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Relationship between the Ocular Dominance and Orientation Maps in Visual Cortex of Monocularly Deprived Cats

Michael C. Crair, Edward S. Ruthazer, Deda C. Gillespie, and Michael P. Stryker*
W. M. Keck Foundation Center for Integrative Neuroscience
Department of Physiology
University of California
San Francisco, California 94143-0444

Summary

The significance of functional maps for cortical plasticity was investigated by imaging of intrinsic optical signals together with single-unit recording in kittens. After even a brief period of monocular deprivation during the height of the critical period, only isolated patches of visual cortex continued to respond strongly to the closed eye. These deprived-eye patches were located on the pinwheel center singularities of the orientation map and consisted of neurons that were poorly selective for stimulus orientation. Neurons in regions surrounding the deprived-eye patches responded only weakly to the deprived eye but were well tuned for the same stimulus orientation that optimally excited them when presented to the open, nondeprived eye. The coincidence of deprived-eye patches with pinwheel center singularities, and the selective loss of orientation tuning within the deprived-eye patches, indicate that the orientation and ocular dominance maps are functionally linked and provide compelling evidence that pinwheel center singularities are important for cortical plasticity.

Introduction

Maps of stimulus orientation and eye preference each fill the primary visual cortex of most primates and carnivores. Stimulation through either eye generates neural responses throughout most of the visual cortex, but local regions are more responsive to one eye or the other, forming a mosaic of eye preference referred to as the ocular dominance map (Hubel and Wiesel, 1969). Most visual cortical neurons also respond only to a small range of stimulus orientations, and neighboring neurons tend to have similar orientation preferences (Hubel and Wiesel, 1974; Hubel et al., 1978). The representation of stimulus orientation changes gradually and progressively across most of the visual cortex, and is punctuated by singularities referred to as "pinwheel centers" (Bonhoeffer and Grinvald, 1991; Blasdel, 1992), around which all orientations are locally represented. Mathematically, a map of a circular function like orientation must have such singularities (Rojer and Schwartz, 1990), unless it consists of perfectly parallel rows.

In the monkey, it has been reported that pinwheel center singularities of the orientation map tend to lie along the center lines of ocular dominance bands (Bartfeld and Grinvald, 1992; Obermayer and Blasdel, 1993). In the cat, the regions of cortex most strongly driven by one eye or the other tend to lie on or near the singularities of the orientation map (Crair et al., 1997). This relationship between the peaks of ocular dominance and orientation pinwheels accounts for the weaker tendency for pinwheels also to lie along the center lines of ocular dominance in the cat (Crair et al., 1997). The link between orientation and eye preference maps in normal animals led us to investigate whether a deep relationship between the different maps might guide cortical plasticity.

Depriving one eye of visual experience early in development causes most of the visual cortex to respond preferentially to stimulation of the remaining open eye, even after the lid-sutured eye has been reopened (Hubel and Wiesel, 1970). Previous studies of the effects of monocular visual deprivation (MD) have shown that even 1 week of deprivation during the peak of the critical period can lead to the apparent elimination of optical responses to stimulation through the deprived eye (DE; Kim and Bonhoeffer, 1994; Godecke and Bonhoeffer, 1996). However, the design of these experiments obscured components of the response that were not selective for stimulus orientation. Indeed, experiments performed with single-unit microelectrodes have noted persistent responses through the DE, even after prolonged periods of MD (Shatz and Stryker, 1978; Sherman and Spear, 1982), though the strong responses were largely confined to the middle cortical layers in patches that, anatomically, were shown to receive geniculate input serving the DE. These single-unit studies also noted that most but not all cells responding to the DE lacked orientation selectivity following prolonged periods of MD. However, the arrangement of selective and nonselective DE responses was not evident in these single-unit studies, and in general it has been difficult to characterize the properties of DE responses since they are, on average, so much weaker than responses through the nondeprived (ND) eye (Sherman and Spear, 1982).

The effects of MD are activity dependent, and correlated pre- and postsynaptic activity is necessary to induce a shift of cortical response toward the open eye (Reiter et al., 1986; Reiter and Stryker, 1988). Such an activity-dependent process might be expected to accentuate the normal linkage between the maps for stimulus orientation and ocular dominance, because these maps reveal the patterns of activity elicited by different visual stimuli.

Using imaging of intrinsic optical signals produced by visual stimulation (Bonhoeffer and Grinvald, 1996) together with single-unit recording in kittens, we determined the relationship between ocular dominance, orientation tuning, and the spatial structure of the orientation map following periods of MD in critical-period kittens. We found that the effects of MD depended strongly on the structure of the orientation map. Regions that remained strongly responsive to the DE were

*To whom correspondence should be addressed.

located at the pinwheel center singularities and consisted of neurons that largely lacked orientation selectivity when driven through the DE, while the majority of visual cortex became poorly responsive to the DE but maintained selectivity for stimulus orientation through both eyes.

Results

Effects of Monocular Deprivation

Experiments were performed on normally reared kittens at or near the peak of the critical period of susceptibility to the effects of MD. In each case, the experiment began between P26 and P31 with a period of 2 days ($n = 9$), 7 days ($n = 3$), or 5 months ($n = 1$) of monocular lid suture. At the end of the deprivation period the lid-sutured eye was opened, and measurements of intrinsic optical signals revealed, as expected, responses to stimulation of the open eye indistinguishable from those in normal animals and a strong and consistent reduction of responses to stimulation of the DE. The weakening of DE responses, however, was far from uniform, in that the bulk of the residual DE responses was located in patches, the optical signals from which were almost equally responsive to stimuli at all orientations, as described below. Outside the DE patches, responses to the DE were small and biased toward the preferred orientation of the cells driven by the ND eye. These findings are consistent with previous descriptions from microelectrode recordings of the effects of MD, although it has been difficult to characterize the arrangement of remnant DE responses using electrophysiological techniques (Wiesel and Hubel, 1963; Shatz and Stryker, 1978; Spear et al., 1980; Sherman and Spear, 1982). Optical recording reveals patches of response dominated by and strongly responsive to the DE, which are not selective for orientation, in a field otherwise dominated by the open eye, in which DE responses are weak but orientation selective.

Optical Intrinsic Signal Response

Figure 1 shows an example of such optical measurements following 7 days MD. All of the raw responses following a particular stimulus are shown on an identical absolute scale of cortical reflectance. This is achieved by dividing the measured reflectance following a particular oriented stimulus by the background reflectance measured in the absence of visual stimulation, using the same background image for normalization of all the stimulus conditions. With this method, the magnitudes of the responses may be observed and directly compared among pixels and images. Since visual stimulation decreases cortical reflectance, the responsive areas are dark in the raw records (Figures 1a–1d, 1f–1m, and 1o–1r). The raw responses from stimulation of the ND eye, shown in Figures 1a–1d and 1f–1i, are patchy and strong, and the patterns of response are different for stimulation at each orientation. Together, these individual responses tile the cortex into an "angle map" of stimulus orientation, which is depicted in a pseudo-color representation in Figure 1e. In this angle map, the color of each point indicates the stimulus orientation that produced the biggest optical response. The color scale for stimulus orientation is shown at the upper right of Figure 1. This pattern of optical response through the ND eye is similar to that observed through either eye in an animal that has not been deprived of vision (Blasdel and Salama, 1986; Bonhoeffer and Grinvald, 1993; Crair et al., 1997). MD drastically alters DE responses. Raw responses from stimulation of the DE, shown in Figures 1j–1m and 1o–1r, are strong within restricted areas, and the few patches of strong response (examples indicated by red arrows in l) are at similar positions regardless of the stimulus orientation presented. This is in contrast to the very distinct patterns of response to stimulation of the ND eye at different orientations (Figures 1a–1d and 1f–1i), and as a result, the angle map through the DE (Figure 1n) is much noisier and less distinct than through the ND eye (Figure 1e).

Features evident in the average of the raw DE responses (Figure 1t) are identical to those of the ocular dominance ratio map described previously (Blasdel and Salama, 1986; Grinvald et al., 1986; Figure 1u, calculated pixel by pixel as the sum of the DE raw responses divided by the sum of the ND eye raw responses to stimulation at all orientations). Essentially identical results were obtained in all 13 animals of the present study. Earlier imaging reports (Kim and Bonhoeffer, 1994; Godecke and Bonhoeffer, 1996) had found strong effects of MD, but the weak residual orientation map from the DE had not been evident, and the patches of strong, nonoriented DE responses reported here had inadvertently been removed by the normalization procedures employed.

Orientation Tuning of DE Response

The orientation tuning of the DE response was not uniform across the cortex. Instead, cells which were strongly responsive to the DE tended to be poorly tuned and clustered, with cells that were better tuned but still responsive to the DE arrayed outside the clusters. This is reflected in HLS maps of the optical response, which provide a more comprehensive representation of the optical signal that reveals the arrangement of orientation tuning in the DE response. In HLS maps, the preferred orientation is depicted by hue (H), the magnitude of the response by increased lightness (L), and the orientation tuning by color saturation (S). For example, the HLS maps of response to stimulation of the ND eye from the kitten described in Figure 1 revealed that most of the cortex was strongly responsive and well tuned for orientation throughout (Figure 2a). A gray-scale map of the orientation tuning through the ND eye, shown in Figure 2b, shows that the cortical response was very well tuned (white) everywhere, with the exception of isolated regions (black) at the singularities in the orientation map. In contrast, the HLS map for the DE (Figure 2c) showed patches of strong DE responses that were not selective for orientation, indicated by bright and unsaturated regions (white patches indicated by red arrows in Figure 2c), surrounded by cortex that is weakly responsive but orientation selective, indicated by the darker saturated colors. The map of orientation tuning through the DE (Figure 2d) shows patches of poorly tuned cells (dark

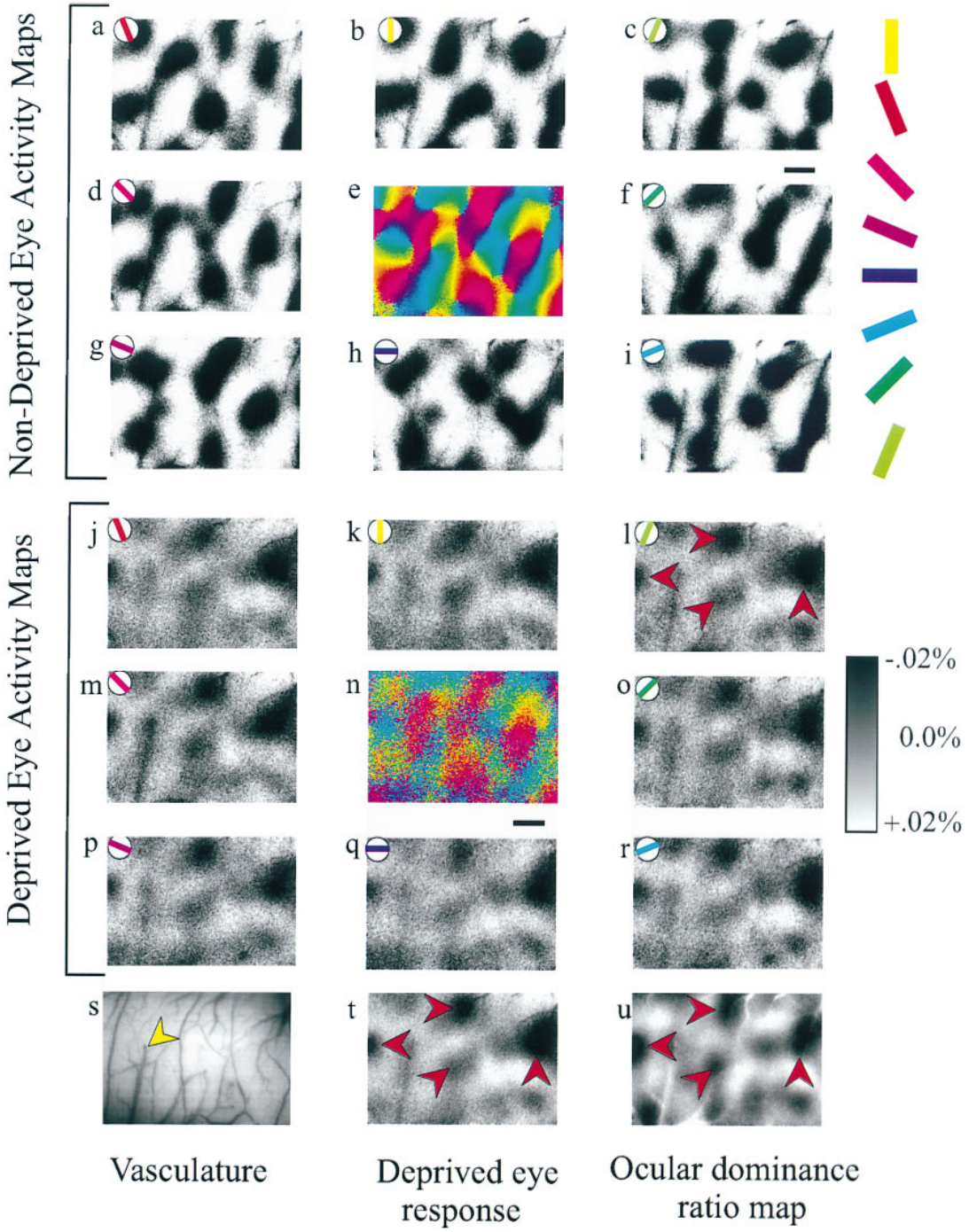

Figure 1. Orientation and Ocular Dominance Maps from the Visual Cortex of a Lid-Sutured Cat

Optical intrinsic signal activity maps from the visual cortex of a kitten, in which one eye was sutured closed for 1 week (P28–P35). Dark areas in the gray scale activity maps (all panels but [e], [n], and [s]) correspond to regions of increased neuronal response, with black being the most responsive. Panels (a) through (i) are responses to stimuli of the nondeprived (ND) eye, and (j) through (r) are for the deprived eye (DE). Visual stimuli were displayed monocularly at the orientations indicated by the color bar inserts. Red arrows (l) indicate patches of DE response that is strong but not orientation-selective. The overlying vascular structure is shown in (s), with a yellow arrow pointing to a blood vessel which causes a visible artifact in the activity maps. Panels (t) and (u) are two related ways of looking at ocular dominance maps. The DE response map (t) is the simple sum of the responses to DE stimulation at all orientations divided by the response to presentation of a blank gray screen. The ocular dominance ratio map (u) is the average of the responses to stimulation of the DE at all orientations divided by the average of the responses to the ND eye. The similarity in the spatial pattern present in (t) and (u) confirms that the DE response pattern is real and not an artifact of the normalization procedure. Scale bar, 500 μm; gray scale ranges for (t) and (u) are ±0.025% and ±0.03%, respectively.

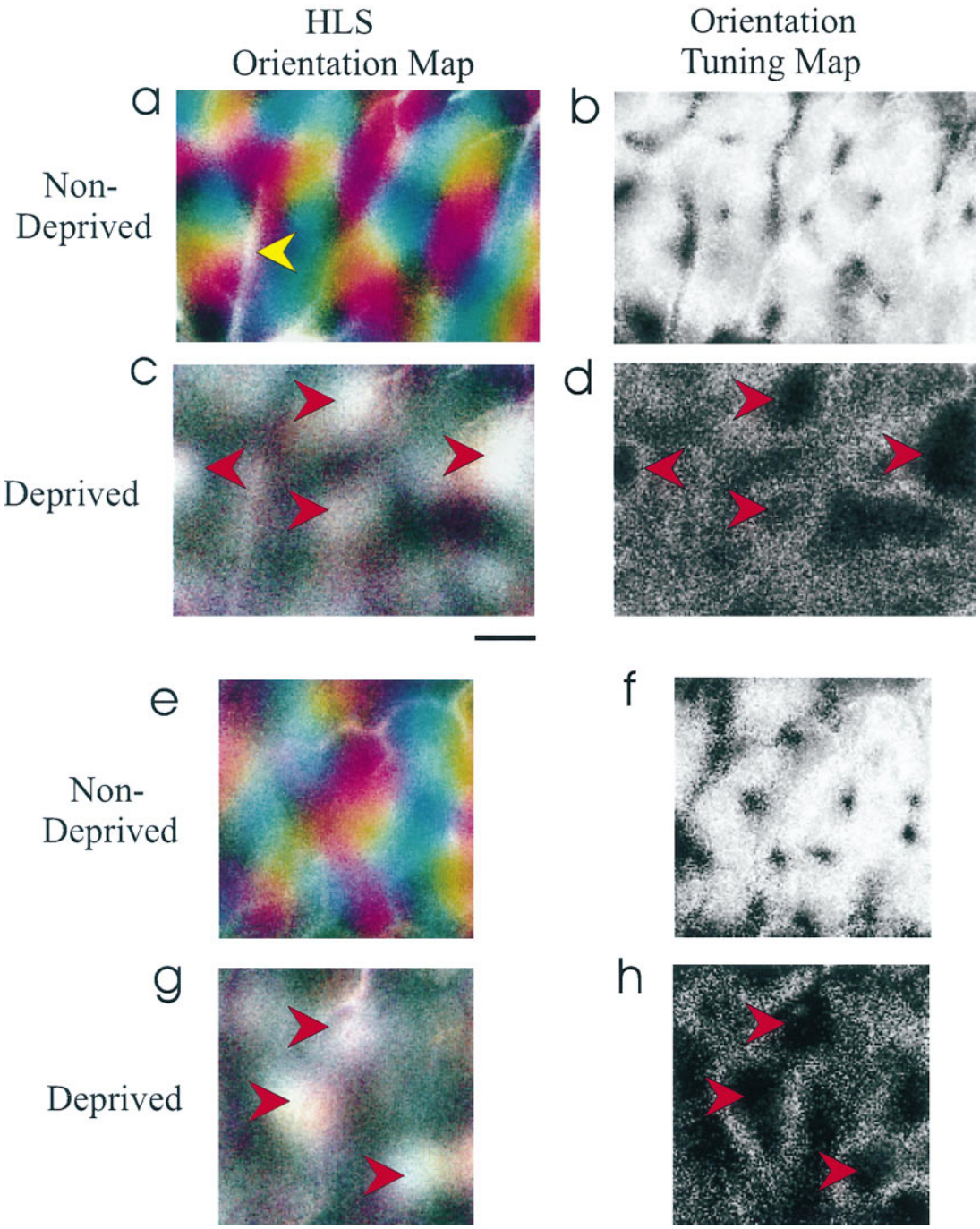

Figure 2. HLS and Orientation Tuning Maps

HLS maps ([a], [c], [e], and [g]) of the optical response and maps of the orientation tuning index ([b], [d], [f], and [h]). For comparison, maps (a) through (d) are from the same kitten depicted in Figure 1, which was deprived for 7 days. Maps (e) through (h) are from another kitten, which was deprived for 2 days. Maps (a), (b), (e), and (f) show responses through the ND eye, and (c), (d), (g), and (h) are through the DE. Orientation tuning through the ND eye ([b] and [f]) is high (white) everywhere except for in small (dark) regions located at pinwheels or associated with blood vessel artifacts or fractures (dark lines). The yellow arrow, as in Figure 1s, points to one of the blood vessels that causes a visible artifact in the activity maps. Scale bar, 500 μm.

patches indicated by red arrows) surrounded by cells that were more weakly responsive to the DE but were better tuned. The spatial segregation of the orientation tuning thus appears in Figure 2d as annuli of bright regions well tuned for orientation surrounding the poorly tuned but more powerfully responsive DE patches. This same segregation of orientation tuning, which looks like craters in the orientation tuning maps from the DE (Fig-

ures 2d and 2h), can be seen in another example from an animal monocularly deprived for 2 days (Figures 2e–2h) and in all other animals tested.

Confirmation of Optical Response with Single-Unit Microelectrode Recording

The responses of single neurons in the visual cortex of monocularly deprived kittens were studied by targeted microelectrode recordings and found to confirm the features noted in the optical maps. First, we checked that single neurons within the DE patches identified from the optical images (Figure 3d) were strongly driven by the DE. Consistent with the optical maps, cells encountered in penetrations targeted for DE patches (labeled 1, 2, and 5 in Figure 3e) were strongly responsive to the DE (red histogram in Figure 3c). This is in contrast to penetrations targeted away from DE patches, where responses to the DE were absent or very weak (penetrations labeled 3 and 4 in Figure 3e; blue histogram in Figure 3c). Summary ocular dominance histograms (Figure 4a) for nine kittens, in which different microelectrode penetrations were targeted both into and far from DE patches, confirmed that strong responses to the DE were confined to the DE patches identified in the optical maps.

Tuning Properties of Single Units

We also determined, using computer-generated stimuli, the orientation tuning properties of single cells in penetrations targeted to DE patches and in penetrations targeted outside the patches. The nonorientation-selective optical responses that were observed in DE patches could, in principle, be due either to individual cells that were themselves poorly tuned or to well tuned cells selective for many different orientations, the responses from which overlapped in the optical image. Single-unit recordings revealed the former: cells responsive to the DE in DE patches were individually very poorly tuned, even following the brief periods of deprivation studied here. For example, polar plots of response as a function of stimulus orientation for single neurons encountered in two penetrations outside the DE patches are shown in Figures 3g and 3h, and for two penetrations inside a DE patch in Figures 3j and 3k. These single-unit responses, which are similar to the corresponding plots of the optical intrinsic signal response to each stimulus orientation (Figures 3i and 3l), show that cells from DE patches are very broadly tuned in comparison to ND eye cells in control penetrations. The quantitative comparison of orientation tuning in single-unit (Figures 4b and 4c) and optical responses (Figure 4c) were similarly consistent in all four animals studied with both techniques.

Neurons that responded to the ND eye were generally sharply tuned for orientation, as illustrated in the open bars of Figures 4b and 4c. Of particular interest was the orientation tuning of responses through the two eyes in binocularly driven cells. Outside the DE patches, very few cells responded at all to the DE (Figure 4a), and the tuning of those that did was difficult to characterize. In penetrations targeted into the DE patches, Figures 4b and 4c show that neuronal responses to the ND eye were nearly always more selective than those to the DE. Results were identical in all animals, when we considered only the neurons for which tuning curves were measured through both eyes. Since neurons in normal animals have similar tuning properties through the two eyes, the orientation tuning of single cells driven through the DE must have become worse as a result of the deprivation.

Many but not all ND eye responses were similar in tuning to those in penetrations targeted outside the DE patches. However, the average tuning even of ND eye responses was poorer in the DE patches than outside. The significance of this will be discussed below.

Coincidence between Pinwheel Singularities and DE Response

A striking effect of the visual deprivation was the consistent coincidence of the DE patches with pinwheel center singularities of the orientation map. The raw response maps to presentation of a particular stimulus orientation were used to calculate, as in Figures 1t and 1e, the average response map from the DE (Figure 5a) and the angle map from the ND eye (Figure 5b). Contour lines drawn to outline the peaks in the DE response maps are shown on the angle map derived from the ND eye (Figure 5c). It is evident in this example, and in the two additional examples illustrated (Figures 5d and 5e), that these DE response peaks occur at the pinwheel centers of the orientation map.

Not all pinwheel centers had DE patches associated with them. At most half would be expected to, since at least half of the pinwheels would lie in territory belonging to the other eye before the onset of the deprivation. In the total of 84 patches found in 12 animals deprived for 2–7 days, there were only 42% as many DE patches as pinwheels.

Through the use of automated procedures to outline DE patches and locate pinwheel centers in maps from the 12 animals deprived for 2–7 days, most DE patch centers were found to fall on or near pinwheel centers (Figure 6a). The coincidence of pinwheels and DE patches is highly significant ($p < 0.00001$, Kolmogorov-Smirnov). If the DE patches were distributed by chance on a field of pinwheels randomly scattered at the measured pinwheel density (2.46 pinwheels/mm), the mean expected separation would be 359 μm. Ninety-four percent (79 out of 84) of the DE patch centers were closer than this mean separation expected by chance, and 80% were closer than half this distance.

Duration and Character of Deprivation

The effects illustrated were found to be true for all deprivations of 2 days duration or longer. Nine animals (examples shown in Figures 2e–2h, 3, and 5; summary data in Figures 4 and 6) were deprived for just 2 days, and their results were qualitatively indistinguishable from those of three animals deprived for 7 days (examples shown in Figures 1 and 2a–2d; summary data in Figures 4 and 6) or from one deprived for more than 5 months. Quantitatively, the colocalization of DE patches and pinwheel center singularities was also similar, with the

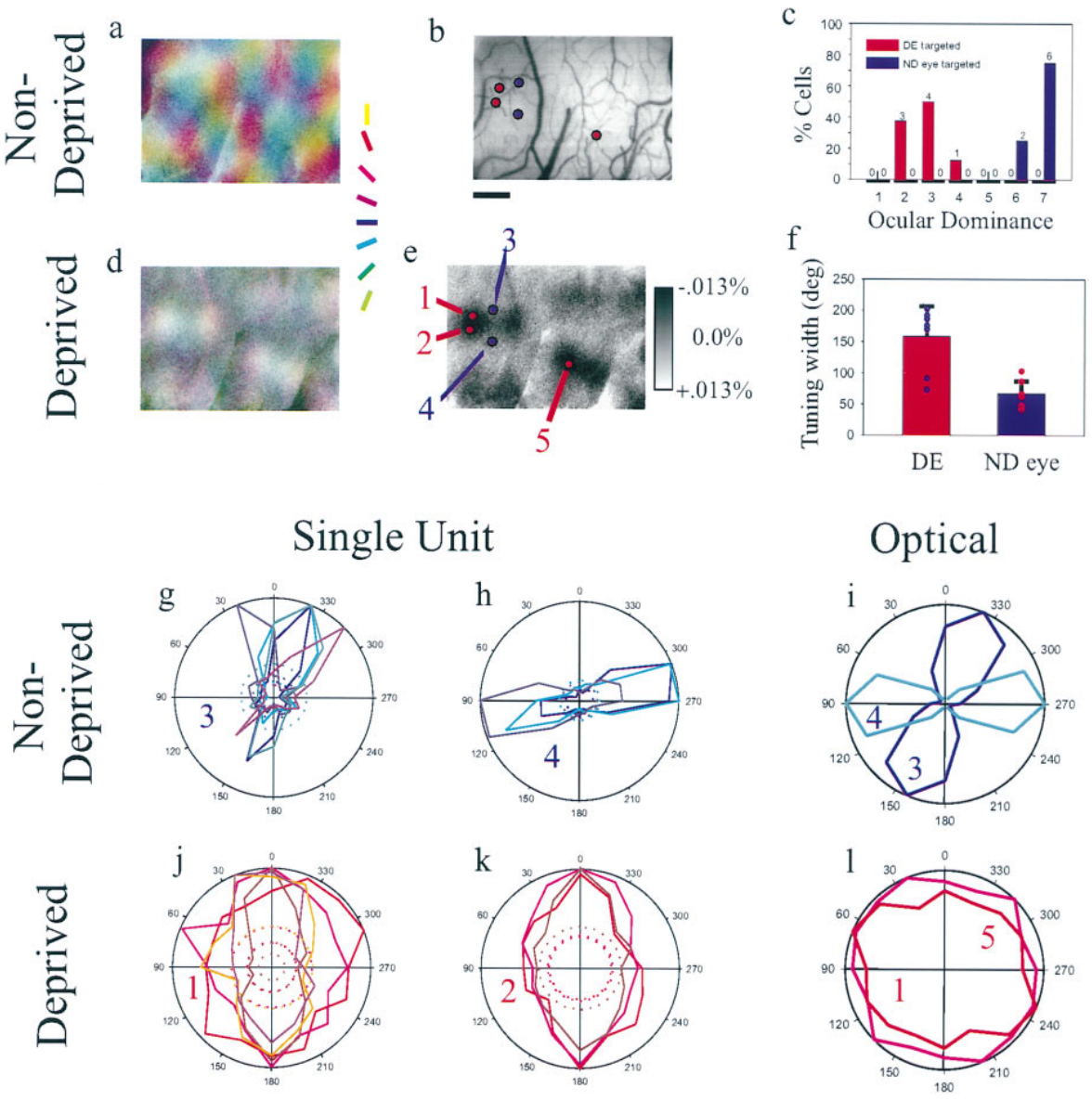

Figure 3. Single-Unit Confirmation of Optical Intrinsic Signal Properties

Optical responses to oriented gratings were used to generate HLS orientation ([a] and [d]) and ocular dominance (e) maps. From these maps, with the aid of the vasculature image (b), patches of nonoriented DE response or ND eye control response were targeted with extracellular single-unit microelectrodes (penetrations marked 1–5 in [e], red for the DE and blue for the ND eye). Cells in penetrations targeted for DE patches in the optical maps were found to be most responsive to the DE as shown in the ocular dominance histogram (c), where ocular dominance is on the conventional 1–7 scale, with class 1 completely DE driven, class 4 binocular, and class 7 responsive only to the ND eye. Measurements of single-unit tuning widths (f) and polar maps of single-unit responses show that cells responsive to the ND eye ([g] and [h]) were well tuned in comparison to DE single units ([j] and [k]). Each solid colored line ([g], [h], [j] and [k]) plots the normalized responses of a single neuron at the indicated orientations; dotted colored lines show the spontaneous activity of the same units. Maximum responses shown for the DE and the ND eye were 8 ([g] and [h]) and 13 ([j] and [k]) spikes/stimulus. Polar plots of the optical responses ([i] and [l]; 0.03% and 0.0375%, respectively) computed from pixels at the same locations as penetrations 3 and 4 in (i) and 1 and 5 in (l) show similar preferred orientations and the absence of tuning in the DE (optical polar plots from 8 bidirectional stimuli; single-unit polar plots from 16 unidirectional stimuli). Numbers above individual bars in (c) indicate the number of cells of that ocular dominance class recorded. Scale bar, 500 μm.

7-day MD animals being only slightly more closely coincident (Figure 6).

In the monocularly deprived kittens, cells that remained vigorously responsive to the DE lost most stimulus selectivity. The poorly selective receptive fields of the cells in the DE patches might be thought to match the pattern of activity produced by vision through a closed eyelid (Loop and Sherman, 1977; Spear et al., 1978). That is, the change in response properties produced by MD could possibly be characterized not as a loss of selectivity but instead as the acquisition of selectivity appropriate for vision through a sutured eyelid. To test this notion, we studied two additional animals in which the MD was produced by completely blocking

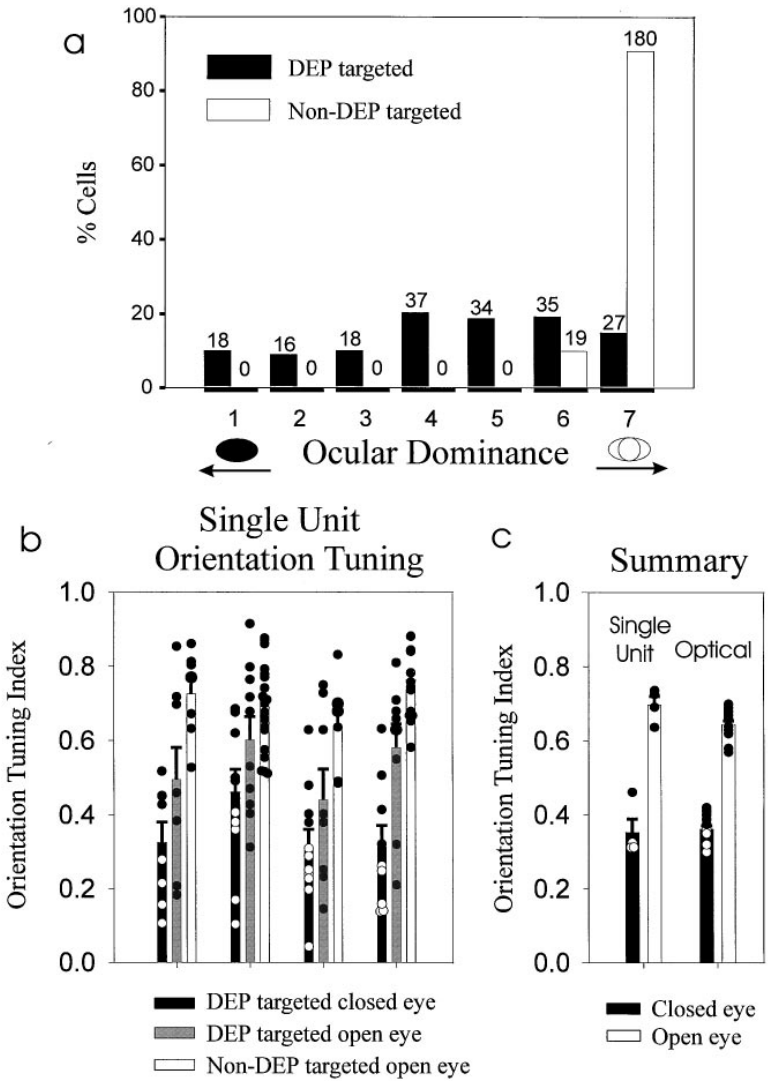

Figure 4. Summary of Single-Unit Ocular Dominance and Orientation Tuning

(a) Summary ocular dominance histogram from 19 penetrations in 9 animals. Single-unit activity in penetrations targeted for deprived-eye patches (DEP), identified from the optical intrinsic signal maps, show robust response to the DE (black histogram). Electrode penetrations targeted away from DEP show no or weak response to the DE (white histogram). Numbers above individual bars indicate the number of cells of that ocular dominance class recorded. One of the 9 kittens was deprived for 5 months, one was deprived with repeated monocular injections of TTX over the course of 6 days, 2 were deprived for 7 days with monocular lid suture, and the remaining 5 were deprived for 2 days with monocular lid suture.

(b) Orientation tuning index of single-unit response. Each set of three histograms (black, gray, and white) are from targeted penetrations in a single animal. The closed circles in (b) are the orientation tuning of individual cells from which the histogram was constructed. Penetrations targeted for DEP show weak tuning, especially through the DE. The orientation tuning in 34 out of 38 neurons in penetrations targeted for a DE patch was characterized through both eyes, and in 32 out of 34 cases the tuning was better in the open eye than in the DE.

(c) Summary orientation tuning index calculated from single units (n = 4 kittens) on the left and from the optical responses (n = 16 kittens) on the right, when stimulated through either the deprived or the ND eye. Closed circles in (c) represent mean data from the individual kittens from which the histogram was constructed.

retinal activity in one eye for 4–6 days with intravitreal injections of tetrodotoxin (TTX). All results for the TTX animals were essentially identical to those in the animals deprived by eyelid suture: patches of cells colocalized with pinwheel centers, with strong and poorly selective responses to the DE, surrounded by an area dominated by the open eye containing weaker but more selective DE responses. Results from the TTX animals are illustrated in qualitative (Figure 5) and quantitative (Figure 6) form. These results indicate that changes in the receptive fields and optical response maps in monocularly deprived kittens do not simply reflect the experience of blurred, low-contrast vision through the lid-sutured eye.

Discussion

These experiments have revealed the arrangement of responses to the two eyes to different stimulus orientations following MD. Most of the visual cortex, with continuous mapping of preferred orientation, responds strongly and selectively to the open eye, with responses to the deprived eye that are weak but are still orientation selective. In patches of cortex that are mainly located on points of discontinuity in the representation of orientation, the cortex responds to the DE strongly but not selectively. These findings have implications for the mechanisms of cortical development and plasticity, and they provide compelling evidence that the pinwheel center singularities in the orientation map play a special role in these processes.

Orientation Tuning through the Deprived and Nondeprived Eyes

The changes in orientation selectivity of cells that respond strongly to stimulation of the DE, and the observed association of DE patches with pinwheel singularities in the orientation map, imply that MD does not simply cause a uniform reduction of DE responses. Rather, cells that remain strongly responsive to DE stimulation do so by sacrificing their orientation selectivity, a process that occurs most readily around pinwheels in the orientation map. In contrast, cells that are still responsive to the DE but farther from clusters of DE

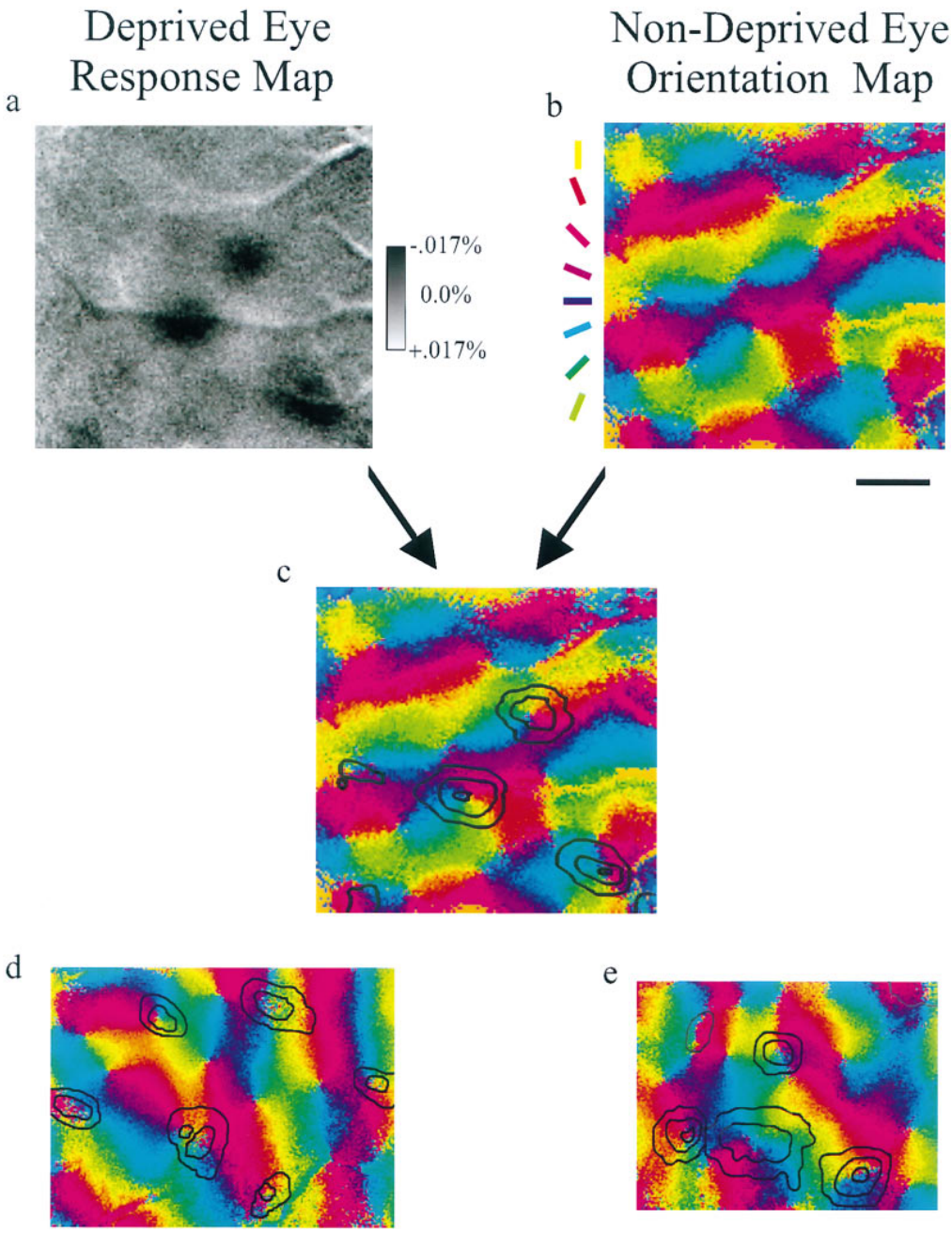

Figure 5. DE Patches Overlie Pinwheel Centers

The average of the DE responses to all orientations is represented in gray scale from a kitten deprived for 2 days in (a). The superposition of the DE response, shown as a contour plot in (c), over the angle map constructed from the ND eye response (b) illustrates the coincidence of the patches of strong DE response with the orientation pinwheel singularities. A similar angle and contour map from a kitten deprived with monocular injections of TTX (d) and a second example from a kitten monocularly lid sutured for 7 days (e) exhibit the same structure. In each case, DE patches coincide with singularities in the orientation map. Scale bar, 500 μm; gray scale range for (a) is ±0.014%.

response maintain their orientation selectivity, in concert with ND eye-responsive cells.

The poor selectivity of neurons driven through the DE must be due to a genuine loss of selectivity, because responses of the same neurons to stimulation of the ND eye were considerably more selective, and selectivity is similar for the two eyes in normal animals (Sherman and Spear, 1982). Our recordings of responses through the ND eye in penetrations targeted to the DE patches revealed that many neurons were as selective as those in penetrations targeted elsewhere, but some had reduced selectivity, lowering the average. Such reduced selectivity may not be due to the deprivation but may represent the normal state of neurons at pinwheel centers in young kittens (Crair et al., unpublished data), an issue discussed further below.

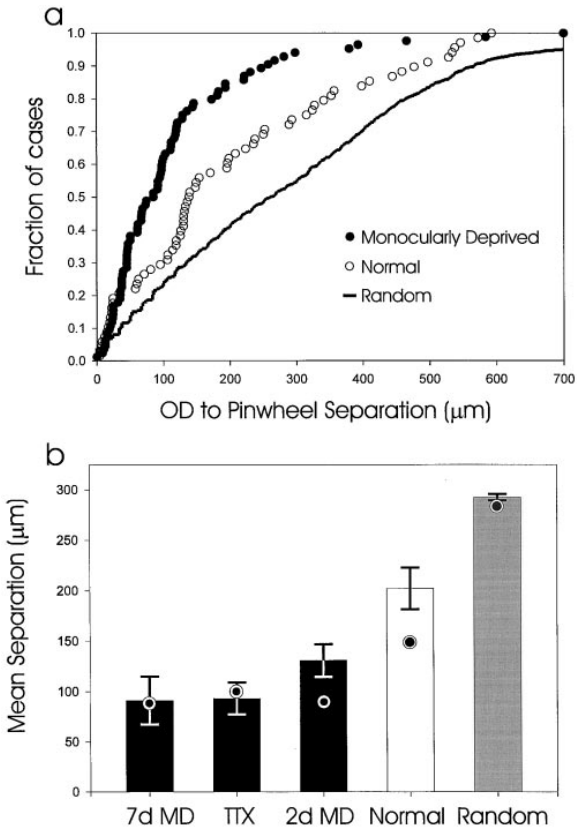

Figure 6. Summary of Separation between Patches of DE Response and Pinwheel Centers

Cumulative probability distributions for the separation between centers of DE patches and the nearest pinwheel singularity are shown with closed circles (a). Similar measurements from animals with normal visual experience for the separation between peaks of ocular dominance and the nearest pinwheel center (Crair et al., 1997) are shown with open circles. Results of a Monte Carlo simulation, in which the positions of both the DE patches and the pinwheel centers have been constrained to lie along the center lines of ocular dominance columns, are shown with a continuous line. All three of these distributions are significantly different from one another ($p < 0.0001$, Kolmogorov-Smirnov), as are their means ($p < 0.0004$). Indicated in (b) are the means (bars) and medians (circles) of the separations between DE patches and pinwheels after 7 days of monocular lid suture (7d MD), monocular TTX injections (TTX), and 2 days of monocular lid suture (2d MD). These are all significantly smaller ($p < 0.0004$) than the mean separation in normal kittens (Normal) or the expected separation if there were no relationship between DE patches and pinwheels, other than the constraint that both lie along the centers of ocular dominance bands (Random).

Comparison with Normal Animals

The coincidence of DE patches and pinwheel centers is much stronger than would be predicted if the DE patches were the result of uniform shrinkage of one eye's ocular dominance columns from their condition before the onset of deprivation. In the monkey, there is a tendency for pinwheel centers to lie on or near the center lines of ocular dominance bands (Bartfeld and Grinvald, 1992; Obermayer and Blasdel, 1993). This tendency is also present in the cat (Hubener and Shoham, personal communication; Crair et al., 1997), though a Monte Carlo analysis shows it is much too weak to account for the present findings. This analysis was carried out by randomly placing the centers of DE patches in relation to pinwheels after constraining both the pinwheels and the DE patches to lie only on the center lines of ocular dominance columns; the probability that the observed coincidence between patches and pinwheels could then arise by chance is <0.0001 (Figure 6a).

In cats with normal visual experience, the extrema or peaks of ocular dominance also tend to lie near the pinwheel center singularities (Crair et al., 1997). However, the coincidence between the patches of DE response and pinwheels reported here is much stronger than that previously noted between ocular dominance peaks and pinwheels in normal animals (Figure 6a; $p < 0.0001$, Kolmogorov-Smirnov), where the mean separation was nearly double what it is in the deprived animals (Figure 6b; ocular dominance peak-to-pinwheel separation is 202 μm in normal animals, and DE patch-to-pinwheel separation is 116 μm). Thus, uniform shrinkage of one eye's ocular dominance columns onto their peaks present before the onset of deprivation cannot account for the much closer observed coincidence of DE patches with pinwheel centers seen after MD.

Possible Mechanisms

The orientation map from the ND eye, measured here, was found by Kim and Bonhoeffer (1994) to be unchanged from its state prior to deprivation. The accentuated colocalization of DE patches with pinwheel centers following MD is thus very likely due to changes in the ocular dominance map in the presence of a fixed orientation map.

Three mechanisms might operate, independently or in concert, to produce the changes observed. The first two mechanisms assume that the outcome of the deprivation process is to leave patches of DE input and responsiveness somewhere in cortex, and they seek to explain both why those places are at the pinwheel centers and why the DE cells in the patches are poorly selective.

Mechanisms Acting within a Cortical Column at the Pinwheel Center

One plausible explanation for the coincidence of DE patches and pinwheel centers depends on the visual response properties of the neurons in the pinwheel centers. While it is believed that single neurons in the pinwheel centers in adult animals are normally selective (Bonhoeffer and Grinvald, 1993; Maldonado et al., 1997), we have found that in kittens, during the critical period, small clusters of cells centered on the pinwheels have much poorer selectivity for orientation and weaker responses to their optimal stimuli than do cells away from the pinwheel centers (Crair et al., unpublished data). These clusters of poorly selective cells in normal kittens are very much smaller than the DE patches seen in MD animals of the present study. In addition, most cells in the DE patches described above are poorly selective through the DE but not through the open eye, unlike the case for the clusters in normal animals, where responses through the two eyes are generally similarly selective. Therefore, the DE patches do not merely represent the preservation of the normal clusters of poorly selective

cells in the absence of visual stimulation to one eye. The difference between the findings in kittens and adult animals suggests that brisk responsiveness and selectivity develop later or less securely at the pinwheel centers than elsewhere.

Earlier work has shown that many neurons in the visual cortex of normal kittens can be driven by stimulation through closed eyelids (Spear et al., 1978), and visual behavior in adult cats can similarly be guided by stimuli through closed eyelids (Loop and Sherman, 1977). The mechanism proposed here posits that, in monocularly deprived animals, the advantage of the open eye over the DE is less in the pinwheel centers than elsewhere. This is because in most of the cortex, oriented contours seen through the open eye drive cells much better than they do through the closed eye; while in the pinwheel centers, where cells are not selective, the same stimuli seen through the open eye do not drive cells well, perhaps little better than diffuse input from the closed eye (or no input at all, in the case of the TTX animals). This proposed explanation would not require the DE to compete on an equal footing with the open eye in driving cells at the pinwheel centers; rather, because input from the DE must end up somewhere in the cortex, it asserts that the responses to the two eyes would be more nearly equal at pinwheel centers than elsewhere. Thus, MD would leave the remnant DE response at the pinwheel centers, since DE responses are at a greater disadvantage elsewhere.

Mechanisms Acting Tangentially across Cortical Columns around the Pinwheel Center

A second plausible explanation for the location of DE patches on pinwheel centers relies on the hypothesis that mutually reinforcing interactions among adjacent cortical columns are important for plasticity (Miller et al., 1989). If so, then elongated visual stimuli seen through the open eye would be effective, because they simultaneously activate many adjacent orientation columns in regions of cortex in which preferred orientation changes slowly and smoothly, since the preferred orientations of these adjacent columns are similar to that of the column that is best activated by any particular elongated stimulus. In contrast, no elongated visual stimulus could activate cells in the adjacent orientation columns on the opposite sides of the pinwheels.

If such mutually reinforcing interactions among adjacent cortical columns, which cause them all to be active simultaneously, are necessary or particularly effective for inducing plasticity, then the advantage of the open eye over the DE would be much greater at regions of continuity in the orientation map than at pinwheel centers. This explanation relies only on the responses to stimuli through the open eye and is therefore in harmony with the similarity between the effects of monocular TTX and monocular occlusion. A recent model of activity-dependent development of orientation and ocular dominance columns shows that such horizontal interactions can cause responses to the DE to tend to be located at the pinwheel center singularities (E. Erwin and K. Miller, personal communication).

Mechanisms Dependent on Unique Plastic Properties of the Pinwheel Center or Its Inputs

A third possibility is that the ocular dominance columns at pinwheel center singularities of the orientation map may, even in normal animals, receive some special input that renders them especially resistant to the effects of MD. In macaque monkeys, at least, cytochrome oxidase (CO) patches or blobs in the supragranular layers receive a unique, koniocellular geniculocortical input in addition to the magno- and parvocellular inputs received by the ocular dominance bands in layer IV (Livingstone and Hubel, 1982; Hendry and Yoshioka, 1994). The CO patches appear to play a special role in plasticity in the monkey, because when ocular dominance bands in layer IV from one eye shrink following MD and ultimately break up into discrete patches, each remnant of the DE's input to layer IV is precisely centered on a CO patch (Horton and Hocking, 1997). In the normal visual cortical representation of the peripheral visual fields, a similar phenomenon is also present: ocular dominance bands serving the ipsilateral eye wax and wane in width, and the wide spots are always centered around CO blobs (Horton and Hocking, 1997). In the extreme periphery, the narrow parts of the ocular dominance bands disappear, leaving patches in layer IV centered around the supragranular CO patches. Thus, in the monkey, the presence of patches of koniocellular input to the supragranular layers is correlated with, and may well cause, maximum resistance to the effects of deprivation within the underlying portion of layer IV in the same column. These anatomical findings would be in harmony with the present report on cat visual cortex if the pinwheel center singularities of the orientation map in monkey visual cortex were coincident with the CO patches. However, current evidence suggests that the CO patches in monkeys may not colocalize with pinwheels, even though both have been reported to lie along the center lines of ocular dominance stripes (Blasdel, 1992; Bartfeld and Grinvald, 1992), or they may colocalize only to a very limited extent (Blasdel, 1989).

In the cat, there are also supragranular CO blobs of unknown function (Dyck and Cynader, 1993; Murphy et al., 1995), which are likely to correspond to the puffs of geniculocortical input to the supragranular layers (LeVay and Gilbert, 1976) that, by analogy to the monkey, may be of koniocellular origin. The geniculocortical input to ocular dominance bands in layer IV also clearly does vary in width and conceivably in density (Anderson et al., 1988; Boyd and Matsubara, 1996), and the pinwheel centers of the orientation map tend to lie close to the most monocular points in cortex (those that respond most nearly exclusively to one eye; Crair et al., 1997). In the cat, however, it is not yet known whether the widest portions of normal ocular dominance bands underlie the supragranular CO patches or whether pinwheel center singularities overlie CO patches. A strong suggestion to the contrary emerges from a recent report that regions of selectivity for low spatial frequencies correspond to CO patches in the cat but not to ocular dominance bands (Shoham et al., 1997).

While the third hypothesis of a distinct anatomical input to pinwheel centers that is especially resistant to the effects of deprivation is attractive, at present there is little evidence to support it. In the monkey, koniocellular inputs to CO patches may well play a special role in preserving DE responses to the underlying ocular dominance columns in layer IV, but their relationship to the pinwheel centers of the orientation map is not clear. In

normal cats, there is no anatomical evidence of a special input to pinwheel centers of the orientation map that might account for the preservation of DE responses around them.

Role of the Pinwheels in Cortical Development

The selective preservation of DE responses at pinwheel centers is consistent with but not predicted by the reported tendency of pinwheel centers to lie along the center lines of ocular dominance stripes in normal monkeys (Bartfeld and Grinvald, 1992; Obermayer and Blasdel, 1993) and is much stronger than the relationship between the peaks of ocular dominance and pinwheels found in normal cats (Crair et al., 1997). The observed changes in orientation selectivity, as well as the reorganization of ocular dominance following deprivation, are likely to be due to the same mechanisms that guide normal development. The selective preservation of DE responses at pinwheels is the first indication that pinwheel singularities are functionally important in development and plasticity, and is consistent with models for the development of orientation and ocular dominance under the guidance of an activity-dependent, correlation-based mechanism (E. Erwin and K. Miller, personal communication).

Each cortical cell participates in all of the maps that tile its region of cortex. Many of the same synapses must simultaneously convey information for the different maps; this is known to be the case, for example, for the geniculocortical synapses to simple cells in layer IV of cat visual cortex, which are both eye specific and orientation specific (Chapman et al., 1991). It should therefore not be surprising that plasticity in the stimulus attributes of one map may be coupled to other maps. The control of plasticity by interactions between orientation and ocular dominance maps in visual cortex is therefore likely to illuminate fundamental rules governing the development of overlapping cortical representations.

Experimental Procedures

Optical Imaging

Optical intrinsic signal responses were measured using standard techniques (reviewed by Bonhoeffer and Grinvald, 1996). Briefly, reflectance of primary visual cortex (−2.0 to 2.0 mm anterior to the interaural line, close to the V1–V2 border, illuminated with 610 nm light) following monocular presentation of moving (1 cycle/s), high-contrast square wave gratings (0.1–0.2 cycles/degree of visual angle), presented on a video monitor placed 40 cm from the cats' eyes, was divided by reflectance following presentation of a blank (gray) screen. This form of "blank screen" normalization eliminates possible artifacts due to uneven illumination or other nonspecific optical effects, but it incorporates no assumptions about the underlying structure of the neuronal response. Stimuli were presented in pseudo-random order. Images were captured through a matching 610 nm filter by a cooled slow scan CCD Camera (Princeton Instruments, NJ). Images were high-pass filtered (1.2 mm kernel) but never smoothed; hence, artifacts due to blood vessels are sometimes apparent. All analyses were carried out in regions of the maps that did not contain such artifacts. Unless otherwise noted, all images in a given figure are shown with identical absolute linear gray scales or color maps applied to both eyes' responses. Cortical responses to the presentation of different stimulus orientations can be combined and summarized using a single pseudo-color representation referred to as a hue-lightness-saturation or HLS orientation map (Bonhoeffer and Grinvald, 1996). In these maps, the color (hue) represents the orientation for which the neurons respond most strongly, the lightness represents response strength, and the color saturation depicts the underlying orientation selectivity, with white the most poorly tuned (least saturated) but strongest response. Maps of the orientation tuning index (Figure 2) show the vector sum of the optical response at a given orientation divided by the sum of the responses to all orientations computed pixel by pixel. These maps are all normalized using the same background reflectance image, so that all responses for both eyes are represented on a common gray scale, in which white corresponds to best tuning. The orientation tuning index (Figure 4c) for a given animal is the mean of the orientation tuning indices at all pixels.

Single-Unit Extracellular Recording

Responses of single units isolated with a window discriminator were assayed with a computer-based visual stimulation and data acquisition system that presented moving high-contrast bars at 16 different orientations (or no stimulus for measurement of spontaneous response) in a pseudo-random sequence. Plots of the neuronal response above spontaneous rate as a function of orientation were fit with a Gaussian function using a nonlinear regression algorithm, and the tuning width of the cell was calculated as the full width at half-maximum height of the fitted Gaussian. The single-unit orientation tuning index plotted in Figure 4 was calculated exactly like the orientation tuning index for the optical maps described in the previous paragraph. Ocular dominance was categorized by hand for each single unit on a seven-point scale following Hubel and Wiesel (1962).

Analysis and Monte Carlo Simulations

To determine unambiguously the position of the DE patches, the centroid within the full-width half-maximum response contour of each DE patch was computed from the DE response map filtered with a three-bin Lee filter. The positions of pinwheel singularities were defined as the points where the integral of the orientation differences around a pixel was $\pm 180^{\circ}$. When smaller optical response necessitated, the angle map was also smoothed with a three-bin Lee filter to prevent the identification of spurious pinwheels. If the pinwheels were distributed randomly (as a two-dimensional Poisson process), and the positions of the DE patches were not associated with the pinwheels, then the mean separation of the pinwheels and DE patches would be $\sqrt{(1/\pi\lambda)}$ or 359 μm, where λ is the density of pinwheels observed. The Monte Carlo simulation results shown in Figure 6 assumed that all DE patches and pinwheels were constrained to lie on the center lines of the ocular dominance stripes as they existed before the onset of MD.

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