SECTION III

Synapse Rearrangement

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CHAPTER 8

Molecular substrates of plasticity in the developing visual cortex

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Abstract: Ocular dominance plasticity may be the paradigmatic in vivo model of activity-dependent plasticity. More than four decades of intense research has delineated the network-level rules that govern synaptic change in this model. The recent characterization of a murine model for ocular dominance plasticity has facilitated rapid progress on a new front, extending our understanding of the molecular mechanisms underlying ocular dominance plasticity. In this review, we highlight recent advances in this research effort, focusing in particular on signaling pathways mediating shifts in ocular dominance, and mechanisms underlying the timing of the critical period.

Keywords: plasticity; cortex; mouse; ocular dominance; critical period; monocular deprivation

Introduction

The brain harbors an extraordinary number of connections in a singularly small volume — by one estimate, 300×10^6 synapses per square millimeter of neocortex (Beaulieu and Colonnier, 1983). In no small part, this welter of connectivity is achieved through activity-driven rearrangements of neural circuits. The rules which govern this activity-dependent plasticity are perhaps best understood in the mammalian visual cortex, where plasticity induced by monocular deprivation — ocular dominance plasticity — has been studied for decades and is relatively well characterized.

In many carnivorous species (including cats, which comprise the most-studied and best-characterized animal model of ocular dominance plasticity), thalamocortical afferents representing the two eyes arise in the lateral geniculate nucleus (LGN) of the thalamus, and converge on layer IV of the primary visual cortex (V1), providing the first anatomical substrate for binocular interactions. In seminal experiments, David Hubel and Torsten Wiesel showed that these neural circuits could be profoundly shaped by manipulations of an animal's visual environment. Brief closure of one eye resulted in dramatic rearrangements of the connections subserving both the deprived and the nondeprived eyes, with the cortical territory devoted to the former shrinking, and those devoted to the latter expanding (Wiesel and Hubel, 1963).

Experiments elaborating on these initial observations have painted a remarkably clear picture of the rules governing activity dependent competition in ocular dominance plasticity. Importantly, activity-dependent change in the cortex is competitive, pitting afferents representing the two eyes against one another in a battle for cortical territory. Synaptic change in ocular dominance plasticity is Hebbian: effective, depolarizing inputs drive stronger synaptic connections, while ineffectual inputs are weakened. In addition, these competitive interactions are largely confined to a developmental critical period, a

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temporally circumscribed window in which synapses are exceptionally plastic.

The successes of carnivore models in elucidating the rules of ocular dominance plasticity have been complemented in recent years by the characterization of a mouse model for ocular dominance plasticity. Unlike larger mammals such as primates and cats, the mouse has been the subject of intense genetic analysis and manipulation. Technologies which enable the stable introduction of genes into the mouse genome, yielding transgenic mice, coupled with complementary protocols which enable discreet DNA sequences to be removed from the genome, producing knockout (KO) mice, have added an immensely valuable tool to the arsenal of pharmacological, electrophysiological, and anatomical methods which have traditionally been used to study ocular dominance plasticity. As a consequence, our knowledge of the molecular processes that govern plasticity is growing rapidly.

Insights gleaned from studies of mutant mice have not been confined to the molecular mechanisms underlying ocular dominance plasticity. Studies of transgenic animals have also generated new understanding of an old question, by shedding light on how the timing of the critical period is controlled.

Murine model of ocular dominance plasticity

Ocular dominance plasticity in mice is robust (the magnitude of plasticity following monocular deprivation in mice is roughly 85% as large as that seen in cats), rapid (with four days required for near-saturating shifts in mice, and two for cats), and regular (ocular dominance plasticity in mice is governed by the same rules which have been identified in carnivores, as detailed further in the chapter).

Key studies by Gordon and Stryker (1996) have demonstrated that ocular dominance plasticity is subject to the same rules first elucidated in higher mammals. Deprivation results in a competition-driven rearrangement of connections subserving the two eyes — while monocular deprivation drives robust plasticity, binocular deprivation has little if any consequence, ruling out simple disuse effects. The effects of monocular deprivation are maximal in mice when deprivation is initiated in a well-defined critical period, which peaks near postnatal day 26. Similar deprivations initiated in adulthood have little effect.

As for higher mammals, monocular deprivation in mice results in both physiological and anatomical changes. The latter are most pronounced in the afferents subserving the ipsilateral projections of the deprived eye, which are much reduced (as revealed by transneuronal labeling) after prolonged deprivation (Antonini et al., 1999).

Signaling pathways

The molecular pathways that underlie ocular dominance plasticity parallel the themes that emerge from other well-studied forms of neuronal plasticity, such as long-term potentiation (LTP). The N-methyl-D-aspartate (NMDA) receptor is critical, and likely initiates a number of second-messenger mediated signaling pathways by allowing influx of Ca^{2+} after glutamate binding and release of Mg^{2+} blockade, which has been demonstrated in vitro. Downstream signaling likely proceeds through activation of the molecule cyclic AMP response element binding protein (CREB), which initiates transcriptional changes, and subsequent translational events. Translational events are likely to facilitate the anatomical changes induced by monocular deprivation. A small but growing number of molecules have been identified which may contribute to these structural changes, which must include processes underlying both proliferation and growth of connections subserving the nondeprived eye, and pruning and retraction of the connections from the deprived eye.

NMDA receptor

Characterization of the NMDA receptor's properties in vitro suggested that the molecule might play a central role in ocular dominance plasticity, acting as the lynchpin for Hebbian plasticity by serving as the "coincidence detector" of convergent depolarizing input. Despite the attentions of a number of investigators, solid evidence of a role for the NMDA receptor in ocular dominance plasticity, apart from a simple role in basal synaptic transmission, was difficult to establish. Numerous pharmacological experiments showed that blockade of the

NMDA receptor prevents ocular dominance plasticity (Kleinschmidt et al., 1987; Gu et al., 1989; Bear et al., 1990), but such manipulations have potent suppressive effects upon normal synaptic transmission (Miller et al., 1989). A technically innovative solution to this problem came in the form of infusion of anti-sense DNA directed against the NR1 subunit of the NMDA receptor, a manipulation which substantially and selectively suppressed NMDA receptor transmission, without affecting visually driven responses in the visual cortex (Roberts et al., 1998). This manipulation also blocks the effects of monocular deprivation, suggesting that once the NMDA receptor's putative correlation detection role is carefully dissected from its role in normal synaptic transmission, the former indeed exists, and is necessary for ocular dominance plasticity.

Metabotropic glutamate receptor (mGluR)

There is little direct evidence for a role of the mGluRs in ocular dominance plasticity, though they are required for various forms of plasticity in vitro (Balschun et al., 1999). Blockade of mGluR function with the antagonist (*RS*)-alpha-methyl-4-carboxyphenylglycine (MCPG) has no effect upon ocular dominance plasticity (Hensch and Stryker, 1996); the latter finding has been challenged on the grounds that MCPG does not adequately block the effects of mGluR action in the visual cortex (Huber et al., 1998). However, further investigation of a role for mGluRs, using a mutant mouse lacking mGluR2, has demonstrated that these mice possess normal visual cortical plasticity when subjected to monocular deprivation (Renger et al., 2002). To date, mGluR2 is the sole receptor subtype that has been tested for a role in ocular dominance plasticity.

Neurotrophins

The properties of neurotrophin molecules — their promotion of axonal growth and dendritic proliferation (McAllister et al., 1995, 1996), activity dependent release (Blochl and Thoenen, 1995), and presence in the visual cortex and regulation by activity there (Castren et al., 1992) — make them compelling targets for investigation of a potential role in ocular dominance plasticity. Indeed, a great number of studies have attempted to elaborate a role for these molecules in mediating ocular dominance shifts following monocular deprivation, and to substantiate a "neurotrophic hypothesis for ocular dominance plasticity" — the attractive notion that thalamocortical afferents might engage in activity-driven competition for neurotrophins, which would in turn promote axonal growth and the formation of new synapses (Berardi et al., 2003). While the precise role of neurotrophin signaling in ocular dominance plasticity is unknown, investigators of the visual system have amassed a substantial amount of data that is consistent with this hypothesis.

Nerve growth factor (NGF)

Early studies in the rat demonstrated that intraventricular infusion of NGF could prevent shifts in ocular dominance following monocular deprivation (Domenici et al., 1991). Subsequent studies confirmed this effect and extended it, demonstrating that intracortical infusion resulted in a similar blockade of ocular dominance plasticity, without detectable effects upon levels of neural activity (Domenici et al., 1992; Lodovichi et al., 2000). Moreover, infusion of antibodies that specifically activate the NGF receptor trkA prevents the shift in ocular dominance responses that normally follows monocular deprivation (Pizzorusso et al., 1999).

In contrast to the robust effects seen in the rat, infusion of exogenous NGF in the cat has little or no effect. Intraventricular infusion of NGF attenuates ocular dominance plasticity and its behavioral consequences (Carmignoto et al., 1993; Fiorentini et al., 1995), but direct cortical infusion leaves plasticity intact (Galuske et al., 2000; Gillespie et al., 2000; Silver et al., 2001). It is not clear what accounts for this species difference. Silver et al. (2001) have noted that in the rat, unlike the cat, cholinergic afferents to the cortex have focal arborizations, which could allow local infusions of NGF to have relatively more profound effects on the activity of these neurons, which would in turn drive greater cortical activity.

Brain derived neurotrophic factor (BDNF)

Intracortical infusion of BDNF partially blocks the effects of monocular deprivation in the rat, but also has significant effects upon spontaneous and evoked neural firing rates, decreasing the signal to noise ratio in cortical neurons (Lodovichi et al., 2000). For this reason, BDNF effects on plasticity should be interpreted with caution, as they may be consequent to changes in cortical activity. Experiments in which BDNF was infused into cat visual cortex (area 18) support this notion, as this manipulation paradoxically results in the expansion of connections subserving the deprived eye (Galuske et al., 2000). It has previously been shown that intracortical infusion of the $GABA_A$ agonist muscimol, which silences cortical neurons, causes a similar reverse plasticity effect (Reiter et al., 1986). Though the activity-suppressing effects of BDNF infusions make the precise role of the molecule in ocular dominance plasticity difficult to interpret, anatomical studies suggest that BDNF has potent growth-promoting effects in primary visual cortex. For instance, BDNF infusion into kitten V1 desegregates ocular dominance columns in normal and deprived kittens (Hata et al., 2000).

Recent studies of a mutant mouse heterozygous for the null allele of BDNF (homozygous mutants die prior to the onset of the critical period) demonstrate that a 50% reduction in the protein product of the BDNF gene has no effects upon ocular dominance plasticity (Bartoletti et al., 2002).

Studies of mutant mice overexpressing BDNF in the cortex have revealed a critical role for the molecule in regulating the timing of the critical period (see "Timing of the critical period"). This may be distinct from any role the molecule might play in mediating the competition between deprived and nondeprived eye arbors.

Neurotrophin 3 (NT-3)

Neither intracortical infusions of NT-3 into the rat (Lodovichi et al., 2000) or the cat (Gillespie et al., 2000) have discernable effects upon ocular dominance plasticity.

Neurotrophin 4 (NT-4)

Like BDNF, intracortical infusion of the trkB ligand NT-4 has potent effects upon ocular dominance plasticity, blocking monocular deprivation-induced changes in both the rat (Lodovichi et al., 2000) and the cat (Gillespie et al., 2000). However, the latter study found that infusion of the NT-4 made cortical neurons generally less responsive, and so caution in interpreting these results is warranted. On the other hand, it is abundantly clear that the effects of NT-4 are not the trivial consequence of changes in neuronal responsiveness. NT-4 infusion degrades orientation selectivity, reverses a pre-existing shift in ocular dominance (Gillespie et al., 2000), and causes anatomical desegregation of ocular dominance columns (Cabelli et al., 1995, 1997), results that are all consistent with growth-promoting effects that have been documented in vitro (McAllister et al., 1995).

Protein kinase A (PKA)

Using pharmacological approaches, the second messenger effector PKA has been implicated in ocular dominance plasticity. Inhibition of PKA in kitten V1 blocks plasticity, with minor effects on neuronal responses (Beaver et al., 2001). Though mice genetically deficient in the PKA R1β subunit are impaired in measures of plasticity in vitro, they show normal shifts in ocular dominance following monocular deprivation (Hensch et al., 1998a). It is known that developmental compensation in this mouse leads to upregulation of the PKA R1α subunit, and this may allow plasticity to proceed.

Alpha calcium-calmodulin kinase II (αCaMKII)

αCaMKII is an abundant protein, comprising between 1 and 2%; of the total protein in the forebrain (Bennet et al., 1983). Eight to twelve subunits of the molecule assemble into a rosette holoenzyme comprised of α and β subunits (in the forebrain the ratio of α to β subunits is roughly 3 to 1: Miller and Kennedy, 1985). Once activated by Ca^{2+}/calmodulin binding, αCaMKII can phosphorylate

numerous substrate molecules, including the NMDA receptor (Omkumar et al., 1996) and the transcription factor CREB (Dash et al., 1991). Importantly, αCaMKII can autophosphorylate at threonine 286. Autophosphorylation switches the molecule into a Ca^{2+}-autonomous state, which allows the molecule to sustain Ca^{2+}-independent kinase activity, in effect providing a mechanism to turn transient Ca^{2+} influx into a sustained plasticity induction signal.

αCaMKII is a central player in inducing numerous forms of synaptic plasticity, and ocular dominance plasticity is no exception. Knock-out mice lacking αCaMKII are impaired in ocular dominance plasticity (Gordon et al., 1996). Puzzlingly, this deficit was evident in only half of the mutant animals studied, and may be the consequence of compensatory upregulation of other isoforms of αCaMKII (A. Silva, personal communication).

More recent studies have confirmed a necessary role for the molecule in ocular dominance plasticity and further shown a specific requirement for αCaMKII autophosphorylation (Taha et al., 2002). Monocular deprivation of mutant mice carrying a single point-mutation — substitution of alanine for threonine 286, which renders αCaMKII unable to autophosphorylate — is ineffective in driving an ocular dominance shift. These mice show neither changes in neuronal responses nor changes in receptive field organization.

While confirming the importance of αCaMKII in ocular dominance, these results also clarify the issue of exactly where synaptic change that underlies ocular dominance plasticity occurs. On the basis of pharmacological experiments, some investigators have argued that MD-induced plasticity creates changes primarily in inhibitory synapses (Mower and Christen, 1989). αCaMKII, however, has a precise synaptic distribution, occurring specifically in excitatory neurons, and where localized to synapses, is found postsynaptically only in the postsynaptic density of asymmetric (presumed) excitatory synapses (Liu and Jones, 1996). This specificity, combined with the demonstration of a requirement for normal αCaMKII function in ocular dominance plasticity, suggests that synaptic changes induced by monocular deprivation occur primarily in excitatory synapses made onto glutamatergic neurons.

Extracellular signal-regulated kinase 1,2 (ERK)

ERK (also called mitogen-activated kinase) is potently regulated both by neural activity and neurotrophins, and recent experiments in rats have demonstrated that ERK signaling is required for ocular dominance plasticity (Di Cristo et al., 2001). Infusion of an ERK inhibitor directly into the visual cortex blocks the effects of monocular deprivation, with little or no effect on neuronal spiking, as well as blocking LTP in cortical layer II/III (Di Cristo et al., 2001).

While ERK is known to be activated by increases in intracellular Ca^{2+}, and therefore may be activated downstream of NMDA receptor activation, additional work from the Maffei lab has demonstrated that neurotrophin signaling can be Ca^{2+}-independent, and mediated by activation of the ERK pathway (Pizzorusso et al., 2000). This raises the tantalizing possibility that the requirement for ERK in ocular dominance could be downstream of neurotrophin signaling, a supposition which remains to be tested.

CREB

PKA, ERK, and αCaMKII signaling ultimately contribute to changes in nuclear events, likely converging upon CREB-mediated transcription. CREB has been implicated in mediating synaptic plasticity in evolutionarily divergent animals (*Aplysia*: Dash et al., 1990; *Drosophila*: Yin et al., 1994; mice: Pham et al., 1999) and widely divergent plasticity paradigms (late phase hippocampal LTP: Silva et al., 1998; courtship conditioning in *Drosophila*: Griffith et al., 1993; activity-dependent development in V1: Pham et al., 2001). In a number of plasticity paradigms, the requirement for CREB activity seems to be confined to late phases of plasticity, which has prompted the suggestion that CREB activity is the molecular bridge linking short-term changes to long-lasting plasticity (Pittenger and Kandel, 1998).

In the developing visual system of the mouse, CRE-mediated transcription is upregulated by monocular deprivation specifically during the critical period for ocular dominance plasticity (Pham et al.,

1999). This upregulation occurs contralateral to the nondeprived eye, and is not found in binocularly deprived or nondeprived animals. This finding is interesting for two reasons: it shows that CRE-mediated transcription can be driven specifically by a pattern of neural activity which leads to competition — only an imbalance in activity is sufficient to induce it — and that this linkage between activity and transcription is present only during the time of the critical period.

CREB is required for ocular dominance plasticity. Using the vaccinia virus to drive expression of a dominant negative form of the molecule in V1, Mower et al. (2002) have shown that blockade of CREB function prevents ocular dominance plasticity. By recording single units at cortical sites both near to and far from the viral injection, the authors demonstrate that plasticity is blocked only in the region in which successful expression of the transgene occurs. Moreover, the effect is reversible. Monocular deprivation starting one week after viral infection, by which time transgene expression had largely dissipated, induced robust ocular dominance shifts.

Structural changes

Ultimately, intracellular communication through signaling pathways yields changes in the nuts and bolts of synaptic architecture — for many forms of plasticity, including ocular dominance plasticity, structural changes in synaptic connectivity are the endstage of mechanisms underlying long-lasting change (Antonini and Stryker, 1993, 1996; Antonini et al., 1999). Our understanding of the processes and molecules that underlie these changes is limited, but a handful of promising candidates have been identified.

Protein synthesis

In many systems, synaptic plasticity is composed of two distinct stages — an early, rapid, and labile stage, followed by a slower, more enduring phase, which is characterized by a requirement for protein synthesis. This requirement for protein synthesis is thought to provide the molecular substrate for structural rearrangements at the synapse, and studies in systems that provide accessible anatomical preparations, such as *Aplysia*, bear out this point of view (Bailey and Chen, 1989; Bailey et al., 1992).

Following monocular deprivation in the mouse, even the earliest apparent plasticity, as measured electrophysiologically, requires protein synthesis (Taha and Stryker, 2002). This requirement for protein synthesis is confined to the cortex: while infusion of protein synthesis blockers into primary visual cortex prevents plasticity following monocular deprivation, infusion into the LGN has no effect on plasticity, though infusions at both sites clearly reduce protein synthesis (to $< 50\%$, of baseline levels).

The timing and locus of the requirement for protein synthesis suggest both that structural rearrangements occur very rapidly following monocular deprivation, and that these changes consist primarily of cortical remodeling, rather than changes in thalamocortical inputs. This model of rapid cortical change is supported by demonstrations that, in the cat, the earliest changes following monocular deprivation occur outside thalamorecipient layer IV (Trachtenberg et al., 2000).

Tissue plasminogen activator (tPA)

Tissue plasminogen activator is a serine protease that has been implicated in remodeling of cellular processes in many contexts, including neurite outgrowth and cell migration. Mice lacking the gene encoding tPA show a specific deficit in the late phase of LTP, which requires transcription and translation, suggesting a role for the molecule in the structural remodeling which may underlie the maintenance of long-lasting synaptic change (Huang et al., 1996). Measures of tPA activity in organotypic slice cultures of visual cortex show that activity of the molecule is tightly coupled to electrical activity (Muller and Griesinger, 1998). Moreover, proteolytic activity in vivo is driven by an imbalance in afferent activity. Mice subjected to monocular deprivation show significant elevation of tPA activity in the visual cortex after even a single day of deprivation (Mataga et al., 2002).

In both mice (Mataga et al., 2002) and cats (Mataga et al., 1996; Muller and Griesinger, 1998), tPA is required for normal ocular dominance

plasticity. The nature of this requirement appears to differ in the two species. In cats, infusion of a tPA inhibitor into V1 has no effect upon plasticity produced by an initial period of monocular deprivation, but blocks the effects of a subsequent reverse occlusion, both physiologically and anatomically. In mutant mice lacking tPA, plasticity induced by monocular deprivation itself is absent. This deficit can be rescued by intraventricular injection of recombinant tPA.

Hensch et al. (2000) propose that these divergent findings may have their roots in differences in the connectivity of the visual system of cats and mice, rather than differences in the mechanisms underlying plasticity. In normal mouse V1, the balance of afferent input is tipped heavily in favor of the contralateral eye. Plasticity induced by monocular deprivation of the contralateral eye is therefore likely to primarily reflect underlying growth and proliferation of the open ipsilateral eye. Cats, in contrast, have relatively balanced ipsilateral and contralateral eye input in normal V1. Consequently, loss of deprived eye input may contribute relatively more to the plasticity seen after monocular deprivation. Subsequent plasticity induced by reverse occlusion is likely the consequence of underlying synaptic strengthening processes.

Other candidate structural proteins

As yet, relatively few molecules have been identified which are known to be directly involved in mediating changes in axonal and synaptic architecture in ocular dominance plasticity. A number of promising candidates have been identified, however. Two of these include the class I major histocompatibility complex (MHC) antigen and candidate plasticity gene 15 (CPG15), both of which were identified in screens targeting genes with activity-driven changes in gene expression (Nedivi et al., 1993; Huh et al., 2000).

To date, neither molecules' role in ocular dominance plasticity has been tested, but studies in other experimental systems suggest that they contribute to structural changes in neural connectivity in the visual system. CPG15 expression in *Xenopus laevis* optic tectum promotes the growth of both retinotectal presynaptic afferents and postsynaptic tectal dendrites (Nedivi et al., 1998; Cantallops et al., 2000). The class I MHC has been implicated in another experimental paradigm, as mutant mice deficient in class I MHC-mediated signaling show abnormal anatomical refinement of retinogeniculate connections (Huh et al., 2000).

Timing of the critical period

Advances in understanding the mechanisms underlying ocular dominance plasticity have accumulated rapidly, offering the promise of a complete understanding of the molecular events linking neural activity to anatomical change. But outstanding issues in understanding activity-dependent plasticity in V1 are not confined to elucidating the mechanisms that underlie strengthening and weakening of neural connections. The issue of how plasticity in V1 is controlled temporally is of considerable interest as well, and until very recently, little was known about the mechanisms underlying this process. The convergence of studies of neurotrophin action in the cortex, and of inhibitory circuits in controlling plasticity, has dramatically increased our understanding of the molecular processes that control the timing of critical period.

Cortical inhibition and timing of the critical period

In an initial study, Hensch and colleagues (Hensch et al., 1998b) demonstrated that mice deficient in inhibitory neurotransmission (KO mice lacking the 67-kD isoform of glutamic acid decarboxylase, or GAD) were impaired in ocular dominance plasticity. Microdialysis and electrophysiological studies showed hyperexcitability of the cortical network, manifested as prolonged V1 neural discharge following visual stimulation. A four day period of monocular deprivation, sufficient to drive near-saturating plasticity in wild-type mice, had no effect in GAD KO mice. More prolonged periods of deprivation resulted in a small, but significant, shift in responses toward the nondeprived eye. Intracortical infusion of the benzodiazepine agonist diazepam rescued plasticity, showing the locus of the deficit

was cortical, and establishing a powerful tool for probing the timing of the critical period in these mice.

A follow-up study revealed, remarkably, that diazepam rescue of GAD KO mice was not confined to the normal murine critical period for ocular dominance plasticity (Fagiolini and Hensch, 2000). Monocular deprivation concurrent with diazepam treatment in adult GAD KO mice resulted in robust plasticity; plasticity could also be driven in adult GAD KOs without diazepam by prolonging the length of the monocular deprivation. Subsequent experiments revealed that diazepam treatment could drive plasticity in WT mice prior to the normal critical period, but not after it. These findings led the authors to propose that the onset of the critical period was gated by a threshold level of intracortical inhibition, with the mechanism for initiation of the critical period tightly coupled to that underlying its closure. This model received powerful support in the form of a two part experiment: in GAD KO mice, diazepam was infused over the course of the normal critical period (postnatal days 23–33), but prolonged monocular deprivation was not performed until adulthood (postnatal days 45–60). Plasticity was absent in these mice — early diazepam treatment occluded subsequent plasticity in response to prolonged monocular deprivation. These results suggest a threshold level of inhibition to open a transient period of susceptibility to experience-dependent plasticity.

BDNF and the development of cortical inhibition

Spurred by in vitro studies suggesting a role for neurotrophins in regulating the development of cortical inhibition, Huang and colleagues engineered a transgenic mouse overexpressing BDNF in cortical pyramidal neurons (Huang et al., 1999). Overexpression of BDNF in these mice accelerated the maturation of inhibitory circuits in the cortex as demonstrated by a number of criteria: GAD expression was elevated at young ages, as were parvalbumin-positive inhibitory interneurons, and inhibitory potentials recorded in cortical slices prepared from these mice were larger than those found in wild-type mice.

Precocious development of inhibitory circuits in these mice altered V1 plasticity in a manner that echoed the findings of Hensch and colleagues. Early monocular deprivation, initiated at postnatal day 21 (and assessed using visually evoked potentials), suggested a trend toward increased plasticity in BDNF overexpressing mice relative to wild-types, though this effect was not significant. Monocular deprivation initiated at older ages (p23 and p28) showed significant differences between wild-types and transgenics, but with effects moving in the opposite direction — significant plasticity occurred in wild-types but not transgenics. These results suggested that BDNF expression accelerated the closure of the critical period.

Single unit-studies of the same mice clarified these findings, by demonstrating that plasticity in the transgenics was precocious, both beginning and ending earlier than in wild-type littermates (Hanover et al., 1999).

Together with the studies of the GAD KO mouse, these results provide compelling evidence that BDNF promotes maturation of cortical inhibitory circuits, which in turn gate the critical period for ocular dominance plasticity. These results suggest cortical development of these circuits is central in initiating experience-dependent plasticity, and raises interesting questions about the laminar and functional classes of interneurons involved.

Other candidate timing mechanisms

Pizzorusso et al. (2002) have recently provided striking evidence that chondroitin sulphate proteoglycans (CSPs), component molecules of the extracellular matrix (ECM), may play an important role in gating ocular dominance plasticity. CSP-rich ECM has been shown to inhibit axon regeneration in vivo (Davies et al., 1999). Thus Pizzorusso et al. (2002) hypothesized that CSPs might play a similar role in the primary visual cortex, suppressing neurite outgrowth to contribute to the closure of the critical period. Consistent with this idea, CSPs in the primary visual cortex increasingly aggregate into perineuronal nets over the timecourse of the critical period. The consequence of enzymatic degradation of CSP chains in the primary visual cortex of adult rats is surprising

and dramatic: when combined with MD, this treatment results in a substantial shift in cortical responses toward the nondeprived eye. In normal adult rats, of course, MD of the same duration has no effect. Thus, in V1, as has been well established in the spinal cord, anatomical plasticity appears to be critically dependent upon a permissive substrate for neurite outgrowth.

An understanding of the specific mechanisms by which CSPs regulate ocular dominance plasticity, and knowledge of how these mechanisms interact with those governed by intracortical inhibition, await further experiments. However, the importance of the CSPs in regulating V1 plasticity underscores the importance of structural changes in serving as a long-lasting storage mechanism for changes that occur during ocular dominance plasticity.

Discussion

Our understanding of the molecular mechanisms underlying ocular dominance plasticity has expanded rapidly in recent years, hastened both by novel molecular techniques (as in Mower et al., 2002) and the characterization of a murine model for ocular dominance plasticity (Gordon and Stryker, 1996). Progress in elucidating the signal transduction mechanisms contributing to ocular dominance plasticity has been particularly rapid, and findings from experiments delineating the factors regulating the timing of the critical period are entirely novel.

Of course, many outstanding questions remain. Two areas are likely to yield significant research advances in coming years. First, too little is known about the many steps that must intervene between rapid electrophysiological plasticity and subsequent anatomical changes. Novel imaging techniques, allowing anatomical changes to be visualized in real time, combined with the use of KO and transgenic mice, offers much promise of a more complete understanding of the mechanisms underlying the complete timeline of evolving synaptic change. Second, more fully harnessing the potential of murine genetics offers the distinct possibility of accelerating the discovery of important molecular players in ocular dominance plasticity. While studies of existing KO mice have been very fruitful, they lean heavily on in vitro studies to identify promising candidate molecules. Forward genetic screens of mice deficient in ocular dominance plasticity offer the hope of rapidly uncovering molecular mechanisms in an unbiased fashion.

Abbreviations

BDNF	brain-derived neurotrophic factor
CPG-15	candidate plasticity gene 15
CREB	cyclic AMP response element binding protein
CSP	chondroitin sulphate proteoglycan
ECM	extracellular matrix
ERK	extracellular signaling related kinase
GAD	glutamic acid decarboxylase
KO	knock-out
LGN	lateral geniculate nucleus
LTP	long-term potentiation
MCPG	(RS)-alpha-methyl-4-carboxyphenylglycine
mGluR	metabotropic glutamate receptor
MHC	major histocompatibility protein
NGF	neurotrophin growth factor
NT-3	neurotrophin 3
NT-4	neurotrophin 4
NMDA	N-methyl-D-aspartate
PKA	protein kinase A
tPA	tissue plasminogen activator
V1	primary visual cortex

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