salient than ends of lines, and may be more relevant to natural textures (see ref. 5, Fig. 6.5). Breaks in dark lines may be computed as small-scale light blobs.

Our computation leaves many questions unanswered. We have not dealt with multiple scales of blob detection or the integration of various attribute boundaries. Nevertheless, we believe that we have extended perceptually based theories of texture vision to natural images by implementing an algorithm which computes textons, and which compares textons to locate texture boundaries, in accordance with human texture capabilities. We have successfully applied the algorithm to natural images, and have shown how it accounts for previous psychophysical results as well. Our algorithm demonstrates the feasibility of a first-order, symbolic approach to texture discrimination in the spirit of Marr's primal sketch[1] and Julesz's texton theory[4]. It is possible that our statistical test applied to the outputs of a sufficient number of arrays of linear filters would also provide acceptable texture segmentation. Such a scheme, similar to Bergen and Adelson's suggestion[6], remains to be elaborated, implemented and tested. It is possible that the main steps of the algorithm could be implemented in a relatively natural way by known neural structures in the retina and the visual cortex.

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A cellular analogue of visual cortical plasticity

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Neuronal activity plays an important role in the development of the visual pathway. The modulation of synaptic transmission by temporal correlation between pre- and postsynaptic activity is one mechanism which could underly visual cortical plasticity[1-4]. We report here that functional changes in single neurons of area 17, analogous to those known to take place during epigenesis of visual cortex[5,6], can be induced experimentally during the time of recording. This was done by a differential pairing procedure, during which iontophoresis was used to artificially increase the visual response for a given stimulus, and to decrease (or block) the response for a second stimulus which differed in ocularity or orientation. Long-term modifications in ocular dominance and orientation selectivity were produced in 33% and 43% of recorded cells respectively. Neuronal selectivity was nearly always displaced towards the stimulus paired with the reinforced visual response. The largest changes were obtained at the peak of the critical period in normally reared and visually deprived kittens, but changes were also observed in adults. Our findings support the role of temporal correlation between pre- and postsynaptic activity in the induction of long-lasting modifications of synaptic transmission during development, and in associative learning.

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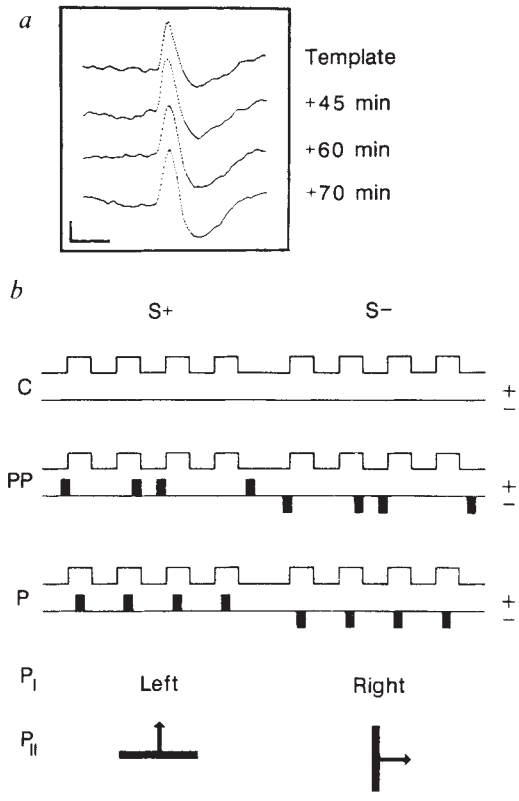

Fig. 1 Imposed temporal correlation between visual afferent activity and postsynaptic firing induced iontophoretically. *a*, For each cell, the shape of the action potential (calibration bars, 1 mV and 1 ms) was continuously monitored on a digital scope to ensure that the same neuron was recorded throughout the experiment. *b*, Each indentation indicates the temporal occurrence of a single visual stimulation, and each filled rectangle that of an iontophoretic pulse of a given polarity (shown at right). During control (C, upper row), two stimuli (S^+ and S^-) were presented by blocks of four trials in succession (upper line), without iontophoretic current (lower line). During pseudo-pairing (PP, middle row), iontophoretic pulses were uncorrelated with visual stimulation. During pairing (P, lower row) iontophoretic pulses were concomitant with the visual response, in such a way as to impose a significant increase (S^+) or decrease (S^-) of the visual response. Cases where no modulation of activity could be produced by iontophoresis are noted S^0 (see right eye stimulation in Fig. 2). The two test stimuli differed in either ocular dominance (P_I), or orientation (P_{II}). The relative preference response between the two test stimuli, given by the normalized ratio of visual responses $S^+/(S^+ + S^-)$, was measured during control periods in which the sequence of stimulation shown in row C was repeated 10-50 times. The two series of values taken from this ratio before and after pairing (or pseudo-pairing) were compared using both parametric (unpaired Student's *t*-test; significance level of $P < 0.005$) and non-parametric tests (Kolmogorov-Smirnov [K.S.]; significance level of $P < 0.05$). To assess non-associative effects, pseudo-pairing procedures (PP) were interposed between controls prior to pairing in some experiments. In the case of the orientation protocol (P_{II}), the full orientation tuning curves were analysed before and after pairing, to reveal possible generalization effects for stimuli other than those used during pairing.

Methods. In addition to standard anaesthesia and electrophysiological procedures detailed elsewhere[16], we used a simple method to artificially control the level of postsynaptic activity by varying the retention/ejection current of the 1-3 M potassium acetate or chloride extracellular recording electrode (2-20 MΩ). Positive current (average value of +4 nA) and increase in the concentration of potassium in the extracellular medium resulted (in 79% of the cases) in a significant increase in spontaneous and/or evoked activity. Negative current (average value of −9 nA) reduced the cells' activity through a field effect[17] (in 76% of the cases) which sometimes led to a total blockade of the response for the preferred stimulus (in 16% of visual cells).

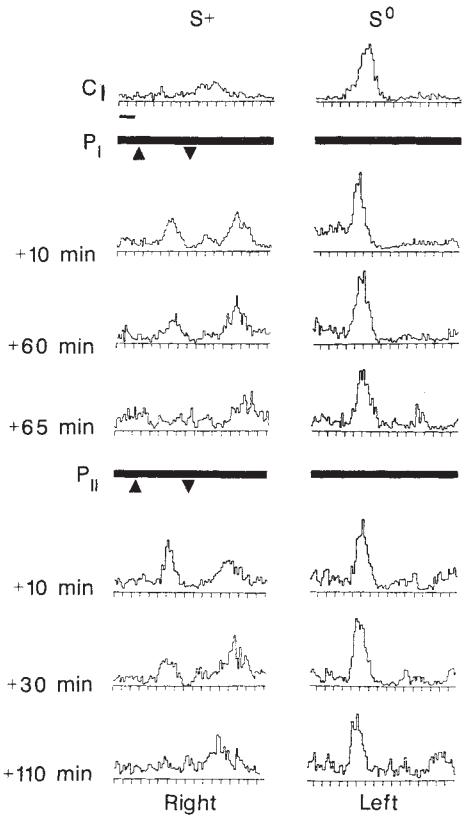

Fig. 2 Ocular dominance change. Recordings were made from a cell in area 17 of a 31-day-old kitten reared normally. PSTHs represent visual response to a bar drifting across the receptive field, using the preferred orientation, through the left eye (left column) and right eye (right column), before and after two pairing periods (thick black lines). Recordings were made for 40 presentations to each eye, interposed in blocks of four as described in Fig. 1. The cell was initially dominated strongly by the right eye (C). During each pairing procedure (not shown), left eye stimulation (S^+) was associated with a +5 nA pulse of current (filled triangles indicate the onset and offset of iontophoresis). No iontophoretic current was applied during stimulation of the right eye (S^0). The first pairing procedure (9 paired stimulations for each eye) resulted in an increase of the visual response to the left eye (+40%) within a restricted zone of the receptive field, and this effect was retained for 60 min (ratio $S^+/(S^+ + S^0)$; K.S., $P < 0.002$) after the end of the pairing. With time the ocular dominance ratio values returned to control levels, but a second pairing procedure (24 paired stimulation for each eye) imposed a 90% increase in firing within the same restricted zone. This second enhancement, confined exclusively to the paired zone, was retained for a 100 min following pairing (K.S., $P < 0.032$). The second zone of discharge of the left eye receptive field (whose activation was outside the iontophoretic pulse) and the response to stimulation of the right eye were unchanged. Calibration bars: 10 action potentials (a.p.) per s; 1 s and 1.5°.

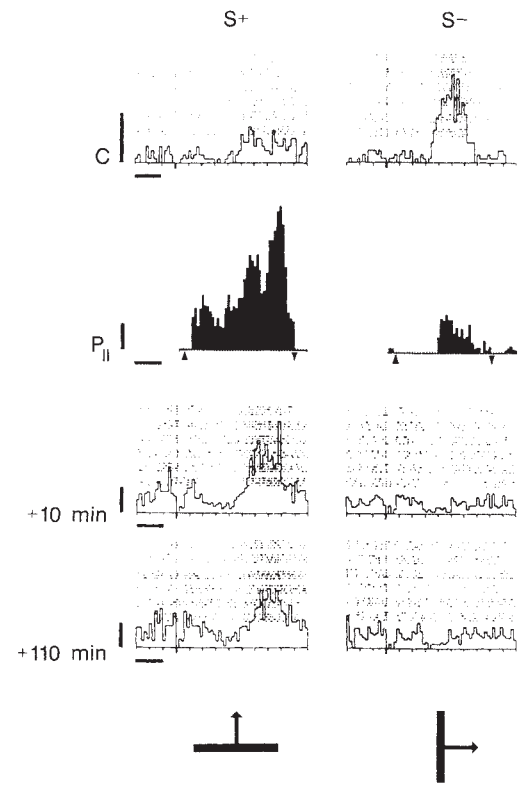

Fig. 3 Orientation preference change. Recordings were made from a cell in area 17 of a 5-week-old kitten reared in the dark for the 3 weeks prior to the experiment. PSTHs represent visual responses to a moving bar (left column, horizontal orientation; right column, vertical orientation). The cell initially showed an orientation bias for a vertical stimulus (upper row, permanent retention current of −3 nA). During pairing (P_{11}, filled histograms), positive current (+3 nA) was applied when the horizontal bar was presented, and interleaved with negative current (−7 nA) when the vertical bar was presented. Iontophoretic pulses produced an enhancement of the visual response for the initially non-preferred stimulus, and a progressive blockade of postsynaptic activity for the initially preferred stimulus which was complete at the end of pairing. The two lower rows show the change in orientation preference observed 10 min (K.S., $P < 0.005$) and 110 min (K.S., $P < 0.005$) after the end of the pairing procedure (60 paired stimulations for each orientation). Calibration bars, 5 a.p. s^{-1}, 1 s and 1.5°. Filled triangles indicate the onset (upwards) and offset (downwards) of iontophoresis.

A refined version of Hebb's neurophysiological postulate[1] of synaptic plasticity is the covariance hypothesis[3,7,8]. Changes in levels of covariation between pre- and postsynaptic activity determine the sign and amplitude of the modification of synaptic efficiency. This hypothesis predicts both increases and decreases in synaptic transmission: synapses whose activation is repeatedly correlated with firing of the target cell would be reinforced[1,9] as the result of positive change in covariance, whereas those which become predominantly silent at the time of postsynaptic firing would lose their efficiency[10] as the result of negative change in covariance. Moreover, a decrease in synaptic efficiency is also predicted in the case where there is a repetitive failure of given synapses to trigger the postsynaptic cell (as the result of a negative change in covariance[3,8]). Such a rule, which implies competition between active and silent synapses, has been applied to the modelling of the development of neuronal selectivity in visual cortex[3].

Functional modifications linked to visual experience have been inferred up to now by comparing distributions of receptive field properties in different populations of neurons in animals raised under different conditions[5,6,11]. Two intrinsic properties of visual cortical organization, ocular dominance, and orientation selectivity, have been examined in this way and their maintenance or development shown to be experience dependent[2,5,6,11–14]. By contrast our approach, using electrophysiological methods applied to anaesthetized and paralysed animals, was devised to induce, during the time of recording from a single neuron, functional changes analogous to those involved during epigenesis. Success in this would demonstrate the ability of neurons to change their integrative properties during functioning, and refute the hypothesis that changes in visual cortical function can be explained on the sole basis of disuse and sampling biases in the populations of neurons recorded at different stages of the critical period[15].

We made recordings from 429 cells in area 17 of cats, aged from 4 weeks to adulthood. Controls, performed on 308 of these cells, assessed the invariance of receptive field properties established for the same cell at different times and at different levels of constant iontophoretic current. The other 121 visual cells were submitted to 202 differential pairings and 33 pseudo-pairings (see Fig. 1).

Changes reached levels of statistical significance in the ocular dominance protocol (P_I; see Fig. 2) in 33% of paired neurons, and they corresponded to a shift in binocularity of 1–2 classes measured on a 5-class scale[16]. In 96% of cases this shift was in favour of the eye whose stimulation was paired with the 'high' level of activity. Changes could be produced both in kittens and in adults. Depending on the cell, changes could last from 15 minutes up to at least several hours. Additional pairings could restore or increase the effect induced by a first pairing procedure, especially in 4–8 1-week-old kittens (Fig. 2).

Ocular dominance changes generally corresponded to differential modifications of the amplitude of the response for the two test stimuli (75% of the cases), and more rarely to a complex reorganization of the temporal structure of the peristimulus time histograms (PSTHs) (but see effects of the first pairing P_I on response to S^+ in Fig. 2). There were sometimes changes in the size of the receptive field, unmasking initially subliminal zones, but no response appeared *de novo*. In particular, four cells initially classified as being activated monocularly, remained driven exclusively by the same eye, in spite of repeated periods of imposed increase in firing concomitant with visual stimulation of the presumptive receptive field of the silent eye.

Similar findings were obtained using the orientation protocol (P_{II}). Forty-three per cent of the paired cells showed a significant change in their relative preference between two stimuli shown through the same eye, but differing in orientation (range 24°–90°). In 94% of cases this shift was in favour of the stimulus paired with the 'high' level of activity. Changes appeared to be largest in kittens deprived of vision (Figs 3 and 4). A new orientation preference could be established in cells which were initially weakly biased or non-selective to orientation (Fig. 4). Modifications in orientation tuning studied on 45 cells showed that responsiveness was in most cases decreased for orientations close to S^- and increased for orientations close to S^+. Shifts of preferred orientation of up to 90° could be obtained in normally reared or deprived kittens up to 12 weeks of age, whereas the largest change observed in adult was a shift of 30° (data not shown). Twenty-five per cent of modified cells maintained their orientation preferences, but adapted their response by significant changes in the polar asymmetry of the tuning profile. As in the case of ocular dominance, the pairing procedure does not induce *de novo* responses. One likely reason for a larger amplitude of effects described for kittens compared with the adult, could be the initially lower level of orientation selectivity and the broader spectrum of orientations through which neurones are activated in younger animals.

These experimentally induced changes in ocular dominance and orientation preference were never observed spontaneously[6] and could not be explained by global modifications in levels of excitability and spontaneous activity, which were occasionally observed following pairing and pseudo-pairing (27% increase and 22% decrease). The minimum number of paired stimulations required to produce a significant effect was eighteen (1st pairing, Fig. 2), but for most cells 80 pairings were performed before a new control. Most changes appeared associative, since only 3% of 32 cells tested modified their relative preference after iontophoretic action uncorrelated with visual stimulation (that is, after pseudo-pairing). Modification was generally activity dependent, since changes were observed in 50% of the cells where a significant control of activity was imposed during pairing, and in only 8% of the cells for which iontophoresis did not affect visual response levels during pairing.

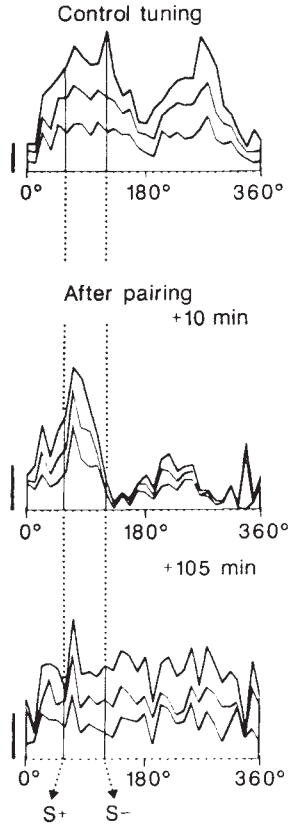

Fig. 4 Orientation selectivity change. Recordings were made from a cell in area 17 of a 5-week-old visually-deprived kitten. Orientation tuning curves were established and repeated three times over a 360° range (with steps of 12°) by randomized exploration of moving bars across the receptive field. Summed orientation tuning curves are shown before pairing (control tuning) and 10 min and 105 min after pairing. The pairing procedure consisted of 40 presentations of S^+ (60°) paired with a +4 nA current, interleaved in blocks of four with 40 presentations of S^- (120°) paired with a −5.6 nA current. The change in relative preference between the two test stimuli in favour of S^+ (iterative protocol), measured during the first hour following pairing (K.S., $P < 0.002$), was correlated with the modification shown in the orientation tuning profile established at the same time (middle row). Note the increased selectivity for S^+ and the loss of response for S^-. This cell, which was initially non-selective to orientation (responded to every direction of stimulation and was activated equally by a bar and a spot of light), acquired after pairing an orientation preference tuned closely with that of S^+. This new functional preference extinguished within 2 hours. Calibration, 20 a.p.

These results are consistent with the hypothesis that changes in covariance levels between pre- and postsynaptic activities control the efficiency of transmission of already existing synapses. The finding that separate zones in the receptive field can be modified differentially (see second pairing in Fig. 2, where increase in visual response is restricted to the sub-zone which has been 'reinforced') is in favour of selective changes in synaptic efficiency. An increase in the efficiency of synapses which are active during S^+ pairing is predicted in hebbian schemes of plasticity, and recent reports from motor cortex[18–20], hippocampus[21–23] and visual cortex[24] demonstrate that forced temporal correlation between electrical activation of afferent fibres and intracellular depolarization of the target neurone above a certain threshold, can induce an increase in the gain of excitatory synapses even in adult animals. By contrast, the decrease in the efficiency of synapses activated during S^- pairing, predicted by the covariance hypothesis and supported by our own data, is less well documented in the literature.

The study reported here is the first demonstration at the functional level, of changes analogous to those described classically during epigenesis of visual cortex[5,6,11]. It is known that unilateral eye-lid closure following normal visual experience[12] or monocular vision in previously deprived kittens[25] results in a global shift of ocular dominance in favour of the eye remaining open. These rearing procedures could correspond to an S^+ situation, where active or passive visuomotor interaction primes neuronal excitability in response to visual stimulation through the open eye[25,26]. Furthermore, it has been shown recently that after monocular vision associated with blockade of postsynaptic activity (induced by intracortical injection of an agonist of GABA) most cells would respond only to the closed eye[27]. This result is predicted by the covariance hypothesis, and is analogous to the effects we found after S^- pairing alone or interleaved with presentation of a neutral stimulus S^0. Similar reasoning applies to the orientational protocol: our data demonstrate the capacity for certain cells to capture the orientation seen during a restricted visual exposure[13,14]. However, it appears that cells can adapt their preference in favour of the imposed orientation, only if this latter initially evokes some response.

Finally, our study suggests that a hitherto unsuspectedly high level of plasticity is retained in the adult cortex. This favours the hypothesis that hebbian-like mechanisms operate both during development, and in adult learning[4,8]. We suggest that, in the intact animal, extraretinal gating signals[4,16,28]—instead of our pairing artifice—produce covariance changes through normal visuomotor experience during a postnatal critical period in kittens[16], or during selective periods of learning in the adult[28,29]. These would allow cortical neurons to undergo transition from a passive relay mode of transmission to an adaptive state reached only below or above certain levels of membrane potential. It still remains to be determined why there is an age-dependency in the expression of this experience-sensitivity in the intact, behaving animal[8]. In the adult animal, predominance of inhibitory intracortical networks and a lesser efficiency of the mechanisms responsible for the detection and transduction of these covariance changes[24,30], might reduce the probability of cortical synapses reaching the threshold for functional plasticity.

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Removal of phosphorylation sites from the β_2-adrenergic receptor delays onset of agonist-promoted desensitization

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Eukaryotic cells have evolved a variety of mechanisms for dampening their responsiveness to hormonal stimulation in the face of sustained activation. The mechanisms for such processes, collectively referred to as desensitization, often involve alterations in the properties and number of cell-surface hormone receptors[1-3]. It has been speculated that phosphorylation–dephosphorylation reactions, which are known to regulate the catalytic activities of enzymes, also regulate the function of receptors[4]. Highly specific receptor kinases, such as rhodopsin kinase[5] and β-adrenergic receptor kinase[6], which show stimulus-dependent phosphorylation of receptors have been described. Direct evidence for a causal relationship between receptor phosphorylation and desensitization has been lacking however. Here we report that prevention of agonist-stimulated β_2-adrenergic receptor (β_2AR) phosphorylation by truncation of its serine and threonine-rich phosphate acceptor segment delays the onset of desensitization. We also show that selective replacement of these serine and theronine residues by alanine and glycine delays desensitization even further. These data provide the first direct evidence that one molecular mechanism of desensitization of G-protein-coupled receptors involves their agonist-induced phosphorylation.

Figure 1*a* depicts how G-protein coupled receptors, in this case the human β_2AR, may be organized within the plasma membrane. Such proteins are thought to cross the membrane seven times[7-9]. Light-dependent phosphorylation of a homologous G-protein coupled receptor, the visual pigment rhodopsin, by an enzyme termed rhodopsin kinase, occurs on multiple serine and threonine residues at its carboxyl terminus[10-12]. A similar concentration of serine and theronine residues at the β_2AR carboxyl terminus has been proposed as a target for agonist-promoted phosphorylation[8].

To evaluate the role of phosphorylation in desensitization we constructed a mutant human β_2AR complementary DNA encoding a protein truncated after amino-acid residue 365 (T-365). As shown in Fig. 1*a*, this mutant lacks most of the serine and threonine-rich carboxyl segment of the receptor. When expressed in Chinese hamster fibroblast CHW cells, both wild-type and mutant (T-365) β_2AR bound the specific βAR ligand ^{125}I-cyanopindolol (^{125}I-CYP) with high affinity ($K_D \approx 40$ pM) and appropriate β_2AR specificity (data not shown). Moreover, photoaffinity labelling of both receptors in the CHW cells confirmed their expected mobility[13] on SDS-PAGE (Fig. 1*b*).

To determine whether T-365 undergoes agonist-promoted phosphorylation, we equilibrated CHW cells expressing wild-type or T-365 β_2AR with $^{32}P_i$, exposed them to the β-agonist isoprenaline (2 μM) for 15 min and then purified the βARs after solubilization by affinity chromatography on alprenolol-Sepharose. The results are shown in Fig. 2. The levels of basal phosphorylation (apparent stoichiometries, see Fig. 2 legend) of the wild-type and mutant receptor were essentially identical. As previously shown in a variety of cell systems[4], phosphorylation of the wild-type β_2AR increased 2-3 fold when cells were exposed to the agonist isoprenaline (Fig. 2*a*). In contrast, no agonist-promoted increase in phosphorylation of the truncated receptor was observed at any time point investigated (15 min,