A STUDY OF OCULAR DOMINANCE PLASTICITY: PROBING THE MOLECULAR MECHANISMS AND EXPLORING ITS EFFECT ON FUNCTIONAL CONNECTVITY

by

Kaiyun Yang

B. Medicine, Southeast University, 2008

A THESIS SUBMITTED IN PARTIAL FULFULLMENT OF THE REQUIREMENTS FOR THE DEGREES OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTGRADUATE STUDIES

(Neuroscience)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

April 2017

ABSTRACT

Ocular dominance plasticity (ODP) is a well-characterized example of experience-dependent plasticity. Multiple molecular mechanisms have been implicated in the studying of ODP. We have previously demonstrated a temporal correlation between long-term depression (LTD) and ocular dominance plasticity. It has also been shown that blockade of LTD abolishes ocular dominance shift during the critical period, suggesting that LTD is necessary for ocular dominance plasticity. Here I go on to explore if LTD is sufficient for ocular dominance plasticity by augmenting it in adulthood. By administering D-serine, an NMDAR co-agonist that selectively enhances LTD in adult visual cortex, I am able to enhance ocular dominance plasticity in adulthood, as evidenced by data collected from single-unit recordings. D-serine operates via an LTD-like mechanism as its effect could be abolished by $GluR2_{3Y}$ peptide, a selective LTD blocker. I therefore argue that LTD plays a key regulatory role in both juvenile and adult ocular dominance plasticity. In addition, D-serine helps facilitate recovery of visual input in long-term monocularly deprived adult mice, suggestive of therapeutic potentials.

In addition, I have examined the functional consequences of monocular deprivation on the rest of primary visual cortex (V1) and cerebral cortex. This is achieved with help of *in vivo* imaging of intrinsic optic signals and calcium imaging. Within the visual cortex, monocular deprivation decreases the correlation between the contralateral monocular zone with the rest of V1. I have also observed transient changes in global functional connectivity correlating with the duration of lid suture during the critical period, in keeping with cross-modal plasticity. However,

this change in functional connectivity is not observed in adulthood, suggesting a sensory period for cross-modal connectivity.

PREFACE

All experiments in this thesis were conducted with the supervision and approval of the University of British Columbia Animal Care Committee. Certificates of approval are A11-0151, A11-0165, and A08-0445.

A portion of this thesis has been published.

Chapter 2 is published in:

Yang K, Xiong W, Yang G, Kojic L, Taghibiglou C, Wang YT, Cynader M (2011) The regulatory role of long-term depression in juvenile and adult mouse ocular dominance plasticity. Sci Rep 1:203.

I conducted experiments reported in Chapter 2 result sections. Dr. Wei Xiong contributed partly to Fig 2.7. The writing, analysis and figure preparation were done by myself, with the advice and assistance from the co-authors.

Chapter 3 is unpublished.

I conducted all experiments in Chapter 3. Jeffrey LeDue helped build the imaging apparatus and helped with data analysis by preparing custom-written scripts. Dr. Matthieu Vanni contributed to data analysis presented in Fig 3.12, 13 &16. Data collection, writing and figure preparation were done by myself, with the advice and assistance from Jeffrey LeDue, Drs. Murphy, Swindale, Vanni, and Cynader.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACSF	artificial cerebrospinal fluid
AMBC	Allen Mouse Brain Connectivity
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP2	activating protein 2
BC	barrel cortex
BDNF	brain-derived neurotrophic factor
BOLD	blood oxygen level dependent
BZ	binocular zone
CBI	contralateral bias index
CMN	contrast modulated noise
dLGN	dorsal lateral geniculate nucleus
E/I	excitatory/inhibitory
ECM	extracellular matrix
EEG	electroencephalography
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
FLS1	forelimb somatosensory cortex
fMRI	functional magnetic resonance imaging
GABA	*gamma*-aminobutyric acid
GAD65	glutamic acid decarboxylase 65
GECIs	genetically encoded calcium indicators
GluR	glutamate receptor
GSR	global signal regression
HLS1	hindlimb somatosensory cortex

ICA	independent component analysis
IOS	intrinsic optical signal
IP	intraperitoneal
LFS	low frequency stimulation
LGN	lateral geniculate nucleus
LTD	long-term depression
LTMD	long-term monocular deprivation
LTP	long-term potentiation
MD	monocular deprivation
mGluR	metabotropic glutamate receptors
MRI	magnetic resonance imaging
MZ	monocular zone
NMDA	*N*-methyl-D-aspartate
NSF	*N*-ethylmaleimide-sensitive factor
OD	ocular dominance
ODI	ocular dominance index
ODP	ocular dominance plasticity
PET	positron emission tomography
PP1	protein phosphatase 1
PV	parvalbumin
RGC	retinal ganglion cell
ROI	region of interest
SC	subcutaneous
SST	somatostatin
SWA	slow wave activity
TTX	tetradotoxin

VIP	vasoactive intestinal peptide
VSD	voltage-sensitive dye

ACKNOWLEDGMENTS

I would like to thank first of all my supervisor Dr. Max Cynader for his guidance, supervision and infectious enthusiasm. Dr. Cynader offered me incredible opportunities and freedom to explore the experience-dependent plasticity and functional connectivity. I am also grateful for his tremendous support during my pursuit of neurosurgery residency training. With his selfless help, I am now following my ambition of becoming a surgeon scientist.

I would like to thank my kind, generous and intelligent committee members, Drs. Yu Tian Wang, Timothy H. Murphy and Nicholas Swindale. Dr. Wang understands my passion for both neuroscience and neurosurgery and believes in me like no other. Dr. Swindale has been an aspiration and a great source of brilliant ideas. I am particularly grateful for Dr. Murphy, who has been kind enough to include me as part of his lab, where I carried out the second part of my thesis. It has been a privilege learning from and collaborating with his incredible team, including Drs. Matthieu Vanni, Allen W. Chan, David McVea, Majid Mahajerani, Jamie Boyd, and Jeffrey LeDue. Pumin Wang and Cindy Jiang have been incredibly generous in lending technical support.

I would also like to thank my family, who has taught me the meaning of love, passion and courage, and blessed me with the liberty to pursue my dreams.

My deepest appreciation goes to my dear friends, Grace Wu, Shengjuan Xu, Shaowei Dong and Jeffrey LeDue, who have been there with me for every success and failure.

DEDICATION

To my family,

near and far,

here and gone.

CHAPTER 1 INTRODUCTION

1.1 Experience-dependent plasticity

Experience-dependent plasticity is a topic of profound interest and extensive study in neuroscience. Although neural development is heavily dependent of genetic influence, the brain is able to wire and rewire itself in response to lasting changes in experience. The capacity of neural circuitry to modify itself according to experience is known as experience-dependent plasticity.

Experience-dependent plasticity is particularly evident early in development, when neuronal connections are being made and broken for the first time (Horng and Sur, 2006). Experience-dependent plasticity is crucial for the development and maturation of the central nervous system. It is fundamental in learning and memory. Better understanding of mechanisms of experience-dependent plasticity will lead to development of therapeutic strategies to facilitate recovery from pathological conditions, including stroke, trauma and neurodegenerative diseases (Nithianantharajah and Hannan, 2006; Kleim and Jones, 2008).

The visual cortex has been one of the preferred systems to study experience-dependent plasticity, as visual experience is easy to manipulate and the consequences of manipulations can be readily measured at the molecular, anatomical, physiological and behavioural levels. Specifically, ocular dominance plasticity (ODP) is a well-characterized example of experience-dependent plasticity.

1.2 Ocular dominance plasticity

1.2.1 Anatomy and physiology of the visual network

Proper understanding of ocular dominance plasticity requires some basic understanding of the visual pathway. Major players include retina, dorsal lateral geniculate nucleus (dLGN), and the visual cortex. Photoreceptors on the retina convert images into spatially distributed neural

activity. Photoreceptors synapse with bipolar cells, which then synapse with retinal ganglion cells (RGCs). The axons of RGCs form optic nerves, which carry visual information intracranially. The two optic nerves continue posteriorly and meet at the optic chiasm. At the optic chiasm, fibers from nasal hemi-retina cross over to the contralateral side, while temporal hemi-retinal fibers continue ipsilaterally. The axons of RGCs are collectively referred to as the optic tract after passing the optic chiasm. Optic tracts terminate at the dLGN. LGN neurons then send their axons to the primary visual cortex (V1), forming the optic radiations. Layer IV of the primary visual cortex receives the geniculocortical axons and relays the input to extragranular layers (layer II, III, V, and VI). Primary visual cortex integrates visual information acquired from each eye, forming the basis of binocular vision (Antonini and Stryker, 1993a; Antonini et al., 1998).

1.2.2 Physiological changes induced by monocular vision

Nobel laureates Hubel and Wiesel have pioneered the studies on ocular dominance plasticity. They started their seminal, long-lasting collaboration by studying the normal physiology in the visual cortex (area 17) in cat (Hubel and Wiesel, 1959). They discovered that the majority of cortical neurons respond to visual stimuli presented to both eyes. The strength of input from both eyes to binocular units can be compared and quantified in ocular dominance (OD) histogram (Wiesel and Hubel, 1963). In this 7-point scale, group 1 neurons are driven only by contralateral eye, and group 7 ipsilateral eye. Contralateral dominance is marked for group 2 and slight for group 3 neurons. Ipsilateral dominance is marked for group 6 and slight for group 5 neurons. Group 4 neurons respond equally to both eyes (**Fig 1.1A**). Overall, the contralateral eye is more influential (Wiesel and Hubel, 1963).

Binocular interaction and functional architecture are present in very young kittens without visual exposure (Hubel and Wiesel, 1963a). However, normal visual experience in early postnatal life is crucial for the normal development of vision function and maturation of the visual network. During the first few months of life, monocular deprivation, visual deprivation with lid suture, causes a dramatic change in the ocular dominance of V1. The large majority of neurons lose responsiveness to the deprived eye and gains responsiveness to the open eye, thus leading to an ocular dominance distribution shift in favour of the open eye (Hubel and Wiesel, 1963a) (**Fig 1.1 B&C**). This shift in ocular dominance distribution is mediated by competition between synapses serving the two eyes, as binocular deprivation by dark rearing does not alter OD distribution (Wiesel and Hubel, 1965).

1.2.3 Anatomical changes induced by monocular vision

In addition to the physiological changes, Hubel and Wiesel also explored structural plasticity induced by monocular deprivation.

V1 in cats and monkeys is characterized by the presence of ocular dominance columns. Thalamocortical inputs representing the two eyes are arranged in alternating patches or stripes in layer IV of binocular segment of V1, in the form of a radial column running from pia to white matter. The columns alternatively receive thalamocortical projections from either eye (Hubel and Wiesel, 1963b; Shatz and Stryker, 1978; LeVay et al., 1980) (**Fig 1.2A**). Ocular dominance columns have been revealed with different anatomical approaches, including the Nauta degeneration method (Hubel and Wiesel, 1972), autoradigraphy following injection of tracer into one eye (Wiesel et al., 1974) and Liesegang stain (LeVay et al., 1975).

One of the hallmarks of ODP in cats and monkeys is the extensive changes induced by monocular deprivation in ocular dominance columns. Monocular deprivation in early life causes

the columns serving the deprived eye to shrink and those serving the normal eye to expand (Hubel et al., 1977; Shatz and Stryker, 1978) (**Fig 1.2 B&C**).

Monocular deprivation also leads to drastic changes in geniculocortical axonal arbors. This is characterized by a dramatic retraction of the branches of deprived geniculocortical axonal arbors, followed much later by a compensatory expansion of the arbors of the open eye (LeVay et al., 1980; Antonini and Stryker, 1996). This phenomenon has been observed at the level of the entire geniculocortical projection (studied by transneuronal labeling) and at the level of single geniculocortical afferent arbors (Shatz and Stryker, 1978; Antonini and Stryker, 1993b, 1996; Antonini et al., 1998) (**Fig 1.3**).

Anatomical changes in LGN have also been observed. In kittens, light and form deprivation leads to marked atrophy of cells in lateral geniculate body, in layers receiving input from the deprived eye (Wiesel and Hubel, 1963; Garey and Blakemore, 1977).

1.2.4 Amblyopia

Ocular dominance plasticity is not only an important model of experience-dependent plasticity; it is also clinically relevant. In essence, it resembles amblyopia, a leading cause of visual impairment that affects approximately 3% in the population (Webber and Wood, 2005). Amblyopia can result from a variety of pediatric optical abnormalities, including congenital cataract, strabismus (ocular misalignment), and anisometropia (a difference in refractive index between the two eyes). It is characterized by permanent impairment of vision through the affected eye, the 'lazy' eye (Webber and Wood, 2005; Webb et al., 2006). Being the number one cause of monocular vision loss in adults, amblyopia is an important socioeconomic problem (Membreno et al., 2002).

Current treatments for amblyopia are limited. The most important and immediate treatment is to correct the underlying cause, such as early removal of congenital cataract and correction of refractive errors. This is followed by occlusion therapy of the normal eye (the 'good' eye), to reinforce the use of the 'lazy' eye. Occlusion can be achieved with the help of patching; or use of opaque contact lenses, occluders, or adhesive tape on glasses. It has been the mainstay of treatment since the 18th century (Webber and Wood, 2005; Webb et al., 2006). Occlusion therapy is rarely effective beyond the age of eight (Vaegan and Taylor, 1979), and compliance in pediatric population has limited its success. Clinicians and researchers have therefore endeavoured to discover new therapeutic approaches (Ding and Levi, 2011; Li et al., 2011; Guo et al., 2016).

1.2.5 Ocular dominance plasticity in mice

Being an effective, clinically relevant experience-dependent plasticity model, ocular dominance plasticity has been intensively studied in the field of neuroscience. Moreover, it offers the chance to study Hebbian synaptic plasticity (discussed in **1.2.7.2**). Since the pioneering work by Hubel and Wiesel in cats and monkeys, ocular dominance plasticity has been demonstrated and explored in several different species, including baboons (Hendrickson et al., 1978), Chimpanzees (Tigges and Tigges, 1979), sheep (Pettigrew et al., 1984), rabbits (Hollander and Halbig, 1980), humans (Horton and Stryker, 1993), rodents (Gordon and Stryker, 1996), and ferrets (Issa et al., 1999).

Over the past couple of decades, the mouse has become increasingly popular as a species for the study of ocular dominance plasticity. Mice exhibit robust ocular dominance plasticity (Drager, 1978; Gordon and Stryker, 1996). Moreover, it is feasible to perform coordinated molecular, biochemical, electrophysiological and behavioural studies. Most importantly, mice

can be genetically manipulated to test molecular and cellular substrates of ocular dominance plasticity. Since the introduction of the mouse model, many molecular mechanisms underlying plastic changes in synaptic strength have been uncovered. For example, it has been shown that GABAergic neurons (Hensch et al., 1998), Nogo receptor (McGee et al., 2005) and BNDF (Huang et al., 1999) all play a critical role in modulating plasticity.

However, there are some fundamental differences between mice and other animal models of ODP. In carnivores and primates with forward facing eyes, the binocular zone spans the central 120 –130 degrees of visual space. In mice, the binocular zone spans only about the central 30 – 40 degrees, due to limited overlap of visual space between two laterally positioned eyes (Trachtenberg, 2015).

Another difference lies in the spatial representation of the monocular and binocular segments of the primary visual cortex. Unlike in cats and monkeys, mice do not have ocular dominance columns. Instead, geniculocortical connections serving the two eyes are completely superimposed (Drager, 1978; Gordon and Stryker, 1996; Antonini et al., 1999). In mice, binocular zone occupies the lateral 1/3 of the primary visual cortex, receiving roughly 80% of visual input from the contralateral eye (Drager, 1978; Drager and Olsen, 1980; Gordon and Stryker, 1996).

Multiple techniques have been used to examine experience-dependent ocular dominance plasticity in rodent visual cortex, including evoked potentials (Sawtell et al., 2003; Pham et al., 2004; Sale et al., 2007), single-unit recording (Antonini et al., 1999; Fischer et al., 2007), behavioural measurements of visual acuity (Prusky et al., 2006; Lehmann and Lowel, 2008), immediate early gene expression (Tagawa et al., 2005), and intrinsic signal optical imaging (IOS) (Hofer et al., 2006b; Lehmann and Lowel, 2008; Sato and Stryker, 2008). With IOS imaging,

ocular dominance plasticity is reflected by a shift in ocular dominance index (ODI). Cortical maps for both contralateral (*C*) and ipsilateral (*I*) eyes are obtained, prior to computing ODI using the equation (*C-I*)/(*C+I*). Four days of MD during the critical period induces a near-maximal shift in ODI, with a reduction from 0.22 to 0.03. Intrinsic signal optical imaging has been gaining popularity in the past decade since its establishment (Cang et al., 2005).

Although OD columns are not present, plasticity similar to that found in cats and monkeys has been observed in mice. Early in postnatal life, brief monocular deprivation shifts the physiological responsiveness of neurons in the binocular zone of V1 towards the open eye (Gordon and Stryker, 1996; Hofer et al., 2006) (**Fig 1.4**). This period in early postnatal life when plasticity is most pronounced is known as the critical period.

1.2.6 Critical period

Experience-dependent plasticity is most pronounced during the critical period, when neural connections are particularly malleable. Critical periods have been described for filial imprinting in several bird species. The first hours after hatching are crucial for fledglings to identify and bond with their mother (Lorenz, 1935).

Critical periods have been extensively studied in the visual system. The timing of critical periods for ocular dominance plasticity is variable in different species. In general, it correlates with the average life expectancy of the species (Berardi et al., 2000). In kittens, ODP in the visual cortex is very low at 3 weeks, rises sharply at 6 weeks, and extends to 6 months of age (Jones et al., 1984; Mower, 1991). The critical period for ODP in the mouse begins around postnatal day19 (P19), peaks around P28, and ends quickly after P32 (Gordon and Stryker, 1996). Beyond the critical period, it becomes very difficult to alter the ocular dominance of visual

cortical neurons, even with extended periods of monocular deprivation (Hubel and Wiesel, 1970; Gordon and Stryker, 1996).

There are different critical periods for different visual functions. For example, in monkeys, the critical period for scotopic spectral sensitivity ends at 3 months, and that for photopic increment threshold spectral sensitivity is over by 6 month. On the other hand, the critical period for spatial vision lasts until about 25 months of age, and that for binocular vision extends beyond 25 months (Harwerth et al., 1986). In general, critical periods for lower levels of function end earlier (Daw and Beaver, 2001).

Critical periods are crucial because they provide a window of opportunity for the brain to recover from developmental defects or acquired injuries. In the case of amblyopia, correction of visual defect in the weak eye has to occur before the age of eight when the critical period for ocular dominance plasticity closes (Vaegan and Taylor, 1979). This is challenging due to poor compliance in pediatric patients. As a result, amblyopia commonly leads to lifelong visual impairment.

Reverse suture in animal models is analogous to patching in children. It involves restoration of the visual input to the deprived eye while occluding input from the initially non-deprived eye. Compared to binocular vision, reverse suture promotes greater physiologic recovery of and better visual acuity in the deprived eye, especially during the critical period (Blakemore et al., 1978; LeVay et al., 1980; Swindale et al., 1981; Mitchell et al., 1984).

Due to the functional significance of the critical period of ocular dominance plasticity, studies have been dedicated to understanding of mechanisms of ODP and treatment measures to extend the critical period.

1.2.7 Molecular mechanism of ocular dominance plasticity

Many theories have been put forward to account for this competition-based plasticity, including excitatory/inhibitory (E/I) balance, synapse formation/elimination and LTP/LTD. Here I will briefly discuss the role of cortical inhibition and synaptic mechanisms as they relate to the results to be presented in this thesis.

1.2.7.1 Role of cortical inhibition

GABAergic (GABA, *gamma*-aminobutyric acid) circuitry represents the primary inhibitory system of the central nervous system. Although GABAergic interneurons make up only 10-20% of neuronal population in rodent brain (Rudy et al., 2011), they play a pivotal role in shaping cortical activity during development (van Versendaal and Levelt, 2016).

Interneurons expressing parvalbumin (PV, Ca^{2+} binding protein) are the largest group of interneurons in the cortex, accounting for 40 % of the total GABAergic population (Butt et al., 2005; Rudy et al., 2011). Basket cells make up the majority of PV + interneurons. They innervate the proximal dendrites and somata of their targets, serving as the main source of somatic inhibition (Somogyi et al., 1983; Kubota and Kawaguchi, 2000). Somatostatin (SST) – expressing interneurons are the second largest group and make up 30% of total cortical GABAergic interneurons. The third largest group of interneuron express the serotonin receptor, 5HT3aR. A subset of this group also expresses vasoactive intestinal peptide (VIP) (Lee et al., 2010). VIP+ interneurons are activated by cholinergic and serotonergic input, thus supporting a role for neuomodulation in ODP (Paspalas and Papadopoulos, 2001).

GABA–mediated inhibition regulates ocular dominance plasticity on multiple fronts. It has been argued that PV+ basket cells are the main regulators of ODP early on, while SST+ and VIP+ interneurons become more prominent regulators during adulthood (van Versendaal and Levelt, 2016).

Specifically, inhibition is necessary to 'open' the critical period for ocular dominance plasticity. Ocular dominance plasticity is absent in mice deficient in glutamatergic acid decarboxylase (GAD65), a GABA synthesizing enzyme. This lack of ODP can be rescued by increasing inhibition pharmacologically with diazepam (Hensch et al., 1998). Increasing the level of GABAergic transmission by benzodiazepine infusion in very young mice promotes early onset of the critical period of OD plasticity (Fagiolini and Hensch, 2000). Precocious critical period have also been induced by genetically increasing cortical BDNF levels, which contributes to the maturation of inhibitory innervation (Hanover et al., 1999; Huang et al., 1999).

The critical period itself is characterized by an optimal excitatory/inhibitory ratio and a gradual increase in inhibition. Inhibitory cells exhibit delayed plasticity during the critical period. Two days of monocular visual deprivation is sufficient to shift the binocularity of excitatory cells, while it takes two more days for inhibitory neurons to shift (Gandhi et al., 2008). V1 is susceptible to any manipulation of E/I ratio during critical period. Intracortical infusion of the $GABA_A$ receptor agonist, muscimol, prevents the ocular dominance shift in MD cat during the critical period (Reiter and Stryker, 1988; Hata et al., 1999).

Lastly, strong somatic inhibition closes the critical period for ocular dominance plasticity. The end of critical period is characterized by structural consolidation, through the maturation of perineural networks of extracellular matrix (ECM) glycoproteins, which surround mature PV+ interneurons (Hartig et al., 1999). Indeed, in mice that lack fully formed perineural nets, the critical period does not close.

In support of modulatory effect of cortical inhibition, some studies have shown continuation of ODP beyond the critical period after modification of the E/I ratio. Transplantation of inhibitory neurons induces ocular dominance plasticity beyond the critical

period (Southwell et al., 2010; Davis et al., 2015). BDNF infusion, coupled with monocular deprivation, is able to re-induce plasticity in adult rats, possibly through a decrease in GABAergic transmission (Maya Vetencourt et al., 2008). Selective serotonin re-uptake inhibitor fluoxetine has been shown to restore ocular dominance plasticity to adults, likely due to a correlative reduction in inhibition (Maya Vetencourt et al., 2008). Disruption of perineural networks, by removal of ECM with protease, reactivates visual cortical plasticity in adult animals (Pizzorusso et al., 2002).

While we acknowledge the seminal researches supporting the functional significance of cortical inhibition, our lab has focused on the synaptic mechanism underlying ocular dominance plasticity, specifically long-term depression (LTD) and long-term potentiation (LTP).

1.2.7.2 Synaptic plasticity, from Hebbian rule, BCM theory to LTP/LTD

Hebbian synaptic plasticity is one of the earliest mechanisms put forward to explain experience-dependent plasticity. According to Hebb, synaptic modification occurs based on the relative rates of neuronal firing of pre- and post-synaptic structures. More specifically, Hebb's rule states "When an axon in cell A is near enough to excite cell B and repeatedly and persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency in firing B is increased" (Hebb, 1949). In other words, "Cells that fire together, wire together". Hebb's hypothesis has received confirmation by the discovery of LTP in hippocampus (Bliss and Lomo, 1973) and many subsequent studies (Lynch, 2004; Herring and Nicoll, 2016).

However, the Hebbian rule taken literally, does not provide stability, as a synapse following the rule can grow in strength indefinitely. Cooper, Liberman and Oja (1979) tried to address this issue by proposing a 'modification threshold, θm' of postsynaptic response. The

polarity of synaptic modification reverses (implying LTD) when postsynaptic activity falls below a threshold θ_m (Cooper et al., 1979).

This subsequently evolved into BCM theory, which proposed an adjustable θ_m (Bienenstock et al., 1982). The value of modification threshold automatically adjusts as a function of the history of integrated postsynaptic activity. The sliding threshold adds both selectivity and stability to the mechanism of synaptic plasticity (Cooper and Bear, 2012). BCM theory is supported by the observation of homosynaptic LTD (Dudek and Bear, 1992), bidirectional synaptic plasticity and metaplasticity (Bear, 2003).

Accumulating evidence favours LTD as a key mechanism for ocular dominance plasticity during the critical period (Kirkwood et al., 1996; Heynen et al., 2003; Frenkel and Bear, 2004; Yoon et al., 2009), in the following way. Visual deprivation initially induces LTD in cortical slices (Heynen et al., 2003; Yoon et al., 2009). Previously, we have shown a temporal correlation between age and the magnitude of LTD induced in visual cortical slices, with LTD diminished in adulthood (Yang et al., 2011). Using a specific LTD blocking peptide $GluR2_{3Y}$ (Ahmadian et al., 2004; Brebner et al., 2005), we showed that *in vivo* blockade of LTD prevents ocular dominance shift induced by monocular deprivation. We believe LTD to be an important molecular substrate of ocular dominance plasticity during the critical period (discussed in detail in Chapter 2).

1.2.8 Ocular dominance plasticity in adulthood

Although experience-dependent plasticity is most pronounced during the critical period, cortical plasticity exists throughout development and extends into adulthood. In the mouse visual cortex, plasticity in V1 in newborns is driven by spontaneous activity that originates in the thalamus and cortex (Siegel et al., 2012), as well as spontaneous retinal activity (Mooney et al., 1996). Spontaneous activity is essential for establishment of thalamocortical and cortical circuitry.

Ocular dominance plasticity becomes prominent during the critical period and gradually wanes thereafter. However, V1 does not abruptly lose its capacity for plasticity at the end of the critical period, but rather that the characteristics of plasticity change over the course of maturation. Although the effects are small, ocular dominance plasticity beyond the closure of the critical period has been observed in ocular dominance columns in cats and monkeys (LeVay et al., 1980; Daw et al., 1992).

In mice, adult ocular dominance plasticity is a slower process, more modest in degree, and takes longer to take effect, compared to that during the critical period (Hofer et al., 2006; Lehmann and Lowel, 2008; Sato and Stryker, 2008) (**Fig 1.5**). Structural plasticity also exists beyond the critical period. Growth and elaboration of terminal geniculocortical arbors continue from P40 to P60, after the peak of the critical period (Antonini et al., 1999). Differences between juvenile and adult ODP are further discussed in Chapter 2 (**section 2.1.4**).

Ocular dominance plasticity in adulthood may have different mechanism than that of the critical period. It is thought that adult OD plasticity is mediated almost exclusively by potentiation of the non-deprived inputs (Sawtell et al., 2003; Hofer et al., 2006b). LTP is a favoured mechanism for ODP in adult rodent brain (Yoshimura et al., 2003). Homeostatic synaptic scaling – in which a prolonged increase in neural activity globally scales down excitatory synaptic responses, while a chronic decrease in activity scales up the responses – has been proposed as a substrate for adult cortical plasticity as well (Turrigiano and Nelson, 2004; Goel and Lee, 2007).

1.2.9 Rationale and hypothesis

As reviewed above, the evidence suggests that LTD plays a key role in ocular dominance plasticity during the critical period (Kirkwood et al., 1996; Heynen et al., 2003; Frenkel and Bear,

2004; Yoon et al., 2009; Yang et al., 2011). However, LTD is developmentally constrained, being difficult to induce in V1 of adult rodents (Dudek and Friedlander, 1996; Jiang et al., 2007; Yang et al., 2011). Thus, I hypothesize that by augmenting the LTD during adulthood, we would be able to induce a state in the visual cortex that resembles that of the critical period; by doing so, we would be able to facilitate ocular dominance shift in adulthood.

D-serine, an endogenous NMDAR co-agonist, has been shown effective in facilitating induction of LTD in adult hippocampal slices. Administration of D-serine augments spatial reverse learning in adult mice (Duffy et al., 2008). Hence, I hypothesize that D-serine application should enhance LTD in adult visual cortex. As we already have a specific LTD-blocking peptide $GluR2_{3Y}$, I set out to examine the functional potential of D-serine in adult visual cortex and the role of LTD in adult ocular dominance plasticity. These results are presented in Chapter 2, in which I focus on the functional significance of LTD in ocular dominance plasticity.

1.3 Global changes in functional connectivity induced by visual deprivation

Monocular deprivation produces physiological and anatomical changes in the binocular zone of V1. However, it is well known that sensory deprivation has striking effects on the development of other modalities (Bavelier and Neville, 2002). These studies will be reviewed in the following sections.

1.3.1 Cross-modal plasticity

Cross-modal plasticity is a form of experience-dependent plasticity that involves the widespread adaptation of brain circuits to sensory deprivation, allowing the brain to navigate its environment with its remaining senses. Cross-modal plasticity involves recruitment of the deprived sensory cortex for processing the remaining senses, as well as experience-dependent refinement of the spared sensory cortices. It has been studied extensively in the context of visual deprivation (Lee and Whitt, 2015) (**Fig 1.6**).

Visual deprivation leads to recruitment of visual cortex by other sensory cortices, often the somatosensory cortex and the auditory cortex. Enucleation in newborn rats leads to expansion of the somatosensory responses into the visual cortex (Toldi et al., 1994a). In monkeys that undergo binocular lid suture in the first year of life, the visual cortex loses its specificity to visual stimuli and gains responsiveness to somatic exploration (Hyvarinen et al., 1981). In cats with prolonged binocular deprivation, anterior ectosylvian visual area, which is normally purely visual, becomes largely driven by auditory and somatosensory stimuli (Rauschecker and Korte, 1993). Primary visual cortex can be driven by auditory input, in naturally blind mole rats (Heil et al., 1991) and visually deprived cats (Yaka et al., 2000). Blind humans display activation of visual areas, including V1, during Braille reading (Sadato et al., 1996; Buchel et al., 1998), and comprehending ultra fast speech (Dietrich et al., 2013). The recruitment of deprived sensory cortex might lead to the abnormal stabilization of usually transient connectivity in the remaining sensory areas (Kato et al., 1993; Negyessy et al., 2000).

Compensatory plasticity of spared sensory modalities has also been widely studied. Enucleation in newborn rats leads to enlargement of receptor fields and an increase in angular sensitivity in the somatosensory cortex. Enucleated rats have better maze performance, which is mediated by somatosensory perception via the whiskers (Toldi et al., 1994b). Binocularly deprived cats have better precision of sound localization than normal cats (Rauschecker and Kniepert, 1994). Prolonged binocular deprivation in both juvenile and adult ferrets lead to a significant improvement in auditory spatial acuity in the lateral sound field (King and Parsons, 1999). Compared to normal subjects, early blind humans can process sounds faster, localize sounds more accurately and have sharper auditory spatial tuning (Lessard et al., 1998; Roder et al., 1999). They have better two-point tactile discrimination skills and superior auditory

recognition memory, as well as larger and faster auditory and somatosensory event-related potentials (Niemeyer and Starlinger, 1981; Kujala et al., 1995; Roder et al., 1996; Roder et al., 1999).

Anatomically, increased spine density and neuron density have been observed in the auditory cortex of rats after early visual or somatic deafferentation (Ryugo et al., 1975). Dark rearing for over three months leads to hypertrophy of the auditory cortex, in both young and adult mice (Gyllensten et al., 1966).

Reciprocal changes have been observed in the visual cortex in response to deprivation of other sensory modalities, such as somatosensory and auditory. Early destruction of cochlear receptors leads to enhancement of visual responses on the primary auditory cortex (Rebillard et al., 1977). Deaf individuals show enhanced tactile accuracy (Levanen and Hamdorf, 2001), and enhanced visual attention, specifically in the peripheral visual field (Rettenbach et al., 1999; Bavelier et al., 2000).

1.3.2 Timing for cross-modal plasticity

Perhaps not surprisingly, the compensatory effect on other sensory modalities induced by sensory deprivation is much greater if deprivation occurs early in life than if it occurs later, indicating that there may be a sensitive period for multimodal plasticity (Bavelier and Neville, 2002).

Auditory localization is better in cats that undergo binocular deprivation from birth than when deprivation is started later in life (Rauschecker and Kniepert, 1994). Cats that are cochleoectomized before the age of three weeks all show visually evoked potentials in A1, whereas cats that are cochleoectomized at a later stage do not (Rebillard et al., 1977). After 10

successive days of bilateral vibrissa clipping, maze performance is poorer in adult rats than newborn rats, indicating more compensatory plasticity in early postnatal life (Volgyi et al., 1993).

However, significant amounts of cross-modal plasticity occur in adulthood (Lee and Whitt, 2015). Visual deprivation in adult mice strengthens thalamocortical synapses in A1 (Petrus et al., 2014). Over three months of dark rearing leads to similar hypertrophy of the auditory cortex occurs in both young and adult mice (Gyllensten et al., 1966). Activation of the primary visual cortex has been observed during Braille reading, in blind subjects who lost their sight after puberty, but not in congenitally blind humans (Buchel et al., 1998). Five days of blindfolding in normal sighted adults, coupled with intensive tactile training, leads to improved performance in Braille character discrimination task. This is accompanied by a transient increased blood-oxygen-level-dependent (BOLD) signal within the occipital cortex in response to tactile stimulation (Merabet et al., 2008).

Like other forms of experience-dependent plasticity, it is probable that cross-modal plasticity is most drastic in early postnatal life, gradually wanes, but persists into the adulthood.

1.3.3 Anatomical basis for cross-modal plasticity – polymodal area

'Polymodal neurons' and polymodal association areas provide a possible basis for cross-modal plasticity and multisensory integration. Instead of working in isolation, primary sensory cortices are influenced by other senses. Multisensory interactions between primary sensory cortices could serve as substrates for cross-modal plasticity in the event of sensory deprivation (Ghazanfar and Schroeder, 2006).

Polymodal neurons have been reported around the 1960s. Auditory and/or somatosensory evoked activity has been observed in neurons of the primary visual cortex (Lomo and Mollica, 1959; Murata et al., 1965; Spinelli et al., 1968; Morrell, 1972). Also, the superficial layers of

primary sensory cortices receive subthreshold inputs originating from other senses (Lakatos et al., 2007; Iurilli et al., 2012). The primary visual cortex is involved in tactile discrimination of grating orientation, as evidenced by transcranial magnetic stimulation study in humans (Zangaladze et al., 1999).

Polymodal association areas refer to part of the cortical network that mediates cross-modal processing in normal individuals (Zangaladze et al., 1999; Calvert, 2001). Polymodal association areas also undergo reorganization in response to sensory deprivation. For example, functional magnetic resonance imaging (fMRI) studies in deaf individuals show increased recruitment of the posterior superior temporal sulcus, an important polymodal association area, in response to moving visual stimuli (Bavelier et al., 2001). After visual deprivation in juvenile rats, cats and monkeys, increased numbers of neurons in polymodal areas respond to somatosensory and auditory information. These areas include the superior colliculus, the anterior ectosylvian region in cats and the parietal cortex in primates (Hyvarinen et al., 1978; Vidyasagar, 1978; Hyvarinen et al., 1981; Rauschecker and Korte, 1993; Rauschecker, 1996). In cats that are visually deprived from birth, the anterior ectosylvian cortex demonstrates improved auditory localization and greater auditory spatial tuning of cells. In addition, the part of this region that typically responds to visual stimuli becomes predominantly auditory or somatosensory in visually deprived animals (Rauschecker, 1995, 1996).

1.3.4 Mechanism of cross-modal plasticity

Earlier studies suggest that cross-modal plasticity is mediated by activity-based competition. This has been followed by theories including changes in local connectivity, changes in subcortical connectivity, changes in cortico-cortical feedback (Bavelier and Neville, 2002), and synaptic mechanisms such as LTP/LTD (Lee and Whitt, 2015).

Cross-modal plasticity may result from changes in local connectivity. Changes in local connectivity persist into adulthood, despite being more pronounced early in development. It is reflected in changes in sensory maps after sensory stimulation or deprivation. This may be a result of local sprouting, unmasking of silent synapses and/or changes in the modulatory effects of lateral connections (Bavelier and Neville, 2002).

Changes in subcortical connectivity may also contribute to cross-modal plasticity. This form of plasticity is more likely to be limited to developing organisms. Auditory colonization of V1 has been observed in congenitally blind mole rat *Spalax ehrenbergi*. In this species, inferior colliculus (an auditory relay) projects to both the auditory thalamus and the visual thalamus (dLGN), leading to the recruitment of V1 by auditory stimuli (Doron and Wollberg, 1994; Rehkamper et al., 1994). Early ablation of areas 17 and 18 leads to expansion of the pathway from the LGN to the middle suprasylvian cortex, which is normally small (Payne and Lomber, 1998; Payne, 1999). Changes in subcortical connectivity may be a result of stabilization of normally transient and redundant pathways (Bavelier and Neville, 2002).

Changes in cortico-cortical feedback may also contribute to cross-modal plasticity. This is largely endorsed by the literature on cross-modal reorganization in the deaf and the blind humans. Cortico-cortical feedback, via polymodal association areas such as parietal cortex, might be a source of cross-modal rearrangement (Bavelier et al., 2000; Weeks et al., 2000). During auditory localization, blind subjects show increased functional connectivity between right posterior parietal cortex and the right occipital region, compared to sighted controls (Weeks et al., 2000). Deaf individuals exhibit enhanced functional connectivity between the parietal cortex and earlier visual areas, when attending to the peripheral visual field (Bavelier et al., 2000).

Primary sensory cortices may also mediate cortico-cortical feedback and cross-modal plasticity. Disrupting function of the occipital cortex with transcranial magnetic stimulation, leads to impaired tactile discrimination in blind humans, but not sighted individuals. This indicates that primary visual cortex mediates tactile performance in the blind (Cohen et al., 1997).

Interestingly, it has been recently proposed that cortical adaptation to sensory loss in cross-modal plasticity involves homeostatic synaptic plasticity and LTP/LTD (Lee and Whitt, 2015). Visual deprivation, by intraocular tetradotoxin (TTX) injection, leads to homeostatic up-regulation of layer II/III neurons in V1 (Maffei and Turrigiano, 2008). It is interesting to note that visual deprivation via lid suture, which permits diffuse light through the closed eyelids, fails to trigger homeostatic scaling (Maffei and Turrigiano, 2008; He et al., 2012). Visual deprivation decreases the neural threshold for sound intensity in A1 neurons, and sharpens the neuronal receptive fields in A1 (Petrus et al., 2014). Visual deprivation by lid suture also leads to sharpening of the functional whisker-barrel map at layer II/III in rat brain (Jitsuki et al., 2011). It is likely that, following visual deprivation, previous sub-threshold inputs carrying auditory and tactile information may become strong enough to summate and cross the threshold to activate V1 neurons (Lee and Whitt, 2015). Also, cross-modal potentiation depends on the spared senses, suggesting an LTP-like mechanism. However, further evidence is required to argue for a convincing role of LTP/LTD in regulating cross-modal plasticity.

In essence, cross-modal plasticity is experience dependent and suggestive of changed functional connectivity in response to sensory deprivation. Understanding the mechanism of cross-modal plasticity has the potential to suggest ways of enhancing plasticity in adulthood and facilitating recovery from injuries. Hence, using intrinsic signal imaging and calcium imaging of the mouse cerebral cortex, I set out to explore the changes in functional connectivity induced by

monocular deprivation in Chapter 3. In line with the evidence revealed above, I hypothesize that monocular deprivation during the critical period can cause compensatory plastic changes across the cerebral cortex in functional connectivity. To study the functional connectivity, I chose to study the spontaneous activity of the brain.

1.4 Spontaneous activity and functional connectivity

1.4.1 Spontaneous activity of the brain

Early studies of cortical neurophysiology were largely focused on the correlates of sensory or motor behaviour. However, in the past couple of decades, spontaneous neural activity has been gaining more attention and recognition as worthy of study (Fox and Raichle, 2007; Snyder and Raichle, 2012). Spontaneous activity, also known as resting-state activity, is brain activity in the absence of an explicit task, such as sensory input or motor output.

Although the brain accounts for only 2% of whole body weight, at rest it consumes 20% energy of the entire body. While only 5% of the energy consumed by the brain is used in response to ongoing sensory or motor tasks (Raichle and Mintun, 2006), 75% is dedicated for active signalling (Attwell and Laughlin, 2001; Howarth et al., 2012). The substantial energy consumption of spontaneous brain activity implies functional significance yet to be better defined.

Spontaneous activity is characterized by rhythmic oscillation. In 1929, Berger reported the first human electroencephalography (EEG) recordings, providing experimental evidence for oscillation on a network level (Berger, 1929). Oscillatory patterns are subsequently recognized as a prominent feature of brain activity (Buzsaki and Draguhn, 2004; Haider and McCormick, 2009). Oscillation occurs at frequencies ranging from infra-slow (< 0.1Hz) to ultrafast (600Hz) (Buzsaki and Draguhn, 2004). The classical EEG frequency bands include

delta (1–3 Hz), theta (4–8 Hz), alpha (9–12 Hz), beta (12–30 Hz), and gamma (>30 Hz) (He et al., 2010). Slow-wave activity (SWA) is used to describe activity in the 0.5-4 Hz range, including both delta and the slow oscillation (defined below) (Mascetti et al., 2011).

On a cellular level, oscillation (<1Hz) is characterized by a relatively rapid switch between a hyperpolarized (by 7-10 mV), silent state and a depolarized state lasting for ~1 s (Steriade et al., 1993a). Both thalamocortical and reticular cells also displayed similar intracellular oscillations (Steriade et al., 1993b). These two states are referred to as UP and DOWN states. The alternation between UP and DOWN is referred to as the slow oscillation (Van Someren et al., 2011).

The UP state is characterized by pronounced excitatory and inhibitory activity and is disrupted by both glutamatergic and GABAergic antagonists (Castro-Alamancos, 2000; Sanchez-Vives and McCormick, 2000; Compte et al., 2003). The DOWN state is triggered by the synchronized withdrawal of synaptic activity of cortical neurons as indicated by increased input resistance during the DOWN state in anesthetized (Contreras et al., 1996b) and naturally sleeping states (Steriade et al., 2001; Timofeev et al., 2001), as well as via modeling of network behaviour under different conditions of background synaptic activity (Compte et al., 2003).

With regard to the origin of spontaneous activity, it is generally believed to be largely cortical with thalamic modulation. Spontaneous activity is preserved in isolated cortical slabs (Timofeev et al., 2000) and slices (Sanchez-Vives and McCormick, 2000; McCormick et al., 2003). While cortical rhythm could survive thalamic lesions (Steriade et al., 1993a), the slow oscillation is not observed in the thalamus following the removal of the cortex in vivo (Timofeev and Steriade, 1996).

On the other hand, it is important to note thalamus plays an important role in shaping the slow oscillation. Both reticular thalamic and thalamocortical neurons display similar intracellular oscillations to those of cortical neurons (Steriade et al., 1993b). Thalamic bursts often precede cortical ones when thalamocortical connections are intact (Contreras and Steriade, 1997; Grenier et al., 1998; Rigas and Castro-Alamancos, 2007). In addition, thalamic input, via stimulation of thalamocortical fibres (Rigas and Castro-Alamancos, 2007; Mann et al., 2009) or sensory stimulation (Petersson et al., 2003), can trigger UP states in the cortex. The rhythm of slow oscillation becomes less regular following thalamic lesions (Crunelli and Hughes, 2010). Intra-thalamic muscimol injection slows the cortical slow oscillation in a dose-dependent fashion (Doi et al., 2007). All this evidence supports a modulatory role for the thalamus.

1.4.2 Functional imaging studies of spontaneous activity of the brain in humans

In human brain, spontaneous activity has been studied with the help of functional magnetic resonance imaging and positron emission tomography (PET). Both methods make it possible to collect signals of brain activity from a wide expanse of cortical and subcortical areas. With fMRI, spontaneous activity in the resting state is measured by fluctuations of the BOLD signal. The BOLD signal is determined by local variations in de-oxyhemoglobin concentration, which in turn is determined by a combination of blood flow, blood volume and oxygen metabolism (Raichle and Mintun, 2006).

Studying spontaneous activity has lead to the discovery of the default mode network (DMN) (Raichle and Snyder, 2007). Using PET, Raichle and colleagues have identified a set of midline cortical areas that are deactivated during performance of a cognitive task, including primarily the medial prefrontal cortex, the precuneus, and the posterior cingulate cortex

(Shulman et al., 1997). These regions, however, are activated at rest in the absence of any task, thus giving name to the default mode network (Raichle et al., 2001). The activity within the default mode network is highly correlated at rest (Greicius et al., 2003). The default mode network helps process memories, plan for the future, or perform other self-referential tasks (Fox and Raichle, 2007).

1.4.3 Spontaneous activity as a means to study functional connectivity

More importantly, spontaneous activity has proved very helpful in studying functional connectivity. Spontaneous fluctuations in brain activity are correlated between functionally and anatomically related regions (Fox and Raichle, 2007). Using these correlations as a measure of connectivity, some argue that the organization of networks strikes an optimal balance between minimizing axon length and maximizing connectivity (Bassett and Bullmore, 2006; Bullmore and Sporns, 2009).

1.4.3.1 Analysis methodology

Studying the BOLD signal has lead to the exploration on functional connectivity, which examines the coherent spatial patterns in neuronal variability. Two techniques have been widely used to study functional connectivity, namely seed based correlation mapping and independent component analysis (ICA) (Fox and Raichle, 2007).

Seed based correlation mapping involves extracting the BOLD time course from a seed region (region of interest, ROI) and calculation of the temporal correlation between this extracted signal and the signals from all other brain voxels (Fox and Raichle, 2007) (**Fig 1.7**). It is widely used due to its inherent simplicity, sensitivity and ease of interpretation (Cordes et al., 2001; Greicius et al., 2003; Fox et al., 2005; Fox et al., 2006b). However, seed based correlation mapping requires *a priori* definition of a seed region, and extensive preprocessing

to minimize the influence of non-neuronal variance (Snyder and Raichle, 2012). In addition, multiple systems cannot be studied simultaneously (Fox and Raichle, 2007).

ICA is another popular technique to study the spatial pattern of spontaneous BOLD data (Kiviniemi et al., 2003; Beckmann et al., 2005; De Luca et al., 2006). The entire BOLD data set is analyzed with sophisticated algorithms and decomposed into components that are maximally independent in a statistical sense. Each component is associated with a spatial map. Some maps reflect neuro-anatomical connection, while others represent noise components. ICA is more advantageous over seed based correlation mapping in that it does not require *a priori* definition of seed regions. Also, it is data driven and automatically isolates sources of noise. However, ICA has its own limitations as well. First, it is less suited to study targeted ROIs. Second, the user must determine the number of components the algorithm is to produce, and which components reflect noise or neuro-anatomical systems, thus introducing room for bias and error. Third, the sophistication of the algorithm poses challenges for data interpretation, especially in disease models in which spontaneous activity is affected (Fox and Raichle, 2007; Snyder and Raichle, 2012).

Global signal regression (GSR) is one of the preprocessing techniques used to improve the spatial specificity of correlation maps. GSR uses the time-series averaged over the whole brain as a nuisance regressor, and thereby reduces the influence of non-neuronal artefact (Birn et al., 2006; Chang and Glover, 2009). One of the most striking features of correlation maps obtained using GSR is anti-correlation between the default mode network and task positive network (Fox et al., 2005). It is important to recognize GSR artificially introduces negative correlations, since GSR entails that the mean value in all computed correlation maps is algebraically constrained to be approximately zero (Fox et al., 2009). Spatial and temporal

smoothing has also been used in preprocessing to improve the spatial specificity of spontaneous BOLD maps (Snyder and Raichle, 2012).

The temporal properties factor into the analysis of spontaneous BOLD signals as well. Spontaneous BOLD follows a 1/f distribution, which means that there is increasing power at lower frequencies (Zarahn et al., 1997). This 1/f distribution has also been observed in studies of spontaneous EEG (Linkenkaer-Hansen et al., 2001), local field potential recordings (Leopold et al., 2003) and cognitive process (Gilden, 2001). Previous studies have indicated that only frequencies below 0.1 Hz contribute to regionally specific BOLD correlations, well away from cardiac or respiratory factors (Cordes et al., 2001). And therefore, a low-pass filter with a cut-off of 0.08 or 0.1 Hz has been frequently applied in analyses of spontaneous BOLD signals (Fox and Raichle, 2007).

1.4.3.2 Functional connectivity revealed by spontaneous activity

Studies of spontaneous BOLD signals have shown that infra-slow activity (< 0.1 Hz) is characterized by synchronous bilateral activity in functional related regions.

In 1995, Biswal and colleagues first demonstrated in the somatomotor cortex that BOLD signals are temporally correlated in the resting state. Spontaneous BOLD fluctuations measured in the left somatomotor cortex are correlated with that in the right somatomotor cortex and with medial motor areas (Biswal et al., 1995). Coherent fluctuations in BOLD signals have subsequently been observed in other related systems of the brain, including visual, auditory, and language area (Lowe et al., 1998; Cordes et al., 2000). In general, studies suggest that brain regions with relevant functions tend to be correlated; while those with opposing function tend to be anti-correlation (Fox and Raichle, 2007).

These findings have been successfully replicated in rodents, with the help of *in vivo* functional imaging techniques, including IOS (White et al., 2011), voltage-sensitive dye (VSD) imaging (Mohajerani et al., 2010), and calcium imaging (Vanni and Murphy, 2014). These methods are compared and contrasted in detail in Chapter 3 (**section 3.1.5**).

A prominent feature of the functional connectivity revealed by spontaneous activity is the symmetrical representation and correlation in the interhemispheric homotopic regions (Biswal et al., 1995; Mohajerani et al., 2010; Vanni and Murphy, 2014). This may be partially mediated by long-range connection via the corpus callosum. The development of the corpus callosum relies on normal experience (Olavarria and Li, 1995; Innocenti et al., 2003; Olavarria and Safaeian, 2006). Absence of the corpus callosum has been observed in mice that have underwent binocular enucleation in infancy (Rhoades et al., 1984). Elimination of sensory inputs to the whisker system in early life impairs formation of the corpus callosum from the corresponding homotopic barrel (Koralek and Killackey, 1990). Interhemispheric correlations are reduced in patients with callosal agenesis (Quigley et al., 2003). In a particular strain of acallosal mice, correlations of SWA between hemispheres are reduced (Vyazovskiy et al., 2004), while the power of SWA within each hemisphere was increased (Vyazovskiy and Tobler, 2005). In other words, there is enhanced intrahemispheric connectivity and reduced interhemispheric connectivity.

Functional connectivity may reflect neuro-anatomical organization. Comparisons of BOLD signal correlations and structural connectivity have shown correlation between these measures (Koch et al., 2002; De Luca et al., 2006; Hagmann et al., 2008; Greicius et al., 2009). Also, computational models based on structural connectivity can predict the functional correlation in the macaque brain, as is shown with fMRI (Honey et al., 2007). Direct

correspondences between structural connectivity and functional connectivity have been demonstrated in healthy brain, during development, and in pathological conditions such as Alzheimer's disease, attention deficit hyperactivity disorder (ADHD) and autism (Honey et al., 2010).

Spontaneous activity can affect the performance of cognitive task, by modulating motor outputs (Fox and Raichle, 2007) or sensory perceptions (Monto et al., 2008). Functional connectivity in motor-planning regions is predictive of impulsivity in juvenile offenders (Brier et al., 2012). Alzheimer's disease is associated with diffuse and progressive loss of intracortical correlation (Brier et al., 2012). On the other hand, learning could modulate spontaneous activity, as is seen in visual perceptual learning (Lewis et al., 2009), acquisition and memory consolidation of sensorimotor tasks (Albert et al., 2009b), learning of visuomotor task (Albert et al., 2009a).

1.4.4 Spontaneous activity reflects prior experience

Spontaneous cortical activity plays a critical role in development (Thompson, 1997; Sur et al., 1999; Chiu and Weliky, 2002), and may also play an important role for processing sensory perception and circuit refinement. Multiple studies have shown that spontaneous activity modulates stimulus-evoked activity (Arieli et al., 1996; Kisley and Gerstein, 1999), and is correlated with behaviour (Compston, 2010).

Patterns of spontaneous activity are very similar to that of activation following sensory stimulation (Watson et al., 2008; Jermakowicz et al., 2009; Luczak et al., 2009). Unilateral somatosensory stimulation during wakefulness increases slow wave activity in the corresponding somatosensory cortex (Kattler et al., 1994). Sensory deprivation by cutting

whiskers causes an interhemispheric shift of slow-wave activity towards the cortex contralateral to the intact whiskers (Vyazovskiy et al., 2000).

Preferential use of a particular forelimb by rats in a food-reaching task induces a local enhancement of slow-wave activity in the contralateral hemisphere during sleep, with a significant power increase in the delta and sigma ranges (Vyazovskiy and Tobler, 2008). Motor learning task increases local SWA (Huber et al., 2004; Hanlon et al., 2009) and leads to improved performance of the task after sleep (Huber et al., 2004). On the other hand, arm immobilization in humans leads to a deterioration in motor performance, a decrease in somatosensory and motor evoked potentials over the contralateral sensorimotor cortex, as well as a reduction of SWA over the same cortical area during subsequent sleep (Huber et al., 2006).

Similar observations have been made within V1. Patterns of spontaneous activity within the visual cortex are similar to those of evoked activity, suggesting the spatial layout of spontaneous activity reflects functional connections in V1 (Kenet et al., 2003; Jermakowicz et al., 2009) (**Fig 1.8**). In addition, there is a spatial colocalization between correlation maps generated from spontaneous activity within V1 and axonal projections from the Allen Mouse Brain Connectivity (AMBC) Atlas (Mohajerani et al., 2013).

Spontaneous activity within V1 is susceptible to visual experiences. Dark rearing leads to decreased slow-wave activity in the visual cortex but normal SWA elsewhere (Miyamoto et al., 2003). The temporal patterns of spontaneous activity of individual cells become more similar to the visually evoked response after repeated stimulation, as revealed by single-unit recordings (Yao et al., 2007). Repetitive presentation of a visual stimulus causes a significant increase in the percentage of spontaneous waves that are similar to the cortical response evoked by the training stimulus. This effect is specific to the training stimulus, and it lasts for several minutes after

training without further stimulation. Such reverberation of spontaneous activity could be a form of short-term memory and help consolidate the transient effects of recent sensory experience into long-lasting cortical modifications (Han et al., 2008).

1.4.5 Rationale and Hypothesis

As reviewed above, slow wave activity is use-dependent and plays a role in consolidating learning and memory (Huber et al., 2004; Vyazovskiy and Tobler, 2008). Slow-wave activity may facilitate synaptic strengthening by grouping the replay of previous neural activity (Sutherland and McNaughton, 2000; Schwindel and McNaughton, 2011). Or, it is possible that the spatial and temporal details of slow-wave activity are involved in synaptic reorganization and downscaling following synaptic potentiation that occurs during waking (Tononi and Cirelli, 2003, 2006).

In chapter 3, I test the hypothesis that monocular deprivation may affect spontaneous activity and functional connectivity within V1 or even on a global scale. Considering the literature supporting a sensitive period for cross-modal plasticity (reviewed in Bavelier and Neville, 2002), I hypothesize that monocular deprivation may cause more changes in spontaneous activity and functional connectivity during the critical period, than in adulthood. This will be achieved with the help of *in vivo* IOS and calcium imaging of spontaneous activity, the results of which will be used to reveal functional connectivity using the seed-based correlation mapping method. The effect of monocular deprivation on spontaneous activity and functional connectivity will be tested in both juvenile and adult mice.

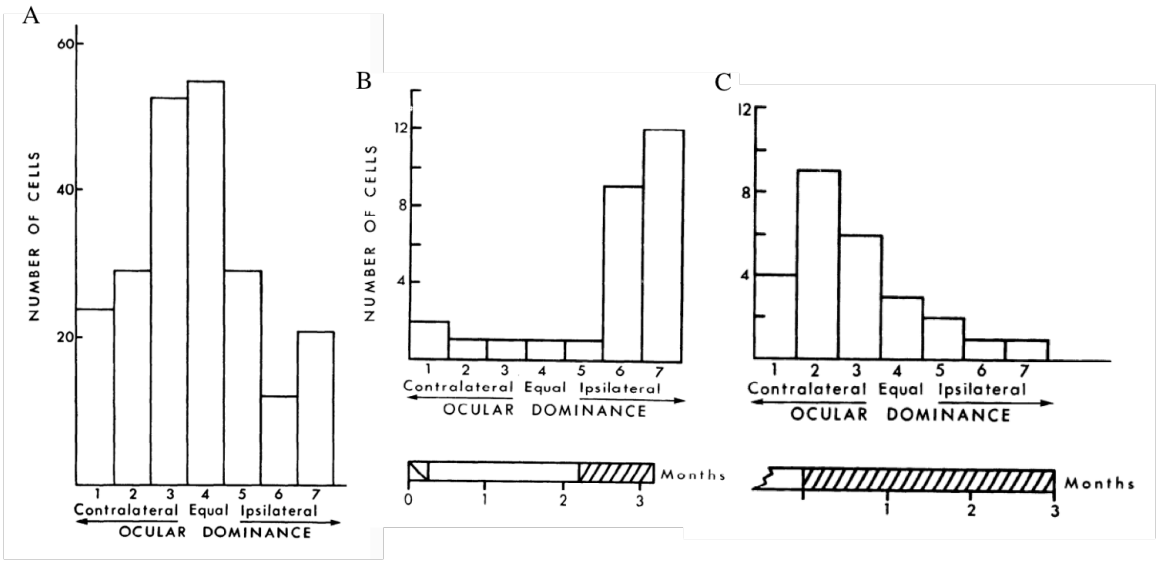


Fig 1.1 Ocular dominance plasticity in striate cortex of the cat, measured by single-unit recordings. (A) Normal ocular dominance distribution in binocular segment of V1 in cats. Group 1 is driven only by contralateral eye while group 7 is only driven by ipsilateral eye. Contralateral dominance is marked for group 2 and slight for group 3. Ipsilateral dominance is marked for group 6 and slight for group 5. Group 4 respond to both eyes equally. (B) One month of monocular deprivation of the right eye starting at 9 weeks of age in a kitten induces a significant OD shift in favour of the open eye. (C) No significant OD shift is observed after three months of monocular deprivation of the right eye in adult cats. From Wiesel & Hubel, 1963, J Neurophysiol, 26: 1003-1017. Used with permission.

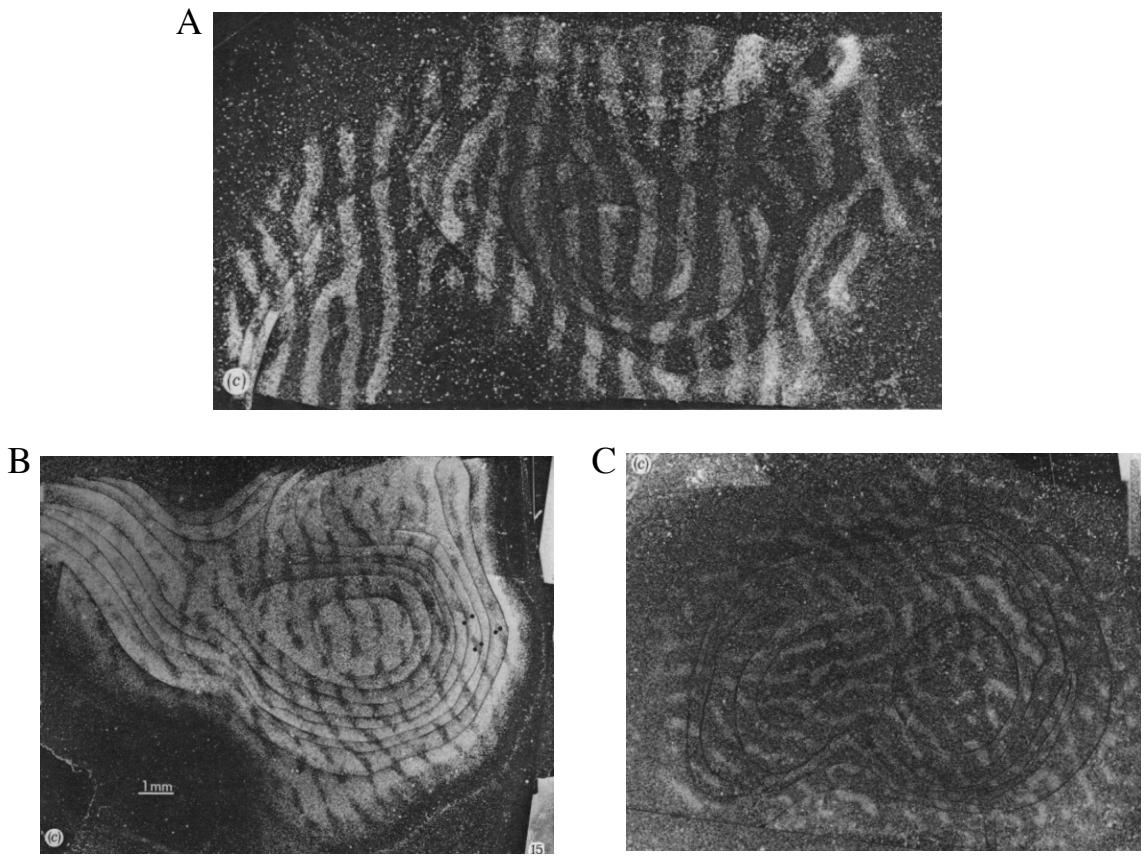


Fig 1.2 Plasticity of ocular dominance columns in monkey striate cortex. (A) Normal ocular dominance columns in monkeys. (B) Ocular dominance columns revealed with injection through the open eye. (C) OD columns revealed by injection via the deprived eye. Both B and C demonstrate expansion of OD columns corresponding to the open eye, in response to monocular deprivation. From Hubel *et al*., 1977, Philos Trans R Soc Lond B Biol Sci, 278(961): 377-409. Used with permission.

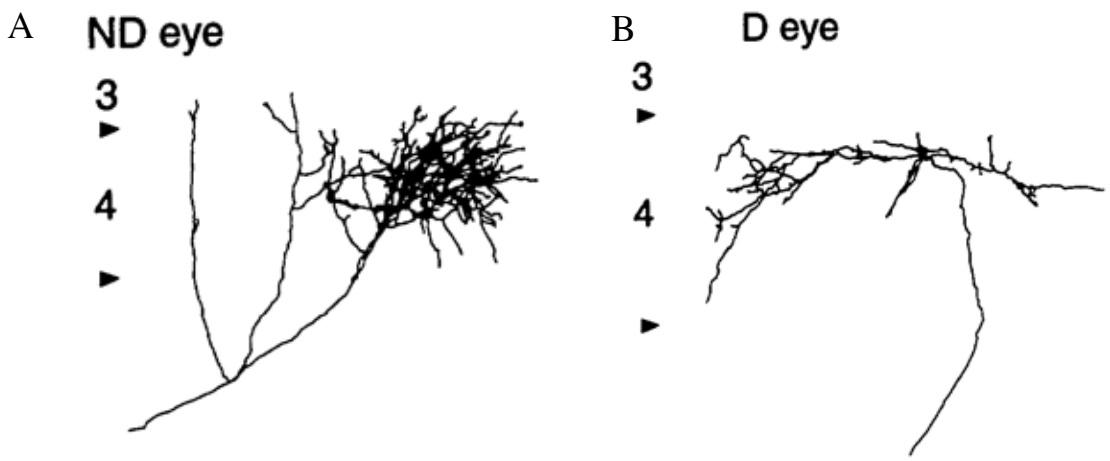


Fig 1.3 Brief monocular deprivation during the critical period leads to rapid rearrangement at single geniculocortical axonal arbors in cats. 6-7 days of monocular deprivation in kittens leads to: (A) Rapid expansion of geniculocortical axonal arbors serving the non-deprived eye, and (B) Rapid withdrawal of the branches of deprived-eye arbors. Arrowheads represent borders of cortical layers 3 and 4. From Antonini and Stryker, 1993, Science, 260(5115): 1819-21. Used with permission.

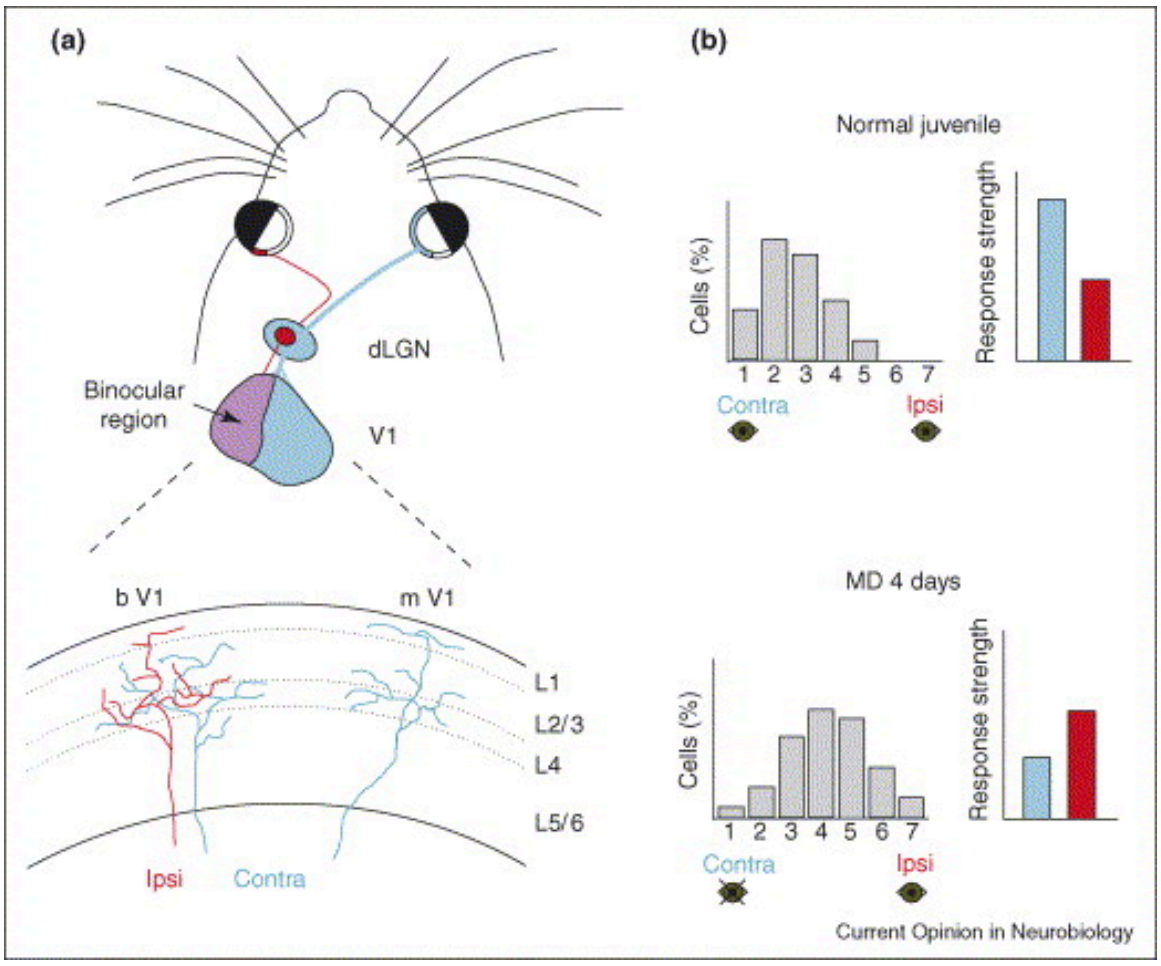


Fig 1.4 Ocular dominance plasticity in mouse during the critical period. (a) An cartoon of the mouse visual system. The binocular region occupies the lateral third of V1. bV1=binocular V1; mV1=monocular V1. (b) Four days of monocular deprivation induces a significant ocular dominance shift during the critical period. From Hofer *et al*., 2006, Curr Opin Neurobiol, 16(4): 451-459. Used with permission.

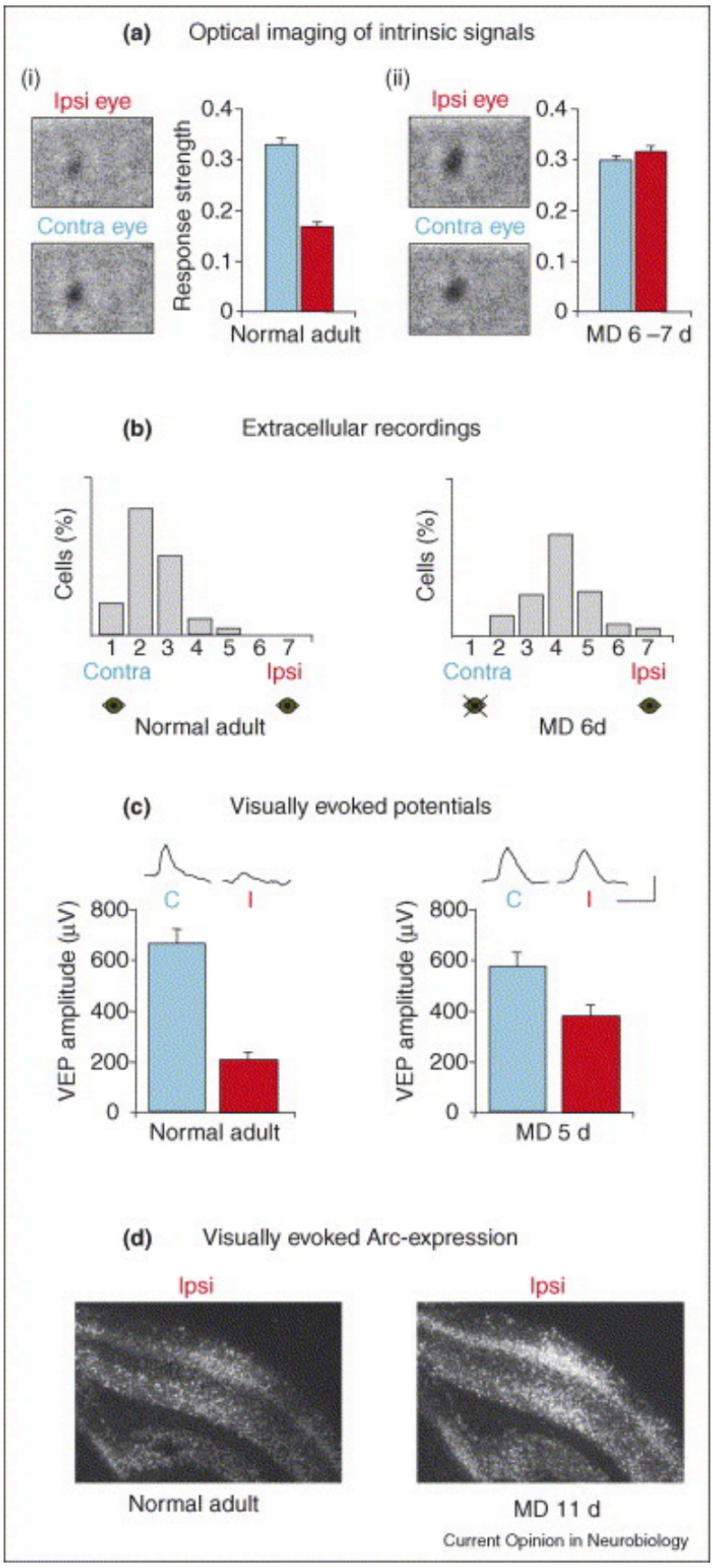

(a) Optical imaging of intrinsic signals
(i)
Ipsi eye
Contra eye
Response strength
0.4
0.3
0.2
0.1
0
Normal adult
(ii)
Ipsi eye
Contra eye
0.4
0.3
0.2
0.1
0
MD 6 –7 d
(b) Extracellular recordings
Cells (%)
1 2 3 4 5 6 7
Contra
Ipsi
Normal adult
Cells (%)
1 2 3 4 5 6 7
Contra
Ipsi
MD 6d
(c) Visually evoked potentials
C
I
VEP amplitude (μV)
800
600
400
200
0
Normal adult
C
I
VEP amplitude (μV)
800
600
400
200
0
MD 5 d
(d) Visually evoked Arc-expression
Ipsi
Normal adult
Ipsi
MD 11 d
Current Opinion in Neurobiology

Fig 1.5 ODP in adult mouse visual cortex. (a) Intrinsic signal imaging shows strengthening of responses to non-deprived, ipsilateral eye stimulation after six to seven days of MD. (b) Single-unit recordings show substantial OD shifts towards the non-deprived eye after adult contralateral MD. (c) 5 days of MD in mice older than P36 lead to an increase in non-deprived eye responses, as is demonstrated by recordings of visually evoked field potential. (d) Arc mRNA expression is substantially stronger after 11 days of contralateral MD than it is in normal adult mice. From Hofer *et al*., 2006, Curr Opin Neurobiol, 16(4): 451-459. Used with permission.

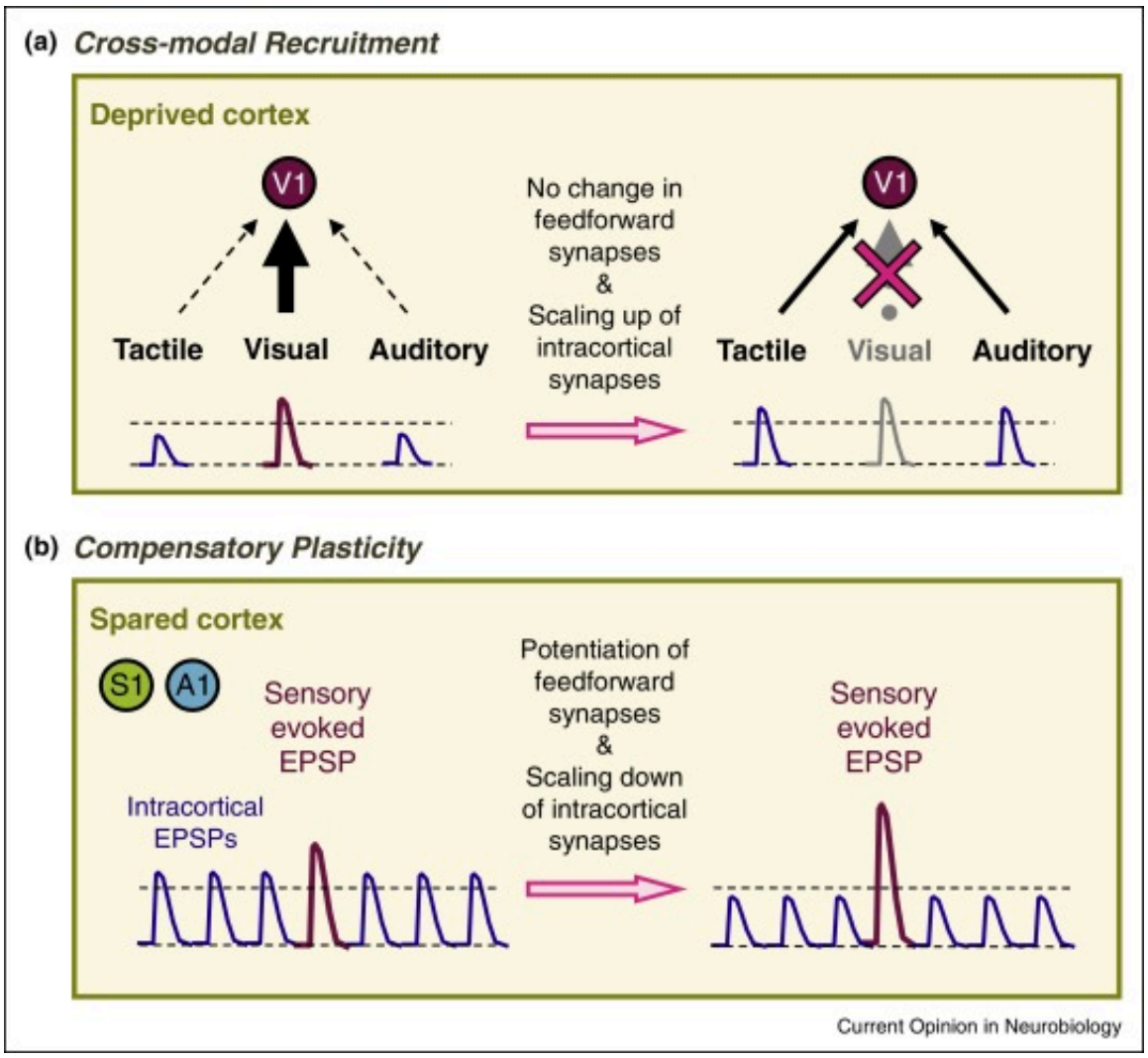


Fig 1.6 Functional implications of cross-modal plasticity. (a) Cross-modal recruitment, i.e., recruitment of the deprived sensory cortex for processing the remaining senses. Demonstrated here is recruitment of V1 by somatosensory and auditory cortices. (b) Compensatory plasticity, manifested as experience-dependent refinement of the spared sensory cortices. In this case, refinement of S1 and A1 in response to visual deprivation. From Lee and Whitt, 2015, Curr Opin Neurobiol, 35: 119-26. Used with permission.

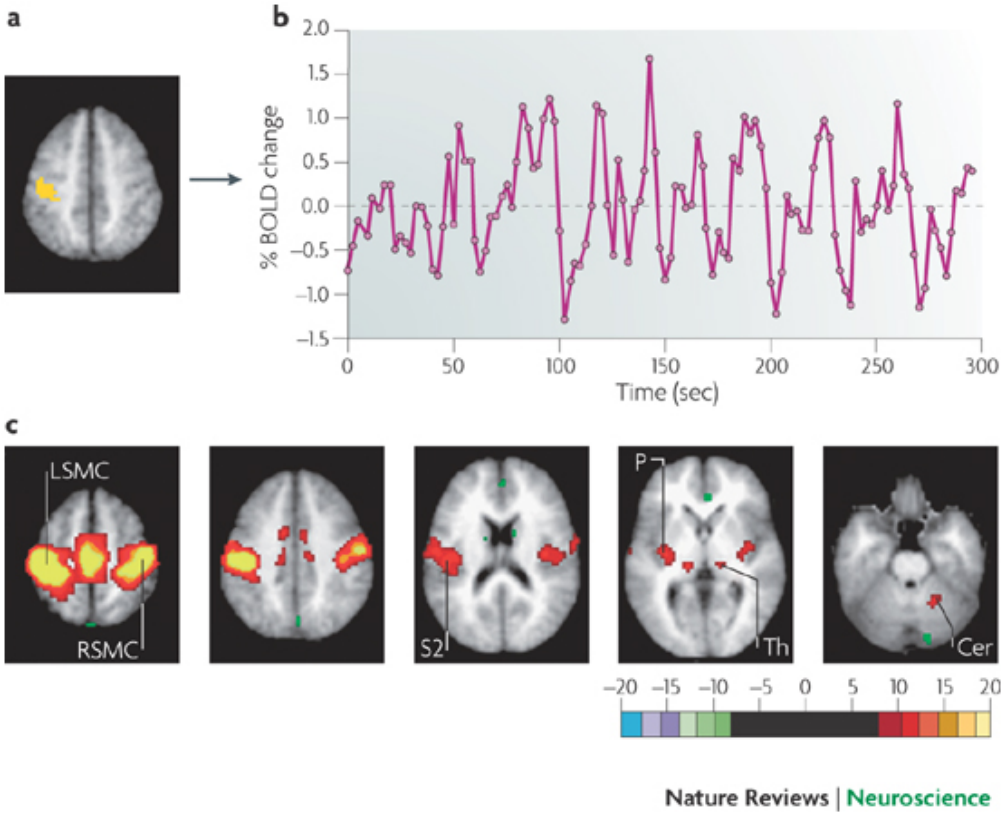


Fig 1.7 Functional connectivity maps revealed by seed based correlation mapping. (a) Seed region in the left somatomotor cortex is shown in yellow. (b) Time course of BOLD activity extracted from the seed region from recordings during resting fixation. (c) Statistical z-score map showing voxels that are significantly correlated with the extracted time course (n=10). Positive correlations are observed in the right somatomotor cortex and medial motor areas, the secondary somatosensory association cortex, the posterior nuclei of the thalamus, putamen, and cerebellum. From Fox and Raichle, 2007, Nat Rev Neurosci, 8(9): 700-11. Used with permission.

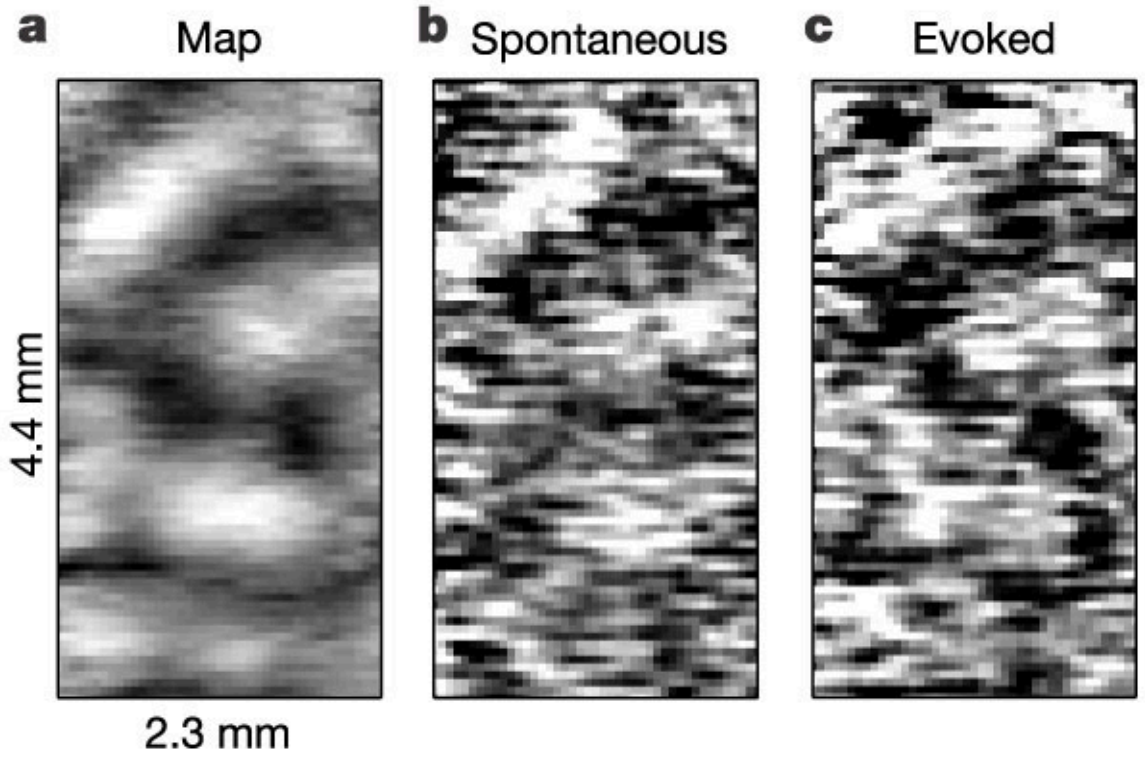


Fig 1.8 Spontaneous and evoked orientation-selective responses in area 18 of the cat. (a) Evoked orientation map, in response to full-field gratings of vertical orientation. (b) A single frame obtained during a spontaneous recording session. (c) A single frame recorded during the presentation of the same orientation as for the evoke map. Note the similarity amongst all three panels, particularly between the spontaneous and evoked response patterns. From Kenet *et al*., 2003, Nature, 425(6961): 954-6. Used with permission.

CHAPTER 2 LONG-TERM DEPRESSION – A KEY MOLECULAR SUBSTRATE FOR OCULAR DOMINANCE PLASTICITY

2.1 Introduction

2.1.1 Ocular dominance plasticity

Ocular dominance plasticity is a well-characterized example of experience-dependent modification of brain circuitry. During the critical period, visual cortical circuitry is extremely susceptible to various forms of deprivation. Monocular deprivation of visual input during the critical period causes life-long impairment in the visual acuity of the deprived eye (amblyopia). The mechanisms for ocular dominance plasticity continue to be subjects of intensive interest, decades after the seminal studies pioneered by Hubel and Wiesel (Hubel and Wiesel, 1963a, 1965, 1970; Hubel et al., 1977).

Ocular dominance plasticity is characterized by remarkable physiological and anatomical changes induced by monocular deprivation during the critical period. Monocular deprivation leads to a reversible weakening of deprived-eye connections and reorganization of intracortical connections in the superficial layers (Trachtenberg et al., 2000; Trachtenberg and Stryker, 2001). This is followed by strengthening of open-eye representations in V1, accompanied by anatomical reorganization of thalamocortical afferents (Shatz and Stryker, 1978; Antonini and Stryker, 1993a, b).

Ocular dominance plasticity in the mouse shares many features with that of higher mammals. Monocular deprivation for as little as 1–2 days during the critical period shifts the physiological responsiveness of neurons in the binocular zone of V1 towards the open eye (Gordon and Stryker, 1996). A reorganization of cortical connections has also been observed in mouse primary visual cortex following monocular deprivation (Antonini et al., 1999). Mice have

been attracting more interest in recent years, due to their robust ocular dominance plasticity as well as the relative ease for genetic manipulation.

2.1.2 LTD

With regards to the mechanisms of ocular dominance plasticity, abundant evidence points to the importance of an appropriate level of cortical inhibition (Hensch et al., 1998), prior visual experience (Cynader and Mitchell, 1980), and a role for axonal sprouting (McGee et al., 2005). In Chapter 1, I discussed role of cortical inhibition (section **1.2.7.1**). In this chapter, I focus on the regulatory role of LTD in ocular dominance plasticity. I will begin this discussion with a brief introduction of glutamatergic receptors, which are crucial in synaptic plasticity.

2.1.2.1 Glutamatergic receptors

Excitatory transmission in the brain is mediated by glutamate-gated α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), *N*-methyl-D-aspartic acid receptors (NMDARs), and metabotropic glutamate receptors (mGluRs). The number and subunit composition of AMPARs and NMDARs regulate membrane depolarization and intracellular calcium levels, and mGluRs regulate downstream signalling events. Each of these receptor types has been implicated in ocular dominance plasticity (Tropea et al., 2009).

NMDARs are composed of GluN1 and GluN2 subunits. GluN2 subunits determine the receptor characteristics and recruit different intracellular signaling molecules. There are four distinct types of GluN2 subunits: GluN2A–D (Cull-Candy et al., 2001). The composition of GluN2 subunit undergoes transitions from low to high GluN2A/GluN2B ratios during normal postnatal development (Sheng et al., 1994; Quinlan et al., 1999). Also, GluN2A-containing NMDARs are preferentially located synaptically, while GluN2B-containing receptors are localized at extrasynaptic sites (Stocca and Vicini, 1998; Rumbaugh and Vicini, 1999; Philpot et

al., 2001). GluN2B-containing NMDARs have been implicated in hippocampal LTD *in vitro* (Liu et al., 2004) and *in vivo* (Fox et al., 2006a).

AMPARs are primarily composed of GluA2 and either GluA1 or GluA3 subunits. Synaptic strength can be effectively modulated by AMPAR number and calcium permeability (Citri and Malenka, 2008). AMPARs are preferentially inserted at synapses that undergo LTP, and removed from synapses that undergo LTD (Shepherd and Huganir, 2007) (**Fig 2.1**). LTP and LTD are experimental phenomena that are widely expressed at almost every excitatory synapse in the mammalian brain. They are important candidates for development, learning and memory, as well as experience-dependent plasticity (Shepherd and Huganir, 2007). NMDAR-dependent LTP and LTD has been investigated extensively in ocular dominance plasticity (Smith et al., 2009; Cooke and Bear, 2014).

2.1.2.2 Glutamatergic receptors in ocular dominance plasticity

It is recognized early on that NMDAR-regulated neurotransmission plays an important role in regulating ocular dominance plasticity. Pharmacological blockade (Bear et al., 1990; Daw et al., 1999b) or genetic deletions (Roberts et al., 1998) of NMDARs prevents MD-induced ocular dominance shift during the critical period.

Ocular dominance plasticity is dependent on GluN1 subunits of NMDARs. GluN1-knockout mice fail to exhibit NMDAR-mediated currents in layer II/III pyramidal cells of V1 (Sawtell et al., 2003). GluN2A/GluN2B ratio increases during development (Sheng et al., 1994; Quinlan et al., 1999). This ratio could be also influenced by visual experience. Dark rearing or lid suture reduces GluN2A/GluN2B ratio, which could be restored by re-exposure to light (Tongiorgi et al., 2003; Chen and Bear, 2007). Visual deprivation decreases GluN2A/GluN2B ratio in adult rats as well (He et al., 2006). Mice with targeted deletion of GluN2A are less

sensitive to the effect of monocular deprivation during the critical period (Fagiolini et al., 2003). These data suggest that NMDAR-mediated synaptic plasticity can regulate the capacity for ocular dominance plasticity.

NMDAR-dependent LTP and LTD are key mediators of ocular dominance plasticity (Smith et al., 2009; Cooke and Bear, 2014). LTP involves AMPAR exocytosis, while LTD involves AMPRA endocytosis (Shepherd and Huganir, 2007; Collingridge et al., 2010). Of note, AMPAR endocytosis is not required for LTD in all cortical layers. Endocannabinoid signalling to the presynaptic terminal is necessary and sufficient to induce LTD in layer II/III of visual cortex (Crozier et al., 2007). Pharmacologic blockade of cannabinoid receptors prevents MD-induced ocular dominance shift in layer II/III during the critical period (Liu et al., 2008).

mGluRs are also involved in ocular dominance plasticity, with distinct roles depending on the receptor subtype and cortical layer (Daw et al., 1999a; Wang and Daw, 2003; Rao and Daw, 2004). In fact, mGluRs are implicated in LTD as well. Chronic down-regulation of mGluR5 signaling impairs NMDAR-dependent LTD *in vitro* and ocular dominance plasticity *in vivo* (Sidorov et al., 2015).

2.1.2.3 Molecular mechanism of LTD

Monocular deprivation in mice during the critical period induces two temporally distinct responses: a rapid, deprivation-induced depression of contralateral (deprived) eye response, occurring over the first 3 days of monocular deprivation; followed by a delayed, deprivation-enabled potentiation of the ipsilateral (nondeprived) eye responses, emerging after 5 days of monocular deprivation (Frenkel and Bear, 2004). It has been proposed that LTP is involved in the open eye potentiation, while LTD is implicated in deprived eye depression (Frenkel and Bear, 2004; Yoon et al., 2009).

LTD could be triggered by synaptic activation of either NMDARs or mGluRs. For the rest of this introduction, I will focus on NMDAR-dependent LTD. NMDAR-dependent LTD is usually induced by low-frequency stimulation (LFS) (Dudek and Bear, 1992). NMDAR-LTD involves the activation of specific NMDAR subtypes (Collingridge et al., 2004).

In NMDAR-LTD, calmodulin detects Ca^{2+} that enters via NMDARs and this leads to activation of protein phosphatase 1 (PP1), a key enzyme in synaptically-induced LTD. PP1 can dephosphorylates multiple substrates, including ser845 on the AMPAR subunit GluA1 and this leads to AMPAR endocytosis (Collingridge et al., 2004).

Multiple studies suggest that AMPAR endocytosis in LTD is dependent on the AMPAR subunit GluA2 (Collingridge et al., 2010). GluA2-containing AMPARs are stabilized on the membrane by *N*-ethylmaleimide-sensitive factor (NSF). NSF is implicated in the constitutive cycling of GluA2 subunit between synaptic and extrasynaptic sites, which is not regulated by neuronal activity (Lee et al., 2004). Clathrin adaptor protein AP2 also binds to the NSF site on the GluA2 subunit, which initiates clathrin coat assembly and receptor endocytosis (Carroll et al., 1999). Hippocalcin (a high-affinity calcium sensor) can target AP2 to GluA2 and therefore displace NSF and initiate clathrin-mediated endocytosis of AMPARs (Collingridge et al., 2010).

The three tyrosine residues located near the end of the GluA2 carboxyl terminal (C-terminus) are also crucial in AMPAR endocytosis. This tyrosine cluster does not influence the constitutive cycling of AMPARs. It is, however, required for insulin- and NMDAR-dependent LTD (Ahmadian et al., 2004).

Also implicated in LTD is the PDZ domain in the C-terminus that binds protein interacting with PICK1 (protein interacting with C kinase 1) and ABP-GRIP (AMPAR-binding protein–glutamate receptor interacting protein) (Collingridge et al., 2004). In some

circumstances, PICK1 may aid the NMDAR-dependent disassociation of AMPARs from ABP–GRIP, potentially via the targeted phosphorylation of ser880 of GluA2 by protein kinase Cα (PKCα) (Collingridge et al., 2010).

The AP2-binding domain, tyrosine-containing cluster, and PDZ domain have been identified as endocytic motifs of the GluA2 C-terminus. Discoveries of these motifs have lead to development of interference peptides that disrupt the interactions between the GluA2 subunit and endocytic machineries, including G2CT and $GluR2_{3Y}$ (Collingridge et al., 2010) (**Fig 2.2A**). Both have proven to be effective blockers of LTD and have been used to explore the functional significance of LTD in development, as well as learning and memory (Ahmadian et al., 2004; Brebner et al., 2005; Kim et al., 2007; Wong et al., 2007; Yoon et al., 2009; Migues et al., 2010).

The G2CT peptide (KRMKLNINPS) is derived from the NSF/AP2 binding region in the GluA2 C terminus (Lee et al., 2002). G2CT disrupts AP2 but not NSF binding, thus preventing AMPAR endocytosis and LTD without affecting basal synaptic transmission (Lee et al., 2002; Griffiths et al., 2008; Yoon et al., 2009).

$GluR2_{3Y}$ (YKEGYNVYG) targets the tyrosine cluster on the C terminus, which is required for regulated GluA2-containing AMPAR endocytosis, and thus preventing induction of LTD (Ahmadian et al., 2004) (**Fig 2.2B**). During my graduate studies, I had the opportunity to explore the regulatory role of LTD in ocular dominance plasticity with the help of $GluR2_{3Y}$.

2.1.3 LTD in ocular dominance plasticity during the critical period

Accumulating evidence favours LTD as a key mechanism for ocular dominance plasticity during the critical period (Kirkwood et al., 1996; Heynen et al., 2003; Frenkel and Bear, 2004; Yoon et al., 2009).

Homosynaptic LTD can be reliably induced between layer IV and II/III in the primary visual cortex during the critical period (Kirkwood et al., 1996). One day of monocular deprivation induces dephosphorylation of the GluA1 subunit, phosphorylation of GluA2 subunit, and a decrease in the cell surface AMPARs, similar to that in electrically induced LTD (Heynen et al., 2003). Deprived eye depression occludes the induction of LTD in cortical slices (Heynen et al., 2003; Yoon et al., 2009). Ocular dominance plasticity is absent in *Arc* knockout mouse, in which LTD and AMPAR trafficking are impaired (McCurry et al., 2010).

LTD is developmentally constrained. LTD is most robust during the critical period for ocular dominance plasticity. It becomes difficult to induce LTD in primary visual cortex of mouse beyond 3 months (Sermasi et al., 1999). LTD in layer IV of V1 is lost shortly after eye opening (Jiang et al., 2007). Consistently, we have previously shown a temporal correlation between age and the magnitude of LTD induced in visual cortical slices (Yang et al., 2011) (**Fig 2.3**). During the critical period (P23-35), LFS effectively induces LTD in mouse visual cortical slices. The amplitude of LFS-induced LTD decreases gradually as the mice age. After the mice reach adulthood, LFS can no longer induce LTD.

In addition, LTD blocking peptides prevent MD-induced ocular dominance shift during the critical period. $GluR2_{3Y}$ blocks LTD *in vivo* and prevents ocular dominance shift during the critical period (Yang et al., 2011) (**Fig 2.4**). Non-deprived mice display a contralateral biased ocular dominance distribution (**Fig 2.4A**). Four days of MD during the critical period induces a characteristic ocular dominance shift toward the non-deprived eye (**Fig 2.4B**). $GluR2_{3Y}$ itself does not influence OD distribution (**Fig 2.4C**) or affect spontaneous activity (**Fig 2.4G**) in non-deprived mice. However, it prevents the MD-induced OD shift during the critical period, which is not observed with control peptide $GluR2_{3A}$ (**Fig 2.4D,E &F**). G2CT, another LTD blocking

peptide, also disrupts AMPAR endocytosis and prevents ocular dominance shift during the critical period (Yoon et al., 2009). Prevention of ODP with G2CT and $GluR2_{3Y}$ provides direct evidence that LTD is crucial in ocular dominance plasticity during the critical period.

2.1.4 Ocular dominance plasticity in adulthood

The visual cortex does not abruptly lose plasticity at the end of the critical period. Some weaker forms of ocular dominance plasticity have been observed in adulthood. A longer period of monocular deprivation is required for ocular dominance shift to occur; and the extent of the shift is weaker than that during the critical period (Hofer et al., 2006a; Karmarkar and Dan, 2006; Prusky et al., 2008).

Like in the critical period, a two-phase process has been described in adult ocular dominance plasticity. Seven days of monocular deprivation produces a significant, saturating decrease in the deprived eye's response, followed by a second phase in which responses to both eyes increase. It is different from plasticity during the critical period in that there is a clear asymmetry between the two hemispheres in the effect of deprivation. More interestingly, the initial decrease in the response of the contralateral eye is of a much smaller amplitude, compared to the drastic decrease brought on by four days of monocular deprivation during the critical period (Sato and Stryker, 2008).

NMDAR-dependent neurotransmission is crucial in adult ocular dominance plasticity as well. Competitive NMDAR antagonists block adult ocular dominance plasticity (Sato and Stryker, 2008). Some believe while LTD is crucial for ocular dominance plasticity during the critical period, LTP contributes more to that of adulthood.

LTD is a key molecular substrate for ocular dominance plasticity during the critical period. Given that LTD is developmentally constrained, I hypothesize that restoration of LTD

could enhance ocular dominance plasticity in adulthood. And therefore I sought methods to enhance LTD in adulthood with the hope of enhancing ocular dominance plasticity beyond the critical period.

2.1.5 D-serine, a potential reagent to enhance LTD in adulthood

2.1.5.1 D-serine, NMDAR co-agonist

Glutamate cannot activate NMDAR, unless the co-agonist binding site on GluN1 subunit is occupied (Johnson and Ascher, 1987; McBain et al., 1989). Initially, it was thought that the NMDAR co-agonist site was occupied by glycine. Thus, the co-agonist site has been referred to as the 'glycine site'. However, it is later discovered that D-serine is a physiological co-agonist of NMDARs (Wolosker et al., 2008).

First of all, there are large amounts of endogenous D-serine in the brain (Hashimoto et al., 1992). D-serine is synthesized from L-serine by serine racemase, a brain-enriched enzyme whose regional localization matches that of endogenous D-serine (Wolosker et al., 1999a; Wolosker et al., 1999b). D-serine is degraded by D-amino acid oxidase enzyme (Horiike et al., 1994; Schell et al., 1997). Although initially suggested as a glia-transmitter (Schell et al., 1995; Wolosker et al., 2002), D-Serine has later been found in the cerebral cortex (Kartvelishvily et al., 2006), some nuclei of the hindbrain (Puyal et al., 2006), and ganglion cells of the retina (Dun et al., 2008).

Second, the distribution of D-serine correlates with that of NMDARs, in hippocampus and cerebral cortex (Schell et al., 1995; Schell et al., 1997). On the other hand, glycine seems to dominate in the caudal areas of the brain, where the density of NMDARs is lower. The extracellular concentration of endogenous D-serine is similar to that of glycine in the cerebral cortex (Hashimoto et al., 1995). This suggests that endogenous D-serine is physically closer to NMDARs than glycine.

Third, the binding affinity of D-serine towards NMDAR is three times higher than that of glycine. This is because D-serine makes three additional hydrogen bonds and displaces a water molecule from the binding pocket (Matsui et al., 1995; Furukawa and Gouaux, 2003). Depletion of D-serine leads to a 60% decrease in the NMDAR-mediated spontaneous activity (Mothet et al., 2000). In the visual system, D-serine is required for the NMDAR-mediated light-evoked responses in the retina (Stevens et al., 2003; Gustafson et al., 2007).

It is interesting to note that functional efficiency of the two co-agonists may be related to their availability at synaptic or extra-synaptic sites (Wolosker et al., 2008). The synaptic glycine concentration is efficiently regulated by GlyT1, a high-affinity glial glycine transporter that limits glycine access to NMDARs (Berger et al., 1998; Bergeron et al., 1998). ALX 5407, a selective inhibitor of hGlyT1 (Atkinson et al., 2001), has been shown to potentiate NMDAR-mediated responses in rat prefrontal cortex (Chen et al., 2003).

2.1.5.2 D-serine regulates NMDAR-mediated synaptic plasticity

Co-agonist binding plays an important regulatory role in NMDAR neurotransmission (Wolosker et al., 2008). Co-agonist binding increases the affinity of NMDAR for glutamate (Fadda et al., 1988), decreases its desensitization (Lerma et al., 1990) and promotes NMDAR turnover by internalization (Nong et al., 2003). NMDAR co-agonist site is not saturated under resting conditions, and therefore co-agonist binding could dynamically modulate NMDAR activity (Wood et al., 1989; Danysz and Parsons, 1998).

Indeed, D-serine plays an important regulatory role in NMDA transmission, synaptic plasticity and development (Wolosker et al., 2008). D-serine is effective in mediating LTP in the hippocampus, as well as learning and memory (Yang et al., 2003; Yang et al., 2005; Junjaud et al., 2006; Mothet et al., 2006). Most interestingly, exogenous D-serine application enhances

hippocampal LTD and spatial reverse learning in adult mice (Duffy et al., 2008). In Chapter 2, I explored the effect of D-serine on LTD and ocular dominance plasticity in adult mouse visual cortex.

2.2 Methods

All experiments were conducted in accordance with protocols approved by the Animal Care Centre, University of British Columbia.

2.2.1 Monocular deprivation by lid suture

Male C57BL/6 mice (Charles River, Quebec, Canada) used for this study were maintained on a 12 h light/dark cycle with *ad libitum* access to food and water. Eyelid suture was performed under anesthesia induced by 3% isofluorane (Abbott, North Chicago, IL) in oxygen and maintained at 1.5% (Gordon and Stryker, 1996). For adult mouse experiments, the left eyelid was sutured around postnatal day 90 (P90). Long-term MD of the left eye started at the beginning of critical period around P23. Eyelid suture was maintained until P80 when the mice were reverse sutured. This was done by opening the left eyelid and closing the right eyelid. During the process, animals were checked daily to make sure that the eyelids were completely sealed. Mice whose eyelid fusion was incomplete and whose corneas showed signs of damage or cataract were excluded from the study.

2.2.2 Local field potential recording

Mice were anaesthetized with urethane (5 mg/kg) and subsequently decapitated for brain extraction. The brains were prepared in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 5 KCl, 1 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 20 glucose. The ACSF was bubbled with 5 % CO_2/95 % O_2. A vibrating blade microtome (Leica, Germany) was used for cutting brain slices. Coronal slices (each 400 μm thick) containing primary visual cortex

were prepared for local field potential recordings. Slices were placed in a submersion recording chamber with carbogenated ACSF and allowed to recover at 30 °C for about 1 h prior to recording.

In situ electrophysiological recordings were conducted in the chamber described above with continuous infusion of carbogenated ACSF at a rate of 1.5 ml/min at 30 °C. Extracellular field potentials were evoked via electrical stimulation through a concentric bipolar stimulating electrode (CBBRC75; FHC, Bowdoinham, ME) placed in the centre of the cortical thickness that corresponded to layer IV, and recorded from layers II/III using glass electrodes (1 MΩ) filled with 1 M NaCl. Slices were evaluated for responsivity every 15 seconds with a constant current pulse of 100 μs duration and 100-200 μA of current, chosen to yield a half-maximal response. This field excitatory postsynaptic potential (fEPSP) was mainly mediated via AMPA receptors as it was completely abolished by the AMPAR antagonist DNQX (20 μM, Sigma). After a stable baseline was achieved, LTD was induced using a low frequency stimulation protocol (LFS) consisting of 900 stimuli at 1 Hz. To induce LTP, three to five episodes of theta-burst stimulation (TBS) were delivered at 10 sec intervals. TBS consists of ten stimulus trains delivered at 5Hz. Each train consisted of four pulses at 100 Hz.

2.2.3 Single unit recordings in the binocular zone

Electrophysiological recordings were performed under urethane anesthesia (50 mg/kg, intraperitoneal injection (IP), Sigma). Atropine (20 mg/kg, subcutaneous injection (SC), Optopics) was injected to reduce secretions and parasympathetic effects of anesthetic agents. Dexamethasone (4 mg/kg, SC, American Reagent Laboratories) was administered to reduce cerebral edema and inflammation. Mice were placed in a stereotaxic frame and a craniotomy was performed over the right side of the visual cortex. Agar was applied to the surface of the cortex

to enhance recording stability and prevent desiccation. The eyelids were resected, and corneas were protected thereafter by frequent application of Ringer's solution. Body temperature was maintained at 37 °C using a homeostatic heating pad (Harvard). Heart rate was monitored continuously with electrocardiography needles. Four to six sites (at least 100 μm apart) through the full thickness of the cortex were evaluated in each of four to six penetrations spaced evenly (at least 200 μm apart) crossing the binocular region (RF centre azimuths <25 degrees from the vertical meridian) of the primary visual cortex to avoid sampling bias. In some cases large individual neuronal responses were isolated while other sites yielded multiunit activity. Sites were assigned to OD categories according to the seven-category scheme of Hubel and Wiesel (Gordon and Stryker, 1996). OD histograms were constructed and contralateral bias index (CBI) scores were calculated for each mouse using the formula: $CBI = [(n_1-n_7)+(2/3)(n_2-n_6)+(1/3)(n_3-n_5)+N]/2N$, where N = total number of cells and n_x = number of cells with OD scores equal to x. The experimenters were blind to the treatment conditions of the mice.

2.2.4 Minipump Implantation

Osmotic minipumps (1007D, Alzet) were implanted for the purpose of local drug application. Mice were anesthetized with 3 % isofluorane in oxygen and mounted in a stereotaxic frame that allowed unobstructed vision. Ophthalmic lubricant was applied to protect the eyes, and body temperature was maintained at 37 °C with a heating pad. The minipumps were filled testing reagents and attached to 30 G stainless steel cannulae. Under aseptic conditions, a longitudinal incision was made in the scalp over the mid-sagittal sinus and the portions of the skull overlying occipital and frontal portions of the brain were cleaned and dried. To avoid any damage to the binocular zone of the primary visual cortex, the location of the infusion was centered in the cortical monocular zone, approximately 2 mm lateral to the midline and 1 mm rostral to lambda

(Drager, 1975; Wagor et al., 1980). A small hole (D < 0.5 mm) was drilled through the skull above the occipital cortex contralateral to the deprived eye. The cannula was inserted to a depth of 1 mm under the surface of the skull and secured with cyanoacrylate adhesive (Alzet). The attached minipump was placed in a subcutaneous pocket at the nape of the neck. The scalp was closed over the implant, and the animal returned to its home cage. Minipump implantation was performed on the day prior to eyelid suture, and infusion continued for the duration of the monocular deprivation, for a total of five days.

2.2.5 Peptide synthesis

$GluR2_{3Y}$ (YKEGYNVYG), $GluR2_{3A}$ (AKEGANVAG), Tat-$GluR2_{3Y}$ (YGRKKRRQRRRYKEGYNVYG), and Tat-$GluR2_{3A}$ (YGRKKRRQRRRAKEGANVAG) peptides were synthesized by the Nucleic Acid and Peptide Service Centre at the University of British Columbia (Vancouver, Canada).

2.3 Results

2.3.1 D-serine selectively enhances LTD in adult mouse cortical slices

I first tested the effect of D-serine on the induction of LTD in visual cortical slices. Low-frequency stimulation (LFS, 1Hz, 900 stimuli) delivered to layer IV could not induce LTD in layers II/III in adult visual cortical slices (P90-P100, 100±0.1% of baseline, n=3), but the induction of LTD was facilitated by co-application of D-serine (**Fig 2.5**). Adult visual cortical slices were incubated in D-serine (20 μM, Sigma, ≥98% (TLC)) for at least 20 minutes of baseline recording before LFS was delivered. D-serine incubation did not affect the baseline activity, but it facilitated the decrease of the fEPSP in the presence of low frequency stimulation (66±12% of baseline, n=4; $p < 0.01$). The effect was stable for more than 30 minutes (**Fig 2.5A**).

However, D-serine did not facilitate the induction of LTP in adult visual cortical slices. Standard LTP-induction protocol theta-burst stimulation did not elicit LTP in adult cortical slices (99±4% of baseline, n=5), nor did any of the concentrations of D-serine from 20 μM up to 200 μM facilitate the induction of LTP (96±6% of baseline, n=5; **Fig 2.5B**). Thus our results suggest that D-serine specifically facilitates the induction of LTD in adult cortical slices without affecting LTP, making it a useful reagent to study the functional significance of LTD in adult ocular dominance plasticity.

2.3.2 D-serine enhances ocular dominance plasticity in adult mice

Next, I examined the effect of D-serine *in vivo* by examining the ocular dominance distribution in monocularly deprived adult mice. Consistent with existing literature, four days of MD (P90-P94) did not induce a significant ocular dominance shift in adulthood, as revealed by single-unit recordings (CBI=0.78±0.01, n=5; p=064, compared to normal adult mice; **Fig 2.6A, B**).

The half-life of D-serine in the rodent brain is around 12 hours (Dunlop and Neidle, 1997). I adopted an effective dose of 600 mg/kg (Lipina et al., 2005), and administered D-serine at the half-life interval for the purpose of prolonging the duration of effective therapeutic dose. Consistent with data showing that D-serine does not affect basal activity in brain slices, D-serine administration *in vivo* (600 mg/kg, SC, twice a day) alone did not affect the ocular dominance distribution (CBI=0.74±0.01, n=5; p=0.03 compared to normal adult mice; **Fig 2.6C**).

However, when challenged with monocular deprivation (**Fig 2.6D**), D-serine treated mice demonstrated a significant ocular dominance shift (CBI=0.56±0.05, n=5; p<0.01, compared to normal mice; **Fig 2.6E**). Control saline injections failed to induce the same effect (CBI=0.77±0.01, n=5, p=0.23, compared to normal mice, **Fig 2.6F**). Quantitative statistical

comparison of CBI among adult mice of different treatment groups showed that D-serine enhanced adult ocular dominance plasticity (**Fig 2.6H**).

2.3.3 D-serine enhances adult ocular dominance plasticity via an LTD-like mechanism

To more closely evaluate whether D-serine enhances adult ocular dominance plasticity through the facilitation of LTD, I gave $GluR2_{3Y}$ to monocularly deprived adult mice treated with D-serine. Mice that received both $GluR2_{3Y}$ (10 nmol/g, IP, daily) and D-serine (600 mg/kg, SC, twice a day) treatment demonstrated a contralateral-biased ocular dominance distribution (CBI=0.68, n=4, **Fig 2.6G**), indicating that $GluR2_{3Y}$ prevented D-serine-induced OD shift. This suggests that D-serine enhances ocular dominance plasticity in adult mice via an LTD-like mechanism.

2.3.4 Intracortical Infusion of D-serine enhances adult ocular dominance plasticity

To rule out potential influences from other components of the visual pathway, we examined the effect of D-serine applied directly to the primary visual cortex by local infusion through osmotic minipumps (**Fig 2.7**). The pumps, containing either D-serine (50 μM) or saline, were implanted one day prior to lid suture (P89). After four days of monocular deprivation (P90-P94), single unit recordings of the D-serine treated group showed a significant ocular dominance shift towards the open eye (CBI=0.47±0.01, n=3; $p<0.01$; **Fig 2.7B**), while saline treatment failed to demonstrate similar effects (CBI=0.78±0.01, n=3; **Fig 2.7C**). Thus, intracortical infusion and systemic administration of the drug induced similar OD shifts (**Fig 2.6E, 2.7B**), indicating that D-serine enhances ocular dominance plasticity via a mechanism operating in the visual cortex.

2.3.5 D-serine administration in adulthood, combined with reverse suture, restores contralaterally-biased visual input in long-term monocularly deprived mice

The studying of visual cortical plasticity is not only important for the understanding of experience-dependent plasticity, but also helps develop new therapeutic approaches to developmental disorders.

Since D-serine facilitated adult visual cortical LTD and ocular dominance shift, I further tested its effectiveness in the amblyopic model, and found that D-serine treatment promoted the re-establishment of the contralaterally-dominated visual input in long-term monocular deprived mice (**Fig 2.8**).

After long-term monocular deprivation starting at the beginning of the critical period (P23) and continuing until adulthood (P80), single-unit recordings revealed a significant ocular dominance shift towards the open eye (**Fig 2.8A,** CBI=0.50±0.05, n=5). Another group of long-term MD mice were reversely sutured at P80, and received treatment with either D-serine (600 mg/kg, SC, twice a day) or saline for two weeks. The effectiveness of D-serine was assessed at P94, and the saline injection group served as controls (**Fig 2.8B**). Single unit recording data in the saline treatment group showed an ocular dominance of the previously open eye (CBI=0.51±0.03, n=4; **Fig 2.8D**), which is similar to the effects observed in the absence of reverse suture. The D-serine treatment group, on the other hand, showed much stronger visual input from the initially deprived eye (CBI=0.77±0.03, n=4; **Fig 2.8C, E**), indicating recovery of visual input in adulthood. This suggests that D-serine has the potential to reverse amblyopia in adulthood.

2.3.6 Facilitation of ocular dominance plasticity by ALX 5407, hGlyT1 inhibitor

To further examine the regulatory effect of LTD, I then tested the effectiveness of another NMDAR co-agonist site modulator, hGlyT1 inhibitor ALX 5407 (Lipina et al., 2005). ALX 5407 has been shown effective in potentiating NMDAR-mediated responses (Chen et al., 2003), possibly by increasing glycine concentration at synaptic sites (Berger et al., 1998; Bergeron et al., 1998). When coupled with brief monocular deprivation, ALX 5407 was also effective in enhancing adult ocular dominance plasticity (**Fig 2.9**).

As shown previously, four days of monocular deprivation is not sufficient to induce ocular dominance shift in adulthood (CBI=0.78±0.01, n=5, **Fig 2.6B**). However, intraperitoneal injection of ALX 5407 (1 mg/kg, daily, Sigma, ≥98% (HPLC)) facilitated ocular dominance shift towards the open eye in adulthood (CBI=0.42, n=4, **Fig 2.9B**).

The effect of ALX 5407 is comparable to that of D-serine (CBI=0.56±0.05, n=5, **Fig 2.6E**). This further suggests that NMDAR co-agonists are effective in modulating NMDAR neurotransmission and ocular dominance plasticity.

2.4 Discussion

Ocular dominance plasticity represents a well-studied example of experience-dependent plasticity. Emerging evidence implicates several overlapping yet distinct mechanisms in the regulation of this plasticity. Among the well-studied phenomena involved in the regulation of visual cortex plasticity are the balance between excitation and inhibition in the cortex (Morishita and Hensch, 2008; Smith and Bear, 2010), the importance of regulation of axonal regenerative and sprouting capabilities via neurotropins and other regulatory molecules (Pizzorusso et al.,

2002; McGee et al., 2005), a role for prior visual exposure (Cynader and Mitchell, 1980), and synaptic processes such as LTP and LTD (Smith et al., 2009; Cooke and Bear, 2014).

2.4.1 LTD and ocular dominance plasticity

Recent studies have closely associated LTD with physiological processes such as learning and memory, and various pathological conditions (Collingridge et al., 2010). Here I focused on the regulatory role of LTD in ocular dominance plasticity. LTD has been proposed as the cellular substrate for the loss of visual responsiveness following MD during the critical period (Bear, 1996; Rittenhouse et al., 1999; Heynen et al., 2003; Yoon et al., 2009). LTD is difficult to induce in V1 of adult rodents (Dudek and Friedlander, 1996; Jiang et al., 2007; Yang et al., 2011). LTD could be blocked in juvenile visual cortical slices by application of $GluR2_{3Y}$, a peptide that regulates AMPA receptor trafficking. Systemic application of $GluR2_{3Y}$ prevents ocular dominance shift after monocular deprivation during the critical period (Yang et al., 2011).

Yoon *et al*. (2009) reported similar findings using a different LTD blocking peptide G2CT. G2CT blocked pair-pulse induced LTD in Layer IV spiny neurons of visual cortical slices and blocked ocular dominance shift *in vivo* after monocular deprivation. Using G2CT as a LTD blocking agent, the same group also suggested that the molecular mechanisms of LTD in layer II/III were different from that of layer IV of the primary visual cortex, being independent of AMPA receptor endocytosis (Crozier et al., 2007). Our *in vitro* data, however, have shown that $GluR2_{3Y}$ inhibited layer II/III LTD. Several differences between the actions of the two different peptides might partially explain the discrepancies between our studies and that of Yoon *et al*. (2009). $GluR2_{3Y}$ specifically targets the tyrosine clusters on GluA2/3, thus avoiding the off-target effects that G2CT has on other endocytosis processes that are mediated by the AP2 adaptor.

In adult mice, we showed that the administration of NMDAR co-agonist D-serine is effective in enhancing ocular dominance plasticity. D-serine operates in an activity-dependent manner. D-serine itself did not affect the baseline activity in adult visual cortical slices, nor did it alter visual responses in *in vivo* recordings. However, D-serine pre-incubation of visual cortical slices facilitated the induction of LTD in the presence of low-frequency stimulation. D-serine supplementation along with the manipulation of cortical activity by brief monocular deprivation caused a significant ocular dominance shift that was otherwise absent in adult mice (**Fig 2.6**). This further supports the idea that exogenous co-agonist application in combination with the manipulation of cortical input can dynamically modulate NMDAR related plasticity. Moreover, D-serine specifically facilitated the induction of layer II/III LTD in adult visual cortical slices, without affecting that of LTP (**Fig 2.5**). Furthermore, the ability of D-serine to enhance ocular dominance plasticity in adult mice was blocked by $GluR2_{3Y}$ (**Fig 2.6G**), again pointing specifically toward LTD as a key mechanism for enhanced adult plasticity. This is also supported by similar effect achieved through the administration of another NMDAR co-agonist site modulator, ALX5407 (**Fig 2.9**).

With local field potential recordings, we focused on the study of layer IV to layer II/III LTD in ocular dominance plasticity. Trachtenberg *et al*. (2000) demonstrated loss of deprived-eye responsiveness in layer II/III prior to that in layer IV after brief monocular deprivation in cats. Studies in cats and ferrets suggest that organization of extragranular layers predicts the anatomical changes in the geniculocortical afferent during development (Ruthazer and Stryker, 1996; Crair et al., 1997; Crair et al., 1998). Data presented here showed that blockade of layer II/III LTD prevented the OD shift in early postnatal life, and enhancement of layer II/III LTD in adult mice facilitated OD shift, suggesting that layer II/III LTD may play a special role in both

juvenile and adult plasticity. At the same time, these results do not rule out a possible contribution of layer IV plasticity in either young or older mice.

2.4.2 A new reagent to promote functional recovery in amblyopic subjects

As for the amblyopic model, recovery from long-term monocular deprivation dating back to the critical period does not occur readily in adulthood. After long-term MD spanning the critical period, reverse suture, or binocular experience alone is not potent in reversing the experimentally-induced permanent visual impairment (Wiesel, 1982; Prusky et al., 2000; Mitchell and MacKinnon, 2002; Prusky and Douglas, 2003). A number of elegant studies have reported effective strategies to promote recovery of visual acuity after long-term monocular deprivation in rodents, such as dark exposure (He et al., 2007), the application of the antidepressant fluoxetine (Maya Vetencourt et al., 2008) and histone deacetylase inhibitors valproic acid and sodium butyrate (Silingardi et al., 2010). Other strategies to enhance adult ocular dominance plasticity include degradation of chondroitin sulphate proteoglycans of the extracellular matrix by chondroitinase-ABC (Pizzorusso et al., 2002), mutation of the Nogo-66 receptor (McGee et al., 2005), transplantation of inhibitory neurons (Southwell et al., 2010), and the reduction of intracortical inhibition of mature visual cortex (Harauzov et al., 2010). Current opinion favors the idea that these effects have been achieved either by resetting the excitatory–inhibitory (E-I) balance to mimic that occurring at critical period onset, or by removing molecular brakes that prevent structural changes after the critical period (Morishita and Hensch, 2008).

I combined reverse suture with D-serine treatment to restore contralaterally biased visual input in long-term monocularly deprived mice (**Fig 2.8**). Reverse suture was used because it potentially allows a greater functional gain, as it shifts the competitive balance in favour of the

initially deprived eye (Mitchell et al., 1977; Mitchell et al., 2001). D-serine administration facilitated the shift in the cortical ocular dominance distribution after reverse suture, perhaps by promoting an LTD-like process altering inputs from the later-sutured eye. The effect of D-serine can be prevented by the LTD-blocking peptide $GluR2_{3Y}$ (**Fig 2.6**). In adult visual cortical slices, D-serine specifically facilitated the induction of LTD while not affecting that of LTP (**Fig 2.5**).

Other than regulating NMDAR transmission, D-serine might conceivably have effects on the reinstatement of adult ocular dominance plasticity through other mechanisms. A previous study suggested that D-serine might decrease GABAergic neurotransmission (Liu et al., 2009), which would contribute to a further increase of the E-I ratio, driving the visual system to a more immature state and making it easier for the recovery of function to occur in the reverse sutured mice. D-serine might also conceivably also have some effect on structural plasticity in addition to functional plasticity, since D-serine is mostly found in astrocytes that ensheathe NMDA-receptor-bearing neurons (Martineau et al., 2006).

2.4.3 Conclusions

Our group has previously demonstrated a temporal correlation between age and the amplitude of LTD induced in visual cortical slices. $GluR2_{3Y}$, a specific LTD blocker, prevented ocular dominance shift in monocularly deprived mice during the critical period (Yang et al., 2011). G2CT, another LTD blocker, was also effective in preventing ocular dominance shift in monocularly deprived mice during the critical period (Yoon et al., 2009). Taken together, these data suggest that LTD is necessary in the induction of ocular dominance shift by monocular deprivation during the critical period.

In this chapter, I provided evidence that NMDAR co-agonist D-serine facilitates induction of LTD in adulthood and enhances ocular dominance plasticity beyond the critical

period via an LTD-like mechanism. NMDAR glycine site modulator ALX 5407 also facilitated ocular dominance shift in monocularly deprived adult mice. This suggests that enhancement of LTD is sufficient in enhancing ocular dominance plasticity in adulthood.

Data presented here suggest that LTD is an important molecular substrate for ocular dominance plasticity. In addition, I have also provided evidence that D-serine is effective in restoring the normal ocular dominance distribution pattern in mice that have undergone long-term monocular deprivation. As an endogenous NMDAR ligand with low toxicity, D-serine seems promising for aiding functional recovery from visual deficits in adulthood. This also holds great promise for the development of new therapeutic strategies in other disease where LTD plays an important regulatory role, such as autism, addiction, schizophrenia, Parkinson's disease and Alzheimer's disease (Wolosker et al., 2008).

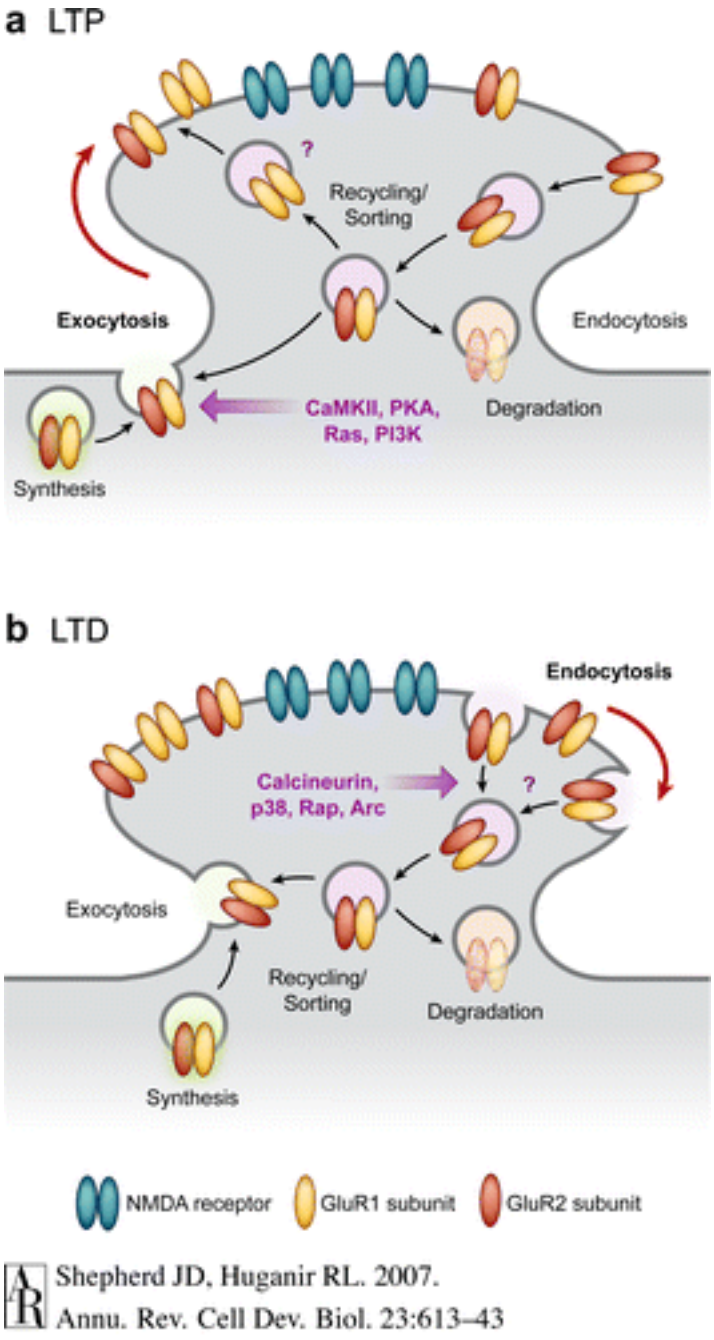


Fig 2.1 AMPAR trafficking during synaptic plasticity. (*a*) AMPAR insertion occurs during long-term potentiation (LTP). Receptors are inserted at extrasynaptic sites or directly at the synapse. (*b*) AMPAR endocytosis occurs during long-term depression (LTD). Internalized receptors are either sent to a recycling/sorting pool or degraded. From Shephered and Huganir, 2007, Annu Rev Cell Dev Biol, 23:613-43. Used with permission.

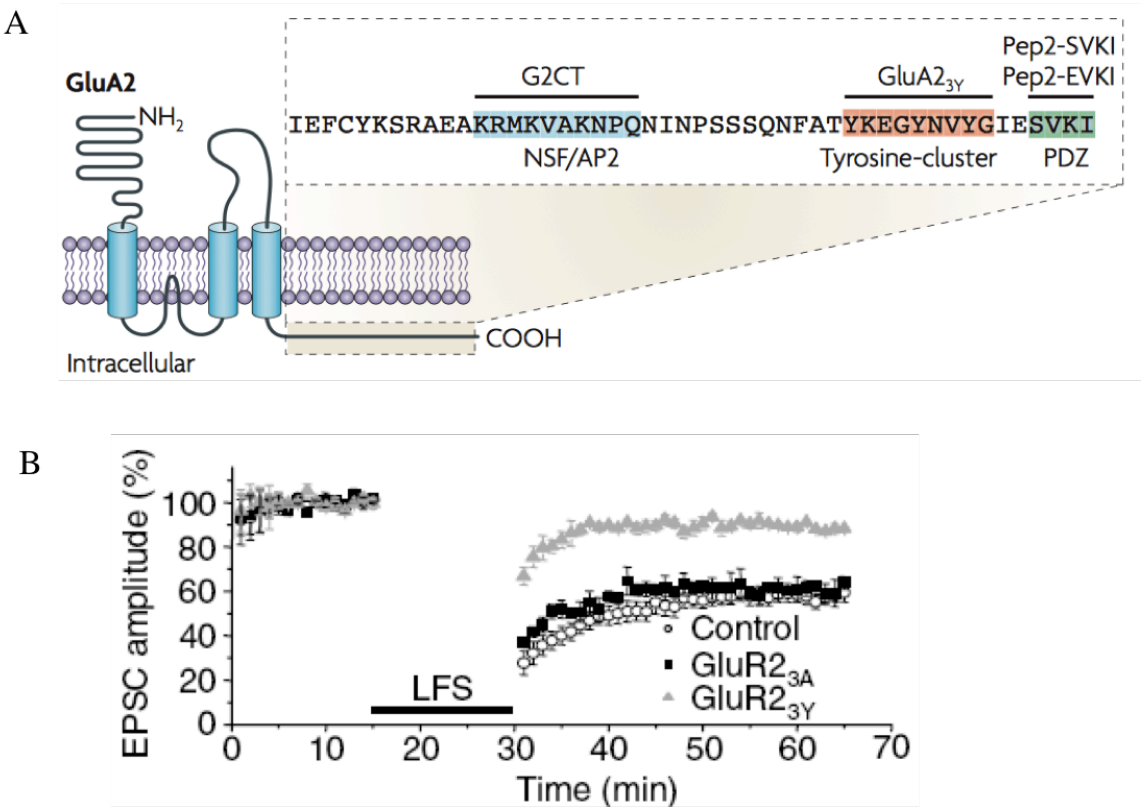


Fig 2.2 GluR2$_{3Y}$ peptide is a specific LTD-blocker. (A) Design of GluR2$_{3Y}$. GluR2$_{3Y}$ targets the tyrosine cluster on GluA2 C-terminus. From Collingridge *et al*., 2010, Nat Rev Neurosci, 11(7): 459-73. Used with permission. (B) Tat-GluR2$_{3Y}$ blocks the induction of LTD in hippocampal slices in the presence of low frequency stimulation (LFS). From Ahmadian *et al*., 2004, EMBO J, 23(5): 1040-50. Used with permission.

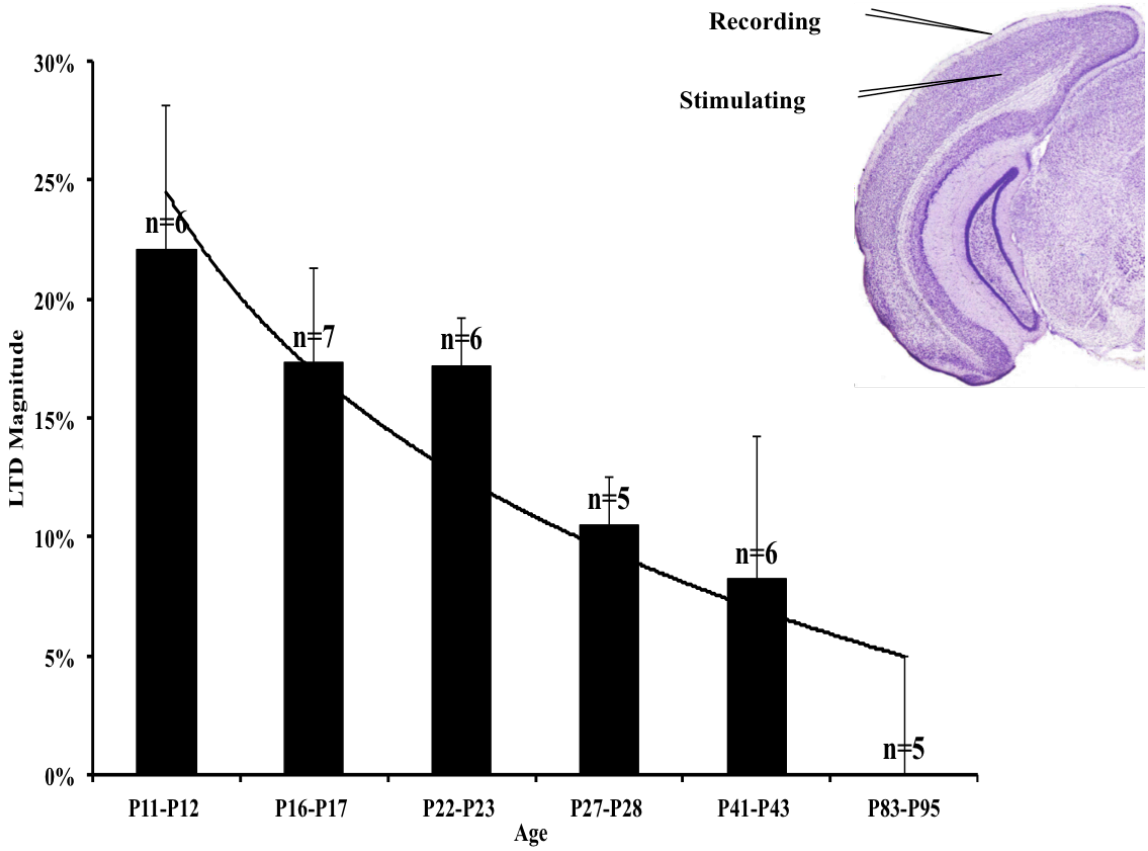


Fig 2.3 LTD is developmentally constrained. Low-frequency stimulation delivered to layer IV induces LTD in layer II/III. The magnitude of LTD induced in visual cortical slices decreases gradually during the critical period. LFS is not effective in inducing LTD in adult visual cortical slices. From Yang *et al*., 2011, Sci Rep 1:203. Used with permission.

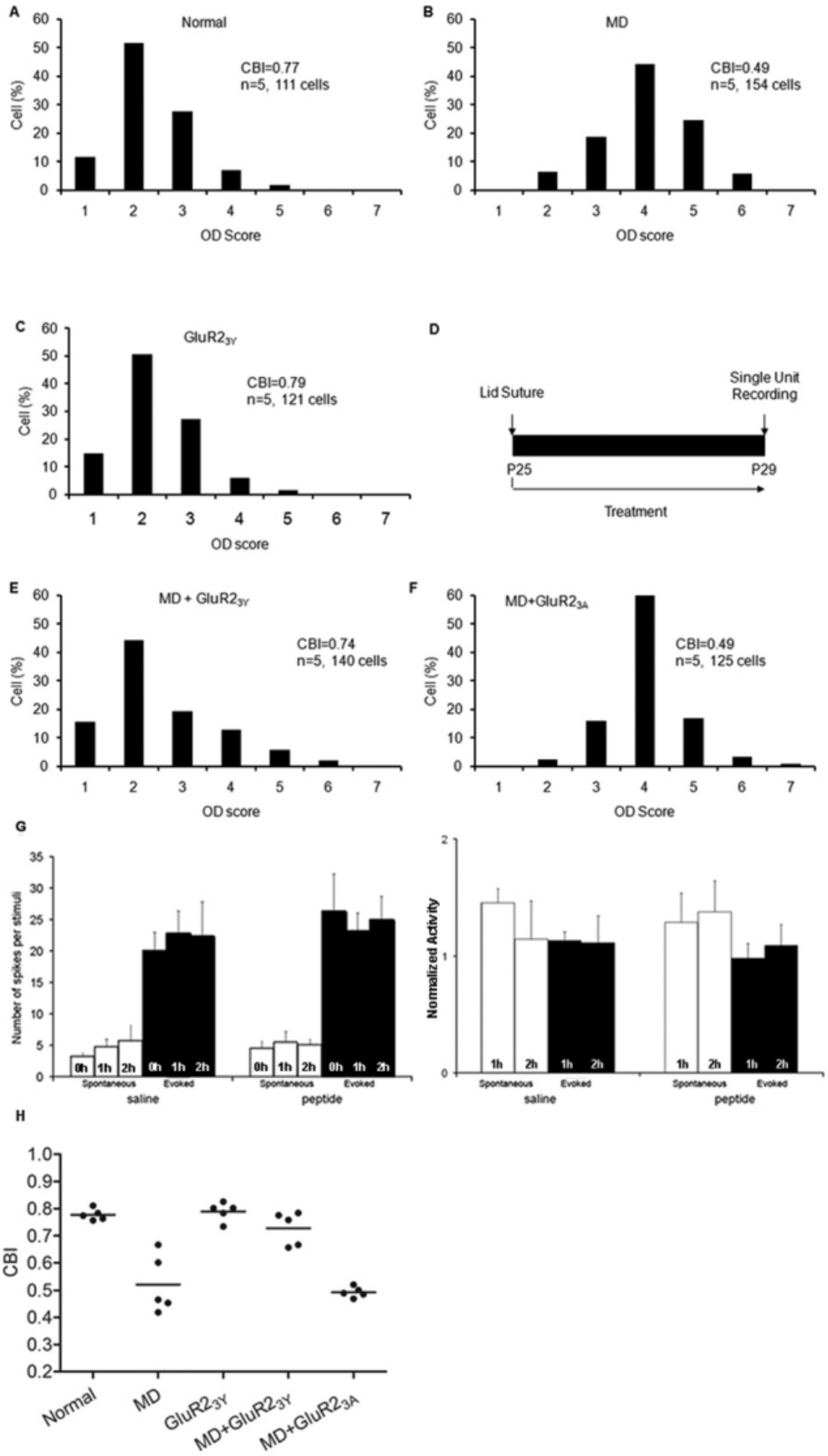
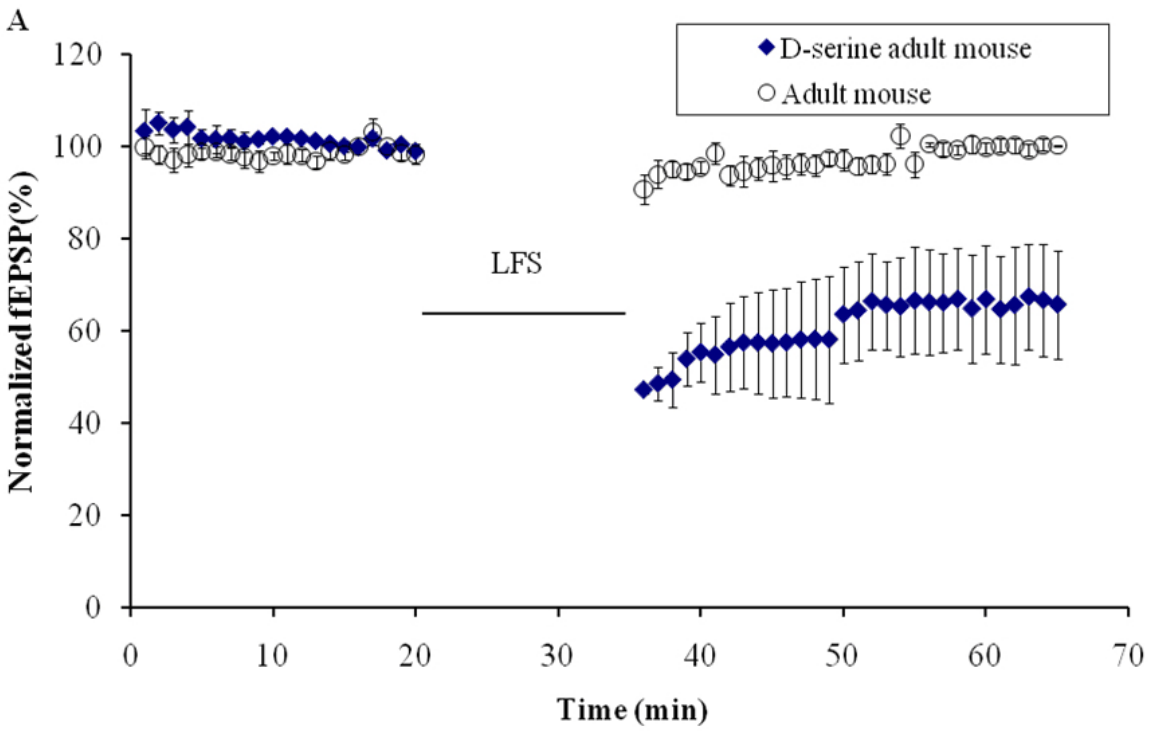

A
Normal
CBI=0.77
n=5, 111 cells
Cell (%)
OD Score
B
MD
CBI=0.49
n=5, 154 cells
Cell (%)
OD score
C
GluR2$_{3Y}$
CBI=0.79
n=5, 121 cells
Cell (%)
OD score
D
Lid Suture
Single Unit Recording
P25
P29
Treatment
E
MD + GluR2$_{3Y}$
CBI=0.74
n=5, 140 cells
Cell (%)
OD score
F
MD+GluR2$_{3A}$
CBI=0.49
n=5, 125 cells
Cell (%)
OD Score
G
Number of spikes per stimuli
0h 1h 2h 0h 1h 2h
Spontaneous Evoked
saline
peptide
Normalized Activity
1h 2h 1h 2h
Spontaneous Evoked
saline
peptide
H
CBI
Normal
MD
GluR2$_{3Y}$
MD+GluR2$_{3Y}$
MD+GluR2$_{3A}$

Fig 2.4 GluR2$_{3Y}$ prevents the ocular dominance shift *in vivo* during the critical period. (A) The OD distribution favors the contralateral eye in normal non-deprived mice. (B) Monocular deprivation shifts the distribution towards the ipsilateral eye during the critical period. (C) GluR2$_{3Y}$ (10 nmol/g, IP, daily) peptide itself does not influence OD distribution in non-deprived mice. (D) Experiment protocol. (E) GluR2$_{3Y}$ (10 nmol/g, IP, daily) prevents the MD-induced ocular dominance plasticity during the critical period. (F) Tat-GluR2$_{3A}$ (10 nmol/g, IP, daily) does not prevent the OD shift following MD. (G) Tat-GluR2$_{3Y}$ (10 nmol/g, IP, daily) does not affect the spontaneous activity and evoked response in the binocular zone of the primary visual cortex. (H) Data summary. From Yang *et al*., 2011, Sci Rep 1:203. Used with permission.


A
D-serine adult mouse
Adult mouse
LFS
Normalized fEPSP(%)
Time (min)
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70

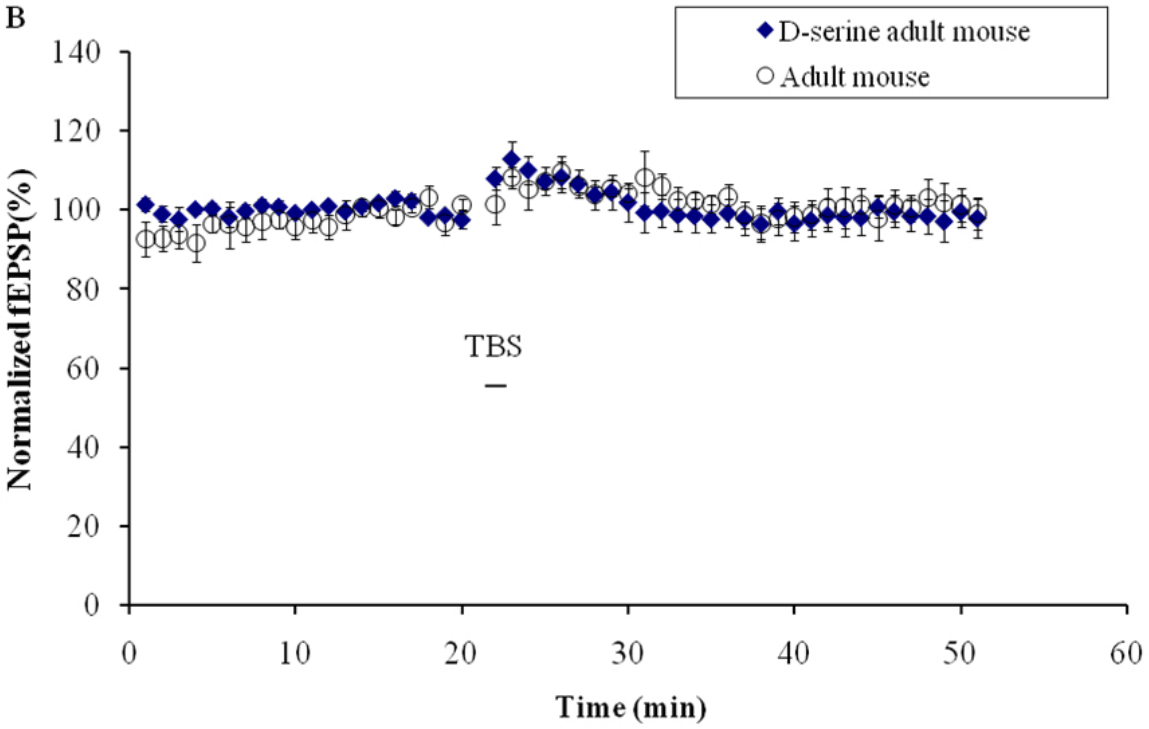

B
D-serine adult mouse
Adult mouse
TBS
Normalized fEPSP(%)
Time (min)
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60

Fig 2.5 D-serine selectively facilitates the induction of LTD in adult visual cortical slices. (A) D-serine (Sigma, ≥98% (TLC)) facilitated the induction of LTD in adult visual cortical slices. Low-frequency stimulation (LFS, 1Hz, 900 stimuli) delivered to layer IV could not induce LTD in layers II/III in adult visual cortical slices (P90-P100, 100 ± 0.1% of baseline, n=3). D-serine (20 μM, 20 minutes) incubation of visual cortical slices did not affect the baseline activity, but it facilitated the decrease of the fEPSP in the presence of low frequency stimulation (66±12% of baseline, n=4; $p< 0.01$). The effect was stable for more than 30 minutes. Error bars reflect SEM. (B) D-serine did not facilitate the induction of LTP in adult visual cortical slices. TBS did not elicit LTP in adult cortical slices (99±4% of baseline, n=5), nor did any of the concentrations of D-serine from 20 μM up to 200 μM facilitate the induction of LTP (96±6% of baseline, n=5).

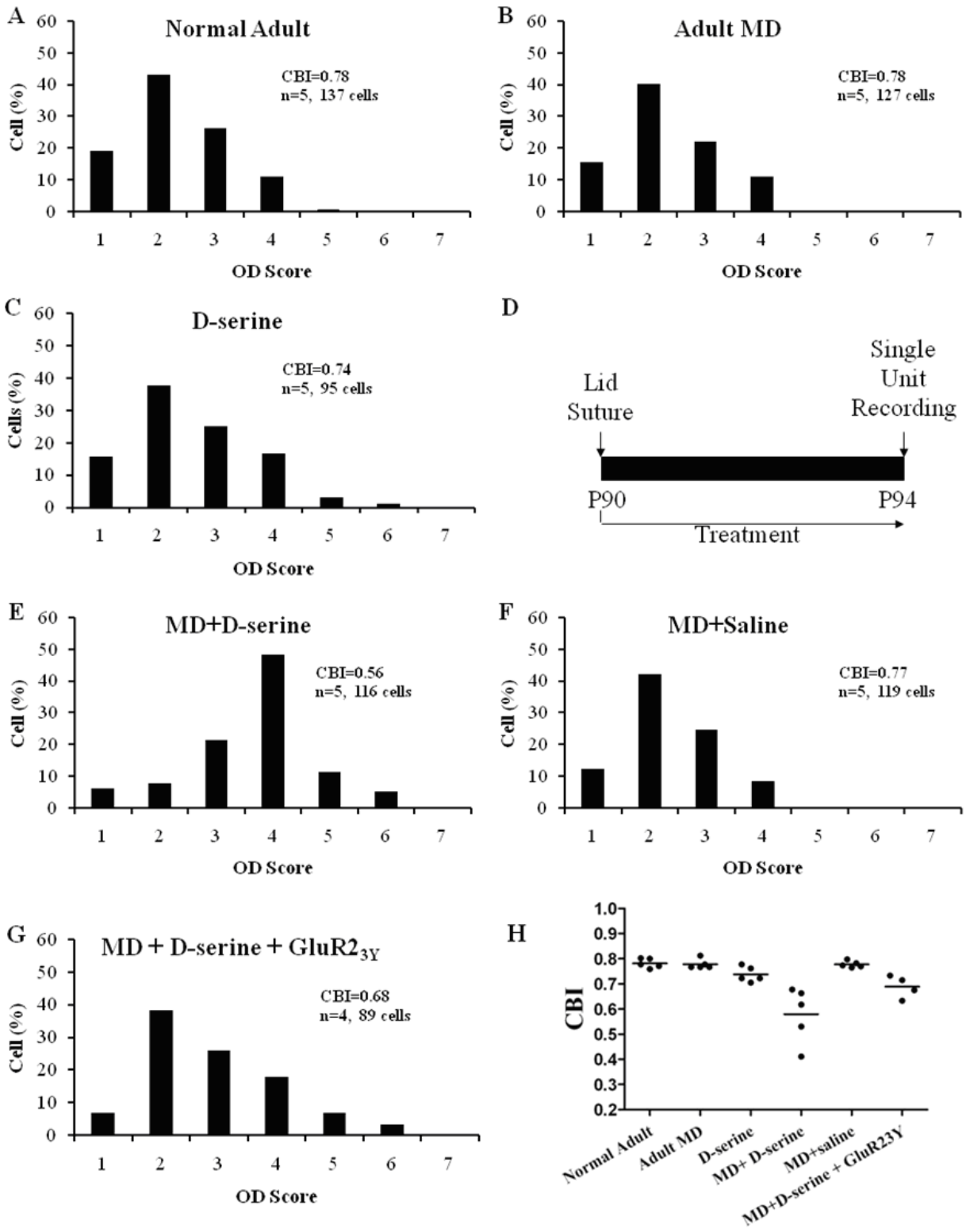

A
Normal Adult
CBI=0.78
n=5, 137 cells
Cell (%)
OD Score
B
Adult MD
CBI=0.78
n=5, 127 cells
Cell (%)
OD Score
C
D-serine
CBI=0.74
n=5, 95 cells
Cells (%)
OD Score
D
Lid Suture
Single Unit Recording
P90
P94
Treatment
E
MD+D-serine
CBI=0.56
n=5, 116 cells
Cell (%)
OD Score
F
MD+Saline
CBI=0.77
n=5, 119 cells
Cell (%)
OD Score
G
MD + D-serine + GluR2 3Y
CBI=0.68
n=4, 89 cells
Cell (%)
OD Score
H
CBI
Normal Adult
Adult MD
D-serine
MD+ D-serine
MD+saline
MD+D-serine + GluR23Y

Fig 2.6 D-serine enhances ocular dominance plasticity in adult mice, via an LTD-like mechanism. (A) Normal adult mice demonstrated a contralateral biased OD distribution (CBI=0.78±0.01, n=5). (B) Single-unit recordings revealed no ocular dominance shift in monocular deprived adult mice (CBI=0.78±0.01, n=5, p=0.64 compared to normal adult mice). (C) D-serine itself (600 mg/kg, SC, twice a day) did not induce an ocular dominance shift compared to untreated adult mice (CBI=0.74±0.01, n=5, p=0.03). (D) Experimental protocol for single-unit recording experiments. (E) D-serine (600 mg/kg, SC, twice a day) reinstated the ocular dominance shift after four days of monocular deprivation in adult mice as is shown in the ocular dominance score distribution (CBI=0.56±0.05, n=5, $p<0.01$ compared to normal mice). (F) The saline treatment group did not show a corresponding shift in OD distribution (CBI=0.77±0.01, n=5, p=0.23 compared to normal mice). (G) $GluR2_{3Y}$ (10 nmol/g, IP, daily) prevented the effect of D-serine (600 mg/kg, SC, twice a day) in monocularly deprived adult mice (CBI=0.68, n=4). (H) Data summary of the binocularity index results in the various conditions studied in adult mice.

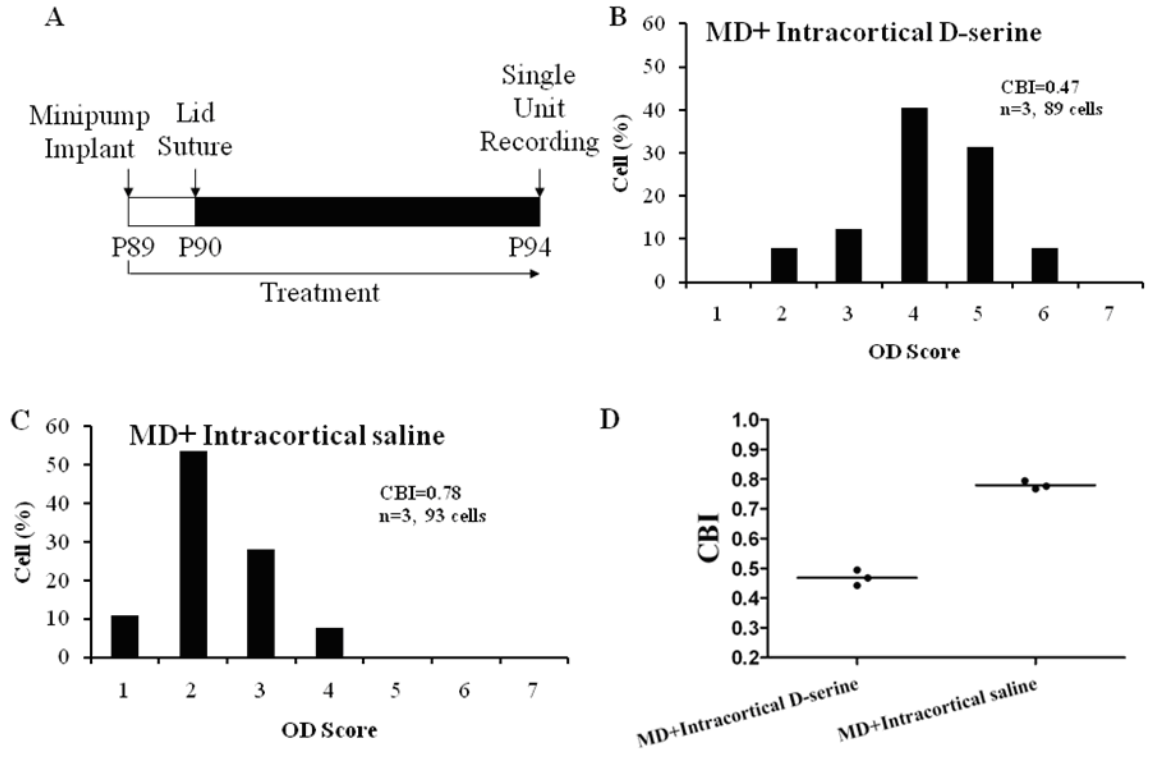


Fig 2.7 D-serine enhances visual cortical plasticity in adult mice. (A) Experimental schedule for single-unit recording experiments in adult mice that received local infusion treatment. (B) D-serine-infused (50 mM) mice showed a significant ocular dominance shift (CBI=0.47±0.01, n=3, $p<0.01$ compared to normal mice). (C) Saline-infused mice demonstrated an OD distribution favoring the contralateral eye (CBI=0.78±0.01, n=3). (D) Data summary of the binocularity index in the D-serine intracortical infusion study.

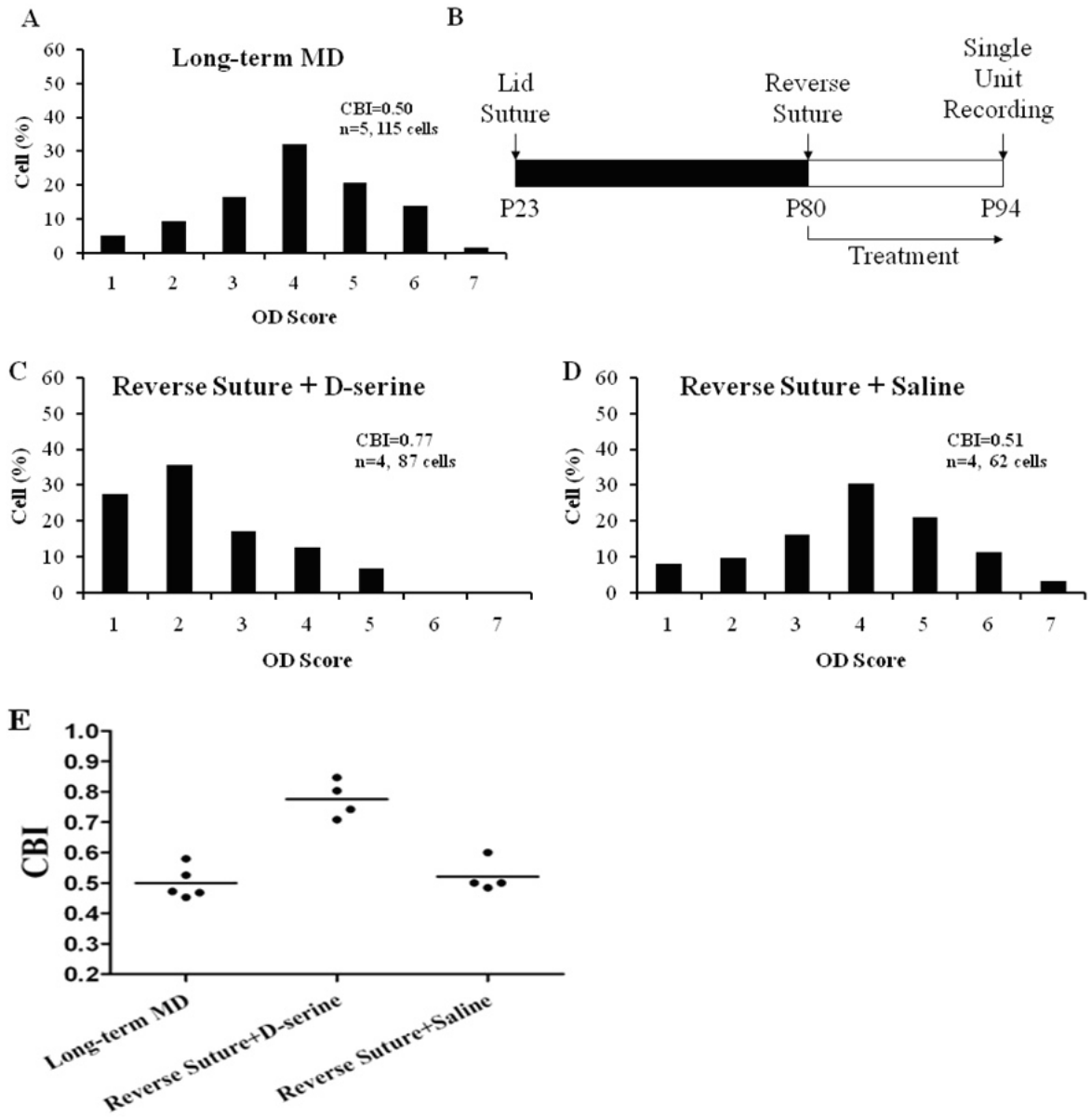


Fig 2.8 D-serine promotes the restoration of normal binocularity in reverse sutured mice.

(A) Long-term monocular deprivation results in an ocular dominance shift favoring the open eye into adulthood (CBI=0.50±0.05, n=5). (B) Experimental schedule for single-unit recording experiments in reversely sutured animals. (C) D-serine (600 mg/kg, SC, twice a day) promoted recovery of visual input from a previously deprived eye in reverse sutured mice (CBI=0.77±0.03, n=4). (D) Saline treated RS mice still showed dominance by the previously open eye

(CBI=0.51±0.03, n=4). (E) CBI summary in long-term deprived mice, and in RS mice treated with D-serine or saline.

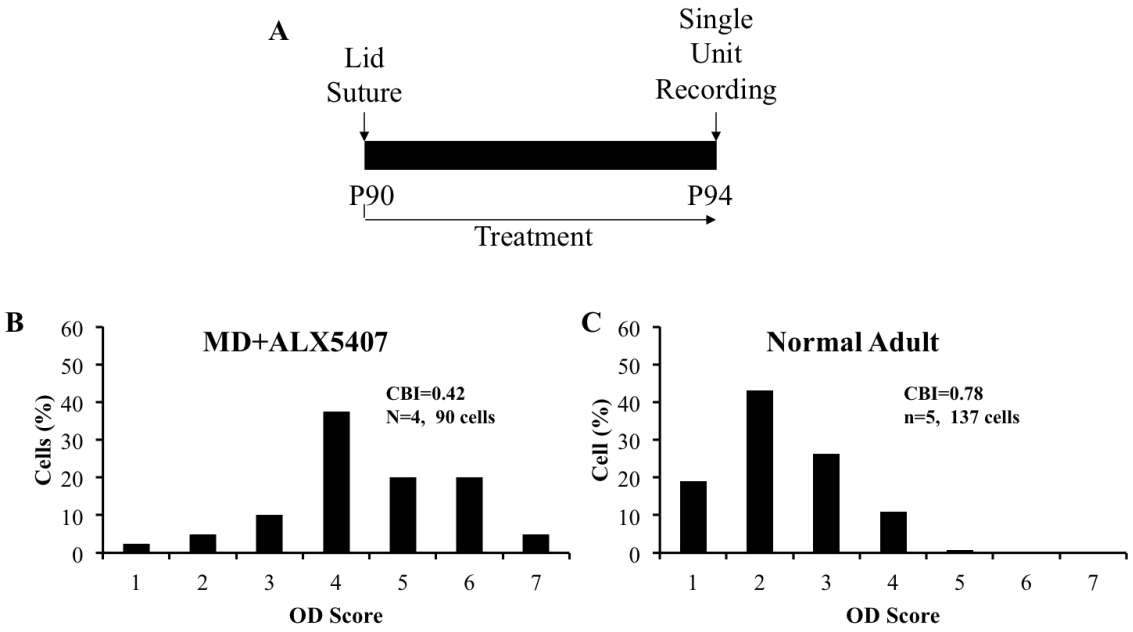


Fig 2.9 ALX 5047, hGlyT1 inhibitor, enhances ocular dominance plasticity in adulthood.

(A) Experimental protocol. Lid suture was performed over the left eye at P90. Single unit recording was performed after four days of monocular deprivation, to examine ocular dominance distribution. (B) Administration of ALX 5407 (1 mg/kg, IP, daily, Sigma, ≥98% (HPLC)), coupled with four days of monocular distribution, resulted in a shift in ocular dominance distribution with a CBI of 0.42, suggesting enhance ocular dominance plasticity in adulthood. (C) Normal adult ocular dominance distribution.

CHAPTER 3 FUNCTIONAL CONNECTIVITY CHANGES INDUCED BY MONOCULAR DEPRIVATION DURING DEVELOPMENT AND IN ADULTHOOD

3.1 Introduction

In the previous chapter, I explored the functional significance of LTD in ocular dominance plasticity. Using the classic monocular deprivation model, with the help of electrophysiological techniques, I provided additional evidence to underscore the importance of LTD in ocular dominance plasticity. Blocking LTD with $GluR2_{3Y}$ prevents ocular dominance shift induced by monocular deprivation during the critical period. Augmenting LTD by D-serine administration enhances ODP in adulthood. My results showed that LTD is not only necessary for ocular dominance plasticity during the critical period, but is also sufficient in enhancing ocular dominance plasticity in adulthood.

To probe the functional consequences of monocular deprivation outside the contralateral binocular zone, in this chapter, I explore the global functional changes with the help of *in vivo* wide-field imaging techniques. This is largely inspired by the rich literature on cross-modal plasticity in response to sensory deprivation, our growing appreciation of spontaneous activity and functional connectivity, and made possible by recent advances in *in vivo* functional imaging techniques.

3.1.1 Cross-modal plasticity

It is well established that sensory deprivation of one modality can lead to behavioural changes in the remaining modalities, as well as a reorganization of cortical functions (for an excellent review, see Bavelier and Neville, 2002). Primary sensory cortices that are associated with the deprived modality can be recruited or colonized by the remaining modalities, while the remaining cortices undergo compensatory changes (Lee and Whitt, 2015). Cross-modal plasticity

has been studied extensively in the association with visual deprivation, both in humans and animal models.

We are no strangers to the fact that early blind humans have sharper pitch discriminations (Gougoux et al., 2004), better spatial localizations of sounds (Lessard et al., 1998), and finer tactile acuity (Goldreich and Kanics, 2003). These behavioural changes may have resulted from recruitment of the visual cortex by auditory and somatosensory cortices, or from compensations by the spared sensory modalities, as suggested by early studies in animal models (Bavelier and Neville, 2002).

Primary visual cortex can be driven by auditory input, in naturally blind mole rats (Heil et al., 1991) and visually deprived cats (Yaka et al., 2000). V1 can also be recruited by somatosensory cortex. Enucleation in newborn rats leads to expansion of the somatosensory responses into the visual cortex (Toldi et al., 1994a). Responsiveness of V1 to somatic exploration has also been observed in monkeys that undergo lid suture in the first year of life (Hyvarinen et al., 1981). On the other hand, compensatory plasticity has been observed in the auditory and somatosensory cortex. Prolonged binocular deprivation in both juvenile and adult ferrets leads to a significant improvement in auditory spatial acuity in the lateral sound field (King and Parsons, 1999). Enlargement of receptor field and increase in angular sensitivity have been observed in enucleated newborn rats (Toldi et al., 1994b). Compared to controls, enucleated rats have better maze performance, which is mediated by somatosensory perception with the whiskers (Toldi et al., 1994b).

As discussed in Chapter 1 (**section 1.3.2**), there appears to be a sensitive period for cross-modal plasticity when the brain is more adaptive to sensory deprivation (Bavelier and Neville, 2002), although cross-modal plasticity does persist into adulthood (Lee and Whitt, 2015).

Inspired by the rich literature on cross-modal plasticity, curious to find out about the global effect of monocular deprivation, I set out to explore the functional consequences of monocular deprivation in the primary visual cortex and other sensory cortices, both during the critical period and in adulthood. I anticipate observing some MD-induced compensatory changes within V1 and globally, with possible recruitment of V1 by somatosensory cortex, during the critical period, if not in adulthood. Considering the invasive and sparse-sampling nature of electrophysiological approaches, I turned to imaging techniques, which would allow for longitudinal studies of the same subjects to track the developmental changes. Also, I studied spontaneous activity to examine functional connectivity.

3.1.2 Spontaneous activity as a means to study functional connectivity

Spontaneous activity results from connections intrinsic to the cortex (Sanchez-Vives and McCormick, 2000; Timofeev et al., 2000), with modulation from the thalamus (Rigas and Castro-Alamancos, 2007; Crunelli and Hughes, 2010). In the past few decades, spontaneous activity has been attracting more attention in neuroscience, in particular, infraslow (<0.1Hz) spontaneous activity. Away from cardiac or respiratory contamination, infraslow activity is thought to represent neuronal activity (Cordes et al., 2001). Infraslow spontaneous activity has been linked with development of neural networks during development (Colonnese et al., 2010; Colonnese and Khazipov, 2012). In humans, infraslow activity synchronizes with higher-frequency brain activity (He et al., 2010). Spontaneous activity modulates behavioural performance and is functionally significant during execution of cognitive tasks (Fox et al., 2007; Monto et al., 2008).

In humans, spontaneous activity can be studied with the help of PET and fMRI. Studies suggest that coherent spontaneous fluctuations in the BOLD signal of fMRI reflect a fundamental or intrinsic property of functional brain organization (Vincent et al., 2007). The

BOLD signal is not a direct measurement of neuronal activity. Instead, it reflects local variations in de-oxyhemoglobin concentration, which is determined by a combination of blood flow, blood volume and oxygen metabolism (Raichle and Mintun, 2006).

Seed based correlation mapping and spatial independent component analysis (ICA) (discussed in more detail in Chapter 1, section **1.4.3.1**) have been used to study spatio-temporal pattern of infraslow spontaneous activity. This has lead to the discovery of the default mode network and inspired tremendous interest in functional connectivity (Raichle and Snyder, 2007).

Several studies have demonstrated similarities between the patterns of infraslow spontaneous activity and those of sensory-evoked activity (Watson et al., 2008; Jermakowicz et al., 2009; Luczak et al., 2009). Moreover, infraslow activity is characterized by synchronous bilateral activity in functionally related regions. This has been observed in visual (Lowe et al., 1998; Cordes et al., 2000), somatomotor (Biswal et al., 1995; Xiong et al., 1999; Cordes et al., 2001) and auditory (Cordes et al., 2000) cortices. Generally speaking, regions of similar functionality tend to be correlated in their spontaneous BOLD activity, while regions of opposing functionality tend to be negatively correlated (Fox and Raichle, 2007).

Spontaneous activity may reflect neuro-anatomical organization, as is supported by the correlation between BOLD and diffusion tensor imaging (Koch et al., 2002; De Luca et al., 2006). Regions linked by white matter fibre tracts are expected to show high functional connectivity (Koch et al., 2002). In recent years, spontaneous activity has gained more attention as a means of studying functional connectivity and architecture of the brain.

3.1.3 Spontaneous activity and functional connectivity in rodent brain

These findings revealed by fMRI and PET in humans have been successfully replicated in rodents, with the help of *in vivo* functional imaging techniques, including IOS (White et al., 2011), voltage-sensitive dye (VSD) imaging (Mohajerani et al., 2010; Mohajerani et al., 2013; Chan et al., 2015), and calcium imaging (Vanni and Murphy, 2014). Spontaneous infraslow activity is regionally distinct, correlates with electroencephalography and local field potential recordings, and shows bilateral symmetry between cortical hemispheres (Chan et al., 2015).

Patterns of spontaneous activity in rodent brain resemble that of evoked activity, as is shown with both VSD (Mohajerani et al., 2013) and GCaMP imaging (Vanni and Murphy, 2014). Infraslow spontaneous activity is dependent on cortical activity. Intracortical application of voltage-gated sodium channel and glutamate receptor antagonists attenuates infraslow activity and abolishes the functional structure revealed by correlation maps (Mohajerani et al., 2013; Chan et al., 2015).

Spontaneous activity is highly synchronized between hemispheres (White et al., 2011; Mohajerani et al., 2013; Chan et al., 2015). This synchrony depends on the corpus callosum, as is evidenced by the attenuation of the synchrony in acallosal mice (Mohajerani et al., 2013). Midline areas, such as anterior cingulate and retrosplenial cortex, exhibit the highest degree of synchrony. These midline hotspots are thought be mouse equivalent of the default mode network (Chan et al., 2015). A similar default mode network has recently been identified with fMRI in rats (Lu et al., 2012).

3.1.4 Spontaneous activity in the visual system

Spontaneous activity has been relatively well explored in the visual system. Previous studies suggest that spontaneous activity plays an essential role in the normal development of the nervous system, acting in concert with genetically driven, molecular determinants to ensure

precise formation of complex neural circuits (Goodman and Shatz, 1993; Pallas, 2001). The following sections briefly review this evidence.

3.1.4.1 Spontaneous retinal waves

Spontaneous activity in the visual system was first studied in the form of retinal waves, waves of spontaneous activity sweeping across the retina. This was initially demonstrated with *in vivo* recordings from the fetal rat retina. Neighbouring retinal ganglion cells periodically exhibit temporally correlated bursts of action potentials. Retinal waves occur in the absence of light stimulation, since photoreceptors are not present in the embryonic rat retina (Galli and Maffei, 1988).

Multielectrode arrays have been used to study the spatial and temporal properties of large populations of cells in the ganglion cell layer in the neonatal ferret retina *in vitro* (Meister et al., 1991). Neighbouring cells exhibit temporally synchronized bursts. One month prior to eye opening, RGCs exhibit bursts of action potentials of 2 to 8 s in duration, followed by relatively long periods of inactivity (30 s to 1 min). Since the burst durations are relatively short compared to the interburst intervals, it is unlikely that the bursting activity from the two eyes will overlap frequently, thus providing temporal cues for activity-dependent segregation of eye inputs in the dLGN (Wong, 1999).

Indeed, retinal cells shift from projecting widely and imprecisely to projecting retinotopically and in an eye-segregated fashion, with the help of spontaneous retinal waves, possibly in a Hebbian-like fashion (Wong, 1999; Firth et al., 2005; Torborg and Feller, 2005). Manipulation of the spatial organization of retinal waves, without changing the overall intensity, impairs eye-specific segregation in the superior colliculus and dLGN (Xu et al.,

2011). Retinal waves play an important role in retinotopic refinement and retino-thalamic maturation.

Retinal activity also plays an important regulatory role in primary and even secondary cortical regions. In ferrets, blocking retinal waves by intravitreal epibatidine injections during the first week of life permanently disrupts the anatomical organization of ocular dominance columns and leads to a dramatic increase in receptive field size for binocular cells in V1 (Huberman et al., 2006). Retinal waves not only serve as the primary source of activity in the midbrain and primary visual cortex, but also modulate ongoing activity in secondary visual areas. In neonatal mice, spontaneous retinal waves are present and propagate throughout the entire visual system before eye opening (Ackman et al., 2012). This suggests that spontaneous retinal activity is transmitted through the entire visual system and carries patterned information capable of guiding the activity-dependent development of complex circuits before the onset of vision (Ackman et al., 2012).

3.1.4.2 Spontaneous activity in the visual cortex

Synchronous fluctuations of spontaneous activity have been observed in the visual cortex (Lowe et al., 1998; Cordes et al., 2000). The spatial layout of spontaneous activity revealed by VSD imaging in the visual cortex reflects functional connections, with patterns of spontaneous activity resembling those evoked by orientated grafting (Kenet et al., 2003). This has also been suggested by data obtained from single-unit recordings (Jermakowicz et al., 2009). In addition, there is spatial colocalization between correlation maps of V1 generated from VSD imaging of spontaneous activity and axonal projections revealed by anterograde tracer injection in the Allen Mouse Brain Connectivity Atlas (AMBC) Atlas (Mohajerani et al., 2013).

Spontaneous activity within V1 is susceptible to visual experience. Dark rearing decreases slow-wave activity in the visual cortex but not elsewhere (Miyamoto et al., 2003). The temporal patterns of spontaneous activity of individual cells become more similar to the visually evoked response after repeated stimulation, as revealed by single-unit recordings (Yao et al., 2007). With VSD imaging in rats, it has been shown that spatio-temporal patterns resembling those of evoked responses appear more often, after repetitive presentation of a given visual stimulus. This effect is transient and lasts for several minutes. Reverberation of recent visual experience could represent a form of short-term memory and help consolidate the transient effects of recent sensory experience into long-lasting cortical modifications (Han et al., 2008). Studies in hippocampus also suggest that infraslow activity may facilitate synaptic strengthening by grouping the replay of previous neural activity (Sutherland and McNaughton, 2000; Schwindel and McNaughton, 2011). Here I tested the hypothesis that monocular deprivation during the critical period affects both spontaneous activity and global functional connectivity as well as within the primary visual cortex.

3.1.5 Imaging techniques used to study spontaneous activity in rodents

In the previous chapter, I explored the molecular mechanism of ocular dominance plasticity with electrophysiological techniques, including single-unit recordings and local field potential recordings. Single-unit recording has been a standard method to study mouse ocular dominance plasticity (Gordon and Stryker, 1996). It allows for direct assessment of neuronal spiking activities. However, only a small proportion of the neurons in a given area could be sampled at a time and the experimental sessions are relatively long. In addition, no anatomical information is revealed about the particular cells responsible for the spikes. With regard to local field potential recording, it is an *in vitro* assessment and is also limited by the region that could be assessed in a

single run. Also, both techniques are invasive in nature. To examine functional connectivity changes longitudinally on a large scale, I turned to *in vivo* functional imaging techniques and relied heavily on studying spontaneous activity. Here I compare and contrast a number of imaging techniques I have experimented with.

3.1.5.1 Optical imaging of intrinsic signals (IOS)

Optical imaging of intrinsic signals has been frequently used to study the visual cortex. Compared to the conventional electrophysiological and anatomical methods, it allows for mapping of a relatively large area over time (Grinvald et al., 1986). Stryker and associates have subsequently adopted this technique in studying ocular dominance plasticity in mice (Kalatsky and Stryker, 2003; Cang et al., 2005).

The IOS signal is biphasic, with an initial fall of reflectance (the 'initial dip') (Frostig et al., 1990; Malonek and Grinvald, 1996), followed by a slower increase of light reflectance. IOS imaging measures changes in cortical light reflectance after local neuronal activation, as indicators of electrical or metabolic activities, caused by the difference in de/oxygenated hemoglobin and changes in local cerebral blood flow. In terms of mechanism, IOS imaging resembles that of functional MRI in humans.

Compared to conventional electrophysiological and anatomical techniques used to study the visual cortex, IOS significantly increases the area of brain that can be studied. It permits imaging over almost the entire dorsal cortical surface. Recently, IOS has been used to study the functional connectivity across the brain in mice (White et al., 2011).

However, IOS imaging is not ideal because of its relatively low temporal and spatial resolutions, compared to imaging counterparts discussed below. In the first paper that used IOS as a measurement of ODP in mice, the absolute response amplitude (i.e. change in reflected light

level) was reported to be around 1.45-2.44 $\times 10^{-4}$ (Cang et al., 2005). The low signal amplitude makes it necessary to do relatively long recordings with repeated trials, thus making recording more time-consuming and challenging.

3.1.5.2 Voltage-sensitive dye (VSD) imaging

Voltage-sensitive dyes bind to cell membranes and change their fluorescence according to the surrounding voltage environment, thereby transducing membrane potential to optical signals (Grinvald and Hildesheim, 2004). A major advance in VSD imaging has been the development of dyes that are excited by wavelengths of light that do not overlap with the wavelengths absorbed by hemoglobin, dramatically reducing the contamination of the signal by heartbeat noise (Shoham et al., 1999).

VSD imaging has been used in a wide range of experimental paradigms, from isolated nerves (Salzberg et al., 1973; Tasaki and Warashina, 1976), to the mammalian cortex (Grinvald and Hildesheim, 2004; Borgdorff et al., 2007; Mohajerani et al., 2013). VSD imaging permits the collection of activity from a large number of regions of the cortex (Seelke and Blumberg, 2010). It has been used to study cortical dynamics (Grinvald and Hildesheim, 2004; Mohajerani et al., 2010), neural development (Borgdorff et al., 2007), as well as functional connectivity (Mohajerani et al., 2010; Mohajerani et al., 2013).

Compared to IOS, VSD imaging has higher temporal and spatial resolution. However, it is associated with side effects, including dye bleaching and enhancement of sensory responses (Grandy et al., 2012). It is also invasive in nature. VSD dye application requires a large craniotomy and durotomy, making it an inappropriate candidate for longitudinal recordings. In order to longitudinally study functional changes in response to monocular deprivation during

development, we sought high-resolution imaging alternatives and turned to imaging with genetically encoded calcium indicators (GECIs).

3.1.5.3 Calcium imaging

GECIs have been successfully used to record cortical response within somatosensory (Minderer et al., 2012) and visual cortex (Andermann et al., 2011) of mice through chronic windows. AAV-viral vector has been used to express calcium indicators, however, only in limited brain regions. Also variable expression levels with viral vectors makes it less appropriate for longitudinal studies (Zariwala et al., 2012). For my study, I used Emx-GCamp3 mice, derived from crossing Emx1-cre and Rosa26-GCaMP3 lines. GCaMP3 is stably expressed within excitatory neurons in all layers across the entire cortex, but not in GABAergic neurons or astrocytes (Gorski et al., 2002; Kummer et al., 2012; Vanni and Murphy, 2014). This provides more specificity over VSD, since VSD stains all membranes, including glia, providing a high background signal. Another difference is that calcium imaging reports predominantly spiking activity (Berger et al., 2007; Tian et al., 2009), while VSD signal incorporates both subthreshold potential changes as well as spiking activity (Stosiek et al., 2003).

In addition, Vanni and Murphy have also demonstrated stability of fluorescence and optical transmission, as well as consistency of spontaneous activity pattern over time (2014). Spontaneous activity in GCaMP3 mice yields connectivity patterns comparable to those of acute VSD imaging (Mohajerani et al., 2010; Mohajerani et al., 2013; Vanni and Murphy, 2014). Although there is contamination from autofluorescence and hemodynamic effects, these contribute to only 3% and 8% of the standard deviation of spontaneous GCaMP3 fluorescence. GCaMP3 imaging has proven accurate and sensitive enough to map long-range functional connections. Moreover, GCaMP3 imaging is superior to IOS imaging in mapping of long-range

connection, because long-range connection pattern demonstrated with IOS imaging has been found to be weaker and less consistent (Vanni and Murphy, 2014). Using an implanted chronic window (Silasi et al., 2013; Silasi et al., 2016), I was able to image the same animal repeatedly over a period of four weeks to follow the developmental changes and study the long-term effect of monocular deprivation.

3.2 Methods

3.2.1 Animal model

With the approval of the University of British Columbia Animal Care Committee, we used C57BL/6 mice for IOS experiments, and Emx-GCaMP3 mice for calcium imaging.

Emx-GCaMP3 mice were produced by crossing homozygous B6.129S2-Emx1tm1(cre)Krj/J strain (Jax no. 005628) with hemizygous B6;129S-Gt(ROSA)26Sortm38(CAG-GCaMP3)Hze/J strain (Jax no. 014538, Ai38) (Ackman et al., 2012; Zariwala et al., 2012). The presence of GCaMP3 expression was confirmed by genotyping each animal with PCR amplification of a 546 bp fragment (forward primer: 5′-CTT CAA GAT CCG CCA CAA CAT CG-3′; reverse primer, 5′-TTG AAG AAG ATG GTG CGC TCC TG-3′).

3.2.2 Rodent surgeries

3.2.2.1 Monocular deprivation by lid suture

This has been described in detail in section **2.2.1**.

3.2.2.2 Tracheotomy

Tracheotomy was performed in some *in vivo* IOS experiments, to reduce motion artefact from breathing and the heartbeat. After induction of anesthesia with 2% isoflurane, the mouse was placed in a supine position. The forelimbs were suspended to the side and fixed with adhesive tapes. The hair on the anterior neck was shaved with an electric razor. Lidocaine was injected

subcutaneously as supplementary local anesthesia. A 1-cm-long median, vertical incision was made, ten minutes after lidocaine injection. This was followed by blunt, anterior neck dissection. The lobes of the thyroid gland were separated at their isthmus. The sternohyoid muscles were retracted laterally. The connective tissue over the exposed trachea was then scrape away and a thin tube was inserted into the trachea via a small opening (Moldestad et al., 2009).

3.2.2.3 Chronic window implantation

Chronic windows were implanted in longitudinal imaging experiments using GCaMP3 mice. Glass covers measuring up to 9mm were cut from No. 1 circular cover glasses (Marienfeld, Lauda-Konigshofen, Germany; Cat #: 0111520), with the help of a high-speed electric drill with a diamond drill bit (ThorLabs, Newton, NJ, USA; Cat#: S90W).

GCaMP3 mice were anesthetised with isoflurane (2% in pure O_2). Body temperature was maintained at 37°C. Mice received an intramuscular injection of 40 μL of Dexamethasone (2mg/mL) and a 0.5mL subcutaneous injection of a saline solution containing buprenorphine (2μg/mL), atropine (3μg/mL) and glucose (20mM). They were then placed in a stereotaxic frame. After injecting lidocaine (0.1mL, 0.2%), the skin covering the skull was removed to expose both hemispheres. Connective tissue was scraped away with care. No craniotomy or thinning of the skull was performed. A thin layer of clear dental acrylic (Parkell, Edgewood, NY, USA; Product: C&B Metabond) (Hira et al., 2009) was subsequently applied over the clean, transparent skull. The pre-cut glass cover was then placed on top and pressed flat into the acrylic. A 4/40 stainless steel setscrew was placed into the drying dental adhesive, posterior to the chronic window. This would later be fixed to a pole mounted on a base-plate during imaging sessions, to help stabilize the head (Silasi et al., 2013; Silasi et al., 2016).

At the end of the procedure, the animal received a second injection of saline (0.5ml, IP) with 20mM of glucose, and recovered in a warmed cage for 30 minutes.

3.2.3 IOS imaging

3.2.3.1 IOS imaging apparatus and signal recording

For *in vivo* IOS experiments, only data from single-time point, i.e., four days after monocular deprivation, were collected. I imaged bilaterally through intact skull. I applied silicon oil was on top of the skull, prior to laying down a glass cover slide on top, to reduce diffraction and improve the signal-to-noise ratio. Tracheotomy (described above) was also performed in long experiments where global spontaneous activity and functional connectivity were examined. We induced anesthesia with isoflurane (2% mixed in oxygen) and recorded at a lighter level (0.5-1%). Body temperature was maintained at 37 ˚C during recordings, using a feedback-regulated heating pad monitored by a rectal thermometer.

The IOS imaging apparatus (**Fig 3.1A**) was located in a darkened room isolated from external light and sound. Images of cortical surface were recorded through a pair of back-to-back photographic lenses (50mm, 1.4 f:135 mm, 2.8 f or 50mm, 1.4 f:30 mm, 2 f) coupled to a 1M60 Pantera CCD camera (Dalsa) (8.4 X 8.4 mm field of view, 65 μm per pixel). Surface vasculature (**Fig 3.1B**) was recorded with a green light-emitting diode (LED) (Luxeon, 530nm). IOS signals were recorded with red LED (Luxeon, 627 nm) with a bandpass filter (620-650nm). I collected images at 10 Hz using a digital camera (1M60 Pantera, Dalsa) and EPIX E4DB frame grabber along with XCAP 3 software (EPIX).

The image of cortical surface and vasculature was used to create a “mask”. This was then uploaded into MATLAB for subsequent data processing and presentation. The same strategy was applied to analysis and presentation of GCaMP data.

3.2.3.2 Visual and somatosensory stimulation

Visual stimuli used to stimulate V1, MZ and BZ included contrast-modulated noise (CMN) and green LEDs (Luxeon, 530nm).

Since neurons in mouse V1 are highly selective for orientation and spatial and temporal frequency (Wagor et al., 1980; Metin et al., 1988; Niell and Stryker, 2008), I used contrast-modulated noise to maximize the stimulation and improve signal-to-noise ratio. The design of contrast-modulated noise takes into consideration of expected range of spatial and temporal response properties of mouse V1 cells. It is effective in stimulating >99% of cells in the visual cortex (Gandhi et al., 2008). In the present experiments, it was presented on a LCD screen (53 cm × 30 cm), positioned 25cm away from the eyes with its midline (defined as 0°) aligned to the midline of the animal. The screen subtended an angle of 94°. For each mapping session, CMN was presented for 5 min (30 cycles, 10s period). The full screen was used to stimulate a large portion of the primary visual cortex, including BZ and part of MZ. CMN was restricted it to the central visual field (-15° to 15°), when it was intended to stimulate BZ only (**Fig 3.1.C**). A light blocker was mounted onto the head plate to prevent contamination of recorded IOS signals. Negative controls (recordings without a mouse) were obtained and confirmed the effectiveness of light blockade.

A flashing green LED light was also used as a stimulus. LEDs were placed in the central visual field to stimulate the binocular zone of the primary visual cortex. To stimulate the monocular zone, they were placed at 45° in the visual field. Black, lightproof tapes were rolled over LEDs to restrict emitted light to prevent scattering and resultant contamination of the imaging signal.

To stimulate the somatosensory cortex, we used piezoelectric bending actuators to stimulate whiskers and the skin of hindlimb or forelimb. To isolate stimulus-related signals, alternate heartbeat-triggered recordings were acquired with and without stimulation. Each run consisted of twenty trials separated by a 10s interval.

3.2.3.3 Data analysis of evoked and spontaneous activity

When periodic contrast-modulated noise was used as a visual stimulus, we performed Fourier transformation to extract visual-evoked response at the modulation frequency of stimuli (Kalatsky and Stryker, 2003). The major components of recorded IOS data include heart rate (2–5 Hz), respiration rate (0.3–1 Hz), vasomotor signal (0.05– 0.1 Hz) (Mayhew et al., 1996), and slow variation of the baseline including anesthesia. I extracted the signal at the frequency of contrast modulation (0.1Hz) as the visual-evoked response (Kalatsky and Stryker, 2003) (**Fig 3.2**).

Examples of IOS maps of V1 in a normal and a monocularly deprived mouse are shown in Fig 3.3. When calculating the size of visually evoked maps, I set a threshold of 30% of response amplitude and quantified the number of pixels above that threshold.

As well as measuring stimulus-evoked activity, I collected spontaneous cortical activity at 10Hz for 15 minutes. I then did temporal filtering of the image stacks with a Chebyshev zero phase-shift band-pass filter (0.01-0.08 Hz). The image stacks were filtered spatially using a Gaussian filter of 25 pixels (65 μm per pixel). Change in reflectance intensity was calculated as $(R - R_0)/ R_0$ where R_0 is the average reflectance. I combined these epochs into sequences representing these 15 minutes of activity for each mouse and conducted data analysis on these aggregated image sets.

I also used seed pixel correlation analysis to generate functional connectivity maps with spontaneous data. Centres of calculated sensory- or visually-evoked maps were used as reference pixels for generation of functional connectivity maps (Illustrated in **Fig 3.5**). The activity of each pixel was extracted along time, and compared to that of seed pixel with the Pearson's correlation method. Then the calculated correlation coefficients were placed at the location of comparison pixel. The generated correlation maps represent functional connectivity of the selected seed pixel with the rest of the cortex.

Several examples of monocularly deprived mice and normal mice were shown in the result section to demonstrate sensory- or visually-evoked maps and correlation maps generated from spontaneous activity.

3.2.4 *In vivo* calcium imaging in Emx-GCaMP3 mice

Emx-GCaMP3 mice of either sex were used for this study. All experiments are longitudinal studies of cortical spontaneous activity up to 28 days in GCaMP3 mice with implanted chronic windows (Silasi et al., 2013; Silasi et al., 2016).

Mice were lightly anesthetized with isoflurane (0.5-1%) during imaging, to ensure a constant level of neural activity. The body temperature was maintained and hydration was ensured by subcutaneous injection of saline (0.5ml) with 20mM of glucose when necessary (experiments running longer than two hours).

3.2.4.1 Stimulation and recordings

The same macroscope setup composed with the same lenses as in IOS imaging were used in GCaMP imaging. A green LED (Luxeon, 530nm) was again used to illuminate the surface of brain. Calcium indicators were excited with blue LEDs (Luxeon, 470nm) with bandpass filters

(467–499nm). Emission fluorescence was filtered using a 510–550nm bandpass filter. I collected 12-bit images at 100ms (10Hz) using XCAP software.

3.2.4.2 Visual and somatosensory stimulation

The stimuli used to evoke cortical activity were the same as used for the IOS experiments descried above (**3.2.3.2**).

3.2.4.3 Data analysis

Spontaneous cortical activity was collected at 1Hz for 10 minutes. The image stacks were filtered temporally using a Chebyshev zero phase-shift band-pass filter (0.1-1 Hz). Changes in fluorescence were calculated as $(F - F_0)/ F_0$ where F_0 is the average fluorescence.

To measure the correlations between cortical regions, multiple sensory cortical areas were examined bi-hemispherically, including forelimb somatosensory cortex (FLS1), hindlimb somatosensory cortex (HLS1), barrel cortex (BC), MZ and BZ. Whenever possible, centres of evoked sensory or visual maps were chosen as seed pixels (centres of ROIs) to calculate functional connectivity maps. Some cortical regions, such as M1 and RS, were identified based on stereotaxic coordinates and their stereotyped position relative to the hindlimb sensory cortex located by stimulation, with the help of mouse brain atlas (Chan et al., 2015; Lim et al., 2015) (**Fig 3.1B**). GCaMP signals for reach region were extracted from seed pixels of each ROI. The correlation values from these ROIs were used to create correlation matrices.

3.3 Results

3.3.1 Monocular deprivation reduces the response in the contralateral V1

To study functional changes induced by monocular deprivation, I first examined visually evoked responses in monocularly deprived young mice with IOS imaging.

Monocular deprivation was induced with lid suture over the left eye during the critical period. After four days (P23-27) of monocular deprivation, I examined visually evoked responses in the visual cortex with IOS imaging through the intact skull. Contrast-modulated noise was used as visual stimulus for optimal response (Gandhi et al., 2008). Full screen stimulation was used to stimulate V1, including BZ and part of MZ. CMN was then restricted to the central visual field (30°) to stimulate BZ (**Fig 3.1C**). I examined the effect of monocular deprivation on the visual cortex by comparing the response amplitude, as well as the area of responsiveness. IOS imaging showed that four days of MD during the critical period reduced both amplitude and the area of responsiveness in contralateral V1. The results will now be presented in more detail.

3.3.1.1 Monocular deprivation reduces the response amplitude in contralateral V1

The response amplitude in the hemisphere contralateral to the deprived eye was compared to that of the ipsilateral hemisphere, in both normal and deprived mice. Considering the small response amplitude and individual variability, I quantified the ratio of response amplitude rather than the absolute values. The contralateral/ipsilateral ratio for each mouse was calculated individually and presented in Fig 3.4.

In normal mice, the responses of ipsilateral and contralateral V1 were largely comparable with an average contralateral/ipsilateral (contra/ipsi) ratio of 0.88 (n=4). After a four-day monocular deprivation starting P23, the average ratio of contra/ipsi response amplitude decreased to 0.55 (n=4, Wilcoxon-Mann-Whitney rank sum test, p=0.02) (**Fig 3.4A**).

The response of the binocular zone was also compared. After a four-day monocular deprivation, the average ratio of contralateral/ipsilateral response of BZ decreased from 1.18 to 0.6 (n=4, p=0.05) (**Fig 3.C**).

3.3.1.2 Monocular deprivation reduces the size of contralateral V1 map

I also looked at the size of evoked visual maps as an indicator of cortical responsiveness. By comparing the size of generated maps of the visual cortex, I found a reduction in the contralateral hemisphere, in both primary visual cortex and the binocular zone.

In normal mice, the size of bilateral V1 (BZ and MZ) is similar with an average ratio of 1.10 (n=4). After four days of monocular deprivation, the responsive area in contralateral V1 became smaller in size compared to the non-deprived hemisphere, with an average contra/ipsi size ratio of 0.74 (n=4, p=0.02, **Fig 3.4B**). The size of responsive contralateral BZ also decreased compared to that of ipsilateral BZ. The average ratio of contralateral/ipsilateral BZ area decreased from 1.10 to 0.64 (n=4, p=0.10, **Fig 3.4D**).

Decreased response amplitude and responsive area in the contralateral hemisphere suggests that the contralateral V1 became less responsive to visual stimuli, after brief monocular deprivation during the critical period.

3.3.2 IOS imaging of spontaneous activity in V1 suggests that monocular deprivation changes functional connectivity within V1

I then started exploring changes in functional connectivity after monocular deprivation. This was done with the help of spontaneous activity, rather than evoked-activity.

I first examined functional connectivity within the visual cortex, in both normal and monocularly deprived mice. Centres of calculated visual-evoked maps were used as seed pixels to create functional connectivity maps. Four regions of interest (ROIs) were studied; the ipsilateral monocular/binocular zone as well as the contralateral monocular/binocular zone. I generated seed-pixel-based correlation maps and examined the effect of brief monocular deprivation on functional connectivity within the visual cortex. Examples of a normal and a

monocularly deprived mouse are shown in Figs 3.6 and 3.7 respectively. Note these calculated functional connectivity maps of MZ and BZ closely resembled patterns of activity evoked by visual stimuli (**Fig 3.8C&D**).

In a normal mouse, selection of mirrored seed pixels generated mirrored correlation maps (**Fig 3.6**). Symmetrical functional connectivity maps were appreciated of both of MZ and BZ seeds. The correlation values were highest around the seed pixels and in functionally related areas. High correlation with MZ was revealed in both BZ (**Fig 3.6A**). BZ showed high correlation with its homotopic region in the other hemisphere (**Fig 3.6B**).

Brief monocular deprivation during the critical period disrupted the symmetry in functional connectivity maps of V1 (**Fig 3.7**). After four days of MD, the contralateral monocular zone demonstrated less correlation with the rest of the visual cortex (**Fig 3.7A**). It became less correlated with not only the contralateral binocular zone, but also V1 in the other hemisphere including both monocular and binocular zones. In a way, the contralateral monocular zone appeared to be 'disconnected' from the rest of the visual cortex. This trend was observed in 2 other monocularly deprived young mice (data not shown). The contralateral binocular zone remained highly correlated with the ipsilateral BZ (**Fig 3.7B**).

3.3.3 Monocular deprivation changes functional connectivity across the cortex

Early studies on cross-modal plasticity have demonstrated global effect of sensory deprivation (Bavelier and Neville, 2002). In order to study global functional connectivity, I took advantage of the large cranial window used for the experiments, which allowed examination of functional connections between the visual cortex and somatomotor cortex. However, examination of the functional connectivity involving auditory cortices was limited, due to the lateral position of the auditory cortices on rodent brains (**Fig 3.1B**).

In order to accurately locate different ROIs of the somatosensory cortex, peripheral stimuli were delivered to map different sensory cortices. With the help of piezoelectric bending actuators, the whiskers and the skin of the forelimbs and hindlimbs were stimulated, to map BC, FLS1 and HLS1 respectively. Fig 3.8 shows montages of IOS signal at 0.5-second intervals in a mouse in response to forelimb, hindlimb, monocular zone and binocular zone stimulation.

Here I show an example of monocular deprivation induced functional changes across the brain (**Fig 3.9**). Functional connectivity maps of each ROI revealed high correlation regionally and with its homotopic connection in the other hemisphere. It is also interesting to note that correlation values were lower in MZ and BZ maps, compared to those of BC, FLS1 and HLS1.

As discussed above, after monocular deprivation during the critical period, compared to the ipsilateral monocular zone, the contralateral MZ became less correlated with the rest of the visual cortex (**Fig 3.9A**). However, looking across the cortex, there was increased correlation of the contralateral monocular zone with the somatosensory cortex, including bilateral BC and FLS1, compared to the ipsilateral MZ (**Fig 3.9A,C&D**). Reciprocal high correlations of bilateral BC and FLS1 with contralateral MZ were also observed (**Fig 3.9C&D**). This finding is consistent with the literature on cross-modal plasticity in which deprived visual cortex became recruited by the somatosensory cortex (Hyvarinen et al., 1981; Toldi et al., 1994a).

One disadvantage of IOS imaging is the rather long recording times required, given the small response amplitude. Combined recording sessions of both spontaneous activity and evoked activity in V1 and somatosensory cortex can take longer than an hour. This made it necessary to perform tracheostomies to reduce motion artefact in long recordings, and in turn made longitudinal studies impractical. It is also an indirect measure of neural activity, mediated by metabolism. At this stage, we started experimenting with different *in vivo* imaging modalities,

including VSD imaging and GCaMP3 imaging, both of which are superior to IOS in terms of spatial and temporal resolutions. I experimented with both imaging modalities and decided to proceed with GCaMP3 imaging since it is non-invasive and allows for longitudinal study of the functional changes through development (Vanni and Murphy, 2014).

3.3.4 Monocular deprivation during the critical period induces global changes in functional connectivity

Using a chronic window (Silasi et al., 2013; Silasi et al., 2016), I imaged the same mice repeatedly through development, from peri-critical period into adulthood. Longitudinal study like this made it possible to track developmental changes, in response to monocular deprivation.

I focused on 12 ROIs in our study of spontaneous activity and functional connectivity. These are bilateral MZ, BZ, BC, FLS1, HLS1 and motor cortex (M1). I chose these ROIs to study the functional connectivity between the visual cortex and somatosensory cortex. Functional correlation maps were calculated for each ROI. Correlation matrices were calculated from these function connectivity maps, for each time point.

Similar to our IOS study, centres of evoked maps and the mouse brain atlas were used to aid the selection of reference pixels. Some examples of functional connectivity maps in a normal mouse were shown in Fig 3.10.

3.3.4.1 Effect of monocular deprivation during the critical period in GCaMP3 mice

First, I explored changes in functional connectivity in monocularly deprived juvenile mice. In order to capture the developmental changes induced by monocular deprivation, chronic windows were implanted at postnatal day 23 (P23), shortly after eye opening. Mice were given two days to recover prior to baseline recording at P25 (D0). MZ, BZ, FLS1, HLS1 and BC were mapped on D0 with the help of peripheral stimuli. This was followed by a brief, two-day monocular

deprivation of the left eye (P25-27), at the peak of the critical period. Seven monocularly deprived mice were then imaged on D2, D4, D7, D14, D21 and D28 after the beginning of lid suture.

Preliminary data suggested that as brief as two days of monocular deprivation were sufficient in altering functional connectivity within V1 (**Fig 3.11**). At baseline, symmetrical functional connectivity calculated from spontaneous activity was observed in juvenile mice, for both monocular and binocular zones (**Fig 3.11A**). After only two days of monocular deprivation, the contralateral MZ demonstrated decreased correlation with the rest of the visual cortex (**Fig 3.11B**). This decrease in the contralateral MZ correlation appeared to persist at D7 (**Fig 3.11C**) and D21 (**Fig 3.11D**) in this particular example.

To quantify the strength of correlation globally and longitudinally, I calculated correlation matrices for the above-mentioned 12 ROIs (6 contralateral and 6 ipsilateral to the MD eye) for all 7 time points in a group of 7 monocularly deprived GCaMP3 mice (**Fig 3.12 intra-hemispheric & Fig 3.13 inter-hemispheric**). Values within the correlation matrices represent mean r-values. R-values of ipsilateral MZ/BZ and contralateral MZ/BZ with BC and the rest of ROIs in V1 were plotted in **Fig 3.14** to highlight the following observations.

Despite monocular deprivation, stable and high correlation values between MZ and BZ were observed throughout development, with intra-hemispheric values higher than inter-hemispheric values. The mean intra-hemispheric MZ-BZ r-value in contralateral V1 was 0.95 on D0 and 0.91 on D28, and that for ipsilateral V1 was 0.98 on D0 and 0.91 on D28 (**Fig 3.12**). The mean contraMZ-ipsiBZ r-value was 0.83 on D0 and 0.67 on D28. The mean ipsiMZ-contraBZ r-value was on 0.83 D0 and 0.69 on D28 (**Fig 3.13**).

Interestingly, we observed a transient change in the functional connectivity within V1 correlating with the duration of monocular deprivation (**Fig 3.14**). For the seven monocularly deprived GCaMP3 mice, the mean intra-hemispheric MZ-BZ r-value for contralateral V1 decreased from 0.95 on D0 to 0.93 on D2, and increased to 0.95 on D4. The mean intra-hemispheric MZ-BZ r-value in ipsilateral V1 decreased to 0.96 on D2 from 0.98 on D0 (**Fig 3.12**). The mean contraMZ-ipsiBZ r-value was 0.83 on D0, 0.79 on D2, and 0.81 on D4. The mean ipsiMZ-contraBZ r-value was 0.83 on D0, 0.76 on D2, and 0.84 on D4. The transient, MD-induced changes in functional connectivity within V1 could be better appreciated in **Fig 3.14**.

In addition, we also observed a gradual decrease of correlation between V1 (including both MZ and BZ) and the somatosensory cortex (including BC, highlighted in **Fig 3.14**). For example, the mean intra-hemisphere MZ-BC r-value in the contralateral hemisphere decreased from 0.79 on D0 to 0.57 on D28. The mean intra-hemispheric MZ-BC r-value in the ipsilateral hemisphere decreased from 0.74 to 0.46 (**Fig 3.12**). The mean contraMZ-ipsiBC r-value decreased from 0.7 on D0 to 0.34 on D28, and the mean ipsiMZ-contraBC r-value decreased from 0.73 to 0.49 from D0 to D28 (**Fig 3.13**). Overall, inter-hemispheric correlation of MZ demonstrated the largest amount of decrease with the somatomotor cortex (highlighted in **Fig 3.14B**, contraMZ-ipsiBC).

3.3.4.2 Prolonged monocular deprivation does not change functional connectivity in adulthood

Chronic windows were implanted on 4 GCaMP3 mice at least three months of age. Baseline imaging was acquired at least four days after implantation of chronic window, after which lid suture was performed over the left eye. As indicated in the literature, plasticity takes longer to

take effect in adulthood, and therefore we tested the effect of seven days of monocular deprivation on spontaneous activity. These mice were imaged on D0, D2 and D7.

An example of an adult mouse that underwent seven days of monocular deprivation is demonstrated in Fig 3.15. At baseline recording, mirrored correlation maps were generated upon selection of mirrored seed pixels within V1 (**Fig 3.15A**). Seven days of monocular deprivation did not disrupt the symmetry of functional connectivity of the visual cortex (**Fig 3.15B**). Also note the correlation between V1 and anterior cingulate (Chan et al., 2015; Lim et al., 2015).

I further analyzed global connectivity within different ROIs (**Fig 3.16**). As in juvenile mice, I observed strong and stable correlation between MZ and BZ. Monocular deprivation did not induce changes in functional connectivity within V1 or globally. Also, we observed a similar degree of correlation between V1 and somatosensory cortex, to that at the end of young mice recording. For example, the mean intra-hemispheric MZ-BC r-value in the contralateral hemisphere was 0.57 (n=7, **Fig 3.12G**) on D28 (P53) for the juvenile group, and 0.54 (n=4, **Fig 3.16A**) on D0 in adult group. This may suggest maturation of long-range connection through development.

3.4 Discussion

In this chapter, I presented results from *in vivo* functional imaging of both evoked and spontaneous activity in the visual cortex, as well as across the entire cerebral cortex. Recording of visually evoked response showed that monocular deprivation during the critical period leads to decreased responsiveness of contralateral V1, compared to ipsilateral V1 (**Fig 3.4**). Examination of spontaneous activity revealed transient changes in functional connectivity within V1 during the critical period (**Fig 3.14**), correlating with the duration of monocular deprivation. In addition, there was a gradual decline through development in the functional connectivity

between V1 and the somatosensory cortex as revealed by GCaMP imaging (**Fig 3.14**). Monocular deprivation in adulthood did not have any significant effect on functional connectivity within V1 (**Fig 3.16**).

3.4.1 Monocular deprivation decreases responsiveness of the contralateral V1

It is well known that monocular deprivation during the critical period leads to impaired responsiveness of the contralateral binocular zone. Here with the help of IOS imaging, which allows for examination of a larger portion of V1, I tested the effect of monocular deprivation on binocular vision.

With IOS imaging, I showed that monocular deprivation decreases the response amplitude, as well as the area of responsiveness, in the contralateral primary visual cortex. Consistent with existing literature (Gordon and Stryker, 1996), I provided additional evidence that monocular deprivation during the critical period leads to decreased responsiveness in the visual cortex contralateral to the deprived eye; and this effect was not only restricted to the binocular zone.

3.4.2 Symmetrical functional connectivity in a normal mouse

Next, I examined spontaneous activity and functional activity in normal and monocularly deprived mice. Unlike previous *in vivo* studies of spontaneous activity which have focused on a single sensory domain, either somatosensory (Marcano-Reik and Blumberg, 2008; Minlebaev et al., 2011) or visual cortex (Hanganu et al., 2006; Colonnese and Khazipov, 2010), I took advantage of large chronic window to image across the entire visible area of cerebral cortex. To the best of my knowledge, this is the first attempt to longitudinally study spontaneous activity and global functional activity in an experience-dependent plasticity mouse model.

One of the immediately notable features is the similarity between functional connectivity maps derived from spontaneous activity and visual-stimulus evoked maps. This was observed in both IOS and GCaMP data, and is consistent with previously reported spatial correlation between functional connectivity maps and sensory evoked maps revealed by VSD imaging (Mohajerani et al., 2013). In addition, our group has also demonstrated spatial colocalization between seed-pixel correlation maps obtained from VSD imaging of spontaneous activity, and axonal projections in the AMBC Atlas, including in V1 (Mohajerani et al., 2013).

Secondly, I observed symmetrical functional connectivity within V1 of normal mice. Both MZ and BZ seeds generated correlation maps characterized by bilateral symmetry (**Figs 3.6, 3.10 & 3.15**). Functional connectivity maps generated by both MZ and BZ seeds revealed high correlations with homotopic regions in the other hemisphere. This symmetry has also been observed within M1 and the somatomotor cortex, including BC, FLS1, and HLS1, as evidenced by GCaMP imaging (**Fig 3.15**).

Overall, correlation values in functional correlation maps are highest around the seed pixel and in functionally related areas. We demonstrated consistently high correlations within the visual cortex between MZ and BZ. This observation holds true during development, even in mice that underwent monocular deprivation during the critical period (**Fig 3.14**).

3.4.3 Transient disruption of functional connectivity within V1 in monocularly deprived mice during the critical period

GCaMP imaging suggested that monocular deprivation did not induce any significant changes in functional connectivity in adult mice. However, brief monocular deprivation during the critical period disrupted the symmetry in functional connectivity within V1. Decreased correlation with contralateral MZ was evident in both IOS and GCaMP data. This change was transient in

GCaMP3 mice, correlating with the duration of monocular deprivation. The transient, MD-induced changes in functional connectivity during the critical period may be a form of short-term memory or Hebbian plasticity.

Our data in a way echo that of Han and colleagues (2008). Repetitive presentation of a visual stimulus leads to a transient increase in the percentage of spontaneous waves that resemble the cortical response evoked by the training stimulus. They have suggested that this reverberation of recent visual experience in spontaneous activity may be a form of short-term memory (Han et al., 2008).

Similar observations have also been made outside the visual system. Studies in the somatomotor cortex also suggest that slow wave activity is experience-dependent and may play a role in consolidating learning and memory (Huber et al., 2004; Vyazovskiy and Tobler, 2008). Unilateral somatosensory stimulation during wakefulness increases slow wave activity in the corresponding somatosensory cortex (Kattler et al., 1994). Motor learning task increases local slow wave activity (Huber et al., 2004; Hanlon et al., 2009) and leads improved performance of the task after sleep (Huber et al., 2004). On the other hand, arm immobilization in humans leads to a reduction of slow wave activity in the somatomotor cortex, and deterioration in motor performance (Huber et al., 2006). These data suggest that spontaneous activity may play a role in regulating plasticity.

On the synaptic level, spontaneous activity is necessary for spatial clustering of co-active synapses (Kleindienst et al., 2011; Takahashi et al., 2012). Synaptic refinement through spontaneous activities prepares the network for sensory processing and is critical for the development of sensory and motor systems (Walsh and Lichtman, 2003; Lee et al., 2014). Spontaneous activity drives local synaptic activity at individual synapse in an “out-of-sync, lose-

your-link" fashion. Synapses that exhibit low synchronization with nearby neighbours become depressed in their transmission frequency. Spontaneous activity may act as a new plasticity mechanism during development (Winnubst et al., 2015).

3.4.4 Exploration of global changes in functional connectivity in monocularly deprived mice

In addition, global changes in functional connectivity were also observed in mice that underwent monocular deprivation during the critical period (**Figs 3.9 & 3.14**). IOS data seemed to suggest that the deprived contralateral MZ became less correlated with the rest of the visual cortex and was perhaps recruited by somatosensory cortex, including BC and FLS1 (**Fig 3.9**). This finding resembles some early experiments on cross-modal plasticity (Bavelier and Neville, 2002; Lee and Whitt, 2015). However, it was not replicated in GCaMP imaging. Instead, we observed a gradual decrease in the correlation between V1 and somatosensory cortex during development in GCaMP mice. It is interesting to note that contralateral MZ did show the largest amount of decrease in its inter-hemispheric correlation. Seven days of monocular deprivation did not induce any significant changes in functional connectivity in adulthood.

There may be a true functional connectivity change that is not clearly detected with GCaMP imaging. One limitation of the seed pixel correlation analysis is that the result is highly dependent on selection of seed pixel. We relied on sensory-evoked maps and the mouse brain atlas (Chan et al., 2015; Lim et al., 2015) to aid the selection of seed pixel. The combined use of functional and anatomical landmark should help reduce error associated with seed selection, since we have previously demonstrated the stability of spontaneous activity and functional connectivity within Emx-GCaMP3 mice (Vanni and Murphy, 2014).

The decrease in functional connectivity between V1 and S1 may reflect cross-modal plasticity and echo similar findings in human fMRI studies. Decreased functional connectivity between visual cortex and somatosensory cortex has been observed in early blind humans (Yu et al., 2008). This is supported by another study in early blind humans demonstrating decreased functional connectivity between visual and somatosensory or auditory cortices. Instead, V1 becomes more heavily incorporated into functional systems instantiating episodic recall and attention to non-visual events (Burton et al., 2014). Similarly, a DTI study has demonstrated reduced or absent axonal connection between V1 and other sensory cortices in early blind humans (Shu et al., 2009).

On the other hand, decreased correlation between the primary visual cortex and somatosensory cortex may simply represent increased selectivity and maturation of the neural network. The extent of correlation between V1 and somatomotor cortex was similar between adult mice and young mice at the last time point. My results may provide additional evidence to support the previously proposed three stages of developmental processes for slow-wave spontaneous activity. 1) Slow-wave spontaneous activity emerges in an activity-dependent manner early in life. 2) In late critical period, it is shaped locally by sensory experience through cortical NMDAR activation. 3) This is consolidated in adulthood (Miyamoto et al., 2003).

3.4.5 Conclusions

Here I presented data on spontaneous activity and functional connectivity to complement results in Chapter 2 on visual-evoked responses. Monocular deprivation induces decreased responsiveness in the contralateral V1, which is not restricted to the binocular zone. Monocular deprivation also leads to transient changes in functional connectivity within V1 that correlate with the duration of lid

suture during the critical period. In addition we also observed maturation of the network evidenced by declining of correlation between visual and somatosensory cortices.

The transient changes in functional connectivity induced by monocular deprivation may represent a form of Hebbian plasticity. Synaptic strengthening could be achieved though slow wave activity grouping the replay of previous neural activity within systems of the brain (Sutherland and McNaughton, 2000; Schwindel and McNaughton, 2011); while synaptic weakening occurs at neighbouring synapse exhibiting low synchronization of spontaneous activity (Winnubst et al., 2015).

Spontaneous activity is a powerful tool that allows for longitudinal examination of functional connectivity of a larger cortical area, independent of peripheral stimulation and responses. *In vivo* functional imaging of spontaneous activity in genetically modified mouse models should prove extremely helpful in studying of disease models in which spontaneous activity and the default network are affected, such as autism and Alzheimer's disease (Honey et al., 2010).

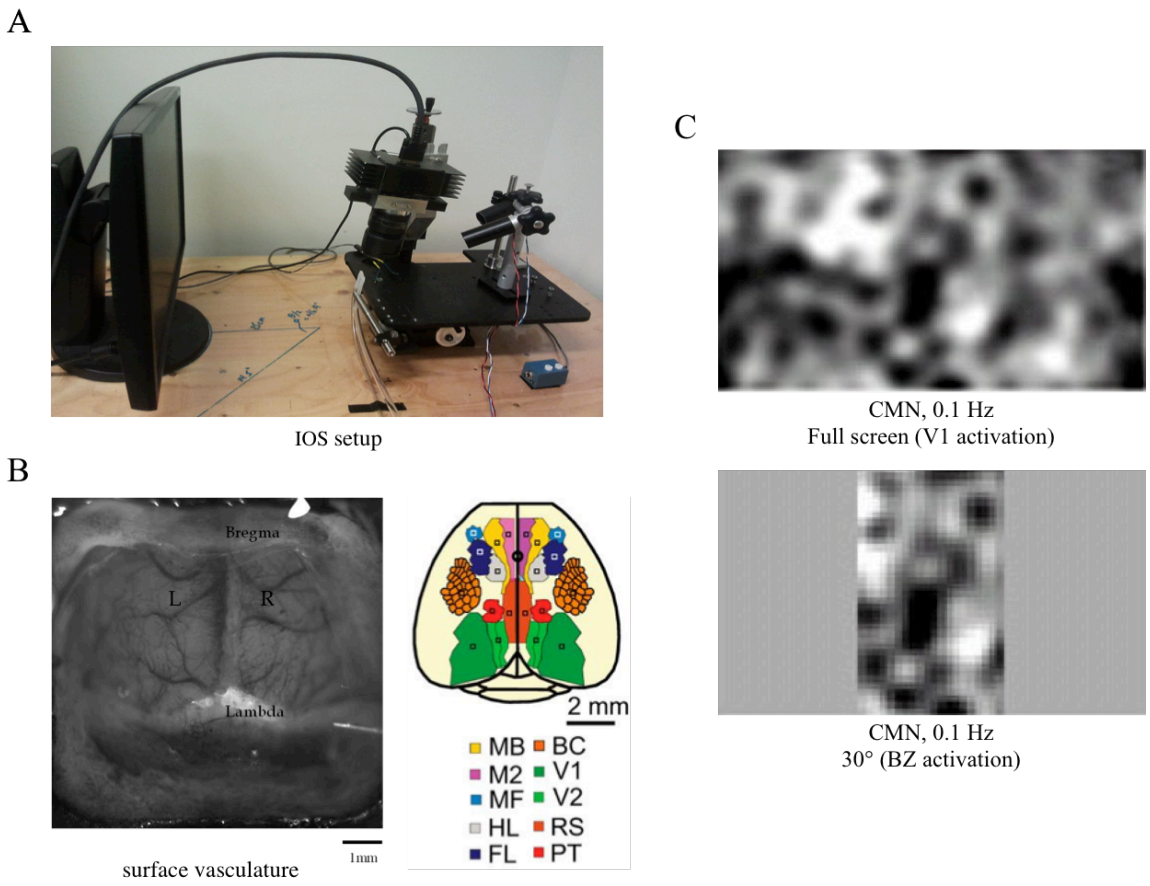


Fig 3.1 IOS imaging setup and visual stimuli. (A) During IOS imaging, the mouse was placed on a base plate mounted under a wide-field macroscope. A red LED (Luxeon, 627 nm) with a bandpass filter (620–650 nm) was used to illuminate the mouse brain. A wide LCD screen was placed 25cm in front of the mouse to present visual stimuli. (B) To visualize the cortex and vasculature, the surface of the mouse brain was illuminated with green light (Luxeon, 530nm). Mouse brain atlas (Chan et al., 2015; Lim et al., 2015) was used to aid localization of some ROIs. MB = motor barrel cortex; M2 = secondary motor cortex; MF = motor forelimb area; FL = forelimb somatosensory cortex; HL = hindlimb somatosensory cortex; BC = barrel cortex; V1 = primary visual cortex; V2 = secondary visual cortex; RS = retrosplenial cortex; and PT = parietal cortex. (C) Contrast modulated noise (CMN, 0.1Hz) was used as a visual stimulus. Full screen

stimulation (94°) was used to stimulate V1, including BZ and part of MZ. Visual stimuli restricted to the central visual field (30°) were used to stimulate the binocular zone.

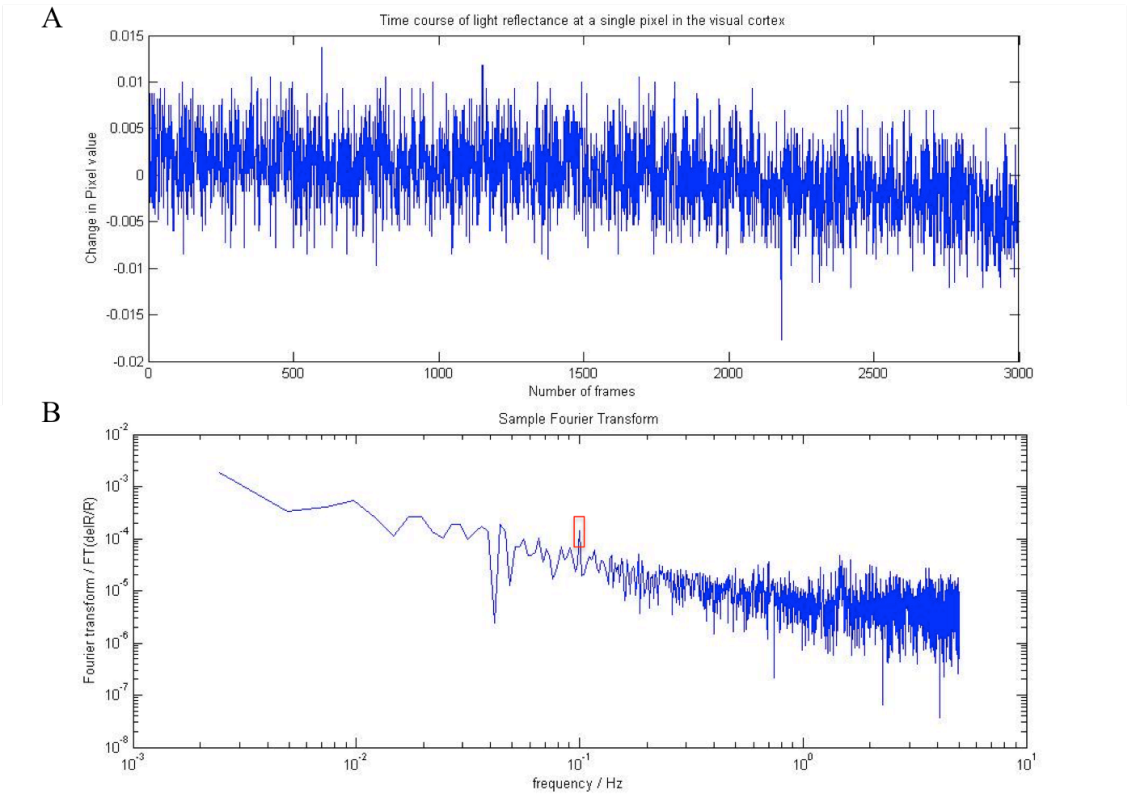


Fig 3.2 Extraction of evoked visual response. (A) An example trace of IOS signals recorded at 10Hz over 5 minutes when the mouse was presented with contrast-modulated noise with a modulation frequency of 0.1 Hz. (B) Fourier analysis was performed to isolate the signal at 0.1Hz (highlighted in red rectangle). This signal was then used to calculate visually evoked IOS maps. 0.1 Hz was chosen as it is away from contamination of non-neuronal signals, such as heart rate (2–5 Hz) and respiration rate (0.3–1 Hz) (Mayhew et al., 1996; Kalatsky and Stryker, 2003).

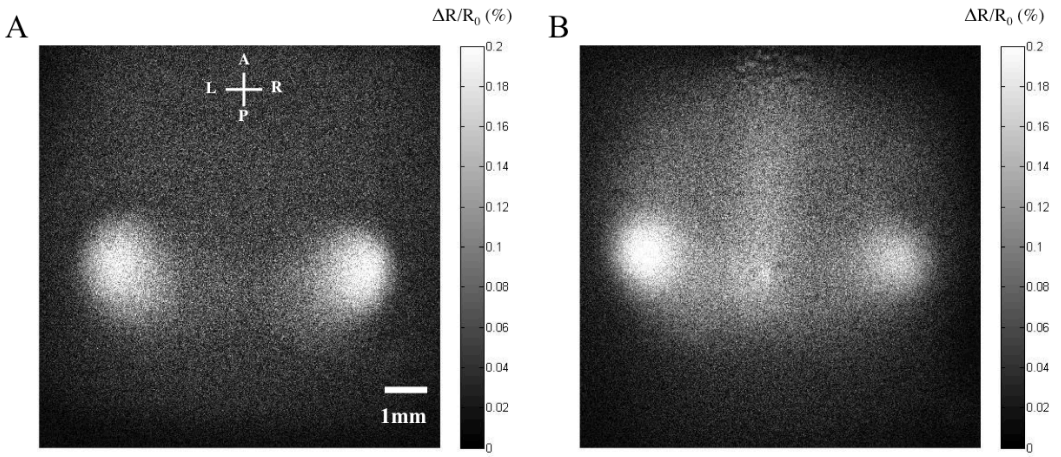


Fig 3.3 IOS maps of V1 in a normal mouse and a monocularly deprived mouse. (A) Example IOS maps of bilateral V1 in a normal mouse. (B) IOS maps of bilateral V1 in a monocularly deprived mouse. The response amplitude and area of responsiveness of normal and MD mice were quantified in Fig 3.4.

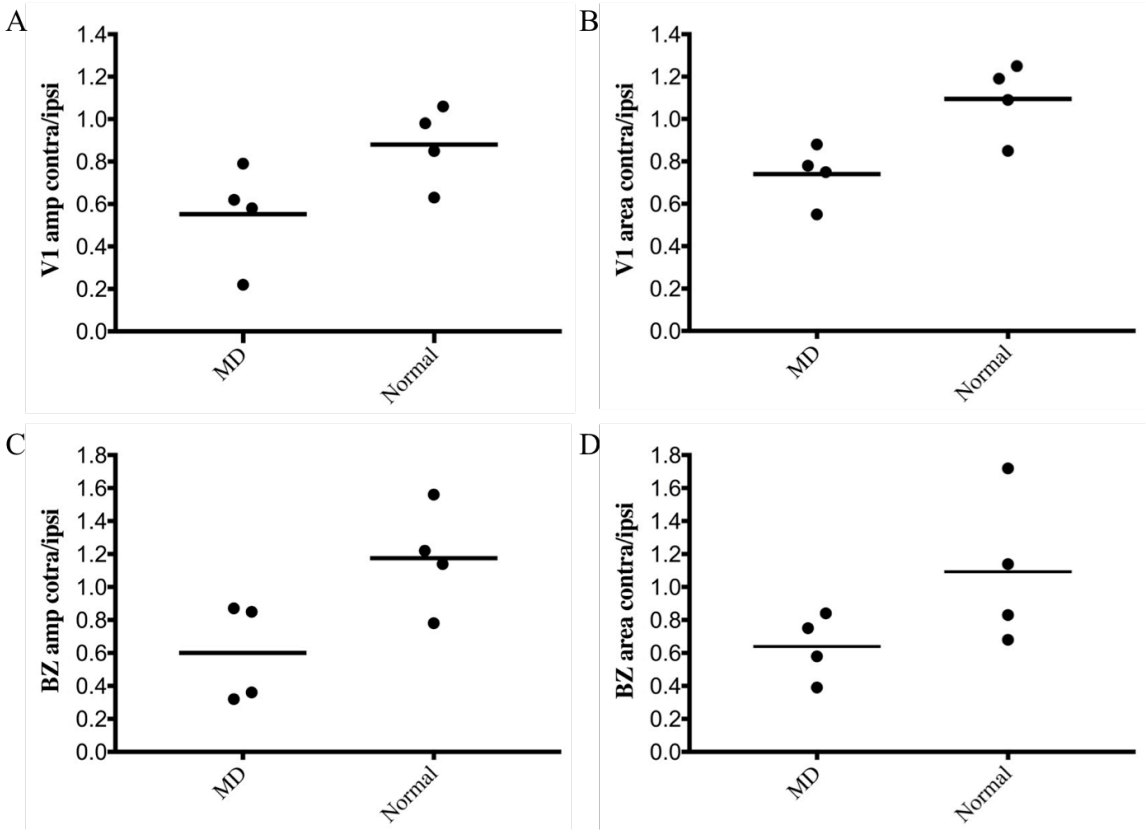


Fig 3.4 Monocular deprivation decreases responsiveness in the contralateral visual cortex.

(A) In normal mice, the average ratio of response amplitude in contralateral/ipsilateral (contra/ipsi) V1 was 0.88 (n=4). This ratio decreased to 0.55 (n=4, Wilcoxon-Mann-Whitney rank sum test, p=0.02) after four days of MD during the critical period. (B) The responsive area within V1 was also largely symmetrical in normal mice. Four days of monocular deprivation lead to a reduction in the contra/ipsi area ratio from 1.10 (n=4) to 0.74 (n=4, p=0.02). (C) Monocular deprivation caused a reduction in the response amplitude of the contralateral BZ. After four days of MD, the average ratio of contra/ipsi BZ response decreased from 1.18 (n=4) to 0.6 (n=4, p=0.05). (D) Brief monocular deprivation reduced area of responsiveness in the contralateral BZ. The ratio of responsive contra/ipsi BZ area was 1.10 (n=4) in normal mice and 0.64 in monocularly deprived mice (n=4, p=0.10).

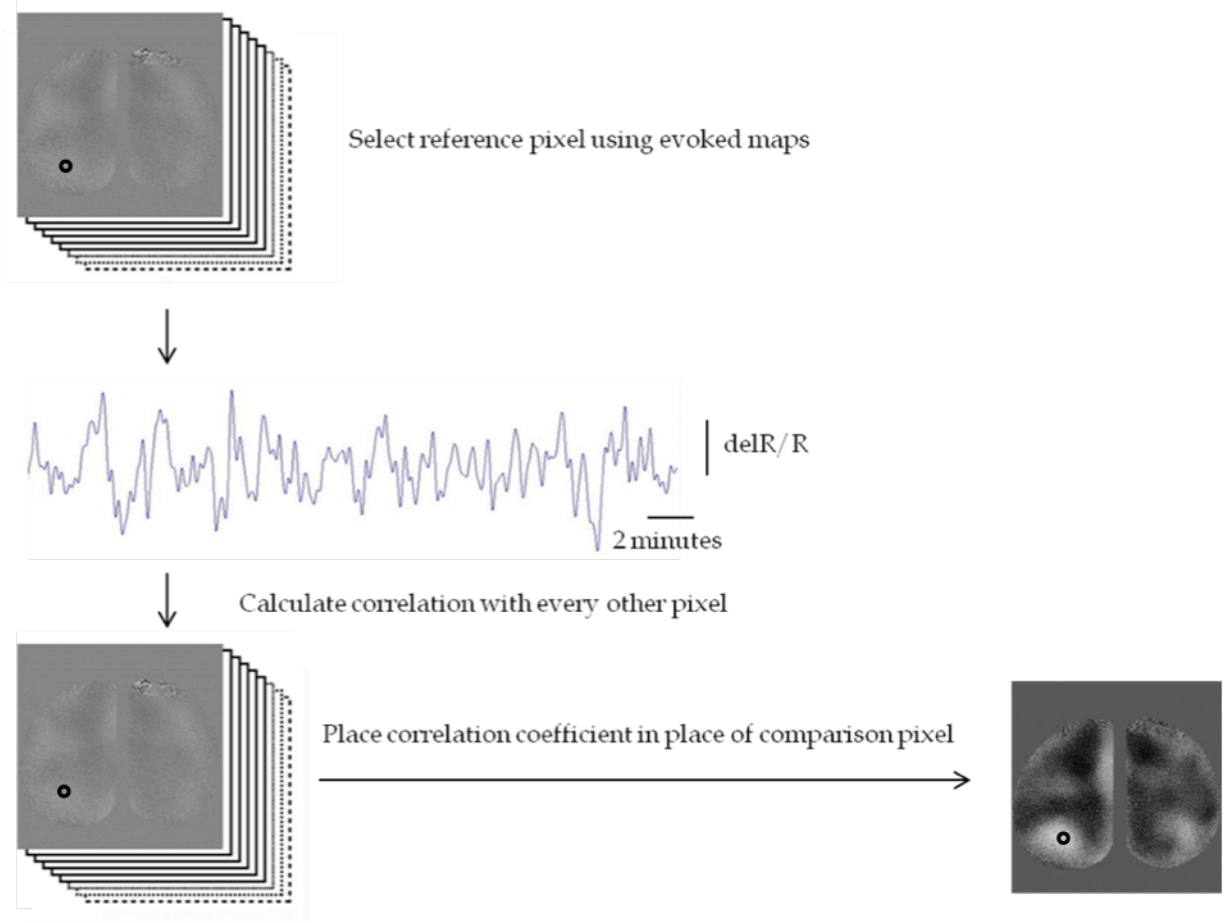


Fig 3.5 Illustration of how a functional connectivity map is generated. Changes in light reflectance (delR/R) were plotted vs. time for each pixel. A seed pixel was selected with the help of sensory-evoked maps and the mouse brain atlas. The activity of every other pixel was compared to that of the seed pixel. A correlation coefficient was calculated using the Pearson correlation method for each comparison pixel. We generated functional connectivity maps by placing the value of correlation coefficient at the location of comparison pixel. Shown here is an example of a correlation map generated with a left MZ seed.

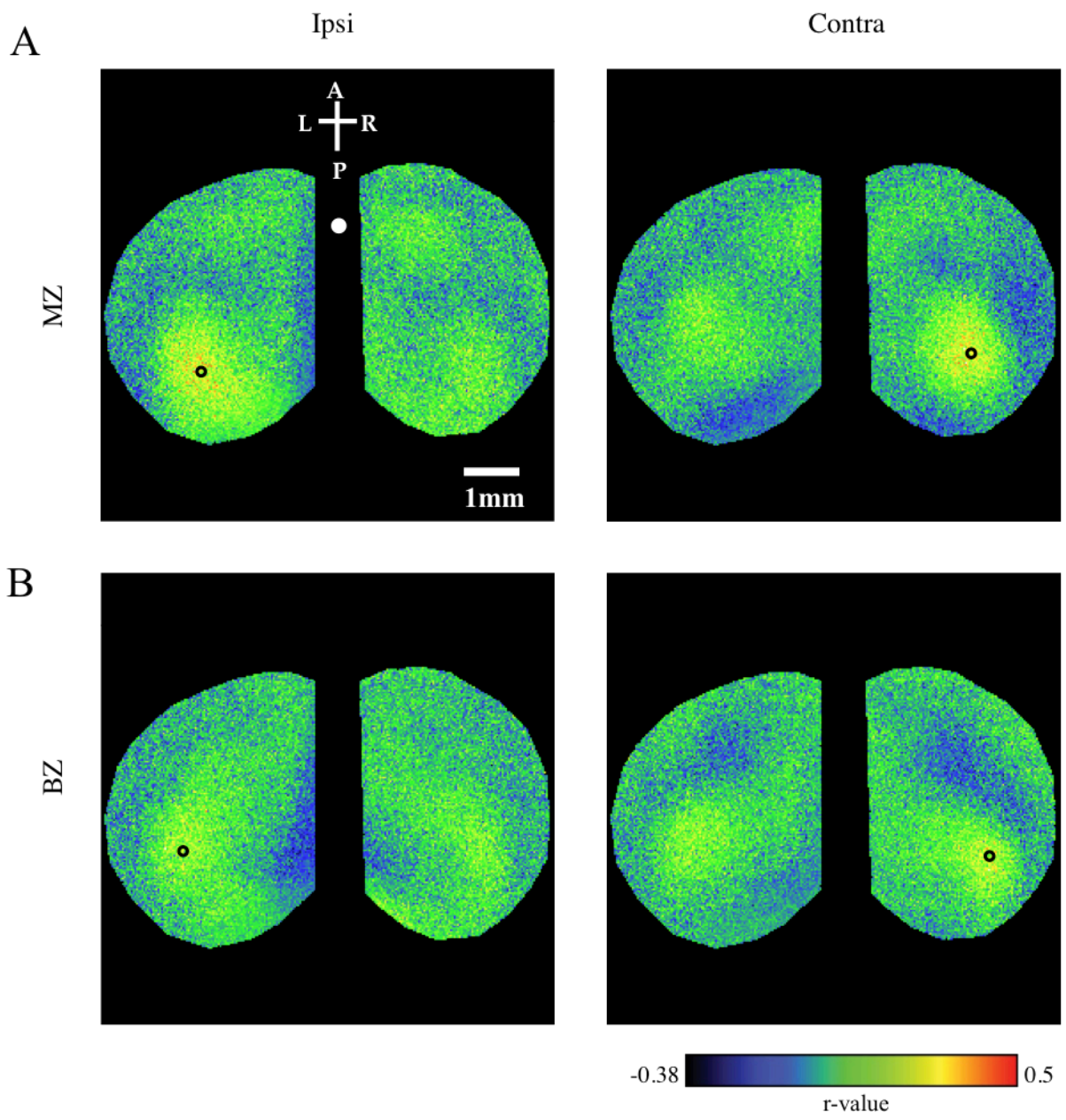


Fig 3.6 Symmetrical functional connectivity maps of V1 in a normal mouse. (A) Mirrored seed pixels generated mirrored functional correlation maps of monocular zone. Solid white circle represented bregma. Centres of evoked MZ maps (black circles) were selected as the seed pixels. MZ demonstrated high level of correlation with BZ intra-hemispherically, and BZ inter-hemispherically. (B) Symmetrical functional connectivity maps of bilateral binocular zones. BZ showed high level of correlation with BZ inter-hemispherically.

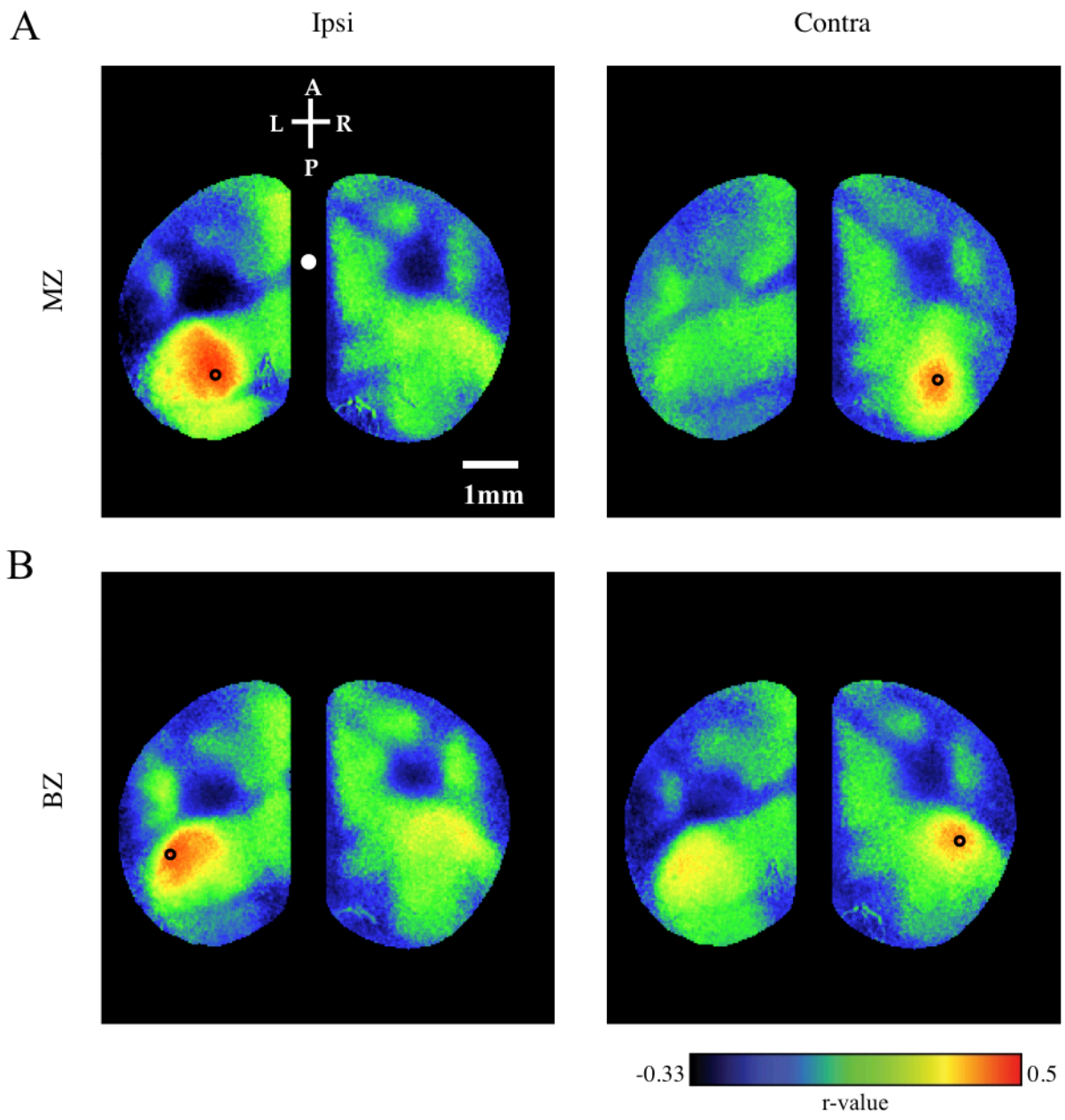


Fig 3.7 Monocular deprivation decreases the correlation of the contralateral MZ with the rest of V1. (A) After four days of monocular deprivation of the left eye during the critical period, the contralateral monocular zone became less correlated with contralateral BZ, as well as the ipsilateral BZ. (B) After brief monocular deprivation, the contralateral binocular zone demonstrated a high level of correlation with the ipsilateral BZ, and increased correlation with the ipsilateral MZ.

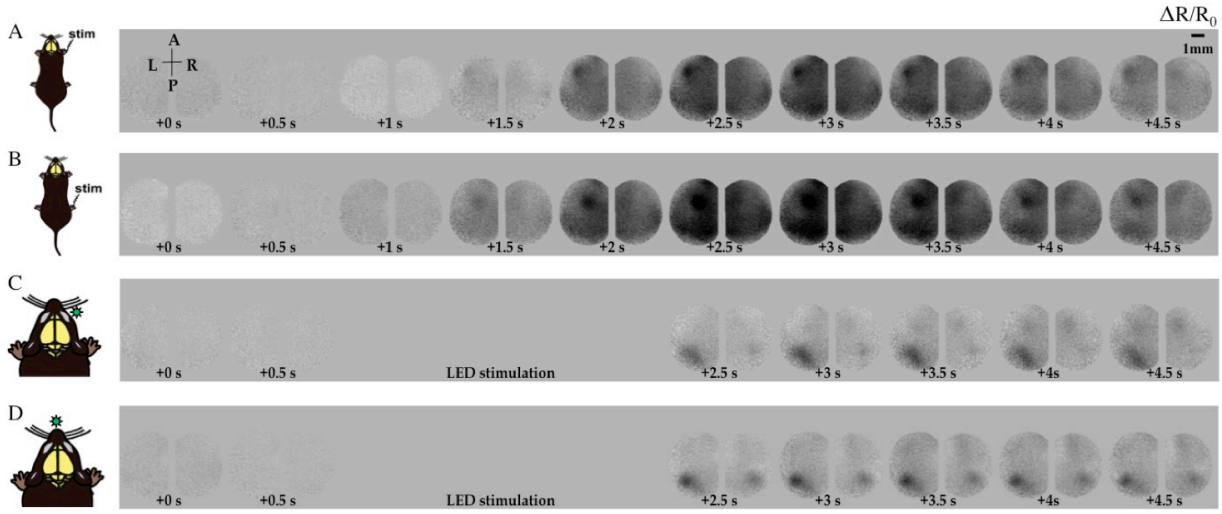


Fig 3.8 Montage of IOS signals at 0.5s interval in response to peripheral sensory stimulation. (A) Piezo stimulators were used to stimulate the forelimb to generate IOS maps of forelimb primary somatosensory cortex (FLS1). (B) Evoked maps of hindlimb primary somatosensory cortex (HLS1) were generated in a similar fashion. (C) Visual stimuli were presented in the temporal visual field for mapping of the monocular zone (MZ). (D) Visual stimuli were restricted to the central visual field to stimulate the binocular zone (BZ). Centres of sensory- or visually-evoked IOS maps were subsequently used as seed pixels while generating functional correlation maps.

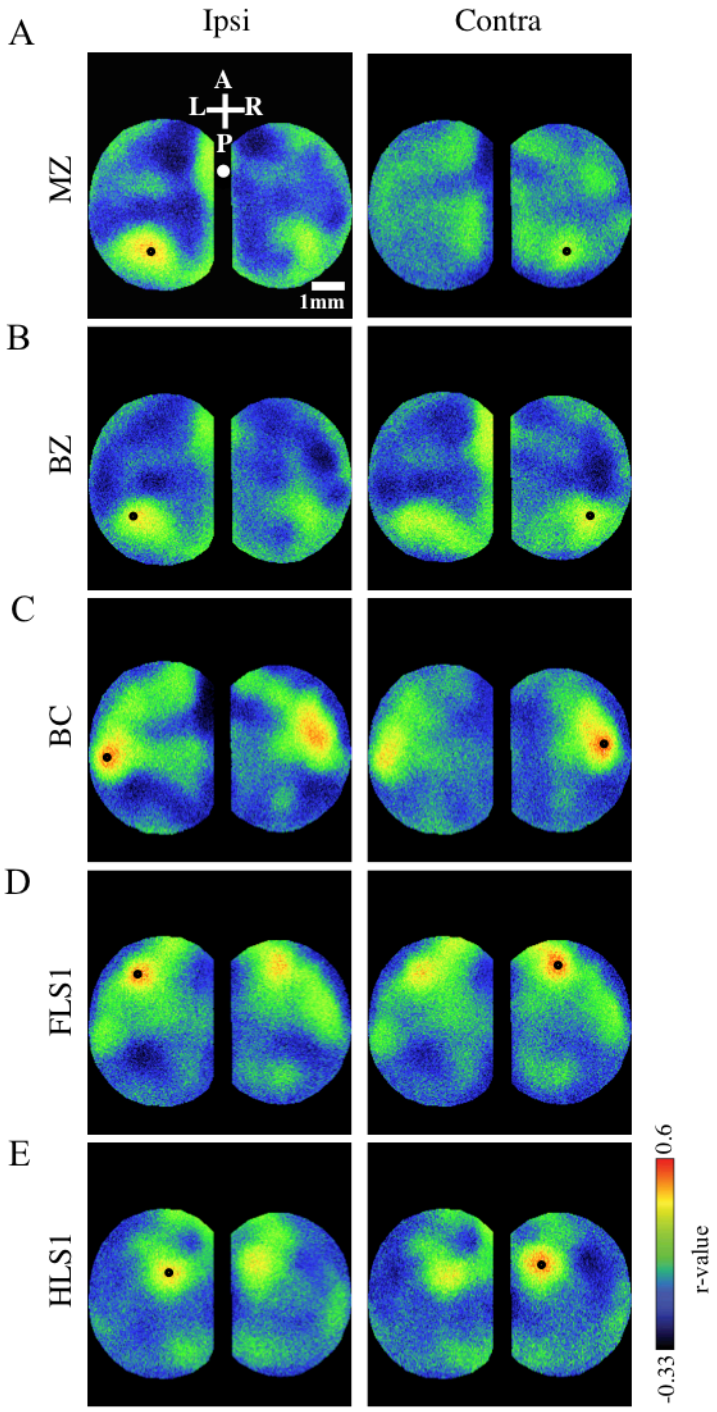


Fig 3.9 IOS imaging suggests global changes in functional connectivity in a monocularly deprived mouse. (A) Compared to the ipsilateral monocular zone, contralateral MZ became less correlated with the rest of the visual cortex, after four days of lid suture over the left eye during

the critical period. (B) The contralateral binocular zone appeared to be more correlated with the ipsilateral MZ after the brief MD. (C) Barrel cortex demonstrated symmetrical connectivity bilaterally. Both BCs appeared to be more correlated with contralateral MZ. (D) FLS1 showed high level of correlation with BC. Bilateral FLS1 appeared to be more correlated with contralateral MZ after brief monocular deprivation. (E) Compared to other regions within the somatosensory cortex, HLS1 was less correlated with the majority of V1.

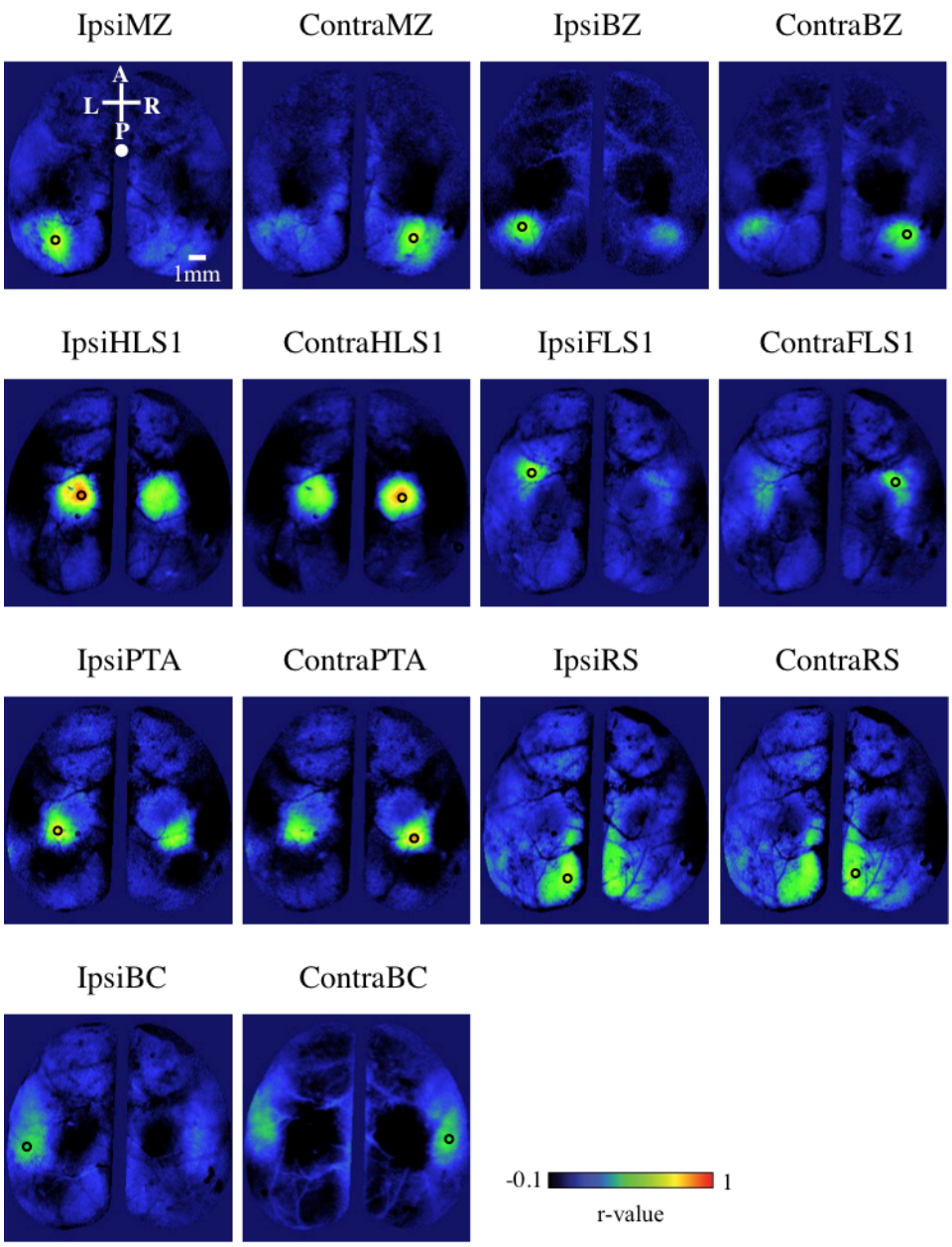


Fig 3.10 Functional connectivity maps of some ROIs in a GCaMP3 mouse. Seed pixels were placed across the cerebral cortex to generate functional connectivity maps of different ROIs. Centres of sensory-evoked maps and the mouse brain atlas were used to aid the selection of seed pixels. Shown here were functional connectivity maps of primary, secondary and associated sensory cortices, including HLS1, FLS1, BC, MZ, BZ, PTA and RS. Symmetrical functional connectivity was appreciated throughout. Midline ROIs showed higher correlation values.

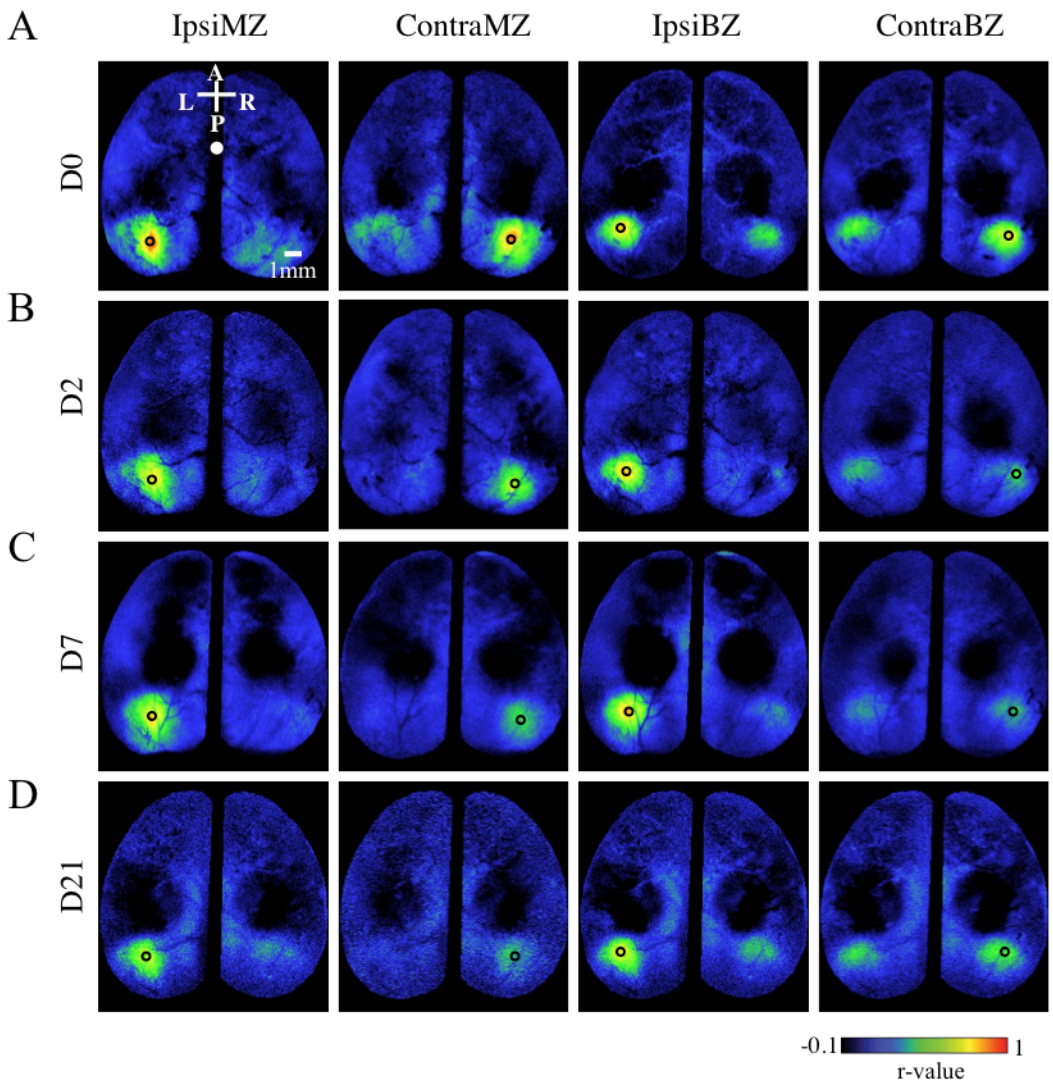
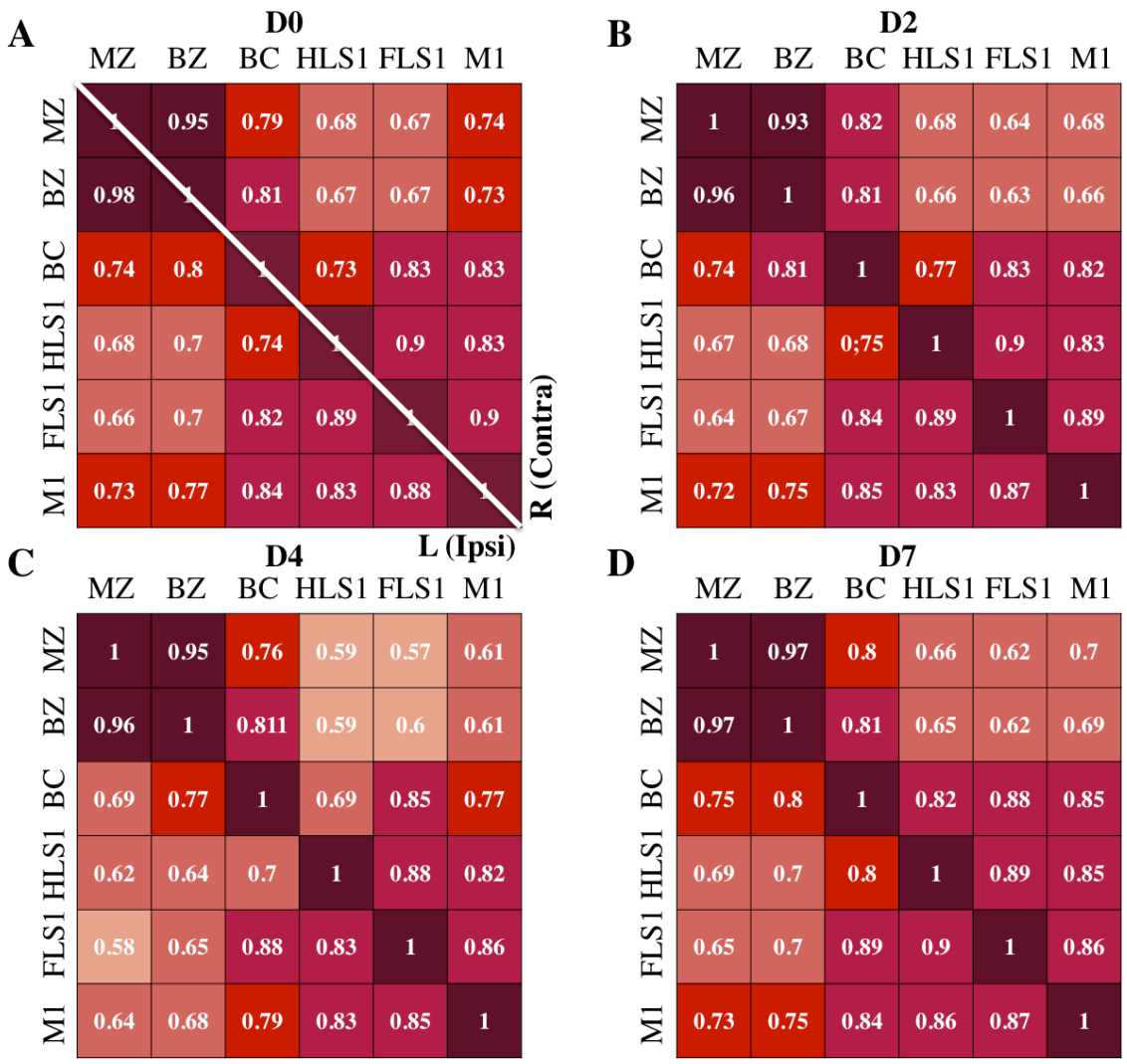


Fig 3.11 Calcium imaging demonstrates functional changes within V1 of a juvenile mouse, after two days of monocular deprivation. (A) At D0 (P25, immediately before MD), baseline recording demonstrated symmetrical functional connectivity of bilateral MZ and BZ. (B) At D2 (the end of MD), after two days of monocular deprivation of the left eye, the contralateral (right) MZ demonstrated decreased correlation with the rest of the visual cortex. The decrease in the contralateral MZ correlation appeared to persist until D7 (C) and D21 (D).


A
D0
MZ BZ BC HLS1 FLS1 M1
MZ 1 0.95 0.79 0.68 0.67 0.74
BZ 0.98 1 0.81 0.67 0.67 0.73
BC 0.74 0.8 1 0.73 0.83 0.83
HLS1 0.68 0.7 0.74 1 0.9 0.83
FLS1 0.66 0.7 0.82 0.89 1 0.9
M1 0.73 0.77 0.84 0.83 0.88 1
R (Contra)
L (Ipsi)
B
D2
MZ BZ BC HLS1 FLS1 M1
MZ 1 0.93 0.82 0.68 0.64 0.68
BZ 0.96 1 0.81 0.66 0.63 0.66
BC 0.74 0.81 1 0.77 0.83 0.82
HLS1 0.67 0.68 0;75 1 0.9 0.83
FLS1 0.64 0.67 0.84 0.89 1 0.89
M1 0.72 0.75 0.85 0.83 0.87 1
C
D4
MZ BZ BC HLS1 FLS1 M1
MZ 1 0.95 0.76 0.59 0.57 0.61
BZ 0.96 1 0.811 0.59 0.6 0.61
BC 0.69 0.77 1 0.69 0.85 0.77
HLS1 0.62 0.64 0.7 1 0.88 0.82
FLS1 0.58 0.65 0.88 0.83 1 0.86
M1 0.64 0.68 0.79 0.83 0.85 1
D
D7
MZ BZ BC HLS1 FLS1 M1
MZ 1 0.97 0.8 0.66 0.62 0.7
BZ 0.97 1 0.81 0.65 0.62 0.69
BC 0.75 0.8 1 0.82 0.88 0.85
HLS1 0.69 0.7 0.8 1 0.89 0.85
FLS1 0.65 0.7 0.89 0.9 1 0.86
M1 0.73 0.75 0.84 0.86 0.87 1

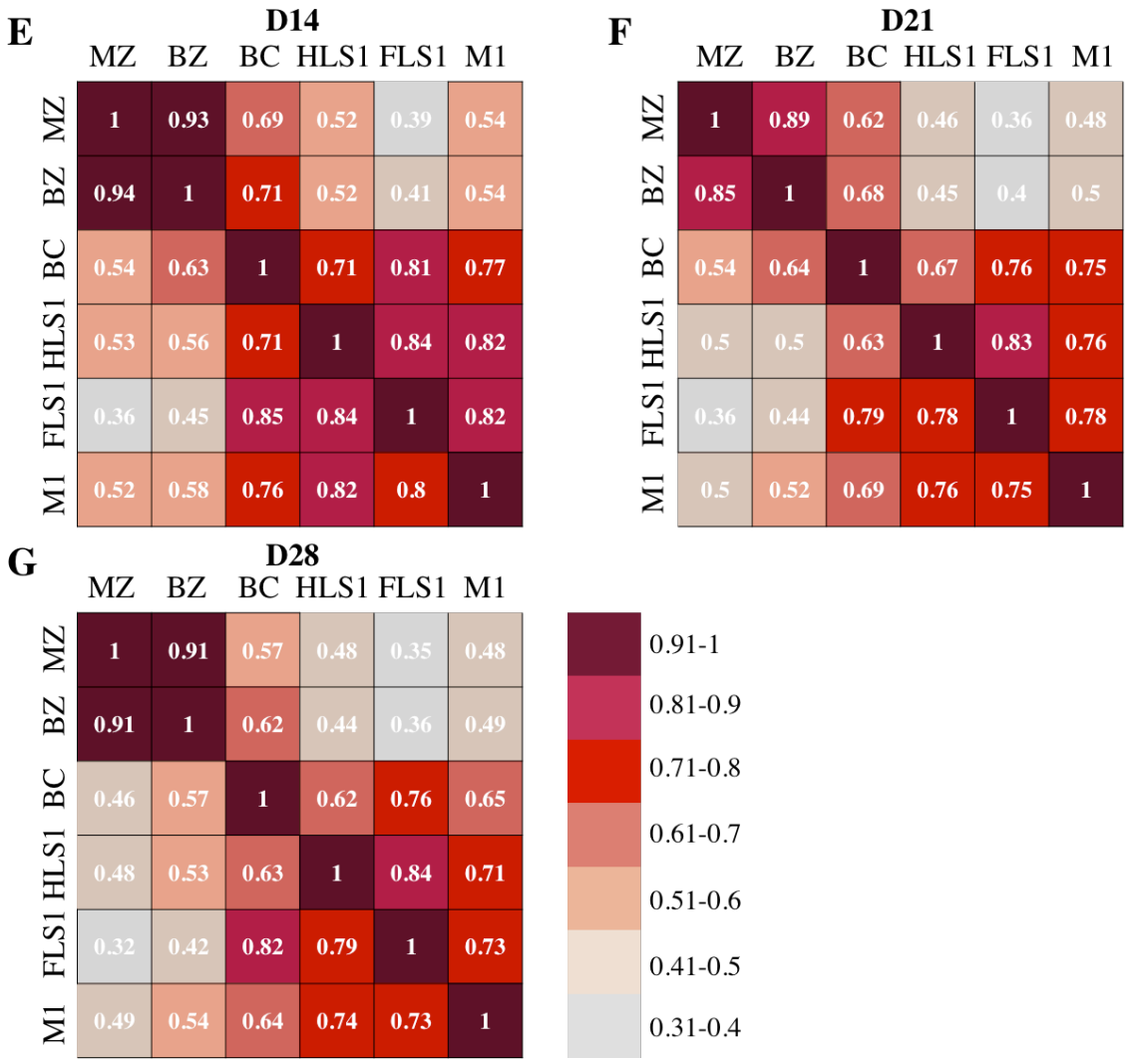
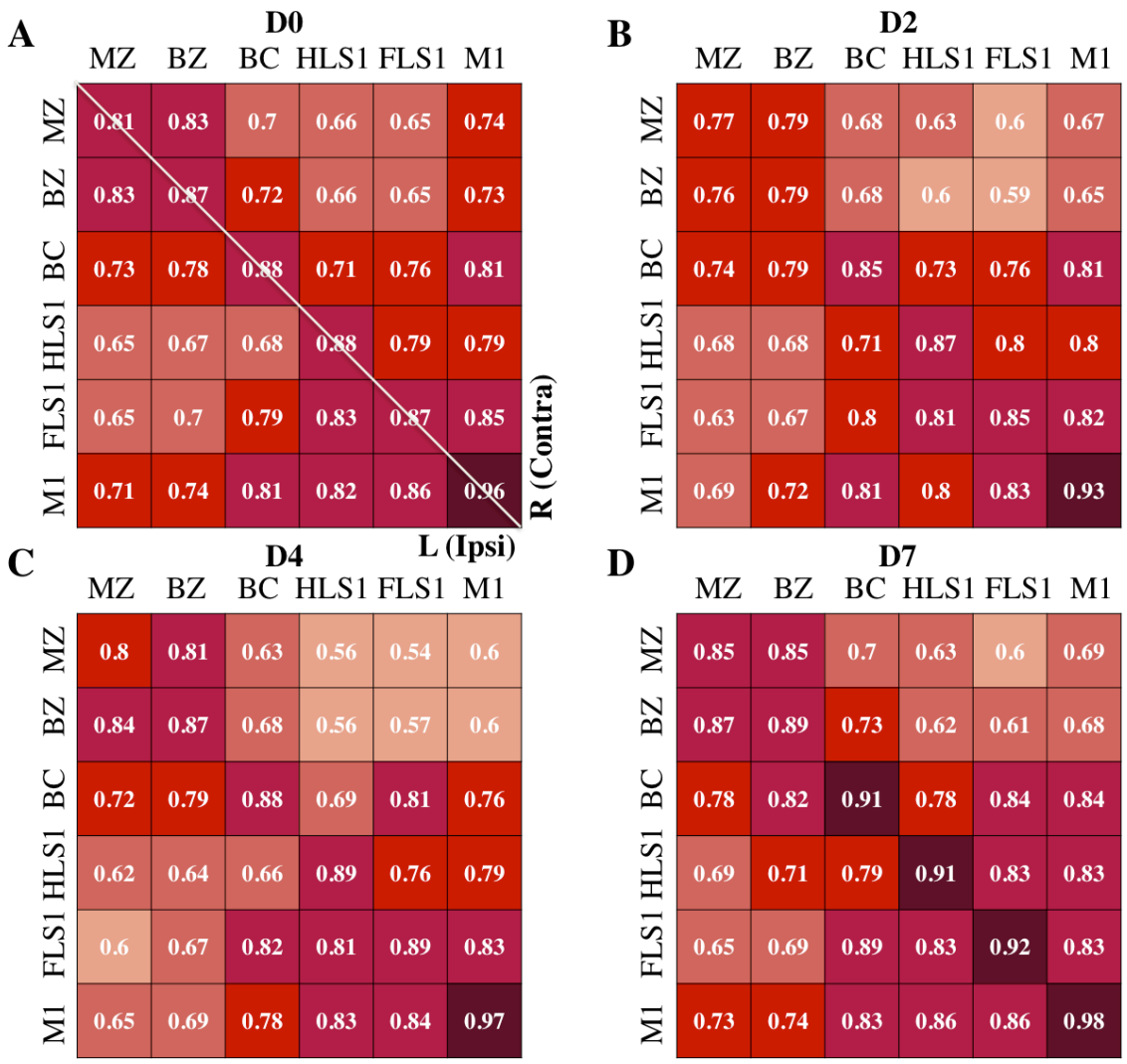


Fig 3.12 Correlation matrices of intra-hemispheric connections of 7 monocularly deprived mice, through development. Time points shown here included D0 (P25, baseline), D2, D4, D7, D14, D21 and D28 (P53). Values within the correlation matrices represent mean r-values. All 7 mice underwent two days of lid suture over the left eye during the critical period. Overall, we observed a strong and stable connection between MZ and BZ intra-hemispherically. The mean MZ-BZ r-value in contralateral V1 was 0.95 on D0 and 0.91 on D28, and that for ipsilateral V1 was 0.98 on D0 and 0.91 on D28. In addition, we also observed a gradual decrease of correlation between V1 (including both MZ and BZ) and the somatomotor cortex (including BC, HLS1,

FLS1 and M1). For example, the mean MZ-BC r-value in the contralateral hemisphere decreased from 0.79 on D0 to 0.57 on D28. The mean MZ-BC r-value in the ipsilateral hemisphere decreased from 0.74 to 0.46.


A
D0
MZ BZ BC HLS1 FLS1 M1
MZ 0.81 0.83 0.7 0.66 0.65 0.74
BZ 0.83 0.87 0.72 0.66 0.65 0.73
BC 0.73 0.78 0.88 0.71 0.76 0.81
HLS1 0.65 0.67 0.68 0.88 0.79 0.79
FLS1 0.65 0.7 0.79 0.83 0.87 0.85
M1 0.71 0.74 0.81 0.82 0.86 0.96
R (Contra)
L (Ipsi)
B
D2
MZ BZ BC HLS1 FLS1 M1
MZ 0.77 0.79 0.68 0.63 0.6 0.67
BZ 0.76 0.79 0.68 0.6 0.59 0.65
BC 0.74 0.79 0.85 0.73 0.76 0.81
HLS1 0.68 0.68 0.71 0.87 0.8 0.8
FLS1 0.63 0.67 0.8 0.81 0.85 0.82
M1 0.69 0.72 0.81 0.8 0.83 0.93
C
D4
MZ BZ BC HLS1 FLS1 M1
MZ 0.8 0.81 0.63 0.56 0.54 0.6
BZ 0.84 0.87 0.68 0.56 0.57 0.6
BC 0.72 0.79 0.88 0.69 0.81 0.76
HLS1 0.62 0.64 0.66 0.89 0.76 0.79
FLS1 0.6 0.67 0.82 0.81 0.89 0.83
M1 0.65 0.69 0.78 0.83 0.84 0.97
D
D7
MZ BZ BC HLS1 FLS1 M1
MZ 0.85 0.85 0.7 0.63 0.6 0.69
BZ 0.87 0.89 0.73 0.62 0.61 0.68
BC 0.78 0.82 0.91 0.78 0.84 0.84
HLS1 0.69 0.71 0.79 0.91 0.83 0.83
FLS1 0.65 0.69 0.89 0.83 0.92 0.83
M1 0.73 0.74 0.83 0.86 0.86 0.98

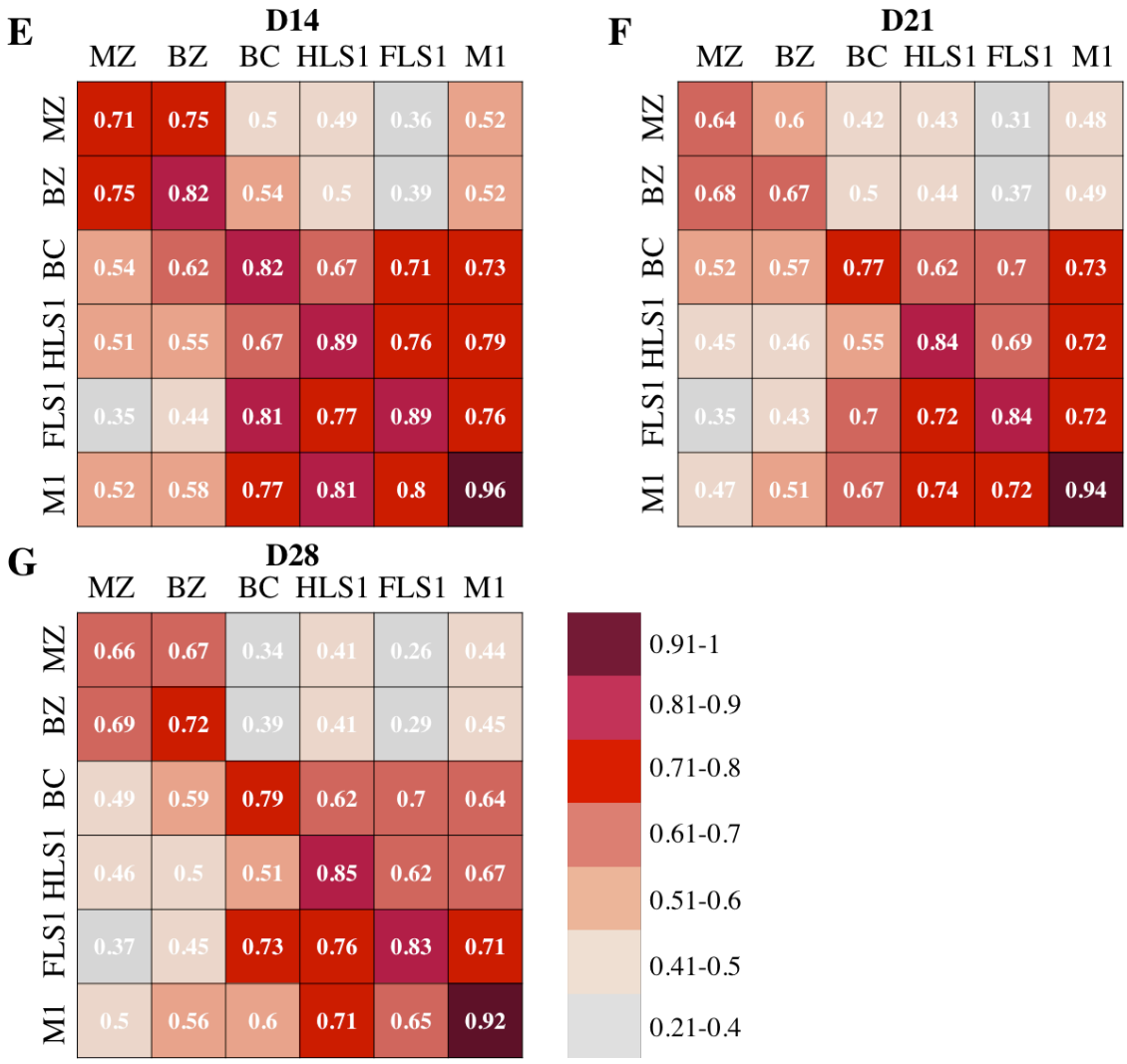
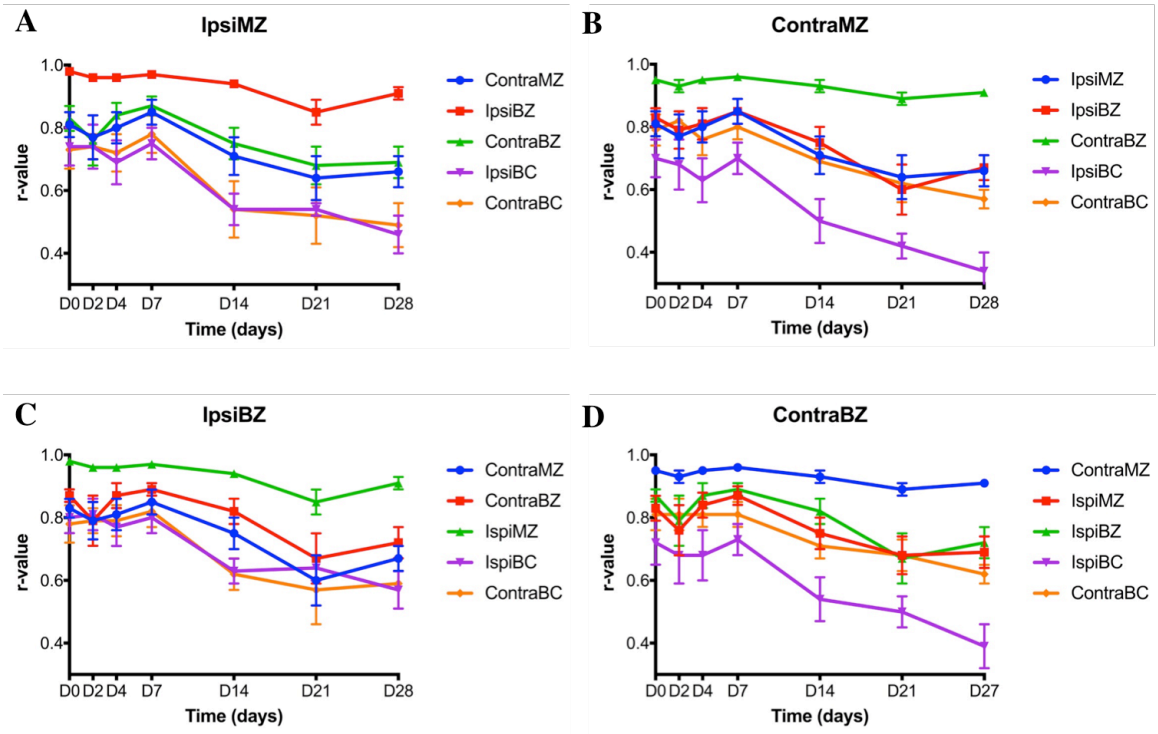


Fig 3.13 Correlation matrices of inter-hemispheric connections of 7 monocularly deprived mice, through development. The same time points as in the previous figure were examined for the 7 MD mice. We again observed a gradual decrease of correlation between V1 and somatomotor cortex, with the greatest decline observed in contralateral MZ. The mean contraMZ-ipsiBC r-value decreased from 0.7 on D0 to 0.34 on D28, and the mean ipsiMZ-contraBC r-value decreased from 0.73 to 0.49 from D0 to D28.



3.14 Functional connectivity changes during development in monocularly deprived young mice. The means (along with SD) in the correlation matrices presented in Figs 3.12 and 3.13 were plotted along time for selected ROIs. For each ROI within V1, the highest correlation was observed in its homotopic region within the same hemisphere. For example, contraMZ demonstrated higher correlation with contraBZ than with any other ROIs. Note the transient changes in r-values within V1 on D2, correlating with the duration of monocular deprivation. Again, there was a gradual decrease in the correlation between the visual cortex and barrel cortex during development. This was most pronounced in contralateral MZ, followed by contralateral BZ. The mean contraMZ-contraBC r-value dropped from 0.79 to 0.57 from D0 to D28, and the mean contraMZ-ipsiBC r-value dropped from 0.7 to 0.34. The mean contraBZ-contraBC r-value dropped fro 0.81 to 0.62, while contraBZ-ipsiBC r-value decreased from 0.72 to 0.39.

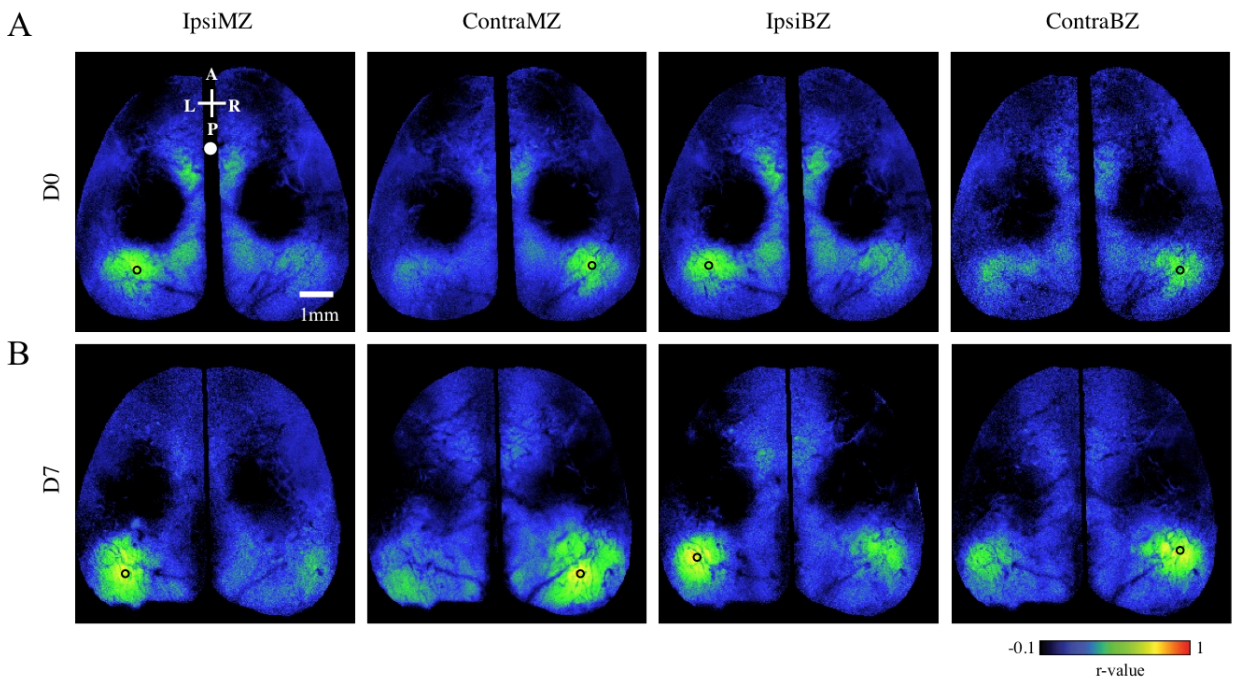


Fig 3.15 Seven days of monocular deprivation does not appear to affect functional connectivity in adulthood. (A) At D0, baseline recording demonstrated symmetrical functional connectivity within both MZ and BZ. (B) After 7 days of monocular deprivation of the left eye, functional connectivity remained unchanged, in both MZ and BZ.

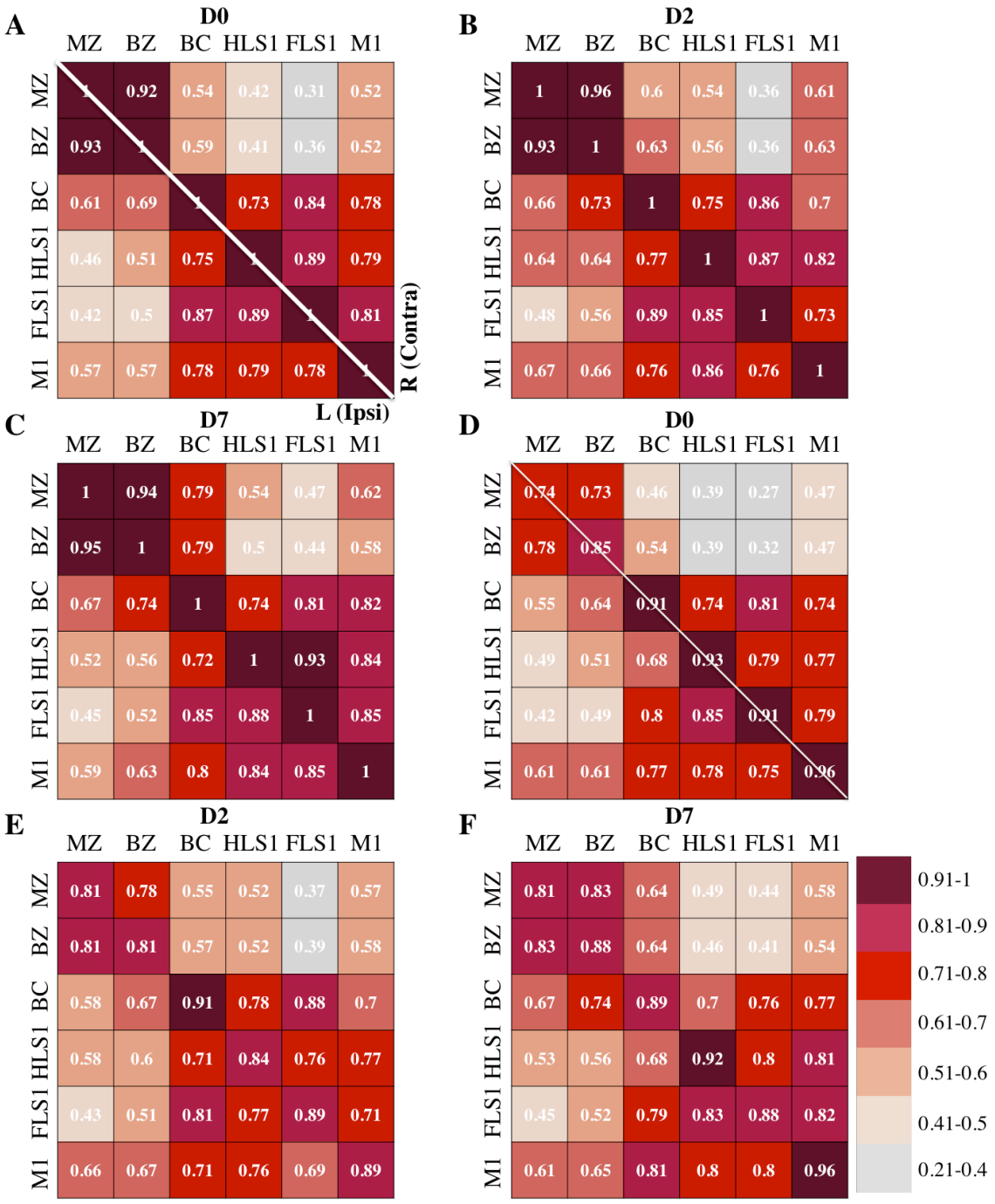


Fig 3.16 Correlation matrices of monocularly deprived adult mice. Four adult mice all underwent seven days of monocular deprivation of the left eye, and were imaged at D0, D2 and D7. (A-C) There were strong and stable intra-hemispheric connections between MZ and BZ. There were stable correlations between MZ and BZ with the rest of somatomotor cortex, similar

to that at the last time point of young mouse recordings on D28 (P53). (D-F) Strong and stable inter-hemispheric correlations were noted, despite seven days of monocular deprivation.

CHAPTER 4 CONCLUSIONS

Experience-dependent plasticity enables the brain to wire and rewire itself in response to lasting changes in experience. It is crucial for development of neural network, learning and memory, as well as recovery from injuries (Nithianantharajah and Hannan, 2006). Ocular dominance plasticity, a well-known example of experience-dependent plasticity, continues to be a subject of intensive studies since its initial discovery (Hubel and Wiesel, 1963a). Using the mouse model of amblyopia, I examined the functional significance of long-term depression in ocular dominance plasticity, and explored the effects of monocular deprivation on spontaneous activity and functional connectivity.

In Chapter 1, I reviewed literature on ocular dominance plasticity, its underlying mechanisms and stressed my particular interest in LTD (**section 1.2**). I hypothesized that ocular dominance plasticity could be enhanced by facilitating LTD in adulthood. This hypothesis is tested in Chapter 2. I also reviewed literature on cross-modal plasticity (**section 1.3**), spontaneous activity and functional connectivity (**section 1.4**). I hypothesized that monocular deprivation may induce global, compensatory changes in functional connectivity during the critical period. This is tested in Chapter 3. Below I summarize the key findings of both result chapters.

In Chapter 2, I presented data supporting functional significance of LTD in ocular dominance plasticity. Being developmentally constrained (Dudek and Friedlander, 1996; Jiang et al., 2007; Yang et al., 2011) (**Fig 2.3**), LTD is difficult to induce in visual cortical slices of adult rodents. $GluR2_{3Y}$, a specific LTD blocker (Ahmadian et al., 2004), prevents ocular dominance shift during the critical period, suggesting that LTD is necessary for ODP (Yang et al., 2011) (**Fig 2.4**). D-serine incubation selectively facilitates the induction of LTD in adult visual cortical

slices (**Fig 2.5**). D-serine also enhances ocular dominance plasticity *in vivo* in adult mice, the effect of which is prevented by $GluR2_{3Y}$ (**Fig 2.6**). This suggests that D-serine operates via an LTD-like mechanism and LTD is sufficient in inducing ODP in adulthood. Moreover, I also showed D-serine is effective in restoring a contralateral-dominated OD distribution in mice that have undergone long-term MD (**Fig 2.8**).

In Chapter 3, I focused on spontaneous activity and functional connectivity, with data obtained from *in vivo* IOS and GCaMP imaging. Spontaneous activity is regionally distinct and highly correlated in functional related regions in mouse brain (Mohajerani et al., 2013; Chan et al., 2015). Examples of IOS (**Fig 3.6**) and GCaMP (**Fig 3.10**) imaging demonstrated symmetry in correlation maps of mirrored seed pixels within V1. This asymmetry is disrupted by monocular deprivation (**Fig 3.7 & 3.11**). Changes induced by brief monocular deprivation were further examined and quantified in a group of 7 GCaMP3 mice. Repeated imaging of the same mice during development revealed a transient change in functional connectivity correlating with the duration of lid suture (2 days) during the critical period (**Fig 3.14**). Monocular deprivation of 7 days fails to induce the same effect in adult GCaMP3 mice (**Fig 3.16**). In addition, I observed a gradual decline in the correlation between V1 and the somatosensory cortex during development (**Fig 3.14**).

In this final chapter, I propose pertinent future experiments; discuss the strengths and weaknesses of my results, and how the conclusions integrate into the current understanding of experience-dependent plasticity and spontaneous activity.

4.1 LTD in ocular dominance plasticity

Previously, our group has demonstrated that LTD is developmentally constrained (Yang et al., 2011) (**Fig 2.3**). Blocking LTD in the visual cortex with $GluR2_{3Y}$ prevents ocular dominance

shift during the critical period (Yang et al., 2011) (**Fig 2.4**). Similar findings have been reported with another LTD blocking peptide, G2CT (Yoon et al., 2009). These data suggest that LTD is crucial for ocular dominance during the critical period (Smith et al., 2009; Cooke and Bear, 2014).

I hypothesized that by enhancing LTD that is otherwise absent in adulthood, ocular dominance plasticity can be enhanced in adult animals. Indeed, this was achieved with NMDAR co-agonist D-serine (**Fig 2.6**) and NMDAR co-agonist site modulator, hGlyT1 inhibitor ALX 5407 (Atkinson et al., 2001) (**Fig 2.9**).

D-serine, an endogenous NMDAR co-agonist (Schell et al., 1995; Wolosker et al., 1999a), selectively facilitates the induction of LTD in adult visual cortical slices (**Fig 2.5**). Systemic administration of D-serine facilitates ocular dominance shift in adult mice after four days of monocular deprivation (**Fig 2.6**). Similar effect is achieved with intracortical infusion of D-serine (**Fig 2.7**), pointing toward direct effect of D-serine on the visual cortex. $GluR2_{3Y}$ effectively prevents the effect of D-serine in adult mice (**Fig 2.6**), again supporting a regulatory of LTD in adult ODP. This, combined our previous findings in juvenile mice, suggests that LTD plays an important regulatory role in ODP in both juvenile and adult mice.

The effect of D-serine (**Fig 2.6**) is similar to that of ALX 5407 (**Fig 2.9**), which potentially increases glycine concentration at synaptic site (Berger et al., 1998; Bergeron et al., 1998), further supporting that manipulating NMDAR co-agonist site is effective in modulating NMDAR neurotransmission in adulthood (Wolosker et al., 2008).

In fact, with a specific LTD blocker $GluR2_{3Y}$ and a specific LTD-facilitator D-serine, we are able to modulate LTD effectively in both directions. This holds great promise in studying disease models in which LTD is implicated, such as drug addiction, stress-induced cognitive

impairment, and psychiatric disorders including depression and schizophrenia (Collingridge et al., 2010).

4.2 D-serine, a potential therapeutic reagent

As discussed in Chapter 1 (**section 1.2.4**), amblyopia is a leading cause of visual impairments in adults and poses an important socioeconomic problem (Membreno et al., 2002; Webber and Wood, 2005). Current treatments for amblyopia include correcting underlying visual defect, and also patching of the 'good' eye during the critical period of visual acuity development. However, children are poorly compliant and do not tolerate patching well. Here, I showed that D-serine is effective in facilitating LTD and enhancing ODP beyond the critical period. Moreover, D-serine, coupled with reverse suture, is effective in reversing the robust shift in ocular dominance brought on by long-term monocular deprivation (**Fig 2.8**). This provides a theoretical basis for treating amblyopia in adulthood.

ALX 5407 is also effective in enhancing ODP in adulthood (**Fig 2.9**). Both ALX 5407 and D-serine have been shown to be beneficial to clozapine in mouse models of schizophrenia (Lipina et al., 2005). It will be interesting to explore the effects of D-serine and ALX 5407 in other LTD-implicated disease processes, such as depression and addiction (Collingridge et al., 2010).

4.3 LTP/LTD is the not only therapeutic target for amblyopia

Although I have emphasized the functional significance of LTD in ocular dominance plasticity, it is by no means the only mechanism underlying this experience-dependent plasticity. As discussed in Chapter 1 (**section 1.2.7.1**), cortical inhibition plays an important role in ocular dominance plasticity (Trachtenberg, 2015; van Versendaal and Levelt, 2016). Elegant studies have also shown that ODP could be enhanced or restored by targeting the inhibitory network and

adjusting the E/I balance (Maya Vetencourt et al., 2008; Southwell et al., 2010; Davis et al., 2015). Homeostatic synaptic scaling may be involved in the activity-dependent refinement of cortical connectivity (Turrigiano and Nelson, 2004). In addition, intrinsic neuronal excitability (Zhang and Linden, 2003) and metaplasticity (Abraham and Bear, 1996) have also been proposed to regulate experience-dependent plasticity.

Nor is LTD/LTP the sole mechanism via which D-serine or ALX5407 enhances ocular dominance plasticity. D-serine may enhance ODP in adulthood via alternative mechanisms. It is possible that D-serine increases the E-I ratio by decreasing GABAergic neurotransmission (Liu et al., 2009), and thereby enhancing ocular dominance plasticity and facilitating recover of visual in reversely sutured LTMD mice. Since D-serine is mostly found in astrocytes that ensheathe NMDAR-bearing neurons (Martineau et al., 2006), D-serine might also takes effect by modulating structural plasticity.

The underlying mechanism of ALX 5407's effect is not tested in this thesis. It will be worth exploring the effect of ALX 5407 on LTP and LTD in visual cortical slices. Further testing with co-injection of ALX 5407 and $GluR2_{3Y}$ in adult mice will help understand if ALX 5407 enhances ODP through an LTD-dependent process.

4.4 Monocular deprivation induces transient changes in functional connectivity during the critical period

In Chapter 3, I explored changes in spontaneous activity and functional connectivity induced by monocular deprivation with *in vivo* imaging techniques. This is the first attempt to study functional connectivity with calcium imaging in an experience-dependent plasticity mouse model. Using GCaMP3 mice, I have directly recorded spiking activity of excitatory neurons in all layers of cerebral cortex, at a high spatial resolution, from large regions of the cortex (Berger et al.,

2007; Vanni and Murphy, 2014). This is made possible with the help of a large chronic window (Silasi et al., 2016). This approach could easily to adapted to study disease models in which spontaneous activity and the default network are affected, including autism and Alzheimer's disease (Honey et al., 2010).

I tested the effect of two days of monocular deprivation, and observed transient changes in functional connectivity correlating with the duration of lid suture in GCaMP mice during the critical period (section **3.3.4.1, Fig 3.14**). Two days of monocular deprivation during the critical period may not have a robust effect (Gordon and Stryker, 1996; Gandhi et al., 2008). It will be interesting to repeat these experiments with longer durations of lid suture, such as 4 or greater than 7 days. Four days of monocular deprivation at the peak of the critical period induces a near maximal shift in ocular dominance (Cang et al., 2005). Longer period of monocular deprivation (greater than 7 days) causes retraction of geniculocortical axon branches representing the deprived eye in mouse V1 (Antonini and Stryker, 1993b; Gordon and Stryker, 1996). It is worth exploring whether functional changes with 4 or 7 days of monocular deprivation get consolidated in spontaneous activity and functional connectivity; and whether changes in spontaneous activity and functional connectivity correlate with those in in structural connectivity.

4.5 Underlying mechanism for changes in functional connectivity during the critical period

Previous studies have suggested that spontaneous activity is dependent on cortical activity (Chan et al., 2015). AMPARs and NMDARs have been implicated in spontaneous activity and functional connectivity (Miyamoto et al., 2003; Chan et al., 2015), as are LTP and LTD (Kimura and Pavlides, 2000; Bliss and Cooke, 2011). Ocular dominance plasticity itself is an LTD-dependent process (Yoon et al., 2009; Yang et al., 2011), it will be interesting to examine

spontaneous activity and functional connectivity in monocularly deprived mice receiving $GluR2_{3Y}$ during the critical period to probe the functional significance of LTD in this process.

I observed changes in functional connectivity in monocularly deprived mice during the critical period, but not in adulthood. This transient change in functional connectivity is more pronounced in the contralateral MZ, but not restricted to within V1. The functional connectivity between V1 and somatosensory cortex was also affected during monocular deprivation (**Fig 3.14**). This may reflect adaptations of brain circuits to sensory deprivation, as depicted in cross-modal plasticity. It is also in agreement with existing literature suggesting a sensitive period for cross-modal plasticity (Bavelier and Neville, 2002).

D-serine enhances ocular dominance plasticity in adulthood and facilitates recovery of visual function in LTMD adult mice. It may affect spontaneous activity and functional connectivity in adult mice as well. Since spontaneous activity reflects prior experience and may represent a form of short-term memory (Huber et al., 2004; Han et al., 2008; Vyazovskiy and Tobler, 2008), it will be interesting to test if facilitation of ocular dominance plasticity by D-serine in adult mice gets translated in changes in spontaneous activity and functional connectivity.

4.6 Gradual decrease in correlation between V1 and somatosensory cortex during development

We observed a gradual decrease in correlation coefficients between V1 (both MZ and BZ) and the somatosensory cortex (**Fig 3.14**). This may reflect increased specificity of distinct cortical regions and maturation of the network during development. The degree of correlation at the end of young mice recording is similar to that at baseline of adult mouse recording (**Figs 3.12, 3.13 & 3.16**). However, without normal control, it is difficult to draw any conclusions. It will be

interesting to pursue longitudinal recordings throughout development in normal mice, from pre- to peri-critical period, through adulthood to explore changes in spontaneous activity and functional connectivity at a network level.

4.7 Concluding remarks

In this thesis, I presented my experiments on mechanism of ocular dominance plasticity, spontaneous activity and functional connectivity, using the classic monocular deprivation model. Hopefully, what I presented here will inspire more interest in and questions about experience-dependent plasticity, spontaneous activity and functional connectivity; and further our understanding of how experience act in concert with genetically driven, molecular determinants of development during the formation of functional neural circuits (Goodman and Shatz, 1993).

REFERENCES

Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. Trends in neurosciences 19:126-130.

Ackman JB, Burbridge TJ, Crair MC (2012) Retinal waves coordinate patterned activity throughout the developing visual system. Nature 490:219-225.

Ahmadian G, Ju W, Liu L, Wyszynski M, Lee SH, Dunah AW, Taghibiglou C, Wang Y, Lu J, Wong TP, Sheng M, Wang YT (2004) Tyrosine phosphorylation of GluR2 is required for insulin-stimulated AMPA receptor endocytosis and LTD. EMBO J 23:1040-1050.

Albert NB, Robertson EM, Miall RC (2009a) The resting human brain and motor learning. Curr Biol 19:1023-1027.

Albert NB, Robertson EM, Mehta P, Miall RC (2009b) Resting state networks and memory consolidation. Commun Integr Biol 2:530-532.

Andermann ML, Kerlin AM, Roumis DK, Glickfeld LL, Reid RC (2011) Functional specialization of mouse higher visual cortical areas. Neuron 72:1025-1039.

Antonini A, Stryker MP (1993a) Development of individual geniculocortical arbors in cat striate cortex and effects of binocular impulse blockade. The Journal of neuroscience : the official journal of the Society for Neuroscience 13:3549-3573.

Antonini A, Stryker MP (1993b) Rapid remodeling of axonal arbors in the visual cortex. Science 260:1819-1821.

Antonini A, Stryker MP (1996) Plasticity of geniculocortical afferents following brief or prolonged monocular occlusion in the cat. The Journal of comparative neurology 369:64-82.

Antonini A, Fagiolini M, Stryker MP (1999) Anatomical correlates of functional plasticity in mouse visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:4388-4406.

Antonini A, Gillespie DC, Crair MC, Stryker MP (1998) Morphology of single geniculocortical afferents and functional recovery of the visual cortex after reverse monocular deprivation in the kitten. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:9896-9909.

Arieli A, Sterkin A, Grinvald A, Aertsen A (1996) Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. Science 273:1868-1871.

Atkinson BN, Bell SC, De Vivo M, Kowalski LR, Lechner SM, Ognyanov VI, Tham CS, Tsai C, Jia J, Ashton D, Klitenick MA (2001) ALX 5407: a potent, selective inhibitor of the hGlyT1 glycine transporter. Mol Pharmacol 60:1414-1420.

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 21:1133-1145.

Bassett DS, Bullmore E (2006) Small-world brain networks. Neuroscientist 12:512-523.

Bavelier D, Neville HJ (2002) Cross-modal plasticity: where and how? Nature reviews Neuroscience 3:443-452.

Bavelier D, Brozinsky C, Tomann A, Mitchell T, Neville H, Liu G (2001) Impact of early deafness and early exposure to sign language on the cerebral organization for motion processing. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:8931-8942.

Bavelier D, Tomann A, Hutton C, Mitchell T, Corina D, Liu G, Neville H (2000) Visual attention to the periphery is enhanced in congenitally deaf individuals. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:RC93.
Bear MF (1996) Progress in understanding NMDA-receptor-dependent synaptic plasticity in the visual cortex. J Physiol Paris 90:223-227.
Bear MF (2003) Bidirectional synaptic plasticity: from theory to reality. Philosophical transactions of the Royal Society of London Series B, Biological sciences 358:649-655.
Bear MF, Kleinschmidt A, Gu QA, Singer W (1990) Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. The Journal of neuroscience : the official journal of the Society for Neuroscience 10:909-925.
Beckmann CF, DeLuca M, Devlin JT, Smith SM (2005) Investigations into resting-state connectivity using independent component analysis. Philosophical transactions of the Royal Society of London Series B, Biological sciences 360:1001-1013.
Berardi N, Pizzorusso T, Maffei L (2000) Critical periods during sensory development. Current opinion in neurobiology 10:138-145.
Berger, H (1929) U¨ ber das Elektrenkephalogramm des Menschen (On the human electroencephalogram). Archiv f. Psychiatrie u. Nervenkrankheiten 87:527–70.
Berger AJ, Dieudonne S, Ascher P (1998) Glycine uptake governs glycine site occupancy at NMDA receptors of excitatory synapses. Journal of neurophysiology 80:3336-3340.
Berger T, Borgdorff A, Crochet S, Neubauer FB, Lefort S, Fauvet B, Ferezou I, Carleton A, Luscher HR, Petersen CC (2007) Combined voltage and calcium epifluorescence imaging in vitro and in vivo reveals subthreshold and suprathreshold dynamics of mouse barrel cortex. Journal of neurophysiology 97:3751-3762.
Bergeron R, Meyer TM, Coyle JT, Greene RW (1998) Modulation of N-methyl-D-aspartate receptor function by glycine transport. Proceedings of the National Academy of Sciences of the United States of America 95:15730-15734.
Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 2:32-48.
Birn RM, Diamond JB, Smith MA, Bandettini PA (2006) Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. NeuroImage 31:1536-1548.
Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med 34:537-541.
Blakemore C, Garey LJ, Vital-Durand F (1978) The physiological effects of monocular deprivation and their reversal in the monkey's visual cortex. The Journal of physiology 283:223-262.
Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. The Journal of physiology 232:331-356.
Bliss TV, Cooke SF (2011) Long-term potentiation and long-term depression: a clinical perspective. Clinics (Sao Paulo) 66 Suppl 1:3-17.
Borgdorff AJ, Poulet JF, Petersen CC (2007) Facilitating sensory responses in developing mouse somatosensory barrel cortex. Journal of neurophysiology 97:2992-3003.

Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S, Phelps L, Phillips AG, Wang YT (2005) Nucleus accumbens long-term depression and the expression of behavioral sensitization. Science 310:1340-1343.
Brier MR, Thomas JB, Snyder AZ, Benzinger TL, Zhang D, Raichle ME, Holtzman DM, Morris JC, Ances BM (2012) Loss of intranetwork and internetwork resting state functional connections with Alzheimer's disease progression. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:8890-8899.
Buchel C, Price C, Frackowiak RS, Friston K (1998) Different activation patterns in the visual cortex of late and congenitally blind subjects. Brain : a journal of neurology 121 (Pt 3):409-419.
Bullmore E, Sporns O (2009) Complex brain networks: graph theoretical analysis of structural and functional systems. Nature reviews Neuroscience 10:186-198.
Burton H, Snyder AZ, Raichle ME (2014) Resting state functional connectivity in early blind humans. Front Syst Neurosci 8:51.
Butt SJ, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G (2005) The temporal and spatial origins of cortical interneurons predict their physiological subtype. Neuron 48:591-604.
Buzsaki G, Draguhn A (2004) Neuronal oscillations in cortical networks. Science 304:1926-1929.
Calvert GA (2001) Crossmodal processing in the human brain: insights from functional neuroimaging studies. Cereb Cortex 11:1110-1123.
Cang J, Kalatsky VA, Lowel S, Stryker MP (2005) Optical imaging of the intrinsic signal as a measure of cortical plasticity in the mouse. Visual neuroscience 22:685-691.
Carroll RC, Beattie EC, Xia H, Luscher C, Altschuler Y, Nicoll RA, Malenka RC, von Zastrow M (1999) Dynamin-dependent endocytosis of ionotropic glutamate receptors. Proceedings of the National Academy of Sciences of the United States of America 96:14112-14117.
Castro-Alamancos MA (2000) Origin of synchronized oscillations induced by neocortical disinhibition in vivo. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:9195-9206.
Chan AW, Mohajerani MH, LeDue JM, Wang YT, Murphy TH (2015) Mesoscale infraslow spontaneous membrane potential fluctuations recapitulate high-frequency activity cortical motifs. Nat Commun 6:7738.
Chang C, Glover GH (2009) Relationship between respiration, end-tidal CO2, and BOLD signals in resting-state fMRI. NeuroImage 47:1381-1393.
Chen L, Muhlhauser M, Yang CR (2003) Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. Journal of neurophysiology 89:691-703.
Chen WS, Bear MF (2007) Activity-dependent regulation of NR2B translation contributes to metaplasticity in mouse visual cortex. Neuropharmacology 52:200-214.
Chiu C, Weliky M (2002) Relationship of correlated spontaneous activity to functional ocular dominance columns in the developing visual cortex. Neuron 35:1123-1134.
Citri A, Malenka RC (2008) Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33:18-41.

Cohen LG, Celnik P, Pascual-Leone A, Corwell B, Falz L, Dambrosia J, Honda M, Sadato N, Gerloff C, Catala MD, Hallett M (1997) Functional relevance of cross-modal plasticity in blind humans. Nature 389:180-183.
Collingridge GL, Isaac JT, Wang YT (2004) Receptor trafficking and synaptic plasticity. Nature reviews Neuroscience 5:952-962.
Collingridge GL, Peineau S, Howland JG, Wang YT (2010) Long-term depression in the CNS. Nature reviews Neuroscience 11:459-473.
Colonnese M, Khazipov R (2012) Spontaneous activity in developing sensory circuits: Implications for resting state fMRI. NeuroImage 62:2212-2221.
Colonnese MT, Khazipov R (2010) "Slow activity transients" in infant rat visual cortex: a spreading synchronous oscillation patterned by retinal waves. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:4325-4337.
Colonnese MT, Kaminska A, Minlebaev M, Milh M, Bloem B, Lescure S, Moriette G, Chiron C, Ben-Ari Y, Khazipov R (2010) A conserved switch in sensory processing prepares developing neocortex for vision. Neuron 67:480-498.
Compston A (2010) The Berger rhythm: potential changes from the occipital lobes in man. Brain : a journal of neurology 133:3-6.
Compte A, Sanchez-Vives MV, McCormick DA, Wang XJ (2003) Cellular and network mechanisms of slow oscillatory activity (<1 Hz) and wave propagations in a cortical network model. Journal of neurophysiology 89:2707-2725.
Contreras D, Steriade M (1997) Synchronization of low-frequency rhythms in corticothalamic networks. Neuroscience 76:11-24.
Cooke SF, Bear MF (2014) How the mechanisms of long-term synaptic potentiation and depression serve experience-dependent plasticity in primary visual cortex. Philosophical transactions of the Royal Society of London Series B, Biological sciences 369:20130284.
Cooper LN, Bear MF (2012) The BCM theory of synapse modification at 30: interaction of theory with experiment. Nature reviews Neuroscience 13:798-810.
Cooper LN, Liberman F, Oja E (1979) A theory for the acquisition and loss of neuron specificity in visual cortex. Biol Cybern 33:9-28.
Cordes D, Haughton VM, Arfanakis K, Wendt GJ, Turski PA, Moritz CH, Quigley MA, Meyerand ME (2000) Mapping functionally related regions of brain with functional connectivity MR imaging. AJNR Am J Neuroradiol 21:1636-1644.
Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, Quigley MA, Meyerand ME (2001) Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. AJNR Am J Neuroradiol 22:1326-1333.
Crair MC, Gillespie DC, Stryker MP (1998) The role of visual experience in the development of columns in cat visual cortex. Science (New York, NY 279:566-570.
Crair MC, Ruthazer ES, Gillespie DC, Stryker MP (1997) Relationship between the ocular dominance and orientation maps in visual cortex of monocularly deprived cats. Neuron 19:307-318.
Crozier RA, Wang Y, Liu CH, Bear MF (2007) Deprivation-induced synaptic depression by distinct mechanisms in different layers of mouse visual cortex. Proceedings of the National Academy of Sciences of the United States of America 104:1383-1388.
Crunelli V, Hughes SW (2010) The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. Nature neuroscience 13:9-17.

Cull-Candy S, Brickley S, Farrant M (2001) NMDA receptor subunits: diversity, development and disease. Current opinion in neurobiology 11:327-335.
Cynader M, Mitchell DE (1980) Prolonged sensitivity to monocular deprivation in dark-reared cats. Journal of neurophysiology 43:1026-1040.
Danysz W, Parsons CG (1998) Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacological reviews 50:597-664.
Davis MF, Figueroa Velez DX, Guevarra RP, Yang MC, Habeeb M, Carathedathu MC, Gandhi SP (2015) Inhibitory Neuron Transplantation into Adult Visual Cortex Creates a New Critical Period that Rescues Impaired Vision. Neuron 86:1055-1066.
Daw NW, Beaver CJ (2001) Developmental changes and ocular dominance plasticity in the visual cortex. Keio J Med 50:192-197.
Daw NW, Reid SN, Beaver CJ (1999a) Development and function of metabotropic glutamate receptors in cat visual cortex. J Neurobiol 41:102-107.
Daw NW, Fox K, Sato H, Czepita D (1992) Critical period for monocular deprivation in the cat visual cortex. Journal of neurophysiology 67:197-202.
Daw NW, Gordon B, Fox KD, Flavin HJ, Kirsch JD, Beaver CJ, Ji Q, Reid SN, Czepita D (1999b) Injection of MK-801 affects ocular dominance shifts more than visual activity. Journal of neurophysiology 81:204-215.
De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM (2006) fMRI resting state networks define distinct modes of long-distance interactions in the human brain. NeuroImage 29:1359-1367.
Dietrich S, Hertrich I, Ackermann H (2013) Ultra-fast speech comprehension in blind subjects engages primary visual cortex, fusiform gyrus, and pulvinar - a functional magnetic resonance imaging (fMRI) study. BMC Neurosci 14:74.
Ding J, Levi DM (2011) Recovery of stereopsis through perceptual learning in human adults with abnormal binocular vision. Proceedings of the National Academy of Sciences of the United States of America 108:E733-741.
Doi A, Mizuno M, Katafuchi T, Furue H, Koga K, Yoshimura M (2007) Slow oscillation of membrane currents mediated by glutamatergic inputs of rat somatosensory cortical neurons: in vivo patch-clamp analysis. The European journal of neuroscience 26:2565-2575.
Doron N, Wollberg Z (1994) Cross-modal neuroplasticity in the blind mole rat Spalax ehrenbergi: a WGA-HRP tracing study. Neuroreport 5:2697-2701.
Drager UC (1975) Receptive fields of single cells and topography in mouse visual cortex. J Comp Neurol 160:269-290.
Drager UC (1978) Observations on monocular deprivation in mice. Journal of neurophysiology 41:28-42.
Drager UC, Olsen JF (1980) Origins of crossed and uncrossed retinal projections in pigmented and albino mice. The Journal of comparative neurology 191:383-412.
Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proceedings of the National Academy of Sciences of the United States of America 89:4363-4367.
Dudek SM, Friedlander MJ (1996) Developmental down-regulation of LTD in cortical layer IV and its independence of modulation by inhibition. Neuron 16:1097-1106.
Duffy S, Labrie V, Roder JC (2008) D-serine augments NMDA-NR2B receptor-dependent hippocampal long-term depression and spatial reversal learning.

Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33:1004-1018.
Dun Y, Duplantier J, Roon P, Martin PM, Ganapathy V, Smith SB (2008) Serine racemase expression and D-serine content are developmentally regulated in neuronal ganglion cells of the retina. J Neurochem 104:970-978.
Dunlop DS, Neidle A (1997) The origin and turnover of D-serine in brain. Biochem Biophys Res Commun 235:26-30.
Fadda E, Danysz W, Wroblewski JT, Costa E (1988) Glycine and D-serine increase the affinity of N-methyl-D-aspartate sensitive glutamate binding sites in rat brain synaptic membranes. Neuropharmacology 27:1183-1185.
Fagiolini M, Hensch TK (2000) Inhibitory threshold for critical-period activation in primary visual cortex. Nature 404:183-186.
Fagiolini M, Katagiri H, Miyamoto H, Mori H, Grant SG, Mishina M, Hensch TK (2003) Separable features of visual cortical plasticity revealed by N-methyl-D-aspartate receptor 2A signaling. Proceedings of the National Academy of Sciences of the United States of America 100:2854-2859.
Firth SI, Wang CT, Feller MB (2005) Retinal waves: mechanisms and function in visual system development. Cell calcium 37:425-432.
Fischer QS, Graves A, Evans S, Lickey ME, Pham TA (2007) Monocular deprivation in adult mice alters visual acuity and single-unit activity. Learn Mem 14:277-286.
Fox CJ, Russell KI, Wang YT, Christie BR (2006a) Contribution of NR2A and NR2B NMDA subunits to bidirectional synaptic plasticity in the hippocampus in vivo. Hippocampus 16:907-915.
Fox MD, Raichle ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nature reviews Neuroscience 8:700-711.
Fox MD, Snyder AZ, Zacks JM, Raichle ME (2006b) Coherent spontaneous activity accounts for trial-to-trial variability in human evoked brain responses. Nature neuroscience 9:23-25.
Fox MD, Snyder AZ, Vincent JL, Raichle ME (2007) Intrinsic fluctuations within cortical systems account for intertrial variability in human behavior. Neuron 56:171-184.
Fox MD, Zhang D, Snyder AZ, Raichle ME (2009) The global signal and observed anticorrelated resting state brain networks. Journal of neurophysiology 101:3270-3283.
Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proceedings of the National Academy of Sciences of the United States of America 102:9673-9678.
Frenkel MY, Bear MF (2004) How monocular deprivation shifts ocular dominance in visual cortex of young mice. Neuron 44:917-923.
Frostig RD, Lieke EE, Ts'o DY, Grinvald A (1990) Cortical functional architecture and local coupling between neuronal activity and the microcirculation revealed by in vivo high-resolution optical imaging of intrinsic signals. Proceedings of the National Academy of Sciences of the United States of America 87:6082-6086.
Furukawa H, Gouaux E (2003) Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core. The EMBO journal 22:2873-2885.
Galli L, Maffei L (1988) Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. Science 242:90-91.

Gandhi SP, Yanagawa Y, Stryker MP (2008) Delayed plasticity of inhibitory neurons in developing visual cortex. Proceedings of the National Academy of Sciences of the United States of America 105:16797-16802.
Garey LJ, Blakemore C (1977) The effects of monocular deprivation on different neuronal classes in the lateral geniculate nucleus of the cat. Experimental brain research 28:259-278.
Ghazanfar AA, Schroeder CE (2006) Is neocortex essentially multisensory? Trends Cogn Sci 10:278-285.
Gilden DL (2001) Cognitive emissions of 1/f noise. Psychol Rev 108:33-56.
Goel A, Lee HK (2007) Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:6692-6700.
Goldreich D, Kanics IM (2003) Tactile acuity is enhanced in blindness. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:3439-3445.
Goodman CS, Shatz CJ (1993) Developmental mechanisms that generate precise patterns of neuronal connectivity. Cell 72 Suppl:77-98.
Gordon JA, Stryker MP (1996) Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:3274-3286.
Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR (2002) Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:6309-6314.
Gougoux F, Lepore F, Lassonde M, Voss P, Zatorre RJ, Belin P (2004) Neuropsychology: pitch discrimination in the early blind. Nature 430:309.
Grandy TH, Greenfield SA, Devonshire IM (2012) An evaluation of in vivo voltage-sensitive dyes: pharmacological side effects and signal-to-noise ratios after effective removal of brain-pulsation artifacts. Journal of neurophysiology 108:2931-2945.
Greicius MD, Krasnow B, Reiss AL, Menon V (2003) Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proceedings of the National Academy of Sciences of the United States of America 100:253-258.
Greicius MD, Supekar K, Menon V, Dougherty RF (2009) Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb Cortex 19:72-78.
Grenier F, Timofeev I, Steriade M (1998) Leading role of thalamic over cortical neurons during postinhibitory rebound excitation. Proceedings of the National Academy of Sciences of the United States of America 95:13929-13934.
Griffiths S, Scott H, Glover C, Bienemann A, Ghorbel MT, Uney J, Brown MW, Warburton EC, Bashir ZI (2008) Expression of long-term depression underlies visual recognition memory. Neuron 58:186-194.
Grinvald A, Hildesheim R (2004) VSDI: a new era in functional imaging of cortical dynamics. Nature reviews Neuroscience 5:874-885.
Grinvald A, Lieke E, Frostig RD, Gilbert CD, Wiesel TN (1986) Functional architecture of cortex revealed by optical imaging of intrinsic signals. Nature 324:361-364.
Guo CX, Babu RJ, Black JM, Bobier WR, Lam CS, Dai S, Gao TY, Hess RF, Jenkins M, Jiang Y, Kowal L, Parag V, South J, Staffieri SE, Walker N, Wadham A, Thompson B, team

Bs (2016) Binocular treatment of amblyopia using videogames (BRAVO): study protocol for a randomised controlled trial. Trials 17:504.
Gustafson EC, Stevens ER, Wolosker H, Miller RF (2007) Endogenous D-serine contributes to NMDA-receptor-mediated light-evoked responses in the vertebrate retina. Journal of neurophysiology 98:122-130.
Gyllensten L, Malmfors T, Norrlin ML (1966) Growth alteration in the auditory cortex of visually deprived mice. The Journal of comparative neurology 126:463-469.
Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, Wedeen VJ, Sporns O (2008) Mapping the structural core of human cerebral cortex. PLoS Biol 6:e159.
Haider B, McCormick DA (2009) Rapid neocortical dynamics: cellular and network mechanisms. Neuron 62:171-189.
Han F, Caporale N, Dan Y (2008) Reverberation of recent visual experience in spontaneous cortical waves. Neuron 60:321-327.
Hanganu IL, Ben-Ari Y, Khazipov R (2006) Retinal waves trigger spindle bursts in the neonatal rat visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:6728-6736.
Hanlon EC, Faraguna U, Vyazovskiy VV, Tononi G, Cirelli C (2009) Effects of skilled training on sleep slow wave activity and cortical gene expression in the rat. Sleep 32:719-729.
Hanover JL, Huang ZJ, Tonegawa S, Stryker MP (1999) Brain-derived neurotrophic factor overexpression induces precocious critical period in mouse visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:RC40.
Harauzov A, Spolidoro M, DiCristo G, De Pasquale R, Cancedda L, Pizzorusso T, Viegi A, Berardi N, Maffei L (2010) Reducing intracortical inhibition in the adult visual cortex promotes ocular dominance plasticity. J Neurosci 30:361-371.
Hartig W, Derouiche A, Welt K, Brauer K, Grosche J, Mader M, Reichenbach A, Bruckner G (1999) Cortical neurons immunoreactive for the potassium channel Kv3.1b subunit are predominantly surrounded by perineuronal nets presumed as a buffering system for cations. Brain research 842:15-29.
Harwerth RS, Smith EL, 3rd, Duncan GC, Crawford ML, von Noorden GK (1986) Multiple sensitive periods in the development of the primate visual system. Science 232:235-238.
Hashimoto A, Oka T, Nishikawa T (1995) Extracellular concentration of endogenous free D-serine in the rat brain as revealed by in vivo microdialysis. Neuroscience 66:635-643.
Hashimoto A, Nishikawa T, Hayashi T, Fujii N, Harada K, Oka T, Takahashi K (1992) The presence of free D-serine in rat brain. FEBS Lett 296:33-36.
Hata Y, Tsumoto T, Stryker MP (1999) Selective pruning of more active afferents when cat visual cortex is pharmacologically inhibited. Neuron 22:375-381.
He BJ, Zempel JM, Snyder AZ, Raichle ME (2010) The temporal structures and functional significance of scale-free brain activity. Neuron 66:353-369.
He HY, Hodos W, Quinlan EM (2006) Visual deprivation reactivates rapid ocular dominance plasticity in adult visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:2951-2955.
He HY, Ray B, Dennis K, Quinlan EM (2007) Experience-dependent recovery of vision following chronic deprivation amblyopia. Nature neuroscience 10:1134-1136.
He K, Petrus E, Gammon N, Lee HK (2012) Distinct sensory requirements for unimodal and cross-modal homeostatic synaptic plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:8469-8474.

Hebb DO (1949) The organization of behavior; a neuropsychological theory. New York,: Wiley.
Heil P, Bronchti G, Wollberg Z, Scheich H (1991) Invasion of visual cortex by the auditory system in the naturally blind mole rat. Neuroreport 2:735-738.
Hendrickson AE, Wilson JR, Ogren MP (1978) The neuroanatomical organization of pathways between the dorsal lateral geniculate nucleus and visual cortex in Old World and New World primates. The Journal of comparative neurology 182:123-136.
Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S, Kash SF (1998) Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science 282:1504-1508.
Herring BE, Nicoll RA (2016) Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking. Annu Rev Physiol 78:351-365.
Heynen AJ, Yoon BJ, Liu CH, Chung HJ, Huganir RL, Bear MF (2003) Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. Nature neuroscience 6:854-862.
Hira R, Honkura N, Noguchi J, Maruyama Y, Augustine GJ, Kasai H, Matsuzaki M (2009) Transcranial optogenetic stimulation for functional mapping of the motor cortex. Journal of neuroscience methods 179:258-263.
Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hubener M (2006a) Lifelong learning: ocular dominance plasticity in mouse visual cortex. Current opinion in neurobiology 16:451-459.
Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hubener M (2006b) Prior experience enhances plasticity in adult visual cortex. Nature neuroscience 9:127-132.
Hollander H, Halbig W (1980) Topography of retinal representation in the rabbit cortex: an experimental study using transneuronal and retrograde tracing techniques. The Journal of comparative neurology 193:701-710.
Honey CJ, Thivierge JP, Sporns O (2010) Can structure predict function in the human brain? NeuroImage 52:766-776.
Honey CJ, Kotter R, Breakspear M, Sporns O (2007) Network structure of cerebral cortex shapes functional connectivity on multiple time scales. Proceedings of the National Academy of Sciences of the United States of America 104:10240-10245.
Horiike K, Tojo H, Arai R, Nozaki M, Maeda T (1994) D-amino-acid oxidase is confined to the lower brain stem and cerebellum in rat brain: regional differentiation of astrocytes. Brain research 652:297-303.
Horng SH, Sur M (2006) Visual activity and cortical rewiring: activity-dependent plasticity of cortical networks. Progress in brain research 157:3-11.
Horton JC, Stryker MP (1993) Amblyopia induced by anisometropia without shrinkage of ocular dominance columns in human striate cortex. Proceedings of the National Academy of Sciences of the United States of America 90:5494-5498.
Howarth C, Gleeson P, Attwell D (2012) Updated energy budgets for neural computation in the neocortex and cerebellum. J Cereb Blood Flow Metab 32:1222-1232.
Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, Tonegawa S (1999) BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98:739-755.
Hubel DH, Wiesel TN (1959) Receptive fields of single neurones in the cat's striate cortex. The Journal of physiology 148:574-591.
Hubel DH, Wiesel TN (1963a) Receptive Fields of Cells in Striate Cortex of Very Young, Visually Inexperienced Kittens. Journal of neurophysiology 26:994-1002.

Hubel DH, Wiesel TN (1963b) Shape and arrangement of columns in cat's striate cortex. The Journal of physiology 165:559-568.
Hubel DH, Wiesel TN (1965) Receptive Fields and Functional Architecture in Two Nonstriate Visual Areas (18 and 19) of the Cat. Journal of neurophysiology 28:229-289.
Hubel DH, Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. The Journal of physiology 206:419-436.
Hubel DH, Wiesel TN (1972) Laminar and columnar distribution of geniculo-cortical fibers in the macaque monkey. The Journal of comparative neurology 146:421-450.
Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. Philosophical transactions of the Royal Society of London Series B, Biological sciences 278:377-409.
Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430:78-81.
Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G (2006) Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nature neuroscience 9:1169-1176.
Huberman AD, Speer CM, Chapman B (2006) Spontaneous retinal activity mediates development of ocular dominance columns and binocular receptive fields in v1. Neuron 52:247-254.
Hyvarinen J, Carlson S, Hyvarinen L (1981) Early visual deprivation alters modality of neuronal responses in area 19 of monkey cortex. Neuroscience letters 26:239-243.
Hyvarinen J, Hyvarinen L, Farkkila M, Carlson S, Leinonen L (1978) Modification of visual functions of the parietal lobe at early age in the monkey. Medical biology 56:103-109.
Innocenti GM, Ansermet F, Parnas J (2003) Schizophrenia, neurodevelopment and corpus callosum. Mol Psychiatry 8:261-274.
Issa NP, Trachtenberg JT, Chapman B, Zahs KR, Stryker MP (1999) The critical period for ocular dominance plasticity in the Ferret's visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:6965-6978.
Iurilli G, Ghezzi D, Olcese U, Lassi G, Nazzaro C, Tonini R, Tucci V, Benfenati F, Medini P (2012) Sound-driven synaptic inhibition in primary visual cortex. Neuron 73:814-828.
Jermakowicz WJ, Chen X, Khaytin I, Bonds AB, Casagrande VA (2009) Relationship between spontaneous and evoked spike-time correlations in primate visual cortex. Journal of neurophysiology 101:2279-2289.
Jiang B, Trevino M, Kirkwood A (2007) Sequential development of long-term potentiation and depression in different layers of the mouse visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:9648-9652.
Jitsuki S, Takemoto K, Kawasaki T, Tada H, Takahashi A, Becamel C, Sano A, Yuzaki M, Zukin RS, Ziff EB, Kessels HW, Takahashi T (2011) Serotonin mediates cross-modal reorganization of cortical circuits. Neuron 69:780-792.
Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529-531.
Jones KR, Spear PD, Tong L (1984) Critical periods for effects of monocular deprivation: differences between striate and extrastriate cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 4:2543-2552.

Junjaud G, Rouaud E, Turpin F, Mothet JP, Billard JM (2006) Age-related effects of the neuromodulator D-serine on neurotransmission and synaptic potentiation in the CA1 hippocampal area of the rat. J Neurochem 98:1159-1166.
Kalatsky VA, Stryker MP (2003) New paradigm for optical imaging: temporally encoded maps of intrinsic signal. Neuron 38:529-545.
Karmarkar UR, Dan Y (2006) Experience-dependent plasticity in adult visual cortex. Neuron 52:577-585.
Kartvelishvily E, Shleper M, Balan L, Dumin E, Wolosker H (2006) Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. J Biol Chem 281:14151-14162.
Kato N, Price DJ, Ferrer JM, Blakemore C (1993) Plasticity of an aberrant geniculocortical pathway in neonatally lesioned cats. Neuroreport 4:915-918.
Kattler H, Dijk DJ, Borbely AA (1994) Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. J Sleep Res 3:159-164.
Kenet T, Bibitchkov D, Tsodyks M, Grinvald A, Arieli A (2003) Spontaneously emerging cortical representations of visual attributes. Nature 425:954-956.
Kim J, Lee S, Park K, Hong I, Song B, Son G, Park H, Kim WR, Park E, Choe HK, Kim H, Lee C, Sun W, Kim K, Shin KS, Choi S (2007) Amygdala depotentiation and fear extinction. Proc Natl Acad Sci U S A 104:20955-20960.
Kimura A, Pavlides C (2000) Long-term potentiation/depotentiation are accompanied by complex changes in spontaneous unit activity in the hippocampus. Journal of neurophysiology 84:1894-1906.
King AJ, Parsons CH (1999) Improved auditory spatial acuity in visually deprived ferrets. The European journal of neuroscience 11:3945-3956.
Kirkwood A, Rioult MC, Bear MF (1996) Experience-dependent modification of synaptic plasticity in visual cortex. Nature 381:526-528.
Kisley MA, Gerstein GL (1999) Trial-to-trial variability and state-dependent modulation of auditory-evoked responses in cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:10451-10460.
Kiviniemi V, Kantola JH, Jauhiainen J, Hyvarinen A, Tervonen O (2003) Independent component analysis of nondeterministic fMRI signal sources. NeuroImage 19:253-260.
Kleim JA, Jones TA (2008) Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. Journal of speech, language, and hearing research : JSLHR 51:S225-239.
Kleindienst T, Winnubst J, Roth-Alpermann C, Bonhoeffer T, Lohmann C (2011) Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites. Neuron 72:1012-1024.
Koch MA, Norris DG, Hund-Georgiadis M (2002) An investigation of functional and anatomical connectivity using magnetic resonance imaging. NeuroImage 16:241-250.
Koralek KA, Killackey HP (1990) Callosal projections in rat somatosensory cortex are altered by early removal of afferent input. Proceedings of the National Academy of Sciences of the United States of America 87:1396-1400.
Kubota Y, Kawaguchi Y (2000) Dependence of GABAergic synaptic areas on the interneuron type and target size. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:375-386.

Kujala T, Alho K, Kekoni J, Hamalainen H, Reinikainen K, Salonen O, Standertskjold-Nordenstam CG, Naatanen R (1995) Auditory and somatosensory event-related brain potentials in early blind humans. Experimental brain research 104:519-526.
Kummer M, Kirmse K, Witte OW, Holthoff K (2012) Reliable in vivo identification of both GABAergic and glutamatergic neurons using Emx1-Cre driven fluorescent reporter expression. Cell calcium 52:182-189.
Lakatos P, Chen CM, O'Connell MN, Mills A, Schroeder CE (2007) Neuronal oscillations and multisensory interaction in primary auditory cortex. Neuron 53:279-292.
Lee H, Brott BK, Kirkby LA, Adelson JD, Cheng S, Feller MB, Datwani A, Shatz CJ (2014) Synapse elimination and learning rules co-regulated by MHC class I H2-Db. Nature 509:195-200.
Lee HK, Whitt JL (2015) Cross-modal synaptic plasticity in adult primary sensory cortices. Current opinion in neurobiology 35:119-126.
Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B (2010) The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:16796-16808.
Lee SH, Simonetta A, Sheng M (2004) Subunit rules governing the sorting of internalized AMPA receptors in hippocampal neurons. Neuron 43:221-236.
Lee SH, Liu L, Wang YT, Sheng M (2002) Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. Neuron 36:661-674.
Lehmann K, Lowel S (2008) Age-dependent ocular dominance plasticity in adult mice. PloS one 3:e3120.
Leopold DA, Murayama Y, Logothetis NK (2003) Very slow activity fluctuations in monkey visual cortex: implications for functional brain imaging. Cereb Cortex 13:422-433.
Lerma J, Zukin RS, Bennett MV (1990) Glycine decreases desensitization of N-methyl-D-aspartate (NMDA) receptors expressed in Xenopus oocytes and is required for NMDA responses. Proceedings of the National Academy of Sciences of the United States of America 87:2354-2358.
Lessard N, Pare M, Lepore F, Lassonde M (1998) Early-blind human subjects localize sound sources better than sighted subjects. Nature 395:278-280.
Levanen S, Hamdorf D (2001) Feeling vibrations: enhanced tactile sensitivity in congenitally deaf humans. Neuroscience letters 301:75-77.
LeVay S, Hubel DH, Wiesel TN (1975) The pattern of ocular dominance columns in macaque visual cortex revealed by a reduced silver stain. The Journal of comparative neurology 159:559-576.
LeVay S, Wiesel TN, Hubel DH (1980) The development of ocular dominance columns in normal and visually deprived monkeys. The Journal of comparative neurology 191:1-51.
Lewis CM, Baldassarre A, Committeri G, Romani GL, Corbetta M (2009) Learning sculpts the spontaneous activity of the resting human brain. Proceedings of the National Academy of Sciences of the United States of America 106:17558-17563.
Li RW, Ngo C, Nguyen J, Levi DM (2011) Video-game play induces plasticity in the visual system of adults with amblyopia. PLoS Biol 9:e1001135.
Lim DH, LeDue JM, Murphy TH (2015) Network analysis of mesoscale optical recordings to assess regional, functional connectivity. Neurophotonics 2:041405.

Linkenkaer-Hansen K, Nikouline VV, Palva JM, Ilmoniemi RJ (2001) Long-range temporal correlations and scaling behavior in human brain oscillations. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:1370-1377.
Lipina T, Labrie V, Weiner I, Roder J (2005) Modulators of the glycine site on NMDA receptors, D-serine and ALX 5407, display similar beneficial effects to clozapine in mouse models of schizophrenia. Psychopharmacology (Berl) 179:54-67.
Liu CH, Heynen AJ, Shuler MG, Bear MF (2008) Cannabinoid receptor blockade reveals parallel plasticity mechanisms in different layers of mouse visual cortex. Neuron 58:340-345.
Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT (2004) Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 304:1021-1024.
Liu YH, Wang L, Wei LC, Huang YG, Chen LW (2009) Up-regulation of D-serine might induce GABAergic neuronal degeneration in the cerebral cortex and hippocampus in the mouse pilocarpine model of epilepsy. Neurochemical research 34:1209-1218.
Lomo T, Mollica A (1959) [Activity of single units of the primary optic cortex during stimulation by light, sound, smell and pain, in unanesthetized rabbits]. Bollettino della Societa italiana di biologia sperimentale 35:1879-1882.
Lorenz, K. Z. (1935). Der Kumpan in der Umwelt des Vogels (The companion in the bird's world). Journal fur Ornithologie, 83, 137-213. (Abbreviated English translation published 1937 in Auk, 54, 245-273.)
Lowe MJ, Mock BJ, Sorenson JA (1998) Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. NeuroImage 7:119-132.
Lu H, Zou Q, Gu H, Raichle ME, Stein EA, Yang Y (2012) Rat brains also have a default mode network. Proceedings of the National Academy of Sciences of the United States of America 109:3979-3984.
Luczak A, Bartho P, Harris KD (2009) Spontaneous events outline the realm of possible sensory responses in neocortical populations. Neuron 62:413-425.
Lynch MA (2004) Long-term potentiation and memory. Physiol Rev 84:87-136.
Maffei A, Turrigiano GG (2008) Multiple modes of network homeostasis in visual cortical layer 2/3. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:4377-4384.
Malonek D, Grinvald A (1996) Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: implications for functional brain mapping. Science 272:551-554.
Mann EO, Kohl MM, Paulsen O (2009) Distinct roles of GABA(A) and GABA(B) receptors in balancing and terminating persistent cortical activity. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:7513-7518.
Marcano-Reik AJ, Blumberg MS (2008) The corpus callosum modulates spindle-burst activity within homotopic regions of somatosensory cortex in newborn rats. The European journal of neuroscience 28:1457-1466.
Martineau M, Baux G, Mothet JP (2006) D-serine signalling in the brain: friend and foe. Trends in neurosciences 29:481-491.
Mascetti L, Foret A, Bourdiec AS, Muto V, Kusse C, Jaspar M, Matarazzo L, Dang-Vu T, Schabus M, Maquet P (2011) Spontaneous neural activity during human non-rapid eye movement sleep. Progress in brain research 193:111-118.

Matsui T, Sekiguchi M, Hashimoto A, Tomita U, Nishikawa T, Wada K (1995) Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. Journal of neurochemistry 65:454-458.
Maya Vetencourt JF, Sale A, Viegi A, Baroncelli L, De Pasquale R, O'Leary OF, Castren E, Maffei L (2008) The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 320:385-388.
Mayhew JE, Askew S, Zheng Y, Porrill J, Westby GW, Redgrave P, Rector DM, Harper RM (1996) Cerebral vasomotion: a 0.1-Hz oscillation in reflected light imaging of neural activity. NeuroImage 4:183-193.
McBain CJ, Kleckner NW, Wyrick S, Dingledine R (1989) Structural requirements for activation of the glycine coagonist site of N-methyl-D-aspartate receptors expressed in Xenopus oocytes. Mol Pharmacol 36:556-565.
McCormick DA, Shu Y, Hasenstaub A, Sanchez-Vives M, Badoual M, Bal T (2003) Persistent cortical activity: mechanisms of generation and effects on neuronal excitability. Cereb Cortex 13:1219-1231.
McCurry CL, Shepherd JD, Tropea D, Wang KH, Bear MF, Sur M (2010) Loss of Arc renders the visual cortex impervious to the effects of sensory experience or deprivation. Nature neuroscience 13:450-457.
McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SM (2005) Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. Science 309:2222-2226.
Meister M, Wong RO, Baylor DA, Shatz CJ (1991) Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. Science 252:939-943.
Membreno JH, Brown MM, Brown GC, Sharma S, Beauchamp GR (2002) A cost-utility analysis of therapy for amblyopia. Ophthalmology 109:2265-2271.
Merabet LB, Hamilton R, Schlaug G, Swisher JD, Kiriakopoulos ET, Pitskel NB, Kauffman T, Pascual-Leone A (2008) Rapid and reversible recruitment of early visual cortex for touch. PloS one 3:e3046.
Metin C, Godement P, Imbert M (1988) The primary visual cortex in the mouse: receptive field properties and functional organization. Experimental brain research 69:594-612.
Migues PV, Hardt O, Wu DC, Gamache K, Sacktor TC, Wang YT, Nader K (2010) PKMzeta maintains memories by regulating GluR2-dependent AMPA receptor trafficking. Nature neuroscience 13:630-634.
Minderer M, Liu W, Sumanovski LT, Kugler S, Helmchen F, Margolis DJ (2012) Chronic imaging of cortical sensory map dynamics using a genetically encoded calcium indicator. The Journal of physiology 590:99-107.
Minlebaev M, Colonnese M, Tsintsadze T, Sirota A, Khazipov R (2011) Early gamma oscillations synchronize developing thalamus and cortex. Science 334:226-229.
Mitchell DE, MacKinnon S (2002) The present and potential impact of research on animal models for clinical treatment of stimulus deprivation amblyopia. Clin Exp Optom 85:5-18.
Mitchell DE, Cynader M, Movshon JA (1977) Recovery from the effects of monocular deprivation in kittens. J Comp Neurol 176:53-63.
Mitchell DE, Murphy KM, Kaye MG (1984) The permanence of the visual recovery that follows reverse occlusion of monocularly deprived kittens. Invest Ophthalmol Vis Sci 25:908-917.

Mitchell DE, Gingras G, Kind PC (2001) Initial recovery of vision after early monocular deprivation in kittens is faster when both eyes are open. Proc Natl Acad Sci U S A 98:11662-11667.
Miyamoto H, Katagiri H, Hensch T (2003) Experience-dependent slow-wave sleep development. Nature neuroscience 6:553-554.
Mohajerani MH, McVea DA, Fingas M, Murphy TH (2010) Mirrored bilateral slow-wave cortical activity within local circuits revealed by fast bihemispheric voltage-sensitive dye imaging in anesthetized and awake mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:3745-3751.
Mohajerani MH, Chan AW, Mohsenvand M, LeDue J, Liu R, McVea DA, Boyd JD, Wang YT, Reimers M, Murphy TH (2013) Spontaneous cortical activity alternates between motifs defined by regional axonal projections. Nature neuroscience 16:1426-1435.
Moldestad O, Karlsen P, Molden S, Storm JF (2009) Tracheotomy improves experiment success rate in mice during urethane anesthesia and stereotaxic surgery. Journal of neuroscience methods 176:57-62.
Monto S, Palva S, Voipio J, Palva JM (2008) Very slow EEG fluctuations predict the dynamics of stimulus detection and oscillation amplitudes in humans. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:8268-8272.
Mooney R, Penn AA, Gallego R, Shatz CJ (1996) Thalamic relay of spontaneous retinal activity prior to vision. Neuron 17:863-874.
Morishita H, Hensch TK (2008) Critical period revisited: impact on vision. Current opinion in neurobiology 18:101-107.
Morrell F (1972) Visual system's view of acoustic space. Nature 238:44-46.
Mothet JP, Parent AT, Wolosker H, Brady RO, Jr., Linden DJ, Ferris CD, Rogawski MA, Snyder SH (2000) D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. Proceedings of the National Academy of Sciences of the United States of America 97:4926-4931.
Mothet JP, Rouaud E, Sinet PM, Potier B, Jouvenceau A, Dutar P, Videau C, Epelbaum J, Billard JM (2006) A critical role for the glial-derived neuromodulator D-serine in the age-related deficits of cellular mechanisms of learning and memory. Aging Cell 5:267-274.
Mower GD (1991) The effect of dark rearing on the time course of the critical period in cat visual cortex. Brain research Developmental brain research 58:151-158.
Murata K, Cramer H, Bach-y-Rita P (1965) Neuronal convergence of noxious, acoustic, and visual stimuli in the visual cortex of the cat. Journal of neurophysiology 28:1223-1239.
Negyessy L, Gal V, Farkas T, Toldi J (2000) Cross-modal plasticity of the corticothalamic circuits in rats enucleated on the first postnatal day. The European journal of neuroscience 12:1654-1668.
Niell CM, Stryker MP (2008) Highly selective receptive fields in mouse visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:7520-7536.
Niemeyer W, Starlinger I (1981) Do the blind hear better? Investigations on auditory processing in congenital or early acquired blindness. II. Central functions. Audiology : official organ of the International Society of Audiology 20:510-515.
Nithianantharajah J, Hannan AJ (2006) Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nature reviews Neuroscience 7:697-709.

Nong Y, Huang YQ, Ju W, Kalia LV, Ahmadian G, Wang YT, Salter MW (2003) Glycine binding primes NMDA receptor internalization. Nature 422:302-307.
Olavarria JF, Li CP (1995) Effects of neonatal enucleation on the organization of callosal linkages in striate cortex of the rat. The Journal of comparative neurology 361:138-151.
Olavarria JF, Safaeian P (2006) Development of callosal topography in visual cortex of normal and enucleated rats. The Journal of comparative neurology 496:495-512.
Pallas SL (2001) Intrinsic and extrinsic factors that shape neocortical specification. Trends in neurosciences 24:417-423.
Paspalas CD, Papadopoulos GC (2001) Serotoninergic afferents preferentially innervate distinct subclasses of peptidergic interneurons in the rat visual cortex. Brain research 891:158-167.
Payne BR (1999) System-wide repercussions and adaptive plasticity: the sequelae of immature visual cortex damage. Restor Neurol Neurosci 15:81-106.
Payne BR, Lomber SG (1998) Neuroplasticity in the cat's visual system. Origin, termination, expansion, and increased coupling of the retino-geniculo-middle suprasylvian visual pathway following early ablations of areas 17 and 18. Experimental brain research 121:334-349.
Petersson P, Waldenstrom A, Fahraeus C, Schouenborg J (2003) Spontaneous muscle twitches during sleep guide spinal self-organization. Nature 424:72-75.
Petrus E, Isaiah A, Jones AP, Li D, Wang H, Lee HK, Kanold PO (2014) Crossmodal induction of thalamocortical potentiation leads to enhanced information processing in the auditory cortex. Neuron 81:664-673.
Pettigrew JD, Ramachandran VS, Bravo H (1984) Some neural connections subserving binocular vision in ungulates. Brain, behavior and evolution 24:65-93.
Pham TA, Graham SJ, Suzuki S, Barco A, Kandel ER, Gordon B, Lickey ME (2004) A semi-persistent adult ocular dominance plasticity in visual cortex is stabilized by activated CREB. Learn Mem 11:738-747.
Philpot BD, Sekhar AK, Shouval HZ, Bear MF (2001) Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. Neuron 29:157-169.
Pizzorusso T, Medini P, Berardi N, Chierzi S, Fawcett JW, Maffei L (2002) Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298:1248-1251.
Prusky GT, Douglas RM (2003) Developmental plasticity of mouse visual acuity. The European journal of neuroscience 17:167-173.
Prusky GT, West PW, Douglas RM (2000) Experience-dependent plasticity of visual acuity in rats. The European journal of neuroscience 12:3781-3786.
Prusky GT, Alam NM, Douglas RM (2006) Enhancement of vision by monocular deprivation in adult mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:11554-11561.
Prusky GT, Silver BD, Tschetter WW, Alam NM, Douglas RM (2008) Experience-dependent plasticity from eye opening enables lasting, visual cortex-dependent enhancement of motion vision. J Neurosci 28:9817-9827.
Puyal J, Martineau M, Mothet JP, Nicolas MT, Raymond J (2006) Changes in D-serine levels and localization during postnatal development of the rat vestibular nuclei. The Journal of comparative neurology 497:610-621.

Quigley M, Cordes D, Turski P, Moritz C, Haughton V, Seth R, Meyerand ME (2003) Role of the corpus callosum in functional connectivity. AJNR Am J Neuroradiol 24:208-212.
Quinlan EM, Philpot BD, Huganir RL, Bear MF (1999) Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex in vivo. Nature neuroscience 2:352-357.
Raichle ME, Mintun MA (2006) Brain work and brain imaging. Annual review of neuroscience 29:449-476.
Raichle ME, Snyder AZ (2007) A default mode of brain function: a brief history of an evolving idea. NeuroImage 37:1083-1090; discussion 1097-1089.
Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001) A default mode of brain function. Proceedings of the National Academy of Sciences of the United States of America 98:676-682.
Rao Y, Daw NW (2004) Layer variations of long-term depression in rat visual cortex. Journal of neurophysiology 92:2652-2658.
Rauschecker JP (1995) Compensatory plasticity and sensory substitution in the cerebral cortex. Trends in neurosciences 18:36-43.
Rauschecker JP (1996) Substitution of visual by auditory inputs in the cat's anterior ectosylvian cortex. Progress in brain research 112:313-323.
Rauschecker JP, Korte M (1993) Auditory compensation for early blindness in cat cerebral cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 13:4538-4548.
Rauschecker JP, Kniepert U (1994) Auditory localization behaviour in visually deprived cats. The European journal of neuroscience 6:149-160.
Rebillard G, Carlier E, Rebillard M, Pujol R (1977) Enhancement of visual responses on the primary auditory cortex of the cat after an early destruction of cochlear receptors. Brain research 129:162-164.
Rehkamper G, Necker R, Nevo E (1994) Functional anatomy of the thalamus in the blind mole rat Spalax ehrenbergi: an architectonic and electrophysiologically controlled tracing study. The Journal of comparative neurology 347:570-584.
Reiter HO, Stryker MP (1988) Neural plasticity without postsynaptic action potentials: less-active inputs become dominant when kitten visual cortical cells are pharmacologically inhibited. Proceedings of the National Academy of Sciences of the United States of America 85:3623-3627.
Rettenbach R, Diller G, Sireteanu R (1999) Do deaf people see better? Texture segmentation and visual search compensate in adult but not in juvenile subjects. J Cogn Neurosci 11:560-583.
Rhoades RW, Mooney RD, Fish SE (1984) A comparison of visual callosal organization in normal, bilaterally enucleated and congenitally anophthalmic mice. Experimental brain research 56:92-105.
Rigas P, Castro-Alamancos MA (2007) Thalamocortical Up states: differential effects of intrinsic and extrinsic cortical inputs on persistent activity. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:4261-4272.
Rittenhouse CD, Shouval HZ, Paradiso MA, Bear MF (1999) Monocular deprivation induces homosynaptic long-term depression in visual cortex. Nature 397:347-350.
Roberts EB, Meredith MA, Ramoa AS (1998) Suppression of NMDA receptor function using antisense DNA block ocular dominance plasticity while preserving visual responses. Journal of neurophysiology 80:1021-1032.

Roder B, Rosler F, Hennighausen E, Nacker F (1996) Event-related potentials during auditory and somatosensory discrimination in sighted and blind human subjects. Brain research Cognitive brain research 4:77-93.
Roder B, Teder-Salejarvi W, Sterr A, Rosler F, Hillyard SA, Neville HJ (1999) Improved auditory spatial tuning in blind humans. Nature 400:162-166.
Rudy B, Fishell G, Lee S, Hjerling-Leffler J (2011) Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev Neurobiol 71:45-61.
Rumbaugh G, Vicini S (1999) Distinct synaptic and extrasynaptic NMDA receptors in developing cerebellar granule neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:10603-10610.
Ruthazer ES, Stryker MP (1996) The role of activity in the development of long-range horizontal connections in area 17 of the ferret. J Neurosci 16:7253-7269.
Ryugo DK, Ryugo R, Globus A, Killackey HP (1975) Increased spine density in auditory cortex following visual or somatic deafferentation. Brain research 90:143-146.
Sadato N, Pascual-Leone A, Grafman J, Ibanez V, Deiber MP, Dold G, Hallett M (1996) Activation of the primary visual cortex by Braille reading in blind subjects. Nature 380:526-528.
Sale A, Maya Vetencourt JF, Medini P, Cenni MC, Baroncelli L, De Pasquale R, Maffei L (2007) Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. Nature neuroscience 10:679-681.
Salzberg BM, Davila HV, Cohen LB (1973) Optical recording of impulses in individual neurones of an invertebrate central nervous system. Nature 246:508-509.
Sanchez-Vives MV, McCormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nature neuroscience 3:1027-1034.
Sato M, Stryker MP (2008) Distinctive features of adult ocular dominance plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:10278-10286.
Sawtell NB, Frenkel MY, Philpot BD, Nakazawa K, Tonegawa S, Bear MF (2003) NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. Neuron 38:977-985.
Schell MJ, Molliver ME, Snyder SH (1995) D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proceedings of the National Academy of Sciences of the United States of America 92:3948-3952.
Schell MJ, Brady RO, Jr., Molliver ME, Snyder SH (1997) D-serine as a neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience 17:1604-1615.
Schwindel CD, McNaughton BL (2011) Hippocampal-cortical interactions and the dynamics of memory trace reactivation. Progress in brain research 193:163-177.
Seelke AM, Blumberg MS (2010) Developmental appearance and disappearance of cortical events and oscillations in infant rats. Brain research 1324:34-42.
Sermasi E, Tropea D, Domenici L (1999) Long term depression is expressed during postnatal development in rat visual cortex: a role for visual experience. Brain research Developmental brain research 113:61-65.
Shatz CJ, Stryker MP (1978) Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. The Journal of physiology 281:267-283.

Sheng M, Cummings J, Roldan LA, Jan YN, Jan LY (1994) Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368:144-147.
Shepherd JD, Huganir RL (2007) The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu Rev Cell Dev Biol 23:613-643.
Shoham D, Glaser DE, Arieli A, Kenet T, Wijnbergen C, Toledo Y, Hildesheim R, Grinvald A (1999) Imaging cortical dynamics at high spatial and temporal resolution with novel blue voltage-sensitive dyes. Neuron 24:791-802.
Shu N, Liu Y, Li J, Li Y, Yu C, Jiang T (2009) Altered anatomical network in early blindness revealed by diffusion tensor tractography. PloS one 4:e7228.
Shulman GL, Fiez JA, Corbetta M, Buckner RL, Miezin FM, Raichle ME, Petersen SE (1997) Common Blood Flow Changes across Visual Tasks: II. Decreases in Cerebral Cortex. J Cogn Neurosci 9:648-663.
Sidorov MS, Kaplan ES, Osterweil EK, Lindemann L, Bear MF (2015) Metabotropic glutamate receptor signaling is required for NMDA receptor-dependent ocular dominance plasticity and LTD in visual cortex. Proceedings of the National Academy of Sciences of the United States of America 112:12852-12857.
Siegel F, Heimel JA, Peters J, Lohmann C (2012) Peripheral and central inputs shape network dynamics in the developing visual cortex in vivo. Curr Biol 22:253-258.
Silasi G, Boyd JD, Ledue J, Murphy TH (2013) Improved methods for chronic light-based motor mapping in mice: automated movement tracking with accelerometers, and chronic EEG recording in a bilateral thin-skull preparation. Frontiers in neural circuits 7:123.
Silasi G, Xiao D, Vanni MP, Chen AC, Murphy TH (2016) Intact skull chronic windows for mesoscopic wide-field imaging in awake mice. Journal of neuroscience methods 267:141-149.
Silingardi D, Scali M, Belluomini G, Pizzorusso T (2010) Epigenetic treatments of adult rats promote recovery from visual acuity deficits induced by long-term monocular deprivation. The European journal of neuroscience 31:2185-2192.
Smith GB, Bear MF (2010) Bidirectional ocular dominance plasticity of inhibitory networks: recent advances and unresolved questions. Frontiers in cellular neuroscience 4:21.
Smith GB, Heynen AJ, Bear MF (2009) Bidirectional synaptic mechanisms of ocular dominance plasticity in visual cortex. Philosophical transactions of the Royal Society of London Series B, Biological sciences 364:357-367.
Snyder AZ, Raichle ME (2012) A brief history of the resting state: the Washington University perspective. NeuroImage 62:902-910.
Somogyi P, Kisvarday ZF, Martin KA, Whitteridge D (1983) Synaptic connections of morphologically identified and physiologically characterized large basket cells in the striate cortex of cat. Neuroscience 10:261-294.
Southwell DG, Froemke RC, Alvarez-Buylla A, Stryker MP, Gandhi SP (2010) Cortical plasticity induced by inhibitory neuron transplantation. Science 327:1145-1148.
Spinelli DN, Starr A, Barrett TW (1968) Auditory specificity in unit recordings from cat's visual cortex. Experimental neurology 22:75-84.
Steriade M, Nunez A, Amzica F (1993a) A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. The Journal of neuroscience : the official journal of the Society for Neuroscience 13:3252-3265.
Steriade M, Contreras D, Curro Dossi R, Nunez A (1993b) The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in

interacting thalamic and neocortical networks. The Journal of neuroscience : the official journal of the Society for Neuroscience 13:3284-3299.
Stevens ER, Esguerra M, Kim PM, Newman EA, Snyder SH, Zahs KR, Miller RF (2003) D-serine and serine racemase are present in the vertebrate retina and contribute to the physiological activation of NMDA receptors. Proceedings of the National Academy of Sciences of the United States of America 100:6789-6794.
Stocca G, Vicini S (1998) Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. The Journal of physiology 507 (Pt 1):13-24.
Stosiek C, Garaschuk O, Holthoff K, Konnerth A (2003) In vivo two-photon calcium imaging of neuronal networks. Proceedings of the National Academy of Sciences of the United States of America 100:7319-7324.
Sur M, Angelucci A, Sharma J (1999) Rewiring cortex: the role of patterned activity in development and plasticity of neocortical circuits. J Neurobiol 41:33-43.
Sutherland GR, McNaughton B (2000) Memory trace reactivation in hippocampal and neocortical neuronal ensembles. Current opinion in neurobiology 10:180-186.
Swindale NV, Vital-Durand F, Blakemore C (1981) Recovery from monocular deprivation in the monkey. III. Reversal of anatomical effects in the visual cortex. Proc R Soc Lond B Biol Sci 213:435-450.
Tagawa Y, Kanold PO, Majdan M, Shatz CJ (2005) Multiple periods of functional ocular dominance plasticity in mouse visual cortex. Nature neuroscience 8:380-388.
Takahashi N, Kitamura K, Matsuo N, Mayford M, Kano M, Matsuki N, Ikegaya Y (2012) Locally synchronized synaptic inputs. Science 335:353-356.
Tasaki I, Warashina A (1976) Dye-membrane interaction and its changes during nerve excitation. Photochem Photobiol 24:191-207.
Thompson I (1997) Cortical development: a role for spontaneous activity? Curr Biol 7:R324-326.
Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, Petreanu L, Akerboom J, McKinney SA, Schreiter ER, Bargmann CI, Jayaraman V, Svoboda K, Looger LL (2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat Methods 6:875-881.
Tigges J, Tigges M (1979) Ocular dominance columns in the striate cortex of chimpanzee (Pan troglodytes). Brain research 166:386-390.
Timofeev I, Steriade M (1996) Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. Journal of neurophysiology 76:4152-4168.
Timofeev I, Grenier F, Bazhenov M, Sejnowski TJ, Steriade M (2000) Origin of slow cortical oscillations in deafferented cortical slabs. Cereb Cortex 10:1185-1199.
Toldi J, Rojik I, Feher O (1994a) Neonatal monocular enucleation-induced cross-modal effects observed in the cortex of adult rat. Neuroscience 62:105-114.
Toldi J, Farkas T, Volgyi B (1994b) Neonatal enucleation induces cross-modal changes in the barrel cortex of rat. A behavioural and electrophysiological study. Neuroscience letters 167:1-4.
Tongiorgi E, Ferrero F, Cattaneo A, Domenici L (2003) Dark-rearing decreases NR2A N-methyl-D-aspartate receptor subunit in all visual cortical layers. Neuroscience 119:1013-1022.
Tononi G, Cirelli C (2003) Sleep and synaptic homeostasis: a hypothesis. Brain Res Bull 62:143-150.
Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10:49-62.

Torborg CL, Feller MB (2005) Spontaneous patterned retinal activity and the refinement of retinal projections. Progress in neurobiology 76:213-235.
Trachtenberg JT (2015) Competition, inhibition, and critical periods of cortical plasticity. Current opinion in neurobiology 35:44-48.
Trachtenberg JT, Stryker MP (2001) Rapid anatomical plasticity of horizontal connections in the developing visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:3476-3482.
Trachtenberg JT, Trepel C, Stryker MP (2000) Rapid extragranular plasticity in the absence of thalamocortical plasticity in the developing primary visual cortex. Science 287:2029-2032.
Tropea D, Van Wart A, Sur M (2009) Molecular mechanisms of experience-dependent plasticity in visual cortex. Philosophical transactions of the Royal Society of London Series B, Biological sciences 364:341-355.
Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. Nature reviews Neuroscience 5:97-107.
Vaegan, Taylor D (1979) Critical period for deprivation amblyopia in children. Trans Ophthalmol Soc U K 99:432-439.
Van Someren EJ, Van Der Werf YD, Roelfsema PR, Mansvelder HD, da Silva FH (2011) Slow brain oscillations of sleep, resting state, and vigilance. Progress in brain research 193:3-15.
van Versendaal D, Levelt CN (2016) Inhibitory interneurons in visual cortical plasticity. Cell Mol Life Sci 73:3677-3691.
Vanni MP, Murphy TH (2014) Mesoscale transcranial spontaneous activity mapping in GCaMP3 transgenic mice reveals extensive reciprocal connections between areas of somatomotor cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:15931-15946.
Vidyasagar TR (1978) Possible plasticity in the rat superior colliculus. Nature 275:140-141.
Vincent JL, Patel GH, Fox MD, Snyder AZ, Baker JT, Van Essen DC, Zempel JM, Snyder LH, Corbetta M, Raichle ME (2007) Intrinsic functional architecture in the anaesthetized monkey brain. Nature 447:83-86.
Volgyi B, Farkas T, Toldi J (1993) Compensation of a sensory deficit inflicted upon newborn and adult animals. A behavioural study. Neuroreport 4:827-829.
Vyazovskiy V, Borbely AA, Tobler I (2000) Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. J Sleep Res 9:367-371.
Vyazovskiy V, Achermann P, Borbely AA, Tobler I (2004) Interhemispheric coherence of the sleep electroencephalogram in mice with congenital callosal dysgenesis. Neuroscience 124:481-488.
Vyazovskiy VV, Tobler I (2005) Regional differences in NREM sleep slow-wave activity in mice with congenital callosal dysgenesis. J Sleep Res 14:299-304.
Vyazovskiy VV, Tobler I (2008) Handedness leads to interhemispheric EEG asymmetry during sleep in the rat. Journal of neurophysiology 99:969-975.
Wagor E, Mangini NJ, Pearlman AL (1980) Retinotopic organization of striate and extrastriate visual cortex in the mouse. The Journal of comparative neurology 193:187-202.
Walsh MK, Lichtman JW (2003) In vivo time-lapse imaging of synaptic takeover associated with naturally occurring synapse elimination. Neuron 37:67-73.

Wang XF, Daw NW (2003) Long term potentiation varies with layer in rat visual cortex. Brain research 989:26-34.
Watson BO, MacLean JN, Yuste R (2008) UP states protect ongoing cortical activity from thalamic inputs. PloS one 3:e3971.
Webb BS, McGraw PV, Levi DM (2006) Learning with a lazy eye: a potential treatment for amblyopia. Br J Ophthalmol 90:518.
Webber AL, Wood J (2005) Amblyopia: prevalence, natural history, functional effects and treatment. Clin Exp Optom 88:365-375.
Weeks R, Horwitz B, Aziz-Sultan A, Tian B, Wessinger CM, Cohen LG, Hallett M, Rauschecker JP (2000) A positron emission tomographic study of auditory localization in the congenitally blind. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:2664-2672.
White BR, Bauer AQ, Snyder AZ, Schlaggar BL, Lee JM, Culver JP (2011) Imaging of functional connectivity in the mouse brain. PloS one 6:e16322.
Wiesel TN (1982) Postnatal development of the visual cortex and the influence of environment. Nature 299:583-591.
Wiesel TN, Hubel DH (1963) Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye. Journal of neurophysiology 26:1003-1017.
Wiesel TN, Hubel DH (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. Journal of neurophysiology 28:1029-1040.
Wiesel TN, Hubel DH, Lam DM (1974) Autoradiographic demonstration of ocular-dominance columns in the monkey striate cortex by means of transneuronal transport. Brain research 79:273-279.
Winnubst J, Cheyne JE, Niculescu D, Lohmann C (2015) Spontaneous Activity Drives Local Synaptic Plasticity In Vivo. Neuron 87:399-410.
Wolosker H, Blackshaw S, Snyder SH (1999a) Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. Proceedings of the National Academy of Sciences of the United States of America 96:13409-13414.
Wolosker H, Panizzutti R, De Miranda J (2002) Neurobiology through the looking-glass: D-serine as a new glial-derived transmitter. Neurochem Int 41:327-332.
Wolosker H, Dumin E, Balan L, Foltyn VN (2008) D-amino acids in the brain: D-serine in neurotransmission and neurodegeneration. The FEBS journal 275:3514-3526.
Wolosker H, Sheth KN, Takahashi M, Mothet JP, Brady RO, Jr., Ferris CD, Snyder SH (1999b) Purification of serine racemase: biosynthesis of the neuromodulator D-serine. Proceedings of the National Academy of Sciences of the United States of America 96:721-725.
Wong RO (1999) Retinal waves and visual system development. Annual review of neuroscience 22:29-47.
Wong TP, Howland JG, Robillard JM, Ge Y, Yu W, Titterness AK, Brebner K, Liu L, Weinberg J, Christie BR, Phillips AG, Wang YT (2007) Hippocampal long-term depression mediates acute stress-induced spatial memory retrieval impairment. Proc Natl Acad Sci U S A 104:11471-11476.
Wood PL, Emmett MR, Rao TS, Mick S, Cler J, Iyengar S (1989) In vivo modulation of the N-methyl-D-aspartate receptor complex by D-serine: potentiation of ongoing neuronal activity as evidenced by increased cerebellar cyclic GMP. Journal of neurochemistry 53:979-981.

Xiong J, Parsons LM, Gao JH, Fox PT (1999) Interregional connectivity to primary motor cortex revealed using MRI resting state images. Hum Brain Mapp 8:151-156.
Xu HP, Furman M, Mineur YS, Chen H, King SL, Zenisek D, Zhou ZJ, Butts DA, Tian N, Picciotto MR, Crair MC (2011) An instructive role for patterned spontaneous retinal activity in mouse visual map development. Neuron 70:1115-1127.
Yaka R, Yinon U, Rosner M, Wollberg Z (2000) Pathological and experimentally induced blindness induces auditory activity in the cat primary visual cortex. Experimental brain research 131:144-148.
Yang K, Xiong W, Yang G, Kojic L, Taghibiglou C, Wang YT, Cynader M (2011) The regulatory role of long-term depression in juvenile and adult mouse ocular dominance plasticity. Sci Rep 1:203.
Yang S, Qiao H, Wen L, Zhou W, Zhang Y (2005) D-serine enhances impaired long-term potentiation in CA1 subfield of hippocampal slices from aged senescence-accelerated mouse prone/8. Neuroscience letters 379:7-12.
Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, Poo M, Duan S (2003) Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. Proceedings of the National Academy of Sciences of the United States of America 100:15194-15199.
Yao H, Shi L, Han F, Gao H, Dan Y (2007) Rapid learning in cortical coding of visual scenes. Nature neuroscience 10:772-778.
Yoon BJ, Smith GB, Heynen AJ, Neve RL, Bear MF (2009) Essential role for a long-term depression mechanism in ocular dominance plasticity. Proceedings of the National Academy of Sciences of the United States of America 106:9860-9865.
Yoshimura Y, Ohmura T, Komatsu Y (2003) Two forms of synaptic plasticity with distinct dependence on age, experience, and NMDA receptor subtype in rat visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:6557-6566.
Yu C, Liu Y, Li J, Zhou Y, Wang K, Tian L, Qin W, Jiang T, Li K (2008) Altered functional connectivity of primary visual cortex in early blindness. Hum Brain Mapp 29:533-543.
Zangaladze A, Epstein CM, Grafton ST, Sathian K (1999) Involvement of visual cortex in tactile discrimination of orientation. Nature 401:587-590.
Zarahn E, Aguirre GK, D'Esposito M (1997) Empirical analyses of BOLD fMRI statistics. I. Spatially unsmoothed data collected under null-hypothesis conditions. NeuroImage 5:179-197.
Zariwala HA, Borghuis BG, Hoogland TM, Madisen L, Tian L, De Zeeuw CI, Zeng H, Looger LL, Svoboda K, Chen TW (2012) A Cre-dependent GCaMP3 reporter mouse for neuronal imaging in vivo. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:3131-3141.
Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nature reviews Neuroscience 4:885-900.