Rapid Remodeling of Axonal Arbors in the Visual Cortex

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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of deprivation, suggesting that synapses become physiologically ineffective before the branches on which they sit are withdrawn.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 geniculo cortical 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 geniculo cortical axonal arbors in animals monocularly deprived for either 4 weeks (long-term) or 6 to 7 days (short-term) during the critical period (17). Geniculo cortical 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 geniculo cortical afferents serving the D or ND eye. Labeled geniculo cortical 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, geniculo cortical 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
axon 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 geniculo-cortical 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 effects of reverse ocuture suture 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, geniculo-cortical 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 geniculo-cortical 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 recovery 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 gluta
tamic 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 neural 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 geniculo-cortical 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 geniculo-cortical affer
ts 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.

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

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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 P36, and from P36 to P42.
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halothane in N₂O/O₂), PHA-L was iontophoreti-
cally injected into the LGN at the stereotactic
coordinates previously identified by the metal
recording electrode. After 10 to 12 days survival,
the animals were perfused transcardially with
ice-cold 0.1 M phosphate buffer followed by
paraformalddehyde fixative. A block of the brain
containing the LGN and the entire caudal pole of
the hemisphere where the visual cortex is located
was cut (80 μm) at the vibratome in the frontal
plane. Sections were processed for standard im-
munohistochemistry. Sections containing the LGN
were stained with cresyl violet for the localization
of the injection sites.
19. Single geniculocortical axons from the medial
aspect of the lateral gyrus (area 17) were serially
reconstructed in three dimensions with a comput-
er and camera lucida system [Neurotrace, A.
(1988)]. Eighteen arbor were obtained from long-
term deprived animals (12 arbor serving the D
eye and 6 arbor serving the ND eye) and 20 from
short-term deprived arbor (13 arbor serving the
eye and 7 arbor serving the ND eye). We refer to
our reconstructions as geniculocortical
t Arbor rather than axons because in the white
matter axonal trunks appeared faintly labeled. It is
possible that axonal trunks gave off other collat-
erals in the deepest portion of the white matter [D.
Ferster and S. M. LeVay, J. Comp. Neurol. 182,
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20. The monocular deprivation per se does not ap-
pear to interfere with the transport of the lectin. For
both D and ND afferevs, 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 one eye, labeled geniculocortical arbor
were even larger and more extensive than in
normal animals (A. Antonini and M. P. Stryker, J.
Neurol., 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 propor-
tion of arbor that straddled 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 arbor 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 determina-
tion 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 geniculo-
cortical arbor 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. Neurol., 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 arbor, as suggested by the tendency of the ND
arbor in long-term MD to have a greater total
length compared to normal arbor at P39 (P
binders on significance in this data set: P =
0.070).
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