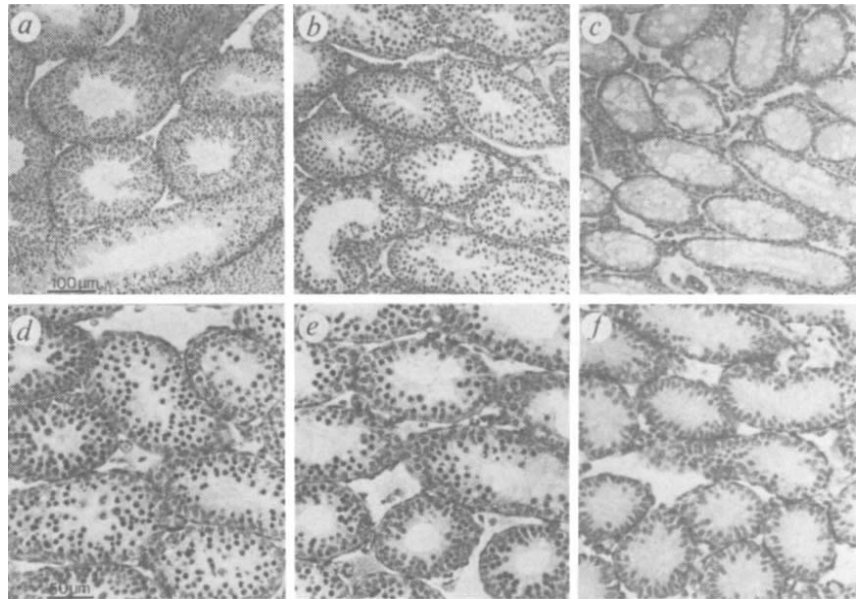


**Fig. 2** Spermatogenic failure in X0 *Sxr'* male mice. *a*, Complete spermatogenesis in a testis from an adult (49-day-old) XY mouse. *b*, Abnormal spermatogenesis with a marked deficiency of condensing spermatid stages in the testis of a 49-day-old X0 *Sxr* mouse (H-Y-positive). *c*, An almost total block of spermatogenesis in the testis of a 49-day-old X0 *Sxr'* mouse (H-Y-negative). Although some spermatogonia are present, almost all tubules lack spermatocytes and spermatids. *d–f*, Testes from 13-day-old XY, X0 *Sxr* and X0 *Sxr'* mice showing that the defect in X0 *Sxr'* spermatogenesis is already present prepubertally when meiosis begins.
**Methods.** X0 *Sxr* and X0 *Sxr'* mice were produced by mating XY *Sxr* or XY *Sxr'* males to females heterozygous for the inversion In(X)1H. These females generate some nullo-X eggs following crossing-over within the inversion. The testes from 15 X0 *Sxr'* and 9 X0 *Sxr* males together with those from their male littermates, at 2–50 days post partum, were examined during this study. All mice were karyotyped. Testes were fixed in Bouin's fixative. The sections were stained with periodic acid-Schiff's reagent and counterstained with haematoxylin.

expression which are tightly linked. It may be pertinent that spermatogonia have been identified as the only adult male cells which type negative for male-specific antigen using serological tests[17]. Later spermatogenic stages are increasingly positive. If serological tests do recognize H-Y antigen, this pattern of expression would be consistent with H-Y antigen being the spermatogenesis gene product.

The possibility that H-Y antigen is the product of the spermatogenesis gene leads to predictions about the H-Y status of human XX males and XY females. A human spermatogenesis gene, which we presume is homologous to that on the mouse Y, is located on the human Y long arm close to the centromere[18]. Human XX males that have acquired testis-determining sequences from the Y short arm[19–21] will lack Y long arm sequences including the spermatogenesis gene, and hence should type H-Y-negative if H-Y is the spermatogenesis gene product. Conversely, XY females that have lost testis-determining sequences from the Y short arm should type H-Y-positive because the spermatogenesis gene on the Y long arm should have been retained. H-Y typing of human sex-reversed individuals is now possible using histocompatibility locus antigen (HLA)-restricted H-Y-specific T-cell clones. All five XX males so far typed are H-Y-negative, in line with our predictions (ref. 22 and E. Goulmy and E. Simpson, personal communication).

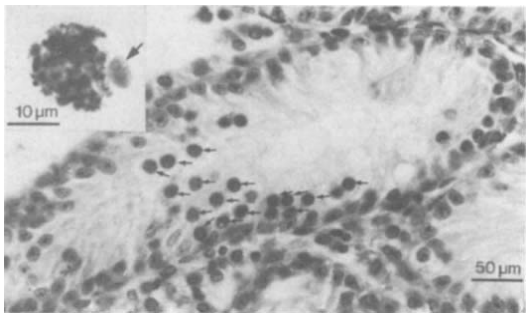


**Fig. 3** Meiotic cells in X0 *Sxr'* testes. Occasionally tubules are seen in X0 *Sxr'* testes which contain patches of meiotic cells (arrowed) that degenerate during the early pachytene stage. These pachytene cells have a clear sex vesicle in air-dried preparations (arrowed in inset), showing that this structure can form in the absence of H-Y antigen.

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# Modulation of visual cortical plasticity by acetylcholine and noradrenaline

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**During a critical period of postnatal development, the temporary closure of one eye in kittens will permanently shift the ocular dominance (OD) of neurones in the striate cortex to the eye that remains open[1]. The OD plasticity can be substantially reduced if the cortex is infused continuously with the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) during the period of monocular deprivation[2–5], an effect that has been attributed to selective depletion of cortical noradrenaline[6]. However, several**

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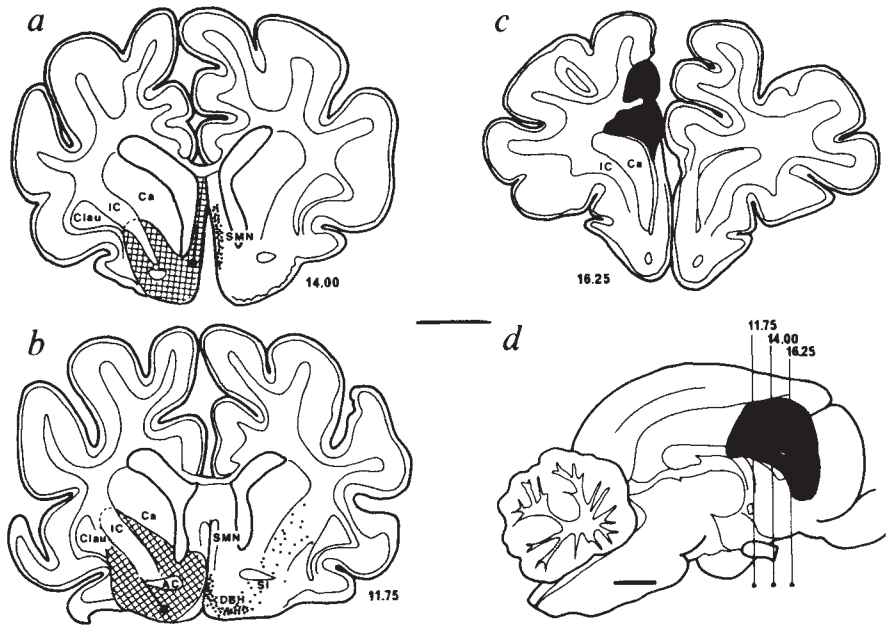


**Fig. 1** *a*, *b*, Coronal sections through the forebrain of a 44-day-old kitten (K125) at the approximate frontal planes A14.0 and A 11.75, respectively. Projected onto the right side of *a* and *b* is the distribution of cells in the basal telencephalon that were back-filled after 1 μl of 30% horseradish peroxidase (HRP) was injected into the visual cortex of a 4-week-old kitten. For each drawing, five contiguous 50-μm thick sections were pooled; each dot represents approximately two HRP-labelled neurones. (????) The extrinsic cholinergic innervation of the striate cortex arises from these cells[20]. This unilateral projection derives from neurones scattered within the internal capsule (IC), the substantia innominata (SI), the diagonal band of Broca and the medial septal nucleus (SMN). Reconstructed on the left side of *a* and *b* are the regions of cell loss (crosshatching) after NMA was injected into the telencephalon of K125. Also indicated (●——) are the two needle tracks along which NMA was delivered, one (*a*) targeting cells in the vertical limb of the diagonal band (50 μg) and SMN (50 μg), the other (*b*) targeting cells in the horizontal limb of the diagonal band (DBH, 125 μg), SI (75 μg) and IC (50 μg). This lesion produced a drastic and widespread depletion of AChE-positive axons in the neocortex. In addition to the cholinergic basal forebrain neurones, cells in the caudate, globus pallidus, ventral pallidum and rostral pole of the dorsal thalamus were also destroyed by the NMA injections. The cell loss in the thalamus was confined to the reticular, ventral anterior and ventral lateral nuclei. The portions of the claustrum (Clau) and intralaminar thalamus that project to the visual cortex were spared by the NMA injections. AC, anterior commissure; Ca, caudate nucleus. *c*, Coronal section through the forebrain of K152 at the approximate frontal plane A 16.25 to illustrate a cingulate gyrus lesion. The blackened region was surgically removed by subpial aspiration. The lesion produced a depletion of AChE-positive axons in the striate cortex comparable to that observed with basal forebrain lesions. Analysis of cortical tissue using HPLC confirmed that NA was also reduced in area 17 to <50% of control levels (Table 1). *d*, Anterior–posterior extent of the lesion in *c* in a mid-sagittal view of a kitten brain, showing the approximate level of each of the sections illustrated. Also indicated is the extent of the cingulate gyrus lesion in K152 (black area). Scale bars, 5.0 mm.

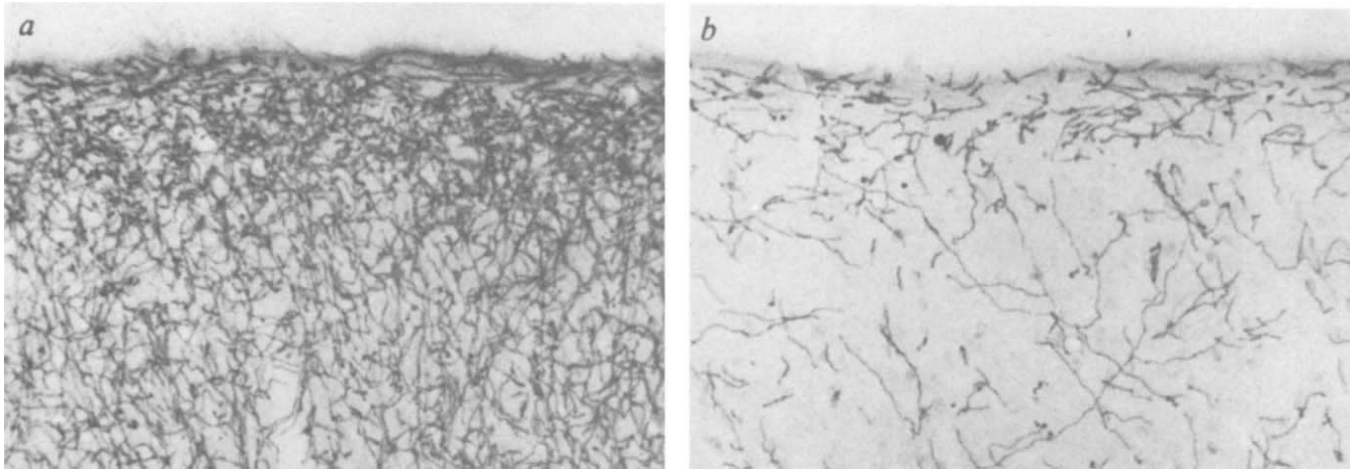


**Fig. 2** Photomicrographs of AChE-containing axons in the striate cortex of a 47-day-old kitten. *b*, Layers I–IV in the hemisphere that had received a chemical lesion of the cholinergic basal telencephalon 18 days earlier. *a*, A homotypic region in the contralateral control hemisphere. A unilateral loss of ocular dominance plasticity accompanies this reduction in the density of cholinergic axons if cortical noradrenaline is depleted concurrently. Magnification, ×150.

**other methods causing noradrenaline (NA) depletion leave the plasticity intact[7–10]. Here we present a possible explanation for the conflicting results. Combined destruction of the cortical noradrenergic and cholinergic innervations reduces the physiological response to monocular deprivation although lesions of either system alone are ineffective. We also find that 6-OHDA can interfere directly with the action of acetylcholine (ACh) on cortical neurones. Taken together, our results suggest that intracortical 6-OHDA disrupts plasticity by interfering with both cholinergic and noradrenergic transmission and raise the possibility that ACh and NA facilitate synaptic modifications in the striate cortex by a common molecular mechanism.**

Our study was designed to assess the contribution of the extrathalamic cortical afferents to OD plasticity in the striate cortex. Our attention was focused initially on the cholinergic projection for three reasons. First, there is converging biochemical[11,12] and anatomical[13] evidence suggesting that the striate cortex receives a dense cholinergic innervation during the critical period. Second, the facilitatory action of ACh on excitatory transmission in striate cortex[14,15] increases the probability of postsynaptic activation, a condition that is required for the experience-dependent modification of many excitatory synapses[16]. Third, the cholinergic projection is thought to be an important component of the ascending reticular activating system[17], and there is evidence that reticular activation of striate cortex may be necessary for OD plasticity[18,19].

The cholinergic innervation of striate cortex arises from cells scattered through the basal telencephalon (Fig. 1*a*, *b*; ref. 20).

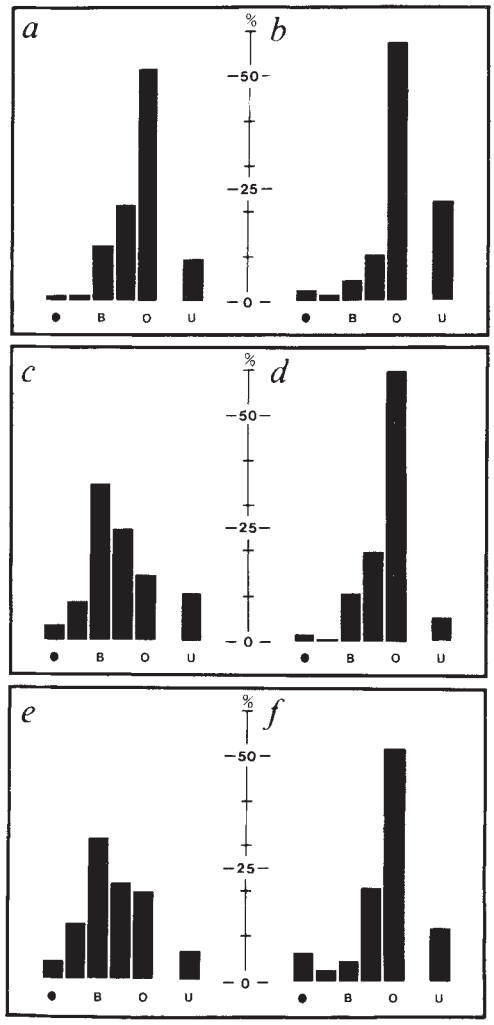


**Fig. 3** Percentages of cells in each of the five ocular dominance groups in the experimental (*a*, *c*, *e*) and control (*b*, *d*, *f*) hemispheres. Open circles, monocular open eye groups; filled circles, monocular closed eye groups; B, strictly binocular groups (group 3); U, % of unclassifiable neurones. *a*, *b*, Composite ocular dominance histograms from kittens that received unilateral NMA lesions of the cholinergic basal forebrain before 7-11 days of monocular deprivation (see Table 1). The ocular dominance distribution is shifted to the open eye both in the ACh-depleted left hemispheres (*a*, 126 cells) and in the control right hemispheres (*b*, 66 cells). *c*, *d*, Composite OD histograms from kittens that received unilateral 6-OHDA lesions of the dorsal noradrenergic bundle 1 week before the NMA lesions of the forebrain (see Table 1). *c*, OD distribution in the experimental hemispheres (110 cells) is significantly more binocular and less shifted than in *d*, showing the distribution in the control hemispheres (97 cells). *e*, *f*, Composite OD histograms from kittens that received unilateral lesions of the cingulate gyrus before 8-9 days of monocular deprivation (see Table 1). The OD histogram from the cingulate-damaged hemispheres (*e*, 94 cells) is also significantly more binocular and less shifted than that from the control hemispheres (*f*, 64 cells). The striate cortex in both the 6-OHDA + NMA-injected and cingulate-damaged hemispheres was depleted of its normal complement of cholinergic and noradrenergic axons. Other than OD, the visual-response properties of the cells recorded in these hemispheres did not differ from the controls. Histological reconstruction of the electrode tracks was not possible because the area centralis representation of area 17 was dissected for HPLC analysis. However, our sampling methods were the same in both hemispheres and no obvious laminar bias was indicated by the relative percentages of simple or complex cells encountered.

**Table 1** Rearing histories of kittens and physiological results

| Animal | Lesion | Age at lesion (days postnatal) | Monocular deprivation (days postnatal) | Control hemispheres N | Control hemispheres B | Control hemispheres OED | Experimental hemispheres N | Experimental hemispheres B | Experimental hemispheres OED | % NA |
|---|---|---|---|---|---|---|---|---|---|---|
| K125 | BF | 29 | 33-43 | 21 | 0.06 | 0.94 | 30 | 0.46 | 0.61 | — |
| K127 | BF | 29 | 40-47 | 20 | 0.25 | 0.81 | 30 | 0.38 | 0.77 | — |
| K128 | BF | 29 | 44-55 | 25 | 0.33 | 0.67 | 36 | 0.52 | 0.59 | — |
| K142 | BF | 32 | 32-40 | — | — | — | 30 | 0.26 | 0.83 | — |
| K149 | DNAB/BF | 32/39 | 39-48 | 30 | 0.39 | 0.71 | 35 | 0.84 | 0.31 | 16 |
| K151 | DNAB/BF | 32/39 | 41-50 | 30 | 0.33 | 0.80 | 35 | 0.78 | 0.39 | 49 |
| K156 | DNAB/BF | 32/39 | 39-49 | 37 | 0.31 | 0.72 | 40 | 0.74 | 0.26 | 40 |
| K152 | C | 39 | 39-47 | 31 | 0.19 | 0.89 | 31 | 0.69 | 0.40 | 30 |
| K157 | C | 39 | 39-47 | — | — | — | 33 | 0.70 | 0.32 | 40 |
| K158 | C | 39 | 39-48 | 33 | 0.42 | 0.60 | 30 | 0.79 | 0.30 | 31 |
| K160 | DNAB | 32 | 39-49 | — | — | — | 30 | 0.33 | 0.82 | 33 |
| K164 | C* | 35 | 35-45 | — | — | — | 30 | 0.33 | 0.64 | — |

BF, basal forebrain; DNAB, dorsal noradrenergic bundle; DNAB/BF, DNAB followed by BF; C, surgical aspiration of the cingulate gyrus; C*, NMA lesion of cingulate cortex. N, number of cells recorded in each hemisphere; B, binocularity; OED, open-eye dominance for each hemisphere. Binocularity is defined as the number of cells in OD groups 2-4 divided by the total number of cells recorded. OED (after Paradiso *et al.*[28]) is defined as the number of cells in group 5 plus 0.5 times the number of cells in group 4 divided by the total number of cells (group 5, by convention, is the monocular group dominated by the open eye). In all cases except K142 the lesions were unilateral so that the opposite hemispheres could serve as controls. The cortical NA content in hemispheres with DNAB and C lesions (expressed as a per cent of the control cortex) is also shown. In these cases, saline-perfused samples of visual cortex were rapidly dissected from both hemispheres and frozen at −70 °C. The samples were subsequently homogenized in 0.1 M perchloric acid containing EDTA (60 mg $l^{-1}$) and sodium metabisulphite (100 mg $l^{-1}$) and then centrifuged to remove denatured protein. Aliquots of the supernatant were applied directly to an HPLC column (ALTEX ODS 5 μm) and the catechol compounds were measured relative to an internal dihydroxybenzylamine standard using electrochemical detection as described elsewhere[7,28]. The NA content of the control visual cortex was 73.67 ± 7.26 (mean ± s.e.m.) ng per g tissue, wet weight.

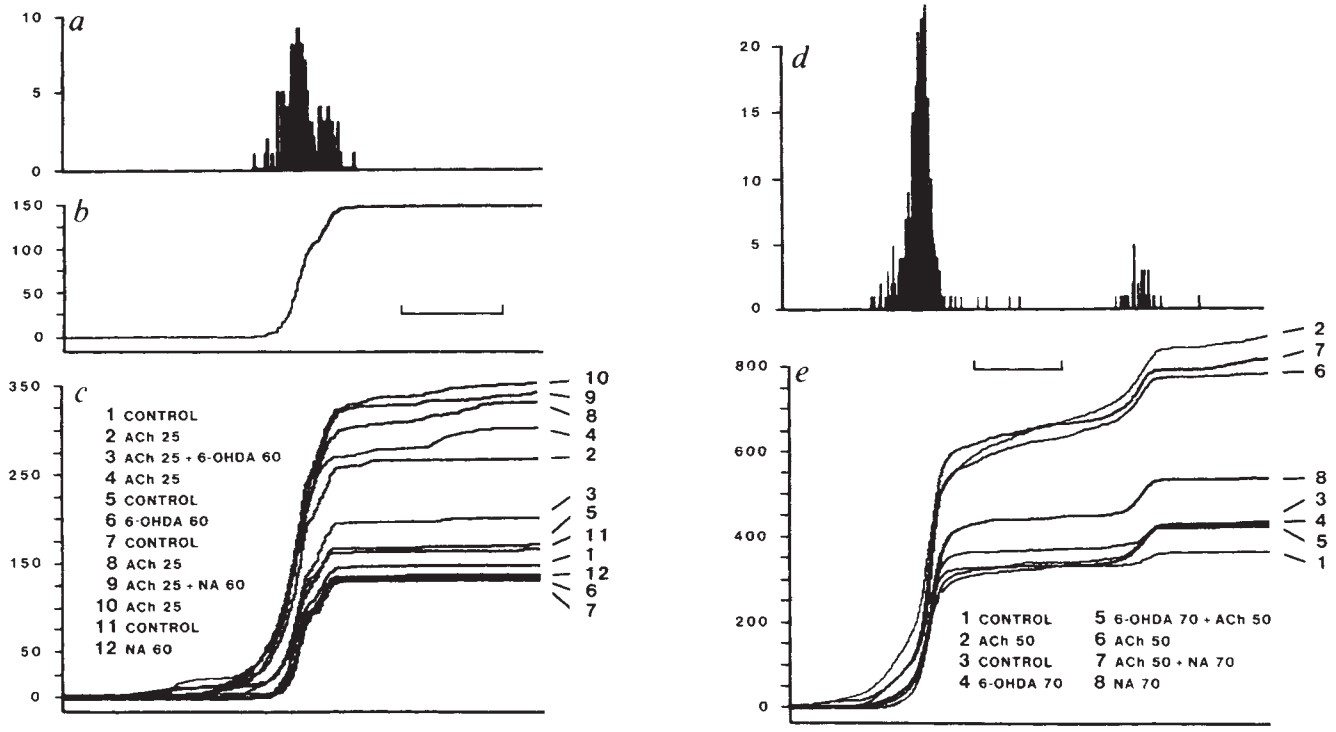


**Fig. 4** Antagonistic effect of iontophoretically applied 6-OHDA on ACh-evoked responses. *a*, Peri-stimulus time histogram of the control response of a simple cell in the striate cortex to an optimally oriented bar of light swept at a constant speed over the receptive field of the neurone. The ordinate shows the number of spikes per bin accumulated in 10 trials; bin width, 20 ms. *b*, To facilitate quantitative comparisons, the same data are expressed as total spikes accumulated as a function of sweep time. In this example, the cell responded to 10 stimulus presentations with 148 spikes. Scale bar, 1.0 s. *c*, Response of this cell to visual stimulation is plotted as in *b*, under the 12 test conditions listed in chronological order. The interval between tests ranged from 1 to 1.5 min. Indicated after each drug tested is the iontophoresis current (nA). The visual response of this cell was reliably enhanced each time ACh was applied by itself (tests 2, 4, 8 and 10). However, this enhancement was reduced considerably when 6-OHDA was applied concurrently (test 3). In contrast, NA applied in the same way had no effect on the ACh response (test 9); 6-OHDA application by itself had little apparent effect (test 6) compared with the control responses (tests 1, 5, 7 and 11). *d*, Peri-stimulus time histogram of the control response of a complex cell in the striate cortex to a bar of light swept first in the preferred direction, then in the opposite direction. *e*, Data from this complex cell expressed as in *b* and *c*. Test conditions are listed in chronological order and the interval between tests ranged from 1 to 7 min. In this example, the facilitation of the visual response by ACh (tests 2 and 6) was completely blocked by 6-OHDA (test 5). Noradrenaline did not have a similar action (test 7) and 6-OHDA had no effect on the cellular response when applied alone (test 4). Scale bar, 1.0 s. Excitation of this cell with glutamate was not antagonized by the concurrent application of 6-OHDA (data not shown).

**Methods.** All the records were made with a micropipette containing 1.5 M potassium citrate ($R = 15$ M) that protruded ~50 μm from an attached 7-barrel micropipette. Two of these barrels contained acetylcholine chloride (2.0 M, *p*H 4.5), two contained 6-OHDA-HCL (0.2 M) in 0.1% ascorbate (*p*H 3), one was filled with noradrenaline-HCl (0.2 M), also in 0.1% ascorbate, and one contained glutamic acid (0.3 M, *p*H 8). Currents of 5 nA were applied to retain the drugs in the barrels; positive for glutamate and negative for all others. Tip potentials were automatically balanced by passing currents of equal and opposite polarity through an additional barrel containing 2.0 M NaCl.

We destroyed these cells with the excitotoxin *N*-methyl-DL-aspartate (NMA) in 4–5-week-old kittens, and then tested for a deficit in the OD shift after brief periods of monocular deprivation. All lesions were made in the left hemisphere. The right eyelids were closed and, 7–10 days later, a standard physiological assay of OD was performed in the striate cortex on both sides[18]. As the normal OD shift is nearly symmetrical in the two hemispheres[8,9], the cortex on the right (unoperated) side served as an internal control. The effectiveness of the lesions was monitored by acetylcholinesterase (AChE) histochemistry. Most, if not all, of the AChE-positive axons in the cat striate cortex arise from cholinergic cells in the basal telencephalon[20].

A typical basal forebrain (BF) lesion, illustrated in Fig. 1*a*, *b* (left side), resulted in a drastic reduction in the density of AChE-stained axons in striate cortex (Fig. 2). Nevertheless, subsequent monocular deprivation shifted the OD of cortical neurones dramatically to the open eye (Fig. 3*a*, *b*, Table 1). These data indicate that a substantial depletion of ACh alone is not sufficient to block plasticity. However, when cortical NA was depleted by >50% (Table 1) before the basal forebrain lesions, the normal OD shift was prevented (Fig. 3*c*, *d*; Table 1). The noradrenergic projection was destroyed unilaterally in these animals by injecting 6–10 μg of 6-OHDA into the left dorsal noradrenergic bundle (co-ordinates A7, L3, DO). Daw *et al.*[9] have shown, and we have confirmed (K160, Table 1), that dorsal noradrenergic bundle lesions do not normally affect the plastic response to monocular deprivation, even with a 70–90% depletion of cortical NA. Thus, although basal forebrain and dorsal noradrenergic bundle lesions alone are ineffective, they cause a significant loss of OD plasticity in the striate cortex when they are combined.

These data suggest that the combined depletion of cortical ACh and NA is a sufficient condition to retard experience-dependent synaptic modifications in the striate cortex. This notion was tested further by using a second experimental approach. Anatomical studies in this laboratory indicate that cholinergic and noradrenergic axons en route to area 17 travel together within the white matter of the cingulate gyrus. Thus, surgical lesions of the cingulate gyrus also effectively deplete striate cortex of both NA and ACh. Such a lesion (Fig. 1*c*, *d*) drastically reduces the density of AChE-positive axons, depletes endogenous NA (Table 1) and prevents the normal OD shift after monocular deprivation in area 17 on the lesioned side (Fig. 3*e*, *f*; Table 1). In one kitten (Table 1, K164), an extensive lesion of the cingulate cortex was made with multiple injections of NMA, which spares axons of passage. This treatment did not significantly alter the normal OD shift after monocular deprivation, thus confirming that the effects of the surgical lesions are

caused by the interruption of pathways passing through the cingulate gyrus.

These results predict that any treatment that simultaneously blocks noradrenergic and cholinergic transmission in the cortex is effective in slowing the OD shift after monocular deprivation. The work of Furness[21] on intestinal smooth muscle indicates that continuously applied 6-OHDA has exactly this effect—noradrenergic denervation *and* blockade of muscarinic cholinergic transmission. We explored this possibility in the striate cortex by assessing the effects of 6-OHDA iontophoresis on the neuronal responses induced by applying ACh from a piggyback microelectrode assembly[22]. We studied 30 ACh-sensitive neurones in the striate cortex of three adult cats. The effects of ACh iontophoresis were generally in agreement with those of Sillito and Kemp[14]. In most cases (26 cells), the visual responses were enhanced by ACh. However, in 85% of these cells the enhancement was significantly attenuated when ACh was applied concurrently with 6-OHDA (Fig. 4).

Our iontophoretic data are consistent with the hypothesis that the continuous application of 6-OHDA to the cortex affects cholinergic transmission, suggesting that intracortical 6-OHDA disrupts cortical plasticity by a combined action on the noradrenergic and cholinergic projections. In this context, note that the threshold concentration of 6-OHDA to block muscarinic transmission in the gut is ~5 μM (ref. 21). This is the same tissue concentration of 6-OHDA that Kasamatsu *et al.*[23] calculated to be the threshold for preventing plasticity in the striate cortex of kittens.

It is not yet clear whether these data indicate a special role for NA and ACh in the control of cortical plasticity. Many experience-dependent synaptic modifications seem to require the activation of cortical neurones[16] and there are indications that this activation must exceed the threshold of voltage-sensitive $Ca^{2+}$ channels in order to be effective[24]. Thus, removal of enough facilitatory extrageniculate inputs could lower cortical excitability below the threshold for synaptic modifications. Both ACh and NA reduce $K^+$ permeability[25,26] and hence both could enhance depolarization in response to visual input[15]. On the other hand, the actions of these neuromodulators may be related more specifically to the control of synaptic plasticity in the cerebral cortex. For example, both stimulate the production of cyclic nucleotides and the mobilization of intracellular $Ca^{2+}$ in target neurones[27]. Thus, it is plausible that ACh and NA regulate, via second-messenger-dependent phosphorylation, a common set of proteins that are involved in the modification of synaptic transmission.

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# T-cell recognition of antigen and the Ia molecule as a ternary complex

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**T-lymphocyte co-recognition of antigen and major histocompatibility complex (MHC)-encoded molecules (such as murine Ia molecules) is thought to be mediated by a single cell-surface receptor, although the molecular mechanism by which this occurs is controversial (reviewed in ref. 1). One possibility is that the antigen molecule and the Ia molecule interact physically, either before or after encountering the T-cell antigen-specific receptor[1,2]. Alternatively, both molecules could bind to the receptor independently of one another, accounting for the dual specificity of the receptor without postulating a physical interaction between a limited number of Ia molecules present in any given animal and the myriad antigens to which T cells can respond. Here, we used a recently described approach for analysing the relative avidity of the T-cell receptor for different ligands[3] to address these two possibilities. We describe a T-cell clone whose response to a single antigen, presented in the context of two different Ia molecules, strongly suggests that the antigen and the Ia molecule interact physically.**

Receptor-bearing cells in culture compete with each other for ligand. For a fixed concentration of ligand, as the number of responding cells in an assay increases, at some point the number of receptors occupied per cell will decrease as the free ligand becomes significantly depleted by binding to the receptor. If the response of the cell to the ligand is proportional to the number of receptors occupied, at least in the dose-sensitive portion of the response, then the average cellular response, or the probability that a cell will respond in the case of a proliferation assay, will also decline[4–6]. Furthermore, the point at which the cells bind sufficient ligand to result in a decrease in the average cellular response is dependent upon two variables: the number of receptors per cell and the affinity of the receptors for the ligand. We have used this phenomenon to analyse the interaction of the T-cell antigen-specific receptor with its ligand, which is a combination of antigen and Ia molecule (antigen-Ia)[3]. The fraction of T cells stimulated by antigen-Ia to incorporate $^3$H-thymidine varied as a function of the number of responding T cells, best displayed by plotting the response of the T cells as a fraction of the maximum response achieved (Fig. 1). In a previous study we used this technique to compare the ability of four synthetic analogues of moth cytochrome *c* to activate a T-cell clone when presented in association with a single Ia molecule, $E^k_\beta E^k_\alpha$ (ref. 3). In the present study, we assessed the effect of changing the allelic form of the Ia molecule while keeping the antigen constant.

The T-cell clone designated F1.A.2 can recognize the moth cytochrome *c* synthetic fragment 86–89; 93–103(93E) (moth fragment) in association with either $E^k_\beta E^k_\alpha$ or $E^b_\beta E^k_\alpha$. These two Ia molecules differ by only four amino acids in the N-terminal domain of their β-chains (ref. 7 and P. Jones, personal communication). The moth fragment was 13-fold more potent (defined in Fig. 1) when recognized in association with $E^k_\beta E^k_\alpha$ than with $E^b_\beta E^k_\alpha$ (one representative experiment is shown in Fig. 2, an