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Plasticity of the developing visual cortex in normal and mutant mice

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Plasticity of the Developing Visual Cortex in Normal and Mutant Mice

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To Poppy and Unc,
who could not read this with their eyes,
but saw it in their hearts.

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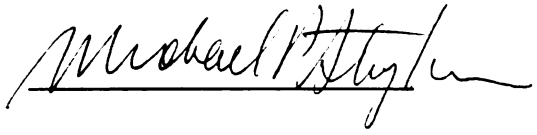
ADVISOR'S STATEMENT

Regarding inclusion of material with multiple authors

Chapter Three has been submitted for publication in its present form. UCSF graduate student Takao Hensch, University of Washington graduate student Eugene Brandon, University of Washington faculty G. Stanley McKnight and Rejean Idzerda, and I will be listed as coauthors. Joshua Gordon performed all the *in vivo* experiments for Figures 3-1B and 4 through 6; Takao Hensch performed the *in vitro* experiments, described in Figures 3-1 A&C, 2 and 3. Both contributed to the discussion of the results. The additional authors besides myself generated and genotyped the mice used in the experiments; my role was as supervisor to both Joshua and Takao. Joshua's contribution represents research and scholarship comparable in scope to a standard thesis chapter.



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Plasticity in the Developing Visual Cortex of Normal and Mutant Mice

by

Joshua A. Gordon

ABSTRACT

Activity-dependent processes fine-tune neuronal connections during the development of the visual system. These processes have been best studied in the primary visual cortex, where inputs from the two eyes compete for synaptic space on their cortical targets. This correlation-based competition occurs only during a well-defined critical period and requires neuronal activity. The cellular mechanisms which implement this competition are poorly understood.

Recently, gene-targeting techniques have enabled the generation of a large and growing number of mouse lines, each possessing specific genetic lesions. These tools are ideal for exploring the roles of particular molecules, and the cellular processes which require them, in complex phenomena which must be studied *in vivo*.

To take advantage of this emerging technology, I have characterized a mouse model for ocular dominance plasticity that in all respects mimics that found in other animals. Brief monocular lid suture maximally shifts cortical responses towards the open eye only during a well-defined critical period;

binocular deprivation has little effect, demonstrating a competitive effect; alternating monocular deprivation, by decreasing correlated activity between the two eyes, decreases the number of binocularly responsive neurons; and monocular deprivation has greater effects in extragranular layers, demonstrating intracortical as well as geniculocortical plasticity.

I have also begun a dissection of the cellular mechanisms underlying ocular dominance plasticity by studying visual cortical responses and plasticity in lines of mice possessing mutations in potential plasticity mediators. Mice with targeted disruptions of the genes encoding the RIβ– and $C\beta_1$-subunits of protein kinase A, Thy-1, Fyn, and the γ-isoform of protein kinase C all have normal visual cortical responses and plasticity. Interestingly RIβ-deficient mice retain normal responses and plasticity despite a lack of a well-studied form of *in vitro* plasticity, theta burst-induced long-term potentiation. Finally, mice lacking the α-isoform of calcium/calmodulin-dependent protein kinase II develop normal visual cortical responses, but a subset of these animals demonstrate reduced plasticity.

These data suggest that theta burst-induced long-term potentiation is not required for, and that CaMKII-dependent processes are involved in, ocular dominance plasticity. Furthermore, they demonstrate the utility of the mouse model of ocular dominance plasticity for investigating the mechanisms underlying activity-dependent development.

Stephen G. Lisberger Michael P. Stryker

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CHAPTER ONE:

Introduction

OVERVIEW

Neuronal connectivity within the mammalian visual system is at once complex and precise. Ocular dominance (OD) columns in animals such as the cat and monkey are particularly striking examples of this precision. In the primary visual cortex of these species, inputs from eye-specific layers of the lateral geniculate nucleus of the thalamus segregate into patches in layer IV (Rakic, 1976, 1977; LeVay et al., 1978, 1980). The specificity of these connections, however, is not present in the immature nervous system, but rather arises through an activity-dependent self-organizing process. Geniculocortical fibers representing the two eyes initially terminate intermingled throughout the extent of layer IV in the primary visual cortex (LeVay et al., 1978; Rakic, 1976, 1977). Over the course of several weeks, the afferents from each eye segregate into their respective columns, and the percentage of cells driven by both eyes decreases (LeVay et al., 1978, Rakic, 1976, 1977). This process requires retinal activity (Stryker and Harris, 1986), and is thought to rely on a Hebbian correlation-based learning rule (Hebb, 1949; Stryker and Strickland, 1984; Stryker, 1986).

Coincident with the period of remodeling of afferent input to the cortex is a period of heightened sensitivity to manipulations of the animal's visual environment. Monocular lid suture (monocular deprivation, MD) during this early critical period causes a dramatic decrease in the spread of geniculocortical arbors from the closed eye, and a corresponding increase in

the spread of arbors from the open eye (Shatz and Stryker, 1978; LeVay et al., 1980). Accompanying this dramatic anatomical change is an equally dramatic physiological one. After even brief periods of MD, the responses of cortical cells to the closed eye nearly disappear (Wiesel and Hubel, 1963, 1970; Olson and Freeman, 1975, 1980; Hubel et al., 1977; Movshon and Dürsteler, 1977; Blakemore et al., 1978; Shatz and Stryker, 1978; LeVay et al., 1980). The rules governing this so-called ocular dominance plasticity are well characterized, and they suggest strongly that the mechanisms underlying this plasticity and those responsible for the segregation of OD columns are the same. Theoretical and experimental evidence supports the hypothesis that OD plasticity results from a competition between inputs from the two eyes for synaptic space on the postsynaptic cell; that the postsynaptic cell acts a coincidence detector, designating inputs whose firing patterns are most correlated with its own; and that the postsynaptic cell then initiates the process of strengthening well-correlated inputs and weakening poorly correlated ones.

While the rules by which active inputs are strengthened and inactive ones weakened are well understood, the cellular mechanisms underlying the transduction of activity patterns into the profound physiological and anatomical rearrangements of these same inputs are quite poorly understood. Much effort has gone into attempts to interfere pharmacologically with cellular processes and thereby disrupt OD plasticity. These efforts have met with little success. This approach is hampered, moreover, by the limitedavailability of effective and specific agonists and antagonists that are

able to function *in vivo*, and the ambiguity of a negative result. These limitations are particularly significant when an attempt is made to intervene with the function of intracellular proteins; in these cases, delivery of the agents to the cytoplasm may be difficult to achieve and equally difficult to verify.

Recently, the development of new and sophisticated techniques for manipulating the genome of the mouse has given rise to a whole new class of tools which have proved useful in examining the cellular mechanisms underlying systems-level processes. Whole animal preparations with alterations in or deletions of a variety of specific proteins are now becoming widely available. These reagents allow one to ask questions regarding the involvement of those proteins, and the cellular mechanisms which require them, in complex phenomena which can only be studied *in vivo*. Of course, this approach presumes the availability of an adequate murine model for the phenomenon of interest.

This thesis describes the characterization a mouse model for ocular dominance plasticity, and the use of specific mutant lines to begin a dissection of the cellular mechanisms underlying this plasticity. Within are described the following: experiments demonstrating the similarity of the mouse model of ocular dominance plasticity to that found in higher mammals such as the cat and monkey and hence the relevance of its cellular basis; the use of a particular mutant line to provide evidence against the hypothesis that a particular form of plasticity *in vitro* underlies OD plasticity *in vivo*; and

studies of a number of other mouse lines with mutations in proteins involved in cellular mechanisms well-situated to play a role in OD plasticity. When considered in the context of the body of work demonstrating the role of OD plasticity in the developing neocortex, these experiments establish the utility of the mouse model for testing hypotheses regarding cellular mechanisms of activity-dependent development.

BACKGROUND

Ocular Dominance Plasticity

The term "ocular dominance plasticity" refers to the effects of various manipulations of visual experience on the responses of binocular neurons in the visual cortex. Manipulations such as monocular lid suture, surgically- or optically-induced strabismus, and pharmacological inhibition of retinal activity all have profound effects on the responses of these neurons. The study of these effects, and the study of the normal development of OD columns and binocular responses, have led to the hypothesis that the mechanisms underlying ocular dominance plasticity are used by the developing nervous system to refine connections during development.

Ocular dominance columns form as a result of an activity-dependent, correlation-based mechanism. The initially widespread, overlapping projections of geniculocortical afferents gradually segregate into ocular dominance columns over the course of several weeks in the visual cortex of cats and monkeys (LeVay et al., 1978; Rakic et al., 1976, 1977). This process can be prevented by blocking retinal activity with intravitreal injections of tetrodotoxin (Stryker and Harris, 1986), demonstrating that neural activity within the visual system itself drives the segregation. The activity does not have to be evoked visually, however, as OD columns mature prenatally in the monkey (Rakic et al., 1976, 1977; Horton et al., 1995), and dark rearing

does not prevent OD column segregation in the cat (Kalil, 1982; Mower et al., 1985; Stryker and Harris, 1986). Rather than any stimulus-evoked responses, therefore, the important component of activity necessary for driving the segregation appears to be the temporal correlation between the firing patterns of inputs from one eye, compared to the other. This has been best demonstrated by experiments in which retinally driven activity was replaced by artificial stimulation of the optic nerve in cats during the time when OD columns are normally segregating. Simultaneous stimulation of both optic nerves prevents OD column formation, while alternating stimulation allows columns to form (Stryker and Strickland, 1984; Stryker, 1986). The interpretation of these experiments, supported by theoretical studies (von der Malsburg, 1979; Miller et al., 1989), is that inputs which are active at the same time stabilize each other within local domains. Since afferents from the same eye should tend to fire together more often than afferents from different eyes, they will be better correlated with each other than with afferents from the other eye, and will form stable domains with each other, excluding afferents from the other eye.

Manipulations of visual experience during ocular dominance column segregation appear to effect changes in visual cortical physiology and anatomy through an identical mechanism. Monocular deprivation in particular causes a profound decrease in both the area of cortex innervated by deprived eye geniculocortical afferents (Shatz and Stryker, 1978; LeVay et al., 1980) and the effectiveness of those afferents in evoking responses in cortical neurons

(Wiesel and Hubel, 1963, 1970; Olson and Freeman, 1975, 1980; Hubel et al., 1977; Movshon and Dürsteler, 1977; Blakemore et al., 1978; Shatz and Stryker, 1978; LeVay et al., 1980). These effects only occur if the deprivations take place during a critical period early in the development of the animal (Hubel and Wiesel, 1970; LeVay et al., 1980; Olson and Freeman, 1980). The decrease is not due simply to atrophy of the deprived eye pathways due to a lack of activity, as binocular deprivations of similar duration produce a much smaller effect on visual responses (Wiesel and Hubel, 1965; Freeman et al., 1981). Furthermore, MD during activity blockade by tetrodotoxin injections into the other causes a shift towards the MD eye, demonstrating conclusively that a competition between the inputs from the two eyes causes the change in cortical responses (Chapman et al., 1986). As is true for OD column development, the shift in responsiveness towards the deprived eye requires neuronal activity. Blocking cortical activity with tetrodotoxin during the deprivation period prevents the shift (Reiter et al., 1986).

As in ocular dominance column formation, a correlation-based mechanism underlies the effects of ocular dominance plasticity. This was initially demonstrated by the effects of various experimental manipulations designed to further decorrelate inputs from the two eyes. Although inputs from the two eyes are sufficiently uncorrelated to cause the segregation of geniculocortical afferents into OD columns within layer IV, enough residual correlation must remain to allow inputs from the two eyes to establish effective connections onto the same cell in other layers. Thus, reducing the

degree of correlation between the two eyes should reduced the number of binocularly driven cortical cells. Accordingly, strabismus and alternating monocular deprivation, manipulations which allow both eyes to receive visual input but prevent the two eyes from seeing the same input, both reduce the number of binocular cortical cells (Hubel and Wiesel, 1965; Blakemore, 1976; Blasdel and Pettigrew, 1979; Crawford and von Noorden, 1979, 1980).

A correlation-based rule explains the decrease in responsiveness to the deprived eye with monocular deprivation. MD prevents patterned activity in the deprived eye inputs, making them less likely activate the postsynaptic cell; as a result, deprived eye inputs become less correlated with the postsynaptic cell than nondeprived eye inputs. A correlation rule therefore strengthens open eye inputs and weaken closed eye inputs. This model is supported by the finding that infusing the $GABA_A$ agonist muscimol into kitten visual cortex during MD causes a reverse shift toward the deprived eye (Reiter and Stryker, 1988; Hata and Stryker, 1994). Muscimol blocks cortical cell activity but does not affect the activity of the presynaptic geniculocortical afferents (Reiter and Stryker, 1988; Chapman et al., 1991). During MD, all activity in the presynaptic cell can be considered uncorrelated with the silenced postsynaptic cell. Since deprived-eye inputs are presumably less active, the correlation rule can explain why deprived eye afferents increase in effectiveness and anatomical spread in the presence of muscimol.

The muscimol experiments directly demonstrate another important property of ocular dominance plasticity. The fact that postsynaptic activity blockade affects the shift seen with MD indicates a role for the postsynaptic cell in the plasticity process. Yet massive rearrangement of the presynaptic geniculocortical arbors is a prominent feature of the MD effect (Shatz and Stryker, 1978; LeVay et al., 1980). Most notably, deprived geniculocortical afferent arbors expand rather than retract after MD with cortical muscimol infusion (Hata and Stryker, 1994). Thus information regarding postsynaptic activity must reach the presynaptic cell in order to affect the profound anatomical rearrangements which occur during MD, implicating retrograde messengers in OD plasticity.

The rules by which the game of OD plasticity is played are therefore well-understood. The correlation between pre- and postsynaptic activity is somehow tabulated and translated into changes in the physiological effectiveness and anatomical arborization of afferents from the two eyes. Although the tabulation and translation could take place pre- or postsynaptically, information regarding postsynaptic activity is eventually translated into anatomical rearrangements presynaptically. These are the rules, but who are the players? Despite much effort, little is currently known regarding the cellular mechanisms underlying ocular dominance plasticity.

Numerous attempts have been made to disrupt ocular dominance plasticity pharmacologically. The NMDA receptor was an early target of investigation. Since its activation requires simultaneous presynaptic glutamate release and postsynaptic membrane depolarization, the NMDA receptor is eminently suitable as a correlation detector (Mayer and Westbrook, 1987; Collingridge and Bliss, 1987). Indeed, cortical infusion of the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (APV) prevents the OD shift induced by MD (Kleinschmidt et al., 1987; Bear et al., 1990). Unfortunately, this result is ambiguous, since NMDA receptor activation appears to be required for visually-evoked activity in V1 (Bear et al., 1990; Miller et al., 1989). The prevention of OD plasticity by APV may be due to a simple activity blockade, rather than a requirement for NMDA receptor

activation. Other pharmacological manipulations, such as interfering with cholinergic and noradrenergic transmission in the cortex by chronic infusion of 6-hydroxydopamine, may also reduce OD plasticity by reducing the visually evoked cortical activity (Bear and Singer, 1986). Still others, such as blockade of nitric oxide synthase (Gillespie et al., 1994; E. S. Ruthazer, manuscript submitted) or metabotropic glutamate receptors (Hensch and Stryker, 1994; T. K. Hensch and M. P. Stryker, manuscript submitted) have failed to prevent the effects of MD.

A manipulation which reduces ocular dominance plasticity without changing cortical responsiveness has recently been described by Maffei et al. (1992). They demonstrated that injection of nerve growth factor (NGF) into the cerebral ventricles prevents the physiological effects of MD in rodents. The NGF receptors, p75 and trkA, however, may not be expressed in cortical neurons (Merlio et al., 1992; M. A. Silver, C. L. Howe, and M. P. Stryker, unpublished observations). Furthermore ventricular NGF has a much smaller effect on MD in cats (Carmignoto et al., 1993). Nonetheless, additional evidence exists to support a role for neurotrophins in OD plasticity. TrkB, the receptor for BDNF and NT4/5, is present in the visual cortex, and appears to be regulated in a time course appropriate for involvement in OD plasticity (Allendorfer et al., 1994). Infusion of BDNF or NT4/5 interferes with ocular dominance column formation and/or maintenance (Cabelli et al., 1995). Finally, cortical NT4/5 prevents the LGN cell shrinkage normally associated with MD (Riddle and Katz, 1995). In addition to neurotrophins, proteases

have been implicated in ocular dominance plasticity in a recent preliminary report (Griesinger and Müller, 1995).

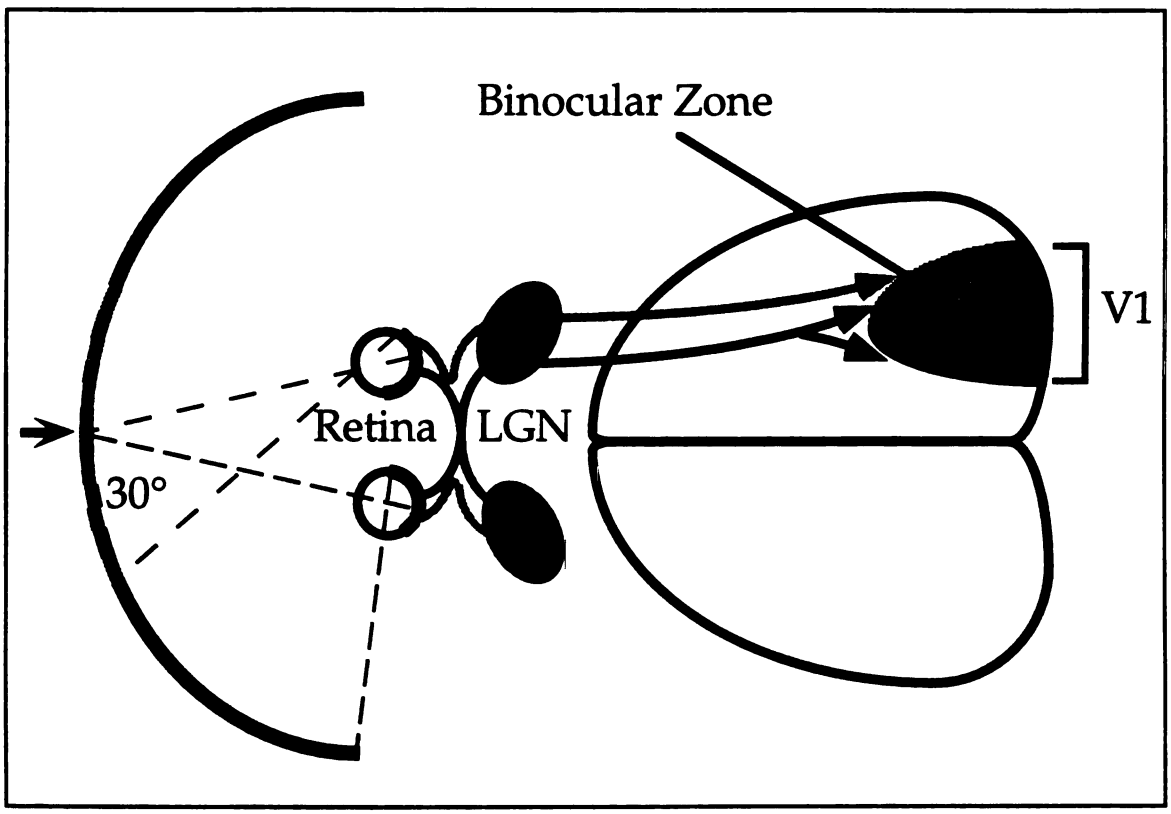
The dearth of direct evidence for cellular mediators of ocular dominance plasticity spurred attempts to develop *in vitro* models. In particular, long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy in slices from visual cortex have been suggested to play a role in the changes seen *in vivo* (Tsumoto, 1991; Kandel and O'Dell, 1992; Bear and Kirkwood, 1993). These models share several properties with OD plasticity. Through high-frequency stimulation, active inputs onto layer III pyramidal neurons are strengthened, while less active inputs, stimulated consistently at low frequency, are weakened (Artola et al., 1990; Kirkwood et al., 1993). The same stimulation results in synaptic strengthening or weakening depending on the level of postsynaptic depolarization, consistent with a correlation-based rule (Artola et al., 1990; Kirkwood and Bear, 1994a). At least some forms of LTP and LTD require NMDA receptor activation (Collingridge and Bliss, 1987; Bear and Kirkwood, 1993; Kirkwood et al., 1993). Finally, LTP induced by stimulation of the white matter cannot be obtained in adult animals without inhibitory neurotransmitter antagonists (Kato et al., 1991; Kirkwood and Bear, 1994a). The time course of this requirement approximates the time course of the critical period for OD plasticity, and dark rearing, which delays the critical period for OD plasticity, also delays the requirement for blockers of inhibition (Kirkwood et al., 1995; Fox, 1995).

While much is known about the cellular mechanisms underlying LTP and LTD, the relevance of this information for OD plasticity is questionable. Pharmacological dissection of visual cortical plasticity *in vitro* has suggested roles for calcium and calcium/calmodulin-dependent protein kinase in LTP (Aroniadou et al., 1993; Kimura et al., 1990; Funauchi et al., 1992) and calcium and calcium/calmodulin-dependent protein phosphatases in LTD (Brocher et al., 1992; Komatsu and Iwakari, 1992; Funauchi et al., 1994; Kirkwood and Bear, 1994b) in addition to the NMDA receptor. Furthermore, by analogy to similar forms of plasticity studied in the hippocampus, roles for several other molecules and processes have been suggested (Tsumoto, 1992). The list of potential mediators of plasticity derived from these *in vitro* models provides a wealth of suggestions for *in vivo* experiments. The expression of several of these molecules and processes change in development with suggestively familiar time courses, providing additional correlative evidence for a potential role in OD plasticity (Hashimoto et al., 1988; Dudek and Bear, 1989; Burgin et al., 1990) Nonetheless, with the possible exception of the NMDA receptor, no direct links between plasticity *in vitro* and *in vivo* have yet been established.

The visual cortex of the mouse

The mouse central visual system is in many key respects similar to the visual system of higher mammals. In the mouse, the frontal 30°-40° of the upper portion of each visual hemifield is seen by the retinas of both eyes

Figure 1-1: Schematic diagram of the mouse central visual system.



(Dräger, 1975; Wagor et al., 1980; see Figure 1-1). Retinal ganglion cell axons representing this region project to eye-specific areas within the dorsal lateral geniculate nucleus (dLGN; Godement et al., 1984). Cells within the dLGN can only be driven by one or the other eye (Métin et al., 1983). These dLGN cells

project their axons to a cytoarchitectonically identifiable area 17 (Dräger, 1974, 1978; Caviness, 1975; Simmons et al., 1982) which contains a complete retinotopic representation of the visual field (Dräger, 1975; Wagor et al., 1980). Surrounding this primary representation are multiple additional representations, prompting Wagor et al. (1980) to define four visual areas: the primary visual cortex, or V1 (corresponding to area 17), V2, V3 and Vm. The lateral one-third of V1 represents the frontal portion of the visual hemifield and receives projections from both eye-specific areas of the dLGN (Dräger, 1978). The majority of cells within this "binocular zone" respond to stimuli presented to either eye (Dräger et al., 1976, 1978; Métin et al., 1988). A key difference with regard to the current work, and a worthy target of future investigation, is the lack of demonstrable OD columns within the binocular zone (Drager 1974, 1978).

Neuronal responses in the primary visual cortex reveal a stimulus selectivity and receptive field structure similar to though markedly less specific than that found in the cat and monkey. Receptive field diameters range from 3-30°, with an average near 15° (Dräger, 1975; Métin et al., 1988). Approximately 40% of cells are orientation selective and can be classified as simple or complex based on the presence or absence of on- and off-subregions; cells in the lower layers are less likely to be orientation selective (Dräger, 1975; Mangini et al., 1980; Métin et al. 1988). The rest of the cells are not orientation selective, although a subset of them are selective for motion (Métin et al., 1988). Interestingly, Métin et al. (1988) reported less variation in preferred

orientation and ocular dominance in cells recorded within radial penetrations as opposed to oblique penetrations, suggesting a columnar arrangement of stimulus specificity like that seen in other animals; others have not seen evidence of such structure.

Monocular deprivation affects the responses of neurons within the binocular zone of mouse primary visual cortex. In the normal animal, the contralateral eye dominates the responses of the great majority of binocular zone neurons (Dräger, 1975, 1978; Métin, 1988). After long periods of MD (6 weeks to 1 year), the physiological responses of binocular zone neurons to stimuli presented to the deprived eye have changed dramatically (Dräger, 1978). In the ipsilateral hemisphere, responses to the deprived eye have nearly disappeared. In the contralateral hemisphere, most cells have become dominated by the ipsilateral, open eye. The relevance of these effects to those seen in cats and monkeys remained unclear, however, for several reasons. First, no effect of these same deprivations on the spread of geniculocortical terminals from either eye was seen using transneuronal transport of tritiated proline to label geniculocortical afferents (the same technique used to label OD columns in cats and monkeys). Second, it is not clear that the effects were due to an underlying mechanism similar to that found to occur in cats and monkeys. Specifically, the dependence of these effects on correlation-based competition was not characterized.

APPROACH

My attempt to describe a mouse model for ocular dominance plasticity was provoked by two factors. First, despite much effort to exploit the well-described cat OD plasticity system, little has been learned regarding cellular mechanisms. Second, recent advances in technology have permitted the introduction of specific genetic lesions into mice, unleashing the power of genetics to approach complex systems *in vivo* in mammals. To use this power to ask questions of cellular mechanism in OD plasticity, it was necessary to develop a mouse model.

As described above, the visual system of the mouse is well-suited for the current investigation. Mouse V1 contains a binocular zone, in which most neurons respond to input from both eyes. Long-term monocular deprivation causes a decrease in the responses of binocular zone neurons to input from the deprived eye. To demonstrate that the changes seen in the mouse resulted from the same underlying mechanism, I set out to determine the following: 1) whether brief deprivations also affect responses to the deprive eye, 2) if these effects are confined to a critical period, 3) if they result from competition between inputs from the two eyes, and 4) if a correlation-based mechanism can account for the effects. Experiments addressing these questions are described in Chapter Two, which includes a discussion of the relevance of the mouse model to other animal models and some more

general conclusions resulting from this and other comparative studies of OD plasticity.

The utility of the mouse model derives from the ability to induce specific mutations in genes of interest and test the effects of those mutations on phenomena of interest. Gene-targeting technology allows one to produce mice with deletions of, or specific mutations in, any cloned gene (Bradley, 1993; Soriano, 1995). Furthermore, a large and growing number of mutant mouse lines are becoming available (Brandon et al., 1995a), and even newer technologies promise tissue-specific and inducible mutations in the near future (Furth et al., 1994; Kuhn et al., 1995; Mayford et al., 1995a). A principle advantage of this technology is that one can compare the effects of identical manipulations on both physiology and behavior, an approach that has already been used to address questions of the cellular processes underlying learning and memory (Silva et al., 1992a, 1992b; Grant et al., 1992; Grant and Silva, 1994). Chapter Three describes the use of a particular mutant mouse line, lacking a regulatory subunit of the cyclic AMP-dependent protein kinase, to conduct a similar analysis of OD plasticity, comparing the effects of the mutation on *in vitro* plasticity to those on *in vivo* plasticity. Chapter Four describes the effects of a number of other mutations on OD plasticity, conducted as part of an ongoing screen aimed at identifying potential cellular mediators of the phenomenon.

CHAPTER TWO:

Experience-Dependent Plasticity of Binocular Responses in the Primary Visual Cortex of the Mouse

ABSTRACT

An activity-dependent form of synaptic plasticity underlies the fine tuning of connections in the developing primary visual cortex of mammals such as the cat and monkey. Studies of the effects of manipulations of visual experience during a critical period have demonstrated that a correlation-based competetive process governs this plasticity. The cellular mechansims underlying this competition, however, are poorly understood. Transgenic and gene-targeting technologies have led to the development of a new category of reagents which have the potential to help address issues of cellular mechanism such as these, provided that these issues can be studied in a mouse model. The current study attempts to characterize a developmental plasticity in the mouse primary visual cortex and to demonstrate its relevance to that found in higher mammals. I found that 4 days of monocular lid suture induced a maximal shift in the responsiveness of cortical neurons toward the open eye, provided the deprivation occurred near the peak of the critical period, at approximately postnatal day 28. Furthermore, binocular deprivation during this critical period had a much smaller effect on visual cortical responses, and alternating monocular deprivation resulted in a decrease in the number of binocularly responsive neurons. Finally, a laminar analysis demonstrated plasticity of both geniculocortical and intracortical connections. These results demonstrate that an activity-dependent,

competitive form of synaptic plasticity which obeys correlation-based rules operates in the developing primary visual cortex of the mouse.

INTRODUCTION

The complex and precise connectivity present in the adult mammalian central visual system arises as a consequence of a remarkable developmental process. Spontaneous and stimulus-evoked activity fine-tune the connections between neurons in the various visual areas, shaping the anatomical and physiological properties of neurons and synapses, eventually honing in on the adult state (Goodman and Shatz, 1993). Much is known about the rules governing this fine-tuning process, thanks to decades of experiments on binocular interactions in the primary visual cortex (area 17) of the cat. During the first few months of life, geniculocortical afferents from the two eyes, initially widespread within the input layer of area 17, segregate into ocular dominance (OD) columns through an activity-dependent competition for synaptic space (LeVay et al., 1978; Stryker and Harris, 1986; Antonini and Stryker, 1993a).

The tremendous plasticity inherent in this developmental reorganization of connectivity can be redirected by manipulating the visual environment of young cats and monkeys. Monocular lid suture (monocular deprivation, MD) during this same early postnatal period results in a pronounced decrease in the area occupied by geniculocortical arbors representing the deprived eye, and a corresponding increase in the area occupied by arbors representing the nondeprived eye (Hubel et al., 1977; Shatz and Stryker, 1978 LeVay et al. 1980). The physiological plasticity is just as

dramatic; after only a few days of MD, the great majority of cortical cells become responsive only to stimuli presented to the non-deprived eye, and only a few cells respond, usually poorly, to stimulation through the deprived eye (Wiesel and Hubel, 1963, 1970; Olson and Freeman, 1975, 1980; Movshon and Dürsteler, 1977; Blakemore et al., 1978; Hubel et al. 1977, LeVay et al. 1980). The rules governing this plasticity are well established. Cortical activity blockade prevents the shift in responsiveness with MD (Reiter et al., 1986). MD later in the life of the animal, at a time after OD columns are normally fully formed, has little or no effect (Wiesel and Hubel, 1970; Olson and Freeman, 1980). Binocular visual deprivation during the critical period has a much smaller effect on responses, demonstrating a requirement for competition between inputs from the two eyes (Wiesel and Hubel, 1965; Freeman et al., 1981). Finally, interventions that prevent coincident activation of corresponding portions of both eyes, such as strabismus or alternating monocular deprivation, decrease binocular responses and cause most neurons to be driven exclusively by one eye or the other, suggesting that OD plasticity follows Hebbian rules (Hubel and Wiesel, 1965; Blakemore et al., 1975; Yinon, 1975; Blakemore, 1976; Changeux and Danchin, 1976; Stent, 1977; Blasdel and Pettigrew, 1979; Crawford and von Noorden, 1979, 1980).

Experiments have suggested that OD plasticity, well described in cats and primates, can also be found in other mammals. Monocular deprivation has been shown to alter the physiological responses of cortical cells in ferrets (B. Chapman and M.P. Stryker, unpublished observations), sheep (Kennedy et

al., 1980), rabbits (Van Sluyters and Stewart, 1974), rats (Maffei et al., 1992; Fagiolini et al., 1994), hamsters (Emerson et al., 1982) and mice (Dräger, 1978). In particular, experiments in rats have shown that even in the primitive rodent primary visual cortex, a critical period-delimited plasticity appears to follow correlation-based rules (Fagiolini et al., 1994; Domenici et al., 1992).

Mice offer an additional advantage to those wishing to manipulate the environment. Powerful transgenic and knockout technologies enable direct control over the genetic makeup of the mouse (Grant and Silva, 1994; Mayford et al. 1995). A large and continuously growing number of mutant and knockout mouse lines are available (Brandon et al., 1995a). Many of these mice may be used to test hypotheses regarding the cellular mechanisms underlying OD plasticity, that to date have remained elusive. Such work requires a simple, well-established murine model. Although Dräger (1978) showed that prolonged MD (6 weeks to 1 year) can induce OD shifts in mice, it was not clear that the effects reported were competitive. Here I describe experiments that demonstrate (1) OD shifts can be reliably induced in mice with brief deprivations, (2) this plasticity is confined to a well-defined critical period, (3) 4 day deprivations at the peak of this critical period have a saturating effect, (4) this plasticity depends on competition between the inputs from the two eyes, and (5) this plasticity appears to result from a correlation-based rule for synaptic reorganization. These results show that an experience-dependent plasticity exists in mouse visual cortex that appears identical to that described in other species.

METHODS

Mouse Strains

Sixty male C57Bl/6 mice were obtained from Simonsen Laboratories (Gilroy, CA) or Banton and Kingman (Fremont, CA). For the laminar analysis and Figure 2-3 only, an additional 12 C57Bl/6 x 129 mice were obtained from A.J. Silva at Cold Spring Harbor Laboratory and G.S. McKnight at the University of Washington. There were no detectable differences in receptive field properties, ocular dominance, or plasticity between these hybrid animals and the inbred mice.

Monocular and Binocular Deprivation

Mice of various ages were anesthetized with 3% Halothane in a 3:2 mixture of nitrous oxide and oxygen. The halothane concentration was reduced to 1.5% after achievement of surgical anesthesia. The area immediately surrounding the eye to be sutured was wiped with 70% ethanol. Lid margins were trimmed and the eye was flushed with saline. In some cases, an antibiotic (chloroptic, Allergan) was placed on the eye, but this practice was discontinued as it did not decrease the incidence of postoperative infection. Two to 3 mattress sutures were placed using 7-0 silk, opposing the full extent of the trimmed lids. Animals were recovered by allowing them to

breathe a mixture of 100% oxygen and room air, and returned to their cages when fully alert.

Animals were checked daily to make sure the eyes remained closed and uninfected. Occasionally, it was difficult to be certain whether or not the lids were closed in the awake animal, in which case the animal was anesthetized briefly with 3% halothane in 3:2 N_2O:O_2. Holes were discovered in 3 animals. In one of these cases, the deprivation was planned for a total of 3 weeks; therefore the hole was repaired and the deprivation continued. The results from this experiment were consistent with similar deprivations in which the lid suture remained intact. The other two animals were to be deprived for 4 days; experiments on these animals were terminated without recording. Only animals whose lid fusions remained intact throughout the 4 day period are considered in the results for 4 day or shorter deprivations. In an additional animal, an infection was severe enough to require drainage, which was carried out under halothane anesthesia. The sutures were removed, and the eye drained and washed with sterile saline, antibiotic applied, and the lids resutured. The results obtained from this animal were also consistent with similarly deprived animals.

Alternating Monocular Deprivation

The right eyelids of 5 mice were sutured at P22 (2 animals) or P23 (3 animals). The procedure followed was identical to that described above for

monocular and binocular deprivation, except that lid margins were not trimmed prior to suturing. Under these conditions, the lids did not heal together, facilitating later opening. At P29, after an initial 6-7 day deprivation, the sutures were removed from the right eye and the left eye was closed. Again the procedure described above was followed, except that the lids were not trimmed and only one mattress suture was placed. This process was repeated daily, alternating the eye closed each day, for the next 7-12 days. Electrophysiological recordings were obtained from the hemisphere contralateral to the initially deprived eye immediately at the end of the period of alternating MD.

Electrophysiology

Electrophysiological procedures were adapted from those of Dräger (1975) and Wagor et al (1980). Mice were anesthetized with an intraperitoneal injection of 50 mg/kg Nembutal (Abott). Additional doses of 0.15 to 0.25 mg were given if necessary to induce surgical anesthesia. A sedative, chlorprothixene (0.2 mg, Sigma), was administered intramuscularly to supplement the Nembutal; this reduced the amount of Nembutal necessary to maintain a sufficiently deep level of anesthesia. Additionally, lidocaine (2 % Anthocaine, Anpro) was applied locally to all incisions. Atropine (0.3 mg, Butler) and Dexamethasone (0.05 mg, Anpro) were injected subcutaneously. The atropine was necessary to reduce secretions and to counter the

parasympathomimetic effect of the anesthetic agents; the dexamethasone helped reduce cerebral edema. The animal's temperature was maintained at 36.5° by a rectal thermoprobe feeding back to a heating pad on which the animal rested throughout the experiment. The animal's eyes were kept closed throughout the ensuing procedures to prevent their drying out. Electrocardiograph leads were attached to the right forelimb and left hind limb, and the heart rate was monitored continuously throughout the experiment using custom software written in LabView (National Instruments) installed on a Macintosh IIfx computer. This enabled us to monitor closely the depth of anesthesia in these animals, that is crucial to maintain optimum visual responsiveness. A low (below 6-7 Hz) or falling heart rate indicated that animal might be overanesthetized, resulting in a dramatic decrease in visual cortical responsiveness. In contrast, a high (greater than 9-10 Hz) or rising heart rate indicated the animal might be getting too light. Additional doses of Nembutal (0.15 to 0.25 mg) were delivered as required through an intraperitoneal catheter.

To keep the animal's airway patent, a tracheotomy was performed, and a bent glass capillary tube (1.0 mm O.D., 0.75 mm I.D. for young animals, larger for adults) was inserted into the open end of the trachea just below the larynx. A plastic tube blowing 100 % oxygen was placed in front of the opening of the trachea tube, so that the animal breathed a mixture of oxygen and room air throughout the course of the experiment. Pulse oximetry in a few representative animals demonstrated that this preparation enabled the

animals to maintain a blood oxygen level of 95 to 98% saturation throughout the course of the physiological recording session. The animal was then placed in a custom-built stereotaxic holder. The head was held in place by ear bars with fine (1.5 mm) tips, and a mouth bar to which the animal's upper teeth were secured by a drop of cyanoacrylate.

Extreme care was required in exposing the visual cortex. A dental drill was used to partially drill through the skull along the suture lines, and the large (5 mm by 5 mm) section of bone was carefully lifted away from the brain with the bent tip of a pair of fine forceps. The dura was left intact, and the exposure was covered with warm agarose (2.8% in saline), which when hardened reduced the cardiac and respiratory pulsations. The eyes were then opened, and lids trimmed until the full extent of the pupil was exposed. The corneas were protected by covering them with silicone oil, which had to be reapplied frequently throughout the course of the experiment. By this point, the eyes were typically tremendously dilated, perhaps a side effect of the anesthetic used. I was therefore able to easily visualize and project optic disk locations onto a tangent screen; the optic disk location varied from animal to animal only slightly (Mean ± S.D.; Elevation = 33.6 ± 5.7, Azimuth = 65.0 ± 6.0).

Resin-coated tungsten microelectrodes (Hubel, 1957) with tip resistances of 2-4 MOhms were used to record single units from the primary visual cortex. Electrodes were positioned under visual guidance, and correspondence between receptive field locations and published maps

(Dräger, 1975; Wagor et al., 1981) were used to locate the binocular zone. I verified that all neurons recorded were in V1, as opposed to extrastriate cortex, in two ways. First, I verified that as the electrode was moved from medial to lateral, electrode field locations moved centrally. Second, lesions placed in 1-2 penetrations per animal were located within V1 using Nissl stained sections, according to established cytoarchitectonic criteria (Caviness, 1976).

Receptive fields of isolated single units were plotted using a hand-held projection lamp on a screen placed 30 cm in front of the animal's eyes. The screen was turned inward at an angle of 58° from a line extending out from the animals' midline to allow it to cover more peripheral areas of the visual field. Bars of light were varied in size and orientation, where appropriate, to obtain a maximal response; for those cells with orientation selectivity, the preferred orientation was noted.

Cells within the frontal 30-40° of the upper portion of each hemifield have the potential to receive input from both eyes (see Figure 2-1A). In our initial recordings from normal animals, I found that cells with receptive fields outside the frontal 25° were much less likely to be driven well by the ipsilateral eye (see Results, Figure 2-3). I therefore conservatively defined the binocular zone as the frontal 25° of each visual hemifield. The vertical meridian was defined as the intersection of the midline of the animal with the tangent screen. The appropriateness of this definition was verified by the consistency of the optic disk projections relative to this midline, and the

reversal of retinotopy seen as the electrode was moved into V2 (see Figure 2-1B & C). The latter typically occurred at azimuths of 0 to -5° (5° into the ipsilateral visual field), consistent with previous reports (Dräger, 1978). Rare deviations in the optic disk projection and retinotopic reversal were consistent and caused us to occasionally revise our estimate of the vertical meridian; in all cases, however, data sets include only those cells within 25° of either estimate, whichever most conservatively estimated the extent of the binocular zone.

Cells within the binocular zone were assigned ocular dominance scores according to the methods of Hubel and Weisel (1962). Optimal stimuli were presented to each eye alternately, and the relative strength of the response was determined. Cells were assigned an OD score of 1 if they responded only to stimuli presented to the contralateral eye, and 7 if they responded only to stimuli presented to the ipsilateral eye. Cells responding equally well to stimuli presented independently to either eye were assigned and OD score of 4. OD scores of 2 or 3 and 5 or 6 were assigned if the cell responded better or was dominated by response to stimuli presented to the contralateral and ipsilateral eye, respectively. The contralateral bias index (CBI) was calculated according to the formula:

$$CBI = [(n_1 - n_7) + (2/3)(n_2 - n_6) + (1/3)(n_3 - n_5) + N]/2N$$

where N = total number of cells and n_x = number of cells with OD scores equal to x.

A cell was evaluated for ocular dominance and other parameters if it could be sufficiently well-driven by a visual stimulus to map its receptive field. If multiple units were encountered at a single site, as was most often the case, the largest unit was studied using an amplitude discriminator. The electrode was advanced a minimum of 50 μm between recording locations. The electrode was moved after 6-7 cells were recorded from a given penetration, or when the electrode was advanced to a point where visually-evoked cell activity was no longer found.

Electrolytic lesions (4.5 mA for 4.5 sec) were placed in 1-3 penetrations per animal. Double lesions were also placed to estimated the degree of shrinkage caused by the histological procedures (see below).

Quantitative Response Evaluation

Custom software running on a 486 PC using a VSG2/2 video card (Cambridge Research Systems, UK) was used to present oriented light bars on a gray scale monitor (Apollo, Chelmstand, MA) placed 25 cm in front of the animal. The length, width and speed of the bars were varied to maximize the response of single units; however, the difficulty of recording from these units for extended periods of time and the need to record from a number of units

per animal precluded attempts to more rigorously quantify optimum stimulus parameters. Most units displayed some preference for a given stimulus orientation, but few were highly selective. Few if any demonstrated strong or reliable direction selectivity. Responses for 8 presentations of each orientation (4 presentations in each direction) were averaged and spontaneous activity subtracted. Peak response rates (firing rates evoked by stimuli of the preferred orientation), though larger than mean response rates (evoked firing rates averaged over all orientations), were modulated identically by the various deprivation patterns. Peak response rates were much more variable. The results from the analysis of mean response rates are therefore discussed. Sampling strategy was as described above, except only larger, more stable units were selected, as each had to remain discriminable for the 15 to 20 minutes required for the quantitative evaluation.

Histology and Laminar Analysis

At the end of each experiment the animal was given an overdose of Nembutal, and perfused transcardially with 0.5 M phosphate-buffered saline (PBS) followed by 10% Formalin in PBS. After postfixation, the brain was removed, cryoprotected in 30% sucrose - 10% Formalin and cut into 40mm sections. Sections were mounted on slides, defatted, and stained with cresylecht violet (Schmid). Cytoarchitectonic borders of Area 17 were determined as described in Caviness (1975). Cortical laminae were well-

defined in this material (see Figure 2-9) and were identified as in Caviness (1975) and Krieg (1946). Cameral lucida drawings of the lesion sites were used to reconstruct electrode penetrations and to determine the laminar location of the neurons recorded in that penetration. Cells within 25mm of a border between cortical layers were not included in the analysis.

RESULTS

Binocular responses in mouse visual cortex

In the mouse, only the frontal 60°-80° of the upper portion of the visual field is seen by the retinas of both eyes (Dräger, 1975, 1978; Wagor et al., 1980; see Figure 2-1A). Information about this portion of the visual world is carried by retinal ganglion cell axons to eye-specific regions of the LGN (Godement et al., 1984; Métin et al., 1983). Geniculocortical afferents project from these regions to the lateral one-third of the primary visual cortex (V1; Dräger, 1975, 1978; Wagor et al., 1980). Within this "binocular zone" individual cells usually respond to inputs from both eyes.

Microelectrode recordings were made from the binocular zone and surrounding monocular area of V1 in 5 C57Bl/6 mice. Each single unit was assigned an ocular dominance score according to the relative activity evoked by a stimulus presented to either eye independently (as in Hubel and Wiesel, 1962; see Methods). The distribution of ocular dominance scores of 27 neurons encountered in the binocular zone of one hemisphere of a normal animal is shown at the top of Figure 2-2. Note the strong bias towards the contralateral eye (that is, towards low ocular dominance scores) of the normal ocular dominance distribution. Even within the binocular zone of a normal animal, cells are more likely to be driven better by inputs from the contralateral eye than from the ipsilateral eye.

Ocular dominance distributions were characterized by the contralateral bias index (CBI), which indicates the degree to which the input from the contralateral eye dominates the activity of the cortical cells (see Methods). The CBI will be closer to 1 if the contralateral input predominates, and closer to 0 if ipsilateral input predominates. The CBI for each OD distribution in Figure 2-2 is shown at the top right of each histogram. The CBIs for 6 hemispheres from 5 normal mice are shown in Figure 2-4.

In order to better define the extent of the binocular zone, I examined the relationship between ocular dominance and receptive field (RF) location for 320 neurons recorded from V1 in the 6 C57Bl/6 and 3 additional hybrid mice. In Figure 2-3A, the OD score of each neuron is plotted against the azimuth of the center of its RF. Cells with RFs within the frontal 25° were very likely to be driven well by stimuli presented to the ipsilateral eye, as can be seen by the large percentage of neurons with OD scores of 2 or greater. In contrast, cells with RFs located between 25 and 40° from the midline were less likely to be driven well by the ipsilateral eye. These qualitative observations were confirmed by calculating the CBIs for OD distributions of cells grouped by RF center azimuth (Figure 2-3C). The CBIs of cells with RFs near 0, 10 and 20° of the vertical midline were quite similar, whereas the CBIs of cells with RFs located more peripherally were much higher, reflecting a greater degree of contralateral dominance.

Effects of brief monocular deprivation

Dräger (1978) has previously shown that long (6-week) periods of monocular deprivation starting at eye-opening (postnatal days 11-12, P11-P12) will result in significant shifts in cortical responsiveness towards the nondeprived eye. In order to determine whether shorter deprivations could induce similar shifts, I deprived several animals for varying lengths of time starting at various ages from P12 to P40. At the end of the deprivation period, extracellular recordings were made from single neurons in the binocular zone. OD scores were assigned as described above.

At the bottom of Figure 2-2 are shown histograms of ocular dominance score distributions from each hemisphere of an animal in which the right eye was sutured shut for 4 days starting at P28. Even after such a brief period of MD, cortical ocular dominance changed dramatically. In the hemisphere ipsilateral to the deprived eye, the contralateral dominance increased markedly; few cells responded to stimuli presented to the ipsilateral eye, and the majority of those that did respond did so poorly. In the hemisphere contralateral to the deprived eye, the open ipsilateral eye dominated responses, and cells driven exclusively by the ipsilateral eye were found. The degree of the shift was quantified by comparing the contralateral bias index in the nondeprived and deprived hemispheres. The CBIs of distributions recorded from ipsilaterally deprived hemispheres were considerably higher,

and contralaterally deprived hemispheres considerably lower, than those from normal animals.

I also examined the effect of receptive field location on plasticity subsequent to monocular deprivation. Recordings were made from the binocular zone contralateral to the deprived eye in 10 mice deprived for four days beginning between P23 and P28. Figure 2-3B shows the OD score for each cell plotted against its receptive field center azimuth. Small differences in the OD distributions for cells of different eccentricities are evident. CBIs were calculated for OD distributions of cells grouped by receptive field center azimuth. A plot of CBI vs. azimuth reveals that cells with receptive fields closest to the vertical midline shifted most (Figure 2-3C).

Critical Period for Monocular Deprivation in the Mouse

Having observed potent effects of a brief, 4 day period of monocular deprivation, I investigated whether ocular dominance plasticity is confined to a critical period in early life, as it is in other species. To delimit the critical period, I sutured shut one eye in each of five mice of the following ages: P19, 23, 28, 32 and 36 (these and all subsequent ages given are ± 1 day). The animals were returned to their cages and microelectrode recordings were performed 4 days later. In this and all subsequent MD experiments, I consider only recordings made from the hemisphere contralateral to the deprived eye, for two reasons. First, an artifactual result of apparent shift towards the

contralateral eye could be obtained if one accidentally records outside the binocular zone. Second, shifts toward the ipsilateral eye reveal cells in ocular dominance classes 6 and 7, which are rarely if ever seen in nondeprived animals. Considering only the contralaterally deprived hemispheres, a decrease in the CBI represents a shift towards the ipsilateral, open eye.

The results shown in Figure 2-4 reveal a well-defined critical period. Average CBIs for the binocular zone contralateral to the closed eye in the deprived animals are plotted together with the CBIs from 6 nondeprived hemispheres in 5 animals. Maximal effects of MD were achieved for deprivations spanning from P28 to P31. Smaller shifts toward the open ipsilateral eye were evident even at the youngest age tested, deprivations extending from P19-P23. The magnitude and reliability of shifts induced by 4-day MD dropped off rapidly after the peak of the period of susceptibility. Although several animals deprived after P32 showed CBIs below the normal range, there were no significant differences between animals deprived at either of the two later time points and nondeprived controls.

4-Day Deprivations are Maximally Effective

One characteristic of the critical period in other species is the surprising finding that brief deprivations have such a strong effect. To determine whether this was true for OD plasticity in mice, I measured the length of time necessary to achieve a maximal shift towards the nondeprived eye at the peak

of the critical period. I therefore deprived a number of animals for varying lengths of time, all approximately centered around P28. Figure 2-5 shows the mean CBIs for deprivations of increasing lengths. Two day deprivations had little effect, while 4 day deprivations were as effective as longer deprivations at inducing a shift in cortical responsiveness towards the open eye.

Effects of Binocular Deprivation

MD produced a dramatic shift in the efficacy of inputs from the two eyes in driving cortical cells. I wished to determine whether this effect was due to simple disuse, a hypothetical degradation in the deprived eye's visual pathway upon lid suture, or whether, as in other species, competition between inputs from the two eyes was required is required. I tested this by suturing shut both eyes (binocular deprivation, BD) of several mice at the peak of the critical period. Short-term BD in animals such as the cat (Wiesel and Hubel, 1965a, Freeman et al., 1981) has been shown to cause only small changes in visual responsivity.

I binocularly deprived 4 mice for 4 days starting at P28, a period identical to that which produced maximal effects in MD animals. I found cortical responses to be remarkably resilient to this manipulation. The data presented in Figure 2-6 demonstrate that all measured aspects of cortical cell responses were normal or nearly so in the BD animals. The distribution of receptive field (RF) sizes in BD animals consistently overlapped those of

normals, although the mean RF size for all BD animals was slightly smaller that that of controls ($p = 0.01$, t-test) (Figure 2-6A). Retinotopy, however, was perfectly preserved in the BD animals. In Figure 2-6B, the receptive field azimuths of cells encountered in successive evenly spaced electrode penetrations are plotted against the lateromedial position of the electrode. I found a consistent a linear relationship between receptive field location and cortical location within the frontal 40° of the visual field, in BD as well as normal animals. The precision of this retinotopic mapping can be estimated by the correlation coefficient of the linear regression, that in normal animals is quite high (0.92 ± 0.6) . There was no significant difference between the mean coefficients of two such sweeps each in BD and normal animals (Figure 2-6B, inset).

The ocular dominance distribution of 131 cells recorded in the binocular zone of the BD animals is shown in Figure 2-6C. The distribution is quite similar to that seen in normal animals (see Figure 2-2 top, Figure 2-8A), although there seems to be a slight reduction in the proportions of cells driven by both eyes in the BD animals. The OD distributions of cells from the BD animals and 5 normals are significantly different by chi-squared analysis ($p < 0.05$). In contrast, there was no shift in OD towards either eye; the CBIs of the 4 BD hemispheres from which sufficient data were obtained were 0.76, 0.67, 0.70, and 0.70, respectively, all well within the normal range. There was no significant difference between the means of these CBIs and those of

nondeprived animals (mean ± S.D. = 0.71 ± 0.04 and 0.71 ± 0.03 for BD and nondeprived, respectively, $p > 0.7$, t-test).

To determine quantitatively the effects of binocular deprivation and to compare them to those of monocular deprivation, I measured firing rates evoked by computer-generated stimuli in 2 normal, 2 BD and 2 MD animals. These measurements are shown in Figure 2-6D. The mean response rates of cells encountered in the binocular zone of nondeprived animals were on average greater for stimuli presented to the contralateral eye than for stimuli presented to the ipsilateral eye, verifying the contralateral dominance seen in the OD histograms. BD resulted in only a small and non-significant decrease in the responsiveness of binocular zone neurons. In contrast, MD resulted in a much greater decrease in the responsiveness of neurons to the deprived eye. Responses of neurons contralateral to the deprived eye were reduced significantly relative to those in nondeprived animals (t-test, $p < 0.05$). Responses of neurons ipsilateral to the deprived eye were nearly eliminated, and were significantly smaller than those in both nondeprived and BD animals (t-test, $p < 0.05$ and 0.03, respectively). In BD animals, as in normals, cells were found which displayed excellent specificity for stimulus orientation, and those which exhibited only a slight preference for a particular orientation specificity. Examples of these cells from two different BD animals are shown in Figure 2-7.

Effects of Alternating Monocular Deprivation

Experiments designed to decorrelate input between the two eyes have implicated a correlation-based mechanism in OD plasticity by causing an increase in the proportion of monocular cells (Hubel and Wiesel, 1965; Blakemore et al., 1975; Yinon, 1975; Blakemore, 1976; Blasdel and Pettigrew, 1979; Crawford and von Noorden, 1979, 1980). I initially attempted to obtain similar evidence for the murine model by inducing exo- or esotropic strabismus, but found it difficult to produce large, maintained deviations in eye position through resection of medial or lateral rectus muscles. I therefore studied the consequences of daily alternating monocular lid suture instead. I first tried alternating MD (AltMD) for approximately 4 to 6 days around the peak of the critical period, that resulted in a shift towards the contralateral eye rather than an increase in monocularity (data not shown). I hypothesized that this contralateral shift might still be consistent with a correlation-based plasticity mechanism, given that cells in the binocular zone of the mouse cortex were quite dominated by the contralateral eye at the time the deprivation began: when put in equal competition, the ipsilateral eye might never have achieved sufficient synaptic efficacy in any cell to come to dominate it completely, and the contralateral inputs would eventually win out in every cell. I reasoned that if I could first balance the efficacy of the two eyes by an initial period of MD before beginning daily AltMD, the strengthened, initially open eye might obtain a sufficient foothold in the

ipsilateral cortex to allow for it to win out in equal competition for some cortical cells.

I monocularly deprived 5 animals for an initial period of 6 or 7 days (P23 to P29, 3 animals, and P22 to P29, 2 animals), immediately followed by a 7 to 12 day period of AltMD. At the end of the AltMD period, I recorded from cortical cells in the binocular zone of V1 ipsilateral to the initially open eye. The resulting ocular dominance distribution of all 257 cells recorded from the 5 cortical hemispheres is shown in Figure 2-8B. Note the J-shaped distribution, with the great majority of cells being monocular, and a considerable number being driven only by the ipsilateral eye. Four of the 5 animals had distributions similar to the total; in one animal, however, only one ipsilateral monocular cell was found, and its OD distribution looked more like an ipsilaterally deprived cortex (the CBI of this animal was 0.90). Note that the overall distribution (CBI = 0.74) and each of the other 4 animals (CBIs = 0.67, 0.70, 0.72 & 0.67) showed no evidence of a net shift towards either eye. Rather the effect of AltMD seemed simply to be a shift towards monocular domination by one eye or the other.

The overall OD histograms for all nondeprived, P28 contra-MD, and all ipsi-MD animals are shown for comparison in Figure 2-8A, C & D. Note once again the effect of AltMD is unlike the effects of contra- or ipsi-deprivation. Pairwise chi-squared analysis demonstrates each of the 4 OD distributions is significantly different from any other ($p < 0.0005$ in all cases). Furthermore, AltMD is unlike a simple linear combination of ipsi- and contra-MD; neither

monocular manipulation results in more cells of OD class 7 than cells of class 4, as occurs after AltMD.

Laminar Analysis

In order to examine more closely the locus of the plasticity induced by monocular lid suture, I analyzed the laminar position of neurons recorded from the binocular zone in a subset of monocularly deprived and control mice. Following single-unit recordings, electrolytic lesions were made in 24 penetrations from 14 monocularly deprived animals and in 14 penetrations from 11 nondeprived controls. The MD animals were all deprived for 4 days beginning between P23 and P28. Cells were assigned to cortical layers based on reconstructions of the electrode paths in Nissl-stained sections. One such reconstruction is illustrated in Figure 2-9. Figure 2-10 shows the ocular dominance distributions for neurons from these penetrations separated according to the cortical layer in which the cells were found. The OD distribution of cells in layers II/III, IV and V/VI from nondeprived animals were nearly identical, each reflecting the normal bias towards the contralateral eye. In contrast, laminar analysis of OD in the MD mice suggested differences in the degree of shift present in each of the cortical layers. The OD distributions of cells in all three groups shifted in favor of the nondeprived eye ($p < 0.005$ for each comparison, chi squared test).

Furthermore, cells in layers V/VI shifted significantly less than those in layer IV, and the cells in layer II/III shifted to an intermediate degree.

Reliability of Contralateral Bias Index

The CBIs varied even between animals subjected to identical periods of monocular deprivation (see Figure 2-4). This variability could arise from genuine variability of the effect of the deprivation in any given mouse, or from variability in our estimate of the true CBI from the sample population of neurons recorded in any given experiment. To discern between these two possibilites, and determine the accuracy of the CBIs obtained given the number of sampled neurons, I calculated CBIs separately for alternate neurons from each of 36 hemispheres from 33 mice. The CBIs from the odd-sampled neurons are plotted against the even-sampled neurons in Figure 2-10. Data include both deprived and nondeprived animals. The correlation coefficient of the linear regression is 0.75, and the slope is significantly different from zero ($p < 0.001$) but not significantly different from 1 ($p > 0.1$). 86% of the residuals were below 0.1. Considering that these CBIs were calculated from half the number of neurons, they should be less accurate than the CBIs calculated from the entire sample in each animal by a factor of the square root of 2. The CBIs reported here for experiments in which 20-30 neurons were sampled should therefore be accurate to approximately within

± 0.05. These findings suggest that only some, but not all, of the variability seen in Figure 2-4 can be accounted for by sampling variability.

DISCUSSION

Although effects of long-term monocular deprivation on ocular dominance in mouse V1 were initially reported by Dräger (1978), this report is the first full characterization of ocular dominance plasticity in this species. The experiments described provide evidence that ocular dominance plasticity in the mouse follows similar rules as in higher mammals in which it has been more completely studied. I found that periods of monocular lid suture as short as four days produced a maximally effective shift in the responsiveness of visual cortical neurons towards the nondeprived eye. These effects were delimited to a critical period early in the development of the animal. Brief periods of binocular deprivation had little or no effect on visual cortical responses, demonstrating the dependence of the plasticity on the competition between the two eyes. Alternating monocular deprivation, by decorrelating the input from the two eyes, decreased the degree of binocularity in the cortex. Finally, a laminar analysis suggested that the plasticity occurs both subcortically and intracortically, as the ocular dominance distribution of cells in layer IV were less shifted that the distribution in infragranular layers; the shift evident in supragranular layers was intermediate between the two. The extent to which these findings support the conclusion that ocular dominance plasticity in the mouse is a valid model for that in other mammals is discussed below.

Effects of brief monocular deprivation

Four days of monocular deprivation at the height of the critical period are necessary and sufficient to maximally shift the responsiveness of mouse V1 neurons maximally toward the nondeprived eye. Two days of monocular lid suture produces an incomplete shift in the hemisphere contralateral to the deprived eye (I have not examined the effects of brief MD on responses in the ipsilateral hemisphere). Studies of brief MD in kittens suggest a similar duration requirement. Although the effects of very brief deprivations (1-2 days) are much more pronounced in the kittens, four day deprivations are still more effective, and 8-10 day deprivations shift cortical responses to the same degree as much longer deprivations (Olson and Freeman, 1975; Movshon and Dürsteler, 1977; Hensch et al. 1995). Very brief MD has not been examined in monkeys or rats.

Time course of the critical period

The critical period for the effects of 4 days of MD in mouse V1 peaks at or near P28, and ends rather abruptly around P32. These findings are in rough agreement with the critical period described by Fagiolini et al. (1994) for the effects of 10 days of MD in the rat. The critical period in rodents appears to be shorter than that of other mammals. Ten days of MD is able to induce a substantial shift in cortical responses from the 2nd to the 11th week

postnatally in kittens (Olson and Freeman, 1980), and longer periods of deprivation can have significant effects throughout the first year of life (Cynader et al. 1980; Daw et al. 1992). MD in the monkey has been shown to be quite effective up until at least 6 weeks of age (Blakemore et al. 1978; LeVay et al. 1980). While longer deprivations might reveal residual plasticity in older mice, it should be noted that rodents mature quite rapidly after birth. A 4-week old mouse is reproductively capable and nearly full-grown. Thus it is not surprising that the critical period should be shorter. Rather it is surprising that it occurs so late and lasts so long.

The dissociation between the time course of visual development and other more general developmental processes has been noted previously for anatomical events. Robinson and Dreher (1990) found that the timing of the establishment of various visual system cell types and pathways in a wide variety different mammals correlates better with cloacal period (the time from conception to eye-opening) than with gestational period. An examination of data from the present study and those of Fagiolini et al. (1994) in the rat and Olson and Freeman (1980) in the cat suggests this is true for activity-dependent physiological processes as well: the time from conception to the peak of the critical period in these animals ranges from 160 to 250% of the gestation period but only 140 to 150% of the cloacal period. Activity-dependent processes appear to be regulated by the same visual system-specific developmental clock as earlier, presumably activity-independent ones.

I do not yet know what aspects of the development of the visual pathways account for the peak of sensitivity to the effects of visual deprivation. One attractive possibility is that the cellular mechanisms responsible for OD plasticity may only be fully functional in the visual cortex near this peak. Another possibility is that plasticity mechanisms that are in place prior to the peak of the critical period may be insufficiently engaged by the differences in activity created by lid suture. This alternative explanation is consistent with our qualitative impression that visual responses were less vigorous in the younger animals. The rapid decrease in susceptibility seen after the peak would then require a different mechanism, such as a decline in the function of some hypothetical cellular mechanism of plasticity. Reductions in NMDA receptor function and loss of susceptibility to long-term potentiation *in vitro* suggest that NMDA-mediated plasticity may be involved in the critical period (Fox et al., 1991, 1992; Carmignoto and Vicini, 1992; Kirkwood et al., 1995; Fox, 1995).

Effects of binocular deprivation

The role of competition in the effects of visual deprivation was established by Weisel and Hubel (1965), who first demonstrated that periods of binocular lid suture do not result in wholesale loss of visual responsivity, in marked contrast to the devastating effects of monocular deprivation. Even more striking is the difference between cats deprived monocularly and

binocularly for brief periods. Dark rearing for 3-6 days has small effects on cortical cell responses in critical-period aged cats (Freeman et al., 1981), whereas similar length monocular lid suture, arguably a more subtle manipulation, completely eliminates responses of most cells to the deprived eye (Olson and Freeman, 1975; Movshon and Dürsteler, 1977). Similar differences between MD and BD effects are reported here for the mouse. In mice binocularly deprived for 4 days at the peak of the critical period for monocular deprivation, visual responses, retinotopy, ocular dominance and receptive field size were very similar to those in nondeprived animals. In MD mice, by contrast, responses to the deprived ipsilateral eye nearly disappear, and responses to the deprived contralateral eye are greatly reduced. These findings argue strongly that in the mouse, just as in the cat, the effects of visual deprivation are largely due to the competition between inputs from the two eyes.

Effects of alternating monocular deprivation

Numerous experiments designed to decorrelate the inputs from the two eyes have demonstrated a correlation-based plasticity operates in the visual cortex of cats and monkeys. Thus, divergent strabismus, alternating monocular occlusion, and rotation of one eye have all been shown to reduce dramatically the percentage of binocular cells in cat visual cortex (Hubel and Wiesel, 1965; Blakemore et al., 1975; Yinon, 1975; Blakemore, 1976; Blasdel

and Pettigrew, 1979). Likewise, surgically or optically induced strabismus in the monkey has similar effects (Crawford and von Noorden, 1979, 1980). After I attempted to compensate to some extent for the initial contralateral bias, daily alternating monocular deprivation for 7-12 days dramatically decreased the number of binocular cells in mouse V1 as well. The ocular dominance distributions for mice subjected to brief periods of AltMD reported here are similar to those reported recently for rats subjected to longer periods of surgically-induced strabismus (Domenici et al., 1992).

Despite initially depriving the animals and recording from the cortex contralateral to the initially deprived eye, I still found a marked contralateral dominance after the period of AltMD. Deprivations similar to those used prior to the AltMD are sufficient to nearly equalize the efficacy of inputs from the two eyes in the contralateral hemisphere (c.f. the effects of deprivation from P23 to P27, Figure 2-4; additional data not shown). The contralateral, deprived afferents must therefore retain some additional advantage at the onset of the period of AltMD, beyond that demonstrated by their physiological efficacy, in order to come to dominate the majority of cells after AltMD. Nonetheless, many group 7 ipsilaterally driven monocular cells were found. These findings demonstrate that the effects of AltMD are altogether different from those of simple monocular deprivation. They support the hypothesis that a correlation-based mechanism underlies plasticity in mouse V1.

Asymmetry of binocular responses

The contralateral inputs' apparent competitive advantage in mouse visual cortex, revealed by alternating monocular deprivation, is confirmed by a comparison of the effects of monocular deprivation in mice to those in other animals. The extent of the shift in cortical responses induced by closing the contralateral eye was considerably less than that seen in comparably deprived cats and monkeys, in which 80-100% of cells fail to respond the deprived eye (Wiesel and Hubel, 1963, 1970; Olson and Freeman, 1975, 1980; Movshon and Dürsteler, 1977; Blakemore et al., 1978; Hubel et al. 1977, LeVay et al. 1980). In the rat, approximately 50% of cells in the binocular zone fail to respond to the contralateral, deprived eye (Maffei et al. 1992; Fagiolini et al. 1994). The less dramatic shift seen in mice is not simply because the deprivations were not long enough. As described here and in Dräger (1978), even in mice deprived for longer periods of time (up to 1 year in the earlier study) only 17-20% of cortical cells fail to respond to the contralateral deprived eye. Cells in the binocular zone ipsilateral to the deprived eye, however, become nearly completely dominated by the contralateral eye, such that the ocular dominance distributions of these cells look quite similar to deprived cats. The significant contralateral bias present in the binocular zone of nondeprived mice appears to prevent the complete domination of the cortex by the ipsilateral eye.

The reason why the initial contralateral bias blocks further domination by the ipsilateral eye is not immediately evident. A review of the effects of monocular deprivation in various species reveals no consistent relationship between the degree of initial contralateral bias in nondeprived animals and the amount of shift after monocular deprivation (Figure 2-12). Note especially the variable degree of shift in rats, rabbits, mice and sheep, all of which start with the same initial contralateral bias. Furthermore, even fully deprived kittens, in which nearly all cells are responsive only to one eye, can be shifted fully back to the other eye if the suture is reversed early enough in the critical period (Blakemore and Van Sluyters, 1974). These data suggest that there is something special about the initial position of the contralateral eye geniculocortical afferents in the mouse binocular zone, besides their initial physiological dominance, that makes them partially immune to the effects of competition. The finding that cells with receptive fields closest to the vertical midline shift greater than those with more peripheral receptive fields raises the intriguing possibility that the monocular area exerts a stabilizing influence on nearby contralateral inputs through horizontal intracortical connections. This influence would be weakest at points furthest from the monocular area, i.e. at the representation of the vertical meridian, precisely where the shift towards the ipsilateral eye is greatest.

Locus of the effects of monocular deprivation

In both cats and monkeys, cortical cells in the geniculocortical input layers tend to be more monocular than in other layers, reflecting the anatomical segregation of inputs from the two eyes (Hubel et al., 1977; Shatz and Stryker, 1978). After a period of monocular deprivation, the dominance of the nondeprived eye is much greater in extragranular layers than in layer IV, even after year-long MD (Shatz and Stryker, 1978). Furthermore, cells in layer V are more shifted than supragranular layers. These findings suggest that intracortical connections are also plastic, in addition to plasticity in the geniculocortical afferents. In the mouse, where geniculocortical afferents appear not to segregate into separate domains within layer IV (Dräger, 1978), I found that layer IV cells had ocular dominance distributions identical to those in extragranular layers. After MD, a shift towards the open eye was found in all layers, but the shift was more pronounced in extragranular layers than in layer IV, with the greatest shift in infragranular cells. This finding suggests that in the mouse, as in other species, intracortical as well as geniculocortical synapses undergo plasticity with monocular deprivation. They also demonstrate the effects of plasticity with increasing layers of intracortical processing, causing the infragranular layers to be most dramatically affected.

Possible anatomical bases of plasticity

The findings of the present study are entirely physiological, but in other species the effects of MD have been shown to be accompanied by major anatomical reorganization of the geniculocortical afferent pathways serving the two eyes. After long periods of monocular deprivation in the cat and monkey, labeling of OD columns by transneuronal transport of amino acids, sugars or lectins into one eye reveal that OD columns belonging to the deprived eye shrink with corresponding increases in the extent of those serving the nondeprived eye (Shatz and Stryker, 1978; Hubel et al., 1977; LeVay et al., 1980). In the initial study of monocular deprivation in the mouse, Dräger (1978) found no change in the extent or intensity of afferent innervation using transneuronal labeling with tritiated proline, the same method as used in the cat and monkey studies. Consideration of the extent to which such label may spill over in the LGN to label afferents serving the other eye makes this finding less than definitive. Alternatively, changes in geniculate cell size have also been used to monitor competitive interactions between the visual pathways of the two eyes (Guillery, 1972). More recently, reconstructions of single geniculocortical afferents have revealed a rapid reorganization of arborization subsequent to brief periods of MD (Antonini and Stryker, 1993b). Similar techniques may reveal whether geniculocortical afferents arbors undergo a reorganization in mouse V1 as well. They might

also reveal the basis of the differential susceptibility of the contralateral eye to the effects of MD.

Conclusion

The characterization of physiological responses in the primary visual cortex of the mouse subsequent to manipulation of visual experience reveals a plasticity remarkably similar to that of other mammals. The findings presented here demonstrate the operation of a competitive, correlation-based plasticity operating during a critical period in the development of the binocular zone of mouse V1. The advantages of a murine model for ocular dominance plasticity are many. OD plasticity is the best-studied form of central nervous system plasticity and serves as a model for the activity-dependent development and reorganization of connectivity throughout the neocortex. Yet I know comparatively little about the cellular mechanisms that underlie this important phenomenon. The mouse is an ideal organism for answering questions regarding molecular and cellular mechanisms of complex biological processes. Targeted disruption of specific genes allows study of the involvement of particular molecules in the development and function of whole systems. Until now, the only form of plasticity amenable to study in rodents has been that described in the cortical barrel fields, that has some similarities to, but many differences from, that in the visual cortex (Fox, 1992, 1994; Shlagger et al., 1993). The development of a mouse model of

ocular dominance plasticity has allowed us to begin a dissection of the cellular mechanisms underlying this better understood form of plasticity (Gordon et al., 1994, 1995; Hensch, 1995; J.A. Gordon et al., unpublished observations). As knowledge of the genome of this species becomes more complete and techniques for manipulating it become more sophisticated, the power of the mouse to answer questions of mechanism will increase. The findings described here demonstrate that ocular dominance plasticity in the mouse is an excellent model for the phenomenon as described in other species, and suggest that explorations of the mechanisms underlying mouse ocular dominance plasticity should help us understand cortical plasticity in mammals generally.

Figure 2-1. The primary visual cortex of the mouse. *A*, Schematic diagram of the central visual pathway in the mouse. The frontal 30°-40° of the upper portion of each visual hemifield is seen by the retinas of both eyes. Retinal ganglion cell axons project to eye-specific regions of the lateral geniculate nucleus (*LGN*). Geniculocortical projections carrying information from the two eyes converge in the lateral one-third of the primary visual cortex (*V1*), in the *binocular zone*. It is within the binocular zone that binocularly responsive neurons are first found. *Arrow*, vertical meridian of the visual field. *B*, Diagram showing the locations of a series of penetrations made into the visual cortex of a normal mouse. *Thin circles*, penetrations in the monocular portion of V1. *Thick circles*, penetrations in the binocular zone of V1. *Dashed circle*, a penetration made into V2. *C*, Retinotopy in the visual cortex of a normal mouse. Representative receptive fields encountered in the penetrations shown in B are plotted here. The numbers correspond to the penetrations in which the receptive fields were found. Within V1, moving the electrode laterally (towards the *right* in B) resulted in finding more centrally located receptive fields (towards the *right* in C). Once the electrode was moved into V2, the retinotopic progression reversed itself, as lateral displacement of the electrode resulted in a peripheral shift in receptive field position. This reversal of retinotopy was used during recording to determine the location of the V1/V2 boundary. The dashed line is the vertical meridian.

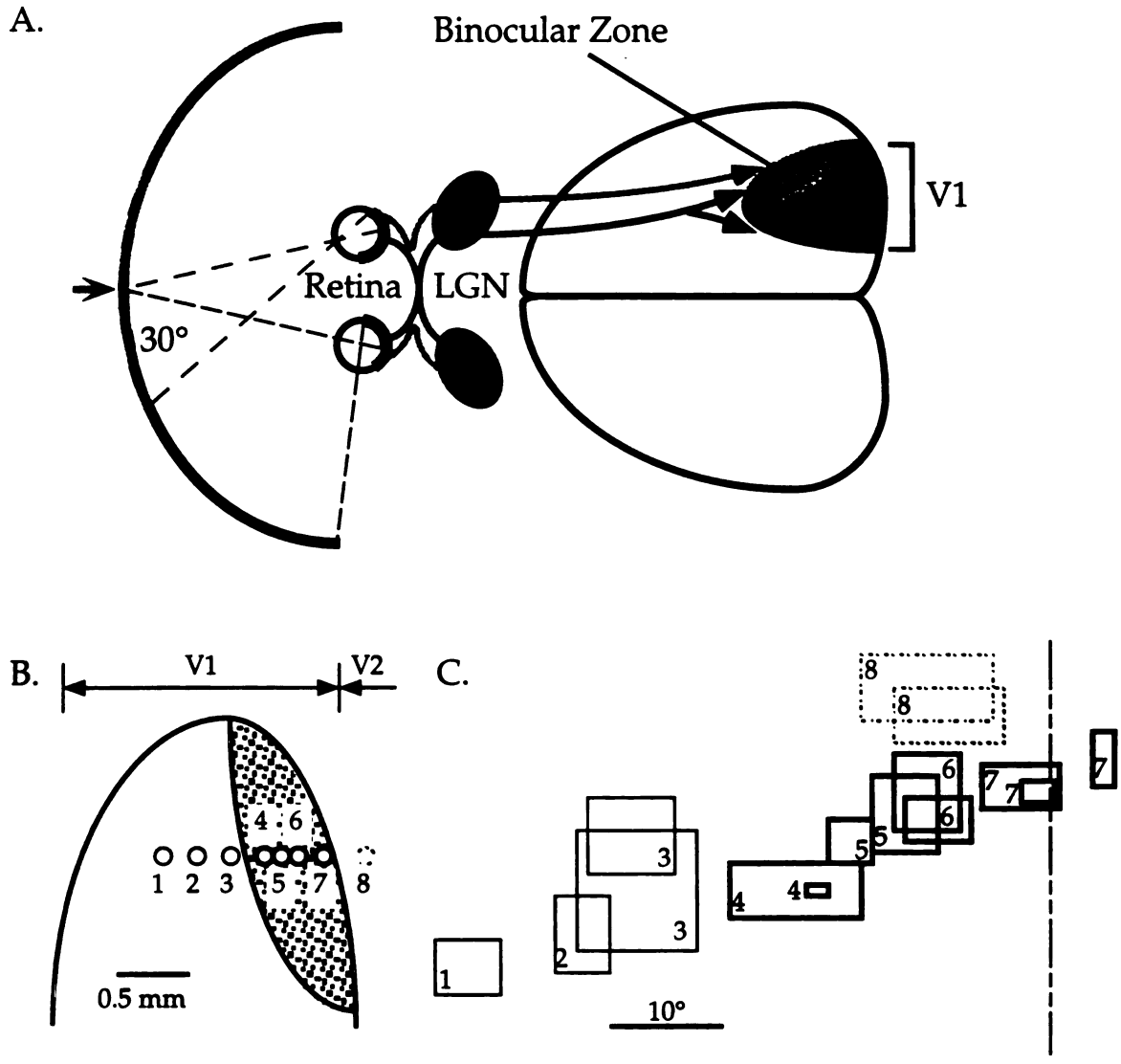


Figure 2-1: The primary visual cortex of the mouse.

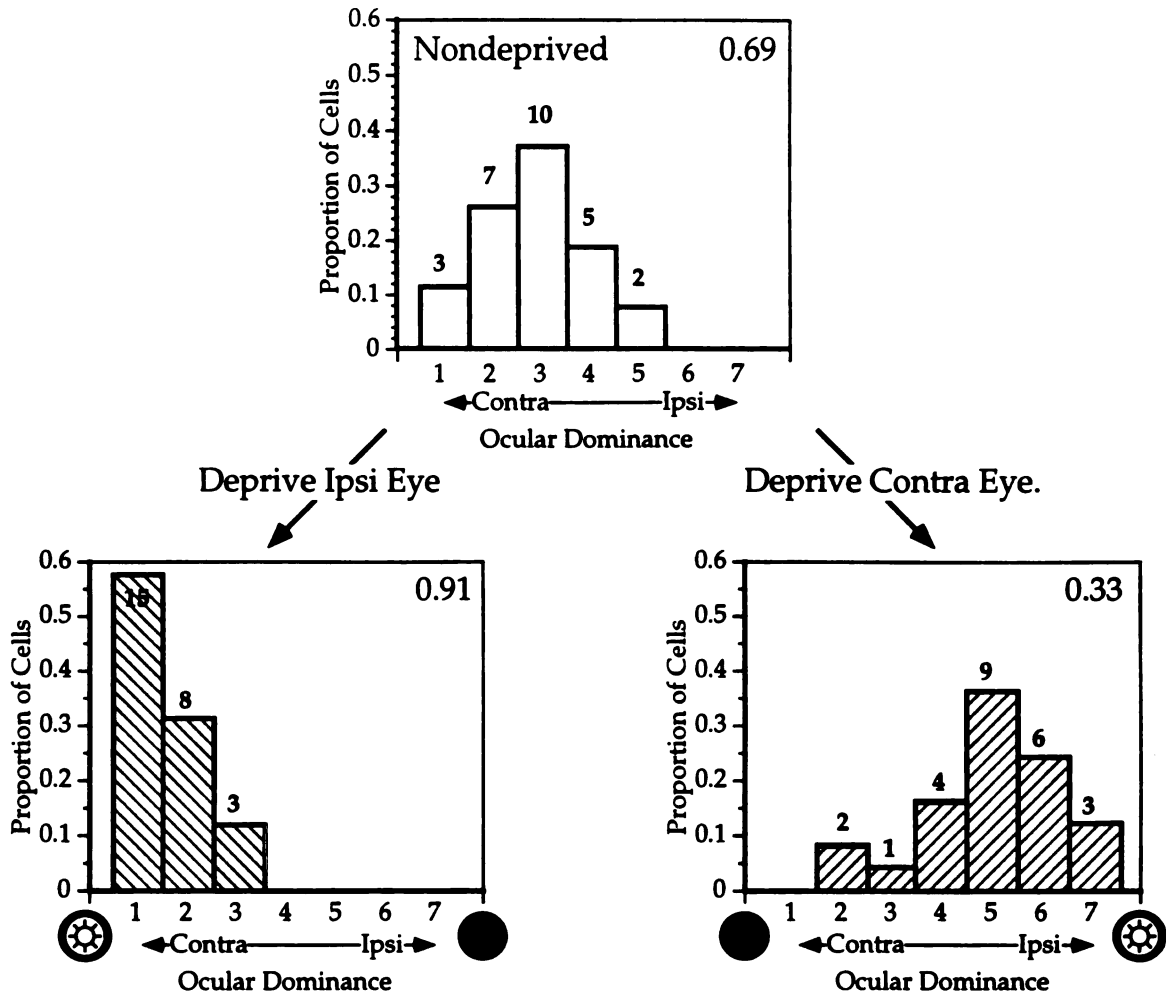


Figure 2-2. Effects of monocular deprivation on ocular dominance in mouse primary visual cortex. *Top,* Distribution of ocular dominance scores (see Results) for 27 neurons recorded from the binocular zone of a normal P24 mouse. *Lower left,* Ocular dominance distribution for 26 neurons recorded in the binocular zone ipsilateral to the deprived eye in a mouse which underwent MD from P28 to P32. *Lower right,* Ocular dominance distribution for 25 neurons from the binocular zone contralateral to the deprived eye; same mouse as lower left. The Contralateral Bias Index (see Methods) is indicated upper right corner of each histogram. The number of neurons of each ocular dominance class is indicated at the top of each bar.

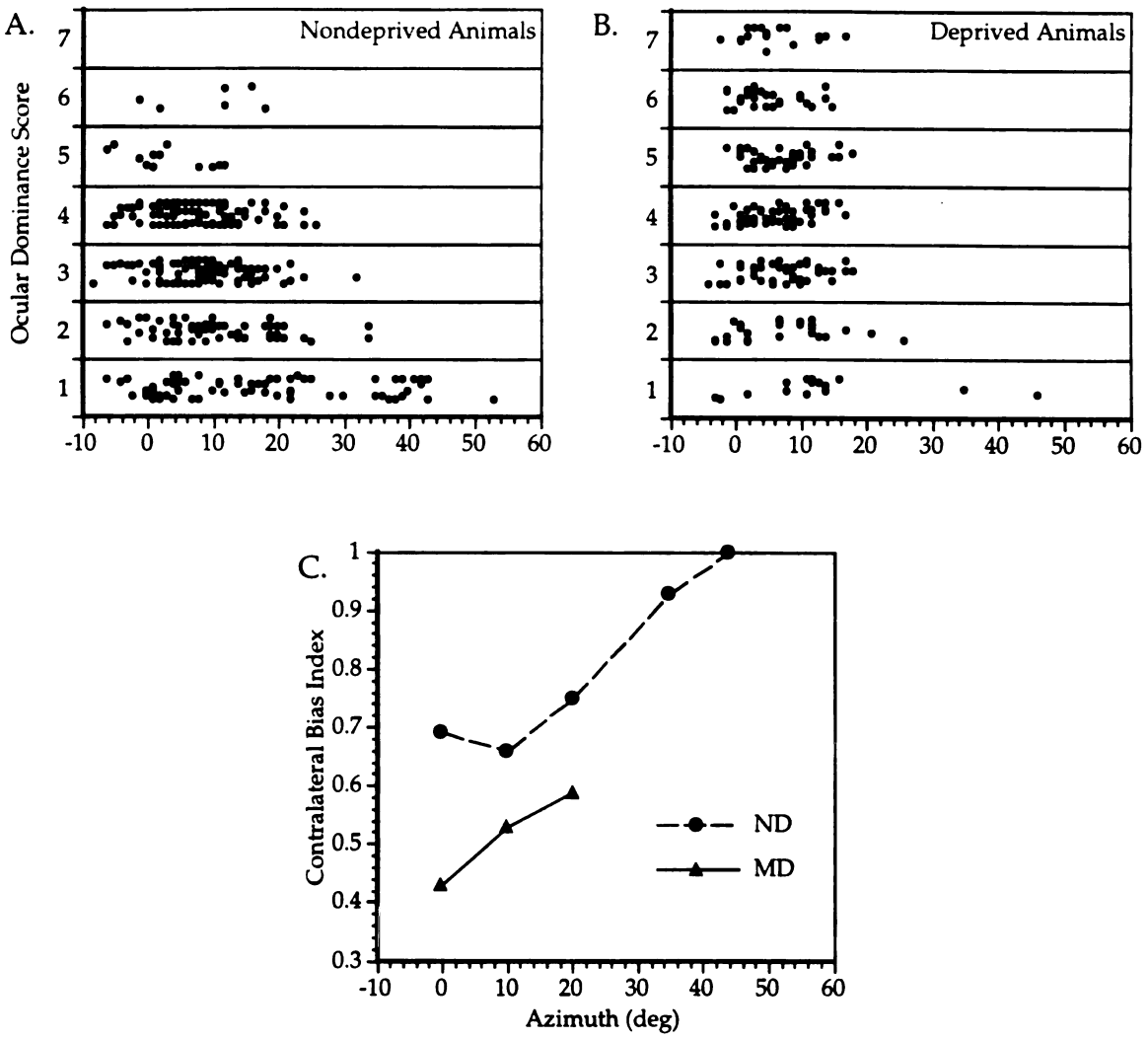


Figure 2-3. Ocular dominance of cells within the binocular zone. *A -C,* Relationship between ocular dominance and receptive field location in nondeprived (A & C) and monocularly deprived (B & C) mice. *A,B,* Ocular dominance scores of 320 cells from 9 ND mice (A) and 234 cells from 10 MD mice (B) are plotted versus the receptive field center azimuth of each cell. Cells from the MD animals were recorded in the cortex contralateral to the deprived eye. *C,* The contralateral bias index was calculated separately for cells with receptive field center azimuths below 0, 0 - 5, 6-10, 11-15, 16-20, 21-30 and 31-45 from ND mice (*circles*) and -5 to 5, 6 to 15, and 16 to 25 for MD mice (*triangles*). MD mice were deprived for 4 days beginning at either P23 or P27.

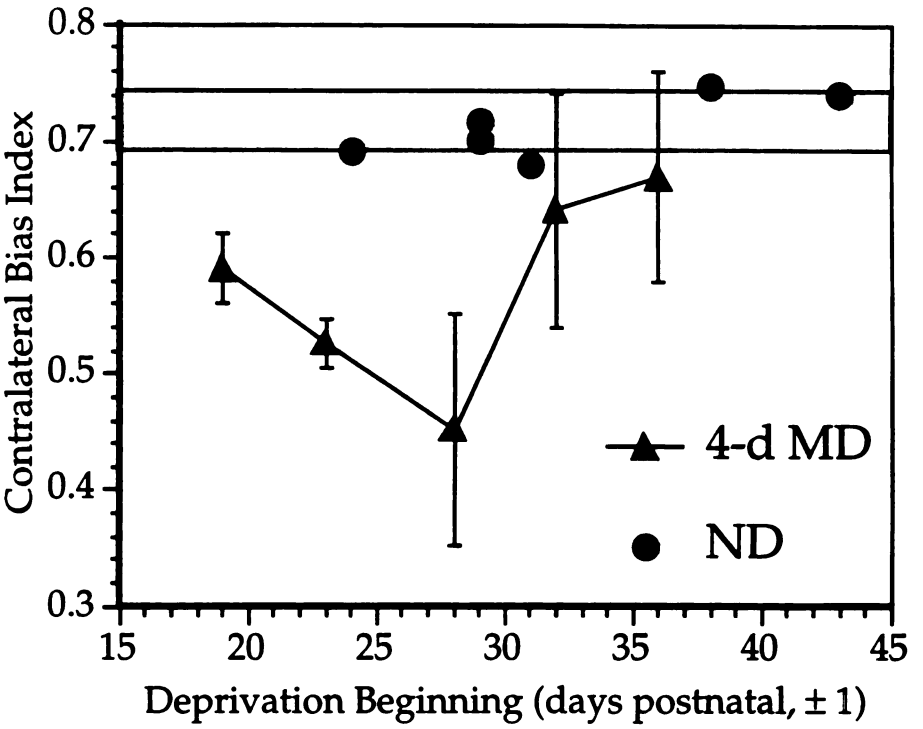


Figure 2-4. Critical period for the effects of monocular deprivation in the mouse. Mean ± s.d. CBIs for ND animals (*dotted bar*) and animals monocularly deprived for four days beginning at various ages (*filled triangles*). *Filled circles,* individual CBIs for OD distributions obtained nondeprived animals. n = 23 to 30 cells per hemisphere, N = 5 hemispheres from 5 animals per time point (MD) and 6 hemispheres from 6 animals (ND).

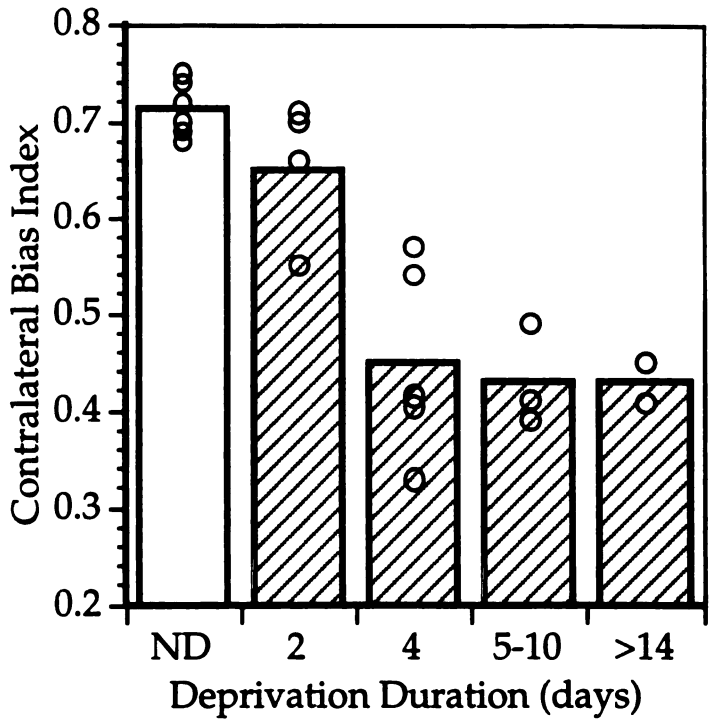


Figure 2-5. Duration of monocular deprivation required for maximal shift. Contralateral bias indices were calculated individually from ocular dominance distributions obtained from nondeprived (*open bar*) mice and mice deprived for increasing lengths of time (hatched bars), centered approximately at P28. Mean (*bars*) and individual CBIs (*open circles*) are shown.

Figure 2-6. Effects of binocular deprivation on visual cortical responses in the mouse. *A*, Receptive field sizes in BD and ND mice. The mean ± s.d. is shown for receptive fields of neurons recorded in each animal (*mo105 - mo326*). The overall means ± s.e.m. are shown for all 4 BD animals (*All BD*) and all 5 ND animals (*All ND*). n = 15-54 cells/animal, n = 137 and 124 cells for All BD and All ND, respectively. *B*, OD distribution of 132 neurons recorded from the binocular zone in the BD mice. Conventions as in Figure 2.C, Retinotopy in BD and normal animals. Series of 3-5 evenly spaced penetrations were made across a portion of the lateromedial extent of V1 in BD and ND animals. The receptive field centeres of 3-5 neurons encounted at each location are plotted here for one representative series of penetrations from a BD (*circles*) and a ND animal (*triangles*). *Solid* and *dotted lines*, linear regressions of receptive field center azimuth on electrode position in the BD and ND animal, respectively. *Inset*, Mean ± s.d. correlation coefficients of 2 and 4 regressions each from BD and ND animals, respectively. *D*, Visual evoked responses in single neurons recorded from the binocular zone in normal (*ND*), *BD* , and *MD* mice. Mean ± s.e.m of responses of neurons to computer-generated stimuli presented to both eyes (*grey bars*), the ipsilateral eye (*white bars*), or the contralateral eye (*hatched bars*) are shown. Responses are calculated seperately for neurons recorded contralateral (*contra MD*) and ipsilateral (*ipsi MD*) to the deprived eye. For each neuron, the response to 8 presentations (4 in each direction) of a bar of the cell's preferred orientation were averaged. n = 7 to 24 cells/category. Both MD and BD mice were deprived from P28 to P32.

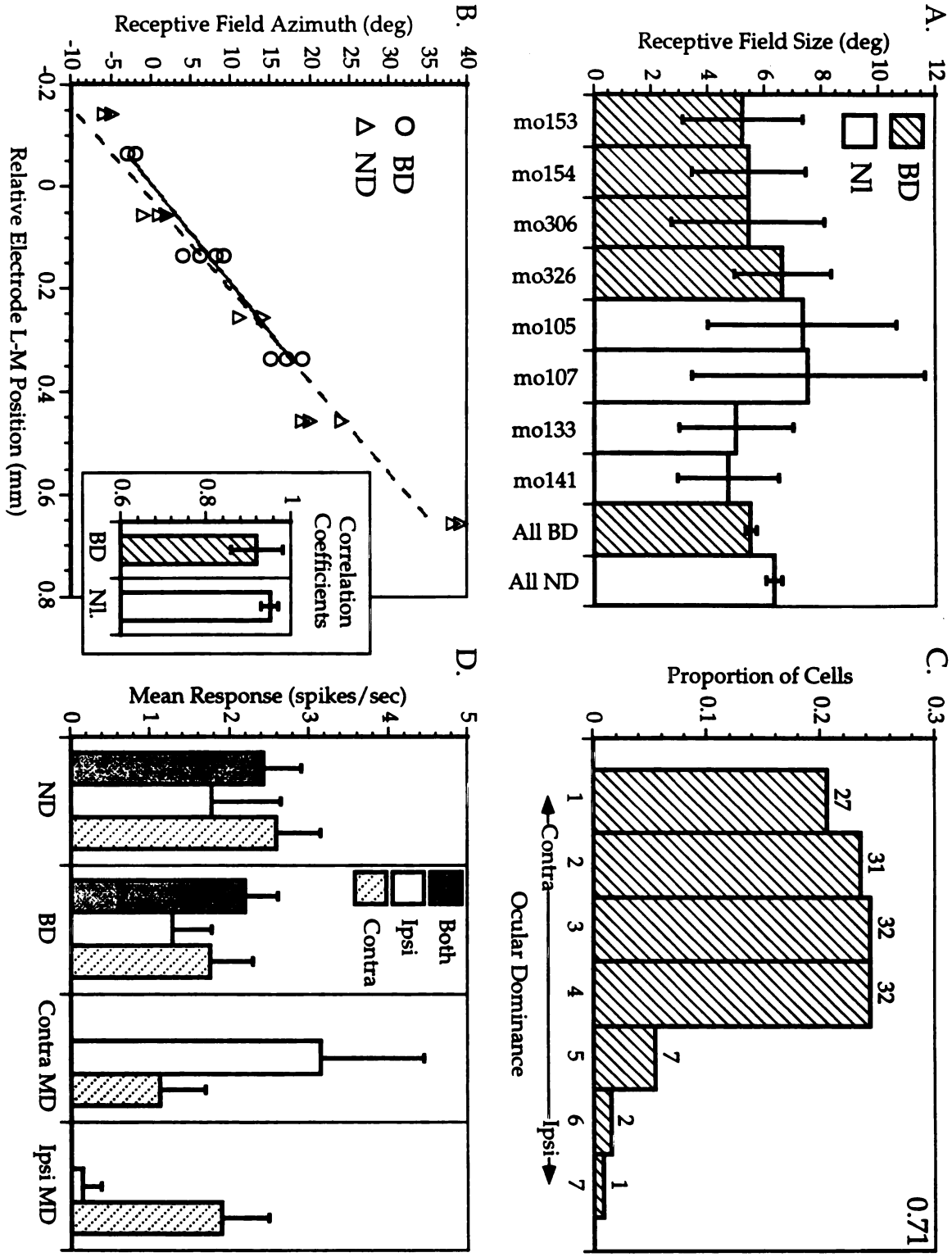


Figure 2-6: Effects of binocular deprivation on visual responses in the mouse

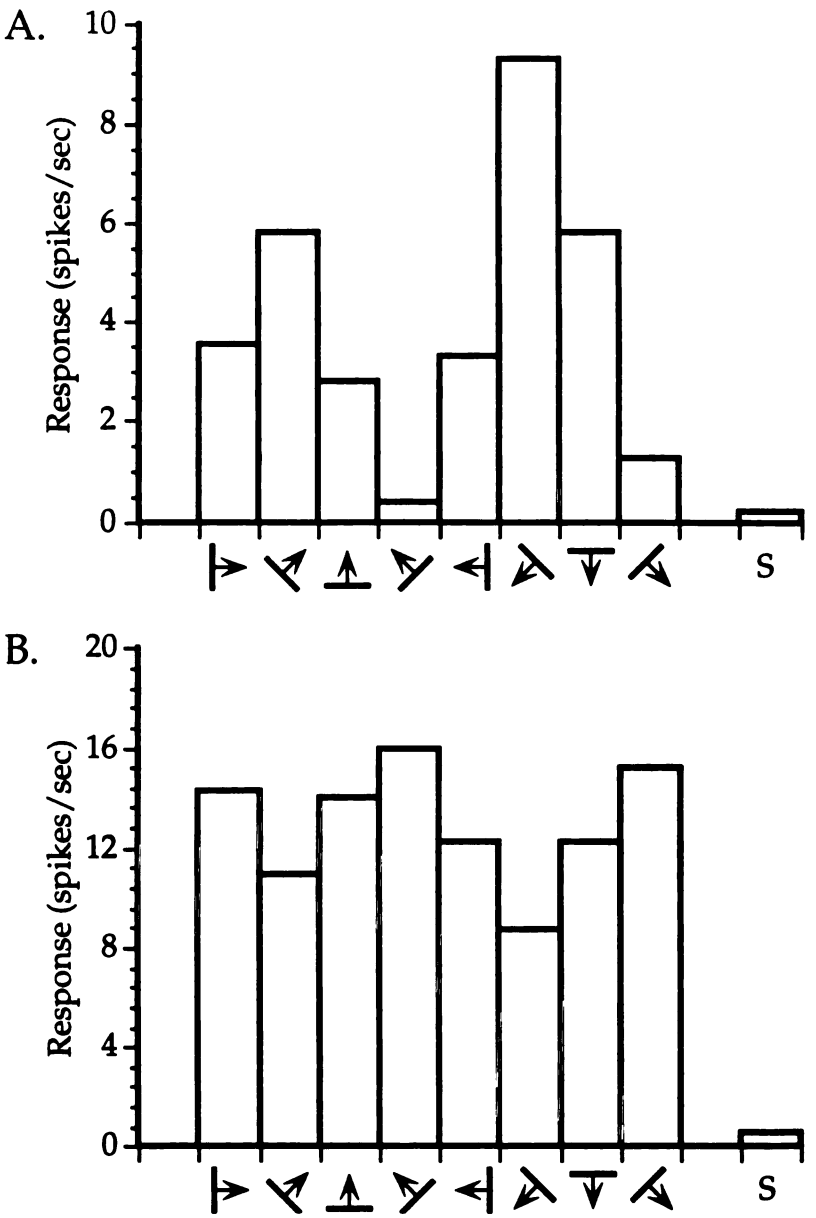


Figure 2-7. Orientation tuning of neurons in binocularly deprived mice. *A*, An orientation-selective cell. *B*, A cell with weak orientation tuning. Averaged responses to 4 presentations of an oriented bar moving in the indicated direction are shown for each of 4 orientations (2 directions each). *S*, spontaneous activity. Both neurons were recorded within the binocular zone of a mouse binocularly deprived from P28-P32.

Figure 2-8. Effects of alternating monocular deprivation on binocular responses. *A - D,* Ocular dominance distributions for cells recorded from mice subjected to various manipulations of their visual experience. Conventions as in Figure 2-2. *A,* Nondeprived mice. 150 cells were recorded from 6 hemispheres in 5 animals. *B,* Alternating monocular deprivation. Mice were initially deprived of vision in one eye from P22 or P23 to P29, then subjected to daily alternating MD for 7 to 12 days. 257 cells were recorded from the hemisphere ipsilateral to the initally deprived eye in 5 mice. *C,* Contralateral monocular deprivation. 130 cells were recorded from the hemisphere contralateral to the deprived eye in 5 MD mice deprived from P28 to P32. *D,* Ipsilateral monocular deprivation. 91 cells were recorded from the hemisphere ipsilateral to the deprived eye in 5 MD mice.

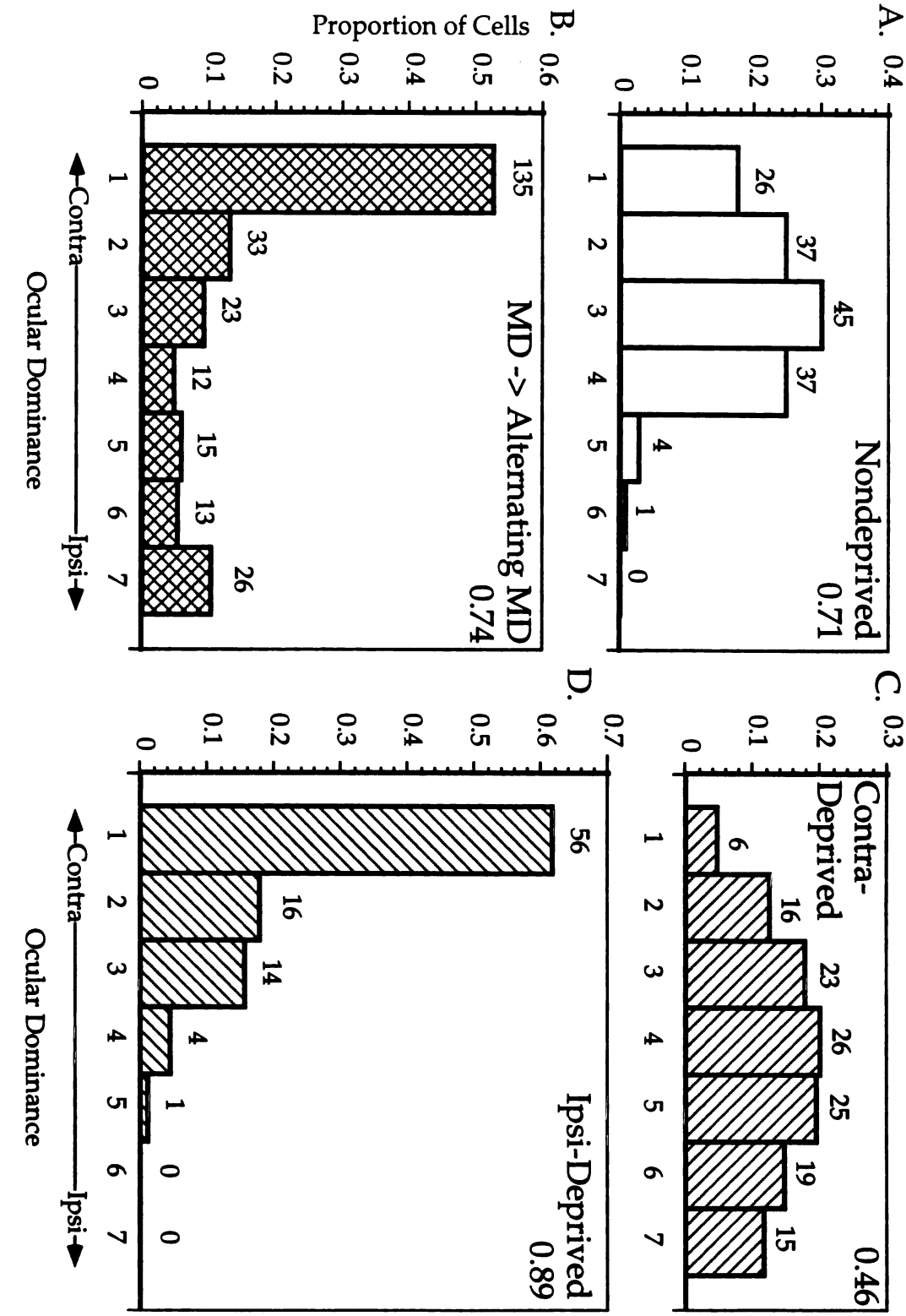


Figure 2-8. Effects of Alt MD on binocular responses.

Figure 2-9. Reconstruction of an electrode track for laminar analysis. *A*, Nissl stained section of the visual cortex in a deprived mouse. Two lesions placed at the end of a penetration into the binocular zone can be seen. *B*, Cameral lucida drawing of section shown in A. Laminar boundaries are drawn, as are the lesions and the electrode track. The locations of each cell encountered in the penetration are indicated by *hash marks* across the electrode track. OD scores are shown to the right of each hash mark for those neurons which could be unambiguously assigned to a cortical layer; numbers to the left of selected hash marks indicated the neurons from which the receptive fields shown in C were obtained. The scale bars and direction key apply to both A and B.*C*, Receptive fields of selected cells obtained in the penetration depicted in A and B. Note that since the electrode penetration was not radial, there was a gradual progression in the receptive field locations toward more central locations as the electrode was advanced deeper into the cortex. The vertical meridian is indicated by the *dashed line.*

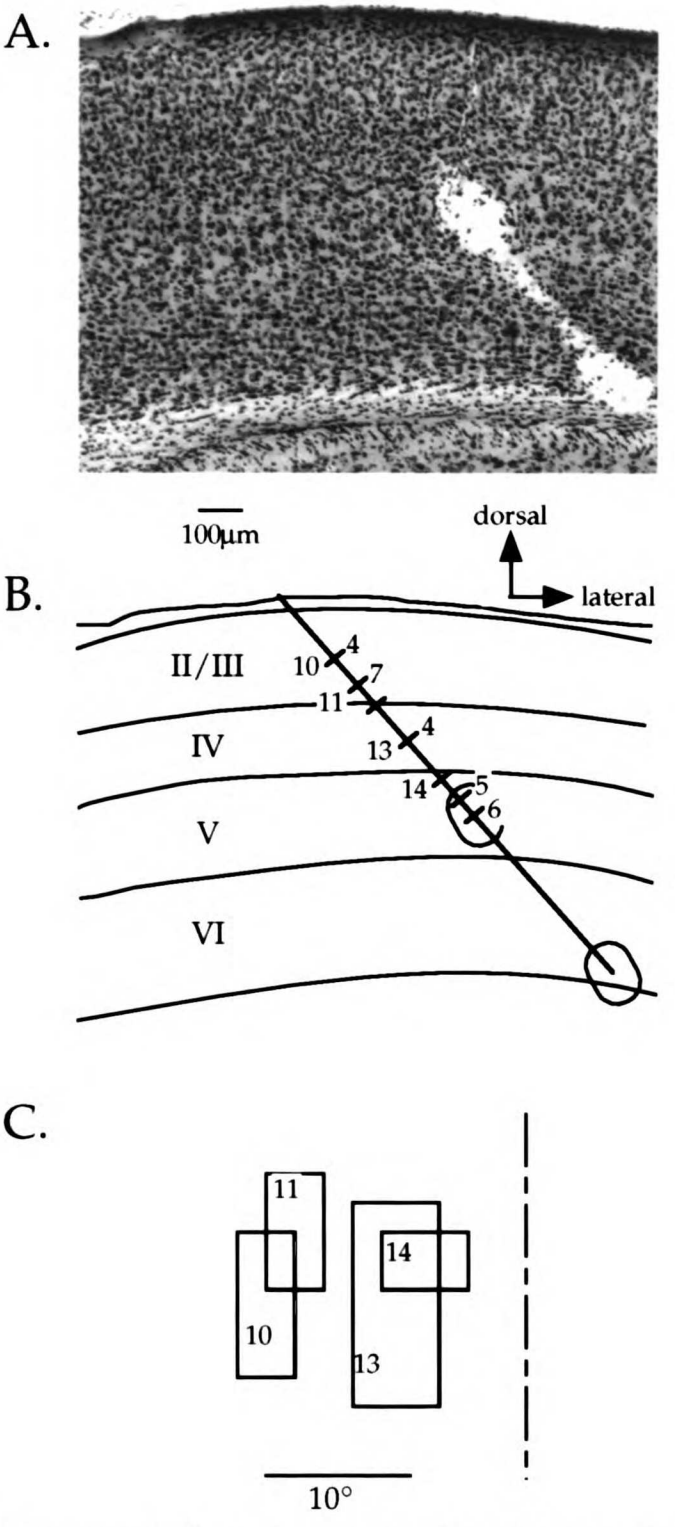


Figure 2-9: Reconstruction of an electrode track for laminar analysis

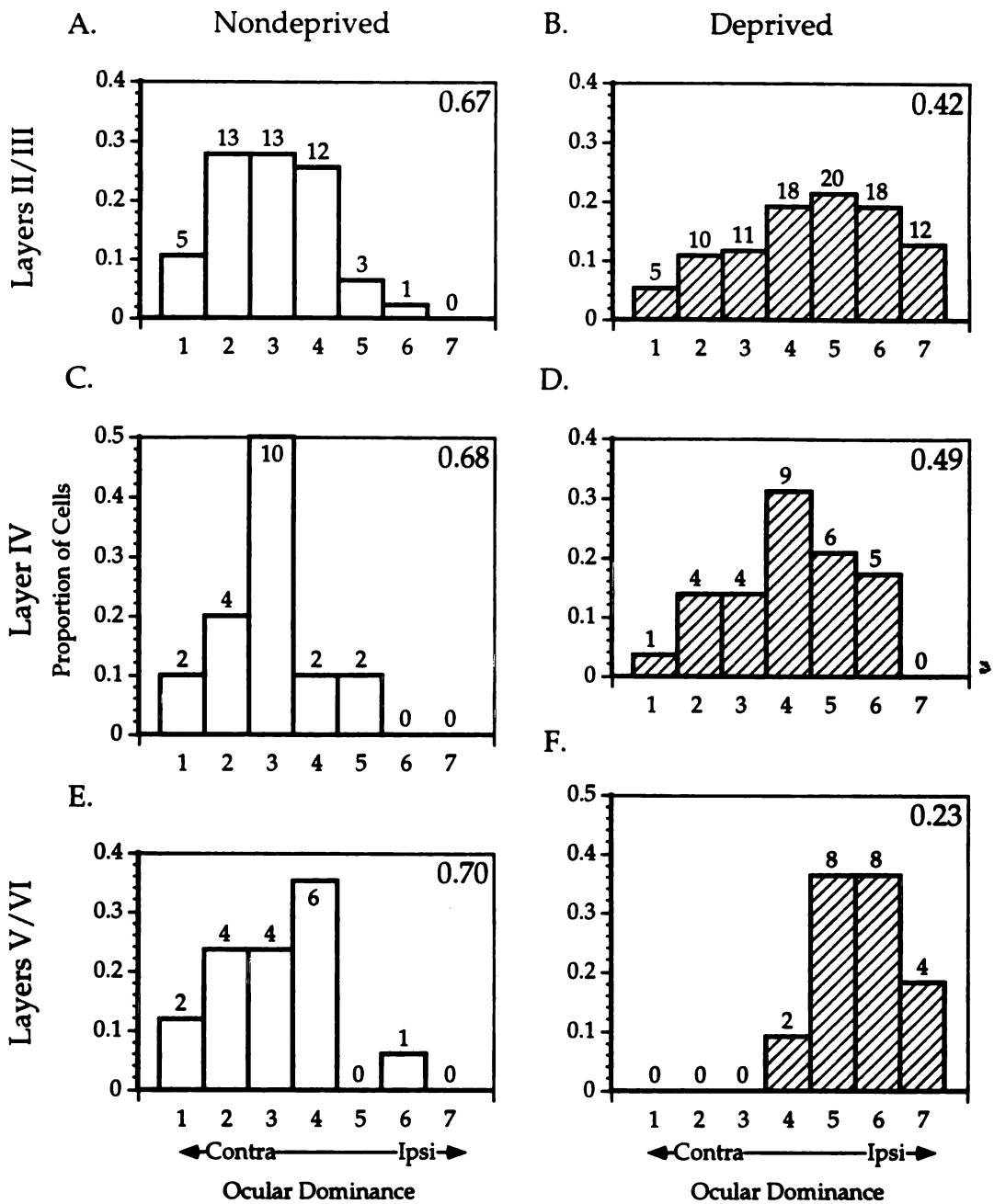


Figure 2-10. Laminar analysis of binocular responses in nondeprived and monocularly deprived mice. *A-F*, Ocular dominance histograms are shown for cells recorded from ND (A, C, E) and MD (B, D, F) mice separated by cortical layer. MD animals were deprived for 4 days beginning between P23 and P28. Conventions as in Figure 2-2.

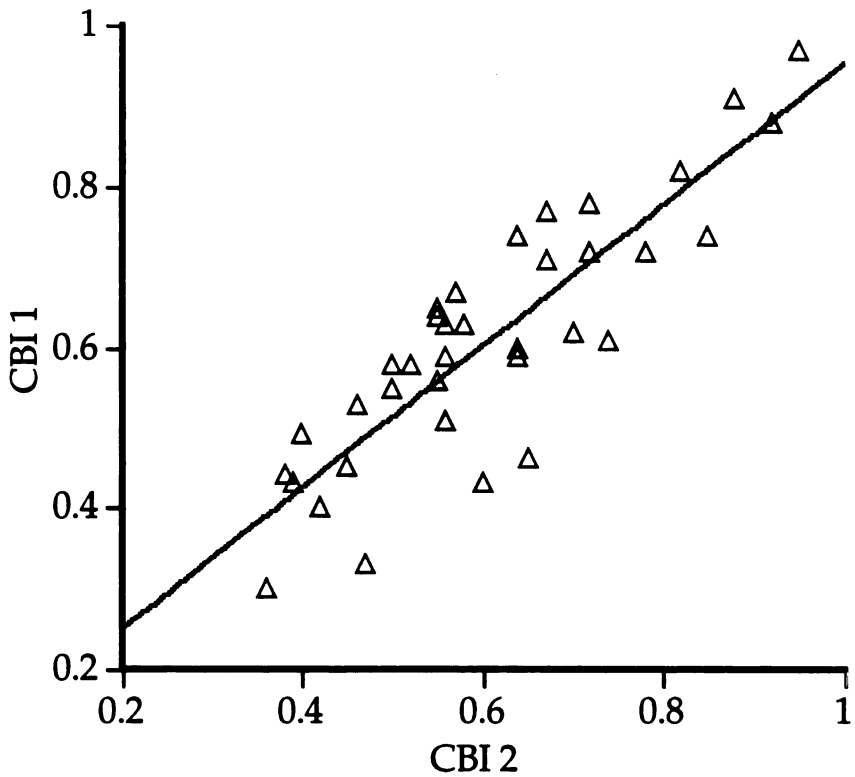


Figure 2-11. Assesment of the reliability of the contralateral bias index. CBIs were calculated seperately from OD distributions of alternately recorded cells from each of 36 hemispheres from 33 mice. The CBI calculated from the odd cells (*CBI 1*) is plotted against the CBI calculated from the even cells (*CBI 2*) for each hemisphere.

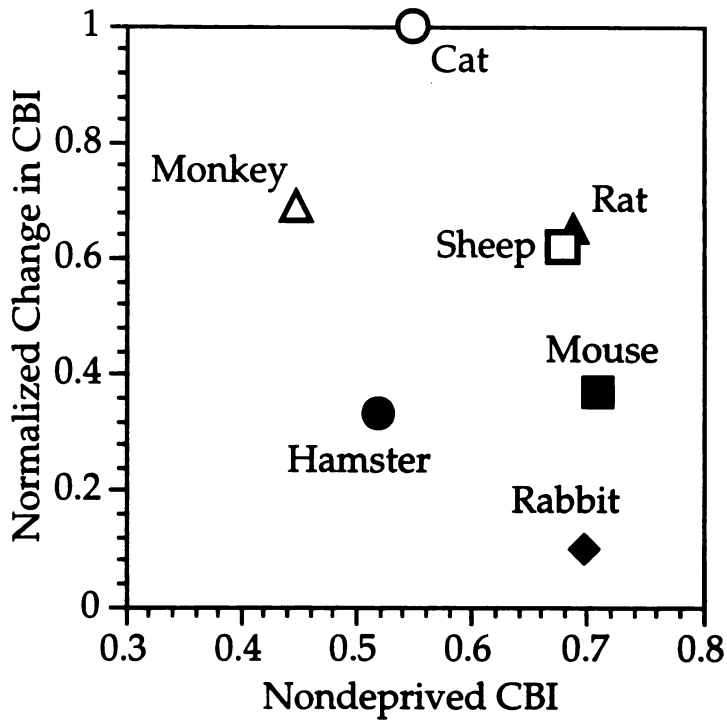


Figure 2-12. Relationship between initial contralateral bias and the degree of shift induced by monocular deprivation. The normalized change in CBI after deprivation of the contralateral eye is plotted as a function of the initial CBI in nondeprived animals for 7 different species. Sources are as follows: sheep, Kennedy et al., 1980; hamster, Emerson et al., 1982; rat, Maffei et al., 1992; cat, Wiesel and Hubel, 1963; monkey, Hubel et al., 1977; rabbit, Van Sluyters and Stewart, 1974. The normalized change in CBI is calculated according to following formula:

$$\text{change} = \frac{\text{(CBI before deprivation) - (CBI after deprivation)}}{\text{(CBI before deprivation)}}$$

CHAPTER THREE:

Normal Ocular Dominance Plasticity Despite Defective Long-Term Potentiation in the Visual Cortex of PKA RIβ-deficient Mice

ABSTRACT

Cyclic AMP signaling through protein kinase A has been implicated in learning and neuronal plasticity. In the visual cortex of mice lacking a neural-specific regulatory subunit of PKA, LTP *in vitro* was absent but plasticity of binocular responses *in vivo* was normal. This dissociation indicates that the activity-dependent rearrangement of synaptic connections in developing visual cortex relies on alternative forms of plasticity.

INTRODUCTION

Activity-dependent mechanisms play a pivotal role in the development of precise connections within the mammalian brain (Goodman and Shatz, 1993). Manipulations of the kitten visual system *in vivo* support the hypothesis that connections between the lateral geniculate nucleus and primary visual cortex (V1) are sculpted by a Hebbian competition between inputs from the two eyes (Shatz, 1990). Thus, synapses of geniculocortical inputs most correlated with the firing of their visual cortical targets are selectively strengthened, and synapses of inputs less correlated with their targets are weakened. At the cellular level, precisely how these coincidences of pre- and postsynaptic activity are detected and translated into signals for synaptic plasticity and axon remodeling remains unknown. One attractive hypothesis is that the cellular mechanisms underlying adult forms of Hebbian synaptic plasticity, such as hippocampal long-term potentiation (LTP) and depression (LTD), are first used by the visual system in development (Kandel and Lynch, 1988).

In particular, theta-burst stimulation to produce LTP and low-frequency stimulation (1 Hz) to elicit LTD in both the hippocampus and visual cortex have been advocated as common, physiologically-relevant models for understanding naturally occurring synapse modifications (Larson and Lynch, 1988; Kirkwood et al., 1993; Kirkwood and Bear, 1994a,b). The induction of homosynaptic changes *in vitro* appears to mimic the functional

strengthening and weakening of visual inputs following monocular deprivation (MD) *in vivo* (Bear et al., 1987; Artola et al., 1990). Intriguing correlations have been observed between the age-dependence for LTP induction in visual cortex and the critical period for ocular dominance plasticity in rodents and kittens (Kato et al., 1991; Berry et al., 1993; Kirkwood et al., 1995). To date, however, there has been no direct test of the hypothesis that LTP reflects a normal cellular mechanism required for experience-dependent changes in the developing mammalian brain.

Advances in gene-targeting technology have made available lines of mice with identical, defined genetic lesions in genes encoding proteins thought to play a role in plasticity. The application of neurophysiological techniques to the mouse permits direct examination of susceptibility to MD *in vivo* and induction of LTP *in vitro* in these mutant animals. We have recently characterized an activity-dependent plasticity in the binocular zone of mouse primary visual cortex that is similar to ocular dominance plasticity as observed in the cat (see Chapter Two). Thus, four days of MD during a critical period shifts cortical responses toward the non-deprived eye, while binocular deprivation has no effect on cortical responsiveness; and alternating MD, by decreasing correlations between the two eyes' input to cortex, results in a decrease in binocularly responsive neurons.

Studies in various model organisms from invertebrates to mammals have suggested a role for cAMP-dependent processes in learning and memory (Frank and Greenberg, 1994; Kandel and Abel, 1994). The cAMP-dependent

protein kinase, PKA, has multiple catalytic and regulatory subunits. Four regulatory subunits are expressed in mouse brain: RIa, RIβ, RIIa and RIIb (Cadd and McKnight, 1989). Mice carrying a targeted disruption of the gene encoding the neural-specific RIβ regulatory subunit (PKA RIβ) have recently been shown to be defective in hippocampal plasticity (Brandon et al., 1995b).

We now report that in slices of visual cortex taken from PKA RIβ-deficient mice, theta burst stimulation in layer IV fails to elicit LTP. Despite this LTP deficit, PKA R1b-deficient mice exhibit normal Hebbian modifications of the visual cortex *in vivo*.

METHODS

Whole cell recording

Mice were generated as previously described (Brandon et al., 1995) and obtained from E.P. Brandon and G.S. McKnight at the University of Washington. Whole-cell patch clamp recordings were made in the binocular zone of 400μm-thick coronal slices of the primary visual cortex continuously superfused with oxygenated (95%O_2/5%CO_2) ACSF, containing (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, 11 Glucose. A bipolar Pt-Ir electrode was used to stimulate layer IV with 100μs pulses. EPSCs were recorded from layer II/III pyramidal cells with a patch electrode (3-8MΩ) in the whole-cell voltage clamp mode (Axoclamp-2B) using either the 'blind' technique or observed with infrared Nomarski DIC optics (Blanton et al., 1989; Stuart et al., 1993). 10μM CNQX (Tocris) or 50μM D-APV (Sigma) were bath applied. The pipette solution contained (in mM): 122.5 KGluconate, 17.5 KCl, 10 HEPES buffer, 0.2 EGTA, 8 NaCl, 2.0 Mg-ATP, 0.3 Na_3-GTP, and 0.15% Biocytin (pH 7.2, 290-300mOsm). Slices were fixed in 4% paraformaldehyde for at least 24 hours, then visualized by FITC-conjugated Avidin (Sigma). Individual filled cells were reconstructed by montages of multiple optical sections captured on a laser confocal microscope (BioRad).

Nissl staining

Mice were perfused transcardially with 0.5M phosphate-buffered saline (PBS) followed by 10% formaldehyde in PBS. After post-fixation, the brain was removed, cryoprotected in 30% sucrose-10% formaldehyde and cut into 40μm sections on a freezing microtome. Sections were mounted on slides, defatted, and stained with cresylecht violet (Schmid).

Field recording of LTP and LTD

Slices were prepared from animals aged P24-P31, at the putative peak of the critical period for MD effects, and maintained in a submersion chamber as described above. Extracellular field EPSPs were recorded with a 1M NaCl electrode inserted into layer II/III. Stimulation was adjusted to yield a half-maximal response. Stable baseline responses were evoked from a bipolar stimulating electrode in layer IV at 0.1 or 0.07Hz. Then 5 episodes of 10 trains at 5Hz of 4 pulses at 100Hz (theta-burst stimulus, TBS) were given once every 10 seconds (Kirkwood et al., 1993; Kirkwood and Bear, 1994), and potentiation was monitored for at least 30 minutes at baseline frequencies. Depotentiation was attempted with 900 pulses at 1Hz and EPSPs were observed for another 25 minutes. Experiments were terminated with bath application of 10mM CNQX/50mM D-APV to determine the synaptic component of the field response by isolating the initial antidromic spike. Measurements of the

initial monosynaptic slope were normalized to the baseline period before TBS and plotted versus running time of the experiment.

In vivo electrophysiology and lid sutures

Electrophysiological and surgical procedures and analysis were conducted as described in Chapter Two. Animals were checked daily to make sure the eyes remained closed and uninfected. If sutures were found to have opened on the day of recording the animal was not included in the data set. Small holes discovered on earlier days were repaired under halothane anesthesia. The 3 animals with repaired holes did not differ from animals deprived completely for a similar duration. For experiments conducted blind to deprivation (Figure 3-5D), the investigator performing the lid suture also prepared the animal for physiological recording, trimming and suturing both eyes so that a second investigator could record blind to the state of deprivation. Nondeprived controls in Figure 3-5D underwent mock lid-suture surgery and were age-matched. All recordings in Figure 3-3 were made from the V1 binocular zone contralateral to the deprived eye. Eyelid trimming was not performed for deprivations in Figure 3-6 to facilitate reopening of the eyes. The reverse suture experiments were carried out blind to genotype; the initial deprivation experiments (Figure 3-6A & B) were carried out blind to which eye had been deprived and to genotype.

RESULTS

Visual Cortical Plasticity *in vitro*

The PKA second messenger pathway via calcium/calmodulin-sensitive adenylyl cyclases is well-situated for involvement in the plasticity process (Cooper et al., 1995) The neural-specific expression of the R1b subunit has led to speculation that it might play a unique role in synaptic transmission and modification (Cadd and McKnight, 1989). We therefore first characterized the morphology and synaptic responses in the visual cortex of R1b-deficient ($R1b^-$) mice *in vitro*. A normal six-layered binocular region of the primary visual cortex was observed with typical supragranular pyramidal cells bearing many post-synaptic spines (Figure 3-1A,B). The influx of calcium through N-methyl-D-aspartate (NMDA) receptor-gated channels on theses spines is known to be essential for the induction of both theta burst stimulation (TBS)-induced LTP and low frequency-stimulated LTD in hippocampus and neocortex (Artola and Singer, 1993; Bear and Malenka, 1994). We confirmed that stimulation of layer IV evoked normal non-NMDA and NMDA-mediated synaptic responses in $RI\beta^-$ pyramidal neurons in layer II/III (Figure 3-1C). Thus, the post-synaptic structures required for the initial steps in LTP induction appear to be present in the visual cortex of $RI\beta^-$ mice.

Since it has been claimed that common forms of plasticity can be induced in supragranular neocortex and hippocampus, we examined LTP induction and de-potentiation in the connection from layers IV to II/III (Kirkwood et al., 1993). Coronal slices (400μm) through the binocular zone of visual cortex were prepared blind to the genotype of the animal at the height of the critical period for monocular deprivation effects (P24-31). In both RIβ$^-$ and genetic background-matched wild-type (WT) control mice, electrical stimulation of layer IV evoked a biphasic field potential in layer II/III, consisting of an initial antidromic spike and a subsequent synaptic response (Figure 3-2 insets). Bath application of glutamate receptor antagonists at the end of each experiment confirmed the identity of these peaks. Initial slope measurements of the monosynaptic component were normalized to a baseline period prior to five episodes of TBS (10 trains at 5 Hz of 4 pulses at 100 Hz; 10 seconds between each TBS). After a post-TBS period of at least 30 minutes, a depotentiating stimulus of 900 pulses at 1 Hz was applied. Upon decoding the genotype, individual records revealed the absence of LTP in RIβ$^-$ slices (**Figure** 3-2C). In contrast, WT slices exhibited a slowly building potentiation, lasting up to 90 minutes after TBS, that could be de-potentiated (Figure 3-2B). As expected from previous results in the rat (Kirkwood and Bear, 1994a), the induction of LTP by TBS in WT slices was blocked by the NMDA receptor antagonist APV (n=3, data not shown). Figure 3-3 shows grouped data for 12 slices from 7 mice of each genotype, illustrating the robust

and statistically significant difference in LTP between RIβ⁻ and WT mice (potentiation 20-30 minutes post-TBS=1±5% vs. 19±5%, $P<0.05$, Student's t-test).

Depotentiation was also reduced in slices taken from the visual cortex of RIβ⁻ mice (Figure 3-3), similar to the deficit found in area CA1 of the hippocampus (Brandon et al., 1995). The interpretation of the depotentiation deficit is complicated, however, by the finding that TBS failed to induce LTP in these cortical slices. A more rigorous comparison of depotentiation to LTD in naive slices from RIβ⁻ visual cortex would be required to address this issue. Of greater relevance to the present work, potentiation differed between hippocampus and cortex in that (with a different tetanic protocol) LTP is normal in RIβ⁻ hippocampal CA1 (Brandon et al., 1995b). This is the first report of a difference between LTP in hippocampal CA1 and neocortical layer II/III (Kirkwood et al., 1993).

Visual Cortical Responsivity *in vivo*

We next determined whether mice lacking PKA RIβ and LTP could nonetheless develop normal visual cortical responses. Extracellular single unit recordings were obtained *in vivo* from V1 of two RIβ⁻ and two WT adult mice blind to genotype. Each neuron was evaluated for receptive field

size and location, ocular dominance, orientation selectivity, and response strength. Qualitatively, neuronal response properties in RIβ⁻ and WT V1 were indistinguishable.

Quantitative comparison of receptive field properties, retinotopy, and ocular dominance confirmed the qualitative impression. Receptive field (RF) size distributions in the RIβ⁻ and WT animals overlapped considerably (Figure 3-4A), and the mean RF size in the RIβ⁻ mice did not differ significantly from WT. Retinotopy was quantified by a linear regression analysis of receptive field azimuth on electrode position. As shown in Figure 3-4B, there was a linear relationship between the cells' RF-center azimuths and lateromedial electrode position in both RIβ⁻ and WT V1. Furthermore, the degree of variance about this relationship was equally low in both RIβ⁻ and WT, as demonstrated by the high correlation coefficients of the linear regressions (Figure 3-4B, inset).

Most of mouse V1 is monocular, but the lateral 600 μm, which represent approximately the frontal 30-40° of the upper portion of each hemifield, receive geniculocortical inputs representing both eyes (Drager, 1975; Wagor et al., 1980). Within this binocular area we found cells responding to input from both eyes; each cell was then evaluated for ocular dominance (OD) on a 7-point scale according to the methods of Hubel and Wiesel (1962). The distributions of OD scores of cells in RIβ⁻ and WT

binocular zones showed the normal bias toward dominance by the contralateral eye and were not significantly different from each other (Figure 3-4C & D). The distributions were quantified and represented as a single parameter called the Contralateral Bias Index (CBI), which is a measure of the degree to which the contralateral eye dominates the cortex. The CBIs for the OD distributions in Figure 3-4C & D are shown. CBIs can also be calculated for each hemisphere individually. The mean CBIs for 3 RIβ$^-$ and 4 WT hemispheres were not significantly different (Figure 3-5C). Thus, despite their visual cortical LTP deficit, RIβ$^-$ animals develop normal receptive field size, retinotopy, and ocular dominance in V1.

Effects of Monocular Deprivation

We next tested the ability of these animals to undergo OD plasticity. Three mice of each genotype underwent MD by lid suture for 4 days at the peak of the critical period, beginning between P25 and P27. At the end of the deprivation period, recordings were made from neurons within the binocular zone of V1 contralateral to the deprived eye. Each cell was evaluated for RF area, location and OD as described above. These experiments were also performed blind to genotype. Figures 3-5A and B show the combined OD histograms for the deprived RIβ$^-$ and WT animals, respectively. In both WT and RIβ$^-$ mice, significant shifts of equal magnitude were seen towards the

nondeprived, ipsilateral eye, which dominated many more cells than it did in the typical nondeprived cortex (chi squared test, $p < 0.0005$). The mean CBIs for WT and RIβ⁻ nondeprived and MD animals, computed individually by hemisphere, are shown in Figure 3-5C. There was a significant difference between CBI means for nondeprived vs. deprived animals of both genotypes (Student's t test, $p = 0.03$ and 0.001 for RIβ⁻ and WT, respectively), and no significant difference between the means for RIβ⁻ vs. WT for either condition.

In order to confirm the ability of RIβ⁻ mice to undergo OD shifts, we recorded from an additional 5 RIβ⁻ mice, blind this time to whether or not the animals had been subjected to monocular deprivation. The mean CBIs for 5 nondeprived and 2 deprived RIβ⁻ hemipsheres from these experiments are shown in Figure 3-5D. Once again, a clearly significant shift towards the nondeprived eye occurred only in the lid-sutured RIβ⁻ mice (Student's t-test, $p = 0.002$). Note also that there were no significant differences between the mean CBIs from deprived and nondeprived animals in this series of experiments and those from the series conducted blind to genotype ($p > 0.7$ for both comparisons).

Reverse Suture: Potentiation *in vivo*

While monocular deprivation is a well-characterized system for studying neural plasticity *in vivo*, it is perhaps not the best paradigm with which to study a link between *in vivo* plasticity and long-term potentiation, since an apparent ocular dominance shift might result solely from a decrease in the efficacy of deprived eye afferents. We therefore turned to a reverse suture paradigm (Wiesel and Hubel, 1965) to demonstrate more rigorously the existence of an efficacy-enhancing mechanism *in vivo*. Because of the bias toward contralateral eye dominance in normal animals, monocular deprivation of the ipsilateral eye results in nearly complete dominance of the cortex by the contralateral eye. This can be seen in Figure 3-6A, which shows the OD distribution of 85 cells recorded from the hemisphere ipsilateral to the deprived eye in 3 RIβ$^-$ animals (compare with the less dramatic effect in the contralateral hemisphere from the same animals, Figure 3-6B). Few cortical cells responded at all to stimuli presented to the deprived eye, and the great majority that did respond did so very poorly. If one then opens the initially deprived eye and sutures shut the initially open eye, the initially closed eye afferents come to drive the cortex effectively (Wiesel and Hubel, 1965). Figure 3-6C shows this for the OD distribution of 106 cells recorded from 4 RIβ$^-$ animals after reverse suture. The significant ($p < 0.0005$, chi squared test) shift back toward the initially deprived, then reopened ipsilateral eye is

underscored by the individually calculated CBIs for each of 4 RIβ$^-$ and 1 WT animals recorded after successively increasing periods following the suture reversal, shown in Figure 3-6D. The data demonstrate a dramatic increase in the efficacy of inputs from the initially deprived eye and thereby establish the existence of an *in vivo* potentiation mechanism in the RIβ$^-$ mice.

DISCUSSION

The mechanism responsible for the loss of LTP in the RIβ$^-$ mice is unclear. Whole-cell recordings from layer II/III pyramidal cells reveal normal morphology and synaptic currents, as well as blockade by norepinephrine of spike frequency adaptation to step depolarizations (data not shown). The latter effect is known to be due to PKA activation (Pedarzani and Storm, 1993), and is fully consistent with the preservation of cAMP-dependent protein kinase catalytic activity measured biochemically in these mice, that presumably is due to a compensatory increase in the ubiquitous RIa isoform of PKA (Brandon et al., 1995). The defect in TBS-mediated LTP induction in the RIβ$^-$ animals suggests a role for some property specific to the neuronal RIβ subunit. The RIβ molecule does have a 3 to 7-fold higher sensitivity to cyclic nucleotides than the other regulatory subunits (Cadd et al., 1990; Solberg et al., 1994). Alternatively, by analogy to the other regulatory isoforms (Coghlan et al., 1993; Skalhegg et al., 1994), RIβ may play a role in subcellular localization of the PKA enzyme that is important for LTP.

Whatever the mechanism by which LTP is disrupted, however, OD plasticity occurred independent of TBS-induced LTP in these mice. The RIβ$^-$ animals were able to develop normal visual cortical responses. Both monocular deprivation and reverse suture led to appropriate shifts in the

relative efficacy of inputs from the two eyes. These results show that PKA RIβ is not essential for development and plasticity of the visual system. More importantly, they demonstrate that TBS-induced LTP *in vitro* and Hebbian modification of visual pathways *in vivo* are dissociable.

The present study examined only one method of studying plasticity *in vitro*, (Bear and Kirkwood, 1993). We chose to study TBS-induced LTP by stimulating layer IV and recording in layer II/III because of the strength of currently available evidence linking this form of LTP with OD plasticity (Kirkwood et al., 1993, 1995; Kirkwood and Bear, 1994). One might argue that the important changes with monocular deprivation occur at the geniculocortical synapse, between white matter and layer IV in the slice. Furthermore, critical period-dependent plasticity is seen when stimulating the white matter and recording in layer II/III, rather than stimulating layer IV. Thus, it might be asserted that we did not study LTP at the appropriate synapse. There are two counterpoints which can be made to these arguments. First, the data presented in Chapter Two argue strongly that intracortical as well as geniculocortical connections undergo plasticity. Therefore, even if our findings on TBS-induced plasticity were relevant only to intracortical synapses, our finding would still be important. Second, the currently held view of TBS-induced plasticity is that stimulating in white matter or layer IV harnesses the same plasticity mechanisms at the same synapses onto layer III cells (Kirkwood et al., 1993, 1995; Kirkwood and Bear, 1994). One would therefore expect that white matter to layer IV LTP would also be absent in

these mutants. Experiments are currently underway to directly test this expectation.

The current data, however, do not directly address the relevance of other *in vitro* models. In particular, it may well prove informative to examine potentiation at other synapses (Stryker, 1995) or to explore completely new models of plasticity *in vitro*, with the aim of finding one that correlates best with the susceptibility to experience-dependent changes *in vivo*. The present work directly tested the hypothesis that TBS-induced LTP induced by stimlating layer IV is an appropriate model for the phenomenon of OD plasticity *in vivo* (Kirkwood et al., 1993, 1995; Kirkwood and Bear, 1994). The findings call into question the relevance of studying this form of LTP for understanding the factors responsible for OD plasticity and suggest that alternative forms of plasticity underlie the activity-dependent rearrangement of connections during development.

Figure 3-1. Normal morphology and synaptic responses in RIβ⁻ mice. *A.* Neurons filled with biocytin in the supragranular layers exhibit normal pyramidal morphology with long apical dendrites extending to the pial surface and profuse basal processes. Scale bar = 40mm. *Inset,* Numerous postsynaptic spines are readily visible. Scale bar = 7mm. *B.* Nissl-stained section through the binocular zone reveals normal gross neuroanatomy of the primary visual cortex. All layers can be identified in coronal sections taken from RIβ⁻ animals. Scale bar = 100mm. *C.* Pyramidal cell synaptic responses to underlying layer IV stimulation exhibit fast non-NMDA and slower NMDA receptor-mediated components. Whole-cell voltage-clamp recordings at -90mV were blocked with 10μM CNQX (middle trace) to reveal NMDA receptor-mediated responses at +50mV. The latter were blocked by 50μM D-APV (not shown). Same cell as in (A).

Figure 3-1

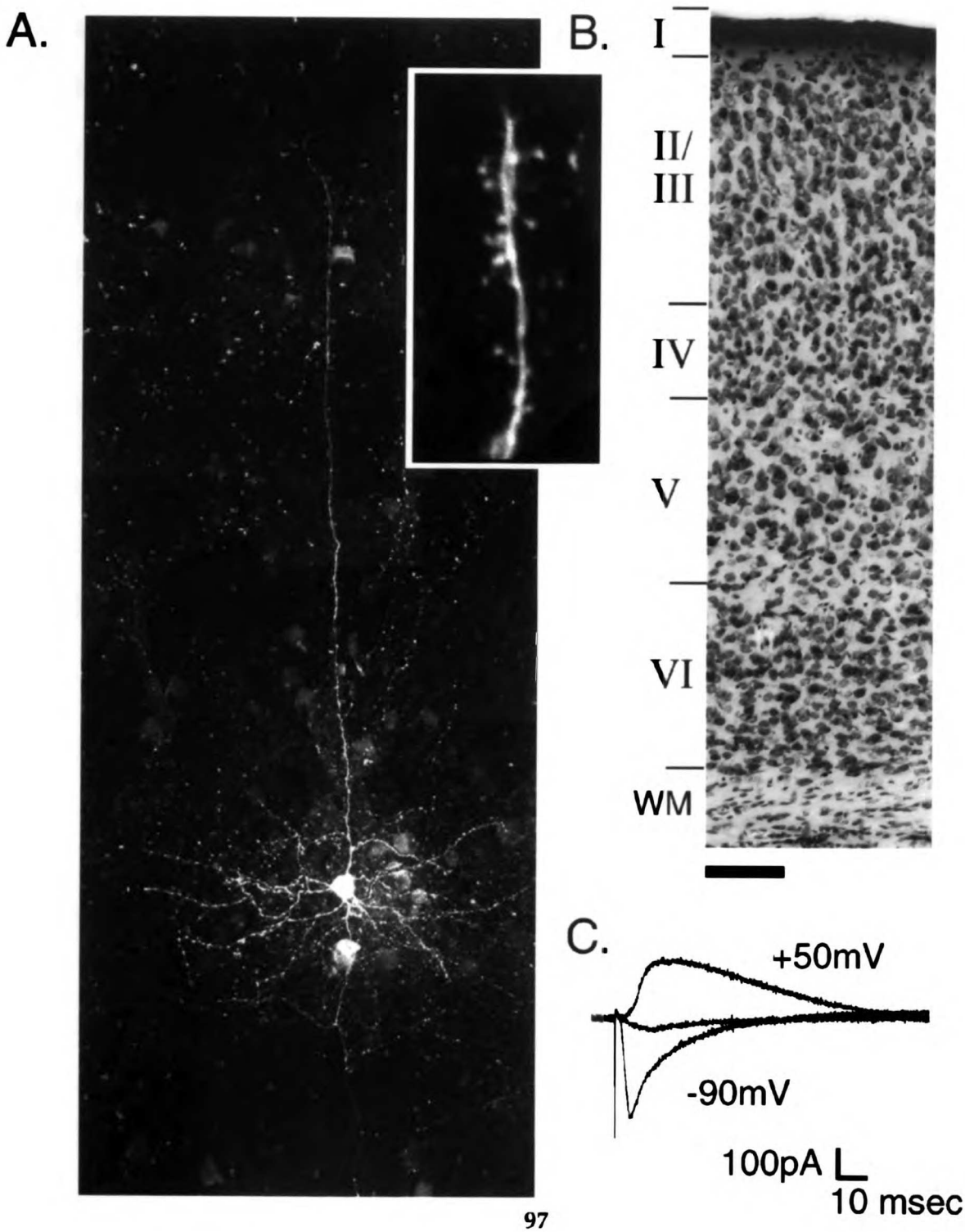


Figure 3-2. Induction of LTP and depotentiation in wild type, but not RIβ$^-$ mice. *A.* Field recordings (R) were made in the supragranular layers of the binocular zone in response to layer IV stimulation (S). All experiments were carried out blind to the genotype in animals at the peak of the critical period. Following a stable baseline recording, TBS (arrow) was applied to assay LTP followed by a prolonged low-frequency stimulation (bar) to induce depotentiation. The individual experiments shown were decoded as *B,* P31 WT, and *C,* P28 RIβ$^-$. *Insets* , (from left to right) Sample field response traces taken before TBS, 25 minutes post-TBS, 25 minutes post-1Hz, and after CNQX/APV bath application (long arrow). Scale bars: 0.1mV/10ms (B), 0.2mV/10ms (C).

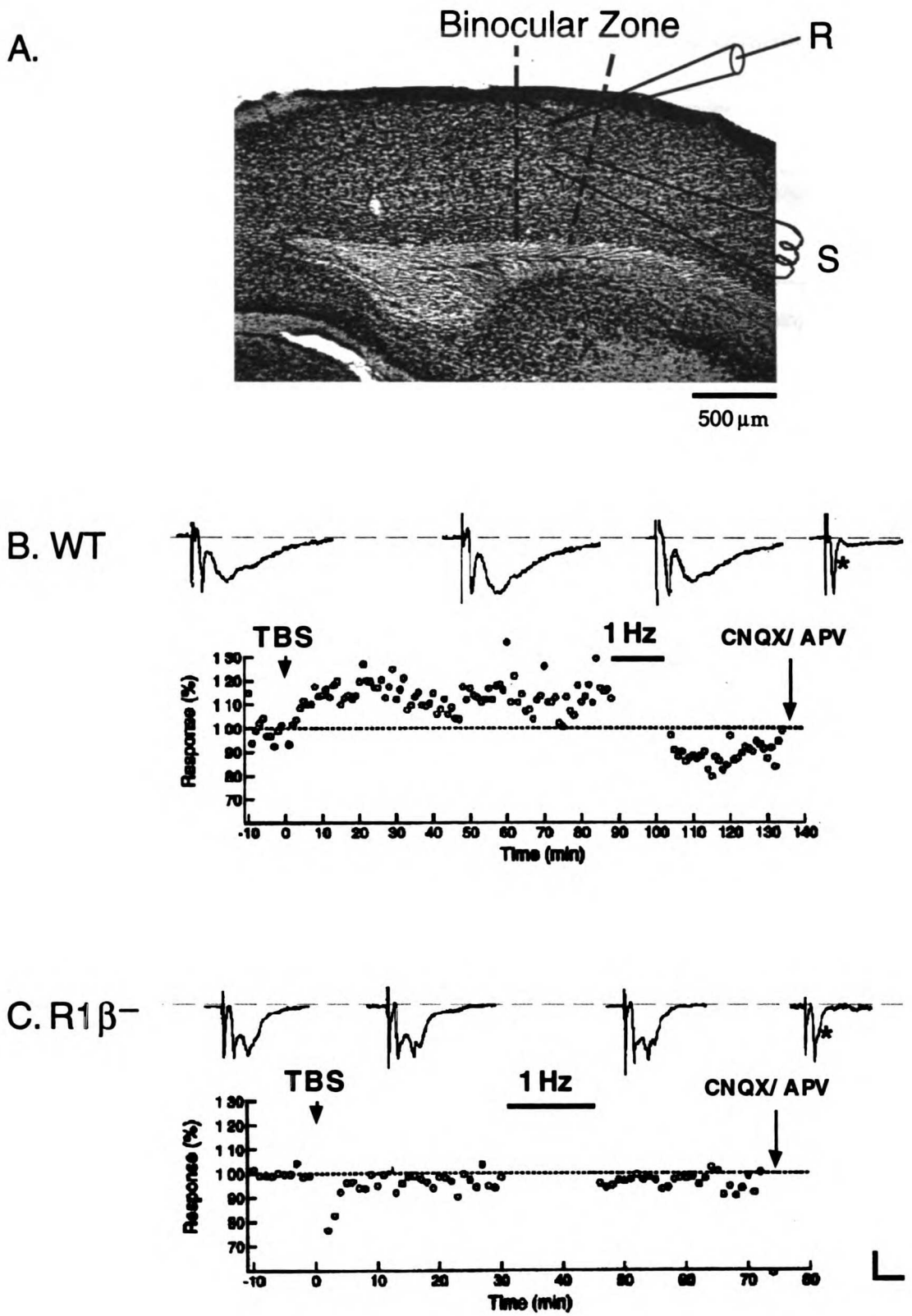


Figure 3-2. Induction of LTP and depotentiation in WT but not RIβ^{-} mice.

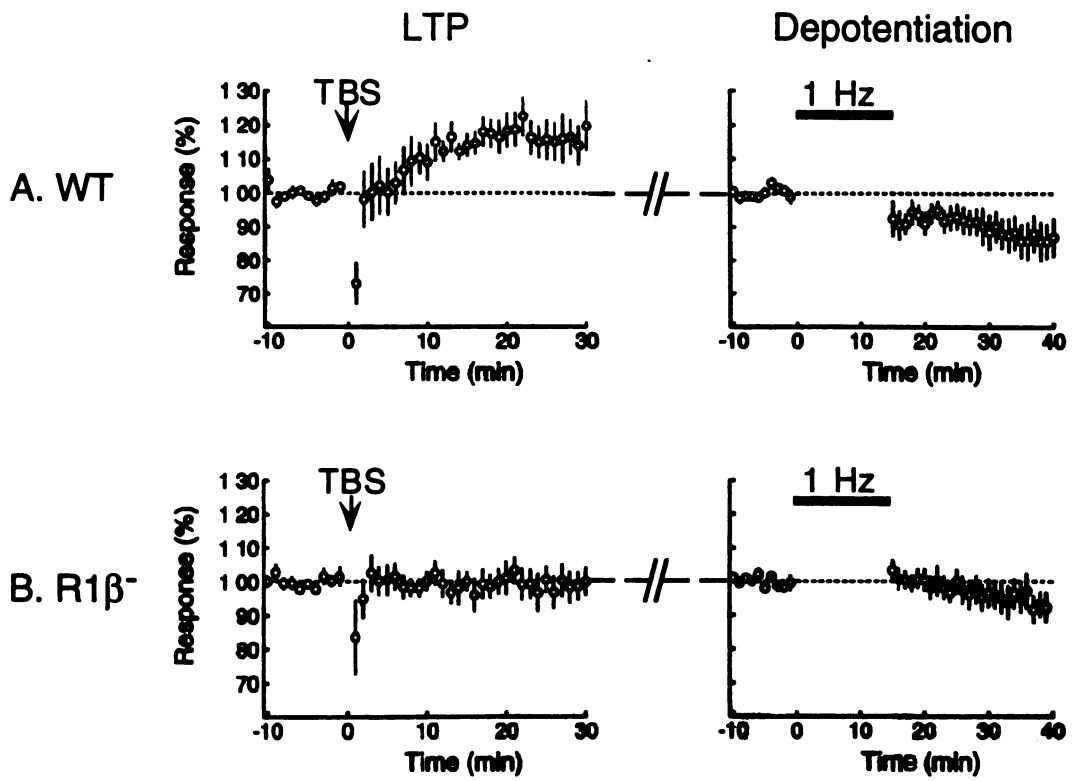


Figure 3-3. Grouped data reveals LTP deficit in RIβ⁻ mice. Mean ± s.e.m., n = 12 experiments from 7 mice for each genotype, performed blind to genotype as in Figure 3-2. Depotentiation curves have been renormalized to the 10 minutes preceding 1 Hz stimulation to adjust for varying lengths of time elapsed after TBS across experiments. The amount of potentiation seen in *A*, WT slices was significantly greater than that in *B*, RIβ⁻ slices ($P<0.05$, Student's t-test).

Figure 3-4. Visual cortical responses are normal in RIβ⁻ mice. *A*, Receptive field size in RIβ⁻ and WT mice. Individual animals: Mean ± S.D, n = 28 - 47 cells/animal. Summaries: Mean ± s.e.m., n = 88 (RIβ⁻) and 104 (WT) cells. Circles, RIβ⁻; triangles, WT. *B*, Retinotopy in RIβ⁻ and WT mice. A series of evenly spaced microelectrode penetrations were made across a portion of the lateromedial extent of V1 in each animal. At least three and up to 7 cortical cell RFs were mapped in each penetration. RF-center azimuths are plotted vs. electrode position. *Inset*, correlation coefficients of 3 RIβ⁻ and 4 WT regressions. *C,D*, Ocular dominance distributions of 77 and 75 cells from the binocular zone of nondeprived adult WT (C) and RIβ⁻ (D) mice, respectively. Contralateral bias indices are in upper right hand corner of each graph.

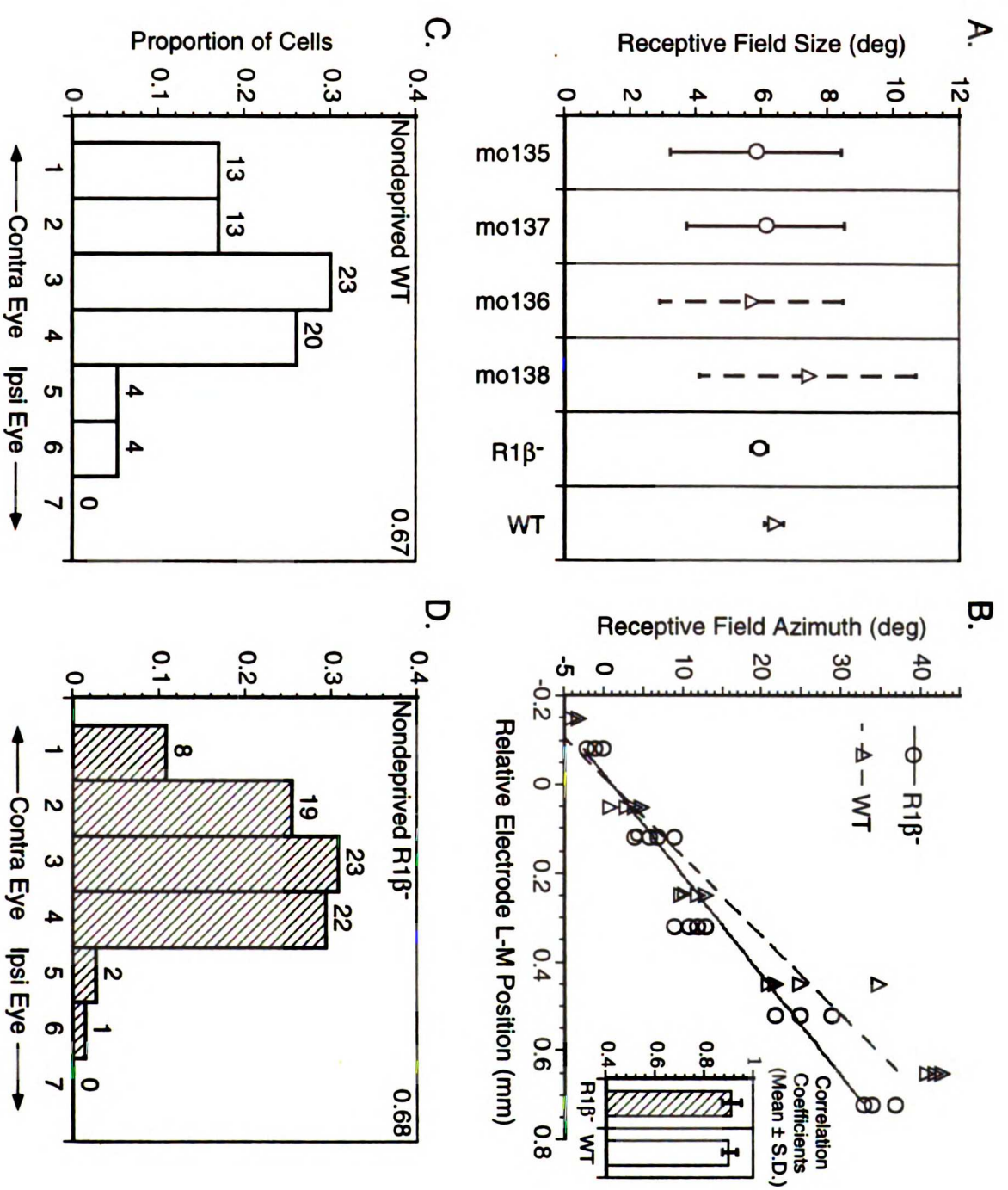


Figure 3-4. Visual cortical responses are normal in RIβ⁻ mice.

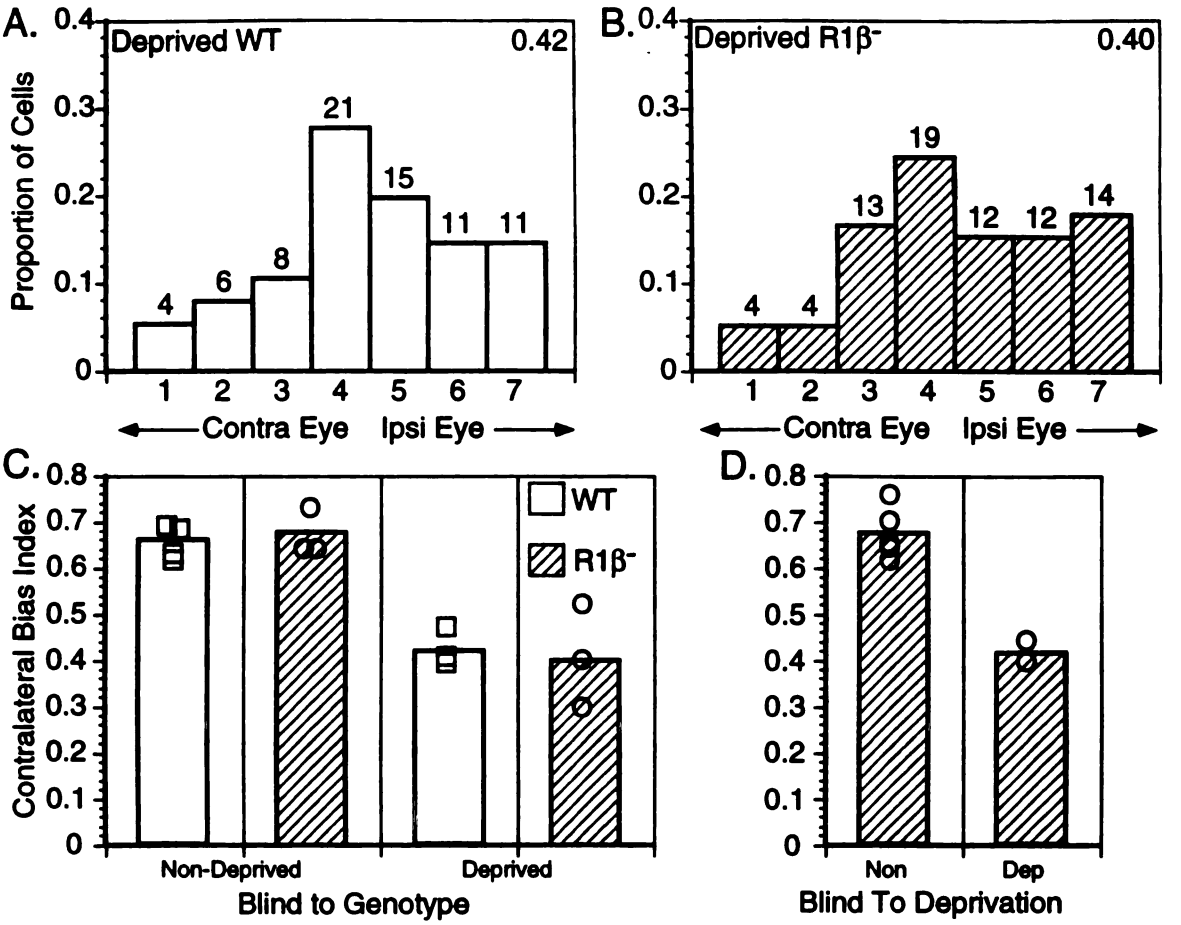


Figure 3-5. Effects of monocular deprivation in RIβ⁻ mice. *A, B,* Ocular dominance distributions from monocularly deprived WT (A) and RIβ⁻ (B) animals. Animals were deprived for 4 days beginning at P25-P27. n = 76 cells from 3 deprived controls and 78 cells from 3 deprived RIβ⁻ mice. *C, D,* Effect of monocular deprivation in WT and RIβ⁻ mice. CBIs calculated individually from single hemispheres (symbols) and mean CBIs for each condition (bars). *C,* Experiments conducted blind to genotype. N = 3 hemispheres except for nondeprived WT, N = 4; n = 14-31 cells/hemisphere. *D,* Experiments conducted blind to deprivation. N = 5 (nondeprived) and 2 (deprived) hemispheres, n = 24-31 cells/hemisphere.

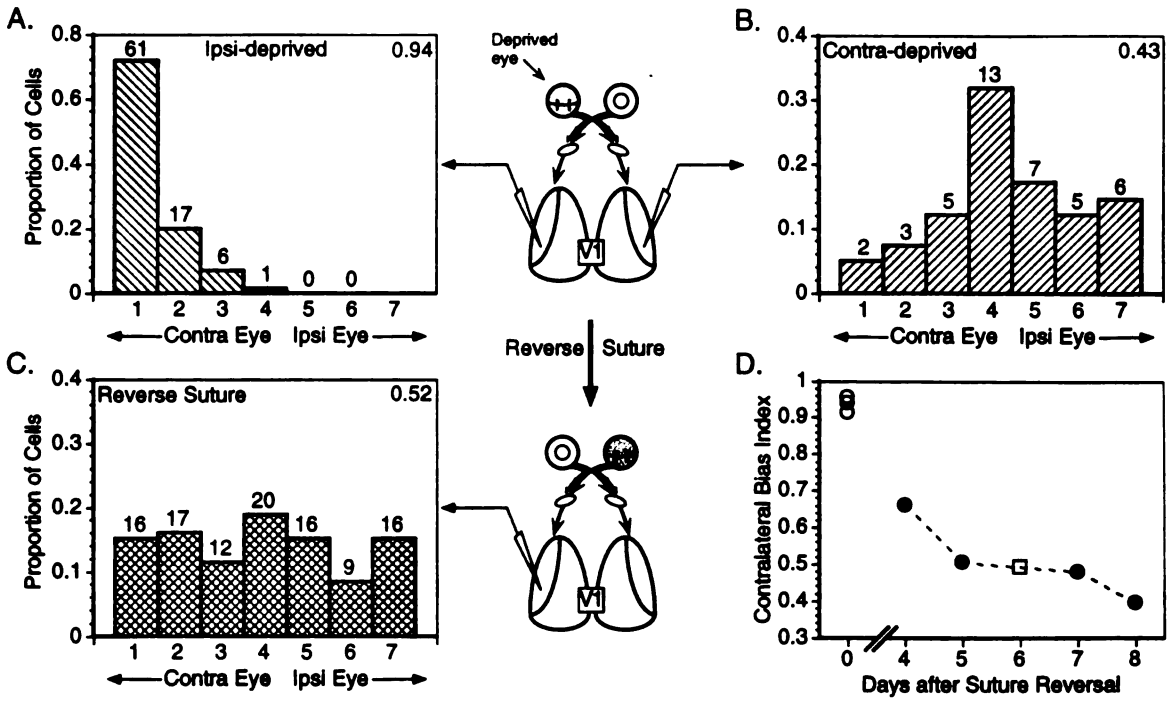


Figure 3-6. Effects of reverse suture in RIβ⁻ mice. *A,B,* Ocular dominance distribution of cells recorded ipsilateral (A) and contralateral (B) to the deprived eye in 3 RIβ⁻ mice. Mice were deprived for 5 days beginning at P20-P22. n = 85 and 41 cells, respectively. *C,* OD distribution of cells recorded ipsilateral to the initially deprived eye in 4 reverse-sutured RIβ⁻ mice. Mice were initially deprived for 5 days beginning at P21; at P26 all the mice underwent reverse suture for an additional 4-8 days. *D,* Individually calculated CBIs of ipsi-deprived and reverse-sutured animals. Open circles, ipsi-deprived RIβ⁻ mice (same animals as in A). Closed circles, reverse sutured RIβ⁻ mice (same animals as in C). Open square, reverse-sutured WT mouse.

CHAPTER FOUR:

Visual Cortical Responses and Plasticity in Mutant Mice

ABSTRACT

Plasticity of binocular responses in the developing visual cortex is perhaps the best studied model of activity-dependent developmental plasticity. The characterization of a mouse model for ocular dominance plasticity permits the use of mutant mice to investigate potential molecular and cellular mechanisms of activity-dependent development. In the present study, mice lacking various molecules implicated in plasticity were examined for their ability to develop normal visual cortical responses and to undergo ocular dominance plasticity. Mice with targeted deletions of the genes encoding the neural adhesion molecule Thy-1, the tyrosine kinase fyn, the neural specific catalytic subunit $C\beta_1$ of protein kinase A, and the neural isoforms γ and α of protein kinase C and calcium/calmodulin-dependent protein kinase II (CaMKII), respectively, were studied. Normal visual cortical responses were found in all mutants examined. Monocular deprivation induced a shift in the responses of binocular zone neurons towards the open eye in all mutants as well. A deficiency of αCaMKII, however, resulted in a slowed shift, such that a longer period of deprivation was required to evoke a consistently maximal response. These data suggest a role for αCaMKII in ocular dominance plasticity.

Thy-1-deficient mice

Thy-1 has long been proposed as a negative regulator of axon outgrowth (Morris, 1992). Initially characterized as an antigen on lymphocytes, the Thy-1 protein is also highly expressed in the adult mouse brain (Reif and Allen, 1964). Thy-1 is member of the immunoglobulin superfamily of cell surface proteins, anchored to the plasma membrane via a glycophosphatidylinositol link (Willams and Barclay, 1988; Homans et al., 1988). A role for Thy-1 in the regulation of axonal outgrowth was first proposed based on its interesting expression pattern (Morris, 1985). Transcription of the Thy-1 gene is turned on in neurons as migration ends, but the protein is excluded from axons until the axon itself has ceased growth (Xue and Morris, 1992; Morris et al., 1992). Direct evidence for a role for the molecule in inhibiting cell growth has come from cell culture experiments, where Thy-1 protein has been shown to inhibit neurite outgrowth, and perturbations which result in the removal of Thy-1 from the cell surface permit neurite outgrowth (Tiveron et al., 1992; Mahanthapa and Patterson, 1992).

Thy-1 protein expression in the cortex increases postnatally through P12 (Xue and Morris, 1992). Although later postnatal data is not available for cortex specifically, whole brain levels of Thy-1 continue to rise until P40 or later (Schachner and Hämmerling, 1974; Barclay, 1979). Because of its putative role in the cessation of neurite outgrowth, we tested whether mice lacking the

gene for Thy-1 could undergo ocular dominance plasticity, and whether this plasticity, if present, was confined to the normal critical period.

A total of 4 Thy-1-deficient (Thy-1$^-$) and 6 genetic background-matched heterozygous mice were studied blind to genotype[1]. Visual responses in V1 neurons were grossly normal. Mean receptive field sizes for V1 neurons in 2 Thy-1$^-$ and 4 hetererozygous mice are shown in Figure 4-1A (RF data from the other two mice were unusable). The RF size distributions from each animal were overlapping, and the overall means for Thy-1$^-$ and hetererozygous receptive fields were not significantly different (t-test: $p > 0.4$, $n = 49$ and 86 neurons for Thy-1$^-$ and hetererozygous mice, respectively). Retinotopy was also intact in these animals (Figure 4-1B), as illustrated by the high correlation coefficients of the linear regressions of receptive field center azimuth on electrode position (Figure 4-1B, inset; t-test: $p > 0.3$, $n = 3$ and 5 regressions from 2 Thy-1$^-$ and 3 hetererozygous mice, respectively).

We examined ocular dominance plasticity in Thy-1$^-$ mice both during and after the end of the critical period. The CBIs of nondeprived and contralateral eye MD hemispheres are shown in Figure 4-2. Nondeprived CBIs for both the Thy-1$^-$ mice and the heterozygote controls were in the normal range, between 0.6 and 0.7. Responses of V1 neurons from mice of

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[1] Thy-1$^-$ mice were generated by Y. Tokugawa, J. Silver and C. Stewart. A paper describing the construction and initial characterization of these animals is currently being prepared (R. J. Morris et al., in preparation). The Thy-1$^-$ mice were bred from homozygous stocks, which necessitated the use of genetic background-matched controls. Both Thy-1$^-$ and heterozygous mice were bred on a pure 129 background and obtained from R.J. Morris at the National Institute for Medical Research, London.

both genotypes deprived for 4-6 days beginning at P28 (the peak of the critical period in normal C57Bl/6 mice) shifted toward the open eye. All the resulting CBIs were within the range of those resulting from 4 day deprivations in normal mice described in Chapter Two. Neuronal responses in animals deprived for 4-5 days beginning at P52 (well after the critical period delineated in Chapter Two) failed to shift towards the open eye, although the heterozygote deprived for 6 days beginning at P52 showed a small shift.

A quantitative analysis of responses or receptive field characteristics was not performed. Moreover, a non-essential role for Thy-1 in the development or plasticity of V1 reponses cannot be ruled out. In fact, one of the two Thy-1$^-$ mice deprived during the critical period showed a small shift, suggesting there might be a quantitative effect of the mutation on plasticity (Figure 4-2). Nonetheless, these data demonstrate that despite a complete lack of Thy-1 protein, Thy-1$^-$ mice develop grossly normal visual responses and retain MD-induced plasticity confined to the normal critical period.

Fyn-deficient mice

The cytosolic protein tyrosine kinase Fyn is widely expressed in the central nervous system during development (Maness, 1992). Fyn expression and activity begins prenatally and rises throughout the first several weeks postnatally (Inomata et al., 1994). The function of Fyn in neurons is presently unknown, although its expression in growing and plastic axon tracts is

suggestive of a role in these processes (Bare et al., 1993; Yagi et al., 1993). Protein tyrosine phosphorylation has been shown to be stimulated by NMDA receptor activation (Bading and Greenberg, 1991), and tyrosine kinase inhibitors block induction of hippocampal LTP (O'Dell et al., 1991), suggesting a possible role for these signalling molecules in neural plasticity.

The development of the hippocampus is disrupted in Fyn-deficient (Fyn^-) mice, resulting in the inability to induce LTP with tetanic stimulation, although LTP induction by a pairing protocol occurs normally (Grant et al., 1992). I examined visual cortical responses and plasticity in Fyn^- mice to determine if the development of the visual cortex was also disrupted. Because of the timing of the availability of these animals, I was able to record from only 2 Fyn^- mice and one wild type littermate[1]; all three animals were deprived for four days during the critical period. Deprivation and physiological procedures were carried out as described in Chapter One. These experiments were not done blind to genotype.

Visual responses and plasticity in V1 neurons were grossly normal in the two Fyn^- mice examined. The average receptive field size for the two Fyn^- and one control animals were within the range of normal mice and are shown in Figure 4-3A. The mean RF size in the Fyn^- mice was larger than that in the control animal (t-test, $p < 0.005$) , but the variability in the quality of responses from animal to animal makes comparisons between groups of

[1] Fyn^- mice were generated as previously described (Grant et al., 1992) and obtained from S.G.N. Grant and E.R. Kandel at Columbia University.

one and two misleading. Retinotopy was conserved in the Fyn⁻ mice, as shown in Figure 4-3B, although this parameter was tested in only one animal. The responses of binocular zone neurons shifted towards the open eye in all three animals. The CBIs are plotted against the period of MD in Figure 4-4. These data suggest that visual cortical development and plasticity occur in the Fyn⁻ mice, although further study would be necessary to rule out quantitative abnormalities.

Protein Kinase Cγ-deficient mice

The many varieties of Protein Kinase C (PKC) are serine-threonine protein kinases which are activated by diacylglycerol in the presence of phospholipids (Schulman, 1991). Recent experiments have implicated this family of kinases in neural plasticity. PKC can directly potentiate NMDA currents (Ben-Ari et al., 1992). PKC inhibitors block LTP induction in the hippocampus (Malinow et al., 1989) and phorbol esters, which directly activate PKC, potentiate synaptic transmission (Malenka et al., 1986). In the cerebellum, LTD of the parallel fiber-Purkinje cell synaptse is blocked by PKC inhibitors and mimicked by phorbol esters (Linden and Connor, 1991).

At least 10 different PKC family members have been identified (Nishizuka, 1988). Many of these are expressed in brain. In particular, PKCγ is a neural-specific subunit that is expressed primarily postnatally in particularly

high levels in the hippocampus (Brandt et al., 1987; Huang et al., 1987, 1988). Furthermore, this isoform is sensitive to calcium, allowing for possible synergism of diacylglycerol and calcium second messenger systems, both of which might be involved in neuronal signalling (Nishizuka, 1988). These properties of the PKCγ isoform and the evidence linking PKC to LTP prompted a characterization of LTP induction in PKCγ-deficient ($PKC\gamma^-$) mice, which were found to require prior low-frequency synaptic stimulation in order to induce LTP with a tetanus (Abeliovich et al., 1993).

PKCγ is also expressed in the neocortex, primarily in layers I, II, V and VI (Tsujino et al., 1990). Although its developmental timecourse has not been characterized in neocortex, PKCγ activity in whole rat brain increases throughout the first four weeks of life, reaching its maximum right at the peak of the critical period for OD plasticity in that species (Hashimoto et al., 1988; Fagiolini et al., 1994). I therefore chose to investigate the role of PKCγ in ocular dominance plasticity.

A total of 10 mice were used for the current study, 5 $PKC\gamma^-$ animals and 5 genetic background- and age-matched wild type controls[1]. Four day deprivations were begun at P24, P26 and P28 in one mouse of each genotype at

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[1] $PKC\gamma^-$ mice were obtained from C. Chen and S. Tonegawa at MIT. The details of their generation have been described (Abeliovich et al., 1993a, 1993b). Since the mice were bred from homozygous mutant stocks, genetic background- and age-matched control animals were used instead of littermates. The mice were bred on a C57Bl/6 x 129 F1 background.

each age. Nondeprived animals were recorded at P38 to P42. Deprivation and physiological procedures were carried out as described in Chapter One. These experiments were performed blind to genotype.

Visual cortical responses were grossly normal in the PKCγ⁻ mice. The receptive fields of V1 neurons in the mutants were the same size as those in the WT mice (t-test: $p > 0.5$; Figure 4-5A). The retinotopic relationship between the visual field and the visual cortex was preserved in the PKCγ⁻ mice, as evidenced by the linear progression of receptive field center azimuths as the electrode was moved across V1 (Figure 4-5B). The correlation coefficients of 4 and 5 linear regressions in control and PKCγ⁻ mice were uniformly high (mean ± s.d. 0.96 ± 0.03 and 0.98 ± 0.02, respectively; t-test: $p > 0.3$; Figure 4-5B inset).

Monocular deprivation caused a profound and equal shift in the responses of neurons in the contralateral binocular zones of both mutant and control V1 (Figure 4-6). The OD distributions of nondeprived hemispheres in PKCγ⁻ and WT mice were similar, as shown by their similar CBIs. The CBI means were not significantly different from each other (t-test: $p = 0.2$). Four days of MD shifted the OD distributions of binocular zone neurons toward the open ipsilateral eye in both WT and PKCγ⁻ mice, demonstrated by the decreased CBI in both groups of deprived animals. The mean CBIs of WT and PKCγ⁻ deprived animals were not significantly different from each other ($p =$

0.4), while both were significantly different from the mean CBI of nondeprived animals of the appropriate genotype ($p < 0.005$ for both comparisons).

These data strongly argue against a requirement for PKCγ in visual cortical development and ocular dominance plasticity. However, the tremendous diversity of PKC isoforms precludes a more general conclusion regarding a role for phospholipid-dependent kinases. Indeed, several other known isoforms share properties with PKCγ that make them candidates for involvement in neuronal plasticity as well, including calcium dependence (Nishizuka, 1988) and neocortical or hippocampal expression (Brandt et al., 1987; Huang et al., 1987, 1988; Tsujino et al., 1990). The expression of these other isoforms in cortex also allows for the possibility of compensation and/or redundancy in these mutants. Thus, the activity of PKCγ might normally contribute to OD plasticity, but in the absence (throughout ontogeny) of the γ-subunit, one of the other subunits may be able to substitute. These caveats do not detract from the definitive finding that PKCγ itself is not required for OD plasticity, but rather point out that future studies of these and other mutants, including double mutants where feasible, are warranted.

Protein Kinase A $C\beta_1$-deficient mice

Considerable evidence exists from many systems linking cAMP-dependent processes to neural plasticity (Frank and Greenberg, 1994; Kandel and Abel, 1994). Signalling via cAMP-dependent Protein Kinase A (PKA) has been shown to modulate hippocampal neuron glutamate responses (Wang et al., 1991, 1993; Greengard et al., 1991). When applied to hippocampal slices *in vitro*, PKA inhibitors block LTD, mossy fiber LTP and a late phase of CA1 LTP (Weisskopf et al., 1994; Huang et al., 1994a, 1994b; Brandon et al., 1995b).

Each PKA holoenzyme is composed of two regulatory and two catalytic subunits. In the mouse, two catalytic subunits and four regulatory subunits have been cloned and characterized: Cα, Cβ, RIα, RIβ, RIIα and RIIβ. Each α-subunit is expressed in numerous tissues, while each β-subunit is primarily expressed in neural tissue (Cadd and McKnight, 1989). In particular, expression of the Cβ-subunit is high in neocortex, is turned on late during gestation, and is expressed in neurons but not glia (Cadd and McKnight, 1989; Massa et al., 1991; Shuntoh et al., 1992). I therefore chose to examine the role of the Cβ-subunit of PKA in ocular dominance plasticity.

A total of 6 animals were used in the current study, 2 PKA $C\beta_1$-deficient ($C\beta^-$) mice and 4 genetic background- and age-matched control animals[1] . All

[1] $C\beta_1^-$ mice were obtained from E. P. Brandon, R. L. Idzerda and G. S. McKnight at the University of Washington Medical School. A paper

animals were monocularly-deprived for 4 days beginning between P25 and P27. Recordings were obtained from the primary visual cortex contralateral to the deprived eye; methods were as described in Chapter Two. These experiments were conducted blind to genotype.

Visual cortical responses were grossly normal in the Cβ⁻ mice. The RF size distributions in the 2 Cβ⁻ animals overlapped those in the 4 controls, although the mean RF size for Cβ⁻ mice was slightly higher than that in the WT animals (t-test, $p = 0.04$; Figure 4-7A). Retinotopy was equally precise in Cβ⁻ animals and controls (Figure 4-7B). The correlation coefficients of the regression of RF center azimuth on electrode position were 0.89 for one series of penetrations from a Cβ⁻ animal and 0.95 and 0.98 for each of two series from a WT control.

Monocular deprivation resulted in a shift in the ocular dominance distribution of binocular zone neurons towards the ipsilateral, open eye. Figure 4-8 shows the CBIs calculated for each of the 6 deprived animals plotted vs. the period of deprivation. The CBIs from the two deprived Cβ⁻ animals demonstrated that MD had its normal, complete effect in these animals.

After these experiments were completed, work from the McKnight laboratory demonstrated that there are three Cβ isoforms, produced from

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describing their construction and initial characterization is currently in press (Qi et al., 1996). $C\beta_1^-$ mice are bred from homozygous stocks, necessitating the

splice variants transcribed from the same gene (Qi et al., 1996). The Cβ$^-$ animals are lacking only one of the three variants, Cβ1.These data strongly argue against a requirement for PKA $C\beta_1$ in visual cortical development and ocular dominance plasticity. The existence of additional PKA catalytic subunit isoforms precludes ruling out a role for PKA-mediated processes in general. In particular, the other Cβ isoforms and the Cα subunit are all expressed in the neocortex (Cadd and McKnight, 1989; Massa et al., 1991; Qi et al., 1996), allowing for the possibility of functional redundancy or compensation. Thus, the activity of $C\beta_1$ might normally be involved in OD plasticity, but in the absence (througout ontogeny) of the β_1-subunit, other subunits might substitute. However, these data demonstrate that $C\beta_1$ itself is not required for OD plasticity. As is the case for the PKCγ experiments, additional studies are warranted; most interesting would be a an animal lacking all three Cβ isoforms or a Cα/Cβ double knockout animal.

α-Calcium/Calmodulin-dependent Protein Kinase II-deficient mice

Calcium/calmodulin-dependent protein kinase II (CaMKII) is one of a family of serine/threonine kinases activated by elevated calcium complexed to its intracellular receptor, calmodulin (Colbran et al., 1989; Braun and

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use of genetic background-matched non-littermate controls.

Schulman, 1995). It is distinguished from other calcium/calmodulin-dependent kinases by its broad spectrum of substrates, including proteins that could play a role in neuronal plasticity such as the AMPA-type glutamate receptor and CREB (Sheng and Greenberg, 1990; McGlade-McCulloh et al., 1993; Braun and Schulman, 1995).

Evidence for a role for CaMKII in neural plasticity comes mainly from studies on long-term potentiation and depression *in vitro*. In the hippocampus inhibitors of CaMKII block LTP induction (Malinow et al., 1989). In the visual cortex, stimulation which normally induces LTP causes LTD instead in the presence of a CaMKII inhibitor (Funauchi et al., 1992). More recently, intracellular injection of active CaMKII has been shown to potentiate synaptic responses (Shirke and Malinow, 1995; Malenka et al., 1995). CaMKII can directly phosphorylate AMPA receptors, resulting in increased responses to glutamate in cultured hippocampal neurons (McGlade-McCulloh et al., 1993), suggesting a possible mechanism for a postsynaptic enhancement of synaptic transmission following CaMKII activation. Indeed, a feasible model for long-term memory storage has been proposed based on the ability of CaMKII to permanently activate itself constitutively via autophosphorylation (Lisman and Goldring, 1988).

There are at least four different classes of CaMKII proteins, each encoded by a separate gene; each class is composed of several splice variants (Braun and Schulman, 1995). The active enzyme is a complex multimer of many subunits, and it is likely that a given multimer contains more than one

type of subunit. The α-isoform (αCaMKII) is predominantly expressed in the adult forebrain, and is higly expressed in the hippocampus and neocortex (Burgin et al., 1990); in hippocampus, αCaMKII makes up as much as 1.4% of the total protein, and is the major component of the postsynaptic density (Erondu and Kennedy, 1985; Godenring et al., 1984; Kelly et al., 1984).

αCaMKII is well-situated to play a role in ocular dominance plasticity. Neocortical αCaMKII expression is first evident postnatally, and grows throughout the first 3-4 weeks of postnatal life, leveling off into adulthood (Kelly et al., 1987; Burgin et al., 1990). Monocular deprivation increases immunocytochemical staining for CaMKII in deprived-eye columns of macaque V1 (Hendry and Kennedy, 1986), demonstrating that expression of the enzyme can be altered by visually-evoked activity. Finally, the α-subunit has been directly implicated in plasticity and learning. Mice deficient in αCaMKII show hippocampal and neocortical LTP deficits as well as spatial learning deficits (Silva et al., 1992a, 1992b; Kirkwood and Bear, 1994c). In light of this evidence, I chose to examine visual cortical responses and plasticity in mice with a targeted deletion of the gene for αCaMKII.

A total of 39 mice were used for this study: 18 homozygote animals deficient in αCaMKII (CK^-), and 11 wild-type (WT) and 10 heterozygous (Het)

littermate controls[1]. Methods were as described in Chapters Two and Three. For monocularly-deprived animals, deprivation was begun between P26 and P33. Recordings were obtained from the primary visual cortex contralateral to the deprived eye. These experiments were conducted blind to genotype.

Visual cortical responses were grossly normal in the CK$^-$ mice. The distributions of receptive field sizes in 17 CK$^-$ and 9 WT mice were overlapping (Figure 4-9A). Overall, the mean RF size of neurons recorded from CK$^-$ mice was slightly smaller than that of neurons recorded from WT animals ($p = 0.2$; $n = 521$ and 327 RFs from CK$^-$ and WT animals, respectively; Figure 4-9B). Retinotopy is intact in the CK$^-$ animals, as shown by the linear relationship between RF center azimuth and electrode position (Figure 4-10). Correlation coefficients of the regressions of RF center azimuth on electrode position were equally high in CK- and WT controls ($p > 0.5$; Figure 4-10, inset). The OD distributions of binocular zone neurons were not different between the CK- and control mice (Figure 4-11A & B).

As an initial attempt to determine whether ocular dominance plasticity was normal in these mutants, I monocularly-deprived 8 CK$^-$ animals and 8 WT or Het controls for 4 days starting between P27 and P32. Summary OD histograms are shown in Figure 4-11; mean and individual CBIs from these experiments are shown in Figure 4-13. MD resulted in significant shifts in the ocular dominance distributions of binocular zone neurons in all 8 control

[1] CK$^-$ mice were generated as described (Silva et al., 1992a, 1992b). CK$^-$ mice and WT and Het littermate controls were obtained from A. Silva at Cold Spring Harbor Laboratory. The mice were bred on a C57Bl/6 x 129 background.

animals. No difference was evident between Het and WT animals (Figure 4-11C vs D).

In contrast, the CK$^-$ animals demonstrated a markedly variable response to 4 days of MD. OD histograms compiled individually for each of the 8 deprived CK$^-$ mice are shown in Figure 4-12. Although a shift in the distribution towards the open ipsilateral eye is evident in each histogram, 4 of the animals appear to have shifted to a much smaller degree than any of the control animals. The summary OD distribution for the 4 partially shifted CK$^-$ animals is shown in Figure 4-11E; this distribution is significantly less shifted than that of the fully shifted CK$^-$ animals shown in Figure 4-11F (chi-square test, $p < 0.0005$) and more shifted than that of nondeprived CK- animals ($p < 0.01$). These data suggest that a subset of the CK$^-$ animals shift less in response to 4 days of MD.

The individually calculated CBIs from the deprived CK- animals show the variable effect of deprivation in these mutants more dramatically (Figure 4-13). The 4 partially shifted animals have CBIs which fall clearly outside the distribution of deprived control animals, demonstrating a blunted effect of MD in these animals. I attempted to determine if longer deprivations would overcome this blunting effect by depriving an additional 5 CK$^-$ and 3 control animals for longer durations. As shown in Figure 4-13A, control mice deprived for more than 4 days show no greater shift than do mice deprived for 4 days (see also Chapter Two). The data from the CK- were ambiguous. Two of 3 CK$^-$ animals deprived for 6-10 days shifted toward the open

eye to an extent intermediate between the fully and partially shifted 4 day animals. Both CK$^-$ mice deprived for more than 10 days shifted to the same extent as 6-10 day-deprived control animals. Although the small sample size precludes a definitive characterization of the deficit, these data are consistent with the idea that the αCaMKII-deficiency slows plasticity in a subset of CK$^-$ animals.

Table 4-1: CBIs from identically deprived CK$^-$ and control mice.

Deprivation Period	CK$^-$	WT/Het
p26-p32	0.58	0.51
p27-p31	0.58	0.45
p28-p32	0.36	0.50
p29-p33	0.56	0.36
p32-p36	0.63	0.47
p33-p37	0.67	0.56
p30-p37	0.52	0.38

The 4-day deprivations described above were begun between P27 and P32. It was conceivable, therefore, that the decreased shift seen in some CK$^-$ animals was due to precisely when these animals were deprived. A careful reexamination of these and other data suggest otherwise, however. When the effects of 4-days of deprivation are examined separately for those mice deprived starting in earlier (between P27 and P29) or later (P30 - P32) epochs, it is clear that at least one CK$^-$ animal in each epoch failed to shift completely

(Figure 4-13B). Considering both short- and long-term deprivations together, an analysis of 7 pairs of CK^- and control animals deprived for identical periods of time starting on the exact same postnatal day supports the hypothesis that CK- shift less for a given deprivation. In 6 out of the 7 pairs, the CK^- member of the pair shifted less (Table 4-1). For these 7 pairs, the CK^- animals were on average significantly less shifted than their identically deprived WT or Het controls (paired t-test, $p < 0.04$).

An apparently smaller shift might have been obtained artifactually if recordings were made from neurons with receptive fields located more peripherally in the visual field in a subset of the CK^- mice. This possibility follows from the finding that neurons with more centrally located RFs tend to shift more towards the open ipsilateral eye (see Figure 2-3). To rule out this possibility, I examined the relationship between OD score and receptive field location in partially and fully shifted CK^- animals deprived for 4 days (Figure 4-14). In both groups, the relative degree of shift was consistent across all azimuths within the binocular zone, demonstrating that differences in RF location of recorded neurons cannot account for the variability of MD effects in CK^- mice.

Other sources of variability considered included RF size, visual responsivity, genetic background, and gender. There were no consistent differences between fully and partially shifted animals across these parameters. An additional possiblity was that the less shifted animals were less active; although a quantitative analysis has yet to be completed, all the

CK^- animals were quite active both in their home cages and in open field tests; indeed, CK^- animals tend to be more active in open-field tests than controls (A.J. Silva, unpublished observations).

It appears, therefore, that ocular dominance plasticity is reduced in a subset of CK^- animals. The finding that longer deprivations result in complete shifts suggests that the effect of a lack of αCaMKII is to slow but not eliminate plasticity in these animals. Why the effect of a targeted deletion of αCaMKII on OD plasticity should be variable is not immediately obvious. It is possible that compensatory mechanisms might fully rescue the deficit in some but not all animals. As a hybrid genetic background was used in these experiments, it is also possible that some independently segregating locus compensates for the lack of αCaMKII.

Alternatively, the αCaMKII deficiency might uniformly slow plasticity in all animals, but a threshold effect controlling the extent of the shift results in an all-or-none effect. Four days of deprivation in these animals would then put the state of the cortex very near the threshold for a complete shift towards the open eye, such that some animals exceed that threshold and shift fully, while others fail to exceed threshold and do not shift. One would then expect to see a similar bimodal distribution of the degree of shift in normal animals deprived for some shorter period, an effect which I have not seen in the mouse but that has recently been seen for 2-day MD in rats (T.K. Hensch, M. Fagiolini and M.P. Stryker, unpublished observations).

Regardless of what causes it, the variability of the effects of MD in CK mice strongly argues for a role for CaMKII in OD plasticity. These data fit well with models of correlation-based plasticity relying on calcium influx through the NMDA receptor as a coincidence detector (Collingridge and Bliss, 1987), and the more controversial evidence for a specific role of the NMDA receptor in OD plasticity (Kleinschmidt et al., 1987; Bear et al., 1990). Nonetheless, the fact that CK$^-$ mice can still undergo OD plasticity proves that that of αCaMKII in particular is not absolutely required for plasticity. Several other isoforms of CaMKII and other mutifunctional calcium/calmodulin-dependent protein kinases, as well as other calcium-modulated kinases such as PKC and PKA, are expressed in visual cortex during the critical period (Burgin et al., 1987; Huang et al., 1987, 1988; Cadd and McKnight, 1989; Thiagarajan et al., 1994; Ikeda and Schulman, 1995). Whether any of these molecules are responsible for the residual ability of CK$^-$ animals to respond to MD remains to be seen.

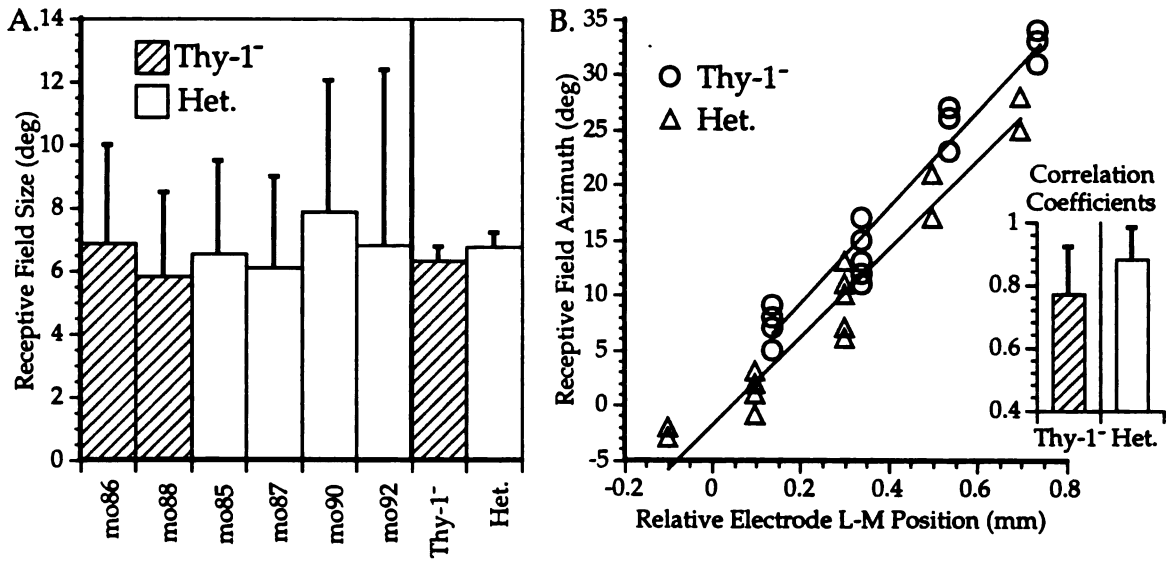

Figure 4-1. Receptive field size and retinotopy in Thy-1-deficient mice. *A*, Receptive field size from Thy-1⁻ (*hatched bars*) and heterozygous control (*open bars*) mice. Mean ± s.d. shown for individual mice (n = 19 to 25 RFs/animal,*mo86* - *mo92*). Mean ± SEM shown for totals (n = 40 and 86 RFs for *Thy-1⁻* and heterozygous [*Het.*] mice, respectively). *B*, Retinotopy in Thy-1⁻ (*circles*) and heterozygous control (*triangles*) mice. Receptive field center azimuths of cells encountered in a series of penetrations spaced across the lateromedial extent of V1 are plotted vs. electrode position. Lines are linear regressions of azimuth on position. *Inset*, mean ± s.d. correlation coefficient of 3 and 4 such regressions from Thy-1⁻ (*hatched bar*) and heterozygous control (*open bar*) mice, respectively.

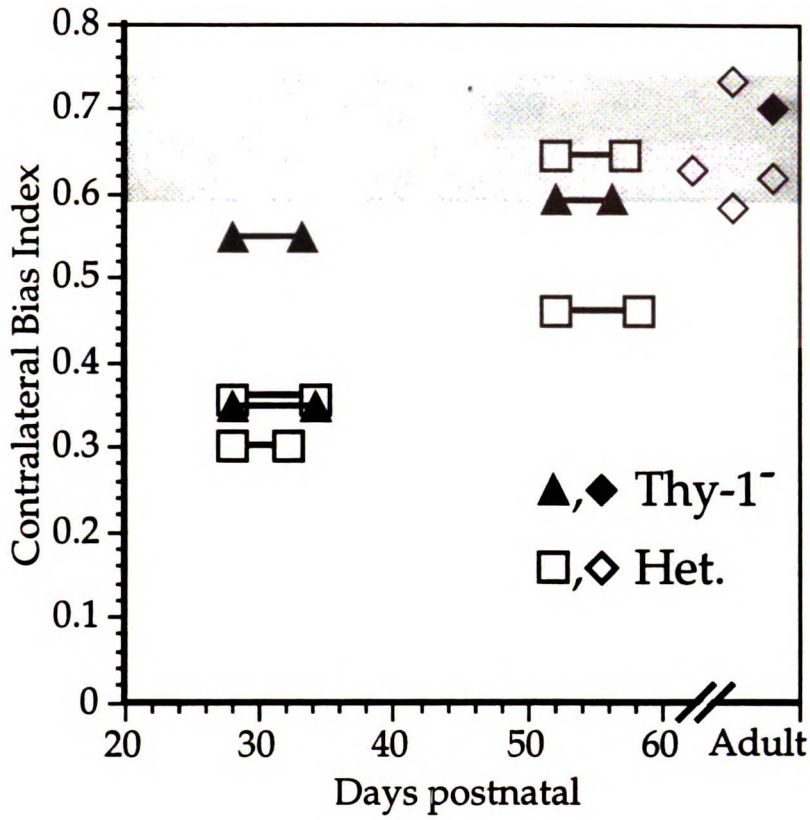

Figure 4-2. Effects of monocular deprivation in Thy-1-deficient mice. The CBIs from OD distributions of neurons recorded contralateral to the deprived eye are plotted vs. the period of deprivation. Each pair of symbols connected by a line represents a single animal deprived for the period indicated by the length of the line: *triangles*, deprived Thy-1⁻ mice; *squares*, deprived heterozygotes (n = 18-25 cells/animal). Individual*open diamonds* are CBIs for 2 hemispheres each from 2 nondeprived heterozygous controls (n = 19-36 cells/hemisphere). The *closed diamond* is the CBI from one hemisphere of a nondeprived Thy-1⁻ mouse (n = 30 cells).

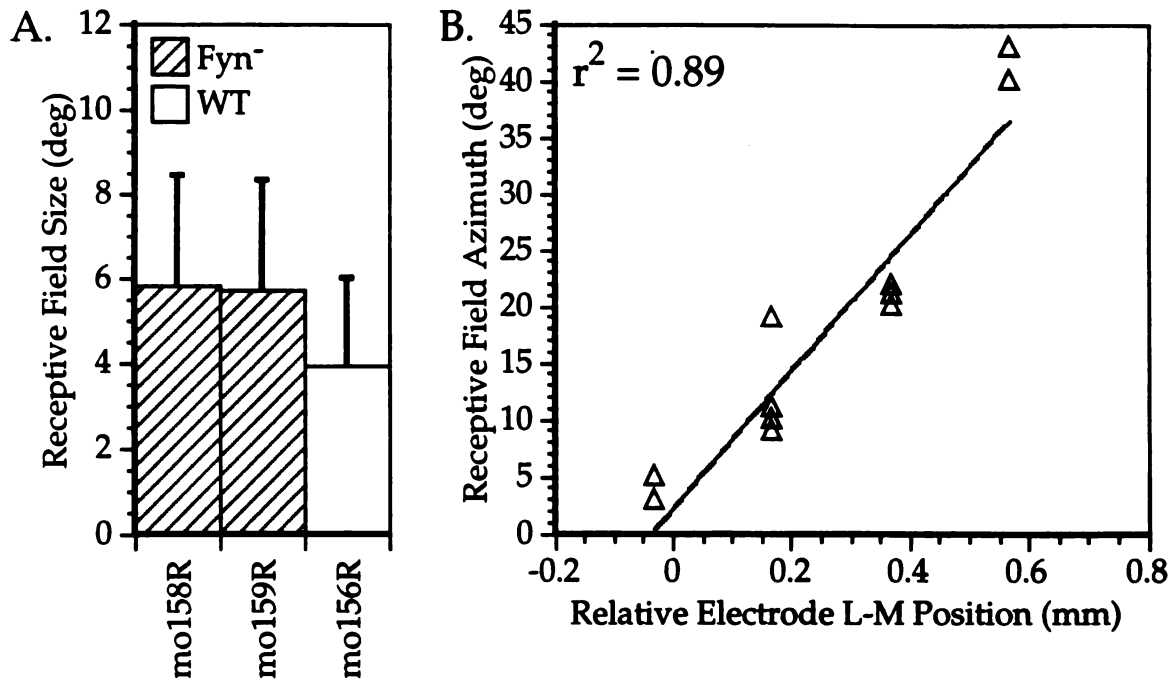

Figure 4-3. Receptive field size and retinotopy in Fyn-deficient mice. *A*, Mean ± s.d. RF size from 2 Fyn⁻ mice (*hatched bars*) and 1 WT littermate (*open bar*). n = 24-35 RFs/animal. *B*, Receptive field center azimuth for cells encountered in a series of penetrations spaced across the lateromedial extent of V1 from a Fyn⁻ mouse, plotted against electrode position. r^2, correlation coefficient of the linear regression of azimuth on position.

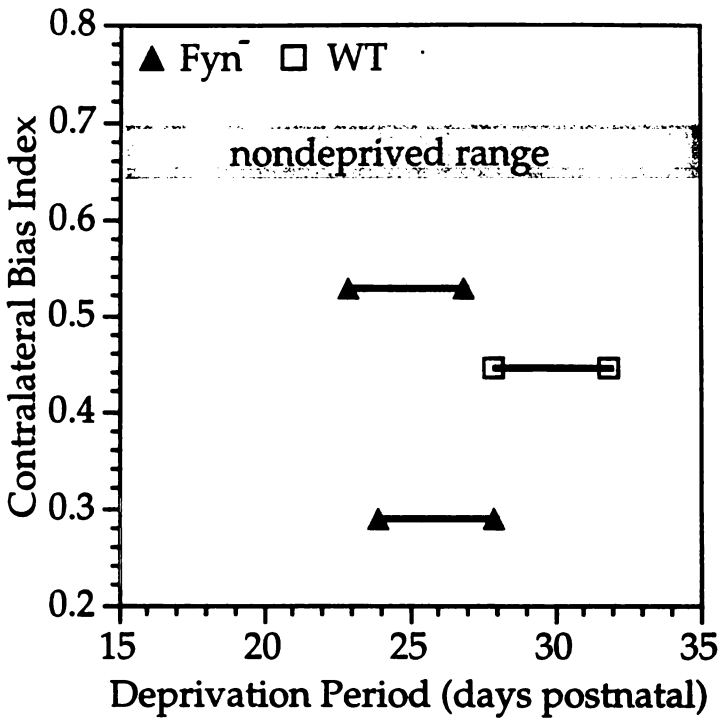

Figure 4-4. Effects of monocular deprivation in Fyn-defiencient mice.

Conventions as in Figure 4-2. *Triangles,* Fyn$^-$ mice. *Square,* WT littermate.

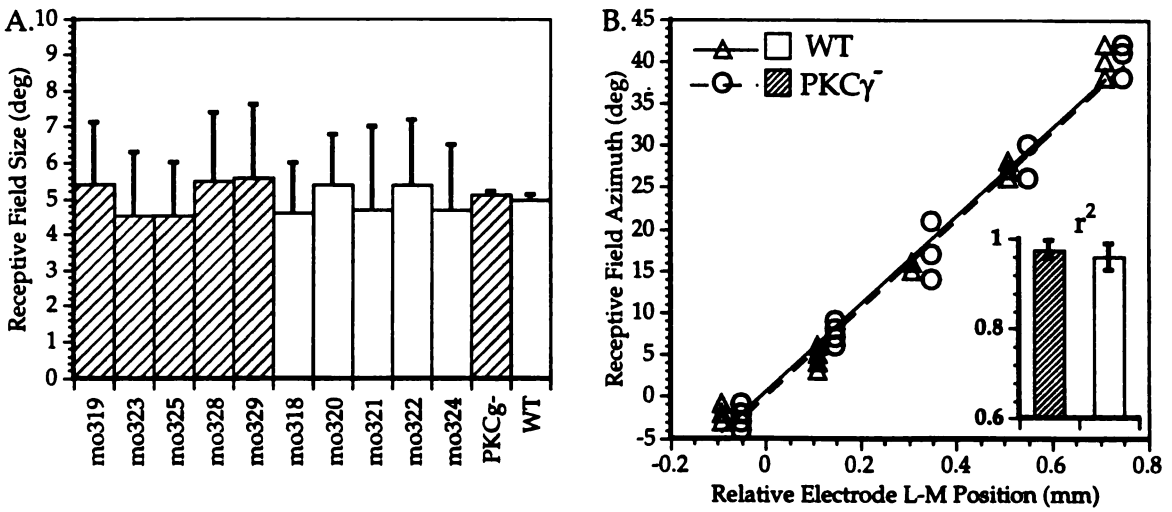

Figure 4-5. Receptive field size and retinotopy in PKCγ-deficient mice. *A,* Receptive field size in PKCγ (*hatched bars*) and WT mice (*open bar*). Mean ± s.d. shown for individual mice (n = 28-80 RFs/animal;*mo318-mo329*). Mean ± SEM shown for totals (n = 207 and 199 RFs for *PKCγ* and *WT* mice, respectively). *B,* Retinotopy in PKCγ (*circles*) and WT (*triangles*) mice. Receptive field center azimuths of cells encountered in a series of penetrations spaced across the lateromedial extent of V1 are plotted vs. electrode position. Lines are linear regressions of azimuth on position. *Inset,* mean ± s.d. correlation coefficients of 5 and 4 such regressions from PKCγ (*hatched bar*) and WT (*open bar*) mice, respectively.

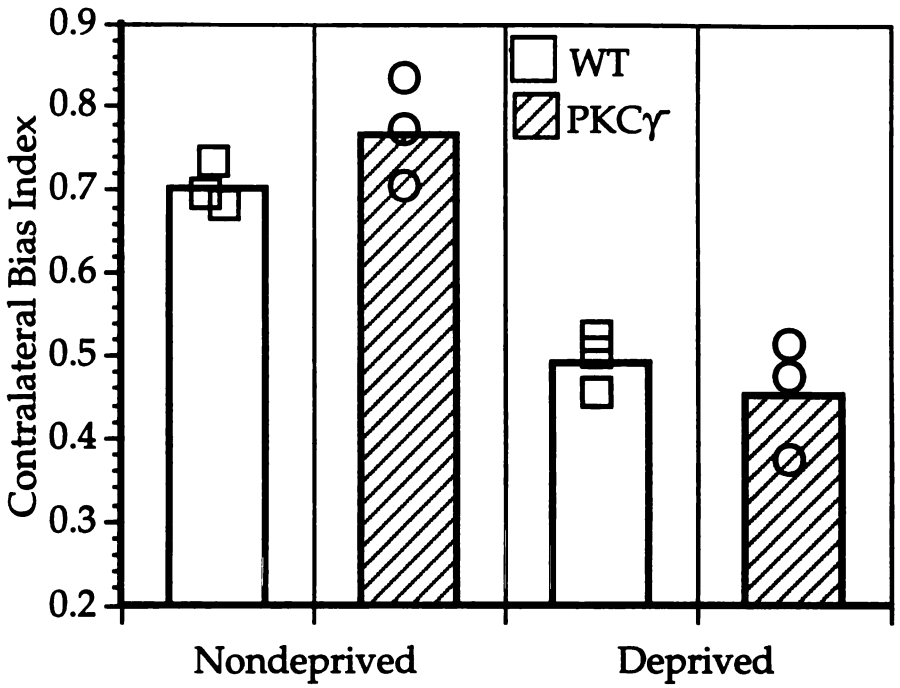

Figure 4-6. Effects of monocular deprivation in PKCγ-deficient mice. CBIs from individual hemispheres (*symbols*) are shown along with the mean CBIs for each condition (*bars*). N = 3 hemispheres from 2 *nondeprived* animals of each genotype, and N = 3 hemispheres from 3 *deprived* animals of each genotype. n = 23-36 cells/hemisphere. *Hatched bars*, PKCγ mice; *open bars*, WT mice.

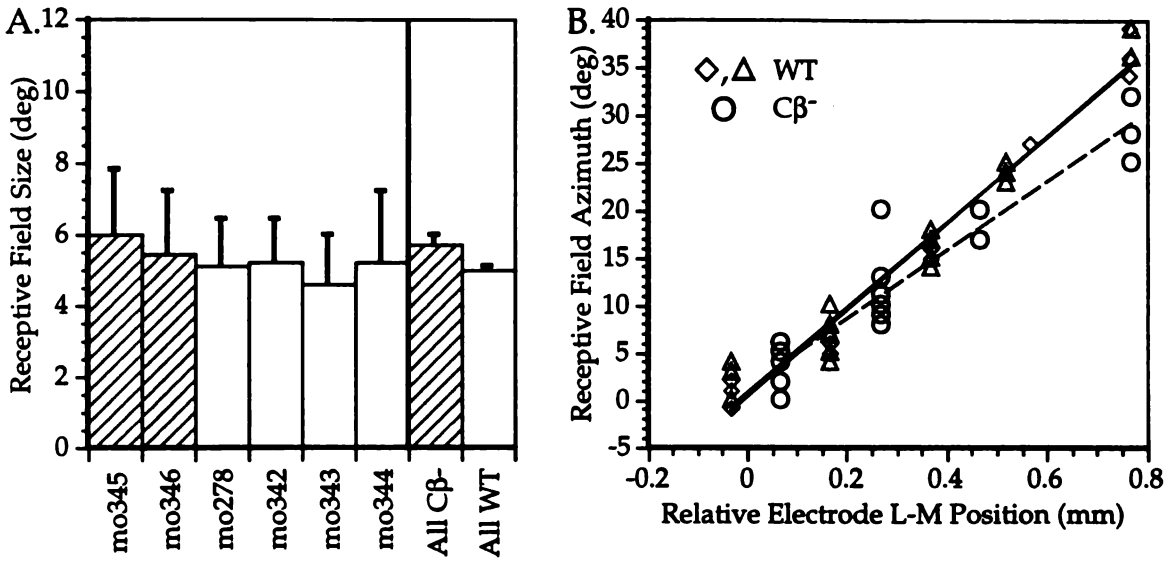

Figure 4-7. Receptive field size and retinotopy in PKA $C\beta_1$-deficient mice. *A*, Receptive field size in $C\beta^-$ (*hatched bars*) and WT mice (*open bars*). Mean ± s.d. shown for individual mice (n = 22-39 RFs/animal;*mo278-mo344*). Mean ± SEM shown for totals (n = 50 and 108 RFs for *Cβ⁻* and *WT* mice, respectively). *B*, Retinotopy in $C\beta^-$ (*circles*) and WT (*triangles, diamonds*) mice. Receptive field center azimuths of cells encountered in a series of penetrations spaced across the lateromedial extent of V1 are plotted vs. electrode position. Lines are linear regressions of azimuth on position. The correlation coefficients are given in the text.

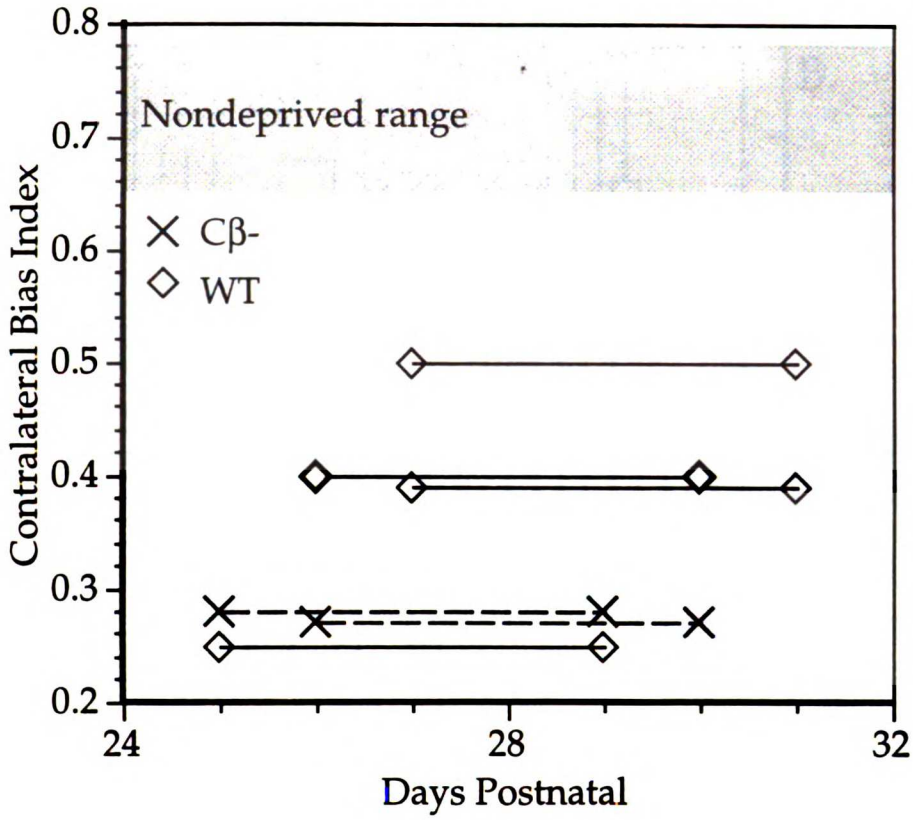

Figure 4-8. Effects of monocular deprivation in PKA $C\beta_1$-deficient mice. Conventions as in Figure 4-2. *Crosses,* $C\beta^-$ mice. *Diamonds,* WT controls.

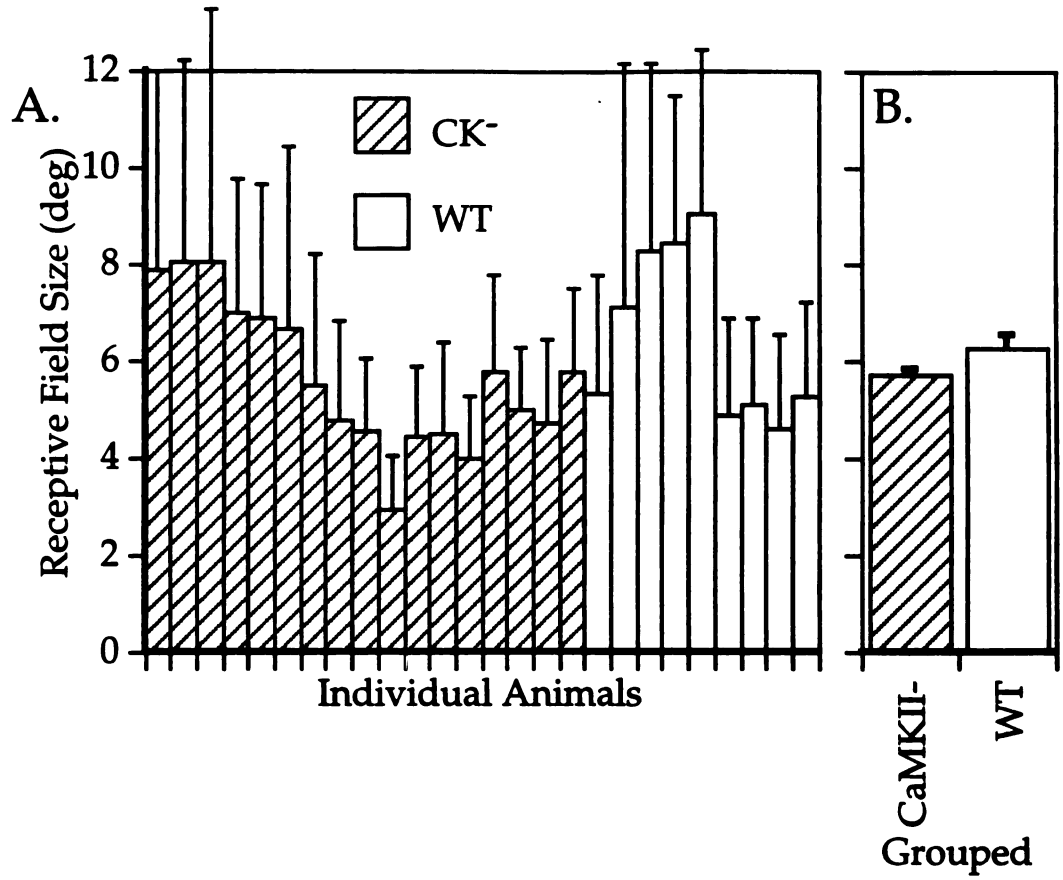

Figure 4-9. Receptive field size in αCaMKII-deficient mice. *A*, Receptive field size in individual CK⁻ (*hatched bars*) and WT mice (*open bars*). Mean ± s.d. shown (n = 23-49 RFs/animal). B, Mean ± SEM RF size for 17 CK⁻ and 9 WT mice. (n = 521 and 327 RFs for *CK⁻* and *WT* mice, respectively).

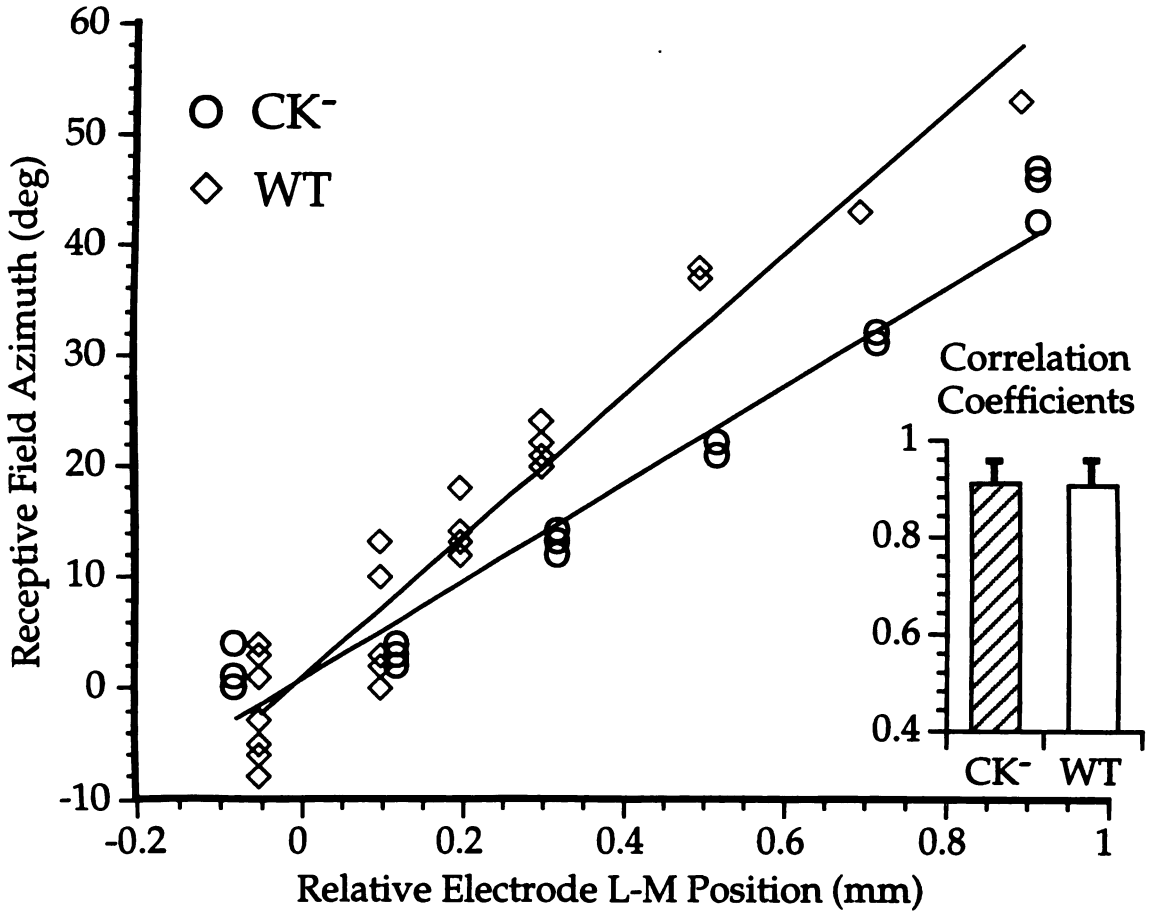

Figure 4-10. Retinotopy in αCaMKII-deficient mice. Receptive field center azimuths of cells encountered in a series of penetrations spaced across the lateromedial extent of V1 in one CK⁻ (*circles*) and one WT (*diamonds*) mice are plotted vs. electrode position. Lines are linear regressions of azimuth on position. *Inset,* mean ± s.d. correlation coefficients of 10 and 5 such regressions from CK⁻ (*hatched bar*) and WT (*open bar*) mice, respectively.

Figure 4-11. Ocular dominance distributions of deprived and nondeprived CK^- and control mice. Conventions as in **Figure 2-3**. *A*, *B*, OD distributions of nondeprived control (*A*) and CK^- (*B*) mice. n = 247 and 74 neurons from 6 (WT/Het) and 3 (CK^-) mice, respectively. *C*, *D*, OD distributions from 5 WT (C) and 3 Het (*D*) mice deprived for 4 days beginning between P27 and P32. Recordings were made contralateral to the deprived eye. n = 158 and 84 neurons for WT and Het distributions, respectively. *E*, *F*, OD distributions from 4 partially shifted (*E*) and 4 fully shifted (*F*) CK- mice deprived for 4 days beginning between P27 and P32. Recordings were made contralateral to the derpived eye. n = 106 and 111 neurons from partially shifted and fully shifted animals, respectively.

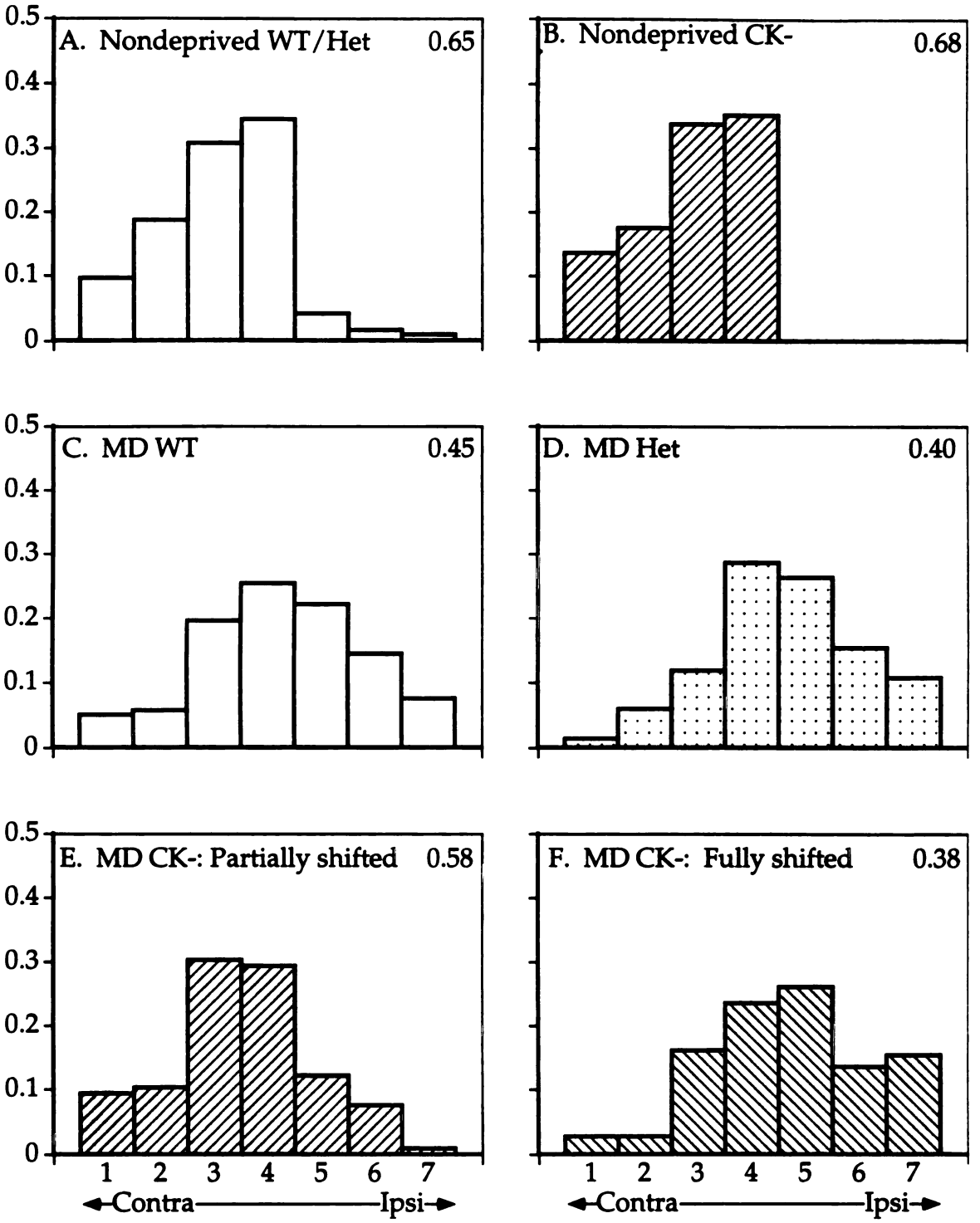

Figure 4-11. Ocular dominance distributions of deprived and nondeprived CK$^-$ and control mice.

Figure 4-12. Individual ocular dominance distributions of 8 CK^- mice deprived for 4 days starting between P27 and P32. Animals which demonstrated a shift towards the open eye equal to that seen in the control mice are shown in the right column (Fully shifted). Animals which shifted less are shown in the left column (Partially shifted). n = 22 to 34 neurons/distribution. All recordings were made contralateral to the deprived eye.

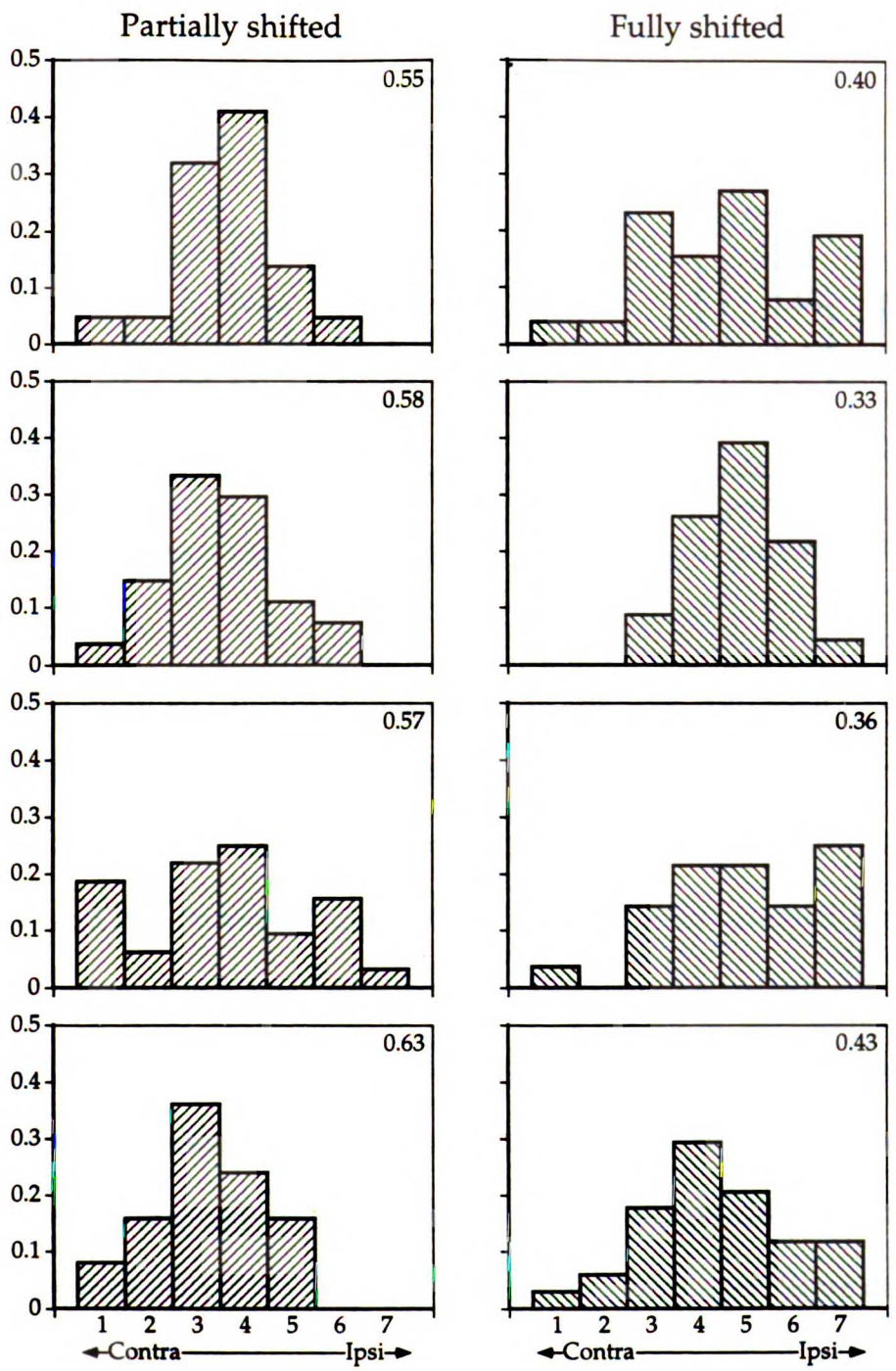

Figure 4-12. Individual ocular dominance distributions of 8 CK$^-$ mice deprived for 4 days starting between P27 and P32.

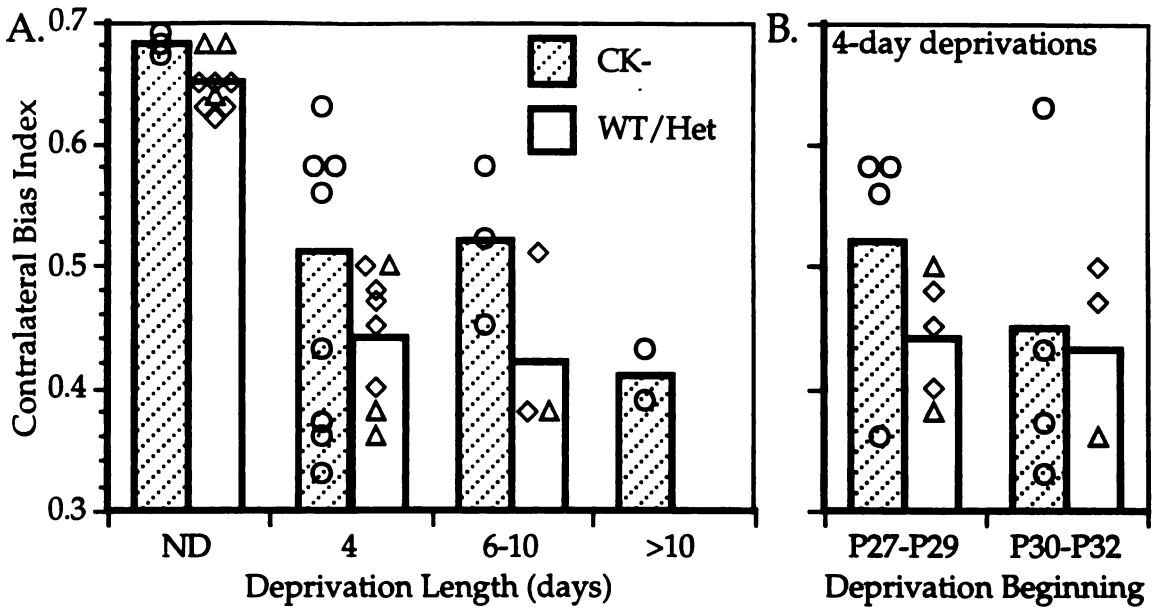

Figure 4-13. Summary of the effects of monocular deprivation in CK$^-$ mice. A, Effects of increasing the duration of the deprivation. Means (*bars*) and individual CBIs (*symbols*) are shown for CK- (*hatched bars, circles*), WT (*diamonds*) and Het (*triangles*) mice. WTs and Hets were grouped together for the control means (*open bars*). *ND,* nondeprived animals. Nondeprived and 4-day deprived animals are the same animals as in Figures 4-11 and 4-12. Longer deprivations were begun between P26 and P30. *B,* Effects of beginning the deprivations at particular ages. Means and individual CBIs for animals deprived for four days beginning between the indicated ages. Same data as in A, 4-day deprivations.

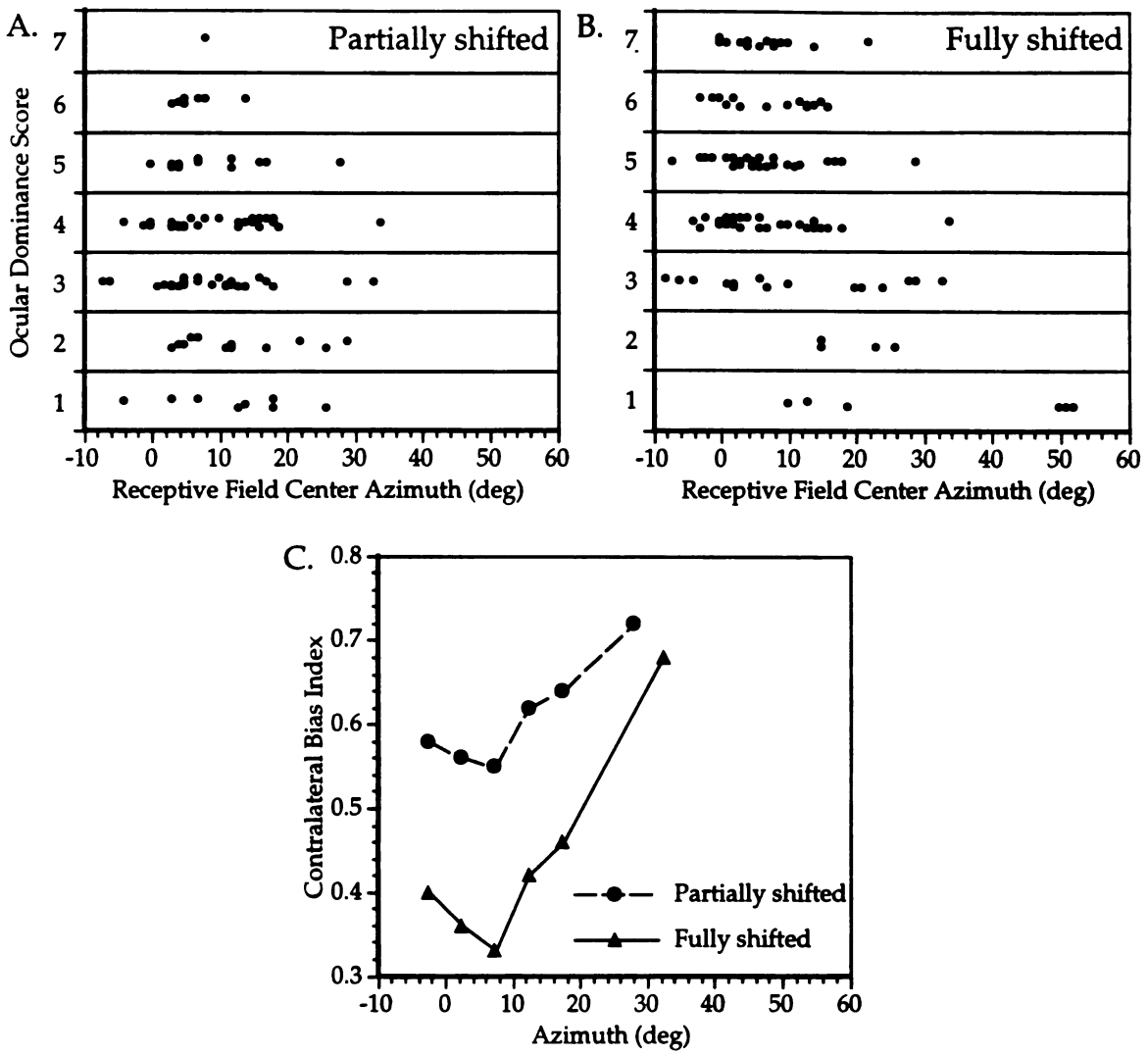

Figure 4-14. Ocular dominance as a function of receptive field position in CK- mice. *A, B,* OD scores of individual cells from 4 partially shifted (*A*) and 4 fully shifted (*B*) CK- mice deprived for 4 days plotted against RF center azimuth. Same animals as shown in Figures 4-11 through 4-13. OD scores from cells from the same animal are plotted along a horizontal line in the box with the appropriate OD score label. *C,* CBI vs. RF center azimuth. The CBIs were calculated separately for cells with RF center azimuths in the following ranges: -5 to 0, 1 to 5, 6 to 10, 11, to 15, 15 to 20, and greater than 20. *Circles,* partially shifted animals. *Triangles,* fully shifted animals.

CHAPTER FIVE:

Conclusions and Future Directions

The cellular mechanisms by which patterns of activity are transduced into patterns of connectivity in primary visual cortex are poorly understood. The current work seeks to make inroads into this unknown area by using the newly developed techniques of genetic engineering. I have demonstrated that in the mouse, as in other mammals, a competitive, correlation-based plasticity exists during a small time window in the development of the visual system. I have also used several mutant mouse lines to probe the role of putative molecular mediators of this plasticity. The data I have presented suggest that TBS-induced LTP and ocular dominance plasticity are separable phenomena, and that calcium/calmodulin-dependent protein kinase II may play a role in ocular dominance plasticity.

In addition to the previously discussed conclusions specific to individual chapters, there are several more general conclusions which deserve brief treatment here. First, the controlled aspects of the molecular lesions in mutant animals permit the comparison of identical manipulations on the ability to induce *in vitro* and *in vivo* plasticity, permitting direct tests of hypotheses raised by *in vitro* experiments. Second, the finding of multiple negative results suggests that OD plasticity may be a robust phenomenon that employs a variety of second messenger and other cellular systems, but that is dependent on no one particular system. Third, the finding that a subset of animals lacking the α-isoform of CaMKII have reduced plasticity in response to monocular deprivation suggests that the calcium/calmodulin second

messenger system would be an ideal starting point from which to begin investigating potential functional redundancy. Finally, the characterization of the mouse model for ocular dominance plasticity should enable the utilization of new and emerging technologies for genetic exploration of its underlying mechanisms.

in vitro/*in vivo* comparisons

The ability to compare the effects of a specific molecular intervention on ocular dominance plasticity and various cellular models thereof is one particularly powerful advantage of the mouse model. An increasingly large number of lines of mice with specific mutations are becoming available. As I have shown in Chapters Three and Four, testing the effects of monocular deprivation in these mutant lines permits a rapid assessment of *in vivo* plasticity in the presence of these lesions. As I have further demonstrated in Chapter Three, *in vitro* plasticity paradigms can be tested in mice with identical lesions. A single mutation was seen to have different effects on *in vivo* and *in vitro* plasticity. These dissociations would argue against a mechanistic link between these phenomena.

The advantages of in vitro and in vivo comparisons are not limited to wholesale dissociations, however, as illustrated by considering the case of the αCaMKII-deficient mice. Kirkwood and Bear (1994c) recently reported a severe but incomplete deficit in LTP in visual cortical slices from these animals.

They found a reduction in both the frequency of obtaining LTP and the magnitude of potentiation evoked by TBS in layer IV while recording field potentials in layer II/III. Nonetheless, in young animals, a statistically significant potentiation was obtained in most slices, leaving open the following question: what is more relevant to the biology of the system - the quantitative deficit, or the residual capability to potentiate?

Questions like these shade the interpretation of most *in vitro* plasticity paradigms, to the extent that they are meant to model the behavior of whole systems. In this case, an examination of ocular dominance plasticity in the CK^- animals provides an answer to the question of relevance at two different levels. At the level of plasticity in vivo, the data presented here suggest that the quantitative reduction in LTP is accompanied by a reduction in ocular dominance plasticity, at least in a subset of the CK^- mice. To explore this relationship further, it would be most informative to study LTP in visual cortical slices from the very same mice in which the effects of MD were measured, to see if there is an animal-by-animal correlation between the two deficits. At another level, OD plasticity itself is a model for developmental processes. The finding that CK^- mice, despite having reduced LTP and slowed plasticity, develop normal visual cortical responses, strongly argues that the finding most relevant to the organism is the residual capability for plasticity. The results from the CK^- studies suggest that if these processes really are involved in development, the system is robust enough to withstand severe (in the case of LTP) and/or subtle (in the case of OD plasticity) deficits.

Functional redundancy in developmental plasticity

The data accumulated so far argue very strongly that activity-dependent development of the visual system is indeed a robust phenomenon resistant to derangement. The data presented in Chapters Three and Four demonstrate that eliminating molecules which are normally highly expressed in the visual cortex and which are thought to play important signaling roles does not prevent plasticity or development. Two of the mutants are particularly informative in this regard. PKA RIβ-deficient mice show a complete lack of TBS-induced LTP, and still develop normally and show normal OD plasticity. Mice lacking αCaMKII also develop normally and retain plasticity, despite the fact that the α-subunit normally makes up nearly 1% of all protein in neocortex, and forebrain calcium/calmodulin protein kinase activity is reduced by nearly 50% in the knockout animals (Erondu and Kennedy, 1985, Silva et al., 1992a).

There are several possible reasons for the apparent resistance of visual system plasticity to these perturbations. One trivial possibility is that the "right" mutant, one that is missing the crucial molecular machinery, has yet to be tested. The tremendous diversity of molecular mechanisms present in neurons lends credence to this possibility, as does the diversity within a given molecular family. There are at least 10 different isoforms of PKC, for instance (Nishizuka, 1988; Tanaka and Nishizuka, 1994), and 4 different isoforms of

CaMKII (Braun and Schulman, 1995). In addition, there are several other calcium/calmodulin-dependent protein kinases with broad substrate specificity (Colbran et al., 1989; Braun and Schulman, 1995). Many of these different molecules are expressed in neurons. Furthermore, whole classes of molecules have yet to be looked at - such as phosphatases, neurotrophins, and eicosanoids - that have been suggested to play roles in plasticity (Nairn and Shenolikar, 1992; Mulkey et al., 1993; Piomelli, 1994; Thoenen, 1995). It may be that one of these molecules is indeed necessary and/or sufficient for the signaling required for OD plasticity to take place.

Another, perhaps more likely, explanation for the resistance of visual cortical plasticity to these perturbations is that this molecular diversity allows a measure of functional redundancy. Multiple isoforms of a given protein might be carrying out overlapping or identical functions in the neuron. Lack of one of these might not lead to a deficit, or might affect plasticity quantitatively rather than qualitatively. Alternatively, altogether different cellular pathways might operate in parallel in the same process, leaving the biology resistant to the loss of one or the other. This form of redundancy is seen in the regulation of immediate early gene expression. Calcium increases affect gene expression through either PKA (activated via the calcium-sensitive adenylate cyclase) or αCaMKII (Ghosh and Greenberg, 1995).

Complicating the suggestion that a particular molecule is not required for plasticity is the possibility that compensation might obscure a role for that molecule in normal animals. Compensation is particularly a problem for

studies involving knockout animals, in which a gene deletion prevents functional expression of a particular protein throughout development of the animal. The effects of the deletion may be complex, difficult to predict or identify. For instance, in the Fyn$^-$ mutants, compensatory increases in the expression of some but not all other nonreceptor tyrosine kinases increases (Grant and Silva, 1994). In the RIβ$^-$ mice, expression of the RIα subunit in the brain is increased, perhaps explaining the lack of a change in biochemically assayed PKA activity (Brandon et al., 1995b). These observations occasion the caveat discussed in Chapter Four, that the lack of an effect of a given gene deletion on OD plasticity demonstrates that a molecule is not necessary for plasticity, but does not demonstrate conclusively that it plays no role in the normal animal.

The role of α-calcium/calmodulin-dependent protein kinase II

Despite the potential for redundancy and compensation, a quantitative slowing of plasticity was observed in a subset of αCaMKII-deficient mice. Although these experiments do not address the specific role of CaMKII, they provide an important clue into the cellular mechanisms of OD plasticity. Consideration of possible roles for the enzyme in the light of the subtle effect of its elimination and sources of functional redundancy suggests several future experiments.

The subtle ocular dominance plasticity phenotype seen in the CK$^-$ mice suggests that CaMKII is involved in, but not required for, plasticity. The reasons for residual plasticity should be investigated. As mentioned in Chapter Four, there are multiple isoforms of CaMKII, as well as other calcium-activated kinases (including PKA and PKC) expressed in the visual cortex (Cadd and McKnight, 1989; Tanaka and Nishizuka, 1994; Braun and Schulman, 1995). A simple experiment would be to check for compensatory increases in the expression and/or activity of these enzymes in the CK$^-$ animals. A more difficult but perhaps more revealing approach would be to study OD plasticity in double-knockouts, animals that have targeted deletions of both CaMKII and one of these other kinases. Alternatively, one might attempt to overexpress one or more of these kinases in the cortex of the CK$^-$ mice. Overexpression of a parallel pathway might eliminate the small deficit seen in these animals.

The deficit seen in the CK^- mice prompts an investigation into the precise role of CaMKII in plasticity. Specifically, one would like to know how its activity is modulated by visual activity, what the consequences of this activation are, and whether perturbing these upstream or downstream events also affects OD plasticity.

How CaMKII might be activated by visual activity deserves some attention. It is known that NMDA receptor activation by glutamate can activate CaMKII in cerebellar granule cells and hippocampal pyramidal neurons (Fukunaga and Soderling, 1990; Fukunaga et al., 1992; Soderling et al., 1994). NMDA receptor activation has been shown to be required for OD plasticity (Kleinschmidt et al., 1987; Bear et al., 1990). It would be interesting to determine if CaMKII can be activated by visual activity by comparing autophosphorylated enzyme levels in dark reared animals to those in dark reared animals recently exposed to light, or some other intense activity-evoking stimulus. The pharmacology of this activation might then be explored by interfering with voltage-gated calcium channels, release of calcium from internal stores, and NMDA receptor activation. The dependency of OD plasticity on calcium influx through the NMDA receptor might be studied in mice with mutant NMDA receptors which cannot pass calcium.

The downstream effects of CaMKII activation have been characterized in a handful of systems. The relevance of these data to OD plasticity has yet to be explored. It is known, for instance, that activated CaMKII phosphorylates

glutamate receptors, potentiating the effects of glutamate on hippocampal neurons (McGlade-McCulloh et al., 1993). It would be interesting to know if this relationship was present in the visual cortex, and if it was important for ocular dominance plasticity. The former question might be asked by looking for light-induced phosphorylation of glutamate receptors in the visual cortex of dark-reared mice. Another approach would be to examine the phosphorylation state of the receptor in the CK^- animals; one could even correlate that state with the ability of the mice to undergo OD plasticity. To answer the question of importance for OD plasticity, one could test the effects of mutations at the phosphorylation acceptor site on the receptor. Both constitutively activated and phosphorylation-incompetent mutants might be studied.

Another well-characterized function of CaMKII is to activate the expression of immediate early genes in neuronal and non-neuronal tissues via the phosphorylation of the transcriptional activator CREB (Ghosh and Greenberg, 1995). Expression of a number of immediate early genes is regulated in the visual cortex by visual activity (Worley et al., 1991; Rosen et al., 1992; Chaudhuri and Cynader, 1993; Chaudhuri et al., 1995). One might test the activity-regulated expression of immediate early genes in the CK- mice, and again correlate this with the ability of the mice to undergo OD plasticity. (Gene expression might also be an excellent assay with which to test the mechanisms of functional redundancy. Pharmacological experiments might reveal that the induction of immediate early gene expression in the

CK- animals depends on a particular enzyme instead of or in addition to CaMKII.) Finally, to determine if immediate early gene expression plays a role in OD plasticity, one could study mice deficient in each of several such genes. The most promising experiments along these lines, however, would be to study visual cortical development and plasticity in animals lacking CREB family members, as these regulate the expression of many genes.

Other more general aspects of the role of CaMKII in ocular dominance plasticity are also amenable to study using the mouse and other models. NGF has been shown to prevent the effects of monocular deprivation when applied intraventricularly in the rat (Maffei et al., 1992). Preliminary results from our lab suggest that NGF also blocks the effects of MD in the mouse (M. Fagiolini and M.P. Stryker, unpublished observations). It would be interesting to see if NGF blocks plasticity in the CK^- mice; the failure to do so would suggest the NGF exerts its effects through αCaMKII. One might also attempt to confirm the involvement of CaMKII in plasticity by examining the physiological and anatomical effects of activating CaMKII in cortical neurons in the cat or ferret. Supplying activated CaMKII to hippocampal neurons enhances the efficacy of synaptic inputs, demonstrating the effectiveness of viral infection or injection of the enzyme (Pettit et al., 1994; Shirke and Malinow, 1995; Malenka et al., 1995). Similar approaches have been used to explore the effects of neurotrophins on cortical neuron dendritic morphology (Lo et al., 1994; McAllister et al., 1995).

A genetic dissection of ocular dominance plasticity

Attempting even a few of the proposed experiments testing the role of CaMKII in ocular dominance plasticity would require the use of an impressively broad array of molecular genetic technologies. Yet most of these experiments rely on currently available reagents or methods. Furthermore, additional tools and techniques are currently being developed that could make experiments like these easier and more definitive. Already, gene-targeting and transgenic techniques have been used to direct tissue-specific and inducible gene expression and gene deletion, that have the potential to dramatically reduce the potential for compensation and allow for more tightly controlled experiments (Furth et al., 1994; Kuhn et al., 1995; Mayford et al., 1995a). A well-characterized mouse model for OD plasticity enables the use of these candidate gene approaches to investigate the roles of potential cellular mediators of plasticity.

In addition to the candidate gene approach, however, much has been learned regarding molecular and cellular machinery from forward genetics: the mapping and cloning of new genes based on functional assays. Behavioral geneticists have long tried to use different mouse strains to map loci responsible for various behavioral traits, with variable success. Recently, however, the development of new mutagenesis methods has allowed the use of genetic screens in mice (Takahashi et al., 1994). Moreover, these methods

have been used for the first time to map a gene based on a behavioral screen (Hotz Vitaterna et al., 1994). While a physiological determination of ocular dominance shifts subsequent to monocular deprivation is too time consuming to use in a screen, developing a simple behavioral assay might allow one to screen a mutagenized population of mice for an ocular dominance plasticity phenotype.

The combined use of all these emerging technologies, from transgenics to gene-targeting to genetic screens, is enabled by a well-characterized mouse model. The current work represents a first step. I have shown that ocular dominance plasticity in the mouse is an excellent model for ocular dominance plasticity in other better-studied animals. I have also begun studies aimed at characterizing the cellular mechanisms underlying this plasticity. The results support some interesting conclusions: TBS-induced LTP may not be an appropriate model for the fine tuning of connections during the development of the visual system, and calcium/calmodulin-dependent protein kinase may play a role in the process. Clearly, the mouse model promises to be a useful tool with which to dissect the cellular mechanisms underlying the plasticity that organizes connections in the developing visual cortex.

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