

Pairing-Induced Changes of Orientation Maps in Cat Visual Cortex

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Summary

We have studied the precise temporal requirements for plasticity of orientation preference maps in kitten visual cortex. Pairing a brief visual stimulus with electrical stimulation in the cortex, we found that the relative timing determines the direction of plasticity: a shift in orientation preference toward the paired orientation occurs if the cortex is activated first visually and then electrically; the cortical response to the paired orientation is diminished if the sequence of visual and electrical activation is reversed. We furthermore show that pinwheel centers are less affected by the pairing than the pinwheel surround. Thus, plasticity is not uniformly distributed across the cortex, and, most importantly, the same spike time-dependent learning rules that have been found in single-cell *in vitro* studies are also valid on the level of cortical maps.

Introduction

The visual cortex of higher mammals contains regular maps for a number of essential stimulus properties, including orientation, ocular dominance, direction of movement, and spatial frequency. The orientation preference map is characterized by mostly smooth changes of the preferred orientation across the cortical surface. In addition, this map exhibits singularities where orientation preference changes rapidly, giving rise to a pinwheel-like arrangement of orientation domains in the visual cortex (Bonhoeffer and Grinvald, 1991).

While many experimental as well as theoretical studies have investigated the development of orientation preference maps, it is still unclear to what extent activity-dependent mechanisms contribute to the formation of this map: on the one hand, it has been shown that orientation preference maps can develop independently of patterned vision (Gödecke and Bonhoeffer, 1996; Weliky and Katz, 1997; Gödecke et al., 1997; Crair et al., 1998). On the other hand, the proper formation of the orientation preference map requires normal levels and patterns of neuronal activity (Chapman and Stryker, 1993; Ruthazer and Stryker, 1996; Chapman and Gödecke, 2000), and its structure can be modified by altering the visual input, e.g., by stripe rearing (Sengpiel et al., 1999). These changes are thought to occur by a “Hebbian” mechanism, in which synaptic strength increases when pre- and postsynaptic neurons are active simultaneously. Cell culture experiments (Debanne et al., 1994; Bi and Poo, 1998; Debanne et al., 1998) and slice studies in

vitro (Markram et al., 1997; Feldman, 2000) as well as single-cell recordings *in vivo* (Zhang et al., 1998), however, suggest that correlated pre- and postsynaptic activity can lead to increases or decreases in synaptic efficacy. It was found that the relative timing between pre- and postsynaptic cells determines the direction of changes in synaptic strength on a millisecond timescale: a synapse becomes potentiated if activity of the presynaptic cell is followed by depolarization of the postsynaptic neuron. In contrast, if the action potential of the postsynaptic cell precedes the synaptic input, this synapse is weakened. Such a spike time-dependent rule, if valid also during the development of cortical maps, would have strong effects on how patterns of neuronal activity shape cortical maps. The effects of relative timing have so far only been investigated in single-cell studies, either *in vitro* or in the frog tectum (Zhang et al., 1998), so that the consequences for cortical map development are unknown. In rearing experiments (Hirsch and Spinelli, 1970), on the other hand, the relatively imprecise control over the stimulus precludes a rigorous study of the effects of millisecond timing on the development of cortical maps.

To address this question, we have adapted and modified an approach first introduced by Fregnac et al. (1988) to induce changes in orientation preference maps: we repeatedly paired a very brief visual stimulus (a grating of one orientation) with electrical stimulation within the visual cortex and investigated the resulting changes in the orientation preference maps by optical imaging of intrinsic signals before and after this pairing procedure. By varying the interval between visual and electrical stimulation, we were able to investigate the effect of the relative timing between activation of thalamo-cortical inputs by the visual stimulus and intracortical electrical stimulation.

We used this well-controlled *in vivo* model of functional plasticity of orientation maps to address the following questions:

- Does the relative timing between pre- and postsynaptic activity determine the changes of the orientation preference maps, i.e., can the cortical response to the paired orientation either be enhanced or depressed depending on the relative timing of electrical and visual stimulation? A positive answer to this question would necessitate a reconsideration of theories of cortical map formation (Miller et al., 1999; Shouval et al., 2000) that are currently based on learning rules not dependent on correlations in the millisecond time domain.
- It has been suggested that specific locations within cortical maps have a higher capacity to undergo plastic changes than other locations (Crair et al., 1997; Kojic et al., 2000; Trachtenberg et al., 2000). These studies have, however, only addressed ocular dominance plasticity and have left open the question whether the plasticity of orientation preference exhibits similar inhomogeneities across the cortical surface.
- Stripe rearing studies (Blakemore and Cooper, 1970;

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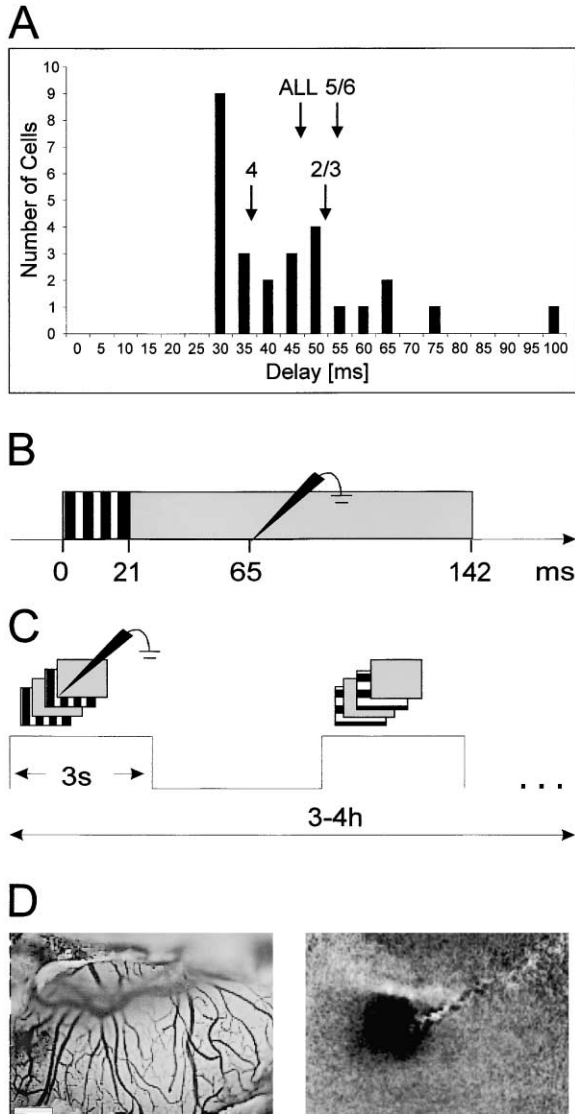


Figure 1. Distribution of Response Latencies and Schematic of the Pairing Paradigm

(A) Distribution of response latencies in area 17. The average latency of all cells as well as of cells in different cortical layers is indicated. (B) An orientated grating is very briefly presented on a screen, followed by an electrical stimulus (symbolized by the electrode) delivered to the cortex 65 ms after the onset of the visual stimulus. This sequence is repeated at 7 Hz. The 65 ms delay of the electrical stimulus ensures that neurons in the primary visual cortex are electrically stimulated immediately after visually evoked activity arrives in the cortex. (C) Pairing at 7 Hz was continued for 3 s, yielding a single pairing trial. Pairing trials were interleaved with similarly flashed presentations of the orthogonal orientation but without concurrent electrical stimulation. The total duration of the pairing was 3–4 hr. (D) Optical imaging (32 repetitions) of cortical activity evoked by electrical stimulation alone, using the same parameters as for pairing (60 μ A, 200 μ s, 7 Hz). The tip of the stimulating electrode was located 400 μ m below the cortical surface in the center of the dark spot, which corresponds to the activated cortical region. The electrode is partly visible to the right of the activated region. Scale bar within corresponding blood vessel image: 1 mm.

Hirsch and Spinelli, 1970; Freeman and Pettigrew, 1973; Sengpiel et al., 1999) have shown that the orientation preference map can be altered by experience. However, due to the fact that no recordings were done before the stripe rearing started, it could not be determined whether these changes affected the general structure of the map or whether preexisting domains were merely enlarged and reduced in the course of the altered rearing conditions.

Results

To induce localized changes in orientation preference maps in kitten visual cortex, we employed a pairing protocol consisting of combined visual and electrical stimulation over a period of 3–4 hr. To this end, electrical stimuli were applied to the visual cortex while the animal was stimulated visually with one of four oriented gratings.

In order to establish a precise timing between visual and electrical stimulation, we determined the response latency of neurons in area 17 to flickering gratings similar to the ones used in the pairing protocol. On average, cells in area 17 responded with a latency of 46.8 ms (± 3.3 ms, $n = 27$ cells; throughout this paper, \pm symbols and error bars denote SEM) after the onset of the visual stimulus. This value is very close to the 46.7 ms found by Ikeda and Wright (1975). As expected, cells recorded at depths corresponding roughly to layer 4 overall had shorter latencies than cells in the supra- and infragranular layers (Figure 1A).

For pairing, we used a flickering grating (7 Hz) of one orientation with only 21 ms on time as visual stimulus (Figure 1B). Based on the measured response latencies, the interval between the visual and electrical stimulus was chosen such that, for cells in the cortex, activation by the visual stimulus either preceded (pairing) or followed (antipairing) the electrical stimulus. As a control, these stimulation trials were interleaved with presentation of the orthogonal orientation (Figure 1C). The effectiveness of the electrical stimulus in driving cortical cells as well as the size and position of the activated cortical region was determined by optical imaging (Figure 1D).

Pairing-Induced Shift in Orientation Preference

In the first set of experiments, we tested whether the response to the paired orientation is enhanced when visual stimulation of neurons in the visual cortex by thalamo-cortical fibers precedes electrical stimulation within the cortex. To measure changes in orientation preference, we imaged maps before and 1–6 hr after the pairing, using the same spatial frequency, which was presented during the pairing procedure. In Figure 2A, examples of single-condition maps of the paired and the orthogonal orientation are presented. The color-coded difference map displays the difference between the single-condition maps imaged before and after pairing. In this map, red codes for an increase of the intrinsic signal after pairing, while blue codes for a signal decrease. A comparison of the single-condition maps as well as the difference maps shows that at the site of stimulation the cortical response to the paired orientation increased substantially after pairing relative to the response of the

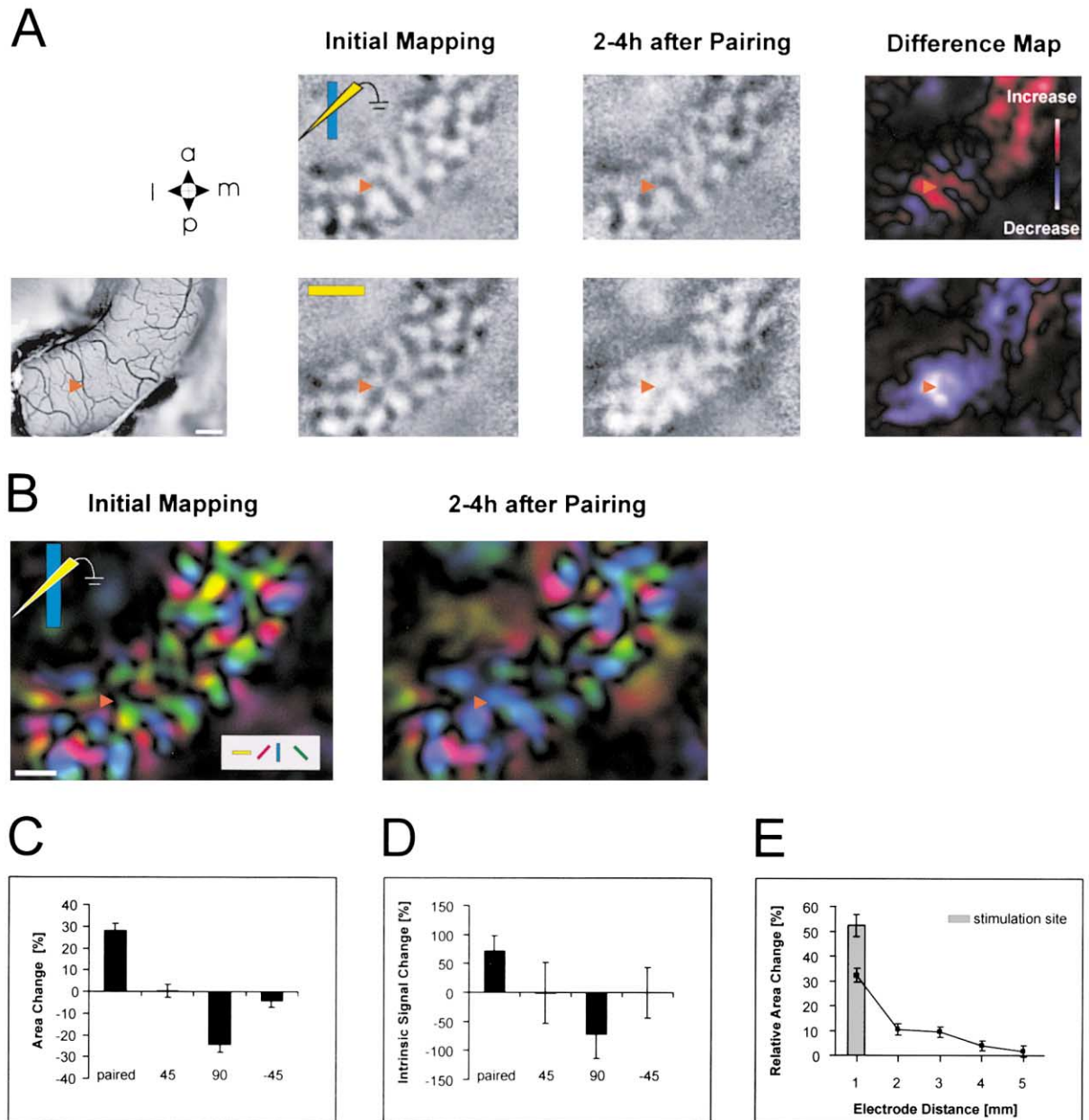


Figure 2. Optical Imaging of Pairing-Induced Shifts in Orientation Preference

(A) Single-condition maps (cocktail-blank corrected) of the paired (top) and orthogonal (bottom) orientation imaged before and after pairing. The arrowhead marks the position of the stimulation electrode. The difference maps show the difference between the single-condition map obtained before and after pairing. Red and blue code for an increase and decrease, respectively, of the intrinsic signal after pairing, i.e., the absorption of light due to neuronal activation. Left: blood vessel pattern and orientation of recorded cortical area (anterior, a; posterior, p; medial, m; and lateral, l).

(B) Polar maps from the same experiment. Color codes for orientation preference and color saturation for orientation selectivity. In the single-condition and the difference maps as well as in the polar maps, an increase in response to the paired orientation and a decrease to the orthogonal orientation occurs around the site of stimulation. Scale bars: 1 mm.

(C) Quantification of the pairing effect in six animals. We measured the pairing-induced change in cortical area responding preferentially to each orientation at the site of stimulation. Error bars are SEM.

(D) Additionally, the corresponding change in the integral of the intrinsic signal was computed for each orientation.

(E) Decay of the effect with increasing distances from the site of stimulation. The relative change in size between paired and orthogonal orientation was quantified in concentric rings around the stimulation site ($n = 6$). Note that for this quantification regions in area 18, which were more distant than 5 mm from the stimulation site, were excluded.

orthogonal orientation. This orientation-specific change becomes even more obvious when comparing the polar maps (Ts'O et al., 1990), which display orientation preference by color and orientation selectivity by saturation (Figure 2B). The polar map taken before pairing shows an equal distribution of all orientations (data not shown). After the pairing procedure, blue patches occur around the site of stimulation, which indicates that the response to the paired orientation is enhanced.

While the shift in orientation preference is visible mainly at the site of stimulation in area 17, a shift can also be detected at some distance within area 18 in the two of six experiments in which the optically recorded region comprised the area 17/18 border. The strong activation of area 18 neurons during pairing could account for this effect: the visual stimulus employed during pairing had a low spatial frequency and was presented at a high temporal frequency (7 Hz); thus, it is likely to drive neurons in area 18 more strongly than cells in area 17 (Movshon et al., 1978). At the same time, it can be expected that the electrical stimulus in area 17 will also activate cells in area 18 via retinotopically organized connections between the two areas. Although we have no direct proof that the changes in area 18 occur at retinotopically corresponding locations, the retinotopic divergence of the 17-18 connections (Ferrer et al., 1988) as well as the size of the electrically stimulated region are at least compatible with this interpretation.

We quantified the pairing-induced changes in cortical area responding preferentially to each of the four orientations at the site of stimulation in six animals (Figure 2C; paired orientation, $28\% \pm 3\%$; orthogonal orientation, $-24\% \pm 3\%$ of total area). The cortical region activated by the electrical stimulus was determined as shown in Figure 1D, and the overall change of the intrinsic signal in this region was computed for each orientation (paired orientation, $72\% \pm 26\%$ of the mean amplitude of the intrinsic signal; orthogonal orientation, $-70\% \pm 52\%$; Figure 2D). In every experiment, both the cortical area and the overall intrinsic signal corresponding to the paired orientation increased, while both measures decreased for the orthogonal orientation, which was presented during the pairing period but without electrical stimulation. It is evident that the variance of the intrinsic signal change is much larger than that of the areal change. The reason for this difference is most likely that the intrinsic signal differs quite strongly between animals, partly due to different physiological and imaging conditions. Moreover, the area measure is normalized, thus leading to smaller interanimal variations. The cortical regions activated by the paired orientation increased by nearly a factor of 2 at the site of stimulation and the difference between the change in area of the paired and orthogonal orientation was highly significant ($p = 0.0013$, Student's *t* test). As substantiated by quantification in concentric rings of increasing diameter around the site of stimulation, the magnitude of change in orientation preference decays with radial distance from the site of stimulation (Figure 2E). In this quantification, we excluded regions in area 18 which were more distant than 5 mm from the stimulation site. The shift in orientation preference did not occur in one control animal where we inserted an electrode and did not stimu-

late electrically but only visually during an otherwise normal "pairing" protocol.

To further validate the pairing-induced shift in orientation preference, we recorded from single units in one animal before (72 cells) and after (76 cells) pairing. To sample from approximately the same cortical sites before and after pairing, we used the cortical blood vessel pattern to position the recording electrode. Comparing all cells recorded before and after pairing a mean shift of $19.3^\circ \pm 2.9^\circ$ toward the paired orientation occurred, which was highly significant ($p = 0.00002$, Wilcoxon Rank test). In four out of six penetration sites, this shift was found to be significant ($p < 0.03$, Wilcoxon Rank test, Figure 3A). To crossvalidate optical and electrical recordings, we correlated the orientation preference as determined from optical and electrical recordings at the recording sites. As expected, both before and after the pairing the values proved to be well correlated (correlation coefficient: 0.96, Figure 3C). Remarkably, only at those five positions where a change toward the paired orientation was detected with the single-cell recordings, optical imaging revealed a shift toward the paired orientation. Interestingly, orientation selectivity, measured as the half-width at half-height of the Fourier-filtered tuning curve, did not change significantly. Likewise, the overall shape of the tuning curves did not exhibit significant differences when we compared the averaged tuning curves aligned by their preferred direction for each orientation. This latter result implies that, for instance, no secondary peaks in orientation preference were induced by the pairing procedure. Also, spontaneous rate and direction preference remained unchanged (all *p* values >0.1 , Student's *t* test). While this makes it likely that the observed changes in the orientation preference maps are caused by a real shift in orientation preference and not by a widening of the orientation tuning curves (Sengpiel et al., 1999), a real proof of this assumption would necessitate recordings from the same cells before and after the pairing, which was not feasible in our experiments. We did however record neurons 5–14 hr after the pairing, implying that the changes remain for this period of time, at least. We further confirmed this in one cat by imaging orientation preferences 14–18 hr after pairing and by observing that the shift was still present.

Taken together, these results show that the pairing paradigm is capable of inducing long-lasting shifts in orientation preference toward the paired orientation. The single-unit data corroborate this finding and demonstrate that the effect observed with optical imaging is neither a subthreshold change in orientation preference nor a mere metabolic change.

Antipairing Causes Shift Away from Antipaired Orientation

In the experiments described above, the delay between electrical and visual stimulation was chosen to mimic the pairing paradigm used in single-cell studies (Debanne et al., 1994; Markram et al., 1997; Bi and Poo, 1998; Debanne et al., 1998; Zhang et al., 1998; Feldman, 2000) in which presynaptic activation followed by postsynaptic depolarization leads to increases in synaptic strength. To investigate whether the relative timing determines the direction of plasticity for orientation maps in the

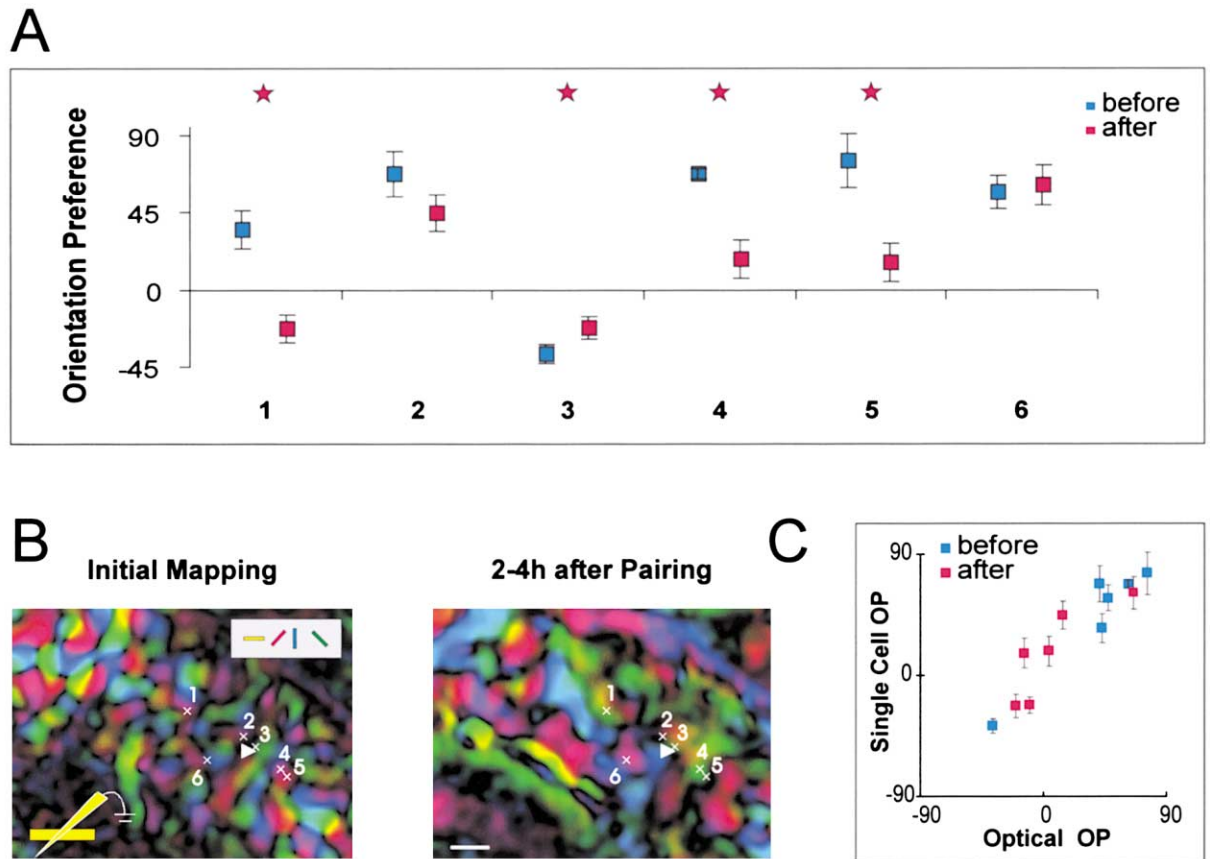


Figure 3. Single-Cell Recordings Before and After Pairing with a 0 Degree Grating

(A) Average orientation preference before and after pairing for each track. The recording positions are marked in the polar maps in (B). Asterisks denote a significant difference before and after pairing ($p < 0.05$); error bars are SEM.

(B) Polar maps obtained before and after pairing. The recording sites are marked with numbers; scale bar: 1 mm.

(C) Scatter plot of optically and electrically measured preferred orientation for each track position before and after pairing. Note that orientation preference recorded after pairing clusters closely around the paired orientation.

visual cortex as well, we decreased the temporal delay between visual and electrical stimulation to 35 ms (Figure 4A). In this antipairing paradigm, most cells in the cortex are electrically stimulated before the visual stimulus can activate them (Figure 1A). In striking contrast to the effects reported above, the single-condition maps of the antipaired orientations are reduced in strength after the antipairing, while cortical neurons respond more strongly to the orthogonal orientation, which was presented during antipairing, without a concurrent electrical stimulus (Figure 4B). Again, this shift is more obvious when comparing the color-coded polar maps (Figure 4C): orientation domains activated by the antipaired orientation decrease both in area and intrinsic signal strength, while there is a corresponding increase for the other orientations at the site of stimulation. Quantification of this effect in four animals reveals a significant decrease of both the cortical area activated by the antipaired orientation (antipaired orientation, $-8.6\% \pm 1.2\%$; orthogonal orientation, $10.6\% \pm 2.4\%$ of total area; $p = 0.009$, Student's *t* test; Figure 4D) as well as its intrinsic signal strength (antipaired orientation, $-43\% \pm 16\%$; orthogonal orientation, $25\% \pm 16\%$ of the amplitude of the intrinsic signal; $p = 0.03$, Student's

t test; Figure 4E). As is the case for pairing, the degree of change in orientation preference decays with radial distance from the site of stimulation (Figure 4F).

Thus, analogous to the effects observed in single-cell studies *in vitro*, antipairing can reduce neuronal responses to a visual stimulus at the level of cortical maps *in vivo*.

Spatial Distribution of Pairing Effects

In addition to investigating the effects of the relative timing of pre- and postsynaptic activity on changes in response properties, the spatial resolution of optical imaging made it possible to study the distribution of plasticity in different regions of cortical maps. As a measure for the magnitude of the orientation shift, we employed the relative change in the intrinsic signal between the paired and the orthogonal orientation at the site of the stimulation. We did not use changes in domain area to quantify the distribution of plasticity across the cortical surface because areal changes are *per se* not equally distributed across the cortex but are rather confined to the borders of orientation domains. Surprisingly, we did not observe any significant differences between the different orientation domains, *i.e.*, the magnitude of the

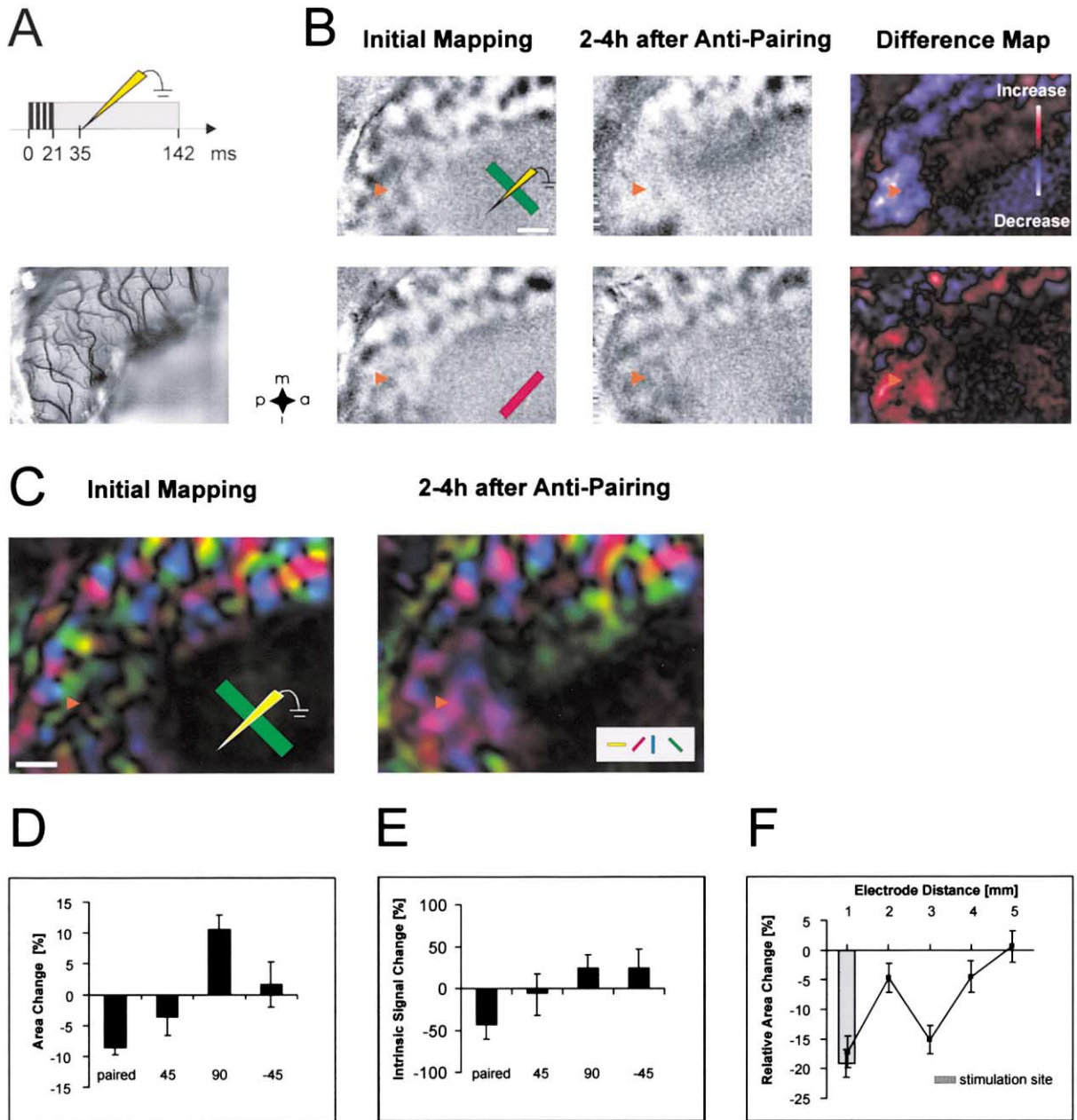


Figure 4. Optical Imaging of the Effect of Antipairing

(A) Schematic illustration of the antipairing paradigm. (B) Comparison of single-condition maps, difference maps, and (C) polar maps obtained before and after antipairing; scale bars: 1 mm. In these maps, the response to the antipaired orientation decreased after antipairing near the stimulating electrode (arrowhead). (D) Quantification ($n = 4$) of the change in cortical area and (E) intrinsic signal at the stimulation site: in contrast to the pairing paradigm, the neural response to the antipaired orientation decreases. (F) Decline of the antipairing effect with radial distance from the stimulation electrode.

change was not significantly different within domains for the paired, orthogonal, or intermediate orientations (pairing, $p = 0.17$; antipairing, $p = 0.27$; ANOVA; Figure 5A). At first glance, this result cannot be explained easily, because neurons, which are already tuned to the paired orientation, should not be capable of shifting their orientation any further toward this orientation. We shall return to the possible mechanisms underlying this result in the Discussion.

As for the different orientation domains, there was

also no significant difference in the degree of change between low and high spatial frequency domains (pairing, $p = 0.09$; antipairing, $p = 0.18$; Student's t test). We did, however, find a systematic variation in the size of the shift in different regions of the orientation map: near pinwheel centers, the magnitude of plasticity was significantly lower than in regions of the cortex further away from the pinwheel centers (pairing, $p = 0.02$; antipairing, $p = 0.03$; Student's t test; Figure 5A). To test whether the inhomogeneous local neighborhood of ori-

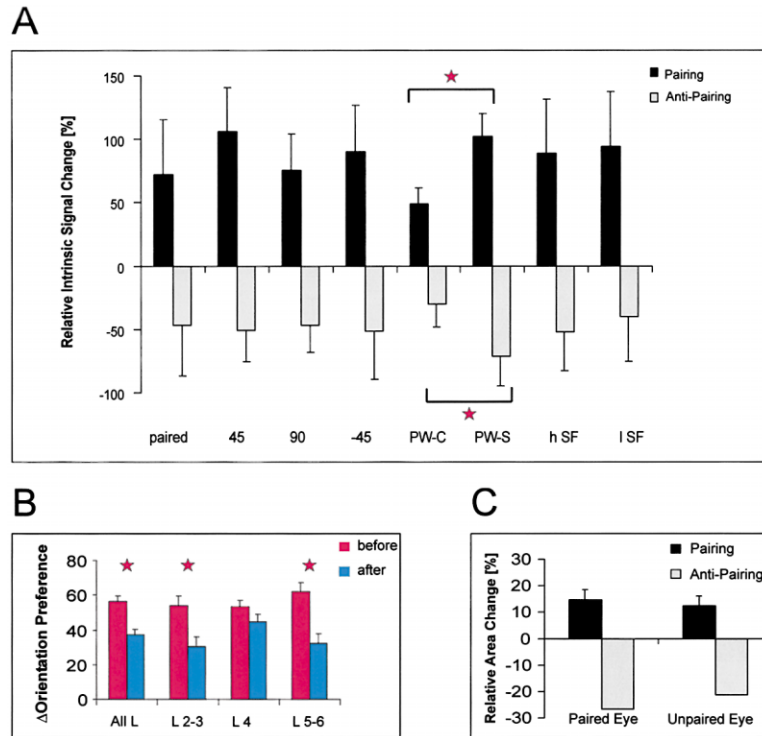


Figure 5. Spatial Distribution of Pairing Effects

(A) Relative intrinsic signal change (between paired and orthogonal orientation) in different regions of functional maps after pairing (black) and antipairing (gray). While there were no significant differences between domains for the paired, control, or intermediate orientations, cortical regions near pinwheel centers (PW-C) showed significantly less change compared to the pinwheel surround (PW-S). No difference was observed between high (h SF) and low spatial frequency (l SF) domains. Asterisks denote significant differences between regions ($p < 0.05$).

(B) Layer-specific distribution of plasticity as assessed with electrical recordings. The histogram displays the average difference between the cells' preferred orientations and the orientation used for pairing before (red bars) and after (blue bars) pairing for all tracks. Layer 2/3 (L 2/3, $n = 20$ and 21 cells before and after pairing, respectively) and layers 5/6 (L 5/6, $n = 27$ and 22 cells before and after pairing, respectively) exhibited a significant shift ($p < 0.05$) toward the paired orientation, while no significant shift occurred in layer 4 (L 4, $n = 25$ and 33 cells before and after pairing, respectively).

(C) Interocular transfer of pairing ($n = 3$ animals) and antipairing ($n = 1$ animal) induced changes. The (anti-)pairing effect was quantified by measuring the relative change in cortical area between paired and orthogonal orientation for the paired and unpaired eye. The changes in the unpaired eye are almost as strong as in the paired eye.

entation preferences at pinwheel centers causes their resistance to shifts in orientation preferences, we measured the dependence of the orientation shift magnitude on the local homogeneity of orientation preference. This homogeneity was formalized as the "orientation similarity index": it quantifies the similarity of orientation preference in the neighborhood of a given point within the cortex. Orientation similarity is high in the pinwheel surround and drops near pinwheel centers. Formally, we determined the orientation similarity index by computing the inverse spatial standard deviation of the orientation maps in a circle with a radius of $300 \mu\text{m}$ around each pixel. We found that the correlation between shift magnitude and orientation similarity index is not significantly different from 0 when computed in the pinwheel surround (correlation index: pairing, -0.21 ± 0.40 , $p = 0.14$; antipairing, 0.02 ± 0.46 , $p = 0.26$; Student's *t* test). This lack of correlation indicates that local neighborhood of orientation preference is not important for induction of plasticity in the orientation domain.

While optical imaging is well suited to study variations in the degree of plasticity across the cortical surface, single-unit recordings are necessary to determine the layer-specific distribution of plasticity. We therefore used the readings on the microdrive advancing the electrode to assign cells recorded at different depths in the cortex to different groups, roughly corresponding to different cortical layers. We found that the most significant shift ($30.8^\circ \pm 6.0^\circ$, $p = 0.001$, Wilcoxon-Rank test,

Figure 5B) toward the paired orientation occurred in lower layers (presumably, layers 5 and 6). It was less pronounced but still significant ($23.8^\circ \pm 5.7^\circ$, $p = 0.004$, Wilcoxon-Rank test) in upper layers (layers 2 and 3), while no significant shift in orientation preference was found in layer 4 ($8.34^\circ \pm 4.2^\circ$, $p = 0.22$, Wilcoxon-Rank test).

This layer-specific effect is unlikely to result from electrode penetrations not being oriented orthogonal to the cortical surface. In this case, one would expect a monotonous distribution of the apparent shifts as a function of recording depth. However, we did find a bimodal distribution, with shifts being high in the upper and the lower layers but small in layer 4.

The most straightforward explanation for this observation is that the shift in orientation preference is not caused by changes of thalamo-cortical but rather of cortico-cortical synapses outside layer 4. To substantiate this interpretation, we studied whether the effect of the pairing is transferred from one eye to the other. To this end, we imaged the monocular orientation maps for both eyes, but paired electrical and visual stimulation only for one eye. If the shift were mainly due to changes of cortico-cortical synapses, a considerable transfer of the effect to the unpaired eye would be expected. Such a transfer did in fact occur, and the degree of shift in the orientation map recorded through the unpaired eye was nonsignificantly different from the shift of the paired eye ($p = 0.17$, Student's *t* test, Figure 5C).

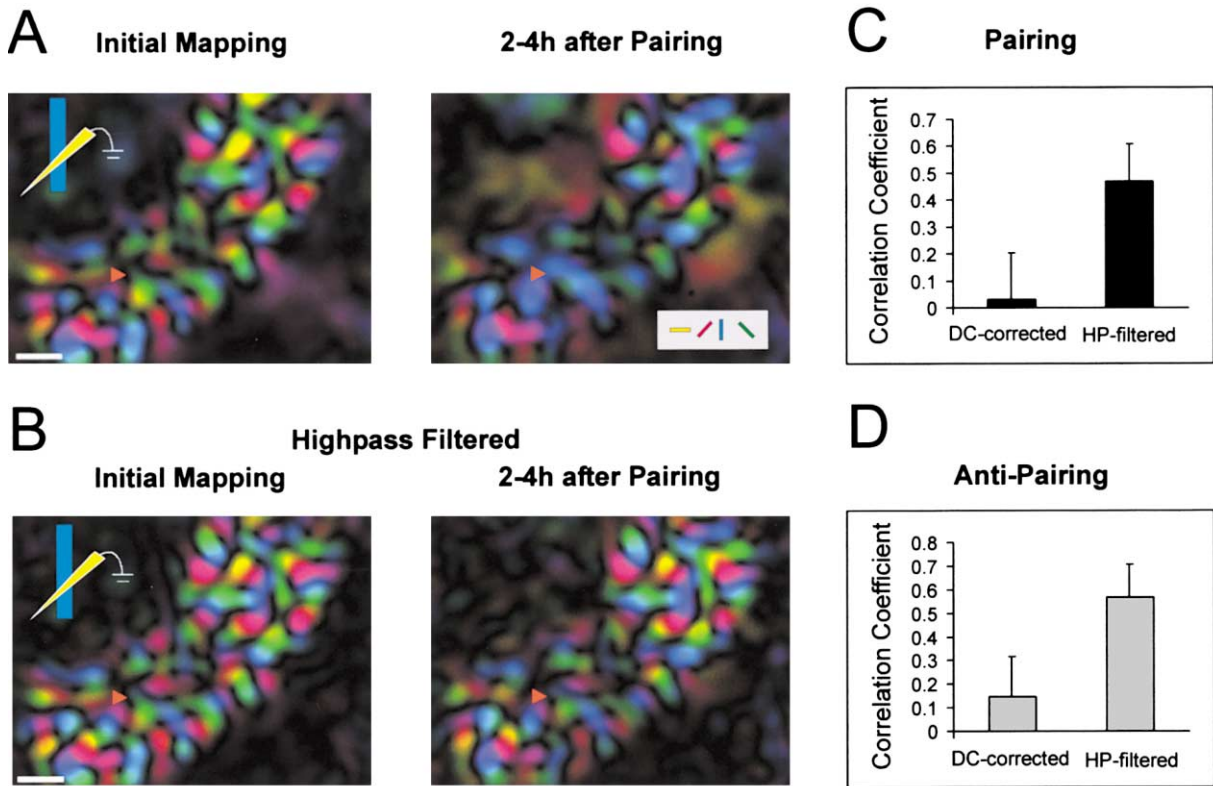


Figure 6. Stability of the General Structure of the Orientation Map

Polar maps computed from (A) DC shift corrected and (B) strongly high-pass filtered (boxcar: 1 mm) single-condition maps obtained before and after pairing. The high-pass filtering does not change the overall layout of the maps as indicated by the virtually identical layout of the two polar maps before pairing. Since the plastic effects are on a scale 3-fold larger than orientation patches, the high-pass filtering extracts the change in orientation preference and leaves the orientation preference map obtained before and after pairing virtually unchanged. Without this filtering, a prominent orientation shift is visible. Scale bars: 1 mm. (C and D) The change in general map structure was quantified by correlating the single-condition maps with and without high-pass filtering at the site of stimulation for (C) pairing and (D) anti-pairing. The high correlation coefficient calculated after high-pass filtering indicates that the general map structure is not changed by (anti-)pairing.

Stability of General Structure of the Orientation Map

The results described above clearly show that prominent changes in orientation preference can be induced by a pairing protocol. Does this manipulation also lead to alterations of the general structure of the layout of orientation in the visual cortex? To address this question, we used a simple method to extract global changes in orientation preference from the maps: since the changes induced by pairing extend over cortical regions much larger than individual orientation domains (Figures 2A and 2E and Figures 4B and 4F), we applied a strong high-pass filter (1000 μm boxcar) to the single-condition maps. This filtering removes any large-scale variations in the activity maps, while it only marginally affects small structures, such as individual orientation domains, as illustrated by the polar maps computed after this high-pass filtering (Figure 6B). Comparing otherwise identical polar maps with and without this spatial filtering before pairing reveals that the layout of the maps is virtually insensitive to the high-pass filter (Figures 6A and 6B). Importantly, when comparing high-pass filtered polar maps before and after pairing, virtually no change in map layout can be detected, suggesting that the general map structure is left unaltered by the pairing. We quanti-

fied the stability of the general map structure by correlating the high-pass filtered single-condition maps obtained before and after the pairing near the site of stimulation (Figures 6C and 6D). The correlation is not significantly different from control regions further away from the stimulation site ($p = 0.09$, Student's *t* test). Without high-pass filtering, however, the correlation is significantly reduced ($p = 0.02$, Student's *t* test). Thus, despite pronounced shifts in orientation preference, the observed changes seem to be only superimposed over an otherwise stable structure of the orientation layout.

Discussion

Plasticity and development of functional maps in the visual cortex have been studied extensively by examining the effects of long-term alterations of the visual input like monocular deprivation (Wiesel and Hubel, 1963; Shtatz and Stryker, 1978; Antonini and Stryker, 1993) or stripe rearing (Blakemore and Cooper, 1970; Hirsch and Spinelli, 1970; Freeman and Pettigrew, 1973; Sengpiel et al., 1999) on the cortical architecture. While a great deal of information on plasticity of cortical maps has been obtained with these studies, rearing experiments have the disadvantage that they do not allow strict con-

control over the stimulus conditions. Relatively precise measurements of determinants of synaptic plasticity in vivo with natural stimuli have only been obtained on the single-cell level in pairing paradigms using stimulation with potassium or pharmacological agents (Fregnac et al., 1988; Fregnac and Shulz, 1999; Shulz et al., 2000). However, with these paradigms it is not possible to assess detailed temporal aspects of the stimulation, like the impact of the relative timing of pre- and postsynaptic activation on the direction of synaptic changes (Debanne et al., 1994; Markram et al., 1997; Bi and Poo, 1998; Debanne et al., 1998; Zhang et al., 1998; Feldman, 2000). In the present study, we have employed a paradigm which made it feasible to induce plasticity on the level of cortical maps under well-controlled stimulation and recording conditions: we used short visual and electrical stimuli in combination with optical imaging of intrinsic signals.

This pairing paradigm is capable of inducing long-lasting shifts in orientation preference in cat visual cortex. While single-cell in vivo studies are essentially restricted to the measurement of plastic changes in the range of an hour (Fregnac et al., 1988; Fregnac et al., 1992), we were able to observe pairing-induced effects on the basis of cortical maps for periods up to 24 hr. Furthermore, in contrast to rearing experiments, we were able to record the orientation preference maps before and after the pairing both electrically and optically. Thence, a more accurate control and interpretation of experimentally induced neuronal response changes become feasible. Additionally, we found that on a millisecond timescale the relative timing between visual and electrical stimulation controls the direction of plasticity within the orientation preference maps. Moreover, our results show that the magnitude of this form of plasticity is strongly reduced at pinwheel centers in comparison to the pinwheel surround.

Pairing and Antipairing

We have shown that orientation preference can be shifted strongly by stimulating the visual cortex first with natural input and immediately afterwards electrically. This type of stimulation can lead to long-lasting changes, and it is analogous to a pairing paradigm, which combines presynaptic stimulation and subsequent postsynaptic depolarization of a single neuron, as it is used in single-cell studies for the induction of synaptic long-term potentiation (LTP; Markram et al., 1997; Zhang et al., 1998; Feldman, 2000). In our experiments, the visual stimulus activates cortical neurons via synaptic potentials, while the electrical stimulus elicits action potentials in cortical neurons, mainly by direct depolarization at the axon initial segment (Rattay, 1998). In contrast, fibers of passage or dendritic trees contribute only little to the activation of cortical cells by electrical stimulation (Rattay, 1998). Therefore, it becomes possible to control the relative timing of presynaptic stimulation and postsynaptic activation by altering the interval between visual and electrical stimulation: given that the electrical stimulus is applied after visually evoked signals arrive within the cortex, postsynaptic activation caused by electrical stimulation is likely to follow activation of presynaptic inputs by the visual stimulus.

In contrast to single-cell studies, not only excitatory but also inhibitory circuitry will be recruited by the stimulation and can influence the pairing effect. The strong shunting inhibition caused by the visual stimulus (Borg-Graham et al., 1998) and the electrical stimulation (Chung and Ferster, 1998) are likely to sharpen the timing relation between the visual and electrical stimulation for pairing and antipairing, respectively.

The hypothesized timing relations, however, will not hold precisely for all neurons but will only be statistically valid for neurons around the stimulating electrode. Therefore, we employ optical imaging which samples the neuronal response from many cells; thence it is suited to detect potential population changes with high variability on the single-cell level.

Our results indicate that if the visual (presynaptic) stimulus precedes the electrical (postsynaptic) stimulus the response to the visual stimulus is enhanced, while it is reduced if electrical precedes visual stimulation. A straightforward interpretation of these results consistent with single-cell studies (Debanne et al., 1994) is that the (anti-)pairing paradigm alters synaptic strength within excitatory pathways: accordingly, pairing strengthens excitatory synapses, which contribute to the generation of orientation preference, while antipairing depresses those synapses. Cortical orientation selectivity, however, is most likely generated by an interplay of excitatory and inhibitory networks (Sillito, 1975; Eysel et al., 1998; Shevelev et al., 1998). Therefore, changes in the inhibitory network could also contribute to the observed shift in orientation preference. It has been shown in slice recordings that GABAergic synapses can also undergo LTP (Komatsu and Iwakiri, 1993) and LTD (McLean et al., 1996), but spike time-dependent plasticity has not been investigated at inhibitory synapses. It is therefore difficult to determine to what extent synaptic changes in inhibitory pathways contribute to the pairing-induced effects seen in our experiments.

Our data suggest that spike time-dependent plasticity can influence activity-dependent refinement of cortical maps. Modeling studies (Song et al., 2000) have shown that spike time-dependent learning enhances short latency inputs and depresses longer latency inputs. In the case of orientation preference, this could lead to a sharpening of the orientation tuning, because nonoptimal synaptic inputs to a weakly orientation-selective neuron will become reduced over time.

Distribution of Plasticity

To unravel the impact of synaptic changes on cortical network activity, it is necessary to pinpoint the site of changes within the functional network. On the one hand, this facilitates the interpretation of the current results; on the other hand, it might further our understanding of the development of cortical orientation selectivity.

Cortico-Cortical but Not Thalamo-Cortical Synapses Are Changed

Our electrophysiological data imply that changes occur predominantly at cortico-cortical but not at thalamo-cortical synapses in layer 4, since layer 4 neurons do not shift their orientation preference, while layer 2-3 cells and, even more pronounced, cells in layers 5 and 6 exhibit strong shifts. The interocular transfer of monocular

lar pairing supports this conclusion: if the observed plasticity were mainly due to thalamo-cortical synapses, one would expect much smaller transfer to the unpaired eye.

These results are in line with studies on the layer-specific distributions of the effects of brief monocular deprivation, in which it was shown that changes are observed first in the upper layers and not in layer 4 (Kossut et al., 1983; Kossut and Singer, 1991; Trachtenberg et al., 2000). The difference found in these studies between granular and extragranular layers with respect to the shift in ocular dominance could in principle be an effect of the difference in the proportion of binocular cells in these layers: a number of studies indicate that binocular cells are more prone to undergo shifts in ocular dominance than monocular cells. It has been shown, for example, that the ocular dominance columns of the deprived eye shrink mainly in binocular zones (Crair et al., 1997). Moreover, it has been reported that strabismic cats, which have fewer binocular cells (Maffei and Bisti, 1976), are less sensitive to monocular deprivation (Mustari and Cynader, 1981) and recover less readily from prolonged monocular deprivation (F. Sengpiel, personal communication). Therefore, it could have been argued that the lower number of binocular cells in layer 4 might cause layer 4 to be more resilient to ocular dominance shifts.

Our findings suggest, however, that the layer-specific potential to undergo plasticity is not due to differences in the number of binocular cells between layers. Since orientation selectivity, in contrast to ocular dominance, is more or less similar across cortical layers in the cat (Berman et al., 1982), a similar argument cannot explain the resistance of layer 4 cells to pairing-induced shifts in orientation preference. Therefore, the lack of plasticity in layer 4 neurons is more likely to be due to layer-specific critical periods (Daw et al., 1992; Kirkwood et al., 1995; Kirkwood et al., 1997; Sermasi et al., 1999).

Another potential explanation for the relatively small effects observed in layer 4 is that layer 4 cells respond to the visual stimulus about 15 ms earlier than neurons in the extragranular layers (Figure 1A). Therefore, the delay between the visual and the electrical stimulus might be too large for these cells to exhibit significant shifts in orientation preference.

Equal Expression of Orientation Shift across All Orientation Domains

We did not detect any significant differences in the degree of shifts between different orientation domains. This was surprising because *prima facie* one would expect that the shift in domains of the paired orientation should be smaller, since neurons in these domains are already tuned to the paired orientation and thus cannot shift their orientation preference any further. A number of reasons might account for this equal distribution: first, since we have used only four different orientations as stimuli, the orientation preferences of neurons within the optically imaged domains of the paired orientation span a range of $\pm 22.5^\circ$. In addition, even at a given location in the visual cortex, orientation preference varies by about 15° (Albus, 1975; Murphy and Sillito, 1986; Hetherington and Swindale, 1999). Moreover, the visual stimulus used in our pairing paradigm most strongly activates domains of the paired orientation. Therefore, cells in these domains might be most sensitive to pair-

ing-induced shifts. Finally, a change in orientation preference measured by optical imaging can either be caused by an actual shift in orientation preference or by a sharpening of the orientation tuning for the paired orientation only. Taken together, these factors could lead to the observed homogeneous distribution of the pairing effect across all orientation domains.

Shift in Orientation Preference Is Reduced at Pinwheel Centers

While the degree of plasticity does not vary between different orientation domains, we observed a significant variation between pinwheel center regions and the pinwheel surround: the most pronounced changes in orientation preference occurred within the pinwheel surround, while significantly smaller shifts were detected near pinwheel centers. This provides direct evidence that plasticity is reduced at the site of pinwheels and other regions of rapid changes in the orientation preference map. This variation could be due to variations in the distribution of plasticity-related molecules across the visual cortex (Trepel et al., 1998; Kojic et al., 2000). It has been found that NMDA receptors are concentrated in the transition zone between ocular dominance columns (Trepel et al., 1998). Since the centers of ocular dominance columns statistically colocalize with pinwheel centers (Crair et al., 1997; Hübener et al., 1997), the NMDA receptor density—and thus possibly plasticity—is expected to be reduced at pinwheel centers. A similar stability of pinwheel centers has been inferred from a recent monocular deprivation study (Crair et al., 1997). Crair et al. found that the small cortical regions maintaining their responsiveness to the deprived eye colocalized with pinwheel centers. In addition to an intrinsic specialization like the suggested NMDA receptor distribution causing the stability of pinwheel centers, Crair et al. speculated that differences in circuitry might contribute to this stability. In fact, very recently, it has been found that the lateral spread of horizontal connections is relatively small at pinwheel centers compared to their surround (Yousef et al., 2001).

Other explanations, e.g., differences in receptive field properties between pinwheel center and pinwheel surround, are less likely because neurons at pinwheel centers are equally selective for orientation as neurons in the pinwheel surround (Maldonado et al., 1997). Additionally, we excluded that the neurons' ability to shift their orientation preferences depends on a neighborhood of similar orientation preference: the strength of plastic changes within the pinwheel surround is not significantly correlated with the similarity index.

Therefore, the most plausible candidate mechanism to explain the differences between pinwheel centers and surround are variations in the expression of plasticity-related molecules. However, to validate this hypothesis further, more molecularly oriented studies will be needed.

In a recent study, Dragoi et al. (2001) have analyzed adaptation-induced changes in orientation preference across the cortical surface. They found that neurons near pinwheel centers showed stronger shifts in preferred orientation than neurons in the pinwheel surround. This finding seems to be at odds with our observation of smaller shifts near pinwheel centers. However, there are important conceptual differences between the

two studies, as well as—most likely—differences in the underlying mechanisms: Dragoi and colleagues looked at the effects of adaptation in the visual cortex of adult cats (generally leading to reduced responses to the stimulus presented during adaptation over a time course of a few minutes), while we have studied the effect of pairing-induced plasticity in young animals (resulting in overall enhanced responses to the paired orientation over a time course of many hours).

Stability of Orientation Preference Maps

Stripe rearing studies (Blakemore and Cooper, 1970; Hirsch and Spinelli, 1970; Freeman and Pettigrew, 1973; Sengpiel et al., 1999) have suggested that specific visual environments can cause quantitative alterations of the layout of orientation domains. The most recent of these studies (Sengpiel et al., 1999) explicitly suggests that the general map structure remains unaltered. However, in none of these studies a direct comparison between the maps before and after induction of shifts in orientation preference was carried out. Comparing the orientation maps obtained in our study before and after pairing revealed clear shifts in orientation preference in large regions of the cortex. To distinguish between mere shifts in orientation preference and change in general structure of the layout, we employed a strong high-pass filter to remove any large-scale changes in orientation preference from the maps and thus demonstrated that the general structure of the maps is preserved despite strong shifts in orientation preference.

In the face of the prominent changes in orientation preference, one might wonder why the general structure of orientation preference maps is so rigid. Our finding that plasticity is inhomogeneously distributed within the cortex provides two possible mechanisms contributing to the observed stability. First, pinwheel centers are less sensitive to the induction of plasticity. Therefore, while the centers of orientation domains can shift their orientation preference, pinwheel centers limit this shift and thus preserve the overall structure of the map. Second, the layer-specific distribution of plasticity can further help to reduce shifts in orientation preference in our paradigm: thalamo-cortical connections have been shown to play a major role in determining orientation preference (Chapman and Stryker, 1993; Reid and Alonso, 1995; Ferster et al., 1996), and we have provided evidence that these connections are unlikely to undergo a pairing-induced change. Therefore, the relative stability of these connections will also limit potential shifts of the orientation preference map and help to maintain its general structure.

Experimental Procedures

The experiments were performed in 8- to 11-week-old kittens; all procedures were carried out in accordance with local government rules and the guidelines of the Society for Neuroscience.

Imaging

Anesthesia was induced with an i.m. injection of ketamine (20–40 mg/kg) and xylazine (2–4 mg/kg). Animals were tracheotomized and artificially ventilated (60% N₂O, 40% O₂, 1.5% halothane during surgery, 0.7%–1.0% during imaging, and 0.4% during pairing). ECG, EEG, end-tidal CO₂, and rectal temperature were monitored continuously. After the EEG recording had been started, the animals were

relaxed with gallamine triethiodide (Sigma, 10 mg/h/kg) added to an infusion of 5% glucose (80%), 0.9% saline (20%), atropine, and corticosteroid. The skull was opened over areas 17 and 18, and the dura was removed. An oil-filled chamber with a small rubber-sealed opening for electrode insertion (Grinvald et al., 1999) or agarose flattened with a coverglass were used to stabilize the cortex for simultaneous imaging and electrical stimulation. The eyes were retracted and focused onto a monitor (Mitsubishi-pro 2020) at a distance of 40 cm, with gas-permeable contact lenses. Visual stimuli were produced by a stimulus generator (VSG Series Three, Cambridge Research Systems, Rochester, UK). Stimuli consisted of high-contrast sine-wave gratings of four different orientations (0°, 45°, 90°, or 135°) and two spatial frequencies (0.2–0.3 and 0.6–0.8 cycles/degree), which drifted back and forth at an angular velocity of 1.5 cycles/degree. In some cases, computer-controlled eye shutters were used for monocular stimulation.

For optical imaging, the cortex was illuminated with light of 707 nm. Images were captured using a cooled slow-scan CCD camera (ORA 2001, Optical Imaging, Germantown, NY), focused about 700 μm below the cortical surface. Between five and ten frames of 600 ms duration were collected during each 3–6 s stimulus presentation, followed by a 9 s interstimulus interval during which the next stimulus was presented stationary.

Pairing Procedure

Flickering gratings of low spatial frequency (0.2–0.3 c/degree) were combined with electrical stimulation in area 17. A short visual stimulus (three frames = 21 ms, followed by 123 ms blank time) was used to evoke a temporally constrained neuronal response in the visual cortex. For each visual stimulus, one brief electrical pulse (60 μA, 200 μs duration) at a fixed latency was applied through the tip of a tungsten electrode positioned ~300 μm below the cortical surface. The paired electrical and visual stimulation was repeated at 7 Hz for 3 s. After a blank interval of 3 s, a grating of the orthogonal orientation was presented in the same fashion but without electrical stimulation. This pairing scheme was continued for 3–4 hr (Figures 1B and 1C).

The size and position of the cortical region activated by the electrical stimulus was determined by imaging the response to electrical stimulation alone (Figure 1D). To ensure that neurons were electrically and visually stimulated during pairing, we also imaged the cortical response during this time.

To investigate the effect of the relative timing between the electrical and the visual stimulus in the millisecond time range, we used two latencies for the electrical stimulus: either 65 ms (visually evoked precedes electrically evoked cortical activity; “pairing”) or 35 ms (electrically evoked precedes visually evoked cortical activity; “anti-pairing”) after onset of the visual stimulus.

Analysis of Functional Maps

Single-condition responses (averages of 48 to 96 trials) were divided by the cocktail blank, i.e., by the averaged response to all stimuli. Twelve-bit digitized camera data were range-fitted such that for the isoorientation maps the 1.5% most-responsive pixels (least-responsive pixels) were set to black (white). The signal amplitude was displayed on an 8-bit gray scale. Some images were postprocessed with a “blood vessel extractor” algorithm to remove artifacts caused by large blood vessels (Schuett et al., 2000). For further analysis, all images were low-pass filtered with a Gaussian (45 μm) and DC shift corrected by subtracting from each image its mean pixel value. Single-condition maps presented in the figures were only DC shift corrected, in order to allow for the estimation of pixel noise by eye. Maps obtained before and after the pairing were aligned using the blood vessel pattern.

The change in neuronal response to a particular visual stimulus was quantified in two ways. To evaluate changes in the overall optical response, we computed the difference between the means of pixel values (reflectance signal) before and after pairing. To account for the variance of the signal amplitude, we normalized the reflectance signal by the standard deviation of the signal amplitude over space for each animal. Amplitude of the intrinsic signal was defined as the mean standard deviation over space of the intrinsic signal across all maps. As a second more intuitive measure, we

thresholded each single-condition map at the mean response and measured the size of the area below the mean. The areal measures were normalized to yield a sum of 1 over all orientations.

These measures were computed in different regions of interest centered on the peak of the optical response to the electrical stimulation in order to determine the magnitude of the pairing effect at different distances from the stimulating electrode.

In addition, we measured the area of induced changes in different regions of functional maps by using masks obtained from the orientation and spatial frequency maps. The mask for one type of orientation domain was obtained by determining those cortical regions where the response to this orientation exceeded the response to all other orientations. The masks for low and high spatial frequency preference were computed by thresholding the spatial frequency map at the mean of the map taken during presentation of a blank screen. Pinwheel center regions, i.e., cortical regions with rapid changes of orientation preference, were determined by computing the angular derivative of the orientation preference map and thresholding at its mean. The inverse was used as a mask for the pinwheel surround.

Electrophysiology

In one animal, in addition to optical imaging, we carried out electrode recordings before and after pairing at approximately the same cortical locations. We recorded quantitative orientation and direction-tuning curves of multiple single neurons recorded on one electrode and discriminated by their waveforms (Brainware, Oxford, UK). Smooth tuning curves were fitted to the data points based on Fourier analysis (Wörgötter et al., 1987), and preferred orientation and half-width of tuning at half-height were determined for these curves.

To measure response latencies in area 17, we recorded the responses to brief (21 ms) presentations of optimally oriented gratings. A neuron's latency was determined by first detecting the peak of the response and then searching backward in time for the first bin (5 ms bin-width) with a spike count less than one standard deviation above the average baseline value. The delay of the following bin was defined as this neuron's latency relative to the onset of the visual stimulus.

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