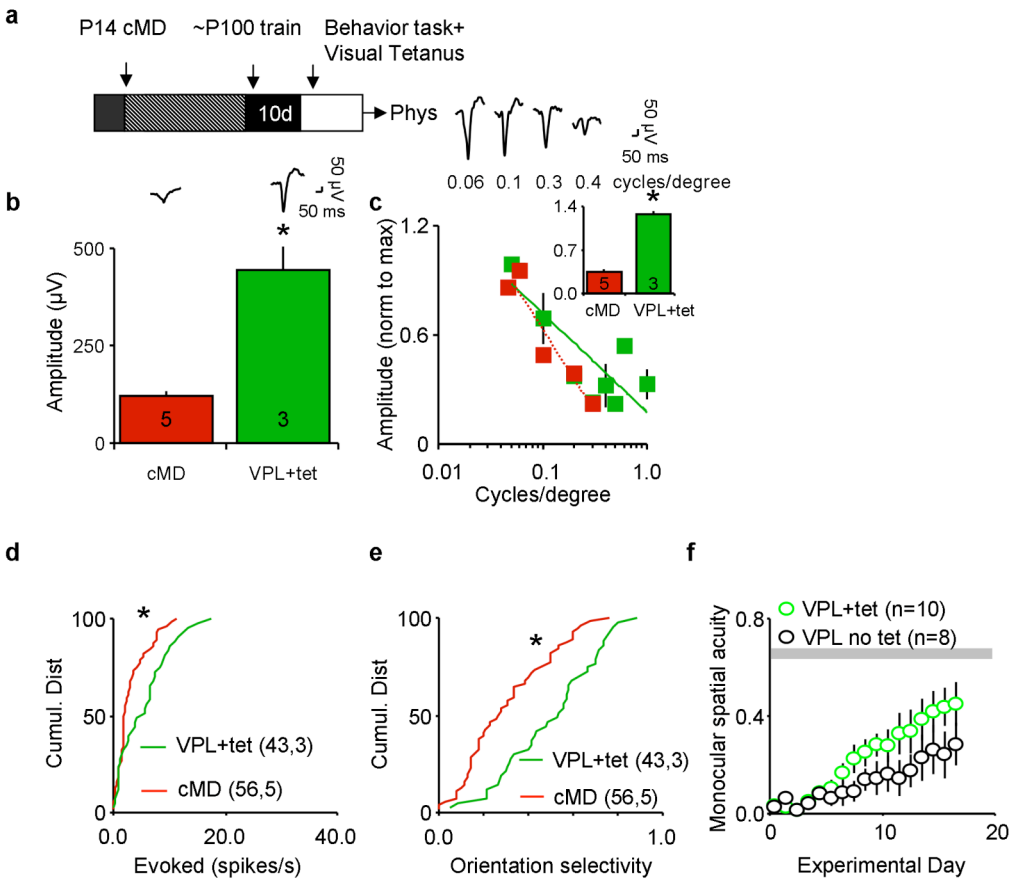

Figure 4: Visual perceptual learning combined with visual tetanus induced a maximal recovery of spatial acuity at a visual task. A. Experimental timeline: All subjects received chronic monocular deprivation (cMD; P14-P100). A subset of subjects received training (train) in the discrimination task with the nondeprived eye. Following reverse deprivation, and 10 days (10d) dark exposure (DE) behavior subjects received visual tetanus prior to performing the discrimination task with the previously deprived eye. B. VEP amplitude of the previously deprived eye significantly increased in visual perceptual learning + tetanus (VPL + tet) subjects relative to chronically deprived controls (average μV ± SEM, *p=0.006, unpaired t-test). C. Significant increase in VEP amplitude across a range of spatial frequencies (average amplitude (norm to max) ± SEM). Inset: Extrapolation of linear regression to 0 VEP amplitude (average amplitude (norm to max) ± SEM) cMD, *p<0.0001, unpaired t-test. Representative VEPs at 0.06, 0.1, 0.3 and 0.4 cycles/degree. Scale bars= 50μV, 50 ms. D. A significant increase in single unit evoked activity (spikes/second) following tetanus and discrimination training at the peak orientation of VPL + tetanus (green line) relative to cMD controls (red line); n=units, subjects, *p=0.007, KS test). E. Significant increase in orientation selectivity following tetanus and discrimination training (VPL + tet, green line) versus cMD (red line) *p=0.001, KS test. F. Visual tetanus and subsequent performance in the visual discrimination task promoted recovery of monocular spatial acuity using the previously deprived eye. Grey bar represents average nondeprived eye spatial acuity ± SEM.

To ask if the potentiation of VEP amplitudes induced by visual tetanus accelerated recovery of spatial acuity, the spatial frequency discrimination task was performed with the previously deprived eye. The initial nondeprived eye acuity did not significantly differ between the two cohorts (monocular spatial acuity of the NDE average ± SEM VPL + tetanus: 0.56±0.06; n=10; VPL no tetanus: 0.57±0.06; n=8; unpaired t-test, p= 0.918, not shown). Subjects received training (~3X/week) at the spatial frequency discrimination task, +/- preceding visual tetanus presented to the opened chronically deprived eye. Spatial acuity emerged by experimental day 4 in subjects that received visual tetanus, and preliminary data suggest that the recovery of spatial acuity may be accelerated in subjects that received visual tetanus (Figure 4f). This suggests that visual tetanus may strengthen synapses in the dark exposed amblyopic visual cortex that can be utilized to promote faster recovery of spatial acuity.

3.5 Discussion

3.5.1 Summary and Conclusions

Dark exposure promotes synaptic plasticity in post-critical period amblyopic adults, which can be recruited by tetanic visual stimulation to enhance the strength of synapses serving the deprived eye. Importantly, unlike many forms of synaptic plasticity in the binocular visual cortex, the rapid potentiation of VEP amplitudes by visual tetanus in dark exposed amblyopes was not stimulus-selective. The absence of input specificity of the visual tetanus likely reflects the reduced neuronal stimulus-selectivity that is characteristic of severe amblyopia. The ability to induce widespread VEP potentiation in dark exposed amblyopes

can be harnessed to drive a generalized recovery of visual response amplitudes. Indeed, the potentiation of VEP amplitudes by visual tetanus improved performance in the spatial discrimination task, demonstrating an activity-dependent strengthening in the pathway serving the recovery of vision while using the deprived eye. While visual tetanus promoted a strengthening of visual responses in dark exposed amblyopic adults, it did not drive the recovery of neuronal orientation selectivity. Increasing the demand on the visual system, by active use in a more complex visual task, may be required to drive the refinement of cortical receptive fields. Indeed, we have previously shown that dark exposure followed by repeated performance in a two-choice spatial frequency discrimination task promoted a modest recovery of spatial acuity in dark exposed amblyopes (He et al., 2007). This suggests that a combination of binocular visual deprivation and monocular visual stimulation could optimize the recovery from severe deprivation amblyopia

3.5.2 Response to visual tetanus is rapid and non-stimulus selective in dark exposed amblyopes

In binocular mice, the potentiation of VEP amplitudes in response to tetanic visual stimulation is first detected ~12 hours after stimulation (Frenkel et al., 2006). However, we observed no acceleration of VEP potentiation in binocular rats with dark exposure. Conversely, in dark exposed adult amblyopes, a similar visual tetanus induced a response potentiation that was rapid and non-stimulus selective. The acceleration of the response to visual tetanus may be due to a deprivation-induced reduction in the threshold for synaptic potentiation

across a large population of synapses. The loss of neuronal stimulus selectivity following chronic monocular deprivation predicts that visual stimulation will allow for widespread activation of neurons in the amblyopic visual cortex in response to a simple oriented grating. The potentiation of a large population of synapses by visual tetanus is revealed in the potentiation of the VEP in response to visual stimuli of various orientations and spatial frequencies.

Chronic monocular deprivation may also promote the response to visual tetanus by decreasing the strength of cortical inhibition. Indeed, chronic monocular deprivation induces a decrease in immunohistochemical markers for inhibitory neurons in the visual cortex contralateral to chronically deprived eye (Mainardi et al., 2009; Hendry and Jones, 1986). Importantly, the decrease in inhibitory neurons was also reflected by a decrease in inhibitory synaptic strength. Even brief (2d) monocular deprivation in juvenile (P14) rats decreased the amplitudes of IPSCs from fast-spiking neurons onto star pyramidal neurons in layer IV of the deprived monocular visual cortex (Maffei et al., 2004). Several subtypes of GABAergic neurons exist in the visual cortex; therefore, the decrease in the strength of one inhibitory neuron subtype may result in a compensatory increase in another GABAergic subtype. However, yellow fluorescent protein (YFP) expression through a vesicular GABA transporter (VGAT) promoter confirmed that this is unlikely. For example, *in vivo* two-photon calcium imaging revealed that monocular deprivation (7 days) in mice (~P58) induced a significant decrease in the response of YFP-VGAT expressing inhibitory interneurons (Kameyama et al., 2010). Others reported that monocular

deprivation induces an initial increase followed by a decrease in the spiking output of fast spiking interneurons in the binocular visual cortex of mice (Yazaki-Sugiyama et al., 2009).

3.5.3 Low temporal frequency oscillations mimic non-REM sleep patterns

It is interesting to consider why low temporal frequency visual stimuli are an effective means of enhancing synaptic responses in rodent visual cortex. One possibility is that the low (0.5 Hz) temporal frequency visual tetanus mimics the slow (<1 Hz) oscillations observed during non-rapid-eye-movement (non-REM) sleep. Enhancements in synaptic plasticity in the visual cortex have been positively correlated with the duration of non-REM sleep. For example, decreased non-REM (0.5-4.0 Hz) sleep, monitored by electroencephalography (EEG) in kittens (~P30), decreased the ocular dominance shift in response to monocular deprivation (Frank et al., 2001). The ability of low frequency stimulation to enhance synaptic plasticity has also been recognized in the field of transcranial stimulation. For example, in humans, low temporal frequency (0.75 Hz) current oscillations applied to the frontolateral cranium during non-REM sleep increased slow wave sleep and enhanced the retention of declarative memories better than higher frequency stimulation (Marshall et al., 2006). Interestingly, application of transcranial direct current stimulation (tDCS) to various sensory cortices can also improve task performance. Weak tDCS of the primary motor cortex increased the amplitude of the motor evoked potential (MEP; Nitsche and Paulus, 2000), improved performance in the acquisition of an

implicit motor learning task (Nitsche et al., 2003), and improved retention times (Reis et al., 2009; Galea and Celnik, 2009). In experimental rodents, simulated anodal DCS applied to vertical fibers of slices of mouse motor cortex (6-8 weeks of age) induced NMDAR-dependent LTP in the superficial laminae (Fritsch et al., 2010). Additionally, tDCS enhanced neurotrophin (BDNF) secretion and tDCS-LTP induction was dependent on tyrosine kinase receptor (TrkB) activation (Fritsch et al., 2010), implicating a signaling cascade previously correlated with enhancement of plasticity in the rodent visual cortex (Maya Vetencourt et al., 2008; Sale et al., 2007; Baroncelli et al., 2010).

Alternatively, 0.5 Hz may be an effective stimulus because rodent visual cortical neurons show a preference for visual stimuli at lower temporal frequencies. Intrinsic signal imaging of superficial laminae in the rodent (2-3 months of age) visual cortex indicates that majority of the neurons respond maximally to 0.5 Hz (Marshel et al., 2011). Likewise, neuronal spiking rates decline in superficial and thalamo-recipient laminae of rodent visual cortex when the temporal frequency of the stimulus exceeds 4 Hz (Niell and Stryker, 2008). Functional magnetic resonance imaging (fMRI) in humans (19-26 years) shows that visual cortical neurons prefer temporal frequencies in the 6-8 Hz range (Mirzajani et al., 2006). Nonetheless, repetitive presentation of a simple visual stimulus at higher (20 Hz) temporal frequency results in an enhancement in vision in normal binocular humans that persists for 10 days (Beste et al., 2011). Repetitive transcranial magnetic stimulation of the visual cortex at 10 Hz transiently (< 1 week) improved contrast sensitivity in human amblyopes

(Thompson et al., 2008). This suggests that the ability to enhance excitability with external stimulation persists in the amblyopic cortex, despite reduced input strength and neuronal stimulus selectivity.

3.5.4 Incremental learning of a visual task in dark exposed amblyopes

The incremental gains acquired during the recovery of spatial acuity in dark exposed amblyopes are reminiscent of the gains observed during visual perceptual learning. The modest gains within a training session may reflect submaximal increases in synaptic strength that accumulate over days to reach a final plateau. Alternatively, the ceiling for strengthening of available synapses may be obtained within a training session, but subsequently increased due to spinogenesis/overnight memory consolidation. Discrimination thresholds of normal-sighted humans often do not improve during a visual perceptual learning session, but improvements are detected on subsequent testing days (Karni and Sagi, 1993). The enhancement of synaptic plasticity may be due to learning-induced spinogenesis, which provides the anatomical basis for subsequent synaptic strengthening. Indeed, two-photon imaging revealed that motor learning induces spinogenesis in the supragranular and infragranular neurons in the mouse (P30) motor cortex (Xu et al., 2009). This suggests that spinogenesis may be an underlying mechanism that plays a key role in memory consolidation. Indeed, extending the duration of motor skill training can raise the ceiling for synaptic strengthening, such that it no longer occludes subsequent LTP (Rioult-Pedotti et al., 2007). Interestingly, this implies that extensive training may

enhance the potential for synaptic plasticity and strengthening.

Visual tetanus presented to the chronically deprived eye of dark exposed amblyopes enhanced VEP amplitudes, which may accelerate recovery of spatial acuity. While preliminary data suggests an acceleration of the recovery rate, additional trials are needed to ask if visual tetanus increases the magnitude of recovery. The recovery of spatial acuity assessed with the spatial frequency discrimination task likely requires recovery of synaptic input, integration, and spiking output throughout the entire visual pathway (Marshel et al., 2011; Andermann et al., 2011). It therefore may not be surprising that a recovery of response properties (VEPs and single units) emerges in the primary visual cortex prior to the recovery of spatial acuity.

3.5.5 Passive versus active visual stimulation

In our experiments, visual tetanus was used to potentiate VEP amplitudes in dark exposed amblyopes that were lightly anesthetized. This demonstrates that the anesthetized visual cortex retains the ability to express activity-dependent synaptic strengthening in the absence of top-down modulation through attention/motivation. Indeed, attention has previously been shown to lower the threshold for relevant feature detection and subsequent perceptual learning; however, it does not appear to be required. Indeed, improvements in discrimination thresholds can be seen for unattended, task-irrelevant features of a visual stimulus (Watanabe et al., 2001).

In contrast, the recovery of neuronal stimulus selectivity was observed only following performance in the spatial frequency discrimination task. The complex demands of the two-choice visual discrimination task may be necessary to drive the recovery of stimulus-selectivity in visual cortical neurons. Alternatively, the sustained visual attention necessary to discriminate between the positive and negative visual stimuli may promote learning. Finally, the physical demands of repeated swimming from the release point to the hidden platform may also promote learning by enhancing cerebral blood flow and inducing the release of neurotrophins (Hillman et al., 2008; Vaynman et al., 2004). Nonetheless, repeated swimming without prior dark exposure does not promote the recovery of spatial acuity from chronic monocular deprivation despite multiple trials (He et al. 2007).

3.5.6 Optimization of recovery from deep amblyopia

An optimal therapy to stimulate the recovery from deep amblyopia must address the cortical 1) loss of neuronal response strength and 2) loss of neuronal response selectivity. We demonstrate that visual tetanus induces widespread synaptic strengthening in the dark exposed amblyopic visual cortex. Preliminary results suggest that the potentiation of VEP amplitudes by visual tetanus may improve the speed of recovery from chronic monocular deprivation. This suggests that dark exposure followed by visual tetanus may be an effective therapy for the recovery of vision in the most severe forms of amblyopia.

Chapter 4: CONCLUSION

4.1 Broader impact/future directions

4.1.1 Broader impact

This work addressed two outstanding questions about the reactivation of ocular dominance plasticity in dark exposed adults.

1) Does dark exposure return the cortex to a juvenile-like state? It had previously been demonstrated that plasticity in thalamocortical synapses was available only during early postnatal life, yet I demonstrated that dark exposure followed by reverse deprivation increased spine density in visual cortical layer IV. In addition, pharmacological isolation of the thalamo-recipient portion of the VEP increased following dark exposure and reverse deprivation implicating that thalamocortical synapses contribute to the recovery from chronic monocular deprivation.

2) Do opportunities exist to accelerate or enhance recovery from deep amblyopia? It had previously been demonstrated that dark exposure followed by reverse deprivation allows for a recovery of many aspects of vision. However, the recovery of spatial acuity assessed with a visual task was slow and incomplete. I showed that visual tetanus presented to the chronically deprived eye in dark exposed amblyopes promoted a generalized enhancement of VEP amplitudes. This suggests that visual tetanus, immediately following dark exposure, may be used to promote a maximal recovery from severe amblyopia.

4.1.2 Further characterization of anatomical response to loss and recovery

In Chapter 2, I demonstrated that the anatomical response to chronic monocular deprivation was robust, and elicited a significant decrease in dendritic spine density in all laminae of the visual cortex. The decrease in dendritic spine density may underlie the decrease in the response to deprived eye visual stimulation. However, dendritic spine density does not necessarily reflect the density of functional synapses. For example, whisker deprivation from birth to ~P9 did not decrease dendritic spine number, but rather increased the fraction of "silent" spines that contain NMDAR currents but lack AMPAR currents (Ashby and Isaac, 2011). This suggests that counting spines may over-represent the number of functional excitatory synapses. Nonetheless, some functional feedforward connectivity must persist following chronic monocular deprivation, as local field potentials (VEPs) and single unit responses can be evoked by visual stimulation presented to the deprived eye. The residual feedforward excitatory synapses are weak (small amplitude) and have lost neuronal stimulus selectivity, but are likely to serve as an anatomical scaffold for building new synapses during recovery from chronic monocular deprivation.

We have interpreted the loss of spines following chronic monocular deprivation as indicative of the loss of excitatory synapses. However, one alternate possibility is that dendritic spines serving the deprived eye may collapse onto dendrites to form shaft synapses. In this case, synapse number would be maintained, despite the loss of dendritic spines. Indeed, synapses on dendritic shafts may be the anatomical basis for the low amplitude VEP evoked by stimulation to the deprived eye. The increase in spine density following dark

exposure and subsequent visual experience is correlated with an increase in the strength of evoked visual responses, implying that the new spines make synapses that participate in the recovery from chronic monocular deprivation. If this represents a transition from shaft to spine synapses, it would be the first description of shaft-spine synapse transition in adult cortex driven by sensory experience. A translation from shaft synapses to spine synapses may occur over development. For example, excitatory synapses in hippocampus are primarily located on dendritic shafts during the first postnatal week, and over the course of the second postnatal week, spine synapses increase while shaft synapses decline (Fiala et al., 1998). Furthermore, the collapse of spine synapses onto dendrites to form shaft synapses has been described in the hippocampi of ground squirrels during hibernation, with a decrease in arousal correlated with a decrease in dendritic spine number (Popov et al., 2007). A future goal is the definitive identification of a shaft synapse, which is possible with serial section electron microscopy that allows for the identification of postsynaptic densities and the characterization of three-dimensional morphology of spines.

4.1.3 Single spine resolution

I showed that dark exposure in adult amblyopes reactivates synaptic plasticity without inducing a change in dendritic spines. This observation was a surprise as we initially expected a scaling up of excitatory synapse number that would be reflected as increased spine density and/or volume. However, the absence of a change in spine density is consistent with the observation that no

change in VEP amplitude or monocular spatial acuity is detected upon cessation of dark exposure. In fact, we have yet to document any anatomical, physiological, or behavioral changes that are induced by dark exposure alone. It has been previously demonstrated that brief (≤ 7 days) dark exposure induces homeostatic scaling of mEPSCs in rodent visual cortex (Goel et al., 2006; Goel and Lee, 2007). The longer duration of dark exposure that we employ may engage compensatory mechanisms that bring mEPSC amplitudes down to control levels. Alternatively, dark exposure may engage different mechanisms in binocular versus amblyopic subjects. Another possibility is that dark exposure increases spine motility rather than spine density. Spine motility reflects structural malleability and has been positively correlated with the level of available plasticity to synapses. Accordingly, spine motility is high in the juvenile visual cortex relative to adults (Holtmaat et al., 2005). Two-photon *in vivo* imaging would be required to test this hypothesis.

Although we have demonstrated a correlation between increased spine density and an increase in the activity that can be evoked by stimulation of the deprived eye, we have yet to demonstrate that the newly emerged spines serve the deprived eye. One way to demonstrate this would be to perform intraocular injections of an anterograde trans-synaptic lectin, such as fluorescent wheat germ agglutinin (WGA) to label thalamic afferents serving the deprived eye. WGA would be taken up by retinal ganglion cells, transported to the axon terminals, and travel across the synaptic cleft to the dorsal lateral geniculate nucleus of the thalamus where thalamic afferents would be labeled in the binocular visual

cortex. If combined with DiOlistic labeling of postsynaptic spines, this would allow for the quantification of colocalized pre and postsynaptic elements serving the deprived/recovering eye.

Recent developments in high-resolution, two-photon functional imaging could also be used to identify synapses serving the deprived eye. Sensory-evoked calcium transients can be detected in single dendritic spines of cortical neurons *in vivo* (Chen et al., 2011). Neurons are dialyzed with a fluorescent calcium indicator and are activated via sensory stimulation (such as visual stimulation). Furthermore, utilizing this technique for time-lapse imaging would allow characterization of the emergence of stimulus selectivity in individual spines. One hypothesis that could be tested with this method is that visual tetanus presented to the chronically deprived eye of a dark exposed amblyope induces spinogenesis and that subsequent visual experience would drive the development of orientation selectivity of the newly formed spine.

4.1.4 Anatomical scaffold for recovery

We hypothesize that some feedforward connectivity must survive chronic monocular deprivation to provide an anatomical framework for recovery of function. One hypothesis is that a deprivation-induced change in the cortical circuitry or molecular composition may promote the maintenance of synapses serving the deprived pathway. Indeed, monocular deprivation (3-7 days) in juvenile mice (P28) modifies the NMDAR subunit composition (decreased NR2A/NR2B ratio) in the deprived visual cortex (Chen and Bear, 2007), which would enhance the duration of NMDAR-mediated currents and increase the

window for correlation-based plasticity. This suggests that deprivation-induced changes in NMDAR subunit composition may promote the maintenance of synapses serving the deprived eye. The deprived eye synapses that persist may then provide the anatomical scaffold for recovery. My data show that dark exposure followed by reverse deprivation promoted a recovery of dendritic spines in proximal (0-75 microns from soma) segments of basolateral dendrites. The dendritic spines that are more proximal to the soma are more likely to be spared by spike-timing mechanisms triggered by backpropagating action potentials. For example, paired pre-post activation of neurons in the superficial laminae of the rat visual cortex induced larger LTP in proximal (<50 microns from soma) than distal segments of the dendrite (Froemke et al., 2005). This distance-dependent reduction in LTP is likely due to attenuation of the backpropagating action potential. In addition, the activity at residual synapses that is engaged by spike-stimulation of the recovering eye may promote recovery of dendritic spine density closer to the soma.

4.1.5 Optimal stimulation promotes a complete recovery

Dark exposure reactivates both physiological and anatomical plasticity in the amblyopic adult visual cortex. However, recovery of evoked physiological activity and dendritic spine density are rapid and complete relative to the slow, incomplete recovery of spatial acuity assessed behaviorally. This disconnection suggests that changes in synaptic structure and function in primary visual cortex may have a lower threshold for recovery than behavior. Alternatively, recovery of

spatial acuity, assessed with the spatial frequency discrimination task, may require recovery of synaptic number and function outside of V1. Nonetheless, in chapter 3, I demonstrated preliminary evidence that the rate of recovery from deep amblyopia may be accelerated by potentiating deprived eye synapses with a visual tetanus. Indeed, visual tetanus presented to the chronically deprived eye of dark exposed amblyopes induced a generalized non-stimulus selective strengthening of visual responses. The same stimulus did not induce a VEP potentiation in dark exposed binocular adults, suggesting that the latter may not serve as appropriate controls for the former. This work also suggests that a combination of approaches (passive and active) may be necessary to drive an accelerated and complete recovery from deep amblyopia. The relatively noninvasive approach of this therapy can potentially translate to humans with deep amblyopia to promote a maximal and permanent recovery.

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