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Rapid Remodeling of Axonal Arbors in the Visual Cortex

Antonella Antonini and Michael P. Stryker

If vision in one eye is blurred or occluded during a critical period in postnatal development, neurons in the visual cortex lose their responses to stimulation through that eye within a few days. Anatomical changes in the nerve terminals that provide input to the visual cortex have previously been observed only after weeks of deprivation, suggesting that synapses become physiologically ineffective before the branches on which they sit are withdrawn. Reconstruction of single geniculocortical axonal arbors in the cat after either brief or prolonged monocular occlusion revealed striking axonal rearrangements in both instances. Rapid withdrawal of the branches of deprived-eye arbors suggests that axonal branches bearing synapses respond quickly to changing patterns of neuronal activity.

During a critical period in early postnatal life neurons in the primary visual cortical area (area 17) of animals with binocular vision are particularly susceptible to an imbalance in the visual experience of the two eyes (1). In cat and monkey, monocular deprivation (MD), that is, depriving one eye of patterned vision by closing the eyelids (2–4) while allowing the other eye normal visual input, leads to physiological and anatomical changes in area 17.

In normal animals, the left and right eyes drive nearly equal numbers of cortical neurons, and the vast majority (>80%) of neurons are binocularly driven. The anatomical basis for this physiology is the division of the major input layer of cortex, layer 4, into nearly equal-sized patches innervated by the afferents that serve the two eyes. After MD, most neurons in area 17 can only be activated through the experienced, nondeprived (ND) eye, and responses to the deprived (D) eye are greatly reduced (2, 5, 6). Anatomical studies based on transneuronal transport of radioactive tracers injected into one eye of kittens monocularly deprived from eye-opening past the end of the critical period have demonstrated that cortical domains devoted to the D eye undergo a substantial shrinkage while those of the ND eye expand (5, 7). This finding suggests an anatomical basis for the functional shift of ocular dominance. The physiological effects of MD are detected after only 2 to 3 days of deprivation (8–13), and the magnitude of the deprivation effect is nearly as great after a week of deprivation as after months. Such plasticity has been thought to take place

too rapidly to be accounted for by anatomical changes; functional bases, such as inhibition of the input or a physiological down-regulation of the efficacy of existing synapses from the D eye, have been suggested (9, 14–16). In this view, the anatomical modifications produced by a brief period of MD would be evident only at the molecular level and would not be detectable in the light microscope.

To study the processes that couple physiological regulation to anatomical changes, we evaluated and compared geniculocortical axonal arbors in animals monocularly deprived for either 4 weeks (long-term) or 6

to 7 days (short-term) during the critical period (17). Genulocortical afferents were anterogradely filled from the lateral geniculate nucleus (LGN) with the phaseolus lectin (PHA-L) (18). The tracer was injected in lamina A of the right and left LGNs, allowing the analysis, in the two hemispheres of the same animal, of geniculocortical afferents serving the D or ND eye. Labeled geniculocortical projections were visualized with standard immunohistochemical techniques (18). A total of 38 arbors were reconstructed in three dimensions (19–21).

Following long-term MD, the labeled afferents serving the D eye showed a reduction in the complexity of the terminal arborization while the afferents serving the ND eye expanded (Fig. 1), consistent with the pattern seen in previous transneuronal labeling experiments (5). Surprisingly, even in the short-term MD experiments, geniculocortical arbors serving the occluded eye were similarly affected (Fig. 2). This result suggests that the physiological ocular dominance shift of cortical neurons produced by short-term MD is associated, at least after 6 days of MD, with a broad restructuring of the terminal arborization and not only with a functional suppression of the weaker input.

To quantify these observations, we measured and compared two parameters of the axonal arborization of LGN neurons in layer 4: (i) the total length of the arborization, obtained from the three-dimensional data, as a measure of growth; and (ii) the total number of branch points, as a measure of arbor complexity. The mean values of

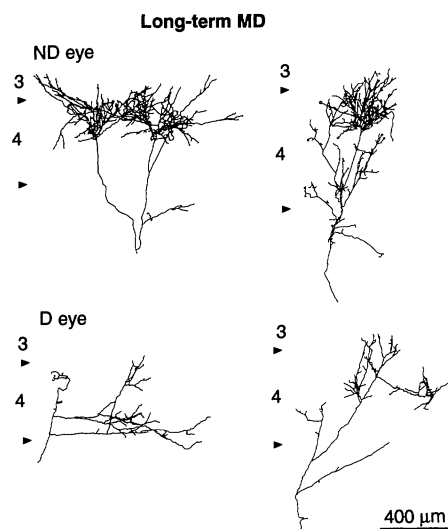


Fig. 1. Coronal view of geniculocortical arbors reconstructed in kittens in which one eye had been occluded for 33 days. The terminal arborization of the deprived (D) eye shows a dramatic reduction in complexity as compared to that of the nondeprived (ND) eye. Cortical layers 3 and 4 are indicated by arrowheads.

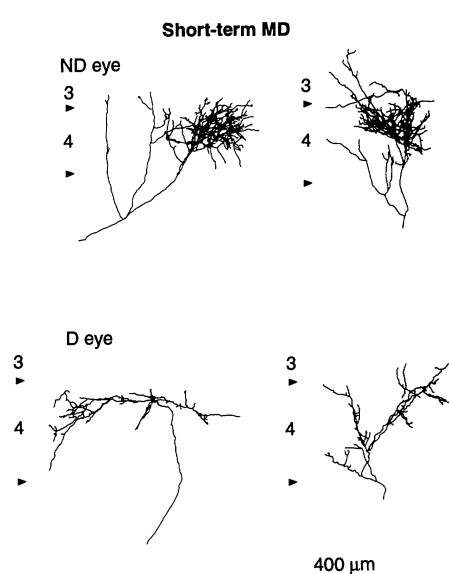


Fig. 2. Coronal view of geniculocortical arbors reconstructed in kittens in which one eye had been occluded for 6 to 7 days. The borders of cortical layers 3 and 4 are indicated by arrowheads.

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axonal length for the D eye (7.7 mm in short-term, 10.1 mm in long-term) were each significantly smaller ($P < 0.002$) (22) than for the ND eye (16.5 mm in short-term, 23.2 mm in long-term, Fig. 3A). Results of long-term deprivation were not statistically different from those in short-term deprivation for either the D eye or the ND eye.

Following short-term MD, the total length of arbors for the D eye was not only smaller than that for the ND eye but was also significantly reduced compared to that of younger normal animals (mean, 13.2 mm; $P = 0.005$) studied before the onset of

short-term MD (23). This finding indicates that brief MD does not merely interfere with growth but induces a rapid elimination of axonal branches. In contrast, the brief period of MD was not sufficient to induce a significant overgrowth of geniculocortical arbors serving the ND eye, as indicated by comparison of corresponding data for the normal animal studied at a similar age (P39: mean, 13.7 mm) (24). These results suggest that destructive neuronal processes that produce the loss of arbors serving the D eye take place more rapidly than constructive ones that expand the ND arbors, consistent with the results of physiological experiments on the responsiveness of cortical neurons (9, 10, 25).

The mean numbers of branch points for the D eye (59.2 in short-term, 54.3 in long-term) were each significantly smaller ($P < 0.003$) than for the ND eye (143.8 in short-term, 207 in long-term, Fig. 3B), indicating that, along with a loss of branches, geniculocortical arbors serving the D eye undergo a significant reduction in the complexity of the terminal arborization. Results of long-term MD were not significantly different from those of short-term MD for either eye. In contrast to the effects on axonal length, the numbers of branch points in both D and ND eye afferents were significantly changed by short-term deprivation from their normal values at P30/31 (mean, 91.2; ND increase, $P < 0.005$; D decrease, $P = 0.02$) (26).

Our findings bear on two aspects of the effects of visual deprivation during the critical period: (i) similarities and differences between short- and long-term deprivation and (ii) the rapidity with which plastic structural modifications can occur in the cat visual cortex. Physiologically the effect of both short-term and long-term MD is characterized by a profound decrease in both the proportion and responsiveness of cortical cells activated through the D eye (2–5, 8–13). In agreement, our results demonstrate a close correlation between this physiological effect of MD and the morphological alterations of geniculocortical afferents serving the D eye in both experimental conditions.

Although the connections of the D eye are similarly reduced after long-term and short-term MD, they appear to differ in their residual plastic ability. After short-term MD, the restoration of D eye responses may occur if the animal is allowed appropriate visual experience (8, 10, 12, 13, 27). After recovery, the receptive fields through the originally D eye have properties comparable to those found in normal kittens, suggesting that a normal pattern of anatomical connections has been restored (12, 13). After long-term MD, some degree of recov-

ery is possible only if the D eye is opened before the end of the critical period (25, 27, 28), but recovered responses are abnormal and binocular receptive fields are mismatched (27). The present results suggest that recovery after both short- and long-term MD entails the physical regrowth of connections from the D eye. However, it is only after brief MD that normally precise functional connections are reestablished.

The most surprising finding is the remarkable rapidity with which gross morphological changes can occur in response to MD. Recent studies have revealed rapid modification in transmitter and receptor function in area 17 after brief environmental manipulation (29–32). In the adult monkey, monocular enucleation or blockade of ganglion cell action potentials by the sodium channel blocker tetrodotoxin induces, within 2 days, changes in immunohistochemically detectable levels of the inhibitory neurotransmitter γ -aminobutyric acid (GABA), its synthesizing enzyme glutamic acid decarboxylase (GAD), GABA_A receptors, and neuropeptides in visual neurons located in the cortical domains of the D eye (29–31). Brief visual experience in dark-reared cats also induces immediate early gene expression in the visual cortex (32). The ability to induce biochemical changes by visual deprivation, such as modulation of neurotransmitter function, is present during development (33) and is maintained through adulthood, but the potential for dramatic remodeling of neuronal structure appears to be specific to the critical period and it is lost thereafter. Therefore, the mechanisms underlying rapid biochemical changes in the adult cannot account for the magnitude of the remodeling of geniculocortical arbors during development.

Plastic changes in the developing visual cortex were as profound and nearly as prompt structurally as they were functionally. We do not know whether there is a time at which the geniculocortical afferents serving the D eye are anatomically normal but functionally ineffective. Our results indicate that this state, if it exists at all, is very brief, lasting no more than a few days.

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7. There is also strong evidence that prolonged MD results in ultrastructural modification of geniculocortical synapses serving the D eye. This suggests a reduced efficacy in the synaptic transmis-

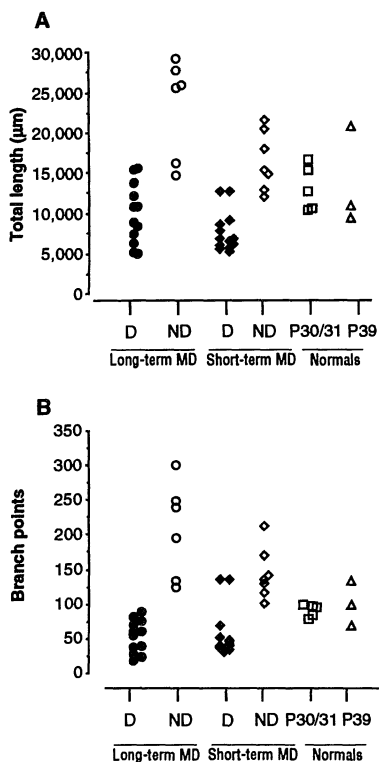


Fig. 3. Analysis of terminal arborization of geniculocortical afferents in layer 4. (A) Scattergram of the total lengths of the terminal arborizations of geniculocortical axons serving D eye (filled symbols) and ND eye (open symbols), in long- and short-term monocularly deprived animals (circles and diamonds, respectively). Each symbol represents one arbor (19). For comparison, data from normal animals at P30/31 (open squares) and at P39 (open triangles) are also plotted (23). (B) Scattergram of the number of branch points. Symbols as for (A). In both the short- and long-term deprived animals, the total lengths and the number of branch points of arbors serving the D eye are significantly reduced compared to those of arbors serving the ND eye. Note also the difference in both parameters between the arbors for the D eye reconstructed in short-term MD and the arbors obtained in normal animals at P30/31, an age close to that at which deprivation was started in the short-term deprived animals; the deprivation induces a significant retraction of branches.

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 17. Long-term MD was carried out in three animals from before eye-opening to P39. Short-term MD in three animals extended from P34 to P40, from P31 to P38, and from P36 to P42.
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 19. Single geniculocortical arbors from the medial aspect of the lateral gyrus (area 17) were serially reconstructed in three dimensions with a computer and camera lucida system [Neurotrace, A. Passera *et al.*, *Soc. Neurosci. Abstr.* **14**, 550 (1988)]. Eighteen arbors were obtained from long-term deprived animals (12 arbors serving the D eye and 6 arbors serving the ND eye) and 20 from short-term deprived animals (13 arbors serving the D eye and 7 arbors serving the ND eye). We refer to our reconstructions as geniculocortical arbors rather than axons because in the white matter axonal trunks appeared faintly labeled. It is possible that axonal trunks gave off other collaterals in the deepest portion of the white matter [D. Ferster and S. M. LeVay, *J. Comp. Neurol.* **182**, 923 (1978); M. J. Friedlander and K. A. C. Martin, *J. Physiol. (London)* **416**, 183 (1989)].
 20. The monocular deprivation *per se* does not appear to interfere with the transport of the lectin. For both D and ND afferents, the axonal arborization appeared completely labeled: Many processes clearly ended in terminal structures, some of which resembled growth cones, and the finest and most superficial terminals crossed the border between layer 3 and layer 4 and extended in the deepest tier of layer 3, with some very thin branches reaching layer 1. Furthermore, even complete blockade of neuronal activity does not compromise labeling by these techniques: In animals given repeated injections of tetrodotoxin into both eyes, labeled geniculocortical arbors were even larger and more extensive than in normal animals (A. Antonini and M. P. Stryker, *J. Neurosci.*, in press).
 21. The large-cell Y-type geniculocortical pathway appears to be more affected by MD than the X pathway [S. M. Sherman, K.-P. Hoffmann, J. Stone, *J. Neurophysiol.* **35**, 532 (1972)]. We could not unequivocally determine whether the labeled afferents were of the Y or X type, but the proportion of arbors that stratified within the upper half of layer 4, a characteristic of normal Y axons, was even greater for the short-term deprived animals than for the long-term ones, suggesting that the small size of the D eye arbors did not result from failure to label Y-type axons. Previous attempts to label D eye geniculocortical afferents to area 17 in cats 6 to 8 weeks old by intracellular filling, a method that would allow physiological determination of cell type, were unsuccessful.
 22. All comparisons were evaluated by means of the Mann-Whitney U test.
 23. We have included in the analysis eight geniculocortical arbors reconstructed in normal animals at P30/31, an age near the beginning of the short-term deprivation period, and at P39 (A. Antonini and M. P. Stryker, *J. Neurosci.*, in press; compare figure 6 with Fig. 2 of the present report).
 24. Longer periods of MD appear to be required for significant elongation of axonal branches in the ND arbors, as suggested by the tendency of the ND arbors in long-term MD to have a greater total length compared to normal arbors at P39 (*P* borders on significance in this data set: *P* = 0.070).
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