EXPERIENCE-DEPENDENT DEVELOPMENT OF THE VISUAL CORTEX

EXPERIENCE-DEPENDENT MODIFICATION OF EXCITATORY AND INHIBITORY PLASTICITY MECHANISMS IN VISUAL CORTEX.

By

BRETT R. BESTON, B.Sc.

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AUTHOR: Brett R. Beston, B.Sc. Hons (McMaster University)

SUPERVISOR: Professor Kathryn M. Murphy

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Abstract

The immature brain is not simply a small adult brain, and normal development involves a complex series of interactions between nature and nurture. For the visual system, normal development is dependent upon visual experience. Occluding one eye of visual experience early in life (Monocular Deprivation) leads to anatomical and physiological changes in the central visual pathways that leave the deprived eye with poor vision. This has become the best model for studying the most common developmental visual disorder -- lazy-eye (amblyopia). Several neural plasticity mechanisms play essential roles in mediating the development of the neural circuits that underlie visual function. In particular, the major excitatory (NMDA, AMPA) and inhibitory receptors ($GABA_A$) play key roles in synaptic plasticity and the emergence of mature anatomical and physiological properties in the visual system. Recent studies, however, have shown that some of these plasticity mechanisms can be affected by abnormal visual experience. This raises the possibility that the balance of these plasticity mechanisms may be affected by visual experience and in turn, may affect the potential for functional recovery from amblyopia. To address these questions, I initiated a comprehensive series of studies to examine experience-dependent changes in the excitatory and inhibitory mechanisms involved in developmental synaptic plasticity. Using quantitative Western blotting, I examined expression of the major excitatory (NR1, NR2A, NR2B, GluR2), and inhibitory ($GABA_A\alpha1$, $GABA_A\alpha3$) receptor subunits in visual cortex with normal development (human and kitten), abnormal visual experience (monocular deprivation - kittens), and following rearing regimens designed to promote visual recovery (kittens). In normal development, there is a gradual increase in the

expression of NMDA, AMPA, and $GABA_A$ receptors that parallels the emergence of functional plasticity in visual cortex. This development is very prolonged in human visual cortex extending well into later childhood. Monocular deprivation leads to significant changes in the balance between these excitatory and inhibitory plasticity mechanisms, and the changes are not uniform across the visual cortex. The portion of visual cortex where the central visual field is represented is most affected. Optimal recovery of plasticity mechanisms following monocular deprivation is promoted by binocular vision, suggesting that good recovery depends upon both binocularly correlated activity and a sufficient level of visually driven activity. Finally, I implemented a novel neuroinformatics approach (Singular Value Decomposition) to quantify the complex multidimensional changes in the global expression pattern of key plasticity mechanisms. This analysis revealed that monocular deprivation leads to a deviation from the normal developmental trajectory but that a short period of binocular vision is sufficient to shift the trajectory back towards the normal direction. The results of these experiments show that there is a delicate balance between excitatory and inhibitory plasticity mechanisms during normal development, and that this balance is dependent upon visual experience.

Preface

This thesis is partly comprised of a paper (Chapter 2) that is published in Developmental Psychobiology. This chapter was a collaboration between myself, Kathryn Murphy, Philip Boley, and David Jones. I was extensively involved in all aspects of the work, including formulating the experiment, performing the Western blotting, conducting the analyses, and preparing the manuscript for publication.

Development of human visual cortex: a balance between excitatory and inhibitory plasticity mechanisms, Murphy KM, Beston BR, Boley PM, Jones DG, Developmental Psychobiology 46:209-221

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I am very lucky to have parents that have never told me what to do, but were always behind me with every decision that I have ever made. It's with that kind of support and independence that I have been able to achieve more than I ever thought possible. They represent the foundations of who I am as a person, and my achievements in life reflect how much they have done for me. I may not say it that often, but I love you both so very much.

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LIST OF ABREVIATIONS

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole
BD	Binocular Deprivation
BV	Binocular Vision
DTT	dithiothreitol
EDTA	ethylene diamine tetraacetic acid
ECL	Enhanced Chemiluminescence
EGTA	ethylene glycol tetraacetic acid
EPSC	Excitatory Post-Synaptic Current
GABA	gamma-amino-butyric-acid
GAD	glutamic acid decarboxylase
HEPES	N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid
IPSC	Inhibitory Post-Synaptic Current
NMDA	N-methyl-D-aspartate
N	Normal
MD	Monocular Deprivation
PBS-T	Phosphate Buffered Saline-Triton X
PCA	Principle Component Analysis
PMI	Post-Mortem Interval
PVDF	polyvinylidine difluoride
RO	Reverse Occlusion
SDS	sodium dodecyl sulfate
SEM	Standard Error of the Mean
SVD	Singular Value Decomposition
V1	Primary Visual Cortex

Chapter 1

General Introduction

Experience Shapes the Development of Visual Cortex

Although newborn infants can perceive basic visual information, multiple measures have shown that visual acuity in infants is roughly 40 times worse than in adults (Mayer and Dobson, 1982; Mayer et al., 1995). During postnatal development, the visual cortex undergoes substantial physiological and anatomical changes that parallel the emergence and maturation of visual abilities. This development is not a singular process, and requires appropriate visual experience. Changes in the quality of visual experience can dramatically and permanently alter the development of visual function. For instance, visual impairment early in life, such as a unilateral congenital cataract can cause permanent deficits in the visual acuity of the affected eye (Ellemberg et al., 2000; Maurer and Lewis, 2001). These changes are restricted to a specific 'critical period' in which the development of the visual system can be altered by experience (Hubel and Wiesel, 1970). Evidence from animal and human studies has helped to define the critical period for humans between 1 month and 10 years of age (Maurer and Lewis, 2001), and between 3 to 12 weeks of age in kittens (Olson and Freeman, 1980). The consequences of monocular deprivation reflect an experience-dependent change in the function of primary visual cortex as monocular deprivation does not affect the anatomical or physiological properties of the retina (Hendrickson and Kupfer, 1976).

In the 1960's, Wiesel and Hubel (Wiesel and Hubel, 1965; Hubel and Wiesel, 1970) discovered that monocular deprivation early in post natal development reduces the number of binocular neurons and shifts the response of neurons in visual cortex in favour of the non-deprived eye. The changes promoted by monocular deprivation have been further broken down into short-term physiological changes in the responsiveness of neurons followed by longer-

term anatomical reorganization of each eye's representation in visual cortex. A short period of monocular deprivation in kittens (2-7 days) induces changes in the physiological responsiveness of neurons in the visual cortex (Olson and Freeman, 1975; Trachtenberg et al., 2000), triggers rapid mobilization of dendritic spines (Oray et al., 2004) and shrinks the geniculocortical axonal arbors of the deprived eye (Antonini and Stryker, 1996). Prolonging monocular deprivation in kittens, induces a large-scale reorganization of geniculocortical afferents including expansion of non-deprived eye arbors (Antonini and Stryker, 1996), that leaves only 10% of neurons in V1 responsive to the deprived eye (Shatz and Stryker, 1978).

Further investigation has shown that other forms of visual deprivation, such as binocular deprivation, have surprisingly little effect on the percentage of binocular neurons remaining in visual cortex (Wiesel and Hubel, 1965). It has been concluded from these studies that functional development of visual pathways do not rely on the absolute amount of activity, but rather on the binocular correlation of visually driven activity. Within this framework, synaptic connections are modified according to the Hebbian rules of synaptic plasticity (Hebb, 1949), whereby neural inputs from the deprived eye are weaker than inputs from the non deprived and will eventually become disconnected in visual cortex. Monocular deprivation, and the disruption of binocular activity has quickly become the central model for studying the neural basis of lazy eye (amblyopia).

Functional recovery of vision is possible if vision is restored in the deprived eye during the critical period. Patching strategies designed to promote recovery of the deprived eye by occluding non deprived eye (reverse occlusion), can lead to good visual recovery in both animal models (Van Sluyters, 1978) and

children with a congenital cataract (Lewis et al., 1995). Physiological studies in kittens have shown that just 18 days of reverse occlusion can promote a nearly complete reversal of the physiological changes in visual cortex (Movshon, 1976). The long-term outcome of patching therapy, however, is often not permanent. In children, the improvement in visual acuity is transient in 20-80% of the cases (Levartovsky et al., 1998; Ohlsson et al., 2002; Simons, 2005). In kittens, restoring binocular vision following reverse occlusion leads to a rapid loss of vision in the initially deprived eye, resulting in permanent visual deficits in both eyes (Mitchell et al., 1984; Murphy and Mitchell, 1986, 1987). The lack of permanent recovery of visual function suggests that the neural plasticity mechanisms mediating changes during reverse occlusion do not support long-term recovery of normal function in the central visual pathways. While the nature of this recovery remains puzzling, new evidence in kittens has uncovered that interleaving a period of 4 days of binocular visual experience between monocular deprivation and reverse occlusion does promote a sustained improvement in the behavioural acuity (Murphy et al., 2002), and physiological representation (Kind et al., 2002; Faulkner et al., 2006) of the deprived eye in visual cortex.

Plasticity Mechanisms in Visual Cortex

There is an emerging consensus that developmental plasticity in visual cortex and therefore, the impact of monocular deprivation on visual development are mediated by the response of excitatory and inhibitory neural plasticity mechanisms to changing patterns of activity and the functioning of those mechanisms (Kirkwood and Bear, 1994; Hensch et al., 1998; Fagiolini et al., 2003). Many studies have elucidated a wide range of physiological and behavioural effects of monocular deprivation. In contrast, less is known about the effect of

abnormal visual experience on the mechanisms that underlie the development of visual cortex.

AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole) and NMDA (N-methyl-D-aspartate) are the two major types of glutamate receptors that mediate the different components of the excitatory post-synaptic current (EPSC). AMPA receptors mediate the fast component of the excitatory response (2ms) (Kleppe and Robinson, 1999), and are composed of multiple subunits, GluR (1-4) (Keinanen et al., 1990). Insertion of the GluR2 subunit affects AMPA receptor maturation and its role in synaptic plasticity by regulating Ca^{2+} permeability and facilitating synaptic transmission (Jonas et al., 1994). GluR2 expression increases during development (Herrmann, 1996), and can be affected by brief monocular deprivation during the critical period, contributing to synaptic depression in the deprived cortex (Heynen et al., 2003).

NMDA receptors mediate the slow component (190-380ms) of the EPSC and have been linked to the formation of nascent synapses, synaptic plasticity and learning and memory (Monyer et al., 1992, 1994; Flint et al., 1997; Roberts and Ramoa, 1999). NMDA receptors form a heteromeric ion channel composed of one obligatory subunit (NR1) combined with one or more NR2(A-D) or NR3 subunits (Monyer et al., 1992). The different NR2 subunits affect the functional and pharmacological properties of the receptor (Kutsuwada et al., 1992). For example, The decay of the EPSC speeds up 2-3 fold when the receptor includes NR2A subunits (Flint et al., 1997).

The NMDA receptor is as a ligand-gated ion channel. In its non-active state, the channel is blocked by a single Mg^{2+} ion. NMDA receptor activation is dependent upon binding of glutamate and the activation of neighboring AMPA

receptors. The resulting cellular depolarization triggers the displacement of the Mg^{2+} ion from the NMDA receptor ion channel allowing an influx of both Na^{+} and Ca^{2+} ions (Bliss and Collingridge, 1993). In developing visual cortex, Ca^{2+} influx is a critical trigger for long-term potentiation and long-term depression (Bear and Kirkwood, 1993; Aroniadou and Teyler, 1991), that are essential components in the development of visual cortical circuitry (Tsumoto et al. 1990; Bear et al., 1996).

During early development, NMDA receptors are composed of primarily more NR1 and NR2B subunits and there is a developmental switch to insert NR2A into the receptor that closely parallels the onset of the critical period for ocular dominance plasticity (Roberts and Ramoa, 1999; Chen and Mower, 2000). Blocking NMDA mediated activity prevents the development of orientation selectivity (Ramoa et al., 2001), and disrupts sensitivity to monocular deprivation in young animals (Kleinschmidt et al., 1987; Roberts and Ramoa, 1998). In addition, expression of NMDA receptor subunits is dependent upon visual experience. Even a short period of total visual deprivation can significantly reduce the NR2A expression (Quinlan et al., 1999). Furthermore, monocular deprivation leads to changes in NR1 expression in visual cortex, with a loss in the central visual field representation where binocular correlation has been reduced, and a homeostatic up-regulation in the monocular region where activity has been reduced (Murphy et al., 2004).

Many studies have also shown that GABAergic (gamma-aminobutyric-acid) mechanisms play a critical role in developmental plasticity in visual cortex (Hensch et al., 1998; Iwai et al., 2003; Fagiolini et al., 2003, 2004; Hensch and Stryker, 2004). $GABA_A$ receptors are the dominant ionotropic inhibitory receptors

in visual cortex, conducting Cl^- ions leading to hyperpolarization of the neuronal membrane. $GABA_A$ is a heteromeric receptor comprised of subunits from at least eight distinct families; α(1-6), β(1-3), γ(1-3), δ, ε, π, θ, ρ (Bonnert et al., 1999; Whiting et al., 1999). Although the composition of $GABA_A$ receptors varies, the majority of $GABA_A$ receptors are composed of 2 α, 2 β, and 1 γ subunits (Araujo et al., 1996; Sieghart et al., 1999). Early in development, the $GABA_A$ receptor is composed mainly of α3 subunits (Laurie et al., 1992; Chen et al., 2001; Murphy et al., 2005) which have a lower binding affinity for GABA (Bohme et al., 2004) and slower IPSC decay (Bosman et al., 2002). During maturation, there is a shift to more α1 expression (Laurie et al., 1992; Hendrickson et al., 1994; Fritschy et al., 1999; Chen et al., 2001) which has a higher binding efficacy and faster inhibitory post-synaptic current (IPSC) decay (Bosman et al., 2002). The expression of α1 is crucial for the onset of the critical period (Fagiolini et al., 2004) and experimental manipulation of GABA-mediated inhibition affects ocular dominance plasticity (Hensch, 1998). Specifically, reducing GABA-mediated inhibition delays the onset of the critical period while enhancing GABA-mediated inhibition advances the critical period (Hensch et al., 1998; Fagiolini and Hensch, 2000; Fagiolini et al., 2003).

During normal development, there are similar developmental changes in NMDA and $GABA_A$ subunits (Chen et al. 2000, 2001), suggesting that there is a developmental balance in the maturation of excitatory and inhibitory receptors. Even small changes in the balance between excitation and inhibition, however, can dramatically alter experience-dependent plasticity in visual cortex (Kirkwood and Bear, 1994; Hensch et al., 1998; Iwai et al., 2003). For example, deletion of the NR2A subunit prolongs NMDA mediated currents and shifts the balance towards

excitation, effectively weakening plasticity in visual cortex (Fagiolini et al., 2003). Plasticity in these animals can be restored by enhancing inhibition with diazepam infusion, thus illustrating the importance of a regulated balance between excitation and inhibition to maintaining functional circuitry and facilitating experience-dependent plasticity (Turrigiano and Nelson, 2004).

These findings demonstrate that changes in AMPA, NMDA, and $GABA_A$ receptor composition play a key role in governing the balance between excitation and inhibition and, therefore, are essential components of developmental plasticity. Accordingly, I examined the effect of monocular deprivation on ocular dominance plasticity by comparing AMPA, NMDA, and $GABA_A$ receptor subunit expression between normal and monocularly deprived kittens during the critical period.

Evaluating how visual experience drives changes in excitatory and inhibitory mechanisms can provide valuable insights toward understanding how visual experience modifies the mechanisms that underlie plasticity in visual cortex. In this thesis, I present a thorough investigation of the development of excitatory and inhibitory receptors in human infant (Chapter 2) and kitten (Chapter 3) visual cortex during normal visual development, after monocular deprivation (Chapter 3), and following treatment paradigms that promote the long-term recovery of visual function (Chapter 4). The main objective of these studies is to determine the normal expression of NMDA, AMPA, and $GABA_A$ receptors and whether the recovery of visual function coincides with the recovery of receptor expression. The results of these studies will provide new insights into the relationship between fundamental mechanisms of experience-dependent plasticity and the development of visual cortex.

Chapter 2

Development of Human Visual Cortex: A Balance Between Excitatory and Inhibitory Plasticity Mechanisms

Abstract

Formation of neural circuitry in the developing visual cortex is shaped by experience during the critical period. A number of mechanisms, including N-methyl-D-aspartate (NMDA) receptor activation and γ-aminobutyric acid (GABA)-mediated inhibition, are crucial in determining the onset and closure of the critical period for visual plasticity. Animal models have shown that a threshold level of tonic inhibition must be reached in order for critical period plasticity to occur and that NMDA receptors contribute to Hebbian synaptic plasticity in the developing visual cortex. There are a number of developmental changes in these glutamatergic and GABAergic mechanisms that have been linked to plasticity. Those changes, however, have only been shown in animal models and their development in the human visual cortex is not known. We have addressed this question by studying the expression of the major glutamatergic receptors, $GABA_A$ receptors, and glutamic acid decarboxylase (GAD) isoforms during the first 6 years of postnatal development of human visual cortex. There are significant changes in the expression of these proteins during postnatal development of human visual cortex. The time course of the changes is quite prolonged and suggests that it may set the pace for the prolonged critical period in human visual development. The changes also affect the nature of spatial and temporal integration in visual cortical neurons and thereby contribute to the maturation of visual functions.

Introduction

The immature brain is not simply a small adult brain and normal development of the brain depends on a complex series of interactions between nature and nurture during the critical period. Studies of the developing visual system have provided many insights into the roles of experience and neural plasticity mechanisms in cortical development. The original studies of Hubel & Wiesel (1965) showed that experience-dependent competitive interactions, following the rules of Hebbian synaptic plasticity (e.g., Miller, 1994; Swindale, 1980), are central components of developmental plasticity in the visual system. Within this framework, activation of cortical neurons by correlated binocular visual activity leads to strengthening of both eyes' connections, but at locations where one eye's inputs are more effective at driving cortical targets, those connections will be strengthened while the other eye's connections will be weakened. Much of the appeal of this model of critical period plasticity comes from the fact that it is supported by evidence from behavioural, anatomical, and physiological studies in a variety of species.

Animal studies have shown that many neural plasticity mechanisms play a role in critical period experience-dependent development of the visual cortex (Katz & Shatz, 1996, Katz & Crowley, 2002). A key postsynaptic mechanism is activation of the ionotropic N-methyl-D-aspartate (NMDA) receptor for the excitatory amino acid glutamate which is the main excitatory neurotransmitter in the visual cortex (Bear et al, 1990; Constantine-Paton et al, 1990; Singer & Artola, 1991; Daw et al, 1994). The membrane voltage-dependence of the NMDA receptor makes it an ideal mechanism for mediating Hebbian synaptic plasticity and responding to correlated patterns of activity such as those driven by binocular

vision (Fox & Daw, 1993; Bear, 1995). Furthermore, the largest response to activation of NMDA receptors occurs during the critical period (Tsumoto et al., 1987). A number of studies have shown that blockade of the NMDA receptor during the critical period reduces ocular dominance plasticity (Kleinschmidt et al, 1987; Bear et al, 1990; Roberts et al, 1998) and stunts the maturation of orientation selectivity (Ramoa et al, 2001). These results suggest that activation of the NMDA receptor is essential for sculpting functional circuits in the developing visual cortex.

Activation of the NMDA receptor, however, is not the sole mechanism involved in experience-dependent developmental plasticity in the visual cortex. Several receptors, ionic channels, second messengers, growth factors, and signalling molecules play essential roles in the development of the visual cortex. There is an emerging view that a fundamental component of visual cortical development is the balance between excitation and inhibition (Turrigiano & Nelson, 2004, Beston et al, 2004). Animal studies of excitatory (NMDA, AMPA) and inhibitory ($GABA_A$) receptors have shown that they appear to function in concert to establish the appropriate level of cortical activity to facilitate experience-dependent plasticity.

The excitatory glutamatergic receptors -- AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA -- play a central role in visual cortex development. AMPA receptors mediate the fast component (a few milliseconds) of excitatory neurotransmission (Kleppe & Robinson, 1999) and expression of AMPA receptors is affected by visual experience. Suturing one eye closed causes reduced AMPA expression (Kumar et al, 1994) and a change in the phosphorylation state of the AMPA receptor that is linked to depression of

synaptic transmission (Heynen et al, 2003). Functional NMDA receptors mediate the slow component of the excitatory postsynaptic current (EPSC) and are composed of multiple subunits (including NR1, and NR2A-D subunits) (Moriyoshi et al, 1991; Kutsuwada et al, 1992; Monyer et al, 1992). Animal studies have shown developmental changes in the expression of the NR1, NR2A, and NR2B subunits (Monyer et al, 1994; Sheng et al, 1994; Laurie et al, 1997). In the immature visual cortex synaptic NMDA receptors are composed of NR1 and NR2B subunits and there is a subsequent inclusion of NR2A subunits (Stocca & Vicini, 1998). The NR2 subunits affect functioning of the receptor largely by changing the opening time of the ion channel and affecting the decay time of the EPSC. A receptor composed of NR1 and NR2B subunits is very sluggish, about 380ms decay, but becomes faster (~190ms) when NR2A is inserted into the receptor (Monyer et al, 1992, 1994; Flint et al, 1997; Roberts and Ramoa, 1999). These developmental changes in NMDA subunit composition occur during the sensitive period for visual cortical plasticity and have been related to synaptogenesis (Tovar & Westbrook, 1999), decline of synaptic plasticity (Sheng et al, 1994), changes in synaptic modification (Quinlan et al, 1999a, 1999b; Philpot et al, 2001), the critical period for ocular dominance plasticity (Roberts & Ramoa, 1999; Chen et al, 2000; Erisir & Harris, 2003; Fagiolini et al, 2003), and the maturation of orientation preference (Fagiolini et al, 2003).

Even small changes in the relative amounts of excitation and inhibition can dramatically alter experience-dependent plasticity (Kirkwood & Bear, 1994; Hensch et al, 1998; Iwai et al, 2003). This exquisite balance between excitation and inhibition is dynamically adjusted during development. The main inhibitory neurotransmitter in the cortex is γ-aminobutyric acid (GABA) and it is synthesized by 2 isoforms of glutamic acid decarboxylase (GAD65 and GAD67).

GAD65 is found mainly in axon terminals bound to synaptic vesicles, while GAD67 is found mainly in cell bodies. In cat visual cortex there are substantial postnatal increases in both isoforms of GAD (Guo et al., 1997). Expression of the GABAergic receptors also change during development. Receptor binding and immunohistochemical studies of macaque and marmoset monkey visual cortex have shown substantial postnatal increases in $GABA_A$ receptor expression (Shaw et al, 1991; Hendrickson et al, 1994; Hornung & Fritschy, 1996), but mRNA levels in macaques do not change dramatically after birth (Huntsman et al, 1999). In the immature cortex the kinetics of the ionotropic $GABA_A$ receptors are relatively slow (Gingrich et al, 1995) because the receptor includes the $GABA_A\alpha3$ subunit (Laurie et al, 1992). This slow decay contributes to a more tonic level of inhibition that is an essential component of ocular dominance plasticity (Hensch et al, 1998; Iwai et al, 2003). The kinetics of the $GABA_A$ receptor speed up threefold during development when the $GABA_A\alpha1$ subunit dominates the receptor complex. Furthermore, the inclusion of $GABA_A\alpha1$ contributes to the transition to shorter lived, more phasic inhibition that results from faster IPSC decay in the cortex (Iwai et al, 2003). This developmental switch in $GABA_A$ subunits occurs in rats (Bosman et al, 2002), cats (Chen et al, 2001) and macaque monkeys (Hendrickson et al, 1994), however, it is correlated with different aspects of the critical period. In rats the switch occurs before the start of the critical period (Heinen et al, 2004), but in cats and macaque monkeys the switch overlaps the critical period for ocular dominance plasticity (Chen et al, 2001; Hendrickson et al, 1994).

Taken together, the animal studies have linked developmental changes in NMDA, AMPA, and GABA receptor expression and the balance between these excitatory and inhibitory mechanisms with plasticity during the critical period.

Each receptor plays a role in experience-dependent developmental plasticity and balancing this cast of excitatory and inhibitory plasticity players is an essential component of functional neural development. But how do these plasticity players change during development of human visual cortex? While it may seem reasonable to extend the observations from animal studies to humans, to date there have been no studies of AMPA receptors, NMDA receptors, GABA receptors and GAD expression during postnatal development of human visual cortex. At birth, many aspects of human visual cortex are more mature than the animal models (mouse, rat, ferret, cat). Yet, in humans there is a very prolonged period of functional plasticity, lasting out to 8-10 years of age, when the human visual system is still susceptible to the effects of visual deprivation (Vaegan and Taylor, 1979; Maurer and Lewis, 2001). These differences make it difficult to extrapolate from the animal studies to human development and leave unanswered basic questions about developmental changes of these neural plasticity mechanisms in human visual cortex. We have addressed these questions by studying the expression of the major glutamatergic and GABAergic mechanisms during the first 6 years of postnatal development of human visual cortex. There are significant developmental changes in some of these plasticity mechanisms but the time course of the changes is slower than would be predicted from the animal studies. A portion of these data have been presented previously (Beston et al, 2002; Boley et al, 2004).

Materials and Methods

Subjects and Tissue

Tissue samples were obtained from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland (Baltimore, MD). The samples were from the posterior pole of the left hemisphere of human primary visual cortex including both superior and inferior portions of the calcarine fissure. This portion of primary visual cortex is where the central visual field is represented (DeYoe et al, 1996). The samples were from 16 individuals ranging in age from 20 days to just under 5 and half years (Table 1). The subjects were divided into 3 groups: less than 1 year of age (n=9), 1-3 years of age (n=3), and over 3 years of age (n=4). All samples were obtained within 23 hours postmortem and the occipital lobe was dissected according to the gyral and sulcal landmarks. At the Brain and Tissue Bank the left hemisphere was fresh frozen after being sectioned coronally in 1 cm intervals, rinsed with water, blotted dry, placed in a quick freeze bath (dry ice and isopentane), and stored frozen (-70°C).

Table 1: Human visual cortex tissue samples

Age (Days)	PMI (hours)
20	9
86	23
96	12
98	16
119	22
120	23
133	16
136	11
273	10
488	21
787	21
805	11
1218	11
1663	15
1718	17
1969	17

Tissue Sample Preparation

A piece of tissue (approximately 100mg) was cut from the frozen block of primary visual cortex and immediately suspended in cold homogenization buffer (0.5 mM DTT, 1 mM EDTA, 2 mM EGTA, 10 mM HEPES, 10 mg/l leupeptin, 100nM microcystin, 0.1 mM PMSF, 50 mg/l soybean trypsin inhibitor). The tissue sample was homogenized in a glass-glass Dounce tissue homogenizer (Kontes, Vineland, NJ) and a portion of the whole homogenate was removed and kept for analysis of the inhibitory GABAergic receptors and transmitter. A subcellular

fractionation procedure (synaptoneurosomes) (Hollingsworth et al, 1985; Quinlan et al, 1999a), was used on the remaining homogenate to obtain protein samples enriched for excitatory glutamatergic synapses (NMDA and AMPA receptors) where PSD-95 is the synaptic anchoring protein. Briefly, the synaptoneurosome was obtained by passing the homogenate through a coarse 100μm pore nylon mesh filter followed by a fine 5μm pore hydrophilic mesh filter (Millipore, Bedford, MA) and then centrifuged at 1000x g for 10 minutes to obtain the synaptic fraction of the membrane. Both the synaptic pellet and the whole homogenate samples were resuspended in boiling 1% SDS and stored at –80°C. Protein concentrations were determined using the BCA assay guidelines (Pierce, Rockford, IL). Samples of the supernate and whole homogenate from each case were used to verify the synaptic enrichment of the pellet.

Immunoblotting

The samples (10-25μg) were separated on polyacrylamide gels and transferred to PVDF membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Each sample was run 3 times. Membranes were preincubated for 4 hours at room temperature in phosphate-buffered saline containing 0.05% Triton X-100 (Sigma, St. Louis, MO) (PBS-T), and 5% (w/v) skim milk, briefly washed in PBS-T, and then incubated in primary antibody overnight at 4°C using the following concentrations: NR1 54.1, 1:1000 (BD Biosciences Pharmingen, San Diego, CA); NR2A, 1:1000; NR2B, 1:1000; GluR2, 1:1000 (Zymed Laboratories, South San Francisco, CA); $GABA_A\alpha1$, 1:200 (Santa Cruz Biotechnology, Santa Cruz, CA); $GABA_A\alpha3$, 1:1000; GAD65, 1:1000; GAD67, 1:500 (Chemicon International, Temecula, CA). The membranes were incubated with the appropriate HRP labeled secondary antibody (1:2000, Cedarlane Laboratories LTD, Hornby, ON)

and washed (PBS-T 6 x 10min, PBS 3 x 10min). Immunoreactivity was visualized using enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech, Piscataway, NJ) and exposed onto autoradiographic film (X-Omat AR, Kodak, Rochester, NY). The blots were stripped and reprobed with additional antibodies using the Blot Restore Membrane Rejuvenation kit (Chemicon International, Temecula, CA).

Analysis

The density of the bands was measured by densitometry. The films were scanned (16 bit, AGFA ArcusII, Agfa, Germany) along with an optical density wedge (Oriel Corporation, Baltimore, MD) and the intensities of the bands were converted to optical densities. The background optical density was subtracted from each band and the band density was quantified using Matlab (The MathWorks, Inc, Natick, MA). Due to the appearance of multiple bands for some antibodies because of postmortem degradation (Wang et al, 2000) (usually within 10kD below expected weight) the sum of all bands was used as the total expression. One of the samples (case 185) was run on all of the blots and the optical density of each sample was measured relative to that sample. The densities were normalized to the total expression of all antibodies. The data was grouped into 3 stages of postnatal development (< 1 year, 1-3 years, 3-6 years) following the groupings used by Law et al (2003) for studying human hippocampal development. Group means were calculated for each antibody and normalized to the mean level of expression for the youngest group. For some of the receptor subunits an important comparison is the relative expression of 2 subunits. To quantify the relative levels of expression the ratios of NR2A:NR2B ((NR2A - NR2B) / (NR2A + NR2B)) and $GABA_A\alpha1$:$GABA_A\alpha3$ (($GABA_A\alpha1$ -

$GABA_A\alpha3$) / ($GABA_A\alpha1$ + $GABA_A\alpha3$)) were calculated for each case and then group means were calculated. Statistical comparisons between the groups were calculated using Kruskal-Wallis non-parametric analysis of variance and planned pair-wise comparisons.

Results

The tissue samples were collected over a range ofpostmortem intervals (Table 1, 9-23 hours) and we first determined whether the length of the postmortem interval affected expression of the various antibodies. There were no significant correlations (all had $p > 0.1$) between the length of postmortem interval and the level of expression of any for the glutamatergic (NR1, R=0.04; NR2A, R=0.09; NR2B, R=0.19, GluR2, R=0.17), or GABAergic (GAD65, R=0.17; GAD67, R=0.27, $GABA_A\alpha1$, R=0.01, $GABA_A\alpha2$, R=0.01, $GABA_A\alpha3$, R=0.41) antibodies.

Glutamate Receptor Expression

Expression of the NR1 subunit during development was quantified for samples of the whole homogenate and synaptoneurosome preparations. Expression of NR1 in the whole homogenate samples was relatively constant during postnatal development (Fig 2.1A). There were no significant differences between the 3 age groups in the level of whole homogenate NR1 expression. The synaptoneurosome samples showed an approximately 3-fold enrichment for the excitatory synaptic anchoring protein PSD-95 compared with the whole homogenate samples (data not shown). This verifies that the synaptoneurosome samples provide information about the level of protein expression at functional

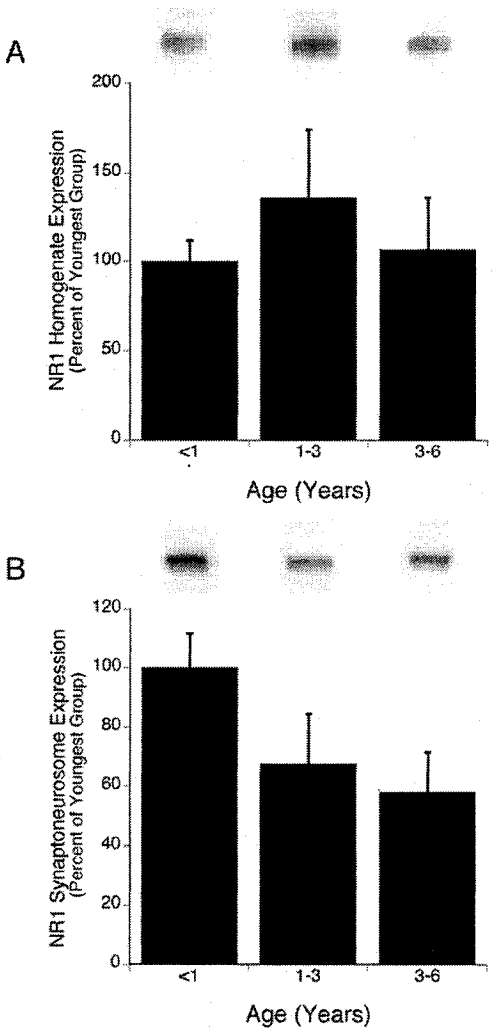


Figure 2.1. NR1 expression in homogenate and synaptoneurosome preparations.
The level of NR1 receptor subunit expression in whole homogenate (A) and synaptoneurosome (B) samples from human visual cortex during the first 6 years of postnatal development. Representative Western blot images are presented above each bar. A, The whole homogenate samples showed no developmental change in NR1 expression. B, There was a steady loss of synaptic expression of the NR1 subunit over the first 6 years ($p<0.02$).

excitatory synapses. The expression of NR1 in the synaptoneurosome samples decreased progressively over the ages studied (Fig. 2.1B). In comparison with the youngest group the level of NR1 expression dropped about 33% by 1-3 years of age ($p < 0.06$) and about 50% by 3-6 years of age ($p < 0.02$). On the one hand, the whole homogenate results show that the total expression of NR1 in human visual cortex does not change substantially during postnatal development. On the other hand, the synaptoneurosome results show that synaptic NR1 was highest at the youngest postnatal ages and declined over the first 6 years of postnatal development of human visual cortex.

There was substantial postnatal change in the level of expression of the NR2A and NR2B subunits in human visual cortex. The synaptic expression of NR2A was lowest for the youngest group and then increased progressively, it increased 3-fold by 1-3 years of age, and 4-fold by 3-6 years of age ($p < 0.01$) (Fig. 2.2A). The opposite pattern of change was observed for the synaptic expression of NR2B. Expression of NR2B dropped rapidly by about 42% between the youngest and the 1-3 year old groups, and by about 50% between the youngest and oldest groups ($p < 0.005$) (Fig. 2.2B). Previous animal studies have shown that the ratio of NR2A:NR2B is an important comparison because it is related to the decay time of the NMDA-mediated component of the EPSC. As the NR2A:NR2B ratio increases, the NMDA receptor decay time decreases (Monyer et al, 1992, 1994; Flint et al, 1997; Roberts and Ramoa, 1999). We calculated the NR2A:NR2B ratio ((NR2A-NR2B)/NR2A+NR2B)) for each case and then plotted the group averages. There was a progressive increase in the ratio of synaptic NR2A:NR2B during development of human visual cortex ($p < 0.005$) (Fig. 2.2C). At the youngest ages synaptic NMDA receptors were dominated by the NR2B subunit, however, by 1-3 years of age there was a significant increase in the ratio and a

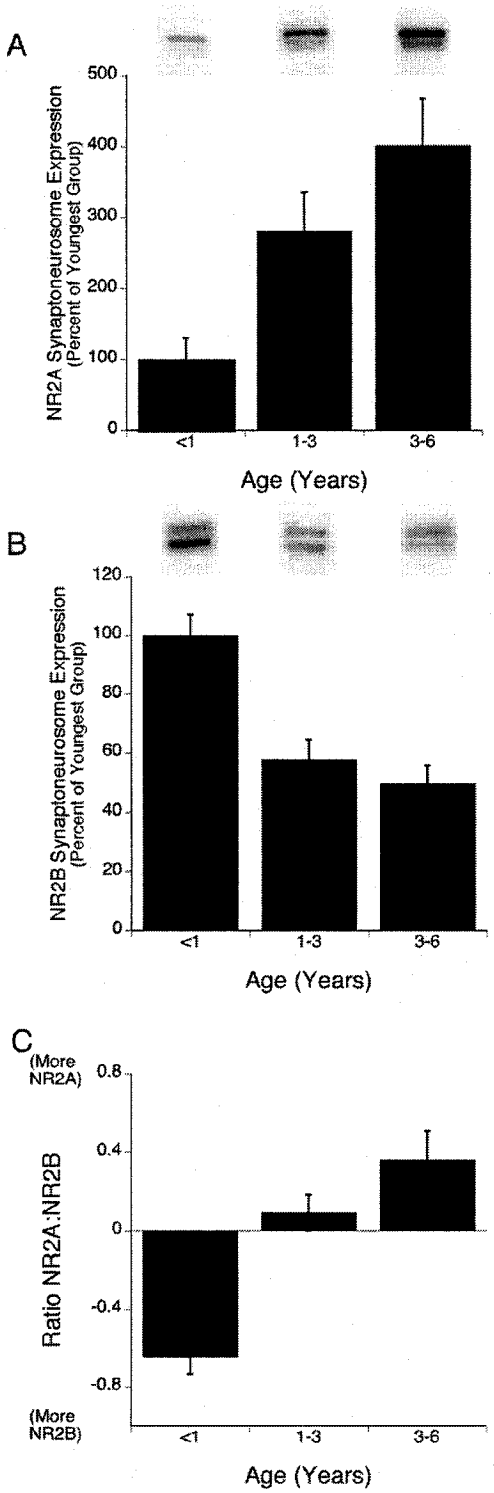


Figure 2.2. Developmental changes of synaptic NMDA receptor subunits.
Representative Western blot images are presented above each bar. A, NR2A expression increases significantly from birth to 1-3 years of age and continued to increase for the 3-6 year old group ($p<0.01$). B, NR2B expression was highest at birth and decreased rapidly by 1-3 years of age ($p<0.005$).C, The increase in the ratio of NR2A:NR2B is related to changes of the speeding up of the NMDA-mediated component of the EPSCs. There was a progressive increase in the NR2A:NR2B ratio over the first 6 years, shifting from dominance by NR2B at the youngest ages to dominance by NR2A at 1-3 years of age ($p<0.005$).

slight bias in favour of the NR2A subunit. The NR2A:NR2B ratio continued to increase with development and by 3-6 years of age the ratio had clearly shifted to be dominated by NR2A expression.

Expression of the non-NMDA glutamatergic receptor AMPA was quantified by examining the expression of the GluR2 subunit during development of the human visual cortex. Activation of the AMPA receptor mediates the fast component of the EPSC and the GluR2 subunit controls Ca^{2+} permeability of the receptor. Synaptic GluR2 expression was relatively constant, there were no significant differences in the level of expression of GluR2 between the 3 age groups (Fig. 2.3A). Comparing the relatively constant expression of GluR2 with the loss of synaptic NR1 suggests that during development of human visual cortex there is a change in the composition of excitatory glutamatergic synapses to include a greater proportion of AMPA relative to NMDA receptors.

$GABA_A$ Receptor and GAD Expression

Expression of 2 subunits of the ionotropic $GABA_A$ receptor ($GABA_A\alpha1$, $GABA_A\alpha3$) was quantified from the whole homogenate samples for each case and group means were calculated. The whole homogenate samples were used because a large percent of GABA receptors are extra synaptic and the synaptoneurosome preparation enriches for excitatory synapses (Hollingsworth et al, 1985). In contrast with the NMDA receptor subunits, expression of these $GABA_A$ receptor subunits was either relatively constant or showed modest changes over the first 6 years of postnatal development (Fig. 2.4). Expression of $GABA_A\alpha1$, which is linked with faster receptor decay times, did not change significantly between the 3 age groups (Fig. 2.4A). There was a slow loss of expression of $GABA_A\alpha3$ with

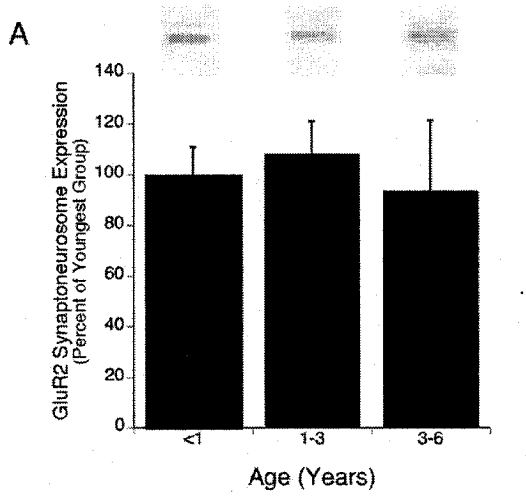


Figure 2.3. Developmental changes in GluR2 expression.
Quantification of GluR2 expression changes during postnatal development. Representative Western Blot images are presented above each bar. GluR2 expression was relatively constant across the ages studied.

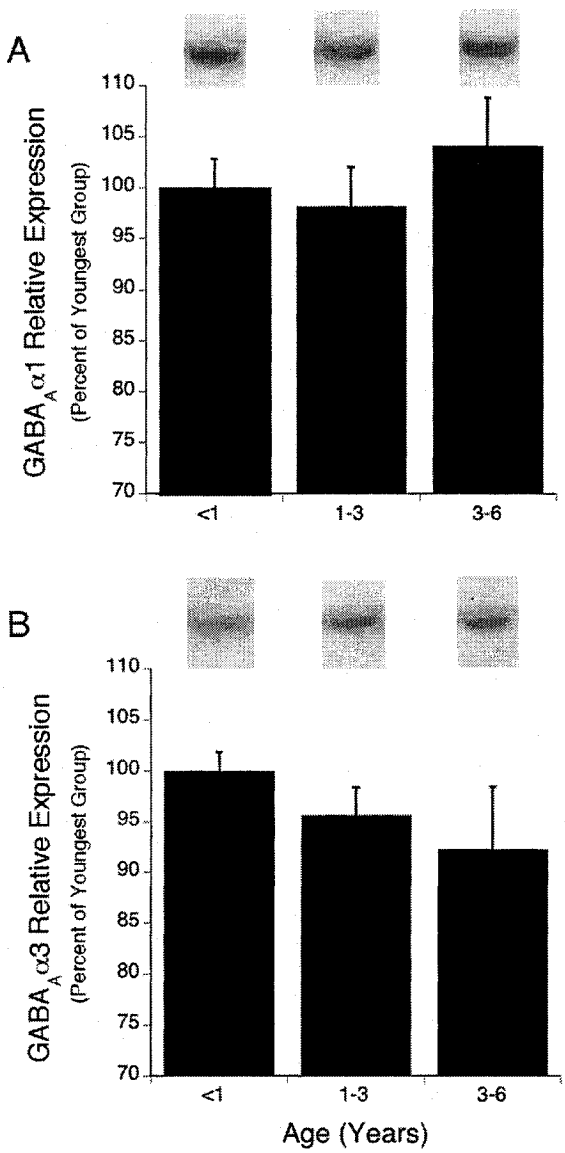


Figure 2.4. Developmental changes in $GABA_A$ subunit expression.
Changes in expression of $GABA_A$ receptor subunits $GABA_A\alpha1$ (A) and $GABA_A\alpha3$ (B) during postnatal development. Representative Western blot images are presented above each bar. A, $GABA_A\alpha1$ expression did not change significantly during development. B, The amount of $GABA_A\alpha3$ expression decreased progressively over the first 6 years.

postnatal development ($p < 0.05$), however, the magnitude of the loss was small in comparison with the changes in NMDA subunit expression. There was only about an 8% loss of $GABA_A\alpha3$ expression between the youngest and oldest groups (Fig. 2.4B). We calculated the ratio of $GABA_A\alpha1$:$GABA_A\alpha3$ expression for each case because the kinetics of the receptor are faster when $GABA_A\alpha1$ dominates the receptor. The change in the mean ratio of $GABA_A\alpha1$:$GABA_A\alpha3$ for the 3 groups was small during postnatal development of human visual cortex (Fig. 2.5), however, by 3-6 years of age the ratio had shifted in favour of $GABA_A\alpha1$ ($p < 0.05$).

To further assess the development of the GABAergic system we quantified the expression of the 2 isoforms of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD65 and GAD67). The GAD65 isoform is associated with synaptic vesicles in axon terminals, while GAD67 is the basal isoform and tends to be found in cell bodies (Esclapez et al, 1994). There was a significant increase in GAD65 expression during development of human visual cortex ($p < 0.01$) (Fig. 2.6A). GAD65 expression increased about 15% during development. The level of GAD67 expression did not change during development (Fig. 2.6B). This difference between the synaptic (GAD65) and basal (GAD67) isoforms suggests that changes in expression of GABA during postnatal development of human visual cortex are largest in axon terminals.

Discussion

This paper presents the first analysis of the postnatal development of AMPA, NMDA and $GABA_A$ receptors, plus GAD expression in human visual cortex. These are key components of the neural mechanisms that underlie

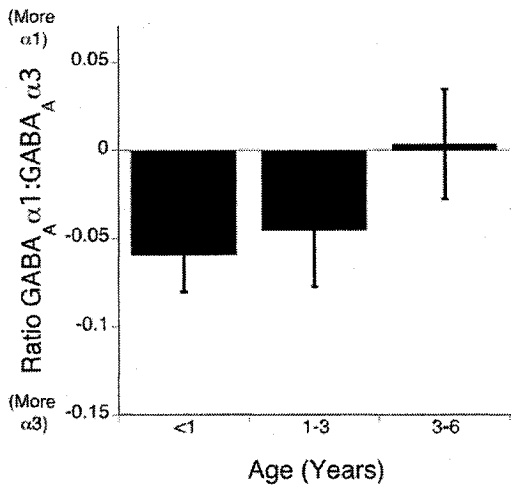


Figure 2.5. $GABA_A$ index.
The ratio of $GABA_A\alpha1$:$GABA_A\alpha3$ is important for determining the kinetics of the $GABA_A$ receptor. During development, there was a significant increase in this ratio in favour of the faster $GABA_A\alpha1$ subunit.

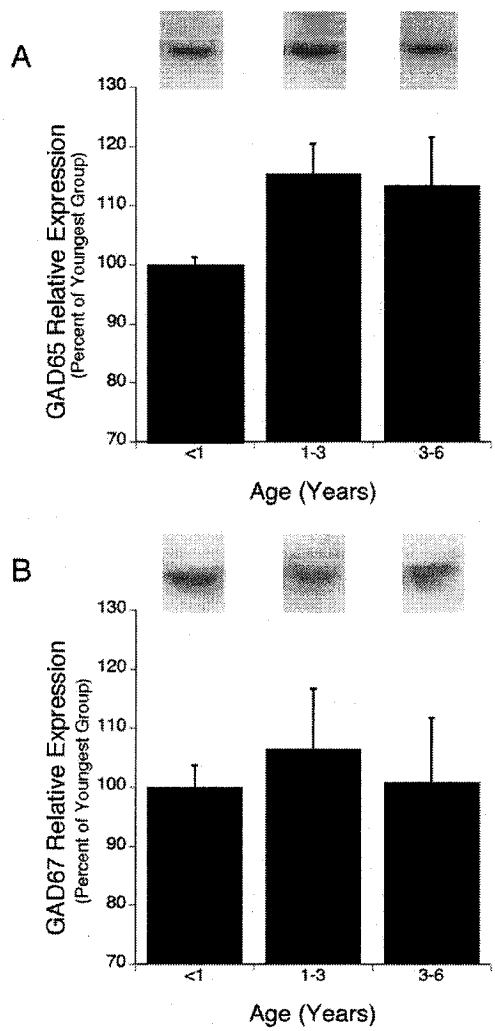


Figure 2.6. GAD65 and GAD67 expression. Developmental changes in expression of the 2 GAD isoforms were found for the synaptic (GAD65) but not the basal pool (GAD67) in human visual cortex. A, The expression of GAD65 increased by 1-3 years of age ($p<0.01$). B, The expression of GAD67 did not change postnatally.

experience-dependent developmental plasticity in the visual cortex and the relative levels of expression will affect critical period plasticity. AMPA receptor expression is relatively constant over the first 6 years, however, there are significant changes in the level of expression of synaptic NMDA receptor subunits. Both NR1 and NR2B expression decline during development. NR2B expression declines quickly, whereas there is a progressive loss of NR1 expression during development of human visual cortex. In contrast, NR2A expression increases and there is a prolonged change in the NR2A:NR2B ratio from being dominated by NR2B at the youngest ages to dominance by NR2A at the oldest ages. The directions of these developmental changes in NMDA receptor subunit expression in human visual cortex are similar to those found in animal studies. The time course of the changes, however, is quite prolonged and much longer than would be predicted from animal models. In particular, the NR2A:NR2B ratio change in human visual cortex occurs too late for it to signal the start of the critical period for ocular dominance plasticity. On the inhibitory side, expression of $GABA_A$ receptor subunits either does not change ($GABA_A\alpha1$, $GABA_A\alpha2$), or shows a modest loss ($GABA_A\alpha3$) and presynaptically there is an increase in synaptic GAD expression (GAD65). The direction of the changes in the $GABA_A$ subunits and GAD65 are similar to those found in the animal models, but, the magnitude of the changes is small and the time course is prolonged.

Taken together, these results show that there are significant changes in the expression of these key components of experience-dependent plasticity during postnatal maturation of human visual cortex. The changes in these glutamatergic receptors and GABAergic mechanism have consequences for synaptic plasticity, the emergence of receptive field properties, and the development of functional

circuits in human visual cortex. For example, the combination of the developmental changes in subunit composition of the receptors, the relative amounts of the receptors, and the GAD isoforms will affect the time constants of EPSCs and IPSCs in human visual cortex. The responses will change from slow at the youngest ages to faster at the older ages. In addition, the changes that we have found suggest that the kinetics of these excitatory and inhibitory mechanisms develop in concert, perhaps to maintain cortical activity within an appropriate level to facilitate experience-dependent plasticity. Finally, the time course of the changes is slow compared with the animal models and suggests a more prolonged period of developmental plasticity in the human visual cortex.

Methodological Considerations

The immunoblotting procedures used in the present study allowed us to quantify the expression of AMPA receptors (GluR2), NMDA receptors (NR1, NR2A, NR2B), GABA receptors ($GABA_A\alpha1$, $GABA_A\alpha2$, $GABA_A\alpha3$) and GAD (GAD65, GAD67) protein in tissue samples from human primary visual cortex. Furthermore, using the synaptoneurosome preparation we are able to quantify the *synaptic* expression of excitatory receptors (Hollingsworth et al, 1985). This subcellular fractionation technique enriches for excitatory synaptic proteins so that the level of expression of GluR2, NR1, NR2A, and NR2B was quantified at the site that is most important for understanding experience-dependent developmental synaptic plasticity. To be able to draw links between physiological changes in synaptic efficacy and the neural plasticity mechanisms that underlie those changes it is essential to know the level of expression of functional receptors in the synapse. The importance of using this subcellular localization is underscored by the difference that we found between the level of NR1 expression

in the whole homogenate versus the synaptoneurosome samples. In the whole homogenate samples there was no change in the level of expression of NR1 during development, but the synaptoneurosome samples showed a significant decline with development. The whole homogenate analysis quantifies the total amount of NR1 protein, the amount inside the cell as well as at the synapse, and can be misleading about the relationship with NMDA-dependent synaptic plasticity (Quinlan et al, 1999a). From the whole homogenate results it would be reasonable to propose that there is no developmental change in NMDA-dependent plasticity in human visual cortex. The synaptoneurosome results, however, tell a different story. There is a significant loss of synaptic NR1 expression, suggesting a loss of functional NMDA receptors that will lead to a progressive decline of NMDA-dependent synaptic plasticity in human visual cortex over the first 6 years of postnatal development. The loss of NR1 found with the synaptoneurosomes fits well with behavioural results where a longer period of visual deprivation is necessary to cause a loss of visual acuity in older children suggesting that there is less neural plasticity (Maurer & Lewis, 2001).

Consideration of Laminar Development

The immunoblotting technique quantifies the amount of protein but does not provide information about laminar development, the tangential arrangement of neurons expressing the proteins, or the types of neurons expressing AMPA, NMDA, or GABA receptors, or GAD. These aspects of visual cortex development will be best addressed using immunohistochemical staining, *in situ* hybridization, or receptor binding on sections from human visual cortex. To date there are no studies using those techniques to examine AMPA or NMDA expression during postnatal development of human visual cortex. Both NMDA and AMPA receptors

are expressed prenatally in the developing human cortex with high levels of both NR1 and NR2B (Ritter et al, 2001), however, that study provided no quantification of laminar changes and did not analyze visual cortex. In animal models there are postnatal changes in the laminar distributions of both AMPA and NMDA receptors. In cat visual cortex NR1 immunostaining is initially darkest in layer IV of kitten visual cortex, but in the adult NR1 immunostaining is lightest in layer IV (Trepel et al, 1998). Similar laminar changes occur for GluR2 expression in developing monkey visual cortex (Wong-Riley & Jacobs, 2002). The tangential arrangement of neurons expressing these proteins also needs to be determined because animal studies have linked NR1 patches with ocular dominance columns (Trepel et al, 1998), GluR2 patches with cytochrome-oxidase blobs (Wong-Riley & Jacobs, 2002), and suggested that NMDA receptors may provide a blueprint for emerging synapses (Aoki et al., 1994; Durand et al., 1996).

GABAergic neurons differentiate early in prenatal development of human visual cortex and attain their mature laminar pattern before birth (Yan et al, 1992). The basic laminar pattern of $GABA_A$ receptor subunit expression in macaque monkey visual cortex emerges prenatally (Shaw et al, 1991; Hendrickson et al, 1994; Huntsman et al, 1999) with a dense band of $GABA_A\alpha 1$ expression in layer IV by birth. This suggests that there may be a shortening of the decay time of IPSCs in layer IV occurring at about the time of birth in macaque monkeys and has important implication for experience-dependent plasticity of thalamocortical connections and the emergence of receptive field properties. While the distribution of $GABA_A$ receptors is very similar in adult human and macaque monkey visual cortex (Hendry et al, 1994) it will still be important to determine the pattern of laminar development in human visual cortex to gain a more

complete understanding of the time course of changes in the circuitry that contribute to critical period plasticity.

Changes in NMDA Receptor Subunit Expression

Animal studies have linked the change in the NR2A:NR2B ratio to a variety of aspects of visual cortical development. In human visual cortex the pattern of change is similar to that found in animals and in human hippocampal development (Law et al, 2003). Initially, NR2B expression is high and NR2A expression is low and then there is an increase in the NR2A:NR2B ratio. The time course of this change is relatively slow and the shift in favour of NR2A occurs only sometime after 1 year of age. This slow change in the ratio of NR2A:NR2B makes it unlikely that it is acting as a switch that signals either the start, the peak, or the end of the critical period for ocular dominance plasticity. Instead the progressive nature of the change in human visual cortex may provide some clues to the significance of the NR2A:NR2B ratio. The increase in NR2A expression has been linked to the formation of synapses because nascent sites are composed of NR1/NR2B subunits and NR2A is added shortly after synapse formation (Tovar & Westbrook, 1999). The prolonged increase in NR2A expression and the slow change in the NR2A:NR2B ratio may reflect the protracted development of intracortical horizontal long-range connections and intercortical feedback connections (Burkhalter et al, 1993; Burkhalter, 1993). The increased NR2A:NR2B ratio has also been linked with a decline in synaptic plasticity (Sheng et al, 1994) and while that fits with the early period of human visual cortex development it suggests that there would be very little plasticity in human visual cortex after 1 year of age when the NR2A:NR2B ratio has already shifted in favour of NR2A. Another hypothesis that builds on the idea of a change in plasticity is that the

NR2A:NR2B ratio affects the threshold for synaptic modification, favouring synaptic potentiation when NR2B is high and the EPSC decay is slow, and synaptic depression when NR2A is higher and the EPSC decay is faster (Quinlan et al, 1999a). This model predicts a continuous rebalancing of the modifiability of synapses as the composition of the NMDA receptor changes and is consistent with a prolonged period of plasticity in human visual cortex where the dynamic range of synaptic modification is gradually reduced during development. In addition to these effects on synaptic plasticity, the development of orientation selectivity is also dependent on expression of the NR2A subunit (Fagiolini et al, 2003). Perhaps the initially low levels of NR2A and progressive increase of expression during postnatal development affects the pace of maturation of orientation selectivity. The result may be a more prolonged period of development for certain visual functions, such as grating acuity (Maurer & Lewis, 2001) and contour integration (Kiorpes & Bassin, 2003), that are thought to depend on orientation selectivity.

The Shift from Tonic to Phasic Inhibition

Animal studies have suggested that the shift from tonic to phasic inhibition that accompanies the developmental switch from $GABA_A\alpha3$ to $GABA_A\alpha1$, and the increase in GAD65, is involved in triggering the critical period for experience-dependent plasticity (Fagiolini & Hensch, 2000; Fagiolini et al 2004; Iwai et al, 2003). In human visual cortex there is a loss of $GABA_A\alpha3$ receptors leading to a shift in favour of $GABA_A\alpha1$, but the change does not occur until 3-6 years of age. This is so late in development that the shift in subunit composition cannot be triggering the critical period. Furthermore, the magnitude of the changes in $GABA_A$ receptor expression in human visual cortex is extremely small in

comparison with the changes found in animal studies (Shaw et al, 1991; Hendrickson et al, 1994; Hornung & Fritschy, 1996). Since previous studies have shown that GABAergic neurons differentiate prenatally in human visual cortex (Yan et al, 1992) perhaps there is a larger prenatal change in the $GABA_A$ receptor composition. A prenatal switch in $GABA_A$ subunits, however, would also be difficult to reconcile with the timing of the critical period. Ocular dominance column segregation in human visual cortex probably happens postnatally since a marker of cortical columns -- cytochrome-oxidase blobs -- is absent in human newborns and emerges over the first few months of postnatal development (Horton, 1984, Wong-Riley et al, 1993). The time course and magnitude of the postnatal changes in $GABA_A$ receptor subunits make it unlikely that it is the sole mechanisms that triggers the critical period in human visual cortex.

The developmental change in GAD expression provides additional support for the notion that there is a shift from tonic to phasic inhibition during the critical period for plasticity in human visual cortex. There is an increase in GAD65 expression in human visual cortex and the larger pool of synaptic GAD will provide the GABA needed to respond to short-term, phasic changes in activity (Feldblum et al, 1993, 1995). Neurophysiological studies of macaque monkey visual cortex have shown a rapid developmental increase in temporal resolution and decrease in latency of cortical neurons, suggesting a developmental shortening of the time window for neural integration (Rust et al, 2002). That physiological change is consistent with a shift towards short-term, more rapid fluctuations in excitation that would need to be balanced by phasic inhibition to maintain an appropriate level of cortical activity. The developmental increase in GAD65 may provide more phasic inhibition in human visual cortex

and help to shorten the window for temporal integration of visual cortical neurons. GAD65 also contributes to synaptic plasticity. GAD65 knockout mice are insensitive to monocular deprivation (Hensch et al, 1998) and have substantially less synaptic plasticity (Choi et al, 2002). Taken together these results suggest a key role for the increased GAD65 expression in human visual cortex affecting both the development of temporal integration and the synaptic plasticity that underlies the maturation of cortical circuits.

Affects on Temporal Dynamics of Neural Transmission and Information Processing

The changes that we have found in the expression of excitatory and inhibitory developmental plasticity mechanisms will affect the wiring up of circuits in the developing human visual cortex. But development is more than just plugging all of the wires into the correct receptacle -- the functioning of the receptacles also changes. This prolonged period of development of receptors and neurotransmitter expression will affect the temporal dynamics of neural transmission and the processing of visual information. During development the response of neurons in macaque monkey visual cortex increases in temporal sensitivity and decreases in latency (Rust et al, 2002). That finding suggests a longer period of neural integration in infant neurons and a speeding up with maturation. The longer period of neural integration is consistent with the immature expression of excitatory and inhibitory receptor subunits and less GAD65 that we have found in infant human visual cortex. The composition of excitatory receptors in infants -- more NR2B and relatively less GluR2 -- will results in a slower time constant for EPSCs. On the inhibitory side, immature IPSCs also have a slower time constant because there is more $GABA_A \alpha 3$ and less

synaptic GAD (GAD65). These developmental changes in AMPA, NMDA and GABA receptor expression lead to an acceleration of receptor decay speeding up both EPSCs and IPSCs. The increase in synaptic GAD expression also contributes to shorter more phasic inhibition (Feldblum et al, 1993, 1995). It seems likely that these changes in excitatory and inhibitory transmission are the mechanisms that underlie the neurophysiological development of temporal resolution in cortical neurons. Taken together, the development of these cellular mechanisms and physiological responses will affect the maturation of visual function by changing spatial and temporal integration in the developing human visual system.

In the animal models, especially rodents, the postnatal development of visual cortex and the length of the critical period is very short with rapid changes in many of the neural mechanisms associated with developmental plasticity. In contrast, the development of visual function and the period of sensitivity to visual deprivation is prolonged in humans even though many aspects of human visual cortex are relatively mature at birth. These differences have posed a problem for linking the neural mechanisms identified in animal studies with the stages of functional development of human visual cortex. We have shown a slow and balanced maturation of glutamatergic and GABAergic plasticity mechanisms in human visual cortex. These results provide new clues for understanding the prolonged period of visual development and critical period plasticity in the human visual system. The slow changes and balance between these excitatory and inhibitory components of neural plasticity appear to set the pace for the functional maturation of human visual cortex.

Chapter 3

Experience-Dependent Changes in Excitatory and Inhibitory Plasticity Mechanisms: Regional Differences in Developing Visual Cortex

Introduction

Disrupting visual input to one eye during the critical period of visual development causes a dramatic reduction in the acuity of the deprived eye. The behavioural consequences of monocular deprivation reflect an experience-dependent competitive loss of inputs (Wiesel and Hubel, 1965; Hubel, and Wiesel, 1970) and a depression of synaptic responses to the deprived eye in visual cortex (Rittenhouse et al., 1999). There is an emerging consensus that these changes in visual cortex are influenced by changes in developmental synaptic plasticity mechanisms and changes in NMDA, AMPA, and $GABA_A$ receptors (Kirkwood and Bear, 1994; Hensch et al., 1998; Iwai et al., 2003). Previous studies have shown that blocking NMDA receptors reduces ocular dominance plasticity (Kleinschmidt et al.,1987; Roberts and Ramoa, 1998) and blocking the receptor or deleting the NR2A subunit prevents the development of orientation selectivity (Ramoa et al., 2001; Fagiolini et al., 2003). In addition, GABAergic transmission is necessary for the initiation of the critical period for ocular dominance plasticity (Hensch, 1998; Fagiolini et al., 2004). While much is known about the importance of these mechanisms for developmental plasticity, less is known about the effects of visual experience on the expression of these mechanisms.

Recent studies have shown that abnormal visual experience leads to changes in NMDA plasticity mechanisms (Quinlan et al., 1999; Chen and Mower, 2001; Murphy et al., 2004). Furthermore, both the amount and pattern of activity can influence experience-dependent changes in visual cortex. In a recent study, we separated these factors by showing that reduced activity promotes a homeostatic increase in NR1 expression, while reduced binocularly correlated activity leads to a loss of NR1 expression (Murphy et al., 2004). We are able to

separate the influence of these factors because binocular correspondence is not uniform across the visual field and, accordingly, I studied changes in visual cortex where there is high, low or no binocularly correlated activity.

Visual information is not processed equally across the visual field and can be broken down into 3 distinct regions of visual processing, the central, peripheral and monocular visual field representations (Tusa et al., 1978). In the central visual field representation, there is a very high degree of binocular correspondence, and information is processed by small receptive fields that respond to fine detail (Hubel and Wiesel, 1962). Away from the central visual field, there is less binocular correspondence because the vertical disparity is larger (Helmholtz, 1925) and there is greater positional jitter of the receptive field (Van Essen et al., 1984). Finally, only one eye provides information in the monocular visual field representation and so there is no binocular correspondence in that region. Developmentally, acuity in the central visual field appears to reach maturity later than the peripheral and monocular visual fields (Adams and Courage, 1995; Allen et al., 1996). Furthermore, vision disorders such as developmental amblyopia in human children are primarily described as a deficit of central vision (Hess and Pointer, 1985). These findings have been supported in physiological studies, showing that the effect of amblyopia spatial resolution sensitivity is more severe in the central visual field representations of V1 than in the peripheral regions (Kiorpes et al., 1998).

Taken together, there is good evidence to suggest that the effects of visual experience are not uniform across the visual cortex. I have extended this notion to address whether there are regional differences in the excitatory and inhibitory mechanisms that underlie synaptic plasticity in the developing visual cortex.

Materials and Methods

Animals

The effect of visual experience on the expression of excitatory and inhibitory receptor subunits was studied in the visual cortex of kittens (age 2 - 32 weeks) reared with either normal visual experience (n = 9), or monocular deprivation (n = 8). Monocular deprivation was initiated at the time of natural eyelid opening by suturing the eyelids closed using aseptic surgical techniques and gaseous anesthetic (2-5% isoflurane mixed in oxygen) following procedures described previously (Murphy & Mitchell, 1987). Kittens were euthanized with Euthanol (165mg/kg), and transcardially perfused with cold 0.1M PBS (4° C; 80-100 ml/min) until the circulating fluid was cleared. The brain was quickly removed from the skull and immersed in cold PBS. To facilitate quantification of regional differences within V1, the area was divided into a series of small tissue samples (n=12, each approx. 2 X 2mm) covering the central, peripheral, and monocular visual field representations (Tusa, Palmer and Rosenquist, 1978) (Fig. 3.1). Each cortical tissue sample was rapidly frozen on dry ice and stored at -80° C.

Synaptoneurosome Preparation

Tissue samples were suspended in 1 ml of cold homogenization buffer (10 mM HEPES, 2 mM EDTA, 2 mM EGTA, 0.5 mM DTT, 10 mg/l leupeptin, 50 mg/l soybean trypsin inhibitor, 100 nM microcystin and 0.1mM PMSF), homogenized in a glass-glass Dounce-tissue homogenizer (Kontes, Vineland, NJ), and a portion of the whole homogenate was kept. The synaptoneurosome was obtained by passing the homogenate through a 5 μm pore hydrophobic mesh filter (Millipore, Billerica, MA) and centrifuging the preparation for 10 min at x1000g. The

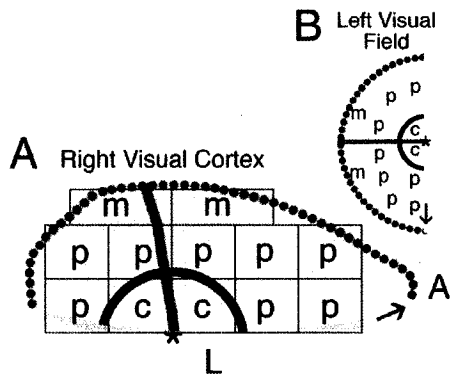


Figure 3.1. Tissue Collection.
The expression of synaptic proteins were quantified from tissue samples collected in the central (c), peripheral (p), and monocular (m) visual field representations of primary visual cortex (A). The location of visual field representation (B) in primary visual cortex (A) was assessed using anatomical markers identified by Tusa, Palmer and Rosenquist (1978).

resulting pellet was resuspended in boiling 1% SDS and heated for 10 min until the sample dissolved. Protein concentrations were determined using the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL). The synaptoneurosome preparations were compared with samples of the supernatant and whole homogenate to verify that the synaptoneurosome had a 2-3 fold enrichment for synaptic proteins.

Immunoblotting

Equal quantities of synaptoneurosomes (20μg) were resolved on 4-20% SDS-PAGE gels (Pierce, Rockford, IL) and transferred to polyvinylidine fluoride (PVDF) membranes (Millipore, Billerica, MA). Membranes were blocked in phosphate buffered saline containing 0.05% triton-x (Sigma, St. Louis, MO) (PBS-T) and 5% skim milk (wt/vol) for 1 hour, and incubated in primary antibody overnight at 4° C using the following concentrations: NR1 1:2000 (BD Biosciences Pharmingen, San Diego, CA); NR2A 1:2000, NR2B 1:2000, GluR2 1:1000, Synapsin I 1:4000 (Zymed laboratories, San Francisco, CA); $GABA_A\alpha1$ 1:500 (Santa Cruz Biotechnology, Santa Cruz, CA); $GABA_A\alpha3$ 1:2000 (Chemicon International, Temecula, CA). The membranes were incubated in the appropriate secondary antibody conjugated to horseradish peroxidase (HRP) for 1 hour (1:2000; Cedarlane laboratories LTD, Hornby, ON). Immunoreactivity was visualized using the enhanced chemiluminescence (ECL) method of detection (Amersham, Pharmacia Biotech, Piscataway, NJ) and exposed to autoradiographic film (X-Omat, Kodak, Rochester, NY). Membranes were stripped and reprobed with additional antibodies using the Blot Restore Membrane Rejuvenation kit (Chemicon International, Temecula, CA).

Analysis

The bands were measured using densitometry by scanning the films (16 bit, AFGA ArcusII, Agfa, Germany) along with an optical density wedge (Oriel corporation, Baltimore, MD), converting the intensity of the bands to optical density units, and quantifying the density of each band using Matlab (The Mathworks, Inc., Natick, Massachusetts). All samples were normalized relative to a control sample that was run on each gel. The mean expression and SEM for each animal and each of the 3 regions were plotted relative to the expression of the normal adult central visual field samples. Developmental curves were plotted using a locally weighted least squares error fit to the data. Changes in NMDA and GABA receptor subunit composition were quantified by calculating 3 indices, NR2A:NR2B [(NR2A-NR2B)/(NR2A+NR2B)] NR1:GluR2 [(NR1-GluR2)/(NR1+GluR2)], and of $GABA_A\alpha1$:$GABA_A\alpha3$ [($GABA_A\alpha1$-$GABA_A\alpha3$)/($GABA_A\alpha1$+$GABA_A\alpha3$)]. Protein expression differences across the groups were compared using nonparametric statistics, Kruskal-Wallis tests were used and post hoc comparisons were made using Wilcoxon tests.

Results

The expression of NR1, NR2A, NR2B, GluR2, $GABA_A\alpha1$, $GABA_A\alpha3$ and Synapsin was quantified in normally reared (n=9) and monocularly deprived (n=8) kittens for each of the 12 tissue samples from V1. First, we present the developmental changes for each of these receptor subunits by analyzing the mean expression for samples from the central, peripheral, and monocular visual field representations. Next, we present analyses of the composition of and balance between excitatory and inhibitory receptor expression.

Glutamate Receptor Expression

Normal Development

In normal animals, there were substantial developmental changes in the expression of the 3 NMDA receptor subunits (Fig. 3.2) and GluR2 (Fig. 3.3a). These subunits were expressed at low levels shortly after eye opening (2 weeks of age), increased 3-8-fold reaching maximum expression levels by 8 weeks, and then decreased substantially to near adult levels by 16 weeks. Adult levels of expression were similar to those found in young animals (< 6 weeks of age) and very similar across each of the 3 regions in V1. There were, however, some regional differences in expression of NR1 and NR2A between 6 and 16 weeks of age. In the central visual field region, maximum expression levels for NR1 and NR2A extended to 12 weeks of age before declining to adult levels (Fig. 3.2a&b). NR2A expression was highest in the central visual field region with the peak extending from 8 to 12 weeks of age (Fig. 3.2b). Expression of NR2A in the peripheral visual field region increased to 8 weeks and then showed a steady decline to adult levels. In contrast to the other glutamate receptor subunits and other regions, the expression of NR2A in the monocular region did not increase after 5 weeks of age (Fig. 3.2b). Between 6-12 weeks of age, NR2A expression in the monocular region was significantly lower than in the other 2 regions ($p<0.01$).

To determine whether the developmental profiles of glutamate receptor expression were matched by presynaptic changes we examined the development of Synapsin I expression in V1 (Fig. 3.3b). Synapsin I is an accurate marker for developing synapses (Moore & Bernstein, 1989) and, thus, changes in Synapsin expression provide information about relative changes in the number of synapses. There was a steady increase in synapsin I expression to the maximum at 12 weeks

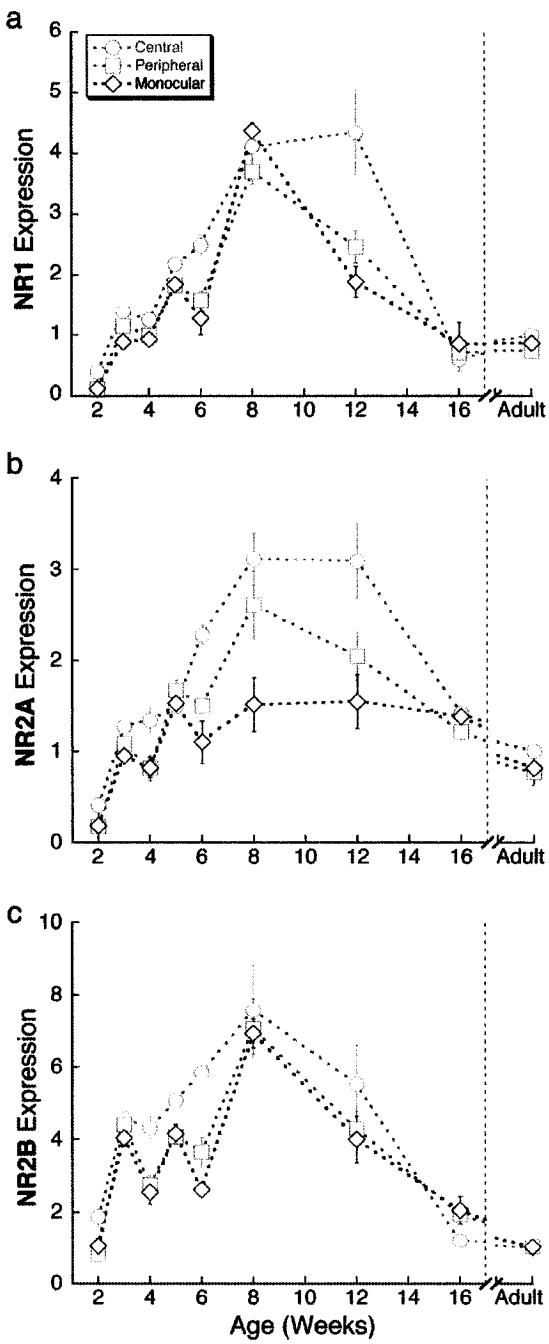


Figure 3.2. NMDA expression during normal development.
Comparison of regional changes in NMDA receptor subunit expression during postnatal development. Expression of NMDA receptor subunits, NR1 (a), NR2A (b), and NR2B (c) were analyzed in the central (red circles), peripheral (green squares), and monocular (diamonds) visual field representations in visual cortex. Expression of each subunit was measured relative to the expression of samples in the central visual field representation of adults. NR1, NR2A, and NR2B expression increased gradually from birth to 8-12 weeks of age, followed by a steady loss to 16 weeks of age. With one exception, the development of NMDA receptor subunits was consistent across all visual field representations. NR2A expression in the monocular representation did not increase beyond 6 weeks of age.

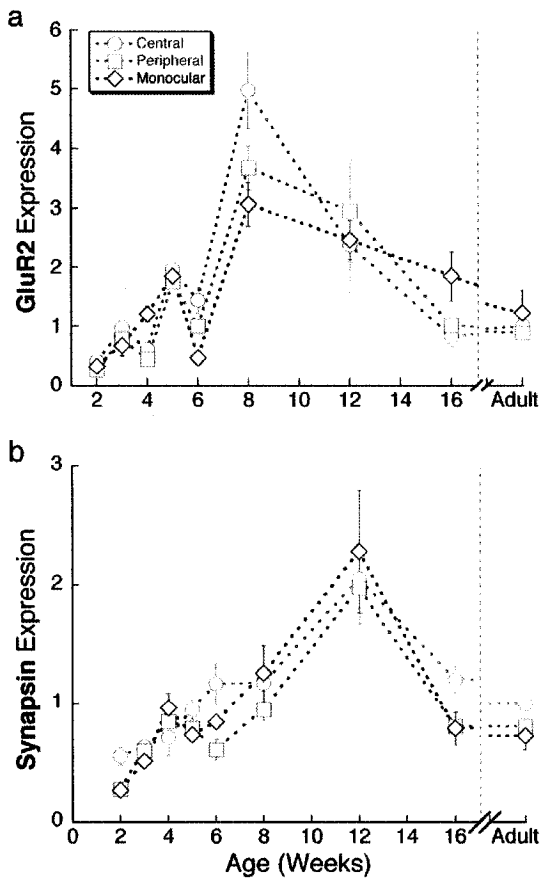


Figure 3.3. GluR2 and Synapsin expression.
Changes in the expression of GluR2 (a) and Synapsin (b) during postnatal development. Expression of each subunit was measured relative to the adult expression in the central visual field representation. Expression of GluR2 increased to as much as 5-fold of that found in adults by 8 weeks of age in the central (red circles), peripheral (green squares), and monocular (black diamonds) visual field representations. Synapsin, a reliable marker of synaptic density in the cortex, increases steadily from eye opening until 12 weeks of age before declining to adult levels of expression.

of age, followed by a sharp decline to reach adult levels by 16 weeks. This pattern of Synapsin expression was similar in all 3 regions of V1 (Fig. 3.3b). The peak of Synapsin I expression occurred later than the peaks for the glutamate receptor subunits suggesting that the initial increase in expression of the glutamate receptor subunits was not simply following an increase in the number of synapses.

Early Monocular Deprivation.

The developmental profile of NR1, NR2A, NR2B, and GluR2 expression in V1 following monocular deprivation was significantly different from normal, with both age-related and regional changes in the expression levels (Figs. 3.4 & 3.5). There was a clear difference in the pattern of expression between early (<6 weeks of age) and later (>6 weeks of age) deprivation and, first, we describe the early changes.

Deprivation to 4, 5, or 6 weeks of age led to changes in expression of the glutamate receptor subunits that paralleled normal development, however, not all regions were affected equally. There was a loss of NR1, NR2A and NR2B in the central visual field region ($p<0.05$), but no change in NR1, NR2A, NR2B, GluR2 in the peripheral visual field region, or NR2A, NR2B, GluR2 in the monocular region. Finally, with early deprivation there was a significant increase in NR1 expression in the monocular region ($p<0.05$).

A summary of the regional changes in NR1 expression after deprivation to 4,5, or 6 weeks of age showed that relative to normal animals there was about half the expression in the central region and a nearly 2-fold increase in the monocular region (Fig. 3.6). This regional pattern of NR1 changes is in good agreement with our previous study that used NR1 immunohistochemistry on unfolded and

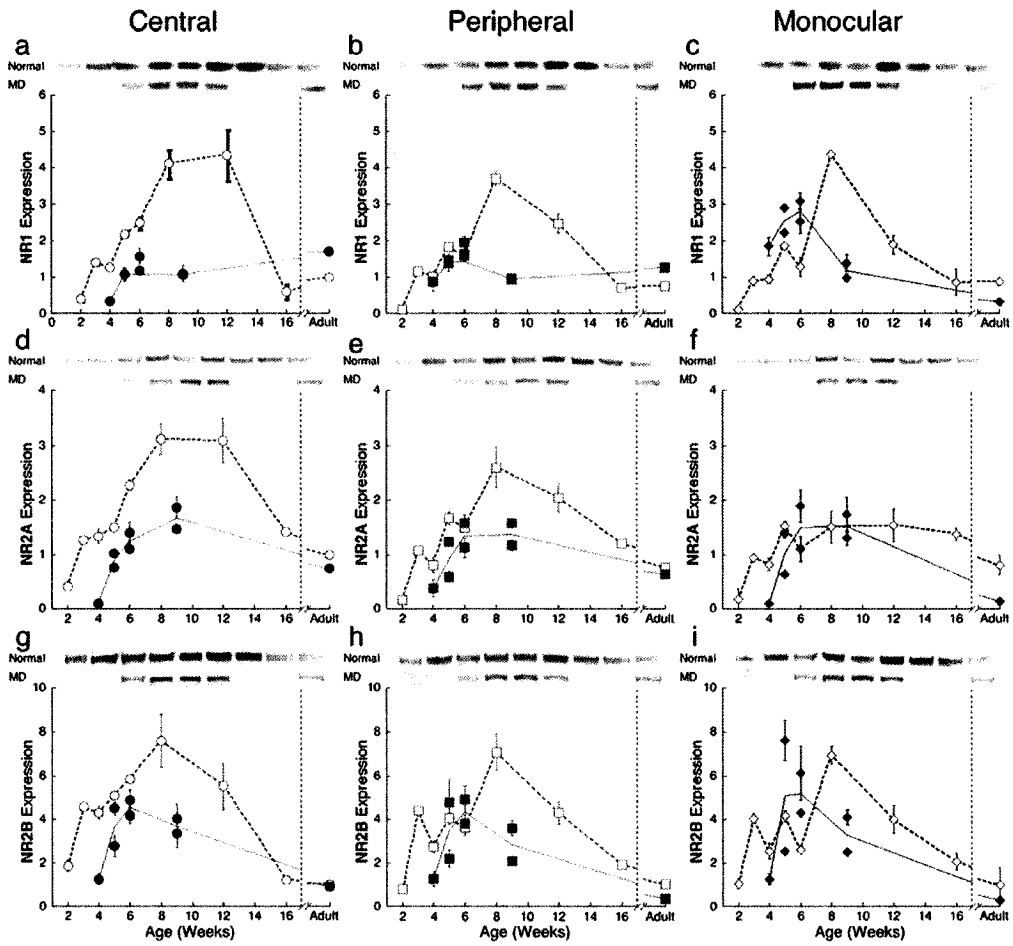


Figure 3.4. NMDA expression after monocular deprivation.
Quantification of NMDA receptor subunits (NR1, NR2A, NR2B) in normal (open symbols) and monocularly deprived (filled symbols) kittens from the central (a, d, g, red circles), peripheral (b, e, h, green squares) and monocular (c,f,i, black diamonds) visual field representations during postnatal development. The expression of each subunit was measured relative to the adult levels of expression in the central visual field representation. Representative blots are shown above each plot. Up to 6 weeks of age, monocular deprivation reduced the expression of NR1, NR2A, and NR2B in the central visual field representation, did not change expression in peripheral visual representation, and increased the expression of NR1 in the monocular regions. Beyond 6 weeks of age, the expression of NMDA receptor subunits was reduced in all regions of visual cortex.

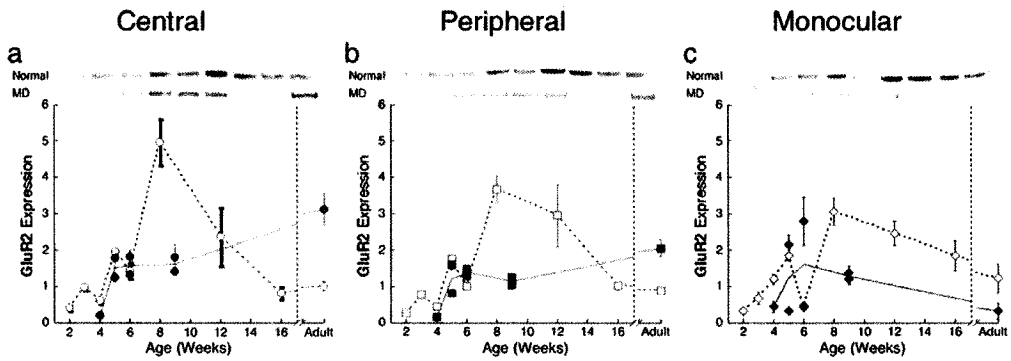


Figure 3.5. GluR2 expression after monocular deprivation.
The expression of GluR2 in the central (a, red circles), peripheral (b, green squares), and monocular (c, black diamonds) visual field representations in visual cortex from normal (open symbols) and monocularly deprived (filled symbols) kittens. The expression of each subunit was measured relative to the normal adult levels of expression in the central visual field representation. Representative blots are shown above each plot. Relative to normals, monocular deprivation does not reduce GluR2 expression within the first 6 weeks of monocular deprivation. In contrast, after 8 weeks of age, GluR2 expression is reduced across all regions of visual cortex.

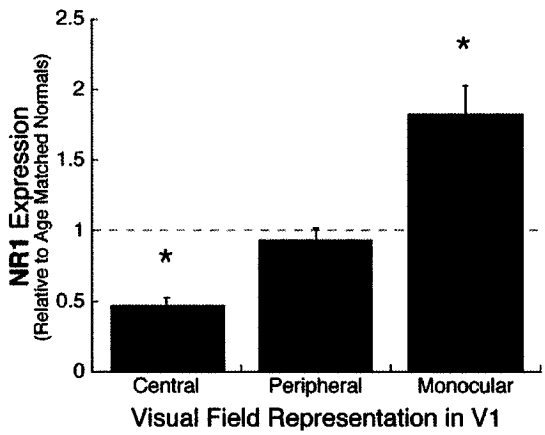


Figure 3.6. Regional expression of NR1.
Regional differences in the NMDA NR1 expression in kittens monocularly deprived up to 6 weeks of age. At each age, the expression in each visual field representation was measured relative to the mean expression of normal kittens. The normal level of expression is denoted by the dotted line. NR1 expression in the central visual field representation was reduced by 50% of normal expression (red), but was elevated to 175% of normal in the monocular regions (black). The relative expression did not change the expression in peripheral visual field representations (green). Asterisks (*) denote significant differences vs. age matched normally-reared kittens ($p<0.05$).

flattened cortical sections and quantified the density of NR1 immunopositive neurons (Murphy et al 2005). The regional changes in NR1, NR2A, and NR2B expression indicate that the effects of abnormal visual experience are not uniform across V1 and that the central visual field region is more vulnerable to the changes promoted by monocular deprivation.

Prolonged Monocular Deprivation.

Extending monocular deprivation beyond 6 weeks of age led to large losses of glutamate receptor subunit expression. Following longer deprivation the expression levels of NR1, NR2A, NR2B and GluR2 either did not increase or went down so that animals deprived to 9 weeks of age had 2-4 times less expression than normal animals (Fig. 3.4 & 3.5) ($p<0.01$). There was one exception to this pattern, at 9 weeks of age NR2A expression in the monocular region was similar between normal and deprived animals. This was because NR2A expression in normal animals does not increase after 6 weeks of age (Fig. 3.4f). The substantial loss of glutamate receptor subunit expression after prolonged period (>6 weeks of age) compared with shorter deprivation suggests that multiple processes underlie the effects of monocular deprivation on expression of these excitatory synaptic components. One process may affect early development (<6 weeks of age) by shifting expression to parallel normal development, and another process affect later development (>6 weeks of age) leading to a large deviation from the normal trajectory and a substantial loss of glutamatergic subunit expression.

$GABA_A$ Receptor Expression

Normal Development

We quantified changes in the expression of two developmentally regulated $GABA_A\alpha$ subunits, $GABA_A\alpha1$ and $GABA_A\alpha3$, because these subunits affect the binding affinity for GABA and the kinetics of the receptor. During normal development, $GABA_A\alpha3$ expression was initially 3-5 fold higher than adult levels and remained high until 8 weeks of age, after which there was a steady decline to reach adult levels by 16 weeks of age (Fig. 3.7a). The changes in $GABA_A\alpha3$ were similar for the 3 regions of V1. Expression of $GABA_A\alpha1$ followed a different developmental pattern and there were regional differences. $GABA_A\alpha1$ was initially low (2 weeks of age) and increased up until 4 weeks for peripheral and monocular regions and up to 8 weeks for the central region, after which expression declined to reach adult levels by 16 weeks of age (Fig 3.7b). The largest developmental increase in $GABA_A\alpha1$ expression was found in samples from the central visual field region where expression increased almost 3-fold between 2 and 8 weeks of age. The development of $GABA_A\alpha1$ expression in the central visual field region (Fig 3.7b) was similar to patterns found for the glutamatergic receptors (Figs. 3.2 & 3.3).

Monocular Deprivation

There were substantial changes in $GABA_A\alpha3$ and $GABA_A\alpha1$ expression following monocular deprivation. In monocularly deprived kittens, $GABA_A\alpha3$ expression was reduced to about half the normal level of expression and the loss was similar in the 3 regions of V1 (Fig. 3.8a-c). Animals monocularly deprived until adulthood had the same level of $GABA_A\alpha3$ expression as normal adults.

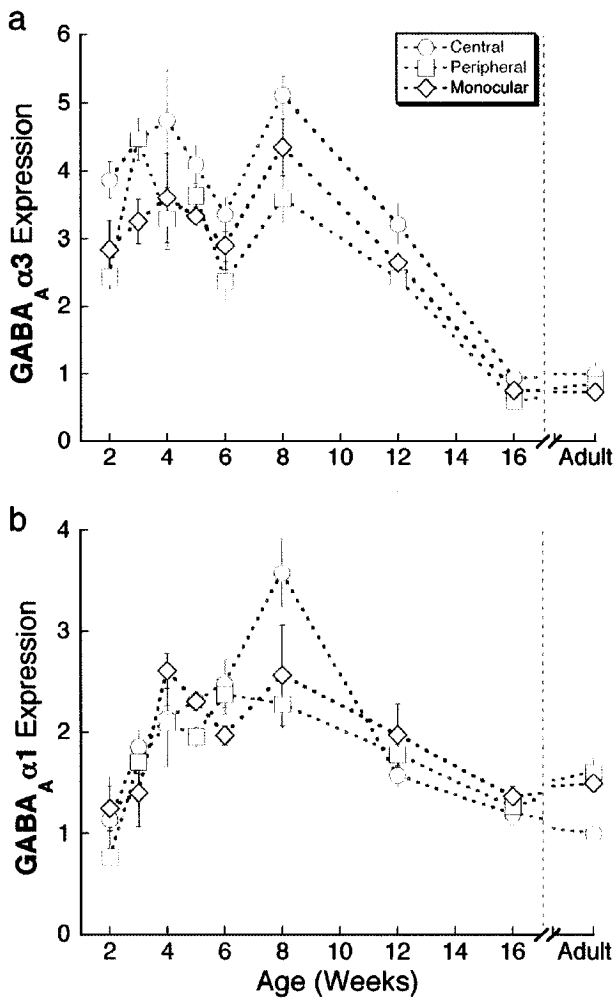


Figure 3.7. $GABA_A$ subunit expression during normal development.
Comparison of the regional changes in $GABA_A$ receptors subunits during postnatal development. Expression of $GABA_A$ receptor subunits, $GABA_A\alpha3$ (a), and $GABA_A\alpha1$ (b) were analyzed in the central (red circles), peripheral (green squares), and monocular (diamonds) visual field representations in visual cortex. The expression of each subunit was measured relative to the adult levels of expression in the central visual field representation. $GABA_A\alpha3$ was expressed at relatively high levels early in development, and remained high until 8 weeks of age. $GABA_A\alpha1$ expression increased gradually after birth to 8 weeks of age, followed by a steady decline to adult levels of expression by 16 weeks of age. The expression of $GABA_A\alpha1$ and $GABA_A\alpha3$ was similar across all regions of visual cortex.

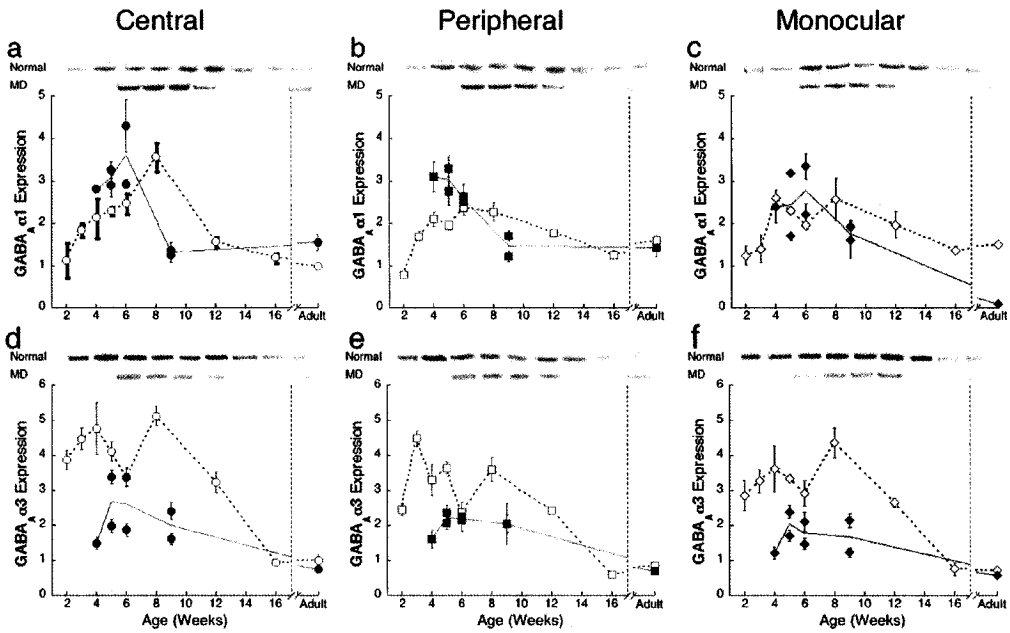


Figure 3.8. $GABA_A$ subunit expression after monocular deprivation.
Comparison of $GABA_A$ subunit expression ($GABA_A\alpha3$, and $GABA_A\alpha1$) in normal (open symbols) and monocularly deprived (filled symbols) kittens from the central (a, d, red circles), peripheral (b, e, green squares) and monocular (c,f, black diamonds) visual field representations during postnatal development. The expression of each subunit was measured relative to the normal adult levels of expression in the central visual field representation. Representative blots are shown above each plot. Monocular deprivation up to 6 weeks of age increased the expression of $GABA_A\alpha1$ in the central and peripheral representations in visual cortex. In contrast, monocular deprivation reduced the expression of $GABA_A\alpha3$ uniformly across the visual cortex.

Monocular deprivation promoted a very different pattern of change in the expression of $GABA_A\alpha1$ (Fig. 3.8d-f) that included both age-related and regional changes. Early deprivation (<6 weeks of age) led to an initial increase in $GABA_A\alpha1$ expression in central and peripheral regions ($p<0.05$), while prolonging deprivation to 9 weeks of age led to a loss of $GABA_A\alpha1$. The difference in $GABA_A\alpha1$ expression between early (<6 weeks) and prolonged (>6 weeks) monocular deprivation was similar to the pattern found for the glutamate receptors and provides additional support for the idea that multiple developmental processes underlie these experience-dependent changes in receptor expression in V1.

Excitatory and Inhibitory Receptor Composition and Balance

Key functional properties of the NMDA receptor, such as the EPSC decay are governed by the NR2 subunits. An increase of NR2A relative to NR2B shifts the NMDA receptor to faster kinetics, and has been linked with various aspects of the critical period for ocular dominance plasticity. We quantified the developmental shift from NR2B to NR2A in the 3 regions of V1 in normal and monocularly deprived kittens (Fig. 3.9). There was initially more NR2B followed by a progressive shift in favor of more NR2A expression in all regions of V1. The shift from NR2B to NR2A also occurred in deprived cases, however, it was delayed relative to normal animals. In normal animals the shift in favor of NR2A occurred by 6 weeks of age but not until 8 weeks in deprived animals. In contrast with this delay, monocular deprivation accelerated the shift in the balance between NR1 and GluR2 expression towards more GluR2 (Fig. 3.10).

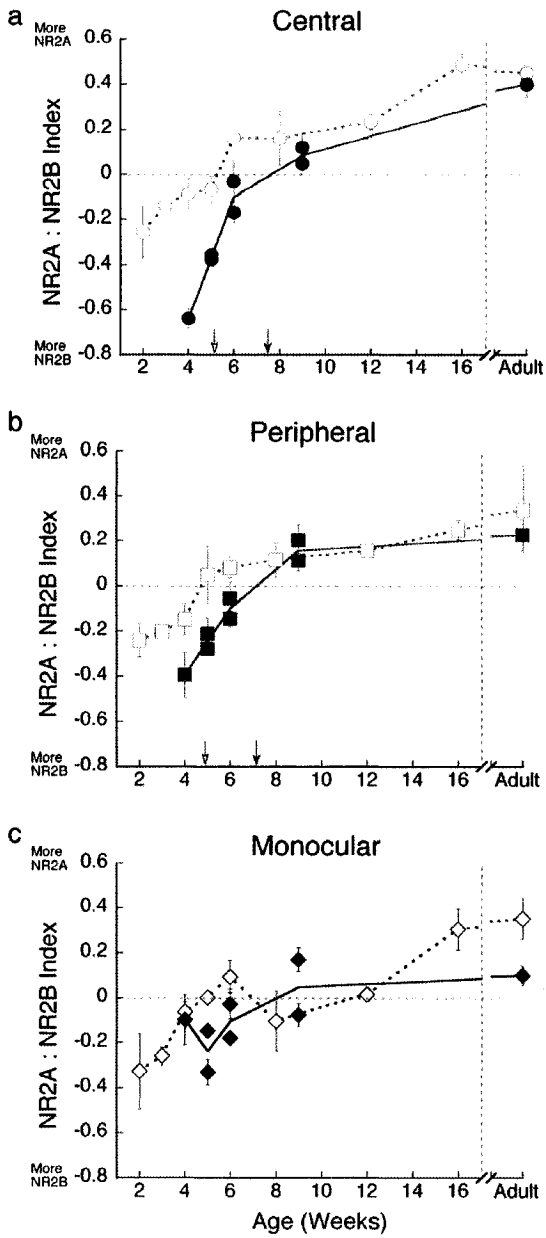


Figure 3.9. Relative index of NMDA receptor subunits.
The relative contribution of NR2A and NR2B is important for determining the kinetics of the NMDA receptor. During normal development (open symbols) there was a shift in the NR2A:NR2B index in the central (a, red circles), peripheral (b, green squares), and monocular (c, black diamonds) visual field representations to include more NR2A expression by 5 weeks of age (open arrow). Monocular deprivation (filled symbols) decreases the NR2A:NR2B index in the central (a, red circles) and peripheral (b, green squares) and delays the maturational switch from NR2B to NR2A by about 2 weeks (filled arrow).

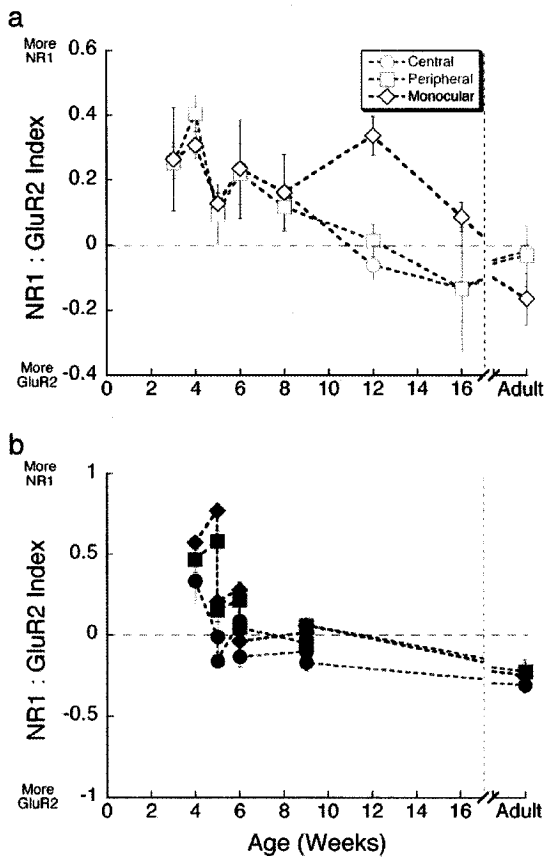


Figure 3.10. Relative shift in NR1: GluR2 index
Developmental changes in the expression of the NMDA receptor subunit NR1 and the AMPA receptor subunit GluR2 index during postnatal development and early monocular deprivation. (a) Early in development excitatory receptors are composed of more NMDA receptors and there is a gradual increase in the relative contribution of AMPA that occurs around 12 weeks of age. (b) Monocular deprivation shifts the balance of NMDA and AMPA receptors to favour AMPA by 5 to 6 weeks of age.

$GABA_A$ receptor function is affected by the composition of the receptor. Previous studies have shown that $GABA_A\alpha3$ expression is highest early in normal development and it has lower affinity for binding GABA. The insertion of $GABA_A\alpha1$ into the receptor speeds up the IPCS decay and increases the binding affinity for GABA. We quantified the relative change in $GABA_A$ receptor composition in V1 of normal and monocularly deprived kittens. In normal kittens the shift from $GABA_A\alpha3$ to $GABA_A\alpha1$ in V1 was similar across the 3 regions, shortly after eye opening $GABA_A\alpha3$ dominated, between 4 to 12 weeks expression was either balanced or slightly in favor of $GABA_A\alpha1$, and by 16 weeks of age there was substantially more $GABA_A\alpha1$ expression. Monocular deprivation led to a very different developmental profile of $GABA_A\alpha1$:$GABA_A\alpha3$ (Fig. 3.11). There was substantially more $GABA_A\alpha1$ relative to $GABA_A\alpha3$, even at the youngest age studied, and this shift in favor of $GABA_A\alpha1$ was maintained during development and into adulthood. Except in the monocular region where there was more $GABA_A\alpha3$ with deprivation into adulthood.

A number of recent studies have highlighted the importance of balance between excitatory and inhibitory mechanisms in maintaining normal developmental plasticity. We determined the relationship between the expression of the excitatory and inhibitory receptor subunits by calculating the correlation between $GABA_A\alpha1$:$GABA_A\alpha3$ and NR2A:NR2B indices (Fig. 3.12). For normal animals these indices were significantly correlated in all 3 regions (central $r=0.96$ $p<0.01$, peripheral $r=0.88$ $p<0.01$, monocular $r=0.79$ $p<0.01$) as well as for all of V1 ($r=0.78$ $p<0.01$). This analysis showed that in normal animals when there was more NR2B there was also more $GABA_A\alpha3$ expression, and when there was more

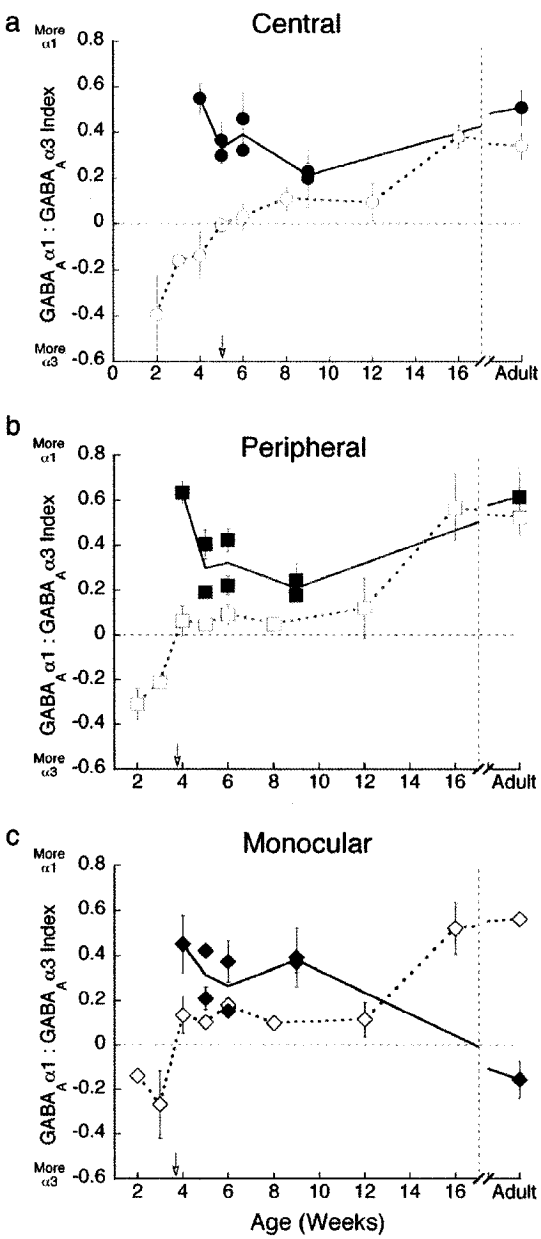


Figure 3.11. Relative index of $GABA_A$ receptor subunits.
The relative contribution of $GABA_A\alpha1$ and $GABA_A\alpha3$ is important for determining the kinetics of the $GABA_A$ receptor. During normal development (open circles), there was a shift favouring $GABA_A1$ expression by 4-5 weeks of age in central regions. Monocular deprivation (filled circles) initiates a rapid increase in the $GABA_A\alpha1$: $GABA_A\alpha3$ index in all regions of visual cortex.

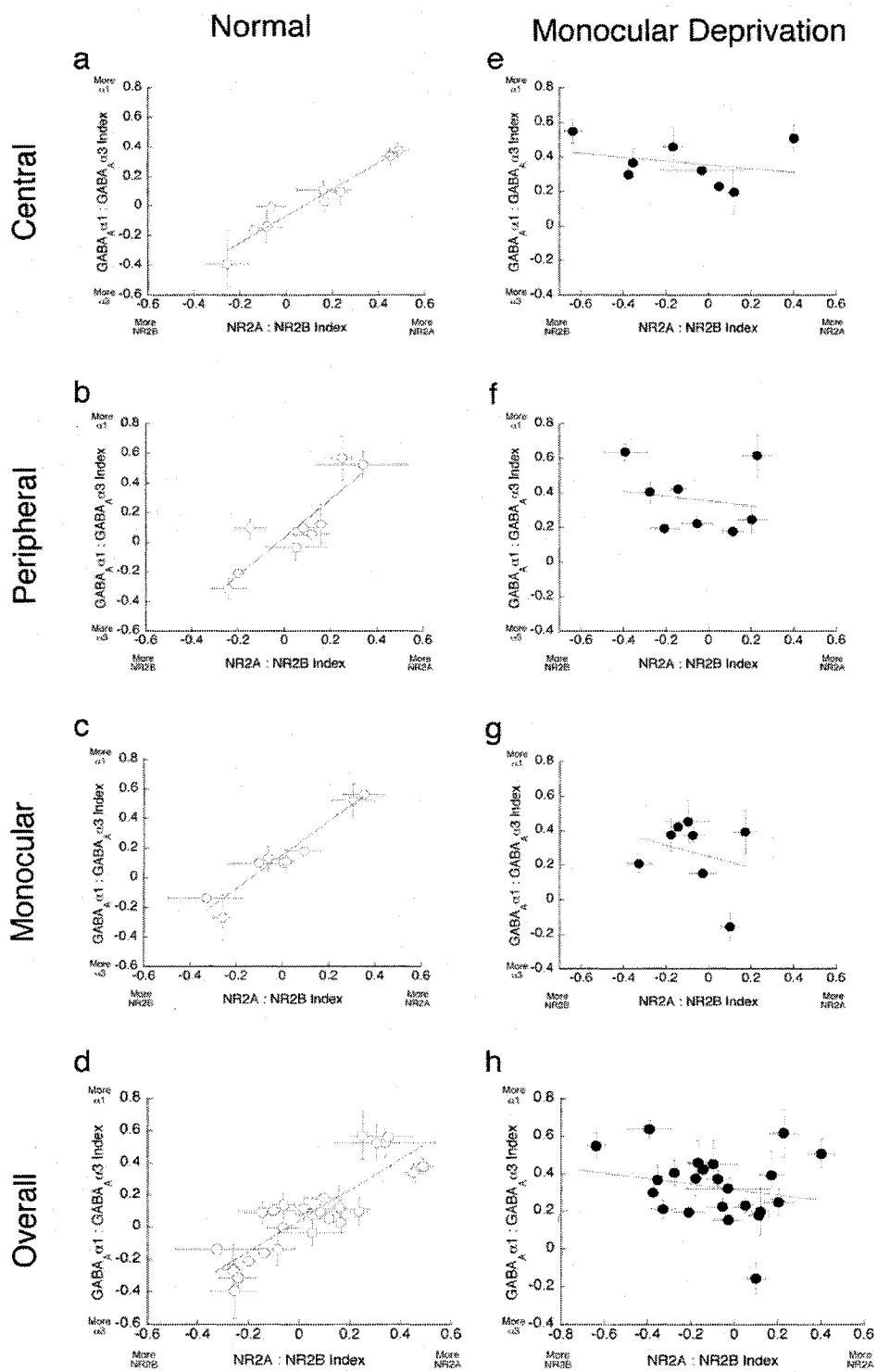

Normal
Monocular Deprivation
Central
Peripheral
Monocular
Overall
a
b
c
d
e
f
g
h
GABA$_A$ α1 : GABA$_A$ α3 Index
More α1
More α3
NR2A : NR2B Index
More NR2B
More NR2A
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8

Figure 3.12. Developmental correlation between NMDA and $GABA_A$ composition.
The maintenance of functional circuits relies on the appropriate balance between excitation and inhibition. The development of NMDA and $GABA_A$ receptors is shown in normal (open circles) and monocularly deprived animals (filled circles) in the central (a,e), peripheral (b,f), and monocular (c,g) visual field representations. Overall differences were also shown for comparison (d and h). During normal development (a-d) there is a significant correlation between the relative expression of $GABA_A$ and NMDA receptor subunits relationship between the maturation of $GABA_A$ and NMDA receptors. In contrast, monocular deprivation (e-h) disrupted the correlation between $GABA_A$ and NMDA receptor subunits.

NR2A there was more $GABA_A\alpha1$. A very different picture emerged in monocularly deprived animals. There were no significant correlations between the $GABA_A$ and NR2 indices showing that monocular deprivation caused a profound changed in the relationship between these excitatory and inhibitory mechanisms. Together, these results provide support for the notion that a balance between excitatory and inhibitory mechanisms is important for normal functional development of V1. In addition, this analysis revealed a relationship between the specific subunit composition of these excitatory and inhibitory receptors and that monocular deprivation disrupted the normal balance.

Discussion

In this study, we examined the normal development of key mechanisms for experience-dependent plasticity and the effect of monocular deprivation on these mechanisms. During normal development, the excitatory receptor subunits, (NR1, NR2A, NR2B, GluR2), increase gradually from eye opening to 8-12 weeks of age before declining to adult levels of expression by 16 weeks of age. On the inhibitory side, the $GABA_A\alpha3$ subunit is initially expressed at near peak levels upon eye opening remaining stable until 8 weeks of age whereas $GABA_A\alpha1$ is initially expressed at low levels and increases to 8 weeks of age followed by a decline to adult levels of expression. This pattern was consistent across the different visual field representations in visual cortex for all subunits except NR2A, which followed a different pattern in monocular visual field representation. Altering visual experience resulted in changes to excitatory and inhibitory subunits that were dependent upon (i) the region in visual cortex and, (ii) the duration of the deprivation. Monocular deprivation up to 6 weeks of age

decreases (NR1, NR2A, NR2B, $GABA_A\alpha3$) or increases ($GABA_A\alpha1$) expression in the central visual field representation, but results in no change, or an increase (NR1, $GABA_A\alpha1$) expression in the peripheral and monocular representations. Beyond 6 weeks of age, there is a reduction of protein expression that is consistent across the entire visual cortex. Taken together, these findings show that monocular deprivation has a significant impact on the expression of NMDA, AMPA, and $GABA_A$ subunits and the composition of NMDA and $GABA_A$ receptors, their functional properties, and synaptic plasticity in visual cortex.

Previous reports on the effect of monocular deprivation on receptor expression used immunohistology on tangential sections from unfolded and flattened visual cortex to identify regional changes in protein expression. While immunohistology offers cellular resolution and detailed tangential and laminar analysis (Murphy et al., 2004, Erisir and Harris, 2003; Chen, and Mower, 2001; Trepel et al, 1998), in practice, it is limited to studying only 1-2 proteins at a time. To overcome this limitation, we used a Western blotting approach to characterize many receptor subunits from multiple samples across visual cortex (Tusa, Palmer and Rosenquist, 1978). This approach provides a rich analysis of many plasticity mechanisms while maintaining sufficient resolution to evaluate regional changes in visual cortex.

Changes in excitatory and inhibitory gene expression following monocular and binocular deprivation have recently been reported in the binocular zone of mice. That study also found an increase in GluR2, but did not find changes in the genes for NR1, NR2A, NR2B, $GABA_A\alpha1$ and it found an increase in the gene for $GABA_A\alpha3$ (Tropea et al., 2006). It is difficult to directly compare protein and gene expression because of the anatomical and functional difference between the

central visual pathways in mice and cats (Drager and Olsen, 1980). Nevertheless, that experiment provides additional support for the notion that visual deprivation causes a complex cascade of changes in the expression of these neural plasticity mechanisms.

Behavioural and physiological studies have identified visuotopic deficits in visual function that are consistent with the regional differences in receptor expression that I have shown here. Behavioural studies on human infants treated for unilateral congenital cataracts have shown that visual acuity and temporal processing in the central visual field representation are more impaired than in the peripheral visual field representation (Bowering et al., 1993; Maurer et al., 1993). Physiological studies in animal models have identified that development of receptive field properties in the central visual field representation develop abnormally after monocular deprivation, but not the peripheral visual field representation (Kiorpes et al., 1998). The loss of NR1, NR2A and NR2B expression in the central visual field representation parallels the behavioural and physiological changes and provides evidence to suggest that reduced NMDA-dependent plasticity may contribute to the functional deficits in amblyopia.

Our findings show that monocular deprivation extending beyond 6 weeks of age truncates further maturation of NMDA, AMPA, and $GABA_A$ receptors. Research has shown that the capacity for recovery of binocular depth discrimination is extremely sensitive to the duration of initial deprivation (Timney, 1983). Monocular deprivation extending beyond 5 weeks of age eliminates any potential for recovery of binocular function in kittens. The substantial change in the developmental trajectories for all of these mechanisms

after 6 weeks of monocular deprivation may signify a critical change in the potential for the plasticity mechanisms to support visual recovery.

Behavioural studies of temporal perception show that children treated for unilateral congenital cataracts exhibit an 8-fold loss in their temporal sensitivity at low temporal frequency (1.5 Hz), but minimal deficits at higher temporal frequencies (Ellemberg et al., 2000). This behavioural change may be related to receptor subunit changes that shifted NR1:GluR2 and $GABA_A\alpha1$:$GABA_A\alpha3$ following monocular deprivation. There was a shift towards more GluR2, which mediates the early fast component, and less NR1 which mediates the later, slow component of the EPSC. Similarly, the shift to more $GABA_A\alpha1$ suggests more brief phasic inhibition. Both the excitatory and inhibitory changes would shorten the window for integrating neural signal and act to filter low frequencies. This suggests a potential mechanism that would preferentially affect the perception of low spatial frequency. In addition, this change in the temporal dynamics would degrade the signal to noise ratio which would alter information processing in visual cortex (Rust et al., 2002).

Previous reports have linked the developmental increase in NR2A expression with ocular dominance plasticity (Roberts and Ramoa, 1999; Chen and Mower, 2001; Fagiolini et al., 2003), maturation of orientation selectivity (Fagiolini et al., 2003) and as a mechanism for binocular coincidence detection (Roberts and Ramoa, 1999). Interestingly, we found that NR2A expression is substantially greater in the binocular regions than the monocular region of V1. This provides further support for the notion that NR2A expression is driven by binocular activity in visual cortex. In addition, the loss of NR1, NR2A and NR2B in the central region after early deprivation provides further support for the notion that

NMDA receptors are involved in binocular visual function. Finally, it has been shown that rearing animals with strabismus, a condition that does not reduce visually driven activity but severely reduces binocular correlations, leads to a significant reduction in NR1 expression (Yin et al., 1996).

A critical level of GABAergic inhibition is necessary to initiate the onset of the critical period in juvenile animals (Hensch, 1998). We found that monocular deprivation triggers an early change in GABAergic mechanisms with an increase in $GABA_A\alpha1$. This change would significantly alter the physiological properties of $GABA_A$ receptors, leading to faster IPSC kinetics (Bosman et al., 2002) and a greater binding efficacy of GABA (Bohme et al., 2004), suggesting that GABAergic inhibition is brief and stronger following early monocular deprivation. Interestingly, this finding is consistent with previous research showing that monocularly deprived cats exhibit a 40% greater response to bicuculline than normally reared cats (Mower and Christen, 1989) and helps to explain why there is a stronger inhibitory response even though the number of GABA neurons is not increased (Bear et al.,1985).

The activation of $GABA_A$ receptors containing the $\alpha1$ subunit plays a key role in plasticity. Selectively disrupting the function of $GABA_A\alpha1$, but not $GABA_A\alpha3$, disrupts ocular dominance plasticity (Fagiolini et al., 2004) and suggest that expression of $GABA_A\alpha1$ plays a specific role in critical period plasticity. In this framework, the increase in $GABA_A\alpha1$ expression following early monocular deprivation might accelerate the onset of the critical period and effectively reduces the available plasticity. Assessments of plasticity in monocularly deprived animals have shown that short-term monocular deprivation reduces plasticity by diminishing the physiological effects of

subsequent periods of monocular deprivation. Those findings are consistent with the presently reported increase in $GABA_A\alpha1$ expression after monocular deprivation which would decrease the capacity for plasticity in the visual cortex.

Many current models of plasticity in visual cortex consider changes in both excitatory and inhibitory processing in the deprived animal (Fagiolini et al., 2003; Turrigiano and Nelson, 2004; Heinen et al., 2004). Even small changes in the balance between excitation and inhibition disrupt sensory responses in primary visual cortex, and alter experience-dependent plasticity (Kirkwood and Bear 1994; Hensch et al. 1998; Heynen et al., 2003). Because changes in the functional properties of NMDA and GABA receptors are largely determined by modifications in the relative expression of NR2A/NR2B and $GABA_A\alpha1$/ $GABA_A\alpha3$ (Monyer et al., 1992; Quinlan et al., 1999; Bosman et al., 2002; Heinen et al, 2004), this measure serves as a good indicator of the excitatory-to-inhibitory balance. We show here that monocular deprivation disrupts the normal relationship between excitation and inhibition by reducing the NR2B:NR2A index (indicative of prolonged NMDA receptor EPSCs), while increasing the $GABA_A\alpha1$: $GABA_A\alpha3$ index (indicative of faster $GABA_A$ receptors IPSPs). The changes in NMDA and $GABA_A$ indices will have a significant impact on the timing of excitatory (Flint et al., 1997) and inhibitory kinetics (Bosman et al., 2002) highlighting the importance of visual experience on the balance between excitation and inhibition.

Chapter 4

Binocular Vision Following Monocular Deprivation Restores Excitatory and Inhibitory Receptor Expression in Visual Cortex

Introduction

Occluding one eye of visual experience early in life reduces acuity and leads to a loss of physiological responses from visual cortical neurons to stimulation through the deprived eye (Wiesel and Hubel, 1965; Dews and Wiesel, 1970; Hubel and Wiesel, 1970). Traditional treatment strategies to promote recovery from monocular deprivation include a prolonged period of reverse occlusion in which the non-deprived eye is patched. Although reverse occlusion can promote an improvement in behavioural acuity (Mitchell et al., 1984a) and complete reversal of physiological representation of the initially deprived eye in visual cortex (Movshon, 1976; Mitchell et al., 1977), the recovery of visual function is labile, and rapidly lost upon the restoration of binocular vision (Mitchell et al., 1984a, 1984b; Murphy and Mitchell, 1986, 1987). The transient nature of this recovery has been a puzzle, however, we have recently provided new insights into potential mechanisms that are essential for experience-dependent plasticity (Murphy et al., 2004; Beston et al., 2004).

Synaptic plasticity in visual cortex is regulated by compositional changes in key excitatory and inhibitory receptors. Hallmark features of experience-dependent development in visual cortex, such as the maturation of orientation selectivity and ocular dominance segregation, are dependent upon activation of the major excitatory (NMDA) (Kleinschmidt et al.,1987; Roberts and Ramoa, 1998) and inhibitory ($GABA_A$) receptors (Hensch, 1998; Fagiolini et al., 2004). In the previous Chapter, we found that the expression of NMDA, AMPA, and $GABA_A$ receptors are affected by visual experience. Monocular deprivation disrupts the binocular correlation among inputs to visual cortex, and leads to a loss of NMDA subunit expression in the central visual field representation of visual cortex

(Murphy et al., 2004). This loss cannot simply be explained by a reduction of visually driven activity, because monocular deprivation promotes a homeostatic up-regulation of NR1 expression in monocular regions of visual cortex. Furthermore, the relative contribution of the $GABA_A\alpha1$ subunit is elevated uniformly across visual cortex following monocular deprivation. These results suggest that regional differences in the pattern of activity and the homeostatic response to the amount of activity operate together to drive changes in NMDA and $GABA_A$ receptor composition. These changes lead to an abnormal balance between excitatory and inhibitory plasticity mechanisms that might limit developmental plasticity.

Emerging treatment strategies have shown that introducing binocular vision is essential to promote optimal physiological (Kind et al., 2002; Faulkner et al., 2006), anatomical and behavioural recovery from early visual deprivation (Murphy et al., 2002). Despite our knowledge that binocular vision is important for behavioural, anatomical, and physiological recovery from deprivation, it is still unclear if binocular vision promotes recovery of key synaptic plasticity mechanisms in visual cortex. To address this question, I have investigated the time course of recovery of AMPA, NMDA, and $GABA_A$ receptor subunits, and for changes in synaptic markers for presynaptic terminals (Synapsin), and dendritic spines (Drebrin) promoted by a brief period of binocular vision.

Methods

The effect of different types of visual experience on the recovery of excitatory and inhibitory receptor expression was studied in the primary visual cortex of kittens (n=7) reared with either normal visual experience, or monocular

deprivation initiated at the time of natural eye opening. At 5 weeks of age, kittens reared with monocular deprivation to 5 weeks of age were followed by either 18 days of reverse occlusion, 4 days of binocular deprivation, or a period of binocular vision (1, 6, 24, 48, 96 hours). The expression levels were compared with those for normal and monocularly deprived kittens presented in the previous chapter.

Surgical procedures, synaptoneurosome preparations, and immunoblotting were performed using the same procedures as described in Chapter 3. In addition to Chapter 3, we assessed the changes in Drebrin expression (1:1000; Research Diagnostics, Inc., Concord, MA) as a marker of spine motility, in homogenate samples taken from visual cortex. Data analysis was also performed in a manner similar to that in Chapter 3, but all data was normalized to the expression of 5 week old normally reared kittens.

Results

We evaluated the effect of different recovery regimens (reverse occlusion, binocular deprivation, binocular vision) after monocular deprivation to 5 weeks of age on the expression of excitatory (NR1, NR2A, NR2B, GluR2) and inhibitory ($GABA_A\alpha1$, $GABA_A\alpha3$) plasticity mechanisms, and synaptic markers (synapses - Synapsin, spines - Drebrin) in visual cortex. The extent of recovery was compared with similarly aged animals that were reared with either normal binocular vision or monocular deprivation.

Reverse Occlusion

18 days of reverse occlusion promoted a complex pattern of change in the expression of excitatory and inhibitory receptor subunits in visual cortex. For

some subunits (GluR2, $GABA_A\alpha1$; Fig. 4.1 c & d) reverse occlusion shifted expression toward normal levels and this was particularly evident for binocular portions of V1 (central and peripheral visual field representations). Alternatively, expression of other subunits (NR2A, $GABA_A\alpha3$; Fig. 4.1 b & e) did not change with reverse occlusion. For one subunit, NR2B (Fig. 4.1 c), there was a substantial loss of expression in all regions of V1 resulting in significantly lower NR2B expression than found for either monocularly deprived or normally reared animals. The obligatory NMDA receptor subunit, NR1 (Fig. 4.1a), showed the most complex pattern of expression after reverse occlusion. In the central visual field there was an increase in NR1 expression towards normal levels, in the peripheral representation there was no change, and in the monocular representation there was a loss of NR1.

Reverse occlusion also promoted changes in synaptic markers. Both Synapsin and Drebrin were significantly greater than in normals, however, changes in Drebrin expression were much higher. In all regions of V1, Drebrin expression rose to about 20 times normal levels which suggests that reverse occlusion promoted a significant increase in spinogenesis.

Previous studies have shown that there are developmental changes in subunit composition of NMDA and $GABA_A$ receptors and the relative expression of NMDA:AMPA receptors. There are developmental shifts from more NR2B to more NR2A and from more $GABA_A\alpha3$ to more $GABA_A\alpha1$. Nascent excitatory synapses include only NMDA receptors and then activity leads to the insertion of AMPA receptors. These changes are linked with different stages of synapse formation and developmental plasticity. To address the influence of reverse occlusion on the relative expression of NR2 subunits, $GABA_A$ subunits, and

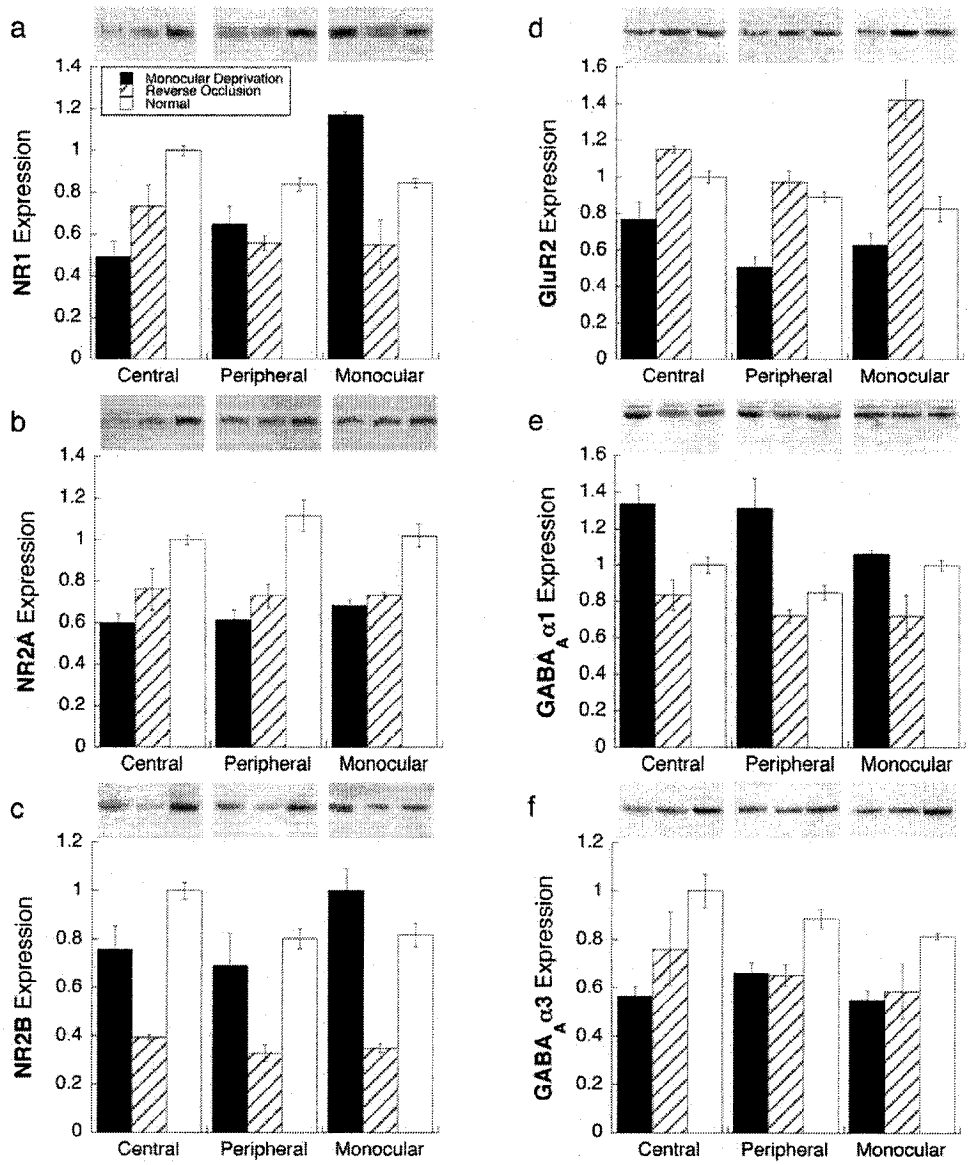


Figure 4.1. Expression of excitatory and inhibitory subunits after reverse occlusion. Expression of excitatory and inhibitory receptors in the central, peripheral, and monocular visual field representations of visual cortex for NMDA (a-c), AMPA (d), and $GABA_A$(e-f) receptor subunits. Representative bands are shown above each plot. The expression of each subunit was measured relative to the expression found in the central visual field representation of 5 week old normal kittens. Results are shown for kittens monocularly deprived to 5 weeks of age (black bars), followed by 18 days of reverse occlusion (striped bars), and age-matched normally reared kittens (open bars). Representative bands are shown above each bar. Reverse occlusion fails to restore the expression of NMDA subunits NR1 (a), NR2A (b), NR2B (c), GluR2 (d) and GABAergic subunits, $GABA_A\alpha1$ (e), and $GABA_A\alpha3$ (f) receptor subunits back to that of normally reared kittens ($p<0.05$).

NMDA:AMPA ratio we calculated 3 indices. Reverse occlusion promoted substantial changes in all of these indices. In all regions within V1, there was a shift to more NR2A with levels that were substantially greater than age matched normal animals and more similar to older normally reared cats (Fig 4.2a). In addition, after reverse occlusion GluR2 dominated all regions of V1 with significantly greater expression than age matched normals (Fig. 4.2b). These results suggest that reverse occlusion accelerates the maturation of NMDA receptor subunit composition and the NMDA:AMPA ratio. In contrast, the relative contribution of $GABA_A\alpha1$ and $GABA_A\alpha3$ shifted towards normal levels of expression (Fig. 4.2c). Taken together, the results for the individual receptor subunits and the indices show that reverse occlusion affects these plasticity mechanisms, but it does not promote recovery of a normal expression pattern.

Binocular Deprivation

To begin to tease apart the factors that underlie the changes in receptor expression after reverse occlusion, we examined changes promoted by a period of binocular deprivation after monocular deprivation. This paradigm leads to a general reduction in visually driven activity but maintains (or even increases) binocularly correlated patterns of activity. This is because the eyelids attenuate the number of photons and act as low-pass filters allowing only very low spatial frequency visual information to reach the retina. Thus, through the closed eyelids the animal is still able to see large, bright, high-contrast objects. Furthermore, there is a much greater likelihood that the low spatial frequency information reaching the 2 retinae through the eyelids will be in correspondence and that will drive binocularly correlated patterns of activity.

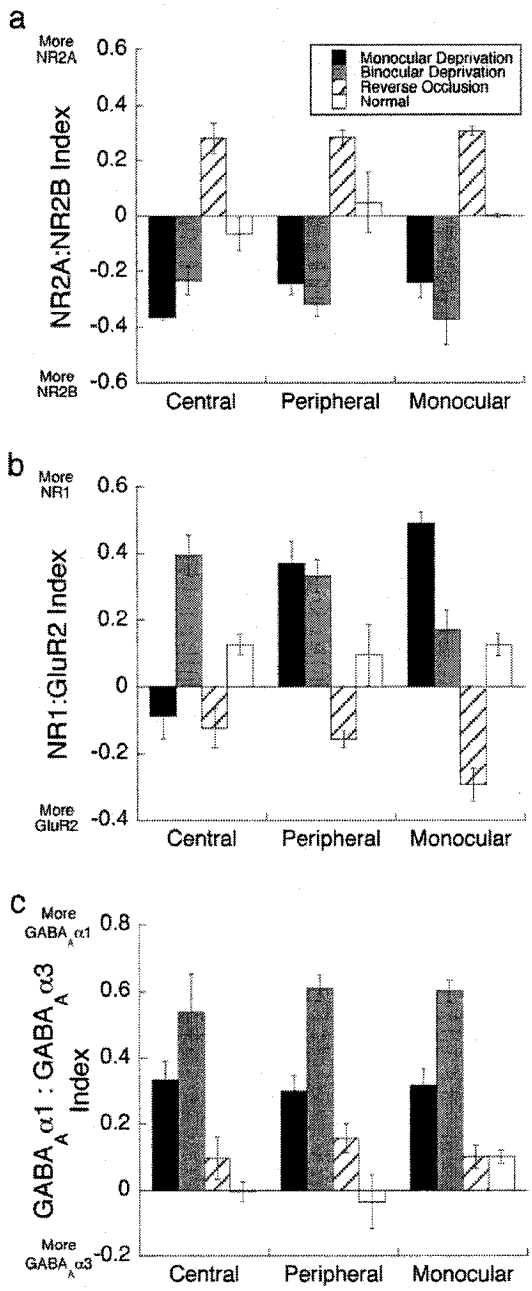


Figure 4.2. Relative changes in excitatory and inhibitory indices. NR2A:NR2B (a), NR1:GluR2 (b), and $GABA_A\alpha1$:$GABA_A\alpha3$ (c) indices following different rearing conditions (black bars = monocular deprivation, grey bars = binocular deprivation, diagonal stripes = reverse occlusion, open bars = normal) in the central, peripheral, and monocular regions in visual cortex. After monocular deprivation and binocular deprivation, there were significantly greater contributions of NR2B, $GABA_A\alpha1$ and NR1 compared to normal. After reverse occlusion there was more NR2A, $GABA_A\alpha1$, and GluR2 expression.

In a previous study, we showed that binocular deprivation leads to a significant increase in NR1 immunoreactivity in visual cortex (Murphy et al., 2004). That finding raised the possibility that binocularly correlated patterns of activity are sufficient to promote recovery. To address this we quantified expression levels of NMDA, AMPA, and $GABA_A$ receptor subunits in animals monocularly deprived to 5 weeks of age followed by 4 days of binocular deprivation (Figure 4.3). In agreement with our previous finding, the 4 days of binocular deprivation led to a 10-35% increase in NR1 expression (Fig. 4.4a). There was, however, no recovery for NR2A, only modest change for GluR2, limited recovery of NR2B only in the central visual field representation and recovery of $GABA_A\alpha3$ only in the monocular representation (Fig. 4.3b, c, d & f). A surprising increase in $GABA_A\alpha1$ expression was found in all regions of V1 with expression levels about 2-3-fold greater than normal (Fig. 4.3e). We calculated the indices described above and found that binocular deprivation did not promote recovery in the composition of NR2A:NR2B, $GABA_A\alpha1$:$GABA_A\alpha3$ receptors or tNR1:GluR2.(Fig. 4.2a & b). The observed increase in $GABA_A\alpha1$ expression skewed the $GABA_A\alpha1$:$GABA_A\alpha3$ index even further away from normal levels (Fig 4.2c). These results show that binocular deprivation does not promote a general return to normal levels of expression and suggests that binocularly correlated patterns of activity alone are not sufficient to support normal expression.

Binocular deprivation promoted no change in the presynaptic marker Synapsin, but an interesting pattern of change in Drebrin expression. There was a very large increase in Drebrin expression (20-30 times normal levels) in the binocular regions of visual cortex, but no change in the monocular region. This

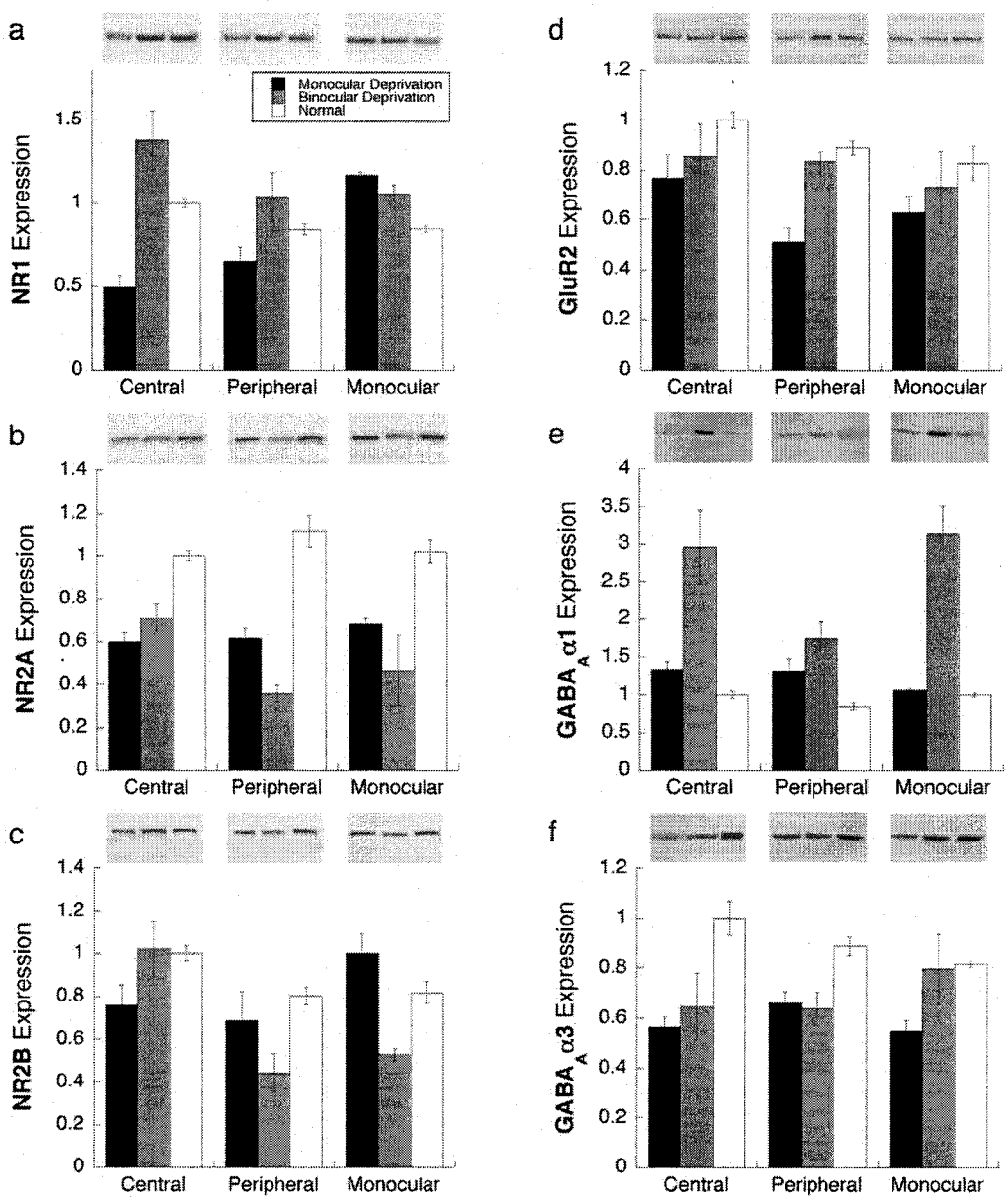


Figure 4.3. Excitatory and inhibitory expression after 4 days of binocular deprivation.
4 days of binocular deprivation (grey bars) fails to promote the recovery of all excitatory and inhibitory receptors to normal levels (open bars). NR1 (a) and $GABA_A\alpha1$ (e) were significantly elevated relative to normal. GluR2 expression was not significantly different from normal. Partial recovery of NR2B and $GABA_A\alpha3$ was observed in the central visual field representation of NR2B and the monocular visual field representation of $GABA_A\alpha3$. No recovery of NR2A expression was apparent. The expression of each subunit was measured relative to expression found in the central visual field representation of 5 week old normal kittens. Representative bands are shown above each plot.

suggests that binocularly correlated patterns of activity were sufficient to initiate a significant amount of spinogenesis in visual cortex.

Reverse occlusion and binocular deprivation promoted opposite changes in NR2A:NR2B, GluR2:NR1, and $GABA_A\alpha1$:$GABA_A\alpha3$ indices (Figure 4.2). In all but one instance, after binocular deprivation these indices remained in the same direction as found for monocularly deprived animals. The one exception was for the GluR2:NR1 index in the central visual field. Here binocular deprivation led to a much greater contribution of NR1, suggesting a change to a more immature ratio of AMPA and NMDA receptors. In general, changes in the indices promoted by binocular deprivation can be described as shifting the excitatory receptors towards less mature and greater plasticity while the $GABA_A$ receptor composition shifted to more mature and less plasticity. These changes are opposite of those produced by reverse occlusion. But, neither of these regimens promoted recovery of a normal excitatory-inhibitory balance. These results provide new insights into why neither of these rearing regimens promote good recovery of vision (Murphy et al., 2002) and suggest that the balance between excitatory and inhibitory mechanisms might be crucial to the recovery of normal vision following monocular deprivation.

Binocular Vision

A number of studies have shown that binocular vision can promote behavioural and physiological recovery after monocular deprivation (Giffin and Mitchell, 1978; Malach et al., 1984; Mitchell, 1988) and that binocularly correlated input is essential for that recovery (Kind et al., 2002). We examined whether binocular visual experience (range 1-96 hours) after monocular deprivation is sufficient to promote recovery of a normal balance between excitatory and

inhibitory mechanisms in visual cortex. Because we studied expression in the different regions of visual cortex, we were able to address whether binocularly correlated activity, which is greatest in the central region and not present in the monocular region, or increased activity as a result of opening the eye, drives recovery of these plasticity mechanisms.

There was no clear pattern of change in Synapsin expression after binocular vision. There were, however, very large changes in Drebrin expression (Figure 4.4). Within 1 hour of restoring binocular vision there was a 50-60 fold increase in Drebrin expression. In the central and peripheral visual regions, a very high level of Drebrin expression was maintained throughout the 4 days (Fig. 4.4a). A different pattern emerged in the monocular region where Drebrin expression subsequently declined back to normal levels after 4 days of binocular vision. This expression pattern suggests that binocular visual experience promoted a rapid and substantial period of spinogenesis that was initially driven by an increase in activity and then maintained in regions where there was an increase in binocularly correlated activity.

Binocular visual experience also promoted changes for all excitatory mechanisms and for $GABA_A\alpha1$, but there was no change for $GABA_A\alpha3$ (Figure 4.5). Some changes were significantly different for the central and monocular regions and in some cases just a few hours were sufficient to promote significant change. Changes that were fitted by either a linear or a log function ($p<0.05$) are plotted to illustrate the progressive nature of the changes.

NR1 expression in the central region doubled, increasing monotonically from 1 hour to 4 days of binocular vision (Fig 4.5a; $R=0.96$). In contrast, NR1 expression in the monocular region dropped to half the level found at the end of

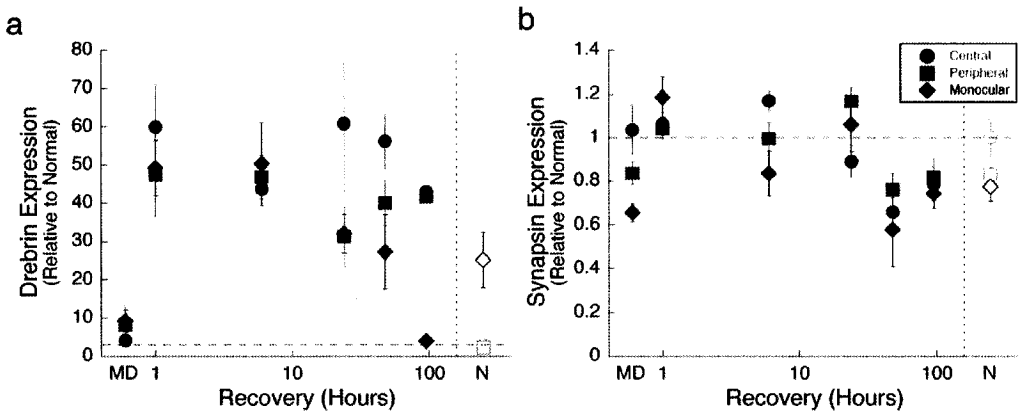


Figure 4.4. Changes in synaptic markers during visual recovery.
The expression of synaptic markers, Drebrin (a) and Synapsin (b) in the central (red circles), peripheral (green squares), and monocular (black diamonds) visual field representations. Expression in normal (N, open symbols) and monocularly deprived kittens (MD, filled symbols) are shown for comparison. Dashed lines represent the normal expression in the central visual field. Binocular vision initiates a rapid increase in the expression of Drebrin within the first hour of binocular vision (a). There was no clear pattern of change in synapsin expression (b).

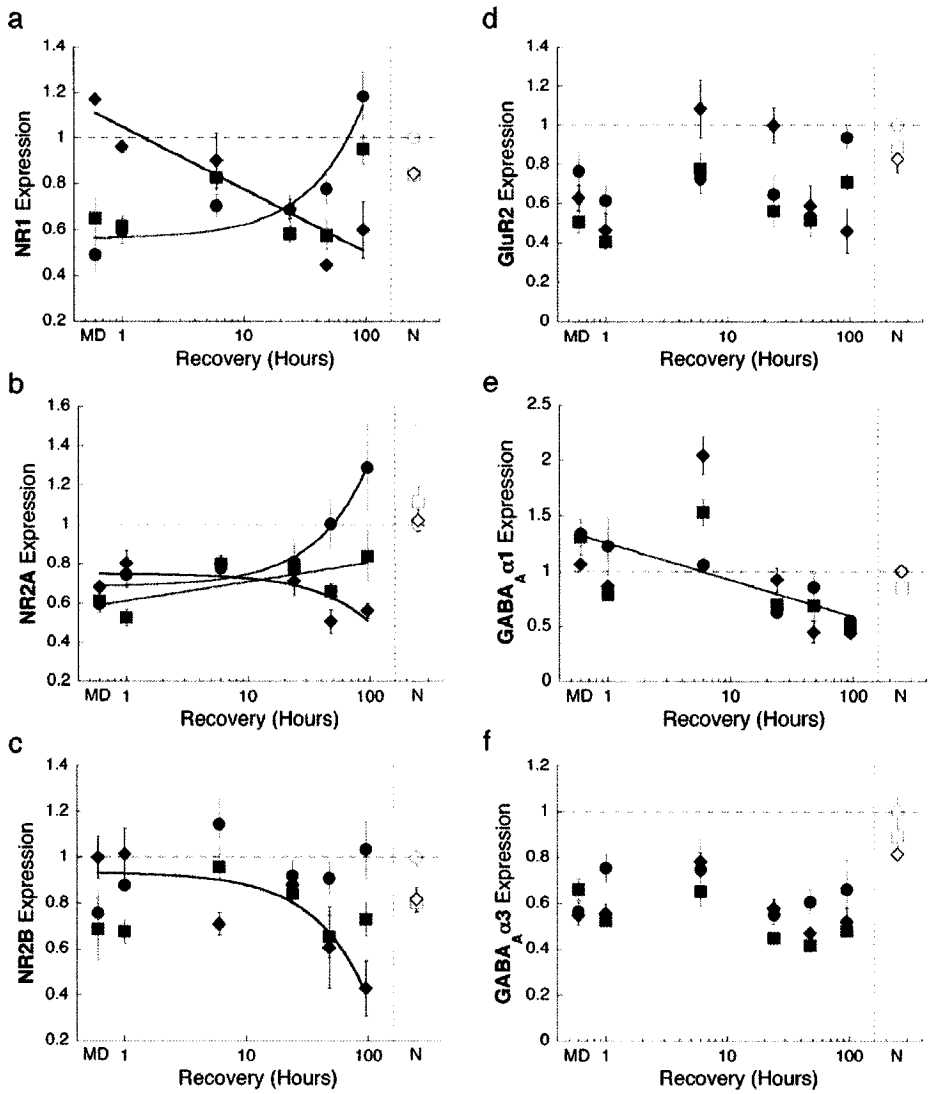


Figure 4.5. Regional recovery of subunit expression following monocular deprivation. Quantification of NMDA (a-c), AMPA (d), and $GABA_A$(e-f) receptor subunits in kittens that received 1 hour to 4 days of binocular visual experience following 5 weeks of monocular deprivation (filled circles). Regional expression is separated by central (red circles), peripheral (green squares) and monocular visual field representations (black diamonds). Values are normalized to normal expression in the central visual field representation (dashed line, open symbols). Fit lines denote a significant trend ($p<0.05$). Binocular visual experience promotes a rapid recovery of NR1 (a), NR2A (b), NR2B (c), and GluR2 (d) in the central and peripheral visual representations, and a decrease in the monocular visual field representation. (e) $GABA_A\alpha1$ expression decreases following 4 days of visual experience, while $GABA_A\alpha3$ expression (f) remains relatively unchanged by binocular visual experience following monocular deprivation.

monocular deprivation. The rate of loss in the monocular region was best fit by a log function (R=0.93) indicating that the loss was rapid during the first few hours of binocular visual experience and then slower over the next few days. Expression of NR1 in the peripheral region was relatively unchanged for the first 2 days but then increased with 4 days of binocular visual experience. These results suggest that increased binocularly correlated activity up-regulates NR1 expression while simply increasing activity levels leads to a homeostatic down-regulation of NR1. NR2A expression showed a monotonic (Fig. 4.5b; R=0.97) increase in the central region, a trend (R=0.72, $p<0.06$) towards a modest increase in the peripheral region, and a modest loss in the monocular region (R=0.77). NR2B expression also showed differences across visual cortex (Fig. 4.5c). In the central region, NR2B expression increased over the first 6 hours and then remained at that level, while in the peripheral region there was an initial increase in NR2B but then it dropped back to levels found at the end of monocular deprivation. NR2B in the monocular region declined monotonically (R=0.88) over the 4 days of binocular vision to reach a level that was less than half of that found at the end of monocular deprivation. GluR2 expression showed modest increases over the 4 days of binocular vision in both the central and peripheral regions (Fig. 4.5d). In contrast, in the monocular region expression initially increased and then decreased to levels that were slightly below that found at the end of monocular deprivation. In all regions, $GABA_A\alpha1$ expression declined after 4 days of binocular vision, dropping to levels that were about half of that attained during monocular deprivation (Fig. 4.5e). The loss in the central regions was best fit by a log function indicating that the loss was rapid during the first few hours and then slower over

the next few days. $GABA_A\alpha1$ expression in peripheral and monocular regions initially increased before declining.

Changes in the individual excitatory and inhibitory mechanisms are summarized in Table 4.1, where the thumbs-up indicate an increase, thumbs-down a decrease, empty cells indicate no change, and the size of the thumb codes for the percent change between monocular deprivation and 4 days of binocular vision (small thumb =10-20% increase, medium thumb =21-49% increase, large thumb =>50% increase). A clear pattern of change emerged for the excitatory receptors with increases in the central region, moderate increases in the peripheral region, and decreases in the monocular region. These regional differences suggest that different patterns of activity might drive theses changes and provide additional support for the notion that experience-dependent plasticity is not uniform across visual cortex. The inhibitory losses, however, were similar across visual cortex which raises the possibility that part of the recovery process might involve a reduction of inhibition

Table 1: Summary of regional changes in receptor subunit composition.

	Central	Peripheral	Monocular
NR1			
NR2A			
NR2B			
GluR2			
$GABA_A\alpha1$			
$GABA_A\alpha3$			

The 3 indices were calculated to determine whether there was recovery in the composition of NMDA, AMPA, and $GABA_A$ receptors to normal levels and if the balance between NMDA:AMPA receptors was restored by binocular vision (Figure 4.6). The NR2A:NR2B index showed a progressive shift from NR2B to NR2A for all regions over the 4 days, reaching values that were similar to those of normal animals (Fig 4.6a). The NR1:GluR2 index changes after 4 days of binocular vision, however, the changes were in the direction of normal expression (Fig. 4.6b). The $GABA_A\alpha1$:$GABA_A\alpha3$ index also showed a progressive change in all 3 regions to reach normal levels after 4 days of binocular vision (Fig. 4.6c). Overall,

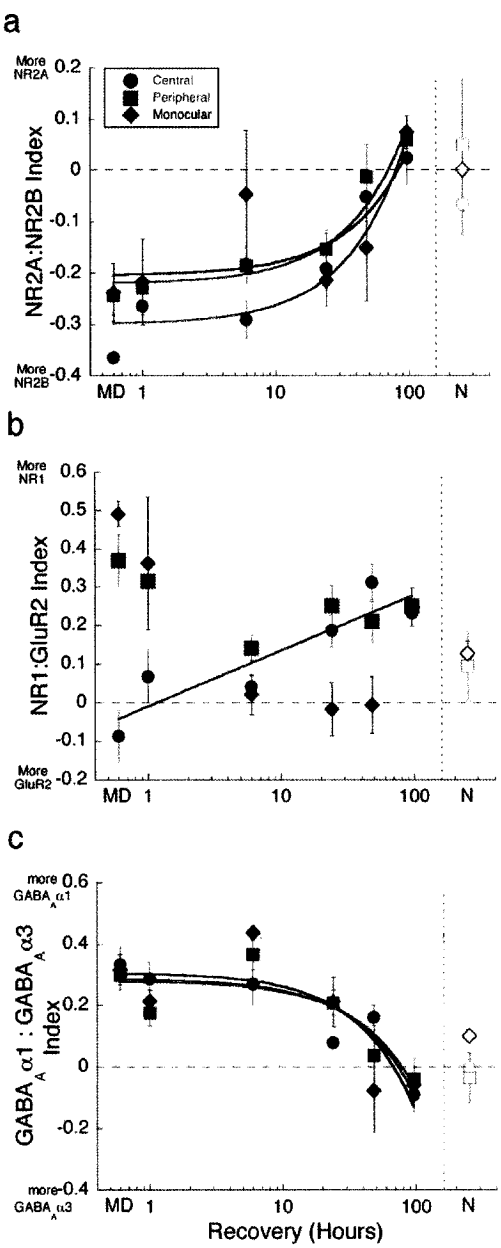


Figure 4.6. Relative changes in excitatory and inhibitory indices after binocular visual experience.
The kinetic and functional properties of NMDA and $GABA_A$ receptors are largely determined by the relative contribution of NR2A:NR2B and $GABA_A\alpha1$:$GABA_A\alpha3$ respectively. 4 days of binocular visual experience is sufficient to promote recovery of NR2A:NR2B (a) and NR1:GluR2 indices (b) in the central (red circles), peripheral (green squares), and monocular (black diamonds) visual field representations. Open symbols represent the index observed in age-matched normally reared kittens. (c) Binocular visual experience promotes regional changes in the $GABA_A\alpha1$: $GABA_A\alpha3$ index.

the period of binocular vision promoted changes in these indices towards normal levels of expression.

Discussion

In this chapter, I have provided a detailed description of changes in NMDA, AMPA, and $GABA_A$ receptor subunits that accompany the restoration of binocular visual experience. Binocular vision promoted changes in the expression of these plasticity mechanisms towards normal levels. Furthermore, the relative expression of NR2A:NR2B, NR1:GluR2, and $GABA_A\alpha1$:$GABA_A\alpha3$ shifted towards the normal balance. These changes were specific to kittens receiving binocular visual experience, since neither reverse occlusion, nor binocular deprivation promoted recovery. These results extend our understanding of the recovery from monocular deprivation and provide new insights to explain why binocular vision is necessary for the physiological and behavioural recovery from amblyopia.

There was a surprising influence of binocular visual experience on Drebrin expression. We observed a rapid, drastic increase in Drebrin expression, suggesting that binocular vision promoted a period of substantial spinogenesis in visual cortex. The increase in Drebrin suggests that binocular vision promotes a period of substantial spinogenesis in visual cortex. The regional differences in Drebrin expression suggest that there is prolonged synaptic motility in the central and peripheral visual field representation, beyond the 4 days of binocular visual experience. Similar observations also demonstrated that monocular deprivation initiates rapid changes in spinogenesis that are greater in the binocular regions of

primary visual cortex (Lund et al., 1991; Oray et al., 2004), indicating that binocular competition is critical for the alterations of structural plasticity.

In the previous chapter, I showed that monocular deprivation to 5 weeks results in a loss of NR1, NR2A, and NR2B expression in the central regions, and an increase in the expression of the NMDA subunits, NR1 and NR2B, in the monocular regions of visual cortex. This regional difference might reflect two separate processes; the reduction of binocularly correlated activity in the central visual field representation and a homeostatic response to a reduction in activity in the monocular regions (Murphy et al., 2004). Consistent with this idea, we found a clear dissociation in the expression of excitatory receptors in the central and monocular regions during the period of binocular visual recovery. Specifically, there was a consistent increase in the expression of excitatory subunits (NR1, NR2A, NR2B, GluR2) in the central regions, but a reduction of expression in the monocular regions. In particular, recent studies have suggested that NR2A expression is thought to play an important role in the detection of binocular activity in visual cortex (Roberts and Ramoa, 1999). In this chapter, I add to those findings by demonstrating that NR2A expression increases in the central and peripheral regions of visual cortex, where there is binocular input, but not in the monocular regions. This provides further evidence to suggest that the regulation and expression of neural plasticity mechanisms are not uniform across the visual cortex and that binocular vision and visual activity influence the expression of those mechanisms in visual cortex.

To further separate the role of visual activity and binocular vision in the recovery of visual function, we evaluated the effect of reverse occlusion and binocular deprivation on the recovery of plasticity mechanisms in visual cortex. Binocular deprivation increases binocular correspondence of visual input, but

reduces visual activity, while reverse occlusion leads to an increase in visually driven activity, but disrupts binocular correspondence. Both reverse occlusion and binocular deprivation lead to changes in expression of some plasticity mechanisms in visual cortex, but not all mechanisms return to normal levels of expression. Behavioural studies have shown that neither of these rearing conditions promote permanent recovery of visual function (Murphy et al., 2002), suggesting that no single mechanism provides the key to visual recovery. These findings help to reconcile why binocular deprivation after monocular deprivation can increase NR1 immunoreactivity in visual cortex, but fail to promote good recovery of visual acuity (Murphy et al., 2002).

Binocular deprivation leads to a homeostatic increase in $GABA_A\alpha1$. It has been proposed that $GABA_A$ mediated inhibitory activity including $GABA_A\alpha1$ is essential for initiating ocular dominance plasticity in the mouse (Fagiolini et al., 2004), and the temporal coding of visual information. The present results suggest that the capacity for experience-dependent plasticity in visual cortex will be influenced by the changes in $GABA_A\alpha1$. As a result, we would predict that binocular deprivation would advance the critical period and thereby reduce the capacity for neural plasticity. These findings might help to explain why binocular deprivation following monocular deprivation leads to poor functional recovery of visual acuity (Murphy et al., 2002).

Changes in NMDA, AMPA, and $GABA_A$ subunit composition are key markers of developmental plasticity including ocular dominance plasticity (Ramoa et al., 2001; Fagiolini et al., 2003; Fagiolini et al., 2004), the development of orientation selectivity (Roberts and Ramoa, 1999; Fagiolini et al., 2003), and synaptic plasticity in visual cortex (Heynen et al., 2003). Our recent finding that

monocular deprivation disrupted the normal developmental balance between NMDA and $GABA_A$ receptor subunit expression led us to consider the possibility that the key to restoring visual function and plasticity in the visual cortex might be to restore the appropriate balance between these excitatory and inhibitory plasticity mechanisms (Beston et al., 2004). This balance may underlie long-term recovery of the physiological representation of the deprived eye (Faulkner et al., 2006), orientation selective tuning (Kind et al., 2002) and visual acuity that is promoted by binocular vision (Murphy et al., 2002). Further evidence in the human literature suggests that children treated for unilateral cataracts do not suffer any negative consequences from an initial period (up to 1 month) of normal binocular vision prior to patching (Maurer and Lewis, 2001). While the nature of this recovery has remained unclear, it has been suggested that an initial increase in binocularly driven visual activity supports a rapid, but non-competitive improvement of visual function in both eyes (Mitchell and Gingras, 1998). The experience-driven changes in NMDA and $GABA_A$ expression that we observed during the brief period of binocular vision will certainly influence the rapid anatomical reorganization and physiological recovery of the deprived eye. Knock-out studies have demonstrated the need for an appropriate balance between excitation and inhibition during development by showing that mice lacking the GABA synthesizing enzyme GAD 65 or the NR2A subunit leads to a significant reduction in experience-dependent plasticity in visual cortex (Hensch et al. 1998, Fagiolini et al., 2003). However, shifting this balance by diazepam infusion restores plasticity in visual cortex as well as the susceptibility to monocular deprivation (Hensch et al. 1998). Our findings that NMDA and $GABA_A$ subunit composition is altered following monocular deprivation and

binocular visual experience suggest that visual experience alone is capable of modifying the relative timing of excitatory and inhibitory mechanisms and modify synaptic plasticity in visual cortex.

It was established in Chapter 3 that monocular deprivation leads to a dissociation between the development of excitatory and inhibitory receptors in visual cortex. We proposed that these differences are likely to underlie the anatomical and physiological changes that lead to poor visual acuity in the deprived eye. We add to those findings in this chapter by showing that binocular deprivation and reverse occlusion promote very different changes in the expression of excitatory and inhibitory receptors, but a similar dissociation in the balance between excitation and inhibition. Both outcomes lead to poor visual acuity and binocular perception illustrating that there are multiple pathways that lead to poor vision. Taken together, these results provide significant insights into the experience-dependent mechanisms that mediate synaptic plasticity and suggest that the relative composition of NMDA, AMPA, and $GABA_A$ receptors are related to the success or failure of recovery from monocular deprivation.

Chapter 5

Experience-dependent Change in the Expression Profile of Excitatory and Inhibitory Plasticity Mechanisms in Developing Visual Cortex.

Introduction

The main theme of this thesis is to understand the experience-dependent development of excitatory and inhibitory plasticity mechanisms in visual cortex. It is clear from the findings presented in Chapters 3 and 4 that there is a complex pattern of change in the expression of these neural plasticity mechanisms in the developing visual cortex. Furthermore, recent gene expression studies have shown that visual experience drives a complex pattern of up- and down-regulation of genes in visual cortex (Majdan and Shatz, 2006; Tropea et al., 2006). Together, this work underscores the importance of studying multiple mechanisms to gain new insights into factors that influence cortical development. The complex nature of theses changes, however, presents a challenge for quantifying and understanding global patterns of experience-dependent development.

A number of sophisticated computational techniques have been applied to analyze large data sets of gene expression and the field of bioinformatics has emerged to tackle the difficult problem of quantifying complex genetic networks. An increasingly common method used to describe changes in expression of multiple genes is the application of Principle Component Analysis (PCA), in particular, Singular Value Decomposition (SVD) to interpret the global pattern of genomic arrays (Alter et al., 2000; Tomfohr et al., 2005) and to identify substances in proteins at the atomic level (Garcia, 1992). However, SVD has not been applied to describe the expression of multiple synaptic proteins during postnatal development. Here, we use SVD as a multivariate approach to analyzing synaptic protein expression patterns during the development of kitten visual cortex.

SVD represents the expression level of all proteins from one kitten as a vector in high dimensional space. The principle component analysis identifies the

direction in "protein expression space" that represents most of the variance in the data across all conditions. When plotted as a 3-dimensional representation of this space, clusters of data near each other, or that are ordered along the same dimension, have similar attributes or classification (Marder and Goaillard, 2006; Taylor et al., 2006). This method provides a useful representation of patterns and similarities among a set of genes or proteins, allowing researchers to explore the global pattern in large sets of data.

I have applied this novel neuroinformatics approach to quantifying global expression patterns and the developmental trajectories for a set of synaptic plasticity mechanisms in normal, monocularly deprived and visual recovery animals. Analysis and visualization of the principle components reveals new insights into the experience-dependent development of the visual cortex.

Methods

Global expression patterns for all synaptic proteins were analyzed using Principle Component Analysis and Singular Value Decomposition (SVD). The principle components were calculated from the expression data for normally reared, monocularly deprived, and recovery animals presented in Chapters 3 and 4. Raw data were organized into an mXn matrix. The m rows represent synaptic proteins (NR1, NR2A, NR2B, GluR2, $GABA_A\alpha1$, $GABA_A\alpha3$, synapsin) for a total of m=7, and the n columns represent protein expression levels for each of the 24 animals (9 normal, 8 monocularly deprived, 7 recovery) in each of the 3 regions (central, peripheral, monocular) for a total of n=72. Data were centered by subtracting the mean column vector, and SVD (Matlab, The Mathworks, Inc., Natick, Massachusetts) was applied to calculate the principle components. This

calculates the principle components and the amount of variance explained by each component of the data. To determine the biological correlates for the principle components, the Pearson correlation coefficient "r" was calculated between each principle component and the expression of various synaptic proteins. Although we used $p<0.05$ as our significance criterion, given the number of correlations, we used a Bonferroni correction criterion of $p<0.007$. The data were visualized in 3-dimensions using a ray-tracing program (Radiance; Ward, 1994).

Results

The Principle Components

We analyzed the pattern of synaptic protein expression in kitten visual cortex using Singular Value Decomposition (SVD). The application of SVD to our data will enable an evaluation of the global protein expression pattern in visual cortex to gain a better understanding of differences between normal development, monocular deprivation, and the recovery from visual deprivation. The first step was to determine the proportion of variance captured by each of the 7 synaptic proteins. The histogram in Figure 5.1 shows that the first component explains the greatest proportion of the variance (57%). The second component describes 19% and the third component describes 9% of the variance. Beyond the third component (components 4-7), gradually less of the variance is explained (6%-2% respectively).

The variance accounted for by each component consists of the linear sum of the signal components plus an additional portion of noise. As the variance explained by each component decreases, it likely consists of noise and very little

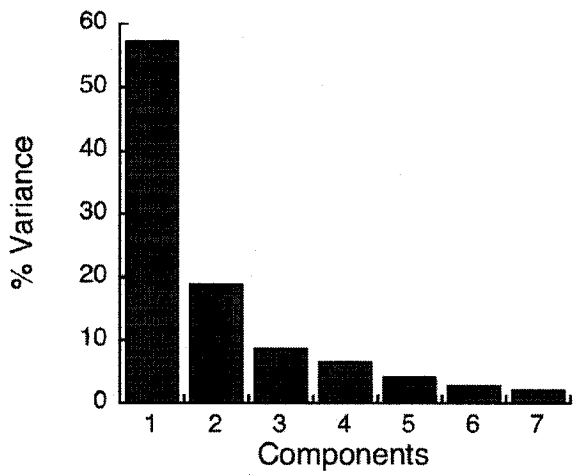


Figure 5.1. Variance plot of the principle components.
Plot of the relative variance captured by each principle component from SVD analysis of excitatory and inhibitory plasticity mechanisms in visual cortex of normal, monocularly deprived, and recovery kittens. The components are organized according to how much variance they capture. Heuristics used to evaluate the principle components indicate that the first 2-3 components represent meaningful differences in the global expression of all plasticity mechanisms.

signal. There are several approaches that can be applied to determine the components of the SVD that are significant and represent signal rather than noise. One heuristic is to ignore components beyond a cumulative variance of 80% (Everitt and Dunn, 2001). Using this approach, the first 3 components, represent 57% 19% and 9% of the variance respectively, accounting for 85% of the cumulative variance and would represent the meaningful components of the SVD. A second heuristic proposed by Everitt and Dunn (2001), suggests using a "threshold", allowing only those components with greater variances to be considered significant. The threshold is calculated as $0.7/n$, where n is equal to the number of vectors (or 7 proteins) that were evaluated by SVD. Using this heuristic, the threshold of significance is 0.1 or 10% of the variance, and both the first and second components reach the threshold of significance, but the third component falls just short. We have chosen to adopt the first heuristic as our measure of significance to evaluate the components further, which identifies the first 3 components of the SVD as significant.

The Relationship Between Principle Components and Protein Expression.

Each component of the SVD represents a linear combination of the expression levels of the excitatory and inhibitory plasticity mechanisms. The influence that each mechanism has on each of the principle components is reflected by the relative amplitude in the basis vector. Evaluating the basis vectors can provide insights into novel patterns of expression. Figure 2 shows the basis vectors for the 3 principle components, illustrating the direction and magnitude for each of the 7 plasticity mechanisms. The first principle component (PCA 1), reflects positive contributions from all mechanisms (Fig. 2a), with the greatest from the excitatory receptors. The second component (PCA 2), shows clear

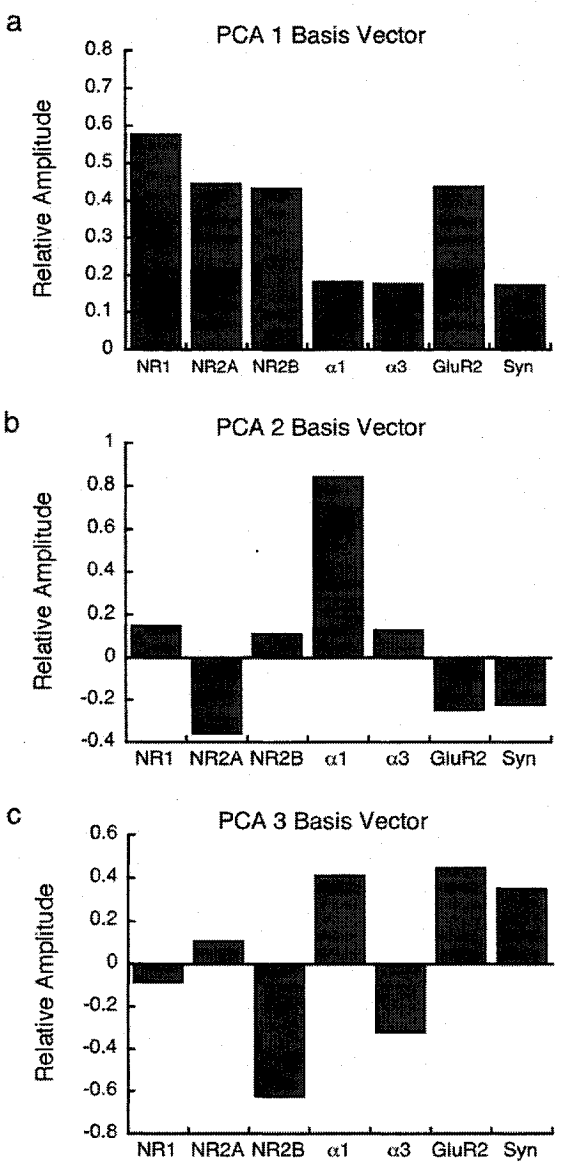


Figure 5.2. Basis vectors of synaptic protein expression for the first 3 principle components
The relative amplitude of all elements of synaptic protein expression (NR1, NR2A, NR2B, $GABA_A\alpha1$, $GABA_A\alpha3$, GluR2, Synapsin) show the influence of individual plasticity mechanisms on the principle component vectors. (a) The relative amplitude of all plasticity mechanisms are high suggesting that the total expression of all mechanisms are driving changes in the first component. (b) In the PCA 2 basis vector, there is a clear increase in $GABA_A\alpha1$, and an opposite change in the relative amplitude of NR2A and NR2B. (c) The PCA 3 basis vector shows an opposite change in the relative amplitudes of $GABA_A\alpha1$:$GABA_A\alpha3$, NR2A:NR2B, GluR2:NR1 and GluR2:NR2B.

differences in the direction and magnitude of the contributions of excitatory and inhibitory receptors. The third principle component (PCA 3) also shows opposite directions for pairs of mechanisms; NR2A:NR2B, $GABA_A\alpha1$:$GABA_A\alpha3$, GluR2:NR1, and GluR2:NR2B (Fig. 2c).

Simply calculating the principle components, however, does not directly address the biological factors that influence each of the principle components. To begin to relate the principle components to the biological mechanisms, we generated a set of correlations based on combinations of mechanisms suggested by the basis vectors in Figure 2. Some combinations have been examined in previous chapters (NR2A:NR2B, $GABA_A\alpha1$:$GABA_A\alpha3$, GluR2:NR1), while the others are novel combinations (Total protein expression, Excitatory expression, Inhibitory expression, GluR2:NR2B) indicated by the basis vectors. Figure 3 shows the correlations between these combinations of excitatory and inhibitory mechanisms and the 3 principle components. This matrix of correlation is colour-coded with significant positive correlations coded in green and negative coded in red ($p<0.007$). This pattern of correlations provides information to describe the biological significance of the principle components. The first principle component is characterized by an increase in the total expression of receptor subunits, and a shift from NMDA to AMPA expression. The second principle component captures the developmental balance between excitatory and inhibitory plasticity mechanisms. Finally, the third principle component captures the maturation from immature to mature subunit expression in visual cortex.

Three Dimensional Visualization of the Principle Components.

To visualize the 3 significant principle components, the data was plotted in 3-dimensional space using a ray-tracing program (Raidance; Ward, 1994; Figure

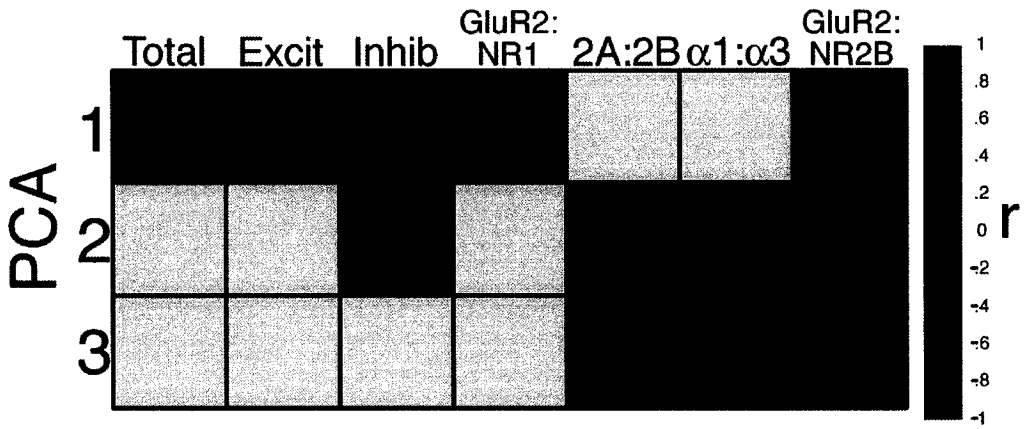
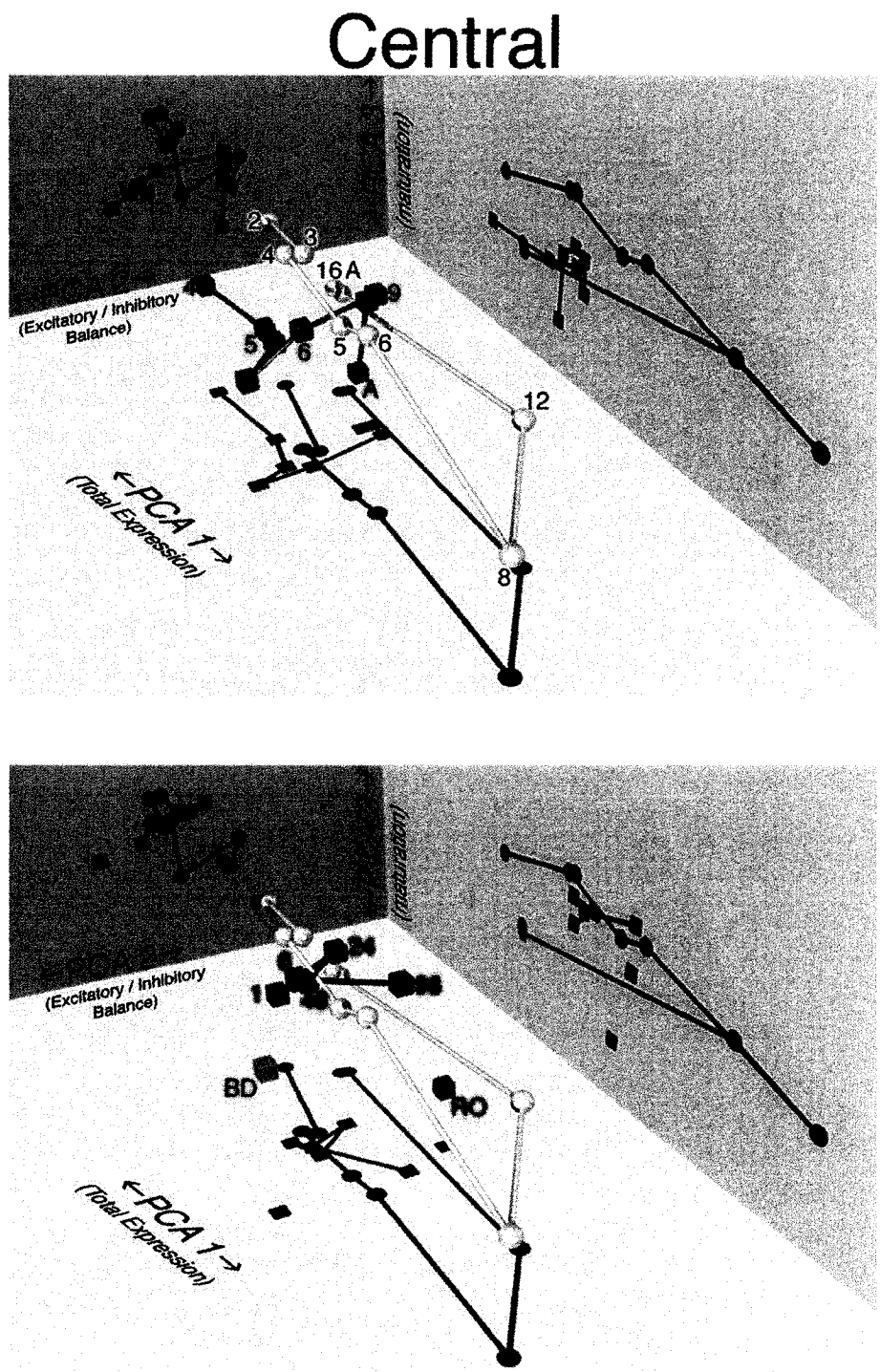


Figure 5.3. Pearson's correlation of the principle components and the biological mechanisms. Pearson's correlation coefficients r between the 3 significant principle components of the SVD and the excitatory and inhibitory mechanisms in visual cortex. Coloured cells indicate significant correlations and the direction of change ($p<0.007$). The first principle component is correlated with an increase in the overall expression of synaptic proteins, and maturation of NMDA and AMPA receptors. The second principle component reflects an increase in the expression of inhibitory mechanisms and a change in the balance between excitation and inhibition. Finally, the third principle component reflects an overall maturation in the composition of excitatory and inhibitory receptors.

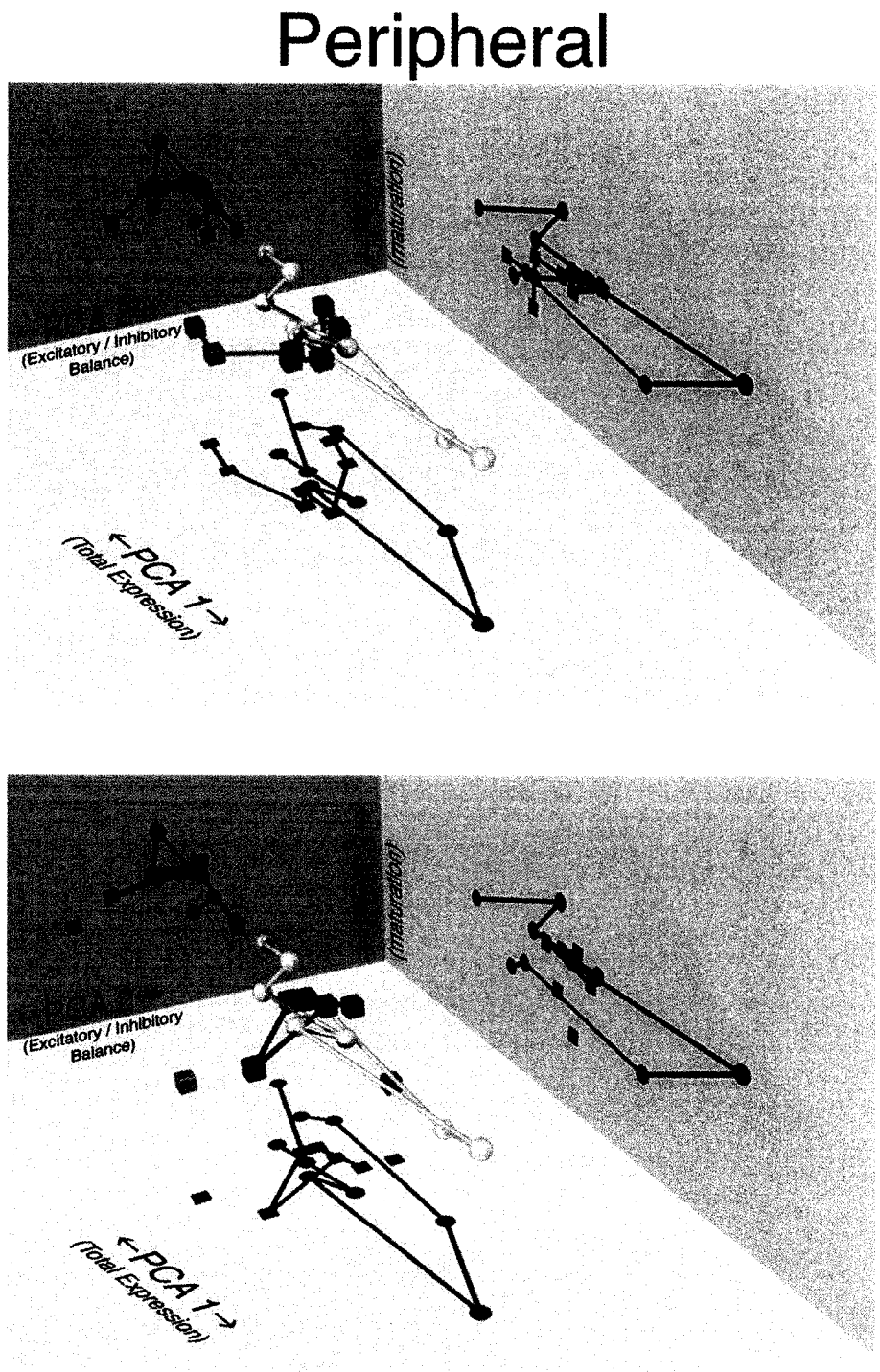
5.2). The projection of SVD components into 3 dimensional space reveals the global structure of the data and can be used for further classification. Each plotted point represents the expression for one kitten. The plots are separated by region (central, peripheral, and monocular), and by condition (normal = gold spheres, monocular deprivation = red cubes, visual recovery = blue cubes, reverse occlusion = magenta cube, binocular deprivation = cyan cube). The connecting lines link the points by age. These plots show distinct developmental trajectories for normal, monocularly deprived, and recovery animals. The most striking difference exists between normally reared and monocularly deprived animals.

In the central region of normally reared kittens (gold spheres) there is an elongated, u-shaped function, that extends along PCA 1 (total expression) from 2 to 8 weeks of age, and then returns back to reach the adult location, suggesting that the maturation of neural plasticity mechanisms is prolonged during normal development (Fig 5.4a). It is important to note that the age of the animals was not part of the analysis and yet there is an orderly, age-dependent, developmental trajectory through this space. There is very little change in PCA 2 between 2 - 8 weeks of age, and the shadows are all clustered together. Then, PCA 2 shifts between 8-12 weeks of age suggesting that the balance between excitation and inhibition changes at this age. Finally, there is a gradual shift of PCA 3 in the direction of more mature expression between 2 -8 weeks of age. Outside of the central region, the magnitude of PCA 1 was progressively less in peripheral (Fig. 5.4b) and monocular regions (Fig. 5.4.c). In addition, PCA 2 collapsed down to within a very small range of values, suggesting that there is no developmental change in the overall excitatory and inhibitory balance in these parts of visual cortex.

a


Central
(Inhibitory)
(Excitatory / Inhibitory Balance)
2
3
4
16A
9
5
6
5
6
A
12
8
←PCA 1→
(Total Expression)
(Inhibitory)
(Excitatory / Inhibitory Balance)
BD
HO
←PCA 1→
(Total Expression)

b


Peripheral
(Interaction)
(Excitatory / Inhibitory Balance)
←PCA 1→
(Total Expression)
(Interaction)
(Excitatory / Inhibitory Balance)
←PCA 1→
(Total Expression)

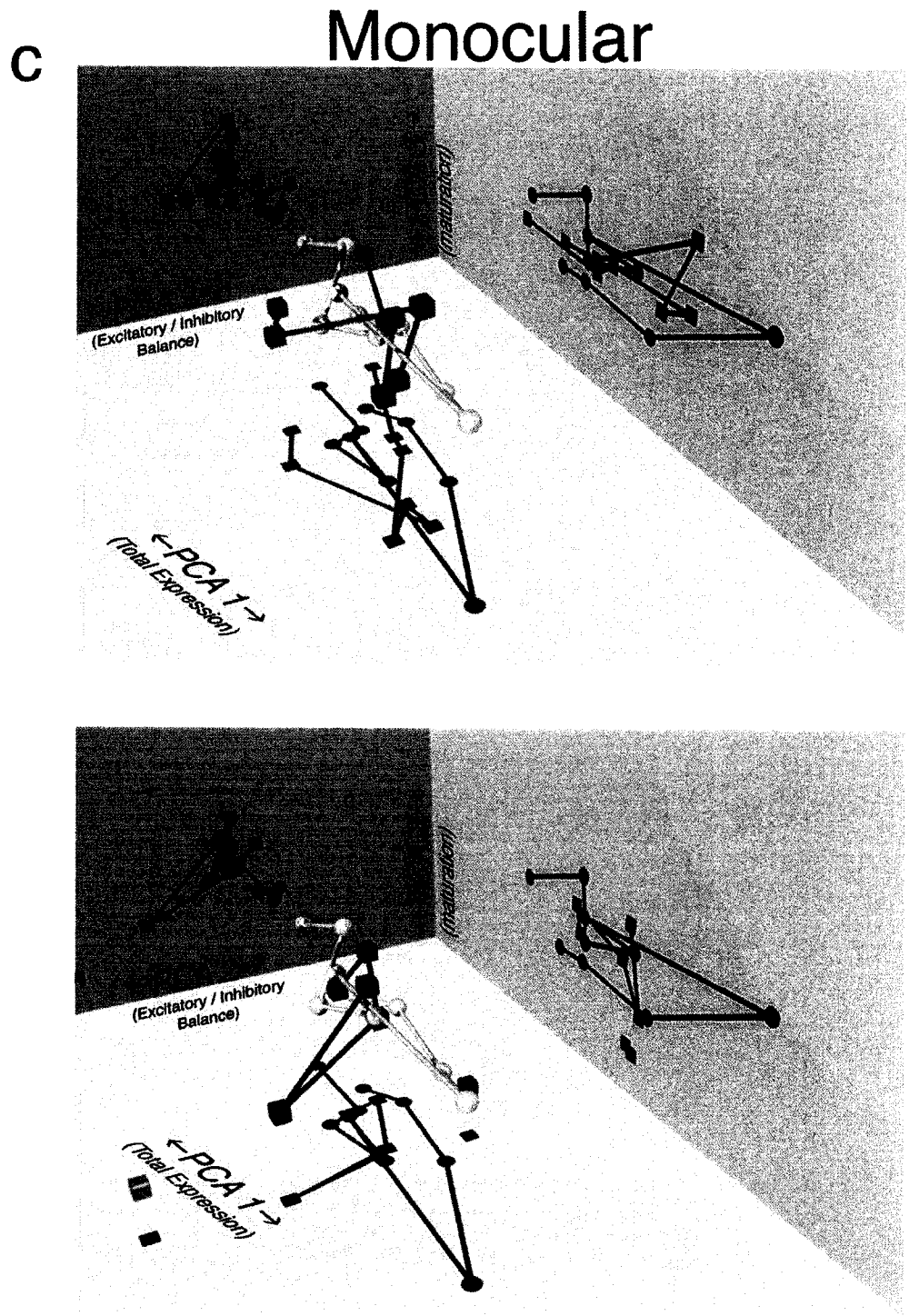

c
Monocular
(Excitatory / Inhibitory Balance)
←PCA 1→
(Total Expression)
(Excitatory / Inhibitory Balance)
←PCA 1→
(Total Expression)

Figure 5.4. SVD analysis of the global expression profile.
Visualization of the principle components are shown for the first, second, and third components of the SVD. The 3-dimensional plots are separated by central (a), peripheral (b), and monocular (c) visual field representations and by rearing condition. Monocularly deprived animals are shown in the top plot (red cubes). kittens that were given reverse occlusion (magenta cube), binocular deprivation (cyan cube), and binocular vision (blue cubes) after monocular deprivation are shown in the bottom plots. For comparison, normal animals are presented in both plots (yellow spheres). Ages ares are shown for normal and monocularly deprived kittens in the central visual field representation. The amount of visual recovery is given in hours. During normal development, there is a prolonged developmental trajectory in the central and peripheral visual field representation. Monocular deprivation truncates the global development of plasticity mechanisms in the central and peripheral regions, beyond 6 weeks of age in visual cortex. Introducing binocular vision after 5 weeks of monocular deprivation rapidly initiates changes in plasticity mechanisms back to normal levels. Neither reverse occlusion or binocular deprivation promoted the recovery of the global profile of the plasticity mechanisms.

A very different pattern emerged when the principle components were plotted for the monocularly deprived animals (red cubes). Monocular deprivation severely truncates the developmental trajectory of principle components in the central and peripheral regions. Monocular deprivation up to 5 weeks of age leads to a separate but parallel developmental trajectory to that of normally reared kittens. Extending deprivation beyond 6 weeks of age, however, causes a dramatic shift in the developmental trajectory away from normal. It is particularly interesting to note that monocular deprivation shifts PCA 3 towards the more mature pattern found in adult cats. This suggests that deprivation changes the cortex to a more mature, less plastic state. In addition, the trajectories for normal and monocularly deprived animals were largely overlapping in the monocular region suggesting that the lack of binocular vision had little impact on the development of this part of visual cortex.

Animals given a brief period of binocular visual experience after monocular deprivation (Fig 5.4a-c; blue cubes) were initially close to monocularly deprived animals, but there was a rapid shift in the developmental trajectory towards the normal animals. In contrast, binocular deprivation (cyan cubes) and reverse occlusion (magenta cubes) resulted in large and opposite deviations away from the normal trajectory.

Discussion

We used Singular Value Decomposition (SVD) to examine the global expression of multiple excitatory and inhibitory proteins in the visual cortex of normally reared, monocularly deprived kittens and the recovery from monocular deprivation. We found 3 main components of the SVD that explained the global

nature of experience-dependent changes in visual cortex. These 3 components are best described by the total expression, the balance between excitatory and inhibitory mechanisms, and the maturation of NMDA, AMPA, and $GABA_A$ receptors. The use of SVD also revealed novel combinations of protein interactions that we had not considered previously. In particular, the basis vector led us to consider the significance of changes in the relative amount of GluR2:NR2B. A recent study has suggested that there is a highly dependent relationship between these subunits and that NR2B plays a distinct role in limiting the insertion of AMPA receptors into the post-synaptic membrane (Hall et al., 2006). Together, these findings reveal the power of this neuroinformatics approach to characterize the developmental trajectory and uncover the biologically relevant interactions between plasticity mechanisms in visual cortex.

The analysis shows that in normal kittens, the development of this set of synaptic plasticity mechanisms in visual cortex is prolonged, extending well out to 16 weeks of age before reaching adult levels. This developmental trajectory is largely accounted for by changes in PCA 1, or total expression of plasticity mechanisms in visual cortex and was consistent in the central, peripheral and monocular regions of visual cortex. Changes in the second (PCA 2) and third (PCA 3) components are significantly correlated to the development of the balance between excitatory and inhibitory mechanisms and the maturation of those receptors. Interestingly, we found that changes in the second and third principle components were smaller in the peripheral and monocular regions of visual cortex and suggest that expression of these fundamental mechanisms for synaptic plasticity is not uniform across the visual cortex.

Trajectories of the principle components were different from normal animals after monocular deprivation. During early monocular deprivation (<6 weeks of age), the trajectory is offset, but parallel to normal animals. However, around 6 weeks of age, a striking difference emerges as the overall development in monocularly deprived animals is truncated, and rerouted to an abbreviated path that is different from normal animals. This change in trajectory suggests an overall reduction in plasticity mechanisms beyond 6 weeks of age that might have several functional consequences. It has been shown that activity of these plasticity mechanisms is important for the formation and stabilization of emerging synapses (Aamodt and Constantine-Paton, 1999). The general loss of plasticity mechanisms beyond 6 weeks suggests that formation of neural circuits and the capacity for neural plasticity might be compromised in visual cortex. The abrupt change in the trajectory occurs at an age when previous physiological studies have shown that there is less capacity for functional recovery (Movshon, 1976). This reduced capacity for functional recovery might be explained by the rapid shift towards the adult pattern of these key synaptic plasticity mechanisms.

Allowing just a few days of binocular vision after monocular deprivation was sufficient to reroute the global expression towards the pattern for normal animals. This was not the case for kittens given binocular deprivation or reverse occlusion, as they end up in opposite positions in the SVD spectrum. Behavioural studies of children with unilateral congenital cataracts have shown that recovery of visual function in the deprived eye is optimal when the cataract is removed early. (Birch and Stager, 1996; Lewis et al., 1995). In addition, we have shown in an animal model that binocular vision is necessary to promote recovery of vision after deprivation (Murphy et al., 2002).

In this Chapter, I have implemented a powerful new tool for analyzing and understanding the experience-dependent expression of multiple molecular markers in visual cortex. Our findings reveal very different developmental trajectories for normal and monocularly deprived animals. Furthermore, implementing a period of binocular vision, but not reverse occlusion or binocular deprivation, promotes recovery towards the normal trajectory. This neuroinformatics approach captures the complex nature of these developmental changes and provides new insights into the experience-dependent development of synaptic plasticity mechanisms in the visual cortex.

Chapter 6

General Discussion

In this thesis, I have made significant advances in our understanding of the experience-dependent expression of synaptic proteins that underlie developmental plasticity in human and cat visual cortex. Results from my studies show that during normal development, there is a gradual maturation of excitatory and inhibitory plasticity mechanisms (Chapters 2 and 5). The prolonged development of these mechanisms in human visual cortex raises the possibility that changes in receptor composition set the pace for the functional development and plasticity of vision in humans. Chapters 3 and 5 show that the development of a set of synaptic plasticity mechanisms is experience-dependent. Monocular deprivation leads to regional changes within visual cortex in the expression of NMDA receptor subunits and advances the maturation of $GABA_A$ receptor subunits. Furthermore, extending monocular deprivation beyond 6 weeks of age leads a significant loss of all synaptic plasticity mechanisms that is reflected by a significant change in the developmental trajectory of the visual cortex. While neither binocular deprivation nor reverse occlusion alone restores the balance of plasticity mechanisms in visual cortex, introducing a period of binocular vision is sufficient to promoted substantial recovery of the mechanism (Chapter 4 and 5). Taken together, my studies of the experience-dependent expression of excitatory and inhibitory plasticity mechanisms provide valuable information about the factors that underlie the development of visual cortex.

My thesis pushes beyond the notion that a single mechanism mediates developmental plasticity in visual cortex and recognizes the importance of evaluating many plasticity mechanisms to gain a deeper understanding of cortical development. There is clear evidence from studies of gene expression (Tropea et al., 2006) and my studies of changes in protein expression, that

expression of proteins are affected by monocular deprivation and visual recovery. Because of the complex nature of these changes, with expression of some genes and proteins going up while others go down, it is necessary to use neuroinformatics to gain a better understanding of the global nature of this development. In Chapter 5, I have taken advantage Principle Component Analysis and Singular Value Decomposition to visualize and analyze changes in this complex data set. This application of neuroinformatics has pulled out important new insights into the development of visual system as a whole. This approach shows that monocular deprivation reduces the overall expression of proteins in visual cortex, truncating the developmental profile and shifting it towards a state of reduced plasticity. Binocular vision, however, is sufficient to drive the overall expression of these plasticity mechanisms back towards the normal trajectory. Unfortunately, I was not able to use this neuroinformatics approach to quantify human visual cortex because the age range was restricted to under 6 years of age. In the future, it will be very interesting to apply this approach to analyze the developmental trajectory of human visual cortex. This will allow us to compare the developmental trajectories between animal and human models and enable comparisons between the functional development and plasticity of the cat and human visual system.

I have taken a comprehensive approach to studying the experience-dependent development by examining a range of excitatory and inhibitory plasticity mechanisms in visual cortex. In addition, I evaluated these mechanisms throughout all regions of visual cortex. While most experiments only use 1 sample to evaluate changes in visual cortex, I used 12 samples representing the central, peripheral, and monocular visual field representations. By studying multiple samples, I found that there are significant regional differences in the

experience-dependent development of visual cortex. This provides strong evidence that the effect of monocular deprivation is not uniform across the visual cortex. Although previous studies have shown that there are clear anatomical and functional differences between the visual field representations, such as smaller receptive field size in the central visual field representation (Hubel and Wiesel, 1962), the over representation of parvocellular ganglion cells in the fovea (Connolly and Van Essen, 1984) and a greater sensitivity to monocular deprivation in the central visual field representation (Bowering et al., 1993), the topographic aspects of the organization of visual cortex have largely been ignored by studies of the molecular mechanisms. On the one hand, it might not be surprising that there are regional differences in the experience-dependent development of plasticity mechanisms in visual cortex. On the other hand, it is surprising that only 1 other study of neural plasticity mechanisms has addressed aspects of visual cortical organization (Murphy et al., 2004).

Taken together, this thesis contributes important new information in the field of visual neuroscience and provides new insights into the experience-dependent development of visual cortex. The relationship between functional recovery of vision and the neural plasticity mechanisms provide a promising direction for future studies to address underlying causes of amblyopia and optimal treatment strategies for children with lazy-eye.

References

Aamodt SM, Constantine-Paton M (1999) The role of neural activity in synaptic development and its implications for adult brain function. Adv Neurol 79:133-144.

Adams RJ, Courage ML (1995) Development of chromatic discrimination in early infancy. Behav Brain Res 67:99-101.

Allen D, Tyler CW, Norcia AM (1996) Development of grating acuity and contrast sensitivity in the central and peripheral visual field of the human infant. Vision Res 36:1945-1953.

Antonini A, Stryker MP (1996) Plasticity of geniculocortical afferents following brief or prolonged monocular occlusion in the cat. J Comp Neurol 369:64-82.

Antonini A, Fagiolini M, Stryker MP (1999) Anatomical correlates of functional plasticity in mouse visual cortex. J Neurosci 19:4388-4406.

Aoki C, Venkatesan C, Go CG, Mong JA, Dawson TM (1994) Cellular and subcellular localization of NMDA-R1 subunit immunoreactivity in the visual cortex of adult and neonatal rats. The Journal of Neuroscience 14:5202-5222.

Araujo F, Tan S, Ruano D, Schoemaker H, Benavides J, Vitorica J (1996) Molecular and pharmacological characterization of native cortical gamma-aminobutyric acidA receptors containing both alpha1 and alpha3 subunits. J Biol Chem 271:27902-27911.

Aroniadou VA, Teyler TJ (1991) The role of NMDA receptors in long-term potentiation (LTP) and depression (LTD) in rat visual cortex. Brain Res 562:136-143.

Bear MF (1995) Mechanism for a sliding synaptic modification threshold. Neuron 15:1-4.

Bear MF (1996) NMDA-receptor-dependent synaptic plasticity in the visual cortex. Prog Brain Res 108:205-218.

Bear MF, Kirkwood A (1993) Neocortical long-term potentiation. Curr Opin Neurobiol 3:197-202.

Bear MF, Schmechel DE, Ebner FF (1985) Glutamic acid decarboxylase in the striate cortex of normal and monocularly deprived kittens. J Neurosci 5:1262-1275.

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. J Neurosci 10:909-925.

Beston, BR, Hessels, SD, Jones, DG, Murphy, KM (2002) Developmental changes in NMDA receptor subunit expression in human visual cortex. Program No. 760.11. Society for Neuroscience.

Beston BR, Jones DG, Murphy KM (2004) Experience-dependent changes in excitatory and inhibitory receptors in developing visual cortex. Program No. 66.7. Society for Neuroscience.

Birch EE, Stager DR (1996) The critical period for surgical treatment of dense congenital unilateral cataract. Invest Ophthalmol Vis Sci 37:1532-1538.

Boley PB, Kubar TJ, Millen KS Pinto, JGA, Latchman SVD, Jones DG, Murphy KM (2004) Development of $GABA_A$ Receptor Expression and GABA synthesizing enzymes in human visual cortex program No. 66.3. Society for Neuroscience.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31-39.

Bohme I, Rabe H, Luddens H (2004) Four amino acids in the alpha subunits determine the gamma-aminobutyric acid sensitivities of GABAA receptor subtypes. J Biol Chem 279:35193-35200.

Bonnert TP, McKernan RM, Farrar S, le Bourdelles B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Brown N, Wafford KA, Whiting PJ (1999) theta, a novel gamma-aminobutyric acid type A receptor subunit. Proc Natl Acad Sci U S A 96:9891-9896.

Bosman LW, Rosahl TW, Brussaard AB (2002) Neonatal development of the rat visual cortex: synaptic function of GABAA receptor alpha subunits. J Physiol 545:169-181.

Bowering ER, Maurer D, Lewis TL, Brent HP (1993) Sensitivity in the nasal and temporal hemifields in children treated for cataract. Invest Ophthalmol Vis Sci 34:3501-3509.

Burkhalter A (1993) Development of forward and feedback connections between areas V1 and V2 of human visual cortex. Cereb Cortex 3:476-487.

Carmignoto G, Vicini S (1992) Activity-dependent decrease in NMDA receptor responses during development of the visual cortex. Science 258:1007-1011.

Chen L, Cooper NG, Mower GD (2000) Developmental changes in the expression of NMDA receptor subunits (NR1, NR2A, NR2B) in the cat visual cortex and the effects of dark rearing. Brain Res Mol Brain Res 78:196-200.

Chen L, Yang C, Mower GD (2001) Developmental changes in the expression of GABA(A) receptor subunits (alpha(1), alpha(2), alpha(3)) in the cat visual

cortex and the effects of dark rearing. Brain Res Mol Brain Res 88:135-143.

Connolly M, Van Essen D (1984) The representation of the visual field in parvicellular and magnocellular layers of the lateral geniculate nucleus in the macaque monkey. J Comp Neurol 226:544-564.

Constantine-Paton M, Cline HT, Debski E (1990) Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. Annu Rev Neurosci 13:129-154.

Daw NW (1994) Mechanisms of plasticity in the visual cortex. The Friedenwald Lecture. Invest Ophthalmol Vis Sci 35:4168-4179.

Dews PB, Wiesel TN (1970) Consequences of monocular deprivation on visual behaviour in kittens. J Physiol 206:437-455.

DeYoe EA, Carman GJ, Bandettini P, Glickman S, Wieser J, Cox R, Miller D, Neitz J (1996) Mapping striate and extrastriate visual areas in human cerebral cortex. Proc Natl Acad Sci U S A 93:2382-2386.

Durand GM, Kovalchuk Y, Konnerth A (1996) Long-term potentiation and functional synapse induction in developing hippocampus. Nature 381:71-75.

Constantine-Paton M, Cline HT, Debski E (1990) Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. Annu Rev Neurosci 13:129-154.

Daw NW (1994) Mechanisms of plasticity in the visual cortex. The Friedenwald Lecture. Invest Ophthalmol Vis Sci 35:4168-4179.

Dews PB, Wiesel TN (1970) Consequences of monocular deprivation on visual behaviour in kittens. J Physiol 206:437-455.

DeYoe EA, Carman GJ, Bandettini P, Glickman S, Wieser J, Cox R, Miller D, Neitz J (1996) Mapping striate and extrastriate visual areas in human cerebral cortex. Proc Natl Acad Sci U S A 93:2382-2386.

Drager UC, Olsen JF (1980) Origins of crossed and uncrossed retinal projections in pigmented and albino mice. J Comp Neurol 191:383-412.

Durand GM, Kovalchuk Y, Konnerth A (1996) Long-term potentiation and functional synapse induction in developing hippocampus. Nature 381:71-75.

Ellemberg D, Lewis TL, Liu CH, Maurer D (1999) Development of spatial and temporal vision during childhood. Vision Res 39:2325-2333.

Ellemberg D, Lewis TL, Maurer D, Brent HP (2000) Influence of monocular deprivation during infancy on the later development of spatial and temporal vision. Vision Res 40:3283-3295.

Erisir A, Harris JL (2003) Decline of the critical period of visual plasticity is concurrent with the reduction of NR2B subunit of the synaptic NMDA receptor in layer 4. J Neurosci 23:5208-5218.

Evritt, BS, Dunn G (1992) Applied multivariate data analysis. Oxford University Press.

Fagiolini M, Hensch TK (2000) Inhibitory threshold for critical-period activation in primary visual cortex. Nature 404:183-186.

Fagiolini M, Fritschy JM, Low K, Mohler H, Rudolph U, Hensch TK (2004) Specific GABAA circuits for visual cortical plasticity. Science 303:1681-1683.

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. Proc Natl Acad Sci U S A 100:2854-2859.

Faulkner, S.D., Vorobyov, V., & Sengpiel, F. (2006). Visual cortical recovery from reverse occlusion depends on concordant binocular experience. J Neurophysiol, 95 (3), 1718-1726.

Feldblum S, Erlander MG, Tobin AJ (1993) Different distributions of GAD65 and GAD67 mRNAs suggest that the two glutamate decarboxylases play distinctive functional roles. J Neurosci Res 34:689-706.

Feldblum S, Dumoulin A, Anoal M, Sandillon F, Privat A (1995) Comparative distribution of GAD65 and GAD67 mRNAs and proteins in the rat spinal cord supports a differential regulation of these two glutamate decarboxylases in vivo. J Neurosci Res 42:742-757.

Feldman DE (2000) Inhibition and plasticity. Nat Neurosci 3:303-304.

Fisher JL (2004) The alpha 1 and alpha 6 subunit subtypes of the mammalian GABA(A) receptor confer distinct channel gating kinetics. J Physiol 561:433-448.

Flint AC, Maisch US, Weishaupt JH, Kriegstein AR, Monyer H (1997) NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. J Neurosci 17:2469-2476.

Fox K, Daw NW (1993) Do NMDA receptors have a critical function in visual cortical plasticity? Trends Neurosci 16:116-122.

Fritschy JM, Kiener T, Bouilleret V, Loup F (1999) GABAergic neurons and GABA(A)-receptors in temporal lobe epilepsy. Neurochem Int 34:435-445.

Garcia AE (1992) Large-amplitude nonlinear motions in proteins. Physical Review

Letters 68:2696-2699.

Gingrich KJ, Roberts WA, Kass RS (1995) Dependence of the GABAA receptor gating kinetics on the alpha-subunit isoform: implications for structure-function relations and synaptic transmission. J Physiol 489 (Pt 2):529-543.

Gu QA, Bear MF, Singer W (1989) Blockade of NMDA-receptors prevents ocularity changes in kitten visual cortex after reversed monocular deprivation. Brain Res Dev Brain Res 47:281-288.

Guo Y, Kaplan IV, Cooper NG, Mower GD (1997) Expression of two forms of glutamic acid decarboxylase (GAD67 and GAD65) during postnatal development of the cat visual cortex. Brain Res Dev Brain Res 103:127-141.

Hebb DO (1949) The organization of Behavior. New York, Wiley.

Heinen K, Bosman LW, Spijker S, van Pelt J, Smit AB, Voorn P, Baker RE, Brussaard AB (2004) GABAA receptor maturation in relation to eye opening in the rat visual cortex. Neuroscience 124:161-171.

Helmholtz, H. von (1925) Helmholtz's treatise on physiological optics. Vol. 3., Edited by JPC Southall, Thoemmes Press.

Hendrickson A, Kupfer C (1976) The histogenesis of the fovea in the macaque monkey. Invest Ophthalmol Vis Sci 15:746-756.

Hendrickson A, March D, Richards G, Erickson A, Shaw C (1994) Coincidental appearance of the alpha 1 subunit of the GABA-A receptor and the type I benzodiazepine receptor near birth in macaque monkey visual cortex. Int J Dev Neurosci 12:299-314.

Hendry SH, Huntsman MM, Vinuela A, Mohler H, de Blas AL, Jones EG (1994) GABAA receptor subunit immunoreactivity in primate visual cortex:

distribution in macaques and humans and regulation by visual input in adulthood. J Neurosci 14:2383-2401.

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.

Hensch TK, Stryker MP (2004) Columnar architecture sculpted by GABA circuits in developing cat visual cortex. Science 303:1678-1681.

Herrmann K (1996) Differential distribution of AMPA receptors and glutamate during pre- and postnatal development in the visual cortex of ferrets. J Comp Neurol 375:1-17.

Hess RF, Pointer JS (1985) Differences in the neural basis of human amblyopia: the distribution of the anomaly across the visual field. Vision Res 25:1577-1594.

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. Nat Neurosci 6:854-862.

Hollingsworth EB, McNeal ET, Burton JL, Williams RJ, Daly JW, Creveling CR (1985) Biochemical characterization of a filtered synaptoneurosome preparation from guinea pig cerebral cortex: cyclic adenosine 3':5'-monophosphate-generating systems, receptors, and enzymes. J Neurosci 5:2240-2253.

Hornung JP, Fritschy JM (1996) Developmental profile of GABAA-receptors in the marmoset monkey: expression of distinct subtypes in pre- and postnatal brain. J Comp Neurol 367:413-430.

Horton JC, Hedley-Whyte ET (1984a) Mapping of cytochrome oxidase patches

and ocular dominance columns in human visual cortex. Philos Trans R Soc Lond B Biol Sci 304:255-272.

Horton JC, Hedley-Whyte ET (1984b) Mapping of cytochrome oxidase patches and ocular dominance columns in human visual cortex. Philos Trans R Soc Lond B Biol Sci 304:255-272.

Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J Physiol 160:106-154.

Hubel DH, Wiesel TN (1963) Receptive Fields Of Cells In Striate Cortex Of Very Young, Visually Inexperienced Kittens. J Neurophysiol 26:994-1002.

Hubel DH, Wiesel TN (1965) Binocular interaction in striate cortex of kittens reared with artificial squint. J Neurophysiol 28:1041-1059.

Hubel DH, Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J Physiol 206:419-436.

Huntsman MM, Munoz A, Jones EG (1999) Temporal modulation of GABA(A) receptor subunit gene expression in developing monkey cerebral cortex. Neuroscience 91:1223-1245.

Huttenlocher PR, de Courten C, Garey LJ, Van der Loos H (1982) Synaptogenesis in human visual cortex--evidence for synapse elimination during normal development. Neurosci Lett 33:247-252.

Iwai Y, Fagiolini M, Obata K, Hensch TK (2003) Rapid critical period induction by tonic inhibition in visual cortex. J Neurosci 23:6695-6702.

Jonas P, Racca C, Sakmann B, Seeburg PH, Monyer H (1994) Differences in Ca2+ permeability of AMPA-type glutamate receptor channels in neocortical

neurons caused by differential GluR-B subunit expression. Neuron 12:1281-1289.

Jones DG, Mitchell DE, Murphy KM (2005) Visual deprivation impairs orientation discrimination in noise. Society for Neuroscience Abstracts 31:978.7

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133-1138.

Katz LC, Crowley JC (2002) Development of cortical circuits: lessons from ocular dominance columns. Nat Rev Neurosci 3:34-42.

Keinanen K, Wisden W, Sommer B, Werner P, Herb A, Verdoorn TA, Sakmann B, Seeburg PH (1990) A family of AMPA-selective glutamate receptors. Science 249:556-560.

Kind PC, Mitchell DE, Ahmed B, Blakemore C, Bonhoeffer T, Sengpiel F (2002) Correlated binocular activity guides recovery from monocular deprivation. Nature 416:430-433.

Kiorpes L, Kiper DC, O'Keefe LP, Cavanaugh JR, Movshon JA (1998) Neuronal correlates of amblyopia in the visual cortex of macaque monkeys with experimental strabismus and anisometropia. J Neurosci 18:6411-6424.

Kirkwood A, Bear MF (1994) Hebbian synapses in visual cortex. J Neurosci 14:1634-1645.

Kirkwood A, Lee HK, Bear MF (1995) Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. Nature 375:328-331.

Kleinschmidt A, Bear MF, Singer W (1987) Blockade of "NMDA" receptors

disrupts experience-dependent plasticity of kitten striate cortex. Science 238:355-358.

Kleppe IC, Robinson HP (1999) Determining the activation time course of synaptic AMPA receptors from openings of colocalized NMDA receptors. Biophys J 77:1418-1427.

Kotak VC, Fujisawa S, Lee FA, Karthikeyan O, Aoki C, Sanes DH (2005) Hearing loss raises excitability in the auditory cortex. J Neurosci 25:3908-3918.

Kumar A, Schliebs R, Bigl V (1994) Postnatal development of NMDA, AMPA, and kainate receptors in individual layers of rat visual cortex and the effect of monocular deprivation. Int J Dev Neurosci 12:31-41.

Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M, et al. (1992) Molecular diversity of the NMDA receptor channel. Nature 358:36-41.

Laurie DJ, Wisden W, Seeburg PH (1992) The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci 12:4151-4172.

Laurie DJ, Bartke I, Schoepfer R, Naujoks K, Seeburg PH (1997) Regional, developmental and interspecies expression of the four NMDAR2 subunits, examined using monoclonal antibodies. Brain Res Mol Brain Res 51:23-32.

Law AJ, Weickert CS, Webster MJ, Herman MM, Kleinman JE, Harrison PJ (2003) Expression of NMDA receptor NR1, NR2A and NR2B subunit mRNAs during development of the human hippocampal formation. Eur J Neurosci 18:1197-1205.

Levartovsky S, Oliver M, Gottesman N, Shimshoni M (1998) Long-term effect of

hypermetropic anisometropia on the visual acuity of treated amblyopic eyes. Br J Ophthalmol 82:55-58.

Levitt JB, Schumer RA, Sherman SM, Spear PD, Movshon JA (2001) Visual response properties of neurons in the LGN of normally reared and visually deprived macaque monkeys. J Neurophysiol 85:2111-2129.

Lewis TL, Chang DHF, Murphy KM, Maurer D, Jones DG (2006) Orientation discrimination in noise: 7-year-olds are noiser than adults. Journal of Vision 6(6):380a http://journalofvision.org/6/6/380/ doi:10.1167/6.6.380

Lewis TL, Maurer D, Brent HP (1995) Development of grating acuity in children treated for unilateral or bilateral congenital cataract. Invest Ophthalmol Vis Sci 36:2080-2095.

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.

Lund JS, Holbach SM, Chung WW (1991) Postnatal development of thalamic recipient neurons in the monkey striate cortex: II. Influence of afferent driving on spine acquisition and dendritic growth of layer 4C spiny stellate neurons. J Comp Neurol 309:129-140.

Majdan M, Shatz CJ (2006) Effects of visual experience on activity-dependent gene regulation in cortex. Nat Neurosci 9:650-659.

Malach R, Ebert R, Van Sluyters RC (1984) Recovery from effects of brief monocular deprivation in the kitten. J Neurophysiol 51:538-551.

Marder E, Goaillard JM (2006) Variability, compensation and homeostasis in neuron and network function. Nat Rev Neurosci 7:563-574.

Maurer D & Lewis TL (1993) In K Simons (Ed.) Early visual development: normal and abnormal. Committee on vision. Commission on behavioural and social sciences and education. National Research Council. Oxford University Press, New York.

Maurer D, Lewis TL (2001) Visual acuity: the role of visual input in inducing postnatal change. Clinical Neuroscience Research 1:239-247.

Maurer D, Lewis TL, Brent HP, Levin AV (1999) Rapid improvement in the acuity of infants after visual input. Science 286:108-110.

Mayer DL, Dobson V (1982) Visual acuity development in infants and young children, as assessed by operant preferential looking. Vision Res 22:1141-1151.

Mayer DL, Beiser AS, Warner AF, Pratt EM, Raye KN, Lang JM (1995) Monocular acuity norms for the Teller Acuity Cards between ages one month and four years. Invest Ophthalmol Vis Sci 36:671-685.

McBain CJ, Mayer ML (1994) N-methyl-D-aspartic acid receptor structure and function. Physiol Rev 74:723-760.

Miller KD (1994) Models of activity-dependent neural development. Prog Brain Res 102:303-318.

Mitchell DE (1988) The extent of visual recovery from early monocular or binocular visual deprivation in kittens. J Physiol 395:639-660.

Mitchell DE, Gingras G (1998) Visual recovery after monocular deprivation is driven by absolute, rather than relative, visually evoked activity levels. Curr Biol 8:1179-1182.

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 (1984a) The permanence of the visual recovery that follows reverse occlusion of monocularly deprived kittens. Invest Ophthalmol Vis Sci 25:908-917.

Mitchell DE, Murphy KM, Kaye MG (1984b) Labile nature of the visual recovery promoted by reverse occlusion in monocularly deprived kittens. Proc Natl Acad Sci U S A 81:286-288.

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12:529-540.

Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH (1992) Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science 256:1217-1221.

Moore RY, Bernstein ME (1989) Synaptogenesis in the rat suprachiasmatic nucleus demonstrated by electron microscopy and synapsin I immunoreactivity. J Neurosci 9:2151-2162.

Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N, Nakanishi S (1991) Molecular cloning and characterization of the rat NMDA receptor. Nature 354:31-37.

Movshon JA (1976) Reversal of the physiological effects of monocular deprivation in the kitten's visual cortex. J Physiol 261:125-174.

Mower GD, Christen WG (1989) Evidence for an enhanced role of GABA inhibition in visual cortical ocular dominance of cats reared with abnormal

monocular experience. Brain Res Dev Brain Res 45:211-218.

Murphy KM, Mitchell DE (1986) Bilateral amblyopia after a short period of reverse occlusion in kittens. Nature 323:536-538.

Murphy KM, Mitchell DE (1987) Reduced visual acuity in both eyes of monocularly deprived kittens following a short or long period of reverse occlusion. J Neurosci 7:1526-1536.

Murphy, K.M., Jones, D.G., & Mitchell, D.E. (2002). Recovery of visual acuity and NMDA expression following monocular deprivation. Program No. 760.1. Society for Neuroscience.

Murphy, K.M., & Mitchell, D.E. (1986). Bilateral amblyopia after a short period of reverse occlusion in kittens. Nature, 323 (6088), 536-538.

Murphy KM, Duffy KR, Jones DG (2004) Experience-dependent changes in NMDAR1 expression in the visual cortex of an animal model for amblyopia. Vis Neurosci 21:653-670.

Ohlsson J, Baumann M, Sjostrand J, Abrahamsson M (2002) Long term visual outcome in amblyopia treatment. Br J Ophthalmol 86:1148-1151.

Okada M, Onodera K, Van Renterghem C, Sieghart W, Takahashi T (2000) Functional correlation of GABA(A) receptor alpha subunits expression with the properties of IPSCs in the developing thalamus. J Neurosci 20:2202-2208.

Olson CR, Freeman RD (1975) Progressive changes in kitten striate cortex during monocular vision. J Neurophysiol 38:26-32.

Olson CR, Freeman RD (1980) Profile of the sensitive period for monocular deprivation in kittens. Exp Brain Res 39:17-21.

Oray S, Majewska A, Sur M (2004) Dendritic spine dynamics are regulated by monocular deprivation and extracellular matrix degradation. Neuron 44:1021-1030.

Oray S, Majewska A, Sur M (2006) Effects of synaptic activity on dendritic spine motility of developing cortical layer v pyramidal neurons. Cereb Cortex 16:730-741.

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.

Poulter MO, Ohannesian L, Larmet Y, Feltz P (1997) Evidence that GABAA receptor subunit mRNA expression during development is regulated by GABAA receptor stimulation. J Neurochem 68:631-639.

Quinlan EM, Olstein DH, Bear MF (1999a) Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. Proc Natl Acad Sci U S A 96:12876-12880.

Quinlan EM, Philpot BD, Huganir RL, Bear MF (1999b) Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex in vivo. Nat Neurosci 2:352-357.

Ramoa AS, Mower AF, Liao D, Jafri SI (2001) Suppression of cortical NMDA receptor function prevents development of orientation selectivity in the primary visual cortex. J Neurosci 21:4299-4309.

Rittenhouse CD, Shouval HZ, Paradiso MA, Bear MF (1999) Monocular deprivation induces homosynaptic long-term depression in visual cortex.

Nature 397:347-350.

Ritter LM, Unis AS, Meador-Woodruff JH (2001) Ontogeny of ionotropic glutamate receptor expression in human fetal brain. Brain Res Dev Brain Res 127:123-133.

Roberts EB, Ramoa AS (1999) Enhanced NR2A subunit expression and decreased NMDA receptor decay time at the onset of ocular dominance plasticity in the ferret. J Neurophysiol 81:2587-2591.

Roberts EB, Meredith MA, Ramoa AS (1998) Suppression of NMDA receptor function using antisense DNA block ocular dominance plasticity while preserving visual responses. J Neurophysiol 80:1021-1032.

Rust NC, Schultz SR, Movshon JA (2002) A reciprocal relationship between reliability and responsiveness in developing visual cortical neurons. J Neurosci 22:10519-10523.

Shatz CJ, Stryker MP (1978) Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. J Physiol 281:267-283.

Shaw C, Cameron L, March D, Cynader M, Zielinski B, Hendrickson A (1991) Pre- and postnatal development of GABA receptors in Macaca monkey visual cortex. J Neurosci 11:3943-3959.

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.

Shirao T (1995) The roles of microfilament-associated proteins, drebrins, in brain morphogenesis: a review. J Biochem (Tokyo) 117:231-236.

Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Hoger H, Adamiker D (1999) Structure and subunit composition of GABA(A) receptors. Neurochem Int 34:379-385.

Silver MA, Stryker MP (2000) Distributions of synaptic vesicle proteins and GAD65 in deprived and nondeprived ocular dominance columns in layer IV of kitten primary visual cortex are unaffected by monocular deprivation. J Comp Neurol 422:652-664.

Simons K (2005) Amblyopia characterization, treatment, and prophylaxis. Surv Ophthalmol 50:123-166.

Singer W, Artola A (1991) The role of NMDA receptors in use-dependent synaptic plasticity of the visual cortex. London: Academic.

Stocca G, Vicini S (1998) Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. J Physiol 507 (Pt 1):13-24.

Swindale NV (1980) A model for the formation of ocular dominance stripes. Proc R Soc Lond B Biol Sci 208:243-264.

Taha S, Stryker MP (2002) Rapid ocular dominance plasticity requires cortical but not geniculate protein synthesis. Neuron 34:425-436.

Takahashi H, Sekino Y, Tanaka S, Mizui T, Kishi S, Shirao T (2003) Drebrin-dependent actin clustering in dendritic filopodia governs synaptic targeting of postsynaptic density-95 and dendritic spine morphogenesis. J Neurosci 23:6586-6595.

Timney B (1983) The effects of early and late monocular deprivation on binocular depth perception in cats. Brain Res 283:235-243.

Tovar KR, Westbrook GL (1999) The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. J Neurosci 19:4180-4188.

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.

Trepel C, Duffy KR, Pegado VD, Murphy KM (1998) Patchy distribution of NMDAR1 subunit immunoreactivity in developing visual cortex. J Neurosci 18:3404-3415.

Tsumoto T, Hagihara K, Sato H, Hata Y (1987) NMDA receptors in the visual cortex of young kittens are more effective than those of adult cats. Nature 327:513-514.

Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci 5:97-107.

Tusa RJ, Palmer LA, Rosenquist AC (1978) The retinotopic organization of area 17 (striate cortex) in the cat. J Comp Neurol 177:213-235.

Vaegan (1979) Critical period for deprivation amblyopia in children. Trans Ophthalmol Soc U K 99:432-439.

Van Essen DC, Newsome WT, Maunsell JH (1984) The visual field representation in striate cortex of the macaque monkey: asymmetries, anisotropies, and individual variability. Vision Res 24:429-448.

Van Sluyters RC (1978) Recovery from monocular stimulus deprivation amblyopia in the kitten. Ophthalmology 85:478-488.

Ward, G (1994) "The RADIANCE lighting simulation and rendering system,"Computer Graphics (Proceedings of '94 SIGGRAPH conference), July 1994, pp. 459-72.

Wang Y, TesFaye E, Yasuda RP, Mash DC, Armstrong DM, Wolfe BB (2000) Effects of post-mortem delay on subunits of ionotropic glutamate receptors in human brain. Brain Res Mol Brain Res 80:123-131.

Whiting PJ, Bonnert TP, McKernan RM, Farrar S, Le Bourdelles B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Thompson SA, Wafford KA (1999) Molecular and functional diversity of the expanding GABA-A receptor gene family. Ann N Y Acad Sci 868:645-653.

Wiesel TN, Hubel DH (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J Neurophysiol 28:1029-1040.

Wong-Riley MT, Jacobs P (2002) AMPA glutamate receptor subunit 2 in normal and visually deprived macaque visual cortex. Vis Neurosci 19:563-573.