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Distributions of Synaptic Vesicle Proteins and GAD65 in Deprived and Nondeprived Ocular Dominance Columns in Layer IV of Kitten Primary Visual Cortex Are Unaffected by Monocular Deprivation

MICHAEL A. SILVER and **MICHAEL P. STRYKER***
W.M. Keck Center for Integrative Neuroscience and Neuroscience Graduate Program, Department of Physiology, University of California, San Francisco, California 94143-0444

Abstract

Two days of monocular deprivation (MD) of kittens during a critical period of development is known to produce a loss of visual responses in the primary visual cortex to stimulation of the nondeprived eye, and 7 days of deprivation results in retraction of axon branches and loss of presynaptic sites from deprived-eye geniculocortical arbors. The rapid loss of responsiveness to deprived-eye visual stimulation could be due to a decrease in intracortical excitatory input to deprived-eye ocular dominance columns (ODCs) relative to nondeprived-eye columns. Alternatively, deprived-eye visual responses could be suppressed by an increase in intracortical inhibition in deprived columns relative to nondeprived columns. We tested these hypotheses in critical period kittens by labeling ODCs in layer IV of primary visual cortex with injections of the anterograde tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L) into lamina A of the lateral geniculate nucleus (LGN). After either 2 or 7 days of MD, densities of intracortical excitatory presynaptic sites within deprived relative to nondeprived ODCs were estimated by measuring synaptic vesicle protein (SVP) immunoreactivity (IR). Because most of the synapses within layer IV of primary visual cortex are excitatory inputs from other cortical neurons, levels of SVP-IR provide an estimate of the amount of intracortical excitatory input. We also measured levels of immunoreactivity of the inhibitory presynaptic terminal marker glutamic acid decarboxylase (GAD)65 in deprived relative to nondeprived ODCs. Monocular deprivation (either 2 or 7 days) had no effect on the distributions of either SVP- or GAD65-IR in deprived and nondeprived columns. Therefore, the rapid loss of deprived-eye visual responsiveness following MD is due neither to a decrease in intracortical excitatory presynaptic sites nor to an increase in intracortical inhibitory presynaptic sites in layer IV of deprived-eye ODCs relative to nondeprived columns.

Keywords

presynaptic terminal; striate cortex; lateral geniculate body; GABA; synaptophysin

Deprivation of pattern vision in one eye for as few as 2 days during a critical period in early life causes a loss of responsiveness of neurons in primary visual cortex to visual stimulation of the deprived eye (Wiesel and Hubel, 1963; reviewed in Hensch, 1996). This phenomenon is known as ocular dominance plasticity, and the mechanisms by which the deprived eye comes to lose its ability to drive cortical neurons remain unknown. The major synaptic inputs to layer IV neurons in primary visual cortex of the cat have been extensively characterized (Winfield, 1983; Beaulieu and Colonnier, 1985; Ahmed et al., 1994), and they consist of excitatory thalamocortical synapses and excitatory and inhibitory intracortical connections. Two days of

*Correspondence to: Professor Michael P. Stryker, Department of Physiology, Room S-762, 513 Parnassus Ave., University of California, San Francisco, CA 94143-0444. E-mail: stryker@phy.ucsf.edu

monocular deprivation (MD) results in no change in the numbers of presynaptic terminals in deprived relative to nondeprived geniculocortical arbors (Silver and Stryker, 1999b). One alternative mechanism to explain the decrease in cortical responsiveness to deprived-eye stimulation is selective loss of intracortical excitatory input in deprived-eye ocular dominance columns (ODCs). Recurrent local excitatory connections in primary visual cortex are thought to amplify sensory signals from thalamocortical inputs in a nonlinear manner (Douglas and Martin, 1991), and even a small decrease in excitatory drive in deprived ODCs could cause a substantial decrease in responses to deprived-eye visual stimulation.

Another possibility is that an increase in intracortical inhibition in deprived ODCs could result in suppression of deprived-eye cortical visual responses. GAD65 is an isoform of the gamma aminobutyric acid (GABA) synthetic enzyme glutamic acid decarboxylase (GAD), which is localized preferentially to inhibitory presynaptic terminals (Kaufman et al., 1991; Esclapez et al., 1994), and disruption of the GAD65 gene in mice prevents ocular dominance plasticity (Hensch et al., 1998). In addition, Jones and colleagues have demonstrated changes in the patterns of expression of GABA and GAD in adult monkey visual cortex following monocular deprivation (reviewed in Jones, 1993; see Discussion).

To test these hypotheses, we have developed a method for measuring the amounts of synaptic vesicle protein (SVP)- or GAD65 immunoreactivity (IR) as a function of position within deprived and nondeprived ODCs. Because the overwhelming majority of layer IV synapses are intracortical excitatory connections (Winfield, 1983; Beaulieu and Colonnier, 1985; Ahmed et al., 1994), measurement of overall SVP-IR is a valid approximation of the density of intracortical excitatory presynaptic sites. Similarly, the levels of GAD65-IR represent a quantitative estimate of the density of intracortical inhibitory presynaptic sites. Our results indicate that following 2 days of MD, both SVP levels and GAD65 levels remain equal in deprived and nondeprived ODCs. Therefore, the loss of deprived-eye visual responsiveness after 2 days of MD is not due to either a decrease in intracortical excitatory presynaptic sites or an increase in intracortical inhibitory presynaptic sites in layer IV of deprived-eye ODCs relative to nondeprived-eye columns.

Seven days of MD is sufficient to cause retraction of deprived-eye geniculocortical axon branches (Antonini and Stryker, 1993b) and loss of presynaptic terminals from these arbors (Silver and Stryker, 1999b). To determine whether these presynaptic rearrangements in the geniculocortical projection are accompanied by similar changes in intracortical connections, we measured SVP- and GAD65-IR in deprived and nondeprived ODCs. Our results again show that 7 days of MD have no effect on levels of either SVP- or GAD65-IR in deprived relative to nondeprived ODCs. These findings constrain possible substrates of activity-dependent plasticity in primary visual cortex during the critical period. Some of these data have been presented in abstract form (Silver and Stryker, 1998, 1999a).

MATERIALS AND METHODS

Most of the anatomical methods used in this study are identical to those in an earlier publication (Silver and Stryker, 1999b) and are therefore described only very briefly here.

Care and use of animals

A total of eight kittens were used in this study. All were from the breeding colony at the University of California, San Francisco, and had normal pigmentation. All procedures involving living animals were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Committee on Animal Research, University of California, San Francisco.

Labeling of ocular dominance columns and monocular deprivation

ODCs in layer IV of primary visual cortex were labeled by injecting the anterograde neuronal tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L; Gerfen and Sawchenko, 1984) into lamina A of the lateral geniculate nucleus (LGN) of both hemispheres in kittens (age 26-30 days). This procedure has been previously described (Antonini and Stryker, 1993a; Silver and Stryker, 1999b). All injection sites used in this study were in the dorsal portion of lamina A within the binocular representation of the visual field. Monocular lid sutures were performed under isoflurane anesthesia for some animals on either postnatal day (P)33 (7-day MD) or P38 (2-day MD).

Perfusion and tissue processing

After recovery from surgery, PHA-L was anterogradely transported by LGN axons to their site of termination in layer IV of primary visual cortex. The transport period was 10-14 days, after which kittens were deeply anesthetized on P40 with an intraperitoneal injection of pentobarbital (100 mg/kg). They were then perfused transcardially, and the LGNs and visual cortex were sectioned coronally as previously described (Silver and Stryker, 1999b).

Immunohistochemistry and immunofluorescence

Standard diaminobenzidine (DAB) immunohistochemical techniques were employed to determine the location of PHA-L-labeled neurons in the LGN. To ensure that the injection sites were confined to the binocular region of lamina A of the LGN, every section of each LGN was processed. Every injection site used in this study was located in the binocular portion of the LGN, and any label resulting from injection sites in either the monocular segment of the LGN or the medial intralaminar nucleus was excluded from further analysis. Because cortical sections containing labeled ODCs were required for double label immunofluorescence, only every seventh section of the visual cortical blocks were reacted with DAB. This allowed an estimate of the anterior and posterior boundaries of the PHA-L-labeled geniculocortical afferents in the visual cortex but still left most of the cortical sections in this region available for immunofluorescence. Descriptions of the immunohistochemical and immunofluorescent techniques used to label PHA-L, SVPs, and GAD65 are in Silver and Stryker (1999b).

Confocal microscopy and image processing

Immunofluorescent sections were imaged by using either an MRC600 or MRC1024 confocal microscope (Biorad, Hercules, CA). Sections with at least two distinct PHA-L-labeled ODCs in layer IV of area 17 in primary visual cortex were selected for further analysis. Stacks of seven SVP or GAD65 optical sections separated by 1 μm were collected through a 60× oil immersion objective lens with a numerical aperture of 1.4. Microscope settings were as follows: zoom setting = 2.0, iris setting = 3 for the MRC600 (corresponding to an aperture size of 2.16 mm) or 1 for the MRC1024 (corresponding to 1 mm), and image size of 768 × 512 pixels (corresponding to 102 μm × 68 μm for the MRC600 and 98 μm × 65 μm for the MRC1024). For each section, gain and black level settings in the SVP or GAD65 channel were chosen to use the full range of pixel intensities (0-255) without saturation at either end of the pixel intensity range. Beginning at the edge of the PHA-L-labeled region, a series of image stacks were collected approximately every 120 μm along a tangential path within layer IV. Once the gain and black level settings were established, they were kept constant for every stack collected from the tissue section. Image collection continued until the PHA-L-labeled region was completely traversed. After each SVP or GAD65 image stack was collected, the field was photobleached to mark its position within the PHA-L-labeled ODCs.

The intensity of SVP and GAD65 label decreases with increasing depth of the optical section within the tissue due to poor antibody penetration (Calhoun et al., 1996) and increased light

scattering. This would introduce variability into the measurements if the image stacks were collected at different tissue depths. To prevent this, stacks of images were always collected at depths where the SVP and GAD65 signal were greatest (invariably at the top face of the tissue section), and the image within each stack that had the highest average pixel intensity was selected for further analysis. After all of the SVP or GAD65 stacks were collected, the tissue section was imaged at low power in the PHA-L channel to visualize the labeled ODCs and the photobleached fields (Fig. 1).

Image processing was performed on a Macintosh computer by using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Statistical analysis

For a given experimental condition, relative fluorescence values representing the amount of label in a single field were expressed as contrast indices (see Results) and graphed on a scatter plot versus distance from ODC center. Coefficients of determination (r^2) were computed to measure the strength of the relationship between the two variables, and simple linear regression was performed to derive the slopes (regression coefficients) of the linear best-fit functions. These were converted to percent change from one ODC center to the next (assuming a column width of 400 μm) by using the following formula:

$$\%\text{change} = 2\frac{(400)(\text{slope})}{[1-(400)(\text{slope})]} * 100$$

t-tests were used to determine whether regression coefficients were significantly different from 0 at the 0.05 confidence level (p. 271-272 of Zar, 1984). Two-tailed tests were carried out for the data from control animals with normal visual experience, whereas one-tailed tests were used for MD animals. *P* values generated by the t-tests were corrected for multiple comparisons using the formula $p_{corr} = 1-(1-p)^n$, where n is the number of comparisons (p. 376 of Hays, 1963) and had a value of 2 for the normal control synaptophysin animal, 6 for the 2-day MD SVP animals, 5 for the 7-day MD SVP animals, and 6 for the GAD65 animals (including all GAD65 deprivation conditions).

For each of the t-tests performed in this study, the null hypothesis H_0 was that there was no relationship between relative fluorescence and position within ocular dominance columns. In general, if a given t-test fails to provide statistical evidence to reject H_0, this conclusion is not equivalent to the positive assertion that there is truly no relationship between the two variables. It is possible that the null hypothesis was not rejected because the sample size was not large enough or because the data were too variable. Statistical power analysis is a technique that provides quantitative estimates of the amount of confidence in a negative result. In the case of simple linear regression, one can compute the minimum value of the regression coefficient (the slope of the linear best-fit function) that could have been detected in a population given the sample data set and chosen confidence levels (p. 75-83 of Cohen, 1977). These minimum detectable regression coefficient values were converted into minimum detectable differences (expressed in units of percent change) to provide estimates of the differences in relative fluorescence values between one ODC center and the next, which could have been detected with our data, if such differences had actually been present in the population.

Laminar analysis of GAD65 immunoreactivity

Differences between cortical layers in GAD65 fluorescence values were assessed by collecting a single line of fields spanning all laminae in the cortical plate. The immunofluorescence procedures described above were performed, except sections were mounted onto glass microscope slides, allowed to dry, cleared in xylenes, and mounted in DPX medium (Electron

Microscopy Sciences, Fort Washington, PA). To avoid potential artifact from any systematic nonstationarities, data collection began at different depths within the cortical plate in the different tissue sections. Of the four tissue sections used for this analysis, one began in the white matter and progressed continuously to the pial surface, one went from the pial surface to the white matter, one began in the center of the cortical plate, progressed to the pia, and then continued from the white matter back to the beginning, and one went from the center of the cortical plate to the white matter and then from the pia back to the beginning. Following image collection and photobleaching, the coverslips were removed by immersion in xylenes, and the sections were stained with cresyl violet. Based on this Nissl stain, laminar boundaries of the sections were determined by using camera lucida. The laminar boundaries were overlaid on the low-magnification photobleached GAD65 images, and fields were assigned to cortical laminae. Fields that fell on the boundaries between cortical laminae were excluded from further analysis.

One-way analysis of variance (ANOVA) was performed to determine whether the amount of GAD65 label varied as a function of cortical layer. For pairwise comparisons, differences between average fluorescence values for 2 laminae (abbreviated as **d**) were converted from units of contrast index to percent differences using the following formula:

$$\%\text{difference} = 2\frac{\text{d}}{1-\text{d}} * 100$$

RESULTS

Labeling of ocular dominance columns in layer IV of primary visual cortex

Cat primary visual cortex is organized into ODCs, which are oriented radially within the cortical plate, span all cortical layers, and are approximately 400- to 500-μm-wide in area 17 (kitten: LeVay et al., 1978; adult cat: Shatz et al., 1977). They are defined physiologically as alternating regions of cortex in which visual responses of cortical neurons are dominated by one eye over the other (Hubel and Wiesel, 1962). Anatomically, ODCs were first visualized by observing degenerating geniculocortical afferents projecting to layer IV of primary visual cortex following lesions of part of one layer of the LGN (Hubel and Wiesel, 1972) and later by transneuronal labeling of the thalamocortical projection with intraocular injections of tritiated proline in the cat (Shatz et al., 1977). There is good agreement between anatomical column boundaries and those defined by using physiological criteria (Shatz and Stryker, 1978).

The three major synaptic inputs to layer IV neurons in primary visual cortex are excitatory geniculocortical, excitatory intracortical, and inhibitory intracortical (Ahmed et al., 1994). Changes in synaptic strength in any of these inputs could account for the rapid loss of deprived-eye visual responses of primary visual cortical neurons following 2 days of monocular deprivation (reviewed in Hensch, 1996). Deprived-eye synaptic inputs could become less effective through the loss of excitatory presynaptic neuro-transmitter release sites and/or the addition of inhibitory presynaptic sites serving the deprived eye. This possibility has been tested for the geniculocortical inputs, where 2 days of MD results in no change in the numbers of presynaptic sites in deprived relative to nondeprived afferents, whereas 7 days causes a 50% decrease in the number of deprived-eye presynaptic sites relative to nondeprived-eye sites (Silver and Stryker, 1999b).

Because the axons making up the geniculocortical projection are strictly monocular, it is straightforward to determine the numbers of presynaptic sites in deprived relative to nondeprived afferents by anatomically labeling afferents serving one eye and counting the number of presynaptic sites labeled with a marker for SVPs. On the other hand, both the intracortical excitatory and intracortical inhibitory inputs are of mixed ocular dominance, and

current techniques do not allow selective labeling of a homogeneous population of intracortical axons of known ocular dominance. However, the response properties of neurons as a function of their position within ODCs suggest that the ocular dominance of intracortical inputs is not randomly distributed in the visual cortex (Shatz and Stryker, 1978). For example, the centers of left-eye ODCs contain primarily synaptic inputs representing the left eye. The fraction of left-eye inputs will decrease as one moves tangentially along layer IV away from the column center. As one moves into right-eye territory, the cortex will contain an increasing fraction of right-eye synaptic inputs until the right-eye ODC center is reached.

The long-range connections between cortical columns, which in the cat are not selective for eye of origin (Löwel and Singer, 1992), are found primarily in the upper layers and therefore do not contribute to the presynaptic sites measured in layer IV in the present study. The excellent correspondence between the physiological ODC boundaries in kitten layer IV and those defined by anatomical labeling of the geniculocortical projection (Shatz and Stryker, 1978) is probably due instead to a combination of the segregation of the monocular geniculocortical afferents (Shatz et al., 1977) and the high density of very short-range excitatory and inhibitory intracortical connections within layer IV which preserve the spatial pattern of the thalamic inputs. Evidence for the latter point comes from cross-correlation analysis of pairs of primary visual cortical neurons (kitten: Hata et al., 1993; adult cat: Hata et al., 1991). In these studies, monosynaptic excitatory as well as monosynaptic inhibitory connections between neurons were most frequently observed at distances of less than 400 μm (approximately the width of one ODC).

In addition, many excitatory layer IV spiny neurons in primary visual cortex have axons whose layer IV projections arborize mostly within 200 μm of the cell body (kitten: Callaway and Katz, 1992; adult cat: Martin and Whitteridge, 1984; Anderson et al., 1994). A similar pattern of short-range axonal arborizations within layer IV has been observed for inhibitory clutch cells located in layer IV of cat primary visual cortex (Kisvárday et al., 1985), although the arbors of inhibitory basket cells can be substantially larger (Somogyi et al., 1983). In conclusion, we assume that by measuring the levels of immunohistochemical markers for intracortical excitatory or inhibitory presynaptic sites as a function of position within ODCs in layer IV, differences in the density of these sites representing either the left or right eye can be estimated.

To determine the effects of MD on the distribution of intracortical excitatory and inhibitory presynaptic sites in deprived and nondeprived ODCs, columns in primary visual cortex were labeled by injections of the anterograde tracer PHA-L in lamina A of the lateral geniculate nucleus, and double label immunofluorescence was performed by using an anti-PHA-L antibody and one of a panel of antibodies raised against either a SVP or the inhibitory presynaptic terminal marker GAD65. PHA-L was taken up by monocular geniculate neurons serving the eye contralateral to the injection sites and transported to geniculocortical arbors. Figure 1 illustrates distinct PHA-L-labeled ODCs in layer IV of primary visual cortex.

The edges of the PHA-L-labeled region (arrowheads in Fig. 1) could represent either the edges of labeled ODCs or portions of ODCs that were not completely labeled because of limited spread of PHA-L at the injection sites in the LGN. However, the presence of two labeled ODCs flanking an unlabeled column serving the eye ipsilateral to the injection sites allows a precise determination of the center of the unlabeled column (arrows in Fig. 1), even if the two flanking columns are only partially labeled. For this reason, all column center designations used in this paper were made in unlabeled ODCs flanked by PHA-L-labeled columns on either side.

Labeling of synaptic vesicle proteins and GAD65

To determine the density of intracortical excitatory and inhibitory presynaptic sites as a function of position within ODCs, quantifiable labels for these types of inputs were required.

Electron microscopic analysis of synaptic density of primary visual cortex has shown that the vast majority of synapses in layer IV are excitatory and of intracortical origin. Approximately 6% of layer IV synapses in adult cat are thalamocortical (Ahmed et al., 1994), although estimates range as high as 28% (LeVay and Gilbert, 1976). Measurements of the fraction of layer IV synapses that are symmetric (and therefore inhibitory) include 15% (adult cat; Beaulieu and Colonnier, 1985) and 1.5% (P40 kitten; Winfield, 1983). Therefore, measurements of total synaptic density in layer IV reflect mostly intracortical excitatory synapses, and the density of overall SVP label can be used to estimate the density of intracortical excitatory presynaptic sites.

To measure this density, one of a panel of anti-SVP antibodies (anti-synaptophysin (Wiedenmann and Franke, 1985), anti-synaptotagmin (Matthew et al., 1981), or anti-SV2 (Buckley and Kelly, 1985)) were used to label presynaptic sites. The density of intracortical inhibitory presynaptic sites was determined with an antibody raised against GAD65 (Chang and Gottlieb, 1988), a protein which is specifically localized to GABAergic presynaptic terminals (Esclapez et al., 1994). Images of SVP or GAD65 immunofluorescent label were collected at high power with a confocal microscope as a series of fields in a tangential trajectory within layer IV of primary visual cortex. Examples of raw images are shown in Figure 2A, C, E, and G. These show the characteristic pattern of presynaptic terminal markers, including punctate neuropil staining and absence of label from non-neuropil structures such as cell bodies and blood vessels. Figure 2B, D, F, and H are examples of SVP or GAD65 label at higher magnification. Punctate structures corresponding to individual presynaptic sites can be distinguished. Following image collection, each field was photobleached to mark its location within the ODCs (dark rectangles in Fig. 1).

Individual images vary in the number and size of cell bodies, blood vessels, and other non-neuropil structures which generally do not contain SVP or GAD65 label. To remove this source of variability from the data set, all non-neuropil structures were manually traced, and the average neuropil pixel intensity was computed for the remaining portion of the field, resulting in a single density measurement for each field (approximately 100 × 70 μm). All image collection and processing was done blind with respect to which eye was represented by the PHA-L-labeled ODCs.

Because gain and black level settings were kept constant for all the images collected from a tissue section, quantitative comparisons of the amount of SVP- or GAD65-IR could be made between fields and correlated with the positions of those fields within ODCs. These relative comparisons remove several sources of variability between tissue sections, including quality of perfusion and antibody penetration. Many fields were collected in each tissue section in a line that spanned several ODCs, and the average SVP density across all of these fields represents the SVP density for the tissue section. Average SVP density measurements from single fields were then compared to the group average in the form of a relative fluorescence metric using the following contrast index:

$$\frac{X_{ind} - X_{avg}}{X_{ind} + X_{avg}}$$

where X_{ind} is the SVP density for an individual field and X_{avg} is the average SVP density across all fields in the tissue section. This contrast index would be positive if the individual field had more label than the average, negative if the individual field had less label than the average, and it would have a value of 0 if the individual field were equal in intensity to the section average.

To correlate these relative fluorescence values with position within labeled ODCs, a line connecting the centers of the measured and photobleached fields was drawn on the low-power

PHA-L montage, and the cumulative distance between the column center and the field center wasmeasured along this line. Fields more than one ODC width from the column center were excluded from analysis. Although Shatz et al. (1977) reported that the average ODC width in adult cats is approximately 500 μm, we have conservatively chosen 400 μm as the ODC width to avoid the possibility that some fields were located beyond the center of the adjacent PHA-L-labeled ODC.

Baseline variability in normal control animals

Before determining the effects of MD on SVP density in deprived and nondeprived ODCs, the relative fluorescence technique described above was employed in one kitten with normal visual experience to establish the baseline variability in the measurements. Because geniculate neurons in lamina A represent exclusively the contralateral eye, injections of PHA-L into lamina A of the LGN in each hemisphere allowed the labeling of left-eye ODCs in the right cortical hemisphere and right-eye columns in the left hemisphere of the same animal. We assume that in undeprived animals, the density of SVP label (and by extension, the density of intracortical excitatory inputs) should be the same in left-eye and right-eye columns. Specifically, a plot of relative SVP fluorescence versus distance from the center of one eye's ODC should indicate no correlation between these two variables. This was observed for synaptophysin immunofluorescence (Fig. 3). Solid lines indicate the linear best fit for the data, and the slopes of these lines were not significantly different from 0 (Table 1).

Two days of MD and relative SVP density

Two days of MD at the height of the critical period is sufficient to cause a saturating shift in responses of visual cortical neurons in favor of the nondeprived eye (reviewed in Hensch, 1996) without any change in the numbers of presynaptic sites in deprived relative to nondeprived geniculocortical afferents (Silver and Stryker, 1999b). As the majority of excitatory current in primary visual cortical cells comes from other cortical neurons (Douglas and Martin, 1991), one hypothesis to explain the rapid loss of deprived-eye responsiveness following MD is a decrease in the number of intracortical excitatory presynaptic sites serving the deprived eye. Because deprived-eye ODCs contain mainly deprived-eye synapses, this hypothesis predicts that the density of intracortical excitatory presynaptic sites (as estimated by overall SVP measurements) should be less in deprived-eye ODCs than in nondeprived-eye columns. Specifically, SVP density should decrease with increasing distance from the center of nondeprived-eye ODCs and increase with increasing distance from the center of deprived-eye ODCs. Figure 4 shows the results of testing this hypothesis for the SVPs synaptophysin, synaptotagmin, and SV2. In all cases, the slope of the linear best-fit function is not significantly different from 0 (Table 1).

Statistical power analysis (Cohen, 1977) was performed to determine the amount of confidence in these negative results. In principle, if there actually were a difference in the amounts of SVP label in deprived relative to nondeprived columns, it is possible that our sample was not large enough or had too much variability to detect this difference. To quantify the likelihood of this possibility, we chose a confidence level of 90% ($\beta = 0.1$) and computed the minimum detectable difference in fluorescence between deprived and nondeprived column centers which could have been observed given these particular sample sizes and variances. Because the 3 SVP markers did not appear to differ from each other, the data were pooled, and the average minimum detectable difference was 24.7%. In addition, qualitative examination of double label immunofluorescent sections at low magnification indicated that SVP density was uniform along layer IV and did not show a pattern of label that correlated with the PHA-L-labeled ODCs (data not shown). This indicates that SVP density is independent of position within either deprived or nondeprived ODCs and that the loss of responses of primary visual cortical neurons

to deprived-eye stimulation following 2 days of MD is not due to elimination of intracortical excitatory presynaptic sites serving the deprived eye.

Seven days of MD and relative SVP density

Seven days of MD causes branch retraction and loss of presynaptic sites in deprived-eye geniculocortical arbors such that both the total arbor length (Antonini and Stryker, 1993b) and the number of presynaptic sites per arbor (Silver and Stryker, 1999b) are approximately 50% of their normal values. If analogous elimination of intracortical excitatory presynaptic sites transmitting deprived-eye signals occurs following 7 days of MD, this should be reflected as a difference in the overall SVP density of deprived and nondeprived ODCs. More precisely, the intensity of SVP label should be maximal in the center of nondeprived ODCs and decrease with increasing distance from the column center, whereas increasing distance from the center of deprived ODCs should be correlated with an increase in immunofluorescence of SVP markers.

Synaptic vesicle protein density in deprived relative to nondeprived ODCs did not change following 7 days of MD (Fig. 5). None of the linear best-fit functions had a slope significantly different from 0 (Table 1). To estimate the power of these negative results, minimum detectable differences were computed for each regression equation. One can be 90% certain that the difference in SVP levels between deprived and nondeprived ODCs is less than 23.4%. Inspection of images at low magnification also indicated that 7 days of MD had no effect on the levels of SVP label in deprived relative to nondeprived ODCs (data not shown). In conclusion, the elimination of deprived-eye geniculocortical presynaptic sites following 7 days of MD (Silver and Stryker, 1999b) is not accompanied by loss of intracortical excitatory presynaptic sites in deprived-eye ODCs.

Monocular deprivation and relative GAD65 density

Another hypothesis to explain the rapid loss of deprived-eye responsiveness following 2 days of MD is an increase in the density of intracortical inhibitory presynaptic terminals in deprived ODCs relative to nondeprived columns. A higher density of inhibitory synapses in deprived-eye columns could suppress deprived-eye visual responses in neurons located within these ODCs. We labeled intracortical inhibitory presynaptic terminals with an anti-GAD65 antibody (Chang and Gottlieb, 1988) and determined whether MD had any effect on the amounts of GAD65 immunoreactivity in deprived relative to nondeprived ODCs.

Levels of GAD65 immunoreactivity in kittens with normal visual experience as well as in animals that under-went 2 or 7 days of MD were measured as a function of distance from either left- or right-eye ODC centers (control animals) or deprived or nondeprived ODC centers (MD animals). In all cases, the slope of the linear best-fit functions was not significantly different from 0 (Fig. 6, Table 1). Average minimum detectable differences at a 90% confidence level were 32.4% for 2-day MD animals and 9.8% for 7-day MD animals. These general conclusions on the pattern of GAD65 density were confirmed by viewing the immunofluorescent sections at low magnification (data not shown). In summary, MD of either 2 or 7 days had no effect on densities of intracortical inhibitory presynaptic terminals in deprived relative to nondeprived ODCs in primary visual cortex, indicating that the rapid loss of deprived-eye visual responsiveness in cortical neurons after 2 days of MD is not due to a increase in the density of inhibitory presynaptic sites in deprived ODCs relative to nondeprived columns. In addition, the density of inhibitory presynaptic sites in deprived and nondeprived columns do not show changes analogous to the documented changes in numbers of presynaptic sites in the geniculocortical projection following 7 days of MD (Silver and Stryker, 1999b).

Validation of the relative fluorescence technique by using laminar analysis of GAD65 density

Because the method employed in this study to quantify relative levels of SVP or GAD65 immunofluorescence has not previously been used to measure the relative densities of presynaptic sites, the finding that all experimental conditions yielded a lack of correlation with position within ODCs calls into question the validity of the technique. To determine whether this method reliably detects differences in presynaptic site density when they are known to exist, we measured relative levels of GAD65 immunofluorescence as a function of location within cortical laminae in primary visual cortex. Image collection was exactly as described above, except instead of collecting fields along a line within layer IV which spanned several ODCs, images were collected in a line that crossed cortical laminar boundaries spanning the pial surface and the white matter. Following photobleaching of the fields, cortical laminae were identified by performing cresyl violet staining on the tissue sections and tracing laminar boundaries with camera lucida. These boundaries were overlaid with the image of the photobleached fields, and individual fields were assigned to a cortical lamina (Fig. 7A). Fields falling on a laminar boundary were excluded from further analysis.

Average relative GAD65 fluorescence levels were plotted as a function of cortical lamina by using the same contrast index used to assess the effects of MD (Fig. 7B). To test whether GAD65 fluorescence varied as a function of location within cortical laminae, a one-way ANOVA was performed, and a highly significant effect of cortical lamina was observed ($F(4,41) = 20.4$, $P < 1 \times 10^{-8}$). Although this result indicates that the technique is able to detect laminar differences in GAD65 label, it does not address whether this laminar profile is related to laminar differences in inhibitory synaptic density. To establish this, the same data were plotted in comparison to measurements from an electron microscopic study of Winfield (1983) in which the density of symmetric synapses was measured inprimary visual cortex of P40 kittens as a function of cortical lamina (Fig. 7C). With the possible exception of layer I, there is general agreement between the data of Winfield (1983) and the GAD65 fluorescence measurements made in this study. Therefore, the technique we have used to assess the effects of MD on SVP and GAD65 density in deprived relative to nondeprived ODCs is able to resolve cortical laminar differences in GAD65 label and is also consistent with the known laminar profile of inhibitory synapses as determined by using a completely independent technique.

Although it is not possible to directly compare results from the techniques employed to assess the effects of MD to those from the laminar analysis, one can ask whether differences in fluorescence between adjacent ODC centers would have been detected if they had been of the same magnitude as the measured laminar differences. Given the standard errors of the mean of the regression coefficients from the GAD65 columnar data (Table 1), the threshold percent difference required to reach significance at the $P = 0.05$ level (following corrections for multiple comparisons) was 20.5 (averaging across all deprivation conditions). Differences of at least this magnitude were observed when comparing GAD65 fluorescence values in layers II/III versus IV, IV versus V, and I versus either II/III, IV, V, or VI. Therefore, the percent difference values obtained from the laminar analysis indicate that if differences of that size had been present in the data set used to assess the effects of MD, they would have been easily detectable.

The reasons for the possible difference between our data and those of Winfield (1983) in lamina I are unknown. The mean density of symmetric inhibitory synapses in lamina I of P40 kitten primary visual cortex might be significantly higher than the mean reported by Winfield (1983), because the standard error of the mean of his layer I measurements is more than 3 times greater than the mean itself. The high level of GAD65 immunofluorescence we have observed in lamina I is due to a band of intense GAD65 immunoreactivity in superficial lamina I. High levels of GAD65 staining in superficial cortical lamina I have also been observed in 5-week-

old kitten primary visual cortex (G. Mower, personal communication), adult cat primary visual cortex (Guo et al., 1997), and adult monkey striate cortex (Hendrickson et al., 1981).

DISCUSSION

Monocular deprivation and intracortical excitatory presynaptic sites

The vast majority of synapses within layer IV of primary visual cortex are excitatory (Winfield, 1983; Beaulieu and Colonnier, 1985). Given that only approximately 6% of layer IV synapses in adult cat are thalamocortical (Ahmed et al., 1994), most of the anatomical synaptic input to layer IV is excitatory input from other cortical neurons. In addition, the majority of excitatory current in visual cortical neurons comes from intracortical connections (Douglas and Martin, 1991). These facts alone make the hypothesis, that loss of intracortical excitatory input to deprived-eye ODCs is responsible for the decrease in deprived-eye visual responsiveness observed after 2 days of MD, an initially attractive one. In addition, long periods of MD result in permanent physiological plasticity that cannot be influenced by opening the deprived eye or by a reverse suture paradigm (Wiesel and Hubel, 1965). This plasticity occurs in all cortical layers, most of which receive little or no direct geniculocortical input compared to the significant geniculocortical projection to layer IV (LeVay and Gilbert, 1976). One possibility is that changes in the density of intracortical excitatory presynaptic sites within deprived and nondeprived ODCs could occur which would result in permanent alterations in synaptic connectivity in primary visual cortex that cause the observed long-lasting physiological changes.

We have tested these hypotheses by labeling intracortical excitatory presynaptic sites in layer IV with anti-SVP antibodies and determining the levels of SVP immunofluorescence as a function of position within deprived and nondeprived ODCs. There is substantial evidence to suggest that the punctate structures labeled by these antibodies are found at synapses defined either physiologically or by electron microscopic criteria. This has been discussed in detail in Silver and Stryker (1999b) and will not be repeated here. Our data indicate that MD of either 2 or 7 days has no effect on the density of intracortical excitatory presynaptic sites in deprived relative to nondeprived ODCs.

Monocular deprivation and intracortical inhibitory presynaptic sites

Several lines of evidence suggest that changes in GABAergic circuitry are critically involved in ocular dominance plasticity. Both intravenous administration (Duffy et al., 1976) and cortical iontophoresis of the $GABA_A$ receptor antagonist bicuculline (Burchfiel and Duffy, 1981) are able to reverse the physiological effects of MD in some neurons, suggesting that at least part of the loss of deprived-eye visual responsiveness is due to increased inhibition of cortical neurons that normally would respond to deprived-eye visual stimulation. Continuous infusion of bicuculline into kitten primary visual cortex during a 7-day period of MD prevents physiological ocular dominance plasticity (Ramoa et al., 1988), and the possibility that the GABAergic changes following MD are presynaptic in origin is supported by the findings of Hensch et al. (1998), in which mice lacking the GAD65 gene show no effect of 4 days of MD on the ocular dominance distribution of primary visual cortical responses.

Unlike many other enzymes that synthesize neurotransmitters, the enzyme that synthesizes GABA, GAD, has two distinct isoforms that have different biochemical properties and subcellular localizations (reviewed in Soghomonian and Martin, 1998). Although activity of both the GAD65 and GAD67 isoforms requires binding to the cofactor pyridoxal phosphate, studies of brain extracts indicate that GAD67 is normally saturated with pyridoxal phosphate and is therefore constitutively active, whereas about half of the GAD65 is not bound to this cofactor (Kaufman et al., 1991). In addition, although GAD67 protein expression is found in

cell somata and dendrites, the GAD65 isoform is localized preferentially to presynaptic terminals of inhibitory neurons (Esclapez et al., 1994). These data raise the possibility that GAD67 plays primarily a metabolic role by providing GABA for the tricarboxylic acid cycle, whereas GAD65 may be specialized for synaptic synthesis of GABA that is subject to rapid regulation (Martin and Rimvall, 1993).

One hypothesis to explain the rapid loss of deprived-eye visual responsiveness following 2 days of MD is an increase in the density of intracortical inhibitory presynap-tic terminals in deprived relative to nondeprived ODCs. Additionally, the anatomical changes in the geniculocortical projection induced by 7 days of MD could be accompanied by long-lasting increases in inhibitory presynaptic terminals in deprived relative to nondeprived ODCs. We have tested both of these hypotheses and conclude that MD of either 2 or 7 days has no effect on density of GAD65 immunoreactivity (and by extension, density of intracortical inhibitory presynaptic terminals) in deprived relative to nondeprived ODCs.

Our data also address the possibility that MD causes complementary changes in the densities of excitatory and inhibitory presynaptic sites. Specifically, MD could result in both a decrease in the number of excitatory intracortical presynaptic terminals and an increase in the number of inhibitory intracortical presynaptic sites in deprived ODCs relative to nondeprived columns. These changes might not be detectable by measuring overall SVP density as a function of position within ODCs, as SVP markers label both excitatory and inhibitory presynaptic terminals, and the complementary changes could cancel each other. However, the lack of either 2 or 7 days of MD on GAD65 density in deprived relative to nondeprived ODCs argues against this possibility, as GAD65 is a selective marker for inhibitory presynaptic sites.

These results, in combination with those of Silver and Stryker (1999b), show that there is little or no change in the densities of any of the excitatory or inhibitory synaptic inputs to deprived relative to nondeprived ODCs in layer IV over a 2-day period during which deprived-eye responses are lost from most of the neurons in the layer. This could be explained by modifications at a molecular level that are not immediately reflected in levels of SVP or GAD65. These include functional changes in presynaptic terminals, resulting in alterations in the probability of neurotransmitter release, or postsynaptic mechanisms which change the size of the excitatory postsynaptic potential evoked by a given amount of released transmitter, and either or both of these could be present at any of the three major synaptic inputs to layer IV.

In addition, the number of excitatory intracortical presynaptic sites representing the nondeprived eye might increase at the same time as deprived-eye sites are withdrawn, such that the overall distribution of excitatory presynaptic sites in layer IV remains unchanged. Similarly, simultaneous addition of deprived-eye inhibitory presynaptic sites and removal of nondeprived inhibitory sites might occur without affecting the overall distribution of GAD65 immunoreactivity in layer IV. Although our data do not exclude these possibilities, the processes of addition and retraction would have to be very tightly coupled in time to preserve the uniform distributions of SVP- and GAD65 immunoreactivity that we have observed after either 2 or 7 days of MD. Although ocular dominance plasticity is clearly the result of competitive interactions between the neural pathways representing the two eyes (reviewed in Shatz, 1990), the processes of synaptic depression or elimination do not necessarily have the same time course as those of synaptic enhancement or elaboration. Substantial retraction of deprived-eye geniculocortical axon branches occurs before any detectable addition of axon branches to nondeprived arbors in kitten (Antonini and Stryker, 1993b, 1996), and chronic recordings of multi-unit activity in kitten primary visual cortex during a period of MD indicate that reduction of responsiveness to deprived-eye visual stimulation precedes enhancement of responses to nondeprived-eye stimulation (Mioche and Singer, 1989).

Finally, there could be changes in the activity of inputs to layer IV from other cortical layers which are more rapidly or powerfully affected by deprivation. This possibility is consistent with recent physiological findings that 1 day of MD causes a physiological ocular dominance shift in neurons in supragranular and infragranular layers at the borders of ODCs in primary visual cortex without corresponding plasticity in layer IV of the same cortical columns (Trachtenberg et al., 2000).

Comparison with previous literature

Monocular deprivation in adult monkeys causes a decrease in the number of GABAergic neurons and GAD-positive somata and puncta in deprived-eye ODCs (Hendry and Jones, 1986,1988). This result is in apparent disagreement with data from the present study, and the changes in GAD-positive puncta observed by Hendry and Jones (1986,1988) are in the opposite direction of those predicted by the hypotheses tested in this paper. Although the distribution of GAD67 mRNA expression in adult cat visual cortex is unaffected by MD (Benson et al., 1989), experiments in adult monkeys suggest that the initial effects of MD on GABAergic markers occur posttranscriptionally and alter protein levels, whereas much longer periods of MD are required to induce analogous changes in GAD67 mRNA levels (Benson et al., 1994). Thus, the lack of effect of MD on GAD67 mRNA expression in adult cat could be due to either species differences between cats and monkeys, or, because the effects of MD on the distribution of GAD protein have not yet been examined in adult cats, to posttranscriptional changes in GAD protein expression following MD that are not reflected at the mRNA level.

Considering only those studies that have measured GAD protein expression, the MD-induced decrease in GAD-immunoreactive puncta that occurs in adult monkey deprived ODCs (Hendry and Jones, 1986,1988) was not observed in critical period kittens either with anti-GAD antibodies which did not discriminate between the 65- and 67-kDa isoforms (Bear et al., 1985), or with presynaptic terminal-specific anti-GAD65 antibodies (present study). One possible explanation for these discrepancies is that the changes in GABA and GAD expression in the adult monkey are the result of a homeostatic compensatory mechanism in which neurons in deprived ODCs correct for the decrease in visual input induced by MD by decreasing inhibitory synaptic transmission. On this hypothesis, MD does not alter GABAergic markers in critical period animals, because physiological plasticity is sufficient to compensate for the initially diminished visual inputs to neurons in deprived ODCs. Because MD of adult monkeys produces no change in response properties of visual cortical neurons (LeVay et al., 1980), the functional changes that occur in critical-period animals are not possible in adult cortex, so an alternative means of compensating for the reduced visual signal coming from the deprived eye may be required.

This hypothesis is also consistent with the apparent age-dependent regulation of $GABA_A$ receptors following MD. Deprivation of adult monkeys results in a decrease in expression of both the receptor complex (Hendry et al., 1990) and individual $GABA_A$ receptor subunits (Hendry et al., 1994) in deprived ODCs, whereas MD of kittens does not cause the distribution of binding sites for the $GABA_A$ receptor agonist muscimol to show a columnar pattern in layer IV of primary visual cortex (Mower et al., 1986). As in the case of the GAD experiments, this set of results might reflect either age or species differences. The definitive experiments to test this model would be to examine the distribution of GABA, GAD, or $GABA_A$ receptors in monkeys deprived during the critical period, and the prediction is that the distributions of these markers in layer IV would be unaffected by MD.

Functional plasticity during the critical period that results in changes in response properties of visual cortical neurons without affecting the overall distribution of either excitatory or inhibitory presynaptic sites may also account for our failure to see any columnar pattern of

synaptophysin or GAD65 label in normal animals despite the well-known physiological dominance of visual cortical neurons by the contralateral eye (Hubel and Wiesel, 1962).

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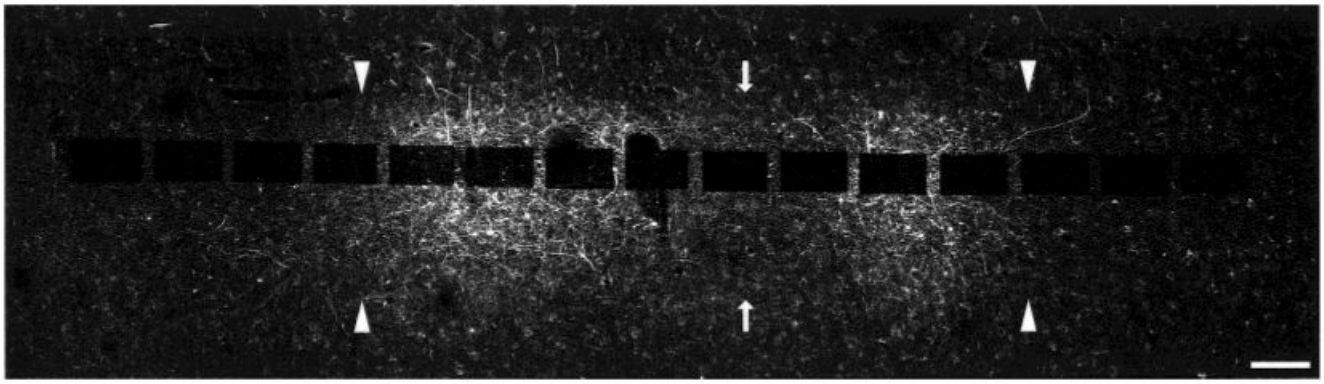

Fig. 1.

Phaseolus vulgaris-leucoagglutinin (PHA-L)-labeled ocular dominance columns (ODCs) can be used to locate synaptic vesicle protein (SVP) or glutamic acid decarboxylase (GAD)65 immunofluorescent fields within an ODC. ODCs in postnatal day (P)40 kitten primary visual cortex were labeled by injections of the anterograde tracer PHA-L into lamina A of the lateral geniculate nucleus. Dark rectangles are fields in which SVP or GAD65 density measurements were made. Following image collection, fields were photobleached to mark their position within the ODC. Because an edge of the PHA-L-labeled area (arrowheads) could represent either the edge of a labeled ODC or the boundary of the PHA-L label, ODC column centers were always defined in an unlabeled column (arrows) flanked by two labeled columns. Scale bar = 100 μm.

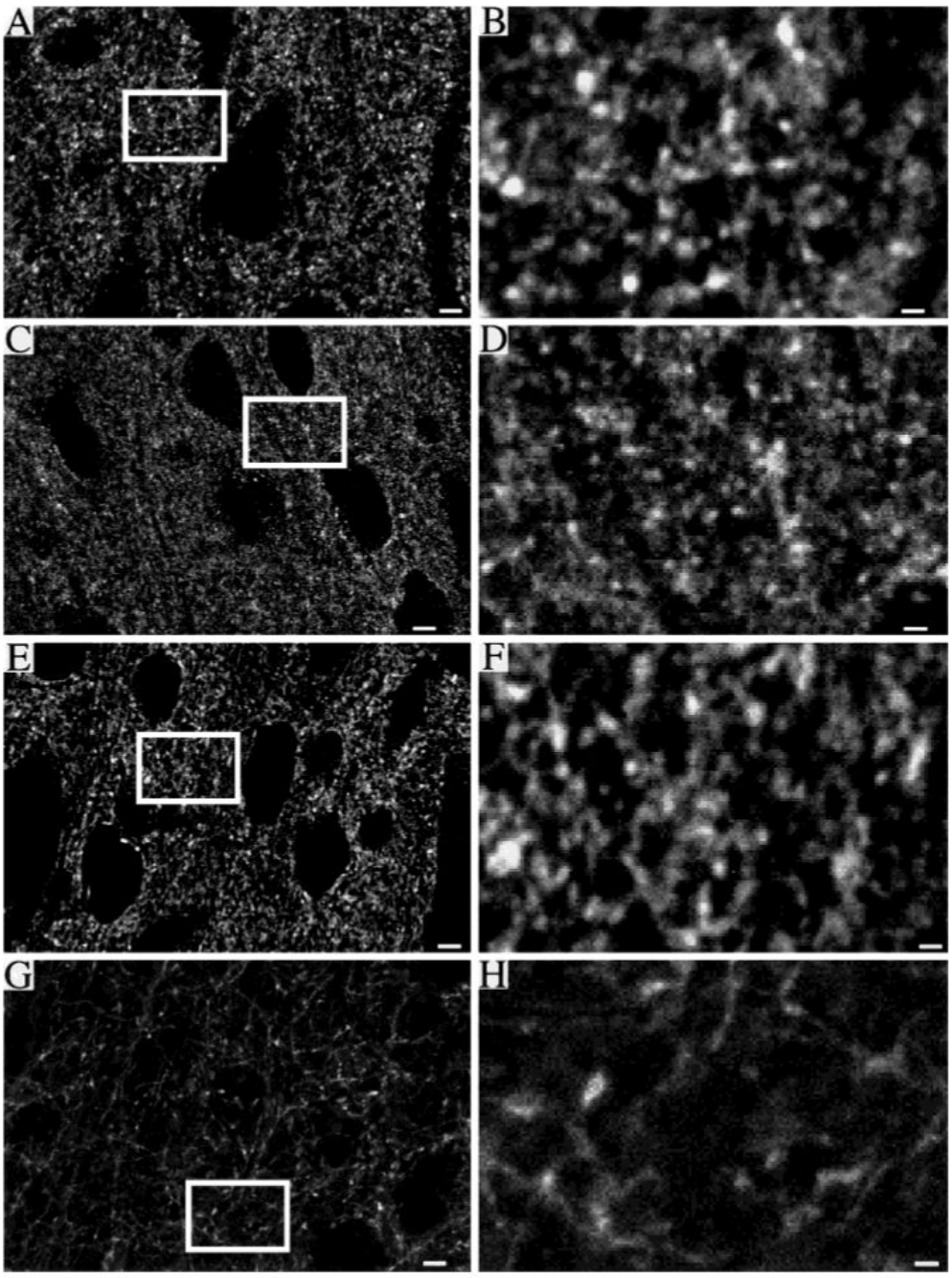

Fig. 2.

Confocal optical sections of synaptic vesicle protein (SVP) and glutamic acid decarboxylase (GAD)65 label. Each of the images in the left column represents a single field (corresponding to one photobleached rectangle in Fig. 1) of the SVP markers synaptophysin (**A**), synaptotagmin (**C**), SV2 (**E**), or the inhibitory presynaptic terminal marker GAD65 (**G**). In all cases, the expected pattern of punctate neuropil label and the absence of label from cell somata and blood vessels were observed. White rectangles indicate areas that are shown at higher magnification in the corresponding images in the right column (**B,D,F,H**). Punctate structures that are likely to be individual presynaptic terminals were observed in all cases. Brightness and contrast levels have not been altered in any of these images; they represent the raw data used

to make SVP and GAD65 density measurements. Scale bars = 5 μm in A,C,E,G; 1 μm in B,D,F,H.

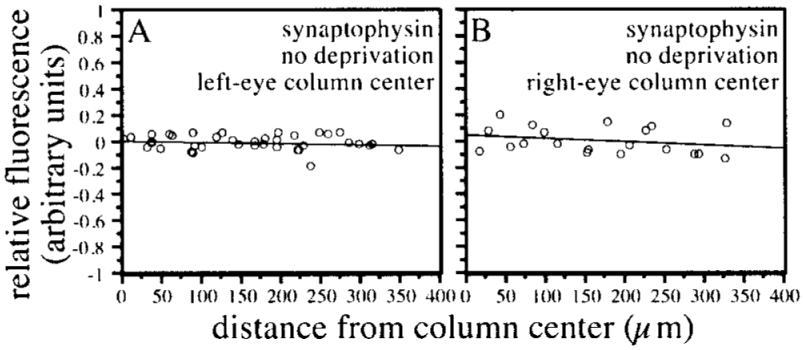

Fig. 3.

Measurements of synaptophysin relative fluorescence in a kitten with normal visual experience as a function of distance from left- or right-eye ocular dominance column (ODC) centers. Each field (corresponding to a single photobleached rectangle in Fig. 1) generated one relative fluorescence value (open circles). Fluorescence measurements were normalized and quantified by using a contrast index (see Results) and plotted versus distance from ODC center. Solid lines indicate the linear best-fit functions derived by using least-squares regression. Only those fields falling within 400 μm of a well-defined ODC center (see Results) were included in the analysis. In both **A** and **B**, the slope of the linear best-fit function was not significantly different from 0 (see Table 1).

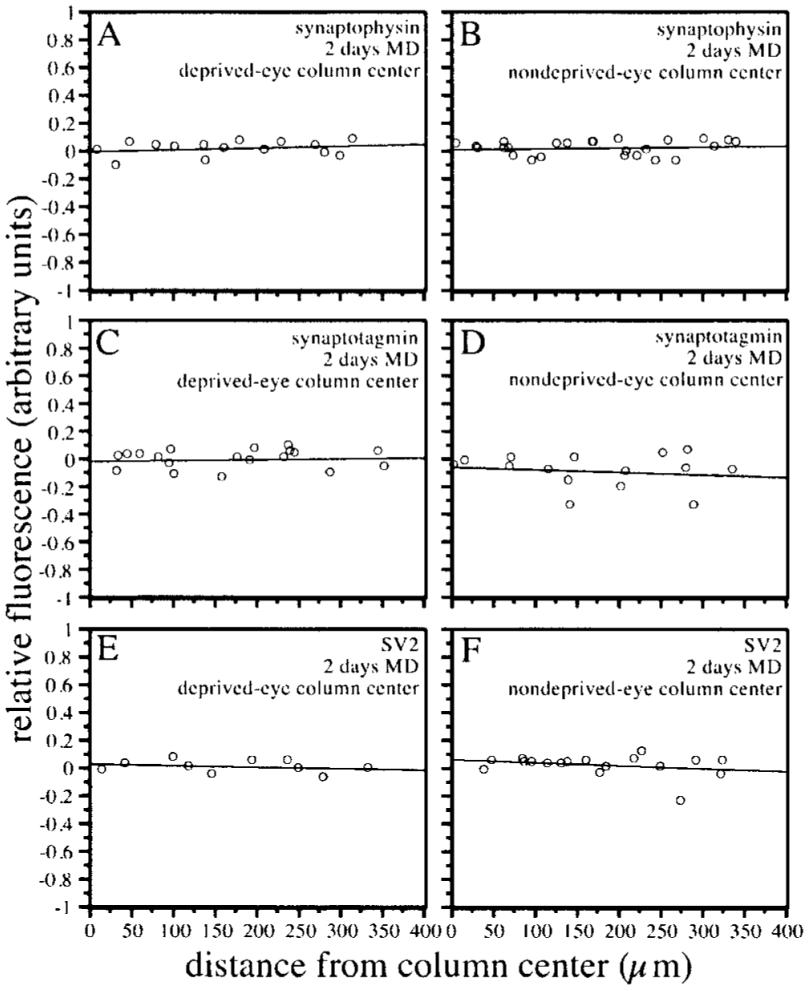

Fig. 4.

Two days of monocular deprivation (MD) has no effect on levels of synaptic vesicle protein (SVP) density in deprived relative to nondeprived ocular dominance columns (ODCs). SVP density measurements for synaptophysin (**A,B**), synaptotagmin (**C,D**), or SV2 (**E,F**) were obtained as described in Figure 3 and plotted versus distance from either deprived (A,C,E) or nondeprived (B,D,F) ODC centers. In all six cases, the slope of the linear best-fit function was not significantly different from 0 (see Table 1).

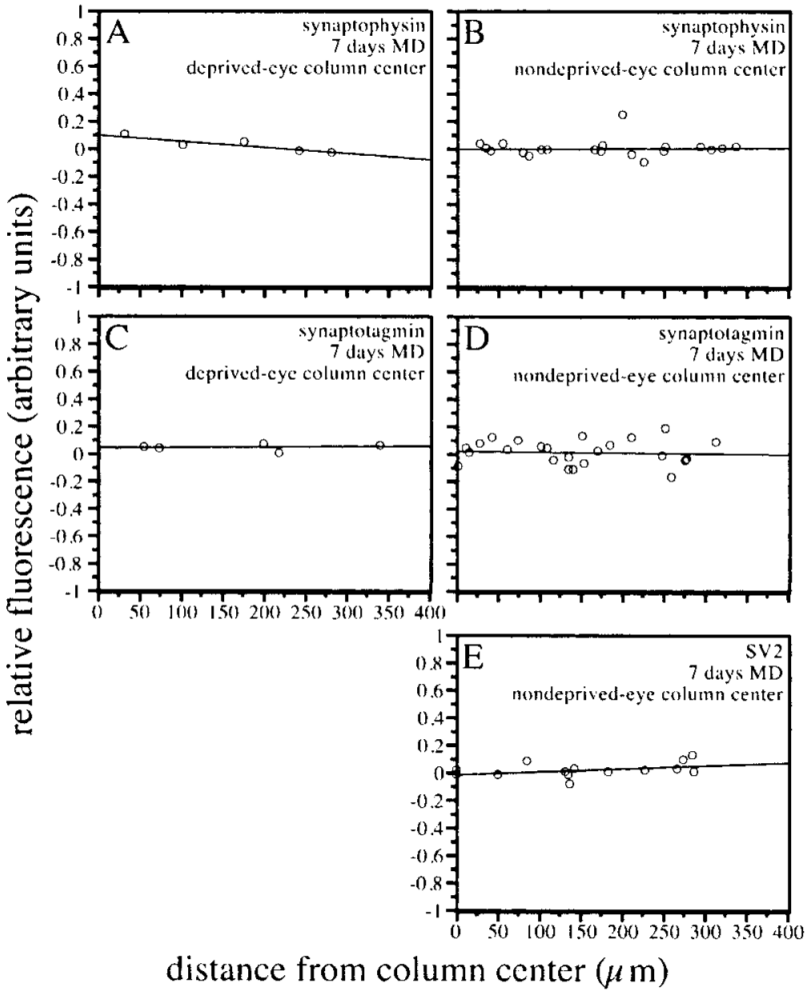

Fig. 5.

Seven days of monocular deprivation (MD) has no effect on levels of synaptic vesicle protein (SVP) density in deprived relative to nondeprived ocular dominance columns (ODCs). SVP density measurements for synaptophysin (**A,B**), synaptotagmin (**C,D**), or SV2 (**E**) were obtained as described in Figure 3 and plotted versus distance from deprived (A,C) or nondeprived (B,D,F) ODC centers. In all five cases, the slope of the linear best-fit function was not significantly different from 0 (see Table 1). No data were obtained for SV2 fluorescence as a function of distance from deprived-eye ODC centers.

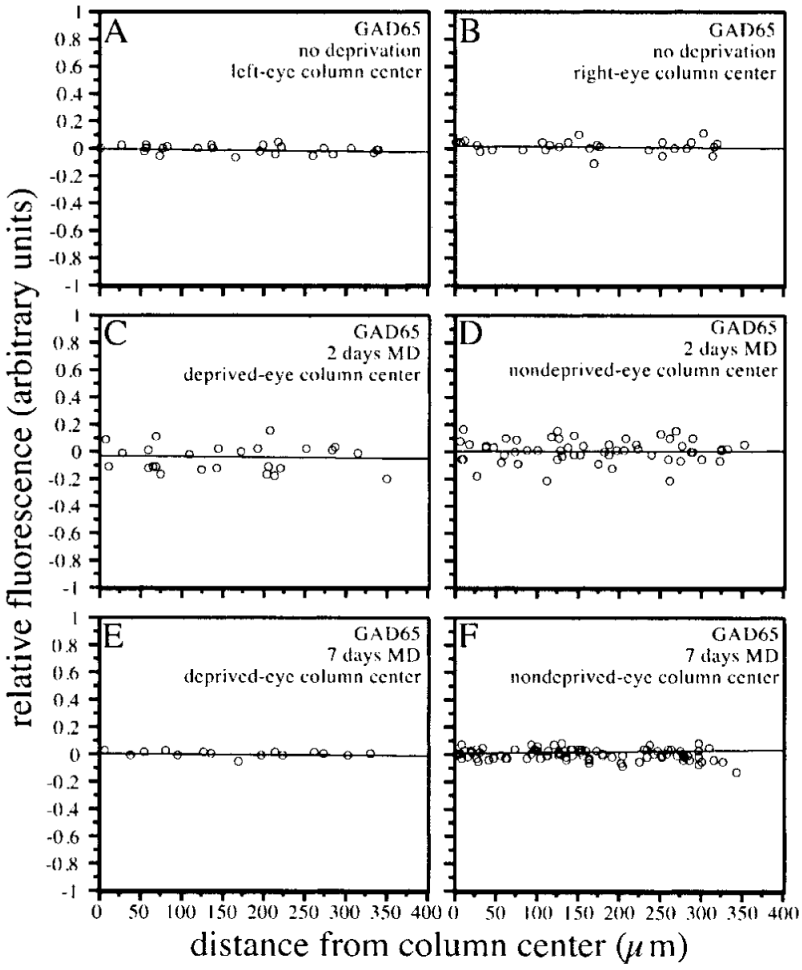

Fig. 6.

Monocular deprivation (MD) of either 2 or 7 days has no effect on levels of glutamic acid decarboxylase (GAD)65 density in deprived relative to nondeprived ocular dominance columns (ODCs). GAD65 density measurements were obtained as described in Figure 3 and plotted versus distance from either left-eye (**A**), right-eye (**B**), deprived (**C,E**), or nondeprived (**D,F**) ODC centers for normal control (A,B), 2-day MD (C,D), or 7-day MD (E,F) animals. In all six cases, the slope of the linear best-fit function was not significantly different from 0 (see Table 1).

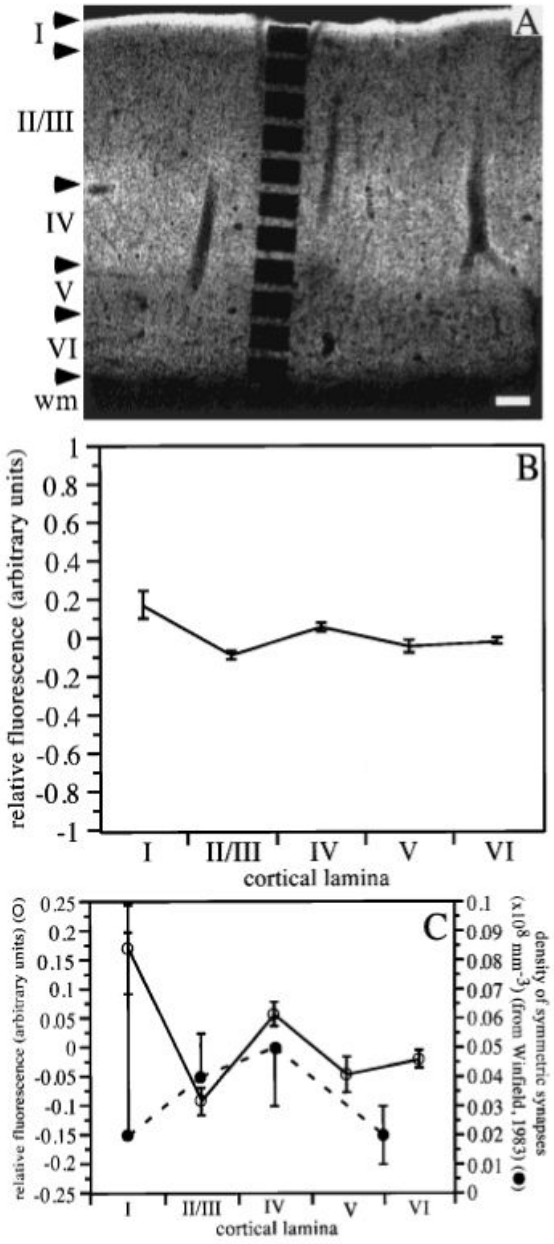

Fig. 7.

Laminar analysis of glutamic acid decarboxylase (GAD)65 fluorescence levels validates the relative fluorescence technique used for plasticity measurements. **A:** GAD65 immunofluorescence in primary visual cortex from a postnatal day (P)40 kitten with normal visual experience. Dark rectangles are photobleached fields from which GAD65 density measurements were made. Arrowheads indicate cortical laminar boundaries assigned by using cresyl violet staining and camera lucida. **B:** Relative GAD65 fluorescence values from fields lying entirely within a single cortical lamina were averaged in the form of a contrast index (see Results) to obtain a mean relative fluorescence value for each cortical lamina (n = 4 tissue sections). One-way analysis of variance (ANOVA) indicated that GAD65 fluorescence varied as a function of cortical lamina. **C:** Data from B were replotted to determine whether the laminar profile of GAD65 fluorescence correlated with the laminar profile of density of symmetric

inhibitory synapses in P40 kitten primary visual cortex from Winfield (1983). With the possible exception of lamina I (see Results), these two independent techniques generate similar laminar profiles of inhibitory synaptic density. Therefore, the relative fluorescence techniques employed in this study can accurately discriminate cortical laminar boundaries and replicate previously published data on inhibitory synaptic density. Error bars represent standard errors of the mean. Roman numerals I-VI, cortical layers I-VI; wm, white matter. Scale bar = 100 μm.

TABLE 1

Statistical Summary of Synaptic Vesicle Protein (SVP) and Glutamic Acid Decarboxylase (GAD) 65 Distributions in Layer IV of Primary Visual Cortex Following Monocular Deprivation (MD)[1]

Experimental group	Sample size	r^2	Slope of linear best fit × 10^4 (regression coefficient)	Percent change
Not deprived; synaptophysin; distance from left-eye column center	38	0.025	-0.9 ± 1.0	-7.1 ± 7.4
Not deprived; synaptophysin; distance from right-eye column center	20	0.066	2.6 ± 2.3	-18.9 ± 16.9
2-day MD; synaptophysin; distance from deprived-eye column center	15	0.046	1.2 ± 1.5	9.8 ± 12.5
2-day MD; synaptophysin; distance from nondeprived-eye column center	25	0.012	0.6 ± 1.1	4.6 ± 8.8
2-day MD; synaptotagmin; distance from deprived-eye column center	19	0.010	0.7 ± 1.6	5.5 ± 14.1
2-day MD; synaptotagmin; distance from nondeprived-eye column center	15	0.025	-1.9 ± 3.2	-13.9 ± 22.7
2-day MD; SV2; distance from deprived-eye column center	10	0.047	-1.0 ± 1.5	-7.3 ± 11.4
2-day MD; SV2; distance from nondeprived-eye column center	18	0.062	-2.0 ± 2.0	-14.9 ± 14.5
2-day MD; pooled SVP markers; distance from deprived-eye column center	44	0.005	0.4 ± 0.9	3.6 ± 7.7
2-day MD; pooled SVP markers; distance from nondeprived-eye column center	58	0.005	-0.7 ± 1.3	-5.4 ± 10.0
7-day MD; synaptophysin; distance from deprived-eye column center	5	0.801	-4.4 ± 1.3	-30.1 ± 9.7
7-day MD; synaptophysin; distance from nondeprived-eye column center	20	0.001	0.2 ± 1.5	1.8 ± 12.8
7-day MD; synaptotagmin; distance from deprived-eye column center	5	0.016	0.3 ± 1.3	2.3 ± 11.0
7-day MD; synaptotagmin; distance from nondeprived-eye column center	24	0.003	-0.5 ± 2.0	-4.0 ± 15.0
7-day MD; SV2; distance from nondeprived-eye column center	14	0.180	2.1 ± 1.3	18.6 ± 11.1
7-day MD; pooled SVP markers; distance from deprived-eye column center	10	0.201	-1.6 ± 1.2	-12.7 ± 9.1
7-day MD; pooled SVP markers; distance from nondeprived-eye column center	58	0.003	0.4 ± 1.0	3.2 ± 8.3
Not deprived; GAD65; distance from left-eye column center	24	0.051	-0.6 ± 0.6	-4.9 ± 4.5
Not deprived; GAD65; distance from right-eye column center	27	0.009	-0.4 ± 0.9	-3.2 ± 7.0
2-Day MD; GAD65; distance from deprived-eye column center	26	0.003	-0.5 ± 2.0	-4.0 ± 14.9
2-day MD; GAD65; distance from nondeprived-eye column center	56	<0.001	0.1 ± 1.1	0.6 ± 9.5
7-day MD; GAD65; distance from deprived-eye column center	15	0.039	-0.4 ± 0.5	-2.8 ± 3.8
7-day MD; GAD65; distance from nondeprived-eye column center	88	0.034	-0.7 ± 0.4	-5.7 ± 3.3

[1] Quantitative analysis of the effects of 2 or 7 days of MD on levels of SVP and GAD65 density in deprived relative to nondeprived ocular dominance columns (ODCs). Sample size is the number of fields (and therefore the number of relative density measurements) for each experimental condition. r^2 is the coefficient of determination and is a measure of the strength of the relationship between relative fluorescence and distance from ODC center. The slope of the linear best-fit function is presented in units of contrast index as well as percent change. Percent change refers to the difference in fluorescence levels between adjacent ODC centers, assuming an ODC width of 400 μm (see Results).