Plasticity of the Developing Visual Cortex in Normal and Mutant Mice

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Connectivity within the mammalian forebrain is at once complex and precise. A well-characterized example of the nature of these connections is the arrangement of inputs from the eye-specific layers of the lateral geniculate nucleus to layer IV of the primary visual cortex. In the adult cat, axons of geniculate neurons carrying information from the left and right eye innervate different patches of cortex, called ocular dominance columns (15). This highly precise arrangement is not found in the immature cortex of young kittens. Instead, axons from the two eyes initially intermingle throughout the input layer of primary visual cortex. Gradually, over the course of the first few postnatal weeks, the axons segregate into their respective patches (15). The segregation of the initially overlapping geniculocortical projections into discrete ocular dominance columns has been shown to require neuronal activity (26), and is thought to rely on a Hebbian correlation-based learning rule (11,25).

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 (1). 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 (21, 22, 29). Similar periods of binocular deprivation have a much smaller effect on responses (6, 30). Strabismus and alternating monocular deprivation, which decrease the degree of correlation between the two eyes’ inputs, decrease the number of cells driven by both eyes (2,13). The effects of these manipulations have led to the conclusion that the same correlation-based competition which underlies ocular dominance column formation also underlies experience-dependent ocular dominance plasticity in the visual cortex.

While the rules governing ocular dominance (OD) plasticity are well understood, the cellular mechanisms underlying it have remained elusive. Efforts to interfere pharmacologically with cellular processes and thereby disrupt OD plasticity have added little to our understanding of the mechanisms involved. This approach is hampered, moreover, by the limited availability of
effective and specific agonists and antagonists that are able to function in vivo. An additional problem of the pharmacological approach is the difficulty of achieving comparable conditions in vitro and in vivo. These limitations are particularly significant when an attempt is made to intervene with the function of intracellular proteins.

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 (10, 17). 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 requires the availability of an adequate murine model. We therefore characterized a mouse model for ocular dominance plasticity (7). Furthermore, we have begun a dissection of the cellular mechanisms underlying this plasticity using several mutant mouse lines with targeted deletions in putative plasticity-mediating molecules (8, 9). 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.

Ocular dominance plasticity in the normal mouse

The mouse central visual system is in many key respects similar to the visual system of higher mammals (Fig. 1A). Visual input from the retinas is received by eye-specific areas of the LGN and relayed to the primary visual cortex. While much of the primary visual cortex of the mouse is monocular, the lateral 500 to 600 μm, representing the central-most 30-40° of the visual hemifield, receives input from both eyes (4, 7, 28). Within this area, called the binocular zone, most cortical neurons respond to stimuli presented to either eye independently. Each neuron can be assigned an ocular dominance score according to the relative activity evoked by a stimulus.
presented to either eye independently (as in ref. 12). The distribution of OD scores for neurons recorded from the binocular zone of normal mice is shown at the top of Fig. 1B.

In the mouse, as in other mammals, monocular deprivation during a critical period decreases the responsiveness of cortical neurons to inputs from the closed eye. This was initially demonstrated by Drager (5), who found that shifts in cortical responses could be induced by long-term (6 weeks to 1 year) deprivations. We have further demonstrated that similar effects can be elicited by brief deprivations (7). Four days of monocular lid suture starting at P28 drives the distributions of ocular dominance scores of cells in the binocular zone towards the open eye in both the ipsilateral and contralateral hemispheres (Fig. 1B). These shifts are reflected in the contralateral bias index (CBI), which indicates the degree to which the input from the contralateral eye dominates the activity of the cortical cells. The CBI is decreased in the hemisphere contralateral to the deprived eye, reflecting the greater influence of the open, ipsilateral eye on cortical neuronal activity.

In order to demonstrate that the effects of MD in the mouse arise from the same underlying mechanisms as in other animals, we examined the critical period of these effects and their dependence on a correlation-based competition between inputs (7). The critical period for monocular deprivation in the mouse is illustrated in Fig. 2A. The CBIs from 6 normal mice are shown for comparison. The effects of MD, represented as the average CBI obtained from 5 monocularly deprived animals, are plotted against the age at which the deprivation began. Maximal effects were obtained by only 4 days of MD; the period of heightened sensitivity to the effects of MD peaked near P28. To demonstrate the requirement for competition, we quantified neuronal response rates in monocularly and binocularly deprived animals. While MD induced a dramatic decrease in neuronal responses to stimuli presented to the deprived eye, binocular deprivation failed to significantly change stimulus-evoked responses (Fig. 2B). Finally, to demonstrate the correlation-based nature of the effects of deprivation, we tested the effects of alternating MD on visual cortical neurons. After compensating for the initial contralateral bias, daily alternating MD,
by decreasing the correlation between inputs from the two eyes, decreased the number of binocular
cells (Fig. 2C).

Ocular dominance plasticity in mice lacking putative plasticity mediators

Having demonstrated the validity of the mouse model of ocular dominance plasticity, we
began a dissection of its underlying molecular mechanisms through the use of mutant mouse lines.
Much of our work has focused on the use of mice with targeted deletions of the genes encoding
molecules thought to play a role in plasticity. Molecules implicated by *in vitro* models of axon
outgrowth and synaptic plasticity, for instance, might play similar roles in developmental plasticity
*in vivo*. The effects of deletions of the genes encoding two such molecules, a neural specific
regulatory subunit of protein kinase A (PKA RII), and the α1-isoform of calcium/calmodulin-
dependent protein kinase II (αCaMKII), will be described in detail elsewhere (8,9).

Briefly, in mice lacking PKA RII, theta-burst stimulation (TBS) of subcortical white
matter fails to induce a long-term potentiation (LTP) of field responses in layer II/III (Fig. 3A, and
ref. 8). (TBS-induced LTP has been proposed as a cellular model for OD plasticity [14]). Despite
this deficit, PKA RII-deficient animals develop normal visual cortical responses and retain normal
ocular dominance plasticity (Fig. 3B, and ref. 8). Mice lacking αCaMKII, in contrast,
demonstrate a deficit in ocular dominance plasticity with variable penetrance. In about 1/2 of
these mutant animals, the shift in cortical responses evoked by brief monocular deprivation is
dramatically reduced (9). The first finding supports the conclusion that LTP induced by theta-burst
stimulation is not an appropriate model for the fine tuning of connections during the development
of the visual cortex. The second finding suggests that calcium/calmodulin-dependent protein
kinases may play a role in the process.
Below we detail studies of an additional mutation on visual cortical development and plasticity. We find that mutations in the gene encoding the neural-specific antigen Thy-1 have no effect on visual cortical development and plasticity. Procedures used were as described previously (7).

Visual cortical responses and plasticity in Thy-1-deficient mice

Thy-1 has long been proposed as a negative regulator of axon outgrowth (18). Initially characterized as an antigen on lymphocytes, the Thy-1 protein is also highly expressed in the adult mouse brain (23). Thy-1 is member of the immunoglobulin superfamily of cell surface proteins, anchored to the plasma membrane via a glycoprophosphatidylinositol link (31). A role for Thy-1 in the regulation of axonal outgrowth was first proposed based on its interesting expression pattern. 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 (20). Direct evidence for a role for the molecule in inhibiting cell growth has come from cell culture experiments. Expression of Thy-1 protein has been shown to inhibit neurite outgrowth, and perturbations which result in the removal of Thy-1 from the cell surface permits neurite outgrowth (16, 27). Finally, mice with targeted deletions in the gene encoding Thy-1 have deficits in hippocampal LTP (19).

Thy-1 protein expression in the cortex increases postnatally through P12 (32). Although later postnatal data is not available for cortex specifically, whole brain levels of Thy-1 continue to rise until P40 or later (24). 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 (19). Visual responses in V1 neurons were grossly normal. The RF size distributions from each animal were overlapping, and the overall means for Thy-1' and heterozygous receptive fields were not significantly different (t-test: p > 0.4, n = 49 and 86
neurons for Thy-1 and heterozygous mice, respectively). Retinotopy was also intact in these animals, as illustrated by the high correlation coefficients of the linear regressions of receptive field center azimuth on electrode position (Figure 4A, inset; t-test: p > 0.3, n = 3 and 5 regressions from 2 Thy-1 and 3 heterozygous 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 Fig 4B. 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 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. Neuronal responses in animals deprived for 4-5 days beginning at P52 (well after the critical period) failed to shift towards the open eye, although the heterozygote deprived for 6 days beginning at P52 showed a small shift.

These data demonstrate that Thy-1 mice develop grossly normal visual responses and retain MD-induced plasticity confined to the normal critical period, despite a complete lack of Thy-1 protein. However, a contributing, though non-essential, role for Thy-1 in the development or plasticity of V1 responses 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 (Fig. 4B). Such detailed quantitative analyses have not yet been performed.

Conclusion

The characterization of the mouse model for ocular dominance plasticity allows one to test specific hypotheses regarding the cellular mechanisms of activity-dependent plasticity using mutant mouse lines. In vitro models such as neurite extension and long-term potentiation often suggest the involvement of particular molecules and cellular pathways in plasticity. While expression and other correlative studies can provide further circumstantial evidence in favor of these hypotheses, direct
proof of the involvement (or lack thereof) of particular molecules in specific forms of plasticity in vivo has been extremely difficult to obtain. The availability of mouse lines with specific genetic lesions, along with the availability of a simple, well-characterized model for plasticity, allows for direct tests of function. Thus, we were able to demonstrate that Fyn and Thy-1, two molecules thought to play a role in plasticity from in vitro experiments, are not required for ocular dominance plasticity in vivo. Similarly, a role for αCaMKII-dependent processes in ocular dominance plasticity is suggested by the finding of a subtle deficit in αCaMKII-deficient mice.

The approach is not limited, however, to molecular questions alone. By comparing the effects of a given mutation in a given protein on both the in vitro models and ocular dominance plasticity in vivo, we can begin to answer long-standing questions regarding underlying cellular and physiological mechanisms. For instance, in PKA RIIβ-deficient mice, normal ocular dominance plasticity persists despite a defect in theta burst-induced LTP. This finding suggests that this particular form of in vitro synaptic plasticity does not underlie ocular dominance plasticity. Multiple levels of analysis, from the molecular to the cellular to the circuit to the system can be applied to the effects of a single highly specific mutation. A large and ever-increasing number of available mutant and knockout lines is available to aid in this analysis (3). 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.

Summary

Activity-dependent processes fine-tune neuronal connections during the development of the mammalian brain. These processes have been best studied in the primary visual cortex, where inputs from the two eyes compete for synaptic space on their targets. The cellular mechanisms underlying this competition are poorly understood.
Recently, gene-targeting techniques have enabled the generation of mutant mouse lines, each possessing specific genetic lesions. To take advantage of this technology, we have characterized a mouse model for ocular dominance plasticity that in its essential aspects mimics that found in other animals: visual experience modulates cortical responses via a correlation-based competition between inputs from the two eyes.

We have therefore begun a dissection of the cellular mechanisms underlying ocular dominance plasticity by studying the effects of mutations in potential plasticity mediators. PKA RB-deficient mice retain normal plasticity deficit in long-term potentiation. A subset of mice lacking αCaMKII demonstrate reduced plasticity. Finally, mice lacking the neural antigen Thy-1 and the tyrosine kinase Fyn have normal visual cortical responses and 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.

References

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Figure 1. Binocular responses in the primary visual cortex of the mouse. 

A. Schematic diagram of the central visual pathway in the mouse. 

B. Monocular deprivation (MD) induces a shift in the responses of binocular zone neurons towards the open eye. 

Top, Distribution of ocular dominance scores of 150 neurons recorded from the binocular zone of 5 normal mice. 

Lower left, The effect of MD on responses of neurons in the binocular zone ipsilateral to the deprived eye. Data are from 5 mice. 

Lower right, The effect of 4 days of MD on responses of neurons in the binocular zone contralateral to the deprived eye. Data are from 5 mice. 

The contralateral bias index 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. Ocular dominance plasticity in normal mice. A. MD exerts its effect during a well-defined critical period. 4-day deprivations between P19 and P28 induced significant shifts towards the ipsilateral, open eye (triangles; mean ± s.d. of 5 animals) compared to normal animals (circles, individual hemispheres). B. Binocular deprivation during the critical period failed to significantly depress cortical responses. Monocular deprivation depressed responses to the deprived eye, and had no significant effect on responses to the open eye. n = 11 to 24 cells. C. Alternating monocular deprivation decreases the number of binocular cells. Note that the number of equally binocular cells (OD score = 4) is less than the number of ipsilateral monocular cells (OD score = 7); this never occurs in normal or monocularly-deprived animals.
Figure 3. Long term potentiation and ocular dominance plasticity in PKA RIβ-deficient mice. A, theta-burst stimulation of the white matter induces a long-lasting potentiation of field responses recorded in layers II/III in slices of primary visual cortex from wild-type (closed symbols) but not mutant (open symbols) mice. B, Monocular deprivation induces a shift towards the open, ipsilateral eye (seen as a decrease in the CBI) in both wild-type (open bars) and mutant (hatched bars) mice.
Figure 4. Visual cortical responses and plasticity in Thy-1- and Fyn-deficient mice. A. The retinotopic mapping of the visual world onto the visual cortex is preserved in Thy-1-deficient mice. Azimuths of individual receptive fields encountered in successive electrode penetrations are plotted vs. the lateromedial location of the penetration. Inset, correlation coefficients of several such linear regressions in both control (Het) and mutant (Thy-1) mice show the precision of this mapping. B, Ocular dominance plasticity is preserved in Thy-1-deficient mice. 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). C, The retinotopic relationship is preserved in this Fyn-deficient mouse. r², correlation coefficient of the linear regression of azimuth on position. D, Normal effects of monocular deprivation in two Fyn-deficient (triangles) and one WT (square) mice. Conventions as in (A).