Activity-Dependent Regulation of Receptive Field Properties of Cat Area 17 by Supervised Hebbian Learning

Yves Frégnac, Daniel E. Shulz

Equipe Cognisciences, Institut Alfred Fessard, CNRS, Avenue de la Terrasse, 91198 Gif sur Yvette, France

Received 14 April 1999; accepted 10 May 1999

ABSTRACT: **Most algorithms currently used to model synaptic plasticity in self-organizing cortical networks suppose that the change in synaptic efficacy is governed by the same structuring factor, i.e., the temporal correlation of activity between pre- and postsynaptic neurons. Functional predictions generated by such algorithms have been tested electrophysiologically in the visual cortex of anesthetized and paralyzed cats. Supervised learning procedures were applied at the cellular level to change receptive field (RF) properties during the time of recording of an individual functionally identified cell. The protocols were devised as cellular analogs of the plasticity of RF properties, which is normally expressed during a critical period of postnatal development. We summarize here evidence demonstrating that changes in covariance between afferent input and postsynaptic response imposed during extracellular and intracellular conditioning can acutely induce selective long-lasting up- and down-regulations of visual responses. The functional properties that could be modified in 40% of cells submitted to differential pairing protocols include ocular dominance, orientation selectivity and orientation preference, interocular orientation disparity, and the relative dominance of ON and OFF responses. Since changes in RF properties can be induced in the adult as well, our findings also suggest that similar activity-dependent processes may occur during development and during active phases of learning under the supervision of behavioral attention or contextual signals. Such potential for plasticity in primary visual cortical neurons suggests the existence of a hidden connectivity expressing a wider functional competence than the one revealed at the spiking level. In particular, in the spatial domain the sensory synaptic integration field is larger than the classical discharge field. It can be shaped by supervised learning and its subthreshold extent can be unmasked by the pharmacological blockade of intracortical inhibition.** © 1999 John Wiley & Sons, Inc. J Neurobiol 41: 69–82, 1999

Keywords: **synaptic plasticity; longterm potentiation (LTP); longterm depression (LTD); adult plasticity; critical period**

Combining experimental and theoretical studies of dynamic changes in cortical function requires the choice of appropriate levels of description. One approach is to go from simple phenomenological rules to complex mechanistic scenarios of synaptic and functional plasticity, and evaluate progressively how each level of complexity can be added to account for the adaptive behavior of the overall network. The present chapter will review our "theory-down-to-experiment" efforts in evaluating the adequation of a simple activity-dependent algorithm of synaptic plasticity in predicting the functional changes of area 17 receptive field properties known to occur during a critical period of postnatal development (Wiesel, 1982; review in Frégnac and Imbert, 1984).

Correspondence to: Y. Frégnac
Contract grant sponsor: CNRS
Contract grant sponsor: PROGRES-INSERM
Contract grant sponsor: AFIRST
Contract grant sponsor: GIS-Cognisciences
Contract grant sponsor: HFSP; contract grant number: RG0103/1998-B

Unsupervised Hebbian processes are thought to participate very early in the development and self-organization of the thalamo-cortical relays (review in Shatz, 1996). Indeed, it is well established that the intrinsic synchronous bursting activity which arises prenatally from the retina before rods and cones are even formed ("dark discharge") exerts a structuring influence on the developing retinofugal pathway. This correlated input takes the form of spatially organized calcium-dependent waves of activity which spread intermittently in random directions across the whole retina. These waves could provide the local correlations necessary for the topographic refinement of retinal projections to the lateral geniculate nucleus (LGN), and perhaps be instrumental in the initial segregation of geniculate afferents in cortex. It is only after the first week of postnatal life, which corresponds in the cat to eye opening and the establishment of mature photoreceptor responses, that this intrinsic pattern-generating mechanism gives way to correlated inputs under the guidance of vision. Eventually, similar activity-dependent rules will apply to the development of ocular dominance bands and intracortical connectivity (review in Callaway and Katz, 1992, Goodhill and Löwel, 1995). One may conclude that the grouping and sorting out of fibers, morphological tuning in the spatial distribution of the terminal boutons of intrinsic and extrinsic axons, functional expression and possible silencing of synapses all could be, at some stage of postnatal development, under the influence of temporal correlation between presynaptic fibers converging onto the same target, or between pre- and postsynaptic partners.

An experimental approach used to demonstrate the functional implication of Hebbian-like mechanisms in visual cortical plasticity relies on the study of the effects of environmental manipulations on feature representation at the cortical level (review in Frégnac and Imbert, 1984). The plasticity protocols reviewed here focus on the consequences of Hebbian rules at the individual cell level. Rather than submitting the cortical network to an environmental "surgery" of the whole visual field (global input clamp mode), we chose to restrict the extent to which cortical activity is modulated to the immediate environment of the recorded cell (local pertubation mode). In the latter case, the experimenter provides a supervision signal which will simulate locally the functional effects of anomalous visual experience during critical periods of development. In collaboration with Bienenstock, Thorpe, Debanne, and Baranyi, we developed over the past 10 years a series of electrophysiological supervised Hebbian paradigms which have allowed us to produce, while recording from a single cell, functional changes analogous to those classically described during visual cortical development (Debanne, et al., 1995, 1998, Frégnac and Debanne, 1993, 1994a,b; Frégnac, et al., 1988, 1992; Shulz, et al., 1993a; Shulz and Frégnac, 1992).

THEORETICAL FRAMEWORK FOR SYNAPTIC PLASTICITY

The major contribution of Hebb to the field of plasticity was to introduce the new concept of cellular assembly, an activity reverberation in "a set of closed pathways" (Hebb, 1949). The neurophysiological postulate of Hebbian synapses was proposed as a way of reinforcing coupling between coactive cells, and thus of growing assemblies. It carries a specific prediction: A period of maintained temporal correlation between pre- and postsynaptic activity will lead to an increase in the efficacy of excitatory synaptic transmission. A symmetric version of Hebb's postulate was later proposed for the case of inhibitory synapses, where functional coupling can be increased by reducing the strength of afferent inhibitory synapses activated at the same time as the postsynaptic cell (Stent, 1973). These two forms of hypothetical plasticity are often confounded in formal models of self-organization of cortical assemblies, where ideal coupling functions are allowed to vary between boundaries of opposite sign (i.e., Bienenstock, et al., 1982). The biological counterpart is to conclude that the synaptic gain defined by modelers represents the balance of dual parallel excitatory and inhibitory pathways [Fig. 1(A)], whose efficacies in transmission are regulated synergistically (review in (Frégnac, 1995; Frégnac and Bienenstock, 1998).

The synaptic gain "divergence" problem caused by a straightforward application of Hebb's principle was solved by modelers by using various rules of normalization which require, in addition to Hebb's rule, depression of the gain of other competing synapses. To account for the striking differences of the effects of monocular versus binocular deprivation in the plasticity of cortical ocular dominance (Wiesel and Hubel, 1963), Stent assumed a selective decrease in the efficacy of synaptic transmission of afferent inputs which were inactive at the time when the postsynaptic neuron was being driven under the influence of another set of inputs (Stent, 1973). Despite the lack of evidence for the proposed biophysical mechanism, Stent first introduced the hypothesis of a threshold for plasticity dependent on the local postsynaptic state at the synapse. Putative mechanisms, later suggested on the basis of cortical effects produced by monocular de-

privation or unbalanced electrical stimulation of optic nerves associated with intraocular tetrodotoxin (TTX) treatment protocols (Rittenhouse, et al., 1999; Tamura, et al., 1992), share similarities with those proposed to account for cross-depression effects in the CA1 field and the dentate area of the hippocampus (Debanne et al., 1994; Levy and Steward, 1983). Both types of data suggest that uncorrelated residual spontaneous activity in the visually deprived pathway might lead to both heterosynaptic and homosynaptic forms of synaptic depression. It remains to be established, in the case of ocular dominance plasticity, whether the involved homosynaptic form of depression is also associative, and depends on the decaying traces of transient calcium bursts evoked by input from the open eye, that may have preceded by a few tens or hundreds of milliseconds the spontaneous activity arising from the closed eye ("dark discharge").

Thus, most algorithms that are currently used to model synaptic plasticity in the developing or adult cortex include both synaptic potentiation and depression rules. They may be summarized by the same general equation in which the change of synaptic efficacy with respect to time is equal to the product of a presynaptic term and a postsynaptic term (review in Frégnac, 1995; Frégnac and Shulz, 1994). The so-called covariance hypothesis introduced by Sejnowski (1977) and applied in visual cortex by Bienenstock et al. (1982) replaces the pre- and postsynaptic terms by the departure of instantaneous pre- and postsynaptic activities from their respective average values over a certain time window. In addition to the Hebbian and Stent rules, the covariance hypothesis predicts an additional form of homosynaptic depression, when presynaptic activity is associated with repetitive failure in synaptic transmission (Blais et al., 1999). *In vivo* tests of such a situation have been carried out in the developing mammalian visual cortex (Bear et al., 1990; Frégnac et al., 1988; Reiter and Stryker, 1988).

In their simplest form, these plasticity algorithms are symmetric in time; i.e., no strict temporal ordering is required between the onset of pre- and postsynaptic activation when they temporally overlap. The temporal contiguity requirement of Hebbian potentiation in cortex was first estimated in the $\pm$50-ms range, both *in vivo* (Baranyi and Feher, 1981, Wigström and Gustafsson, 1985) and *in vitro* (Frégnac et al., 1994a; Harsanyi and Friedlander, 1997). However, recent work using dual patch recordings *in vitro* suggests an even tighter temporal contingency rule (10-ms range) and a temporal order between the test PSP and the backpropagating spike deciding whether potentiation or depression occurs (Markram et al., 1997).

MATERIALS AND METHODS

The rationale that we applied to implement the covariance plasticity rule is summarized in Figure 1. Opposite changes were imposed in the temporal correlation between two test sets of synaptic inputs on the one hand and the output signal of the cell on the other hand. Here, an external supervisor (i.e. the experimenter) helped the cell to respond to one input (S^+ pairing), and blocked the cell's response to another separable input (S^- pairing). The control postsynaptic activity was imposed in two ways: (a) for extracellular pairings, with the application of a iontophoretic current (in most cases, $<\pm$10 nA, which allowed the cell's activity to be recorded even during pairing (Frégnac et al., 1988; see also Andrew and Fagan, 1990) through the recording electrode (KCl 3 *M*, 10–20 *M*Ω); and (b) for intracellular pairings, by applying a brief pulse of depolarizing or hyperpolarizing current (in most cases $<\pm$3 nA for 50–200 ms) through the intracellular electrode k-methylsulfate (KMS, 50–70 MΩ). The common outcome predicted by the covariance hypothesis was that the relative preference between the two test stimulus features should be displaced towards that which had been paired with imposed increased visual responsiveness.

RESULTS

The differential pairing protocols presented in Figures 1 and 2 were considered cellular analogs of epigenesis since they reproduce functional changes occurring during development or following early manipulation of the visual environment: orientation-biased environment [Fig. 2(A)], monocular deprivation [Fig. 2(B)], optically induced interocular orientation disparity (data not shown), rearing restricted to a fixed phase and spatial frequency [Fig. 2(C)]. Surprisingly, the probability of inducing functional changes was comparable in the kitten during the critical period and in older kittens and adults, suggesting that the cellular potential for plasticity might extend well beyond the classical extent of the critical period. Local supervised learning procedures applied at the cellular level might bypass systemic control which normally blocks the expression of plasticity in the mature brain. However, the largest effects were induced in the youngest animals at the peak of the critical period. The major findings are detailed below.

Orientation Selectivity Plasticity

Early studies on the effects of visual exposure restricted to a fixed orientation (Blakemore and Cooper, 1970; Hirsch and Spinelli, 1970) showed the induc-

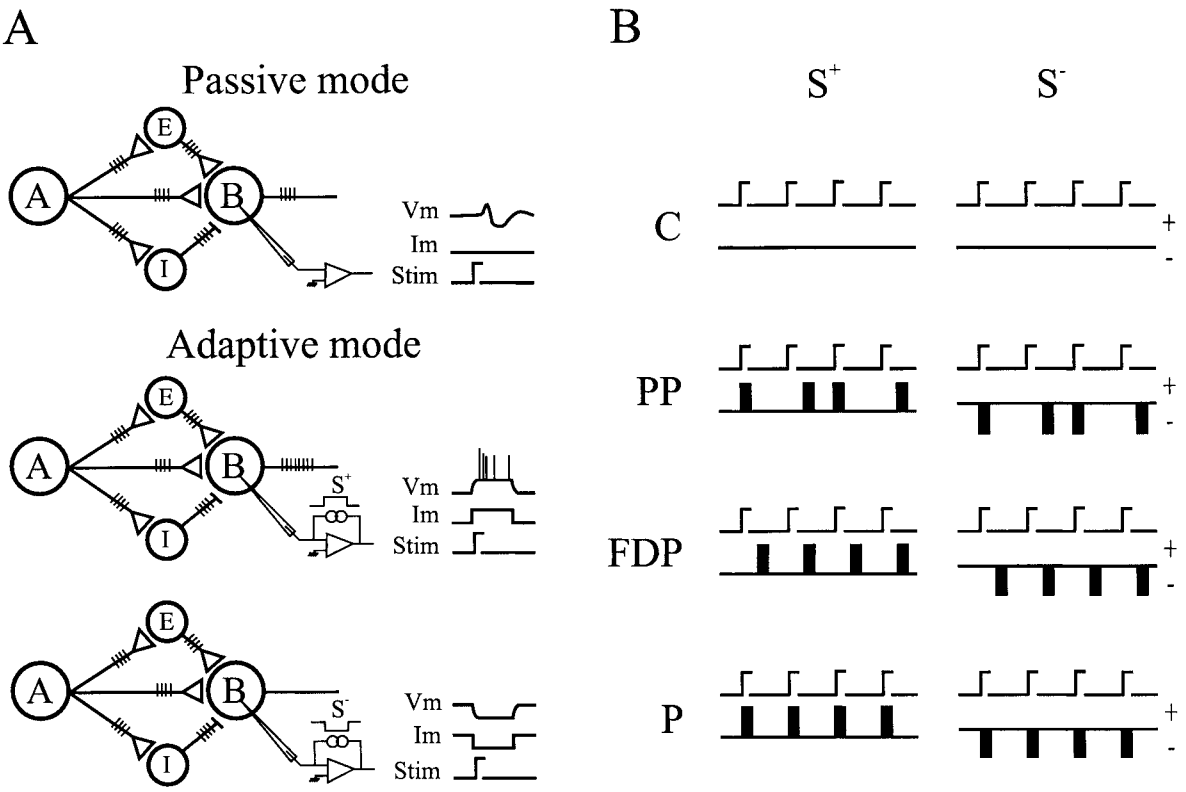

Figure 1 Supervised learning procedures applied to a local network involving mono- and polysynaptic connections. (A) Top: Passive mode of transmission. The functional coupling of two neurons, A and B, results from monosynaptic excitatory contacts and polysynaptic circuits [involving both excitatory (E) and inhibitory (I) interneurons]. Right: Intracellular voltage deflection Vm recorded in cell B in response to the visual or electrical stimulation of cell A (Stim). Bar density is proportional to the firing frequency of the considered pathway. Middle and lower: Adaptive mode of transmission. The temporal correlation between the afferent message and the postsynaptic state of the target cell (B) are directly controlled by the experimenter either by depolarizing (S^+ pairing, first row) or hyperpolarizing (S^- pairing, second row) the postsynaptic cell concomitantly with the activation of the input neuron (A). (B) Differential pairing protocols (C = control; PP = pseudopairing; FDP = fixed delay pairing; P = contiguous pairing). Left column: S^+ protocols. Right column: S^- protocols. For each condition, four repetitions of the test afferent stimulation (upper line) at a low temporal frequency (<0.3 Hz) are associated or not with iontophoretic or intracellular current pulses of a given polarity (±, filled rectangles on bottom line). The same elementary sequences for both current polarities are interleaved 5–50 times

tion of a significant bias in the cortical representation in favor of the orientation to which kittens had been exposed. Two different interpretations concerning the processes involved in these effects were proposed: namely, selective versus instructive mechanisms. However, in view of the inherent limitations of analysis based on the comparison of populations of neurons recorded in different animals, no definitive answer could be given. We applied our protocol of associative conditioning to demonstrate plasticity of orientation selectivity during the time of recording of single cortical cells (Frégnac, et al., 1988, 1992). The response of the recorded neuron was artificially reinforced during the presentation of a given orientation (S^+) and suppressed while presenting a different (but fixed) orientation (S^-) through the same eye [Fig. 2(A1)]. The quantification of the modifications in orientation tuning produced by pairing was used to measure the generalization of the effects to stimuli other than those used during the conditioning [Fig. 2(A2)]. A significant polar asymmetry favoring the S^+ region or even a displacement of the peak of preferred response toward the reinforced orientation was observed. These changes in tuning selectivity appear to be linked to the competitive imbalance imposed between the two orientations presented dur-

ing pairing: As a general rule and independently of their angular separation, a gain in responsiveness was observed around the positively reinforced stimulus, whereas a loss of competence was observed around the negatively reinforced one. However the amplitude of the orientation shift was related to the initial selectivity of the neuron: The probability of observing large changes in orientation preference was significantly higher in initially poorly oriented neurons than in already selective ones. Our findings were replicated in kitten (Greuel et al., 1988) and more recently in adult cat cortex (McLean and Palmer, 1998) using a pharmacological control of postsynaptic activity.

Ocular Dominance Plasticity

Unilateral eye closure by lid suture performed from the third postnatal week quickly produces a dramatic change in cortical binocularity; i.e., most visual cortical neurons respond exclusively to the open eye (Wiesel and Hubel, 1963). Binocular competitive interaction between visual inputs for dominance of central connections appears to be a major mechanism involved in the monocular deprivation effects at the cortical level. We simulated the effect of imbalance between the two eyes by alternately driving with moving stimuli the same cell to a high level (S^+) through one eye, and a low level (S^-) through the other eye, and studying the effects on ocular dominance after 15–80 imposed associations (Shulz and Frégnac, 1992). Figure 2(B) illustrates a case in which the spatiotemporal profile of the response to the reinforced eye—in addition to its magnitude—was altered: A new peak appeared as the result of an increase in responsiveness and was restricted to the previously unresponsive flank of the receptive field [delineated by arrows in Fig. 2(B)], where iontophoresis had been applied concomitantly with visual stimulation.

Interocular Orientation Disparity Plasticity

Another remarkable example of functional adaptation during the critical period comes from experiments imposing some degree of restriction in the binocular correspondence of monocular images (Shinkman and Bruce, 1977). Shinkman and colleagues extensively studied the cortical effects of rearing kittens with prisms which rotated the optical axis of the visual fields of the left and the right eye in opposite directions. Subsequent electrophysiological characterization of visual cortical receptive fields (RFs) analyzing the representation of the center of gaze showed that the distribution of the difference between the preferred orientation [interocular orientation disparity (IOD)] seen through each eye was centered on the disparity imposed by the prism. To test the activity dependence of such functional changes more directly, we developed a cellular analog of IOD plasticity (Shulz and Frégnac, 1992). During binocular viewing, the response of the recorded cell to the dichoptic presentation of two monocular orientated stimuli rotated symmetrically away from the preferred orientations of the two receptive fields—i.e., corresponding to a fixed orientation disparity—was artificially increased. Alternately, the response for an opposite orientation disparity was artificially decreased or even blocked. In agreement with the covariance hypothesis prediction, the relative IOD preference conditions shifted in most cases in favor of the positively reinforced disparity. Remarkably, in 50% of the modified cells this adaptive effect was only expressed under the binocular viewing condition, i.e. that imposed during pairing, whereas monocular evoked responses remained unchanged. This induced effect is reminiscent of behavioral state–dependent learning, where the change is expressed only if the context during which learning took place is recalled.

Spatial Organization of ON–OFF Responses

More recently, we developed an electrophysiological approach to test the possible role of neuronal coactivity in controlling the plasticity of the spatial ON–OFF organization of visual cortical RFs (Debanne et al., 1998). Iontophoresis was used alternately to boost the ON (or OFF) response to a high level of firing (S^+ pairing) and to reduce the opponent response (respectively OFF or ON) in the same RF position to a low level (S^- pairing). These differential pairings resulted in long-lasting changes of the ON versus OFF balance, which favored the response (ON or OFF) which had been paired with the high level of imposed activity. Modifications consisted mostly in the strengthening and/or weakening of short and long-latency responses (100–800 ms), and the amplitude change was on average half that imposed during pairing. In a few cells, the de novo expression of a suprathreshold response was induced for an initially ineffective visual stimulation. Most modifications were observed in the paired position and restricted to that sole region of the RF, suggesting that they likely result from selective changes in the transmission gain of the synapses which were activated during pairing. In a few cells, a fixed delay pairing procedure [FDP in Fig. 1(B)] was

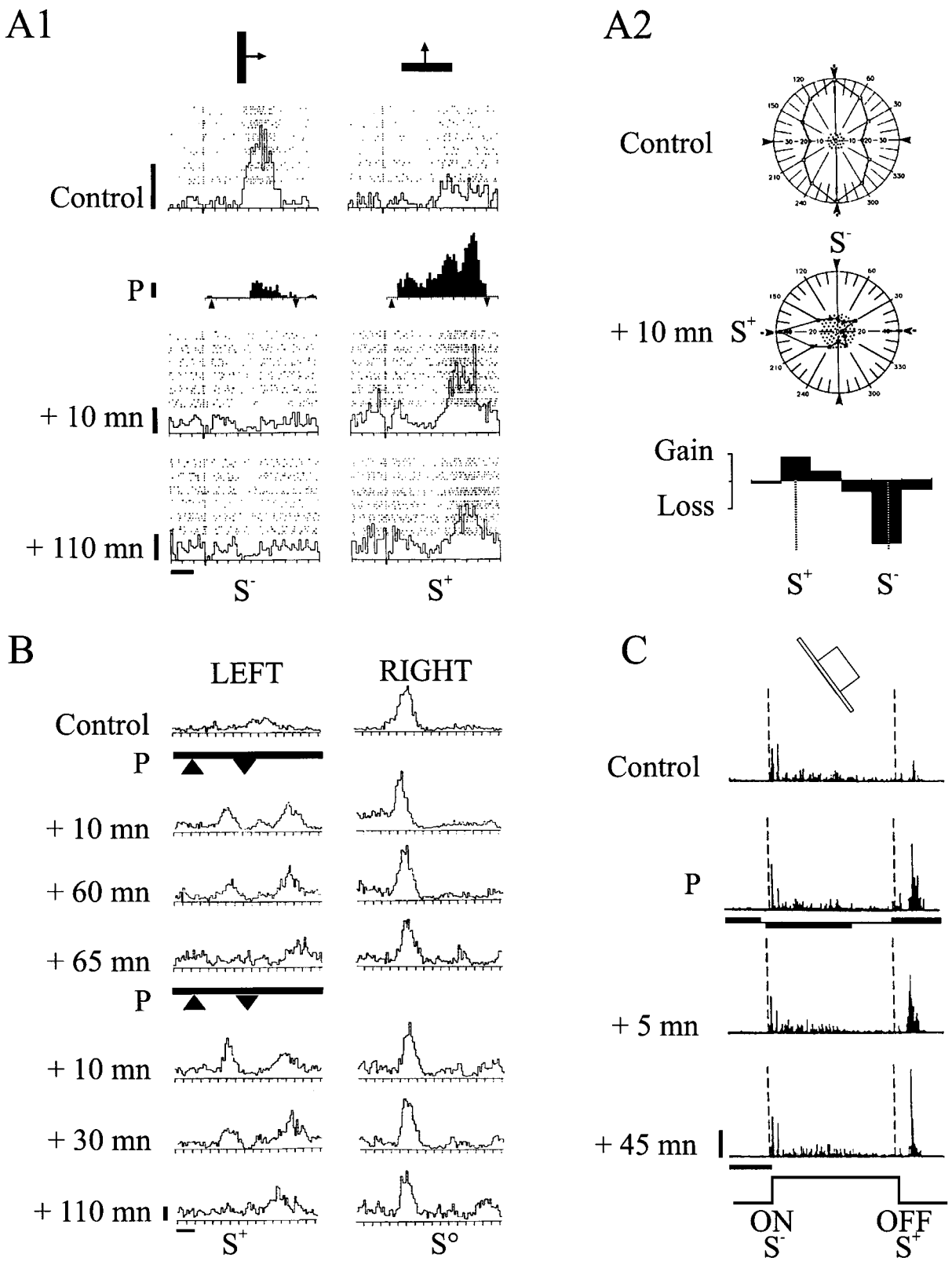
A1
Control
P
+ 10 mn
+ 110 mn
S-
S+
A2
Control
S-
+ 10 mn
S+
Gain
Loss
S+
S-
B
LEFT
RIGHT
Control
P
+ 10 mn
+ 60 mn
+ 65 mn
P
+ 10 mn
+ 30 mn
+ 110 mn
S+
S°
C
Control
P
+ 5 mn
+ 45 mn
ON
S-
OFF
S+

Figure 2

applied in which the iontophoretic current pulse application lagged behind the presentation or the end of the visual stimulus by a few hundred milliseconds (data not shown). Some of the conditioned cells retained for several tens of minutes a temporal pattern of activity with a phase lag reproducing that imposed during pairing. Our findings of Simple cells conditioned to become Complex were also corroborated by a recent study using a phase conditioning protocol (McLean and Palmer, 1998) in which the authors observed the induction of counterphased modulated responses to stimuli presented at the spatial phase which initially did not evoke a response ("null" phase).

For a Synaptic Basis of Functional Plasticity

Extracellular pairing protocols present strong limitations in their interpretative power at the mechanistic level, however, since we do not have access to the subthreshold synaptic events which may be modified by the pairing procedure. In addition, the iontophoretically induced changes in extracellular potassium levels, imposed to modulate the excitability of the conditioned cell, could also have affected presynaptic release and activity. To measure changes of visually evoked synaptic potentials directly, and thus interpret the functional changes in terms of plasticity

Figure 2 Long-lasting modifications of orientation preference (A), ocular dominance (B), and ON/OFF organization (C) of area 17 receptive fields following Hebbian supervised pairing protocols. (A1) Long-lasting change in the orientation preference during the critical period, in a cell recorded in area 17 of a visually deprived kitten (adapted from Frégnac et al., 1992). PSTHs represent visual responses to a moving bar for two different orientations (40 presentations each). Dot displays superimposed on the histograms represent responses for each individual trial. Left column: Responses for the initially preferred stimulus (vertical orientation). Right column: Initially nonreferred stimulus (horizontal orientation). Top to bottom: Evolution of the relative preference as a function of time before (Control), during differential pairing (P), and following pairing. During pairing (P, filled histograms, 60 associations), a positive current pulse (+3 nA) was applied during the sweep of the horizontal bar across the discharge field (arrowheads; S^+), and interleaved with a negative current pulse (−7 nA) when the vertical bar was presented (arrowheads; S^-). The visual response became respectively potentiated for the S^+ stimulus and depressed for the S^- orientation (+10 min). The effects were still present 110 mn after pairing. Calibration = vertical 5 ap/s, horizontal 1s and 1.5°. A2. Polar orientation tuning curves for the same cell were established before (Control) and after pairing (+10 min) using pseudorandom exploration sequences over a 360° range (steps of 30°), averaged over five runs. The normalized firing level observed for a given stimulus direction/orientation is given by the distance of each data point to the center along that radius. The mean spontaneous activity level is shown by the stippled area. The orientations used during pairing are indicated by the arrowheads and the S^+/S^- symbols. The lower graph represents the differences between the normalized tuning curves before and after pairing (folded on a 180° scale) expressed as gains and losses as a function of the orientation of the stimulus (calibration = ±20%). Following pairing, the cell changed its orientation preference by 90° and became tuned to the positively reinforced orientation. (B) Ocular dominance change induced in a 4.5-week-old, normally reared kitten (adapted from Shulz and Frégnac, 1992). PSTHs represent visual responses to stimulation of the left (left column) and the right (right column) eyes, before and after two pairings (thick lines). The increase of the visual response to the left eye (+40%), imposed during the first pairing (nine S^+ trials) was retained for 60 min. After extinction (+65 min), this effect was reinstated by a second pairing (imposing a 90% increase in firing during 24 S^+ trials), which was retained for 100 min. The response to stimulation through the unpaired (S°) right eye was unchanged. Calibration = vertical 10 ap/s, horizontal 1s and 1.5°. (C) Change of the ON/OFF balance in a Simple cell recorded in a 5-week-old kitten (adapted from Debanne et al., 1998). PSTHs represent the cell's response to the presentation (ON) and extinction (OFF) of an optimally oriented bar shown in a fixed position of the RF (schematized above). Before pairing (Control) a strong dominant ON response was observed. The pairing protocol consisted of 50 associations of a negative current pulse (−4 nA, 2000 ms) with the presentation of the test stimulus (ON: S^-), and of a positive current (+4 nA, 2000 ms) with the extinction of the same stimulus (OFF: S^+). The onset of the current pulses preceded the ON and OFF transitions of the visual stimulus by 100 ms. A significant reinforcement of the OFF response was imposed during pairing, whereas the negative current was ineffective in reducing the ON response. Five minutes after pairing, the OFF response was significantly potentiated. This effect lasted for at least 45 mn until the cell was lost. Calibration bars = horizontal 1s, vertical 10 ap/s.

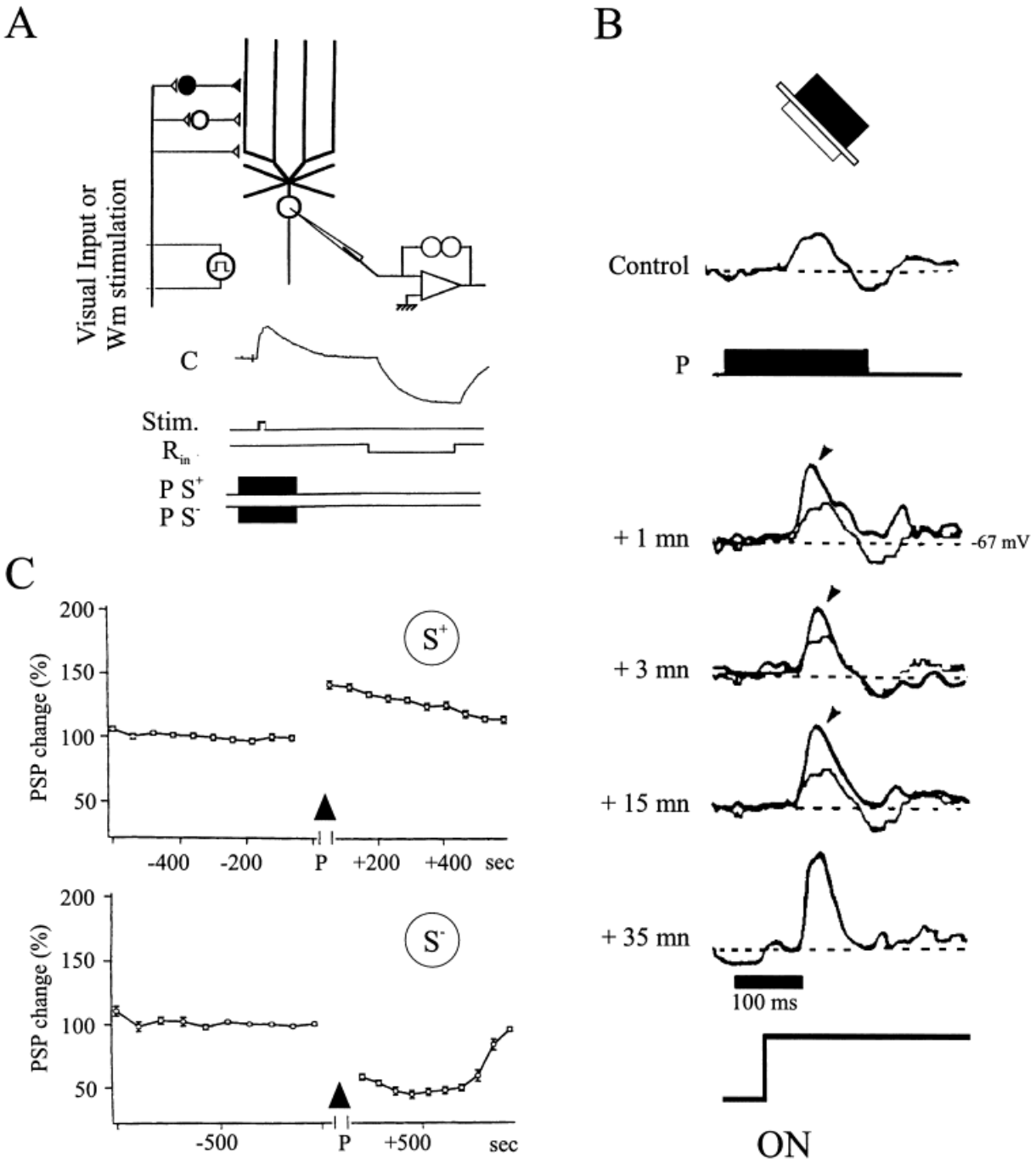

Figure 3 Effects of Hebbian supervised intracellular pairings. (A) Schematic representation of the electrophysiological experiment. Top: Intracellular recording of a visual cortical neuron and concomitant activation of afferent inputs [*in vitro* by white-matter electrical stimulation (WM) or *in vivo* by visual stimulation]. Dendrites and axons are represented by thick and thin lines, respectively, and somas and synapses by circles and triangles. The presynaptic input feeding the impaled target neuron is assumed to be mixed, monosynaptic, and polysynaptic through excitatory (open circles) and/or inhibitory interneurons (filled circles). Lower, from top to bottom: PSP recording as a function of time, in response to the test stimulation followed by an hyperpolarizing pulse (−0.1/−0.4 nA; delay 140 ms; duration 100 ms) to assess membrane resistance (Rin) values during the

of synaptic transmission, in collaboration with Baranyi, we made intracellular recordings and used direct current injection to control the postsysnaptic state of activation. This allowed a more selective pairing procedure during which the visually triggered PSP was temporally associated with concomitant depolarizing or hyperpolarizing current pulse injection into the target cell (Debanne et al., 1995, Frégnac et al., 1994b). Similar experiments were attempted *in vitro* in rat and kitten visual cortical slices by Frégnac in collaboration with Friedlander and colleagues (Frégnac et al., 1994a), in which the visual input was replaced by the electrical stimulation of optic radiations or layer II–III axons. In the majority of conditioned cells, both *in vivo* and *in vitro* the sign of the change (potentiation or depression) of the composite postsynaptic potential was predicted by the sign of the imposed change of the membrane potential during pairing (Fig. 3). The effects appeared associative, since they were not observed when the current pulse was applied unrelated to visual stimulation.

Unmasking Complexity in Simple RFs

The last series of experiments led us to propose that the spatial specificity of intracortical inhibition results in the shaping of spatially separate ON and OFF regions in Simple cells, which arises from an initially mixed convergence of excitatory ON and OFF inputs of the kind found in Complex cells. This schema predicts the unmasking of Complex-like synaptic potentials in Simple RFs during the blockade of GABAergic transmission. This view is in agreement with previous extracellular studies showing ON and OFF spiking responses through the entire RF of Simple cells during iontophoresis of bicuculline (Sillito, 1975; Pernberg et al., 1998; Murthy and Humphrey, 1999). In those experiments, however, it is not possible to conclude whether the effect was due to the blockade of $GABA_A$ receptors on the recorded cell or elsewhere. We tested in collaboration with Bringuier, these two possibilities directly by locally blocking GABAergic inhibition while recording intracellularly the subthreshold antagonist responses of Simple cells. Such experiments required intracellular recording and simultaneous juxtacellular iontophoresis with combined electrodes for periods of up to 3 h (Shulz et al., 1993b; in preparation). For each cell, the smallest effective current for the iontophoresis of bicuculline methiodide sufficient to antagonize the effects of the exogeneously applied GABA was first determined. This current level was then used to antagonize the endogenous GABA supposedly released during visual stimulation. In the example shown in Figure 4(A), before the application of bicuculline methiodide, the presentation of the stimulus in the OFF zone evoked a strong opponent response, corresponding to fast and slow hyperpolarizations [lower trace, third row from top in Fig. 4(A)]. Under bicuculline methiodide, the cell fired in bursts of action potentials which were tightly time-locked to both ON and OFF transitions of the stimulus whatever its position in the RF [second row, BIC 60 in Fig. 4(A)]. The early hyperpolarization was completely abolished for the opponent response and a depolarizing potential of up to 3.6 mV in amplitude was unmasked [upper trace, third row from top in Fig. 4(A)]. This EPSP reached firing threshold [PSTHs in BIC60 in Fig. 4(A)] and evoked a high frequency of spiking tightly phase-locked to the stimulus OFF transition. By the end of the iontophoresis,

Figure 3 (*Continued*) control (C) trials. During pairing (P) an intracellular depolarizing (P_{s+}; upward deflection) or hyperpolarizing (P_{s-}, downward deflection) current pulse (filled rectangle, 50–80 ms *in vitro*, 50–200 ms *in vivo*) applied in the postsynaptic neuron preceded by 10 ms the test stimulation such that its action on spike activity temporally overlapped with the time course of the evoked composite PSP. (B) Simple cell recorded intracellularly *in vivo* with a k-methylsulfate (KMS)-filled electrode in a 10-week-old kitten (adapted from Debanne et al., 1998). Average composite potential evoked by the onset of the stimulus in the ON subfield (Control, 21 trials). During pairing (P, black line) the stimulus onset was paired with a depolarizing pulse (200 ms, +2 nA, 30 associations). A significant potentiation of the PSP (arrowheads) was induced after pairing (thin line: control PSP, thick lines: after pairing at +1, +3, +15, and +35 min). The unpaired OFF response in the OFF subfield (not shown), the resting membrane potential (−67 mV, dotted lines) and the input resistance (30 MΩhm) were unchanged following pairing. Calibration bars = 1 s and 1 mV. (C) Time course of the induced changes in PSP amplitude for S^+ and S^- pairing protocols *in vitro* (adapted from Frégnac et al., 1994a). The dynamics of the changes of the normalized peak amplitude of the PSP evoked by stimulation of the S^+ paired (upper) and S^- paired (lower) pathways were averaged across cells that were significantly affected (KS test; $p < .05$) by the pairing protocols (S^+ paired: $n = 8$; S^- paired, $n = 12$).

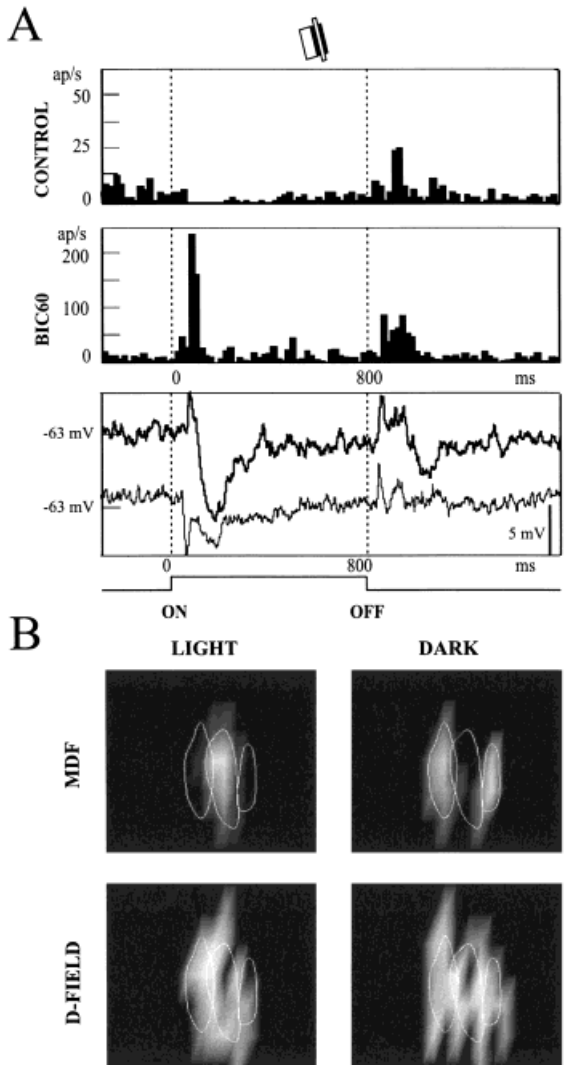

Figure 4 Hidden opponent excitatory input in Simple receptive fields, unmasked by intracortical bicuculline iontophoresis (A) or detected at the subthreshold level (B). (A) Unpublished data from Shulz, Bringuier, and Frégnac. Spike activity (two upper PSTHs) and corresponding intracellular subthreshold response profiles (lower box) in an S2 cell. This cell was recorded with a k-methylsulfate (KMS)-filled combined electrode (a three-barrel pipette glued to the intracellular recording electrode) in a 20-week-old kitten. A decrease in the ongoing spike activity and a complex hyperpolarizing profile in the membrane potential (bottom box, lower thin trace) were evoked by the ON presentation of a light bar in the OFF subfield (CONTROL, 36 trials). During bicuculline iontophoresis (BIC) (60 nA, 31 trials) the initial hyperpolarization (−5.5 mV mean amplitude) was replaced by a depolarizing potential (mean amplitude: +3.6 mV) which gave rise a phasic burst of spikes (ON response in the BIC60 PSTH) followed by a strong after-hyperpolarization (lower box, upper thick trace). This effect disappeared as soon as the application of bicuculline was stopped (data not shown). Note the phase lag of the unmasked EPSP observed during bicuculline application with respect to the IPSP onset recorded in the control condition. Calibration bar = 2 mV. (B). Reverse-correlation maps of the synaptic integration field (adapted from Bringuier et al., 1999). Two different receptive field maps (each shown in a 4° × 4° square) were established for the same neuron (S3 OFF-ON-OFF cell), on the basis of the detection of spikes (discharge field: MDF), and of subthreshold depolarizing events (D-fields). The resting potential of the cell here is above the chloride reversal potential; depolarizing events indicate the presence of dominant excitatory input. Short-duration (33-ms) punctate stimuli of positive or negative contrast were sequentially flashed in random positions in the visual field, and reverse correlation techniques were applied (Bringuier et al., 1999). The gray level shows for all statistically significant pixels ($p < .01$) the z score of the optimized reverse correlation count (z_{max} scores of 10 and 8.5, respectively, for the MDF and D-fields) measured in a 10-ms slice. The discharge zones (upper row, significant response for the whole stimulus duration indicated by a continuous white contour) and the D-fields (lower row) were plotted in response to light stimuli (ON response, left column) and dark stimuli (OFF response, right column). Note that the subthreshold depolarizing fields extend over both ON and OFF discharge areas, whatever the polarity contrast of the stimulus.

all the parameters described above returned to control levels.

DISCUSSION

The study of self-organizing networks and associative memories benefits from the use of simple putative elementary principles of plasticity, operating at a local level (the synapse) and uniformly across the cell assembly. The large number of experimental attempts to demonstrate the validity of Hebb's postulate prediction during the last 50 years should inevitably have narrowed its fields of application. Surprisingly, Hebbian schemes have survived to become the symbol of an ever-renewed concept of synaptic plasticity, open for more generalization. In the case of the field of cortical plasticity, striking uniformity can be found in the application of Hebbian-like principles to visual (Bear et al., 1999; Stryker, 1991), auditory (review in Edeline, 1999), and somatosensory (review in Buonomano and Merzenich, 1998) networks. In particular, the effects induced by the supervised Hebbian learning paradigms that we developed more than 10 years ago have now been replicated in both adult visual (McLean and Palmer, 1998) and auditory (Cruikshank and Weinberger, 1996) cortex.

Two possibilities may explain why our experi-

ments show a much larger potential for plasticity than currently admitted, even with refined tools for visualizing the differential metabolic activity of the entire network in animals reared in various conditions (Gödecke and Bonhoeffer, 1996). One is linked to the local supervised versus global unsupervised nature of the conditioning protocols. Applying a local pertubation will induce a regional reorganization in a weakly coupled network, whereas a global clamp of the input will have no effect when the strength of local and lateral interactions are maximized. In our protocols, the constraints from the network in stabilizing the columnar preference, hence the conditioned cell preference, may be weaker, allowing the cell's response to escape from the global assembly behavior.

Another explanation may be derived from the observation that similar covariance rules tend to hold in the awake animal, whether supervision is imposed externally by the experimenter (Calhusac et al., 1991) or is mediated via self-generated attention related modulatory signals as shown in auditory (Ahissar et al., 1992) and somatosensory (Wang et al., 1995) cortex. In particular, Ahissar and collaborators elegantly applied cross-correlation techniques to the study of plasticity of "functional connectivity" between pairs of neurons in the auditory cortex in awake monkeys performing a sensory discrimination. The correlation of activity between two neurons was artificially controlled by activating the target cell of the pair (the postsynaptic cell), by the presentation of its preferred auditory stimulus every time (and immediately after) the other cell fired spontaneously. Under these Hebbian conditions, reversible changes in functional coupling could be induced only when the animal was attentive to the tone used to control the activity of the postsynaptic cell. These changes lasted for a few minutes and followed the covariance hypothesis predictions: Potentiation of the functional link was induced when the effective coupling was increased during the pairing protocol; conversely, depression was observed when coupling was reduced during the Hebbian association period. These results indicate that Hebb's requirement is necessary but not sufficient for cortical plasticity in the adult cortex to occur: Internal signals indicating the behavioral relevance, and which probably implicate noradrenergic and cholinergic neuromodulation (Ahissar et al., 1996), are also required. We conclude that functional changes produced by supervised Hebbian learning seem to result from strengthening or weakening of functional geniculo-cortical and intracortical links, not only expressed at the level of excitatory synapses, but implying the composite action and activity-dependent regulation of both excitatory and inhibitory polysynaptic circuits.

Hebbian plasticity of intracortical recurrent circuits may thus be implicated in the genesis of the spatial organization of visual cortical receptive fields. The experimentally induced transformation of a Simple RF into a Complex one and the loss of the spatial separation of antagonist ON and OFF subfields by the iontophoresis of bicuculline methiodide reported in this study, and already shown by Sillito (1975), depart from the classical concept of Simple receptive fields in layer IV of the primary visual cortex. In the original pioneering model by Hubel and Wiesel (1962), the separation of simple RFs into elongated ON and OFF subfields results from the convergence of excitatory inputs from principal relay geniculate cells whose RFs centers are of the same type (ON-center and OFF-center, respectively) and are aligned in the visual field (Reid and Alonso, 1995). Intracellular observations limited to layer IV Simple cells support a push–pull organization where Simple inhibitory interneurons showing a mirrorlike organization are responsible for the antagonism between ON and OFF subzones in Simple RFs (Ferster, 1988; Heggelund, 1986). However, none of these studies predict or account for the conditioning-induced modifications of the RF profile reported here, or the unmasking of excitatory responses during blockade of GABAergic inhibition. Our view is that the spatial organization of Simple RFs, at least outside layer IV, and despite clear segregation of ON and OFF zones at the spiking level [Fig. 4(B), upper row], results from a complex temporal interaction of excitatory and inhibitory events that are evoked for both the ON and OFF transitions of the visual stimulus whatever the tested subfield. Our results strongly indicate that most recorded Simple cells indeed receive direct ON and OFF excitatory afferents over the whole extent of the RF from feedforward and/or recurrent excitatory inputs that can be detected with appropriate techniques (Bringuier et al., 1999) even in the absence of blockade of intracortical inhibition [Fig. 4(B), lower row].

Moreover, the up- and down-regulations of subthreshold responses induced in our study by the control of the temporal contingency between the afferent message and postsynaptic activity demonstrate that the primary visual cortex can sustain Hebbian-like plasticity during epigenetic development and adulthood. We propose that the spatial segregation of ON and OFF subfields could be achieved by a selective cross-inhibition between Simple cells with complementary RFs, superimposed on a complex-like excitatory input distributed across the whole extent of the RF. This connectivity model (Simplex model) sup-

poses in addition the presence of adaptive excitatory and/or inhibitory synapses whose activity-dependent stabilization could be responsible for the emergence of two classes of RFs in the adult (namely, Simple and Complex) from a common pool of Simplex cells (Debanne et al., 1998). Interestingly, a related class of receptive field with mixed properties, called Duplex, has been recently described in the striate cortex of alert monkey (Kagan et al., 1998). These cells, which are similar to Complex cells with overlapping incrementing and decrementing fields, modulate, like Simple cells, their firing in response to the stimulus temporal frequency. Others have recently proposed a similar model in which Simple and Complex cell responses are formed by the same cortical circuit (Chance et al., 1999). The difference in our model is that inhibition makes Complex cells Simple, whereas Chance et al. proposed that recurrent excitation makes Simple cells Complex. Technical advances in the measure of the balance between excitation and inhibition indicate that inhibition is more prominent in Simple cells than in Complex ones (Borg-Graham et al., 1998; Carandini et al., 1998), and a detailed study of the functional role and plasticity of inhibition may help to solve the still unknown genesis of the spatial organization of visual cortical receptive fields outside the recipient layer of the feedforward geniculate input.

This work was supported by CNRS, PROGRES-INSERM, AFIRST, GIS-Cognisciences, and HFSP (RG0103/1998-B) grants to YF.

REFERENCES

Ahissar E, Haidarliu S, Shulz D. 1996. Possible involvement of neuromodulatory systems in cortical Hebbian-like plasticity. J Physiol (Paris) 90:353–360.

Ahissar E, Vaadia E, Ahissar M, Bergman H, Arieli A, Abeles M. 1992. Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. Science 257:1412–1415.

Andrew RD, Fagan M. 1990. A technique for controlling the membrane potential of neurons during unit recording. J Neurosci Methods 33:55–60.

Baranyi A, Feher O. 1981. Synaptic facilitation requires paired activation of convergent pathways in the neocortex. Nature 290:413–415.

Bear M, Kleinschmidt A, Gu Q, Singer W. 1990. Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J Neurosci 10:909–925.

Bear MF, Rittenhouse C. 1999. Molecular basis for induction of ocular dominance plasticity. J Neurobiol 41:83–91.

Bienenstock E, Cooper LN, Munro P. 1982. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 2:32–48.

Blais BS, Shouval HZ, Cooper LN. 1999. The role of presynaptic activity on the ocular dominance shift in monocular deprivation: comparison of homosynaptic and heterosynaptic mechanisms. Proc Natl Acad Sci USA 96:1083–1087.

Blakemore C, Cooper GF. 1970. Development of the brain depends on the visual environment. Nature 228:419–478.

Borg-Graham LJ, Monier C, Frégnac Y. 1998. Visual input evokes transient and strong shunting inhibition in visual cortical neurons. Nature 393:369–373.

Bringuier V, Chavane F, Glaeser L, Frégnac Y. 1999. Horizontal propagation of visual activity in the synaptic integration field of area 17 neurons. Science 283:695–699.

Buonomano DV, Merzenich MM. 1998. Cortical plasticity: from synapses to maps. Annu Rev Neurosci 21:149–186.

Calhusac PMB, Rolls ET, Marriott FHC. 1991. Potentiation of neuronal responses to natural visual input paired with postsynaptic activation in the hippocampus of the awake monkey. Neurosci Lett 124:39–43.

Callaway EM, Katz LC. 1992. Development of axonal arbors of layer 4 spiny neurons in the cat striate cortex. J Neurosci 12:570–582.

Carandini M, Anderson J, Ferster D. 1998. Tuning of membrane conductance changes in simple cells of the cat striate cortex. Soc Neurosci Abstr 24:766.

Chance FS, Nelson SB, Abbott LF. 1999. Complex cells as cortically amplified simple cells. Nature Neurosci 2:277–282.

Cruikshank SJ, Weinberger NM. 1996. Receptive field plasticity in adult auditory cortex induced by Hebbian covariance. J Neurosci 16:861–875.

Debanne D, Gähwiler BH, Thompson SM. 1994. Asynchronous presynaptic and postsynaptic activity induces associative long-term depression in area CA1 of the rat hippocampus in vitro. Proc Natl Acad Sci USA 91:1148–1152.

Debanne D, Shulz D, Frégnac Y. 1995. Temporal constraints in associative synaptic plasticity in hippocampus and neocortex. Can J Physiol Pharmacol 73:1295–1311.

Debanne D, Shulz DE, Frégnac Y. 1998. Activity dependent regulation of on- and off-responses in cat visual cortical receptive fields. J Physiol 508:523–548.

Edeline JM. 1999. Learning-induced physiological plasticity in the thalamo-cortical sensory systems a critical evaluation of receptive field plasticity map changes and their potential mechanisms. Prog Neurobiol 57:165–223.

Ferster D. 1988. Spatially opponent excitation and inhibition in simple cells of the cat visual cortex. J Neurosci 8:1172–1180.

Frégnac Y. 1995. Comparative and developmental aspects of Hebbian synaptic plasticity. In: Arbib M, editors.

Handbook of brain theory and neural networks. Cambridge, MA: MIT Press. p 459–464.

Frégnac Y, Bienenstock E. 1998. Correlational models of synaptic plasticity: develoment, learning and cortical dynamics of mental representation. In: Carew T, Menzel R Shatz CJ, editors. Mechanistic relationships between development and learning: beyond metaphor. Chichester: Wiley. p 113–148.

Frégnac Y, Burke J, Smith D, Friedlander MJ. 1994a. Temporal covariance of pre and postsynaptic activity regulates functional connectivity in the visual cortex. J Neurophysiol 71:1403–1421.

Frégnac Y, Debanne D. 1993. Potentiation and depression in visual cortical neurons: a functional approach to synaptic plasticity. In: Ono T, Squire L, Raichle ME, Perrett DI, Fukuda M, editors. Brain mechanisms of perception and memory: from neuron to behavior. New York: Oxford University Press. p 533–561.

Frégnac Y, Debanne D, Shulz D, Baranyi A. 1994b. Does post synaptic membrane potential regulate functional plasticity in kitten visual cortex? In: Baudry M Davis JL, editors. Long-term potentiation. Cambridge, MA: MIT Press. p 227–264.

Frégnac Y, Imbert M. 1984. Development of neuronal selectivity in the primary visual cortex of the cat. Physiol Rev 64:325–434.

Frégnac Y, Shulz D. 1994. Models of synaptic plasticity and cellular analogs of learning in the developing and adult vertebrate visual cortex. In: Casagrande V Shinkman P, editors. Advances in neural and behavioral development. Norwood, NJ: Ablex. p 149–235.

Frégnac Y, Shulz D, Thorpe S, Bienenstock E. 1988. A cellular analogue of visual cortical plasticity. Nature 333: 367–370.

Frégnac Y, Shulz D, Thorpe S, Bienenstock E. 1992. Cellular analogs of visual cortical epigenesis: I. Plasticity of orientation selectivity. J Neurosci 12:1280–1300.

Gödecke I, Bonhoeffer T. 1996. Development of identical orientation maps for two eyes without common visual experience. Nature 379:251–254.

Goodhill GH, Löwel S. 1995. Theory meets experiment: correlated neural activity helps determine ocular dominance column periodicity. Trends Neurosci 18:437–439.

Greuel JM, Luhmann HJ, Singer W. 1988. Pharmacological induction of use-dependent receptive field modifications in the visual cortex. Science 242:74–77.

Harsanyi K, Friedlander MJ. 1997. Transient synaptic potentiation in the visual cortex. I. Cellular mechanisms. J Neurophysiol 77:1269–1283.

Hebb DO. 1949. The organization of behavior. New York: Wiley. 337 p.

Heggelund P. 1986. Quantitative studies of the discharge fields of single cells in cat striate cortex. J Physiol (Lond) 373:277–292.

Hirsch HVB, Spinelli DN. 1970. Visual experience modifies distribution of horizontally and vertically oriented receptive fields in cats. Science 168:865–871.

Hubel DH, Wiesel TN. 1962. Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J Physiol 160:106–154.

Kagan I, Gur M, Snodderly DM. 1998. "Duplex," not simple, cells are the major cell type in striate cortex of alert monkeys. Neurosci Lett 51(Suppl):S20–S21.

Levy WB, Steward O. 1983. Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. Neuroscience 8:791–797.

Markram H, Lübke J, Frotscher M, Sakmann B. 1997. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science 275:213–215.

McLean J, Palmer LA. 1998. Plasticity of neuronal response properties in adult cat striate cortex. Vis Neurosci 15: 177–196.

Murthy A, Humphrey AL. 1999. Inhibitory contributions to spatio-temporal recetive-field structure and direction selectivity in simple cells of cat area 17. J Neurophysiol 81:1212–1214.

Pernberg J, Jirmann KU, Eysel UT. 1998. Structure and dynamics of receptive fields in the visual cortex of the cat (area 18) and the influence of GABAergic inhibition. Eur J Neurosci 10:3596–3606.

Reid RC, Alonso J-M. 1995. Specificity of monosynaptic connections from thalamus to visual cortex. Nature 378: 281–284.

Reiter HO, Stryker MP. 1988. Neural plasticity without postsynaptic action potentials: less-active inputs become dominant when kitten visual cortical cells are pharmacologically inhibited. Proc Natl Acad Sci USA 85:3623–3627.

Rittenhouse CD, Shouval HZ, Paradiso MA, Bear M. 1999. Monocular deprivation induces homosynaptic long-term depression in visual cortex. Nature 397:347–350.

Sejnowski TJ. 1977. Statistical constraints on synaptic plasticity. J Theoret Biol 69:387–389.

Shatz CJ. 1996. Emergence of order in visual system development. Proc Natl Acad Sci USA 93:602–608.

Shinkman PG, Bruce CJ. 1977. Binocular differences in cortical receptive fields of kittens after rotationnally disparate binocular experience. Science 197:285–287.

Shulz D, Bringuier V, Frégnac Y. 1993b. Complex-like structure of simple visual cortical receptive fields is masked by $GABA_A$ intracortical inhibition. Soc Neurosci Abstr 19:638.

Shulz D, Debanne D, Frégnac Y. 1993a. Cortical convergence of ON- and OFF-pathways, and functional adaptation of receptive field organization in cat area 17. Prog Brain Res 95:191–206.

Shulz D, Frégnac Y. 1992. Cellular analogs of visual cortical epigenesis: II. Plasticity of binocular integration. J Neurosci 12:1301–1318.

Sillito AM. 1975. The contribution of inhibitory mechanisms to the receptive field properties of neurones in striate cortex of the cat. J Physiol 250:305–329.

Stent G. 1973. A physiological mechanism for Hebb's postulate of learning. Proc Natl Acad Sci USA 70:997–1001.

Stryker MP. 1991. Activity-dependent reorganization of

afferents in the developing mammalian visual system. In: Lam DMK, Shatz CJ, editors. Development of the visual system. Cambridge, MA: MIT. p 267–287.

Tamura H, Tsumoto T, Hata Y. 1992. Activity-dependent potentiation and depression of visual cortical neurons to optic nerve stimulation in kittens. J Neurophysiol 68: 1603–1612.

Wang X, Merzenich MM, Sameshima K, Jenkins WM. 1995. Remodelling of hand representation in adult cortex determined by timing of tactile stimulation. Nature 378:71–75.

Wiesel TN. 1982. Postnatal development of the visual cortex and the influence of the environment (Nobel lecture). Nature 299:583–591.

Wiesel TN, Hubel DH. 1963. Single-cell responses in striate cortex of kittens deprived of vision in one eye. J Neurophysiol 26:1003–1017.

Wigström H, Gustafsson B. 1985. On long-lasting potentiation in the hippocampus: a proposed mechanism for its dependence on coincident pre- and postsynaptic activity. Acta Physiol Scand 123:519–522.