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6. With the currently available selective ligands, both D-1 and D-2 receptor subtypes have been implicated in the reinforcing properties of cocaine [G. F. Koob, H. T. Le, I. Creese, *Neurosci. Lett.* **79**, 315 (1987); J. Bergman, J. B. Kamien, R. D. Spealman, *Behav. Pharmacol.* 1, 355 (1990); D. R. Britton *et al.*, *Pharmacol. Biochem. Behav.* **39**, 911 (1991); C. B. Hubner and J. E. Moreton, *Psychopharmacology Berlin* **105**, 151 (1991)].
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9. Each infusion contained 0.25 mg of cocaine in a volume of 100 μl of saline. Baseline rates were defined as three consecutive sessions with less than 10% variation in the total number of self-injections. Various doses of dopamine agonists were combined with cocaine according to a Latin-square design to randomize the order of doses. Other details as previously described [S. B. Caine, R. Lintz, G. F. Koob, in *Behavioral Neuroscience: A Practical Approach*, A. Sahgal, Ed. (Oxford Univ. Press, Oxford, in press)].
10. Drug self-administration in animals exhibits a characteristic inverted U-shaped dose-response curve, and manipulations that shift this curve to the left can be interpreted as increasing the reinforcing potency of the drug. Pretreatments with dopamine agonists before the session have been shown to produce this effect by decreasing the frequency of cocaine self-injections [C. B. Hubner and G. F. Koob, *Neuropsychopharmacology* **3**, 101 (1990)]. Alternatively, this effect can be interpreted as enhancing the rate-decreasing effects of cocaine [S. Herling, D. A. Downs, J. H. Woods, *Psychopharmacology Berlin* **64**, 261 (1979)].
11. Relative potency is the ratio of the amounts of each drug needed to produce the specified effect; a higher numerical value indicates a weaker relative potency. The relative potencies of the agonists to decrease cocaine self-administration were calculated from comparisons of the dose (micromoles) response; 7-OHDPAT was assigned unit potency (1.0). The relative potencies were 3.22 for quinpirole and 8.97 for apomorphine (the correlation coefficients of the regression lines for the potency estimates were $r = 0.79$, $r = 0.85$, and $r = 0.88$, respectively) [R. J. Tallarida and R. B. Murray, *Manual of Pharmacologic Calculations with Computer Programs* (Springer-Verlag, New York, 1987), pp. 35–38].
12. The regression analysis indicated a significant correlation of $r = 0.99$ [$F(1,2) = 414.4$, $P < 0.05$].
13. The regression analyses indicated no significant positive correlation between the relative potencies of the three agonists to decrease cocaine self-administration and their $K_i$ values for the D-2, D-1, or D-4 receptor. Indeed, the correlation coefficients for the regression lines were negative ($r = -0.31$, $r = -0.68$, and $r = -0.75$, respectively, $P > 0.1$ in each case).
14. Low doses of the agonists were not sufficiently reinforcing to produce self-administration in two of the three animals tested with each drug (4 μg of 7-OHDPAT: 2, 2, and 258 injections in 3 hours; 4 μg of quinpirole: 2, 2, and 164 injections in 3 hours; and 8 μg of apomorphine: 0, 11, and 58 injections in 3 hours), but as the dose was increased, characteristic patterns of self-administration were produced in all animals such that the dose per injection was inversely related to the total number of self-injections.
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17. Total number of self-injections in 3 hours (mean ± SEM, $n = 3$, same subjects): cocaine (0.25 mg) alone, 37.4 ± 3.5; apomorphine (16 μg) in combination with cocaine (0.25 mg), 21.2 ± 0.8; apomorphine (32 μg) alone, 21.4 ± 0.7. A double dose of cocaine (0.5 mg) has been shown to reduce the total number of cocaine self-injections by about half [G. F. Koob, F. J. Vaccarino, M. Amalric, F. E. Bloom, in *Brain Reward Systems and Abuse*, J. Engel and L. Oreland, Eds. (Raven, New York, 1987), pp. 35–50].
18. As in (*17*): cocaine (0.25 mg) alone, 46.8 ± 3.0; 7-OHDPAT (4 μg) in combination with cocaine (0.25 mg), 24.0 ± 2.4; 7-OHDPAT (128 μg) alone, 45.0 ± 6.5.
19. As in (*17*): cocaine (0.25 mg) alone, 43.4 ± 0.8; quinpirole (4 μg) in combination with cocaine (0.25 mg), 23.2 ± 1.9; and quinpirole (128 μg) alone, 48.3 ± 16.8.
20. $F(3,15) = 1.1$, $P > 0.1$, main effect, analysis of variance (ANOVA) ($n = 5$).
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28. We thank R. Amstutz, D. Römer, E. Rissi, and P. Seiller of Sandoz Inc., Basel, Switzerland, for providing 7-OHDPAT. We also thank F. E. Bloom for comments throughout these studies and B. Everitt, L. Gold, G. Schulteis, A. Markou, and B. Baldo for comments on the manuscript. Supported by National Institute on Drug Abuse (NIDA) grant DA04398 to G.F.K. and NIDA predoctoral fellowship DA05478 to S.B.C. All animal procedures conformed to the *Guide for the Care and Use of Laboratory Animals* endorsed by the National Institutes of Health. This is publication 7732-NP from The Scripps Research Institute.

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# Molecular Cloning of an Apolipoprotein B Messenger RNA Editing Protein

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**Mammalian apolipoprotein B (apo B) exists in two forms, each the product of a single gene. The shorter form, apo B48, arises by posttranscriptional RNA editing whereby cytidine deamination produces a UAA termination codon. A full-length complementary DNA clone encoding an apo B messenger RNA editing protein (REPR) was isolated from rat small intestine. The 229-residue protein contains consensus phosphorylation sites and leucine zipper domains. HepG2 cell extracts acquire editing activity when mixed with REPR from oocyte extracts. REPR is essential for apo B messenger RNA editing, and the isolation and characterization of REPR may lead to the identification of other eukaryotic RNA editing proteins.**

Apolipoprotein B is a critical structural component of circulating lipoproteins and a major etiologic factor in atherosclerosis susceptibility (*1*). The liver synthesizes a 550-kD form of apo B, apo B100, whereas a smaller form, apo B48, is produced from the small intestine by posttranscriptional editing of a CAA (glutamine) to a UAA (stop) codon in apo B mRNA (*2*, *3*). The truncated apo B48 protein lacks the receptor-binding domains for low-density lipoprotein present in apo B100 and is therefore catabolized by a different receptor pathway (*4*, *5*). Intestinal apo B mRNA editing is developmentally regulated in humans and other mammals (6–8) and appears to be mediated by a protein factor or factors that are sequence-, tissue-, and species-specific (*9–18*).

We developed a functional complementation assay for apo B RNA editing on the basis of our recent observation (*18*) that chicken enterocyte S100 extracts enhance in vitro editing of mammalian apo B RNA (Fig. 1) in spite of the fact that chicken apo B mRNA is not itself edited. Rat intestinal polyadenylated [poly(A)$^+$] RNA was size-fractionated by sucrose gradient ultracentrifugation (*19*) and injected into *Xenopus* oocytes. One fraction (fraction 4, Fig. 1) yielded editing activity in oocyte extracts, a function dependent on the addition of chicken enterocyte S100 extract. This functionally active mRNA fraction was used to construct a plasmid cDNA library that contained ~1 × $10^6$ cDNA clones. Screening

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was carried out with the use of sib selection (*20*), which led to the isolation of a single positive clone (131) designated apo B mRNA editing protein (REPR). In the presence of chicken enterocyte S100 extracts, oocyte homogenates expressing this protein edit more than 50% of a synthetic rat apo B RNA template (Fig. 1).

The DNA and deduced amino acid sequence of REPR are shown in Fig. 2A. The nucleotide sequence is 833 base pairs (bp) long and contains a polyadenylate tract of more than 100 bases. The single open reading frame encodes a 229–amino acid protein with a calculated molecular mass of 27,277 daltons. A search of the National Center for Biotechnology Information peptide sequence database (release 73.1) did not show any other proteins with significant sequence similarity. REPR contains consensus phosphorylation sites for cyclic AMP–dependent kinase (residue 33), protein kinase C (residues 13, 58, and 72), and casein kinase (residue 145). Two leucine zipper motifs were also identified at residues 182 to 203 and residues 189 to 210.

A 1.0-kb transcript encoding REPR is present at highest abundance in RNA from rat small intestine and at lower levels in colon, whereas a 1.24-kb transcript is found at high levels in liver and at lower abundance in kidney, spleen, and lung (Fig. 2B). No hybridization signal was found in brain, heart, stomach, or testis.

Oocyte extracts expressing REPR edit apo B mRNA only in the presence of either chicken (Figs. 1 and 3) or rat intestinal S100 extract (*21*), suggesting an interaction of REPR with another factor or factors. Additionally, a synthetic rat apo B RNA template was not edited after coinjection with REPR into oocytes (*21*). Recent estimates of the size of proteins involved in apo B RNA editing range from 40 to 125 kD, suggesting that REPR may be a component of a larger complex that may include specific apo B RNA binding proteins (*10, 14, 22*). However, no protein of the anticipated size of REPR was identified among several apo B mRNA binding proteins recently reported (*22*). We investigated the importance of the leucine zipper domains in REPR in mediating interactions with another factor or factors because this motif has been postulated to stabilize the interaction of homo- and heterodimeric protein complexes (*23*). Linearization at a Sty I site located at nucleotide 543, upstream of the leucine zipper domains, yielded an in vitro translation product with an apparent molecular mass of 20 kD, as opposed to 25 kD for the full-length translation product (Fig. 3A). This elimination of the leucine

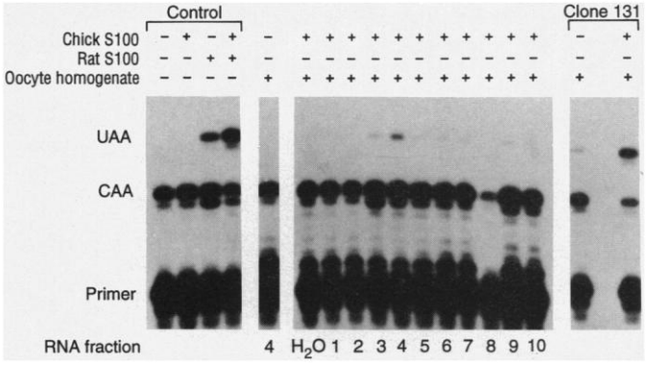


**Fig. 1.** Expression cloning of apo B mRNA editing protein. In vitro RNA editing assays were done with S100 extracts prepared from rat or chicken enterocytes (*27*), demonstrating cooperative interaction as determined by primer extension analysis (control). *Xenopus* oocytes injected (*28*) with $H_2O$ or 100 ng of size-fractionated rat intestinal poly(A)$^+$ RNA (*19*) demonstrate a single fraction (number 4) with in vitro editing activity in the presence of chicken intestinal S100 extract. This RNA fraction was used for cDNA library construction. Functional screening with sib selection (*20*) yielded a single positive clone (number 131). Synthetic RNA (100 ng) transcribed from this clone produced over 50% editing of synthetic rat apo B RNA when assayed in the presence of chicken intestinal S100 extract.

**A**

```
                                                        1
                                                  Met Ser Ser Glu
CCACGCGTCCGAGGAAGGAGTCCAGAGACACAGAGAGCAAG       ATG AGT TCC GAG
                        10
Thr Gly Pro Val Ala Val Asp Pro Thr Leu Arg Arg Arg Ile Glu
ACA GGC CCT GTA GCT GTT GAT CCC ACT CTG AGG AGA AGA ATT GAG
 20                                             30
Pro His Glu Phe Glu Val Phe Phe Asp Pro Arg Glu Leu Arg Lys
CCC CAC GAG TTT GAA GTC TTC TTT GAC CCC CGG GAA CTT CGG AAA
                        40
Glu Thr Cys Leu Leu Tyr Glu Ile Asn Trp Gly Gly Arg His Ser
GAG ACC TGT CTG CTG TAT GAG ATC AAC TGG GGA GGA AGG CAC AGC
 50                                             60
Ile Trp Arg His Thr Ser Gln Asn Thr Asn Lys His Val Glu Val
ATC TGG CGA CAC ACG AGC CAA AAC ACC AAC AAA CAC GTT GAA GTC
                        70
Asn Phe Ile Glu Lys Phe Thr Thr Glu Arg Tyr Phe Cys Pro Asn
AAT TTC ATA GAA AAA TTT ACT ACA GAA AGA TAC TTT TGT CCA AAC
 80                                             90
Thr Arg Cys Ser Ile Thr Trp Phe Leu Ser Trp Ser Pro Cys Gly
ACC AGA TGC TCC ATT ACC TGG TTC CTG TCC TGG AGT CCC TGT GGG
                       100
Glu Cys Ser Arg Ala Ile Thr Glu Phe Leu Ser Arg Tyr Pro His
GAG TGC TCC AGG GCC ATT ACA GAA TTT TTG AGC CGA TAC CCC CAT
110                                            120
Val Thr Leu Phe Ile Tyr Ile Ala Arg Leu Tyr His His Ala Asp
GTA ACT CTG TTT ATT TAT ATA GCA CGG CTT TAT CAC CAC GCA GAT
                       130
Pro Arg Asn Arg Gln Gly Leu Arg Asp Leu Ile Ser Ser Gly Val
CCT CGA AAT CGG CAA GGA CTC AGG GAC CTT ATT AGC AGC GGT GTT
140                                            150
Thr Ile Gln Ile Met Thr Glu Gln Glu Ser Gly Tyr Cys Trp Arg
ACT ATC CAG ATC ATG ACG GAG CAA GAG TCT GGC TAC TGC TGG AGG
                       160
Asn Phe Val Asn Tyr Ser Pro Ser Asn Glu Ala His Trp Pro Arg
AAT TTT GTC AAC TAC TCC CCT TCG AAT GAA GCT CAT TGG CCA AGG
170                                            180
Tyr Pro His Leu Trp Val Arg Leu Tyr Val Leu Glu Leu Tyr Cys
TAC CCC CAT CTG TGG GTG AGG CTG TAC GTA CTG GAA CTC TAC TGC
                       190
Ile Ile Leu Gly Leu Pro Pro Cys Leu Asn Ile Leu Arg Arg Lys
ATC ATT TTA GGA CTT CCA CCC TGT TTA AAT ATT TTA AGA AGA AAA
200                                            210
Gln Pro Gln Leu Thr Phe Phe Thr Ile Ala Leu Gln Ser Cys His
CAA CCT CAA CTC ACG TTT TTC ACG ATT GCT CTT CAA AGC TGC CAT
                       220                             229
Tyr Gln Arg Leu Pro Pro His Ile Leu Trp Ala Thr Gly Leu Lys
TAC CAA AGG CTA CCA CCC CAC ATC CTG TGG GCC ACA GGG TTG AAA
OP
TGA CTTCTGGGAGTTGGGGATGGATGAAATGACTCCTTGTATGTCTTGACAGCAGCAAT
TGATTACCCACTAAAGAGCGACTGCCACAAGGAATCTAGAAGTCGAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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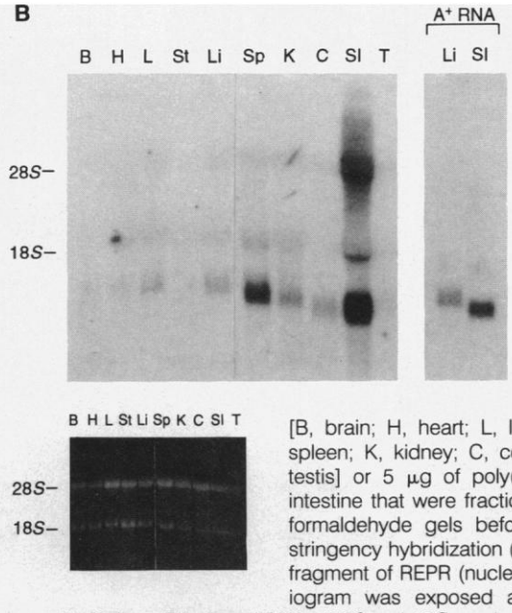


**Fig. 2.** Sequence and tissue distribution of REPR. (**A**) Nucleotide and predicted amino acid sequence of REPR. The Sty I site used to generate the truncated REPR is underlined, as are the two leucine zipper domains. The cDNA sequence has been deposited with GenBank (accession number L07114). (**B**) Tissue distribution of REPR mRNA. We prepared Northern (RNA) blots by using either 20 μg of total RNA from adult rat tissues [B, brain; H, heart; L, lung; St, stomach; Li, liver; Sp, spleen; K, kidney; C, colon; SI, small intestine; and T, testis] or 5 μg of poly(A)$^+$ RNA from liver and small intestine that were fractionated through 1% agarose–6% formaldehyde gels before capillary transfer and high-stringency hybridization (*18*) with a 422-bp Sma 1–Kpn 1 fragment of REPR (nucleotides 129 to 551). The autoradiogram was exposed at −80°C with two intensifying screens for 6 days. Ribosomal RNAs (28*S* and 18*S*) are indicated. The lower portion shows the ethidium bromide–stained gel of total RNA.

zipper domains from REPR completely abolished the editing activity (Fig. 3B) in oocyte extracts, suggesting that this region is necessary for the editing function of the protein.

We investigated the interaction of REPR with other sources of cytoplasmic S100 extract to provide insight into the cell-specific distribution of the apo B mRNA editing machinery. G-292 cells have previously been shown to edit a transfected, chimeric apo B–apo E RNA, although like CHO cells, they do not express endogenous apo B mRNA (*11*). S100 extracts were also prepared from HepG2 cells, in which apo B mRNA is unedited (*3*), and $McA_{7777}$ cells, in which apo B mRNA is edited at a low level (9). At the concentrations used, no editing activity was detectable with these S100 extracts either alone or together with chicken enterocyte S100 extracts (Fig. 4, left and middle). However, addition of REPR increased editing with HepG2 and $McA_{7777}$ extracts to 23% and 30%, respectively, whereas G292 and CHO cell extracts failed to complement REPR activity (Fig. 4, right). The failure of G292 extracts to complement in vitro editing activity in spite of the ability of this cell to edit a transfected apo B–apo E chimera may be attributable either to structural differences in the apo B RNA template (*24*) or to the very low levels contained by (cytoplasmic) S100 extracts of the factor or factors responsible for editing of the (nuclear) transcript (*25*).

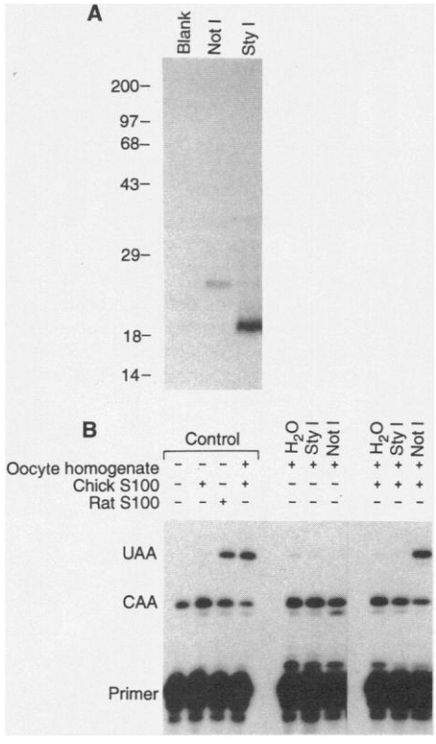


**Fig. 3.** Apo B mRNA editing protein activity is abolished after elimination of the leucine zipper domains. (**A**) In vitro translation analysis of full-length apo B mRNA editing protein (linearized with Not I) and a truncated apo B mRNA editing protein that lacks the leucine zipper domains (linearized with Sty I, Fig. 2A). Approximately 1 μg of capped synthetic RNA was transcribed and translated with [$^{35}$S]methionine. The products were analyzed by 12.5% SDS–polyacrylamide gel electrophoresis and stained with Coomassie blue; the fluorogram is shown. Molecular size markers are indicated at left. (**B**) In vitro RNA editing assays demonstrate the requirement for additional factors for effective conversion by REPR (left and middle). REPR expressed in oocyte homogenates is ineffective in the absence of either chicken or rat (*21*) enterocyte S100 extract. The importance of the leucine zipper domains (right) is suggested by the abolition of in vitro apo B RNA editing with the Sty I truncated protein. In vitro editing assays were performed as in Fig. 1 and used similar amounts of oocyte homogenate.

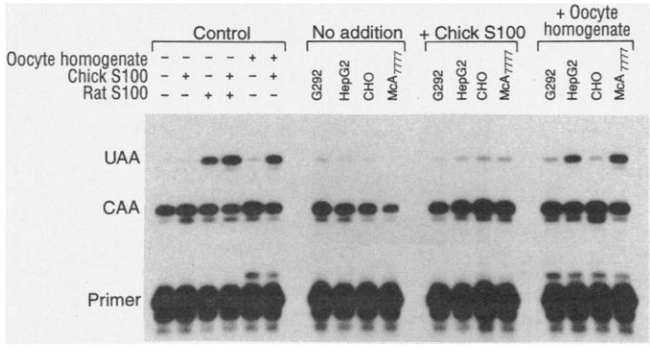


**Fig. 4.** Interaction of S100 extracts prepared from G292, HepG2, CHO, and $McA_{7777}$ cells with oocyte homogenates expressing REPR. Control in vitro editing reactions are shown, with conditions as described in Fig. 1. S100 extracts were prepared from G292, HepG2, CHO, and $McA_{7777}$ cells and assayed alone (no addition) or with 10 μg of chicken enterocyte S100 extracts (+ chick S100). In neither of these conditions was in vitro editing observed (<5%). In the presence of 10 μg of oocyte homogenate expressing REPR (+ oocyte homogenate), effective in vitro conversion with both HepG2 and $McA_{7777}$ extracts was observed. Apo B RNA editing was assayed by primer extension, and the primer, unedited (CAA), and edited (UAA) products are shown.

We have thus isolated and characterized a cDNA encoding a protein essential to intestinal apo B mRNA editing. This clone will facilitate the identification of other components of the apo B RNA editing machinery that appear to be widely distributed in human tissues (6). In addition, the clone may help identify other genes involved in sequence-specific RNA editing such as occurs in a differential manner among the brain glutamate receptor subunits (*26*).

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the homogenate stored at −80°C.
29. We thank M. Kwon, V. Trieu, D. K. Bonen, F. Giannoni, C. Hadjiagapiou, G. I. Bell, and A. D. Sniderman for helpful discussions. Supported by a grant-in-aid (to B.T.) from the American Heart Association (Chicago affiliate) and awards HL-38180, KO-4 HL-02166, and DK-42086 (to N.O.D.) from NIH.

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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*). Geniculocortical 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

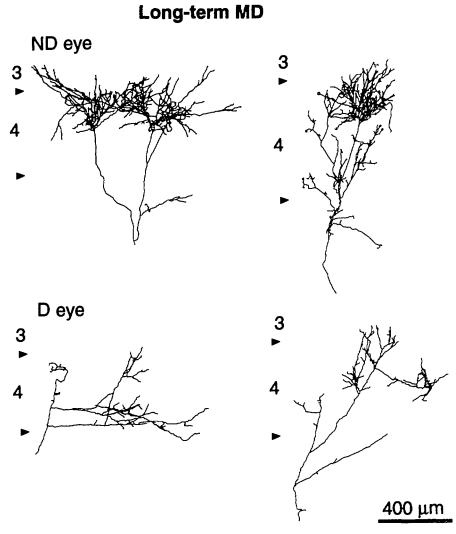


**Fig. 1.** Coronal view of geniculocortical arbors reconstructed in kittens in which one eye had been occluded for 33 days. The terminal arborization of the deprived (D) eye shows a dramatic reduction in complexity as compared to that of the nondeprived (ND) eye. Cortical layers 3 and 4 are indicated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

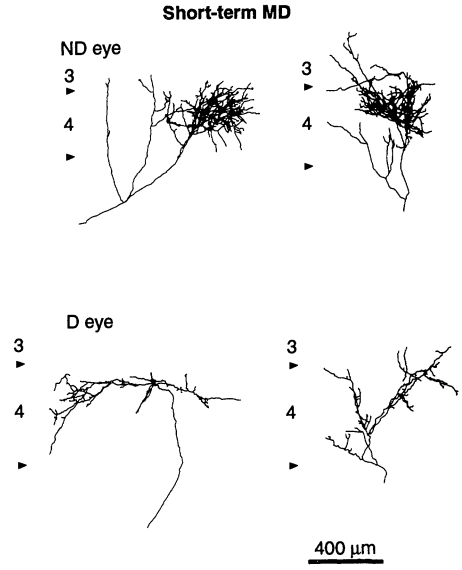


**Fig. 2.** Coronal view of geniculocortical arbors reconstructed in kittens in which one eye had been occluded for 6 to 7 days. The borders of cortical layers 3 and 4 are indicated by arrowheads.

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