THE ROYAL SOCIETY

Restoration of ocular dominance plasticity mediated by adenosine 3′,5′-monophosphate in adult visual cortex

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Noradrenaline (NA)-stimulated β-adrenoreceptors activate adenylate cyclase via excitatory G-proteins (G_s). Activated adenylate cyclase in turn promotes the production of cAMP. Critical roles of cAMP-dependent protein kinase A (PKA) in divergent cellular functions have been shown, including memory, learning and neural plasticity. Ocular dominance plasticity (ODP) is strongly expressed in early postnatal life and usually absent in the mature visual cortex. Here, we asked whether the activation of cAMP-dependent PKA could restore ODP to the aplastic visual cortex of adult cats. Concurrent with brief monocular deprivation, each of the following cAMP-related drugs was directly and continuously infused in the adult visual cortex: cholera toxin (a G_s-protein stimulant), forskolin (a G_s-protein-independent activator of adenylate cyclase) and dibutyryl cAMP (a cAMP analogue). We found that the ocular dominance distribution became W-shaped, the proportion of binocular cells being significantly lower than that in respective controls. We concluded that the activation of cAMP cascades rapidly restores ODP to the adult visual cortex, though moderately. The finding further extends the original hypothesis that the NA–β-adrenoreceptors system is a neurochemical mechanism of cortical plasticity.

Keywords: ocular dominance plasticity; monocular lid suture; β-adrenoreceptor–cAMP cascades; second messenger; protein kinase A

1. INTRODUCTION

Developmental plasticity of the mammalian visual cortex has attracted considerable attention, both as a model for neural plasticity and because of the clear clinical consequences of altered visual experience, such as human amblyopia. The possibility that this form of plasticity (Wiesel & Hubel 1963, 1965) might be modulated by exogenous inputs from the brainstem was first raised in 1976 by experiments impairing the catecholaminergic innervation of the kitten visual cortex (Kasamatsu & Pettigrew 1976). The involvement of noradrenaline (NA)-activated β-adrenoreceptors in plasticity has been directly shown (Pettigrew & Kasamatsu 1978; Kasamatsu *et al.* 1979; Kasamatsu & Shirokawa 1985; Shirokawa & Kasamatsu 1986, 1987; Shirokawa *et al.* 1989).

In later experiments, the roles of GABAergic (Ramoa *et al.* 1988; Mower & Christen 1989; Hensch *et al.* 1998), muscarinic cholinergic (Bear & Singer 1986; Imamura & Kasamatsu 1989; Gu & Singer 1993), serotoninergic (Gu & Singer 1995) and glutamatergic (*N*-methyl-D-aspartate (NMDA) type) receptor systems (Kleinschmidt *et al.* 1987; Rauschecker & Hahn 1987; Bear *et al.* 1990; Roberts *et al.* 1998; see also Fox & Daw 1993; Kasamatsu *et al.* 1998) were also explored. In most of these experiments, the emphasis was on demonstrations that neurochemical lesions of, or receptor blockade of, the neuromodulator system under study caused deficits in plasticity. Such experiments, however, have raised doubts about the extent to which the interference may have affected the normal cortical function that is required for the expression of plasticity, rather than the impairment of plasticity itself.

There was one class of experiments that obviated this difficulty, namely measuring the effects on plasticity of direct activation of the neuromodulator system in the adult visual cortex, after the time when developmental plasticity has essentially ceased to be detectable. If the chemically identified, non-specific sensory system was involved in some way with cortical plasticity, one would expect such experiments to be involved in a restoration of cortical plasticity. Modest plastic changes of this kind have been observed in the normal visual cortex of young adults, the assay for plastic change being ocular dominance of cortical neurons after a prolonged period of monocular lid suture (e.g. four weeks in 26- or 35-week-old cats (Jones *et al.* 1984) and three months in 8–12-month-old cats (Daw *et al.* 1992)). While a wholesale

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ocular dominance shift was generally not observed in these experiments, significant changes in binocularity took place compared with the unaltered pattern of ocular dominance observed in a control cortex. These plastic changes in ocular dominance in the adult cortex are rapidly facilitated, within one week or even less when the eye occlusion is accompanied by NA activation of the modulating system (Pettigrew & Kasamatsu 1978).

To date, plasticity restoration experiments have only been successful by manipulations of the NA–β-adrenoreceptor system (Kasamatsu *et al.* 1985; Heggelund *et al.* 1987; Imamura & Kasamatsu 1988, 1991*a*,*b*; Mataga *et al.* 1992). Since this system is linked to intracellular second messenger pathways through adenosine 3′,5′-monophosphate (cAMP) accumulation, it belongs to a well-recognized class of modulators for plasticity whose mechanisms have been explored at many levels including gene expression controlled by cAMP response element-binding proteins (CREBs; e.g. Frank & Greenberg 1994; Yin & Tully 1996).

In the present study, the paradigm of plasticity restoration was used to investigate these second messenger systems downstream of the β-adrenoreceptor. We found significant restoration of visual cortical plasticity if brief monocular deprivation of adult cats was combined with administration of agents that stimulate the cAMP cascades. Although modest, the plastic changes in ocular dominance that we report here are both consistent and significant and strongly support the thesis that the NA system acts to facilitate visual cortical plasticity. Preliminary reports have appeared elsewhere (Kasamatsu 1980, 1986).

2. MATERIAL AND METHODS

A total of 25 normal adult cats were used (table 1). The surgical procedures were in accordance with NIH guidelines for the care of experimental animals (National Institutes of Health Committee on Care and Use of Laboratory Animals 1985). The experimental protocols were approved by the institutional animal care and use committees.

Two types of cholera toxins, subunits A and B (ChT-A and ChT-B), forskolin hydrochloride and dibutyryl cAMP (dbcAMP) were purchased from Sigma Chemical Co. (St Louis, MI) and water-soluble forskolin, i.e. forskolin 7β-deacetyl-7β-[γ-(morpholino) butyryl] hydrochloride, from Research Biochemicals International (Boston, MA). Their concentrations were as follows: 0.01 or 0.1 mg ml^{-1} ChT-A and ChT-B in 0.4% ascorbate saline (pH 3), 1.0 mM forskolin in 20% dimethyl sulphoxide (DMSO) solution, 100 μM water-soluble forskolin in Ringer's solution and 10 or 100 μM dbcAMP in Ringer's solution.

Each of these solutions or respective vehicles was continuously infused (Alzet minipump, 1 μl h^{-1}) through a cannula made of a hypodermic needle (30 G) implanted in the visual cortex (stereotaxic coordinates: posterior 5.0 mm, lateral 2.0 mm and subcortical depth < 1.5 mm) under ketamine anaesthesia (20 mg kg^{-1}) following the method described previously (Kasamatsu *et al.* 1981*a*; Kasamatsu & Schmidt 1997). For adult cats infused with forskolin for four weeks, the implanted cannula was kept at the same site and the discharged minipump was replaced with a freshly loaded minipump three times.

The eyelids of one eye of the experimental animals were sutured shut to induce changes in ocular dominance (Wiesel & Hubel 1963). The timing of lid suture coincided with the implantation of a cannula–minipump assembly containing the experimental–vehicle solution. The animals were i.m. injected with an antibiotic (4 mg kg^{-1} gentamicin or ampicillin) every 24 h for five days after the surgery.

Following the standard procedures in our laboratory (e.g. Kasamatsu *et al.* 1981*b*, 1985, 1998; Imamura & Kasamatsu 1991*a*), single-unit recording was carried out mostly with tungsten-in-glass microelectrodes (Levick 1972) and occasionally with glass capillaries filled with 2% pontamine sky blue in 0.5 M sodium acetate (Imamura *et al.* 1993). During physiological recordings the animals were anaesthetized with a gas mixture (N_2O:O_2:CO_2 = 75:22.5:2.5), supplemented by 2 mg kg^{-1} h^{-1} pentobarbital (Pentothal i.v.) and paralysis was maintained by continuous i.v. infusion of 10 mg kg^{-1} h^{-1} gallamine triethiodide (Flaxedil). In addition, the infusion solution contained 70 mg kg^{-1} h^{-1} glucose, 0.1 mg kg^{-1} h^{-1} dexamethasone, distilled water (two-thirds of the total volume) and sterile lactated Ringer's (one-third of the total volume). The infusion rate was 2.4–3.3 ml h^{-1}. The vital signs of the animal were continuously monitored and maintained as follows: rectal temperature 37.5 °C, partial pressure of CO_2 of the expired gas 3.5 ± 0.5%, heart rate < 200 min^{-1} and synchronized cortical EEG. The animal also received a bolus i.m. injection of an antibiotic (100 mg streptopenicillin or 4 mg kg^{-1} gentamicin) every 24 h.

The implanted cannula–minipump assembly was removed at the beginning of recordings. Microelectrode penetrations were usually started in a region *ca.* 1.5 mm from the midline and 1.5–3.5 mm anterior to the cannulation site. Each microelectrode track was angled 5° medially and 10° anteriorly from the vertical to allow the microelectrode to cross many laminar and ocular dominance boundaries.

Thirty visually active cells, with a few exceptions (table 1), were recorded, usually along a single recording track (*ca.* 3.5 mm or longer), but occasionally two. We also kept records of visually unresponsive cells and axonal units of lateral geniculate nucleus origin that were encountered along a given recording track. This practice helped us to evaluate the normality of the recorded cortical region. Receptive-field properties of single cells were studied with stationary flashing or moving light slits or dark bars of various sizes. They were projected onto a tangent screen, at 57 cm from the cat's eye, through a rear projection system. The intensity of the light slits was 0.5–1.0 log units above the background luminance, which was kept at a mesopic level (*ca.* 1.0 cd m^{-2}).

First, the orientation range, best orientation, direction selectivity, velocity preference and the location and size of the minimum response fields (Barlow *et al.* 1967) were determined manually. Then, the ocular dominance of each cell was determined according to the seven-group scheme of Hubel & Wiesel (1962). We repeatedly compared responses evoked by stimulation of the two eyes separately before reaching a final decision as to the cell's ocular dominance group. It usually took *ca.* 10 h to obtain a sample of 30 visually responsive cells. Finally, we measured both the spontaneous spike discharge per 10 s for at least 1 min and visually elicited activity every 200 ms for five trials. The ocular dominance index (ODI), as defined by the following equation (Macy *et al.* 1982), was calculated from the peak response of each of 115 cells recorded from two cats (table 1, experiment E):

$$\mathrm{ODI} = \text{ipsilateral response}/(\text{contralateral response} + \text{ipsilateral response}).$$

Table 1. *Animals in the five types of experiments* (*A–E*)

(Age refers to that at the start of each experiment. Infusion (1 μl h^{-1}) of the drug plus vehicle or the vehicle solution alone lasted for one or four weeks, concurrently with monocular lid suture, if applicable. *n* and *B* refer, respectively, to the total number of visually responsive cells recorded in each experimental condition and the corresponding binocularity index, i.e. the proportion of the number of binocular cells (groups 2–6 of Hubel & Wiesel (1962)) to the total number of visually responsive cells per histogram. WS refers to the weighted shift index (see text for calculation).)

		eyelid suture recorded				cortical infusion		measurements		
experiments	animal	age	eye	duration	hemisphere	drug	concentration	*n*	*B*	WS
A	S272	23 weeks	right	1 week	left	ChT-A	0.01 mg ml^{-1}	30	0.43	0.32
A	S262	31 weeks	right	1 week	right	ChT-B	0.01 mg ml^{-1}	30	0.57	0.58
A					left	ChT-A	0.01 mg ml^{-1}	30	0.37	0.39
A	S132	>2 years	left	1 week	right	ChT-A	0.1 mg ml^{-1}	30	0.37	0.61
A					right (remote)	ChT-A	0.1 mg ml^{-1}	30	0.67	0.55
A					left	ascorbate saline	0.4%	30	0.67	0.37
B	922	>1 year	right	1 week	left (remote)	forskolin	1 mM	30	0.77	0.57
B					left	forskolin	1 mM	30	0.27	0.61
B	923	>1 year	right	1 week	right	—	—	30	0.67	0.52
B					left	forskolin	1 mM	30	0.37	0.58
B	A3	>9 years	right	1 week	left (remote)	forskolin	1 mM	30	0.67	0.59
B					left	forskolin	1 mM	30	0.23	0.72
B	S60	>1 year	right	4 weeks	left (remote)	forskolin	1 mM	30	0.63	0.58
B					left	forskolin	1 mM	30	0.17	0.56
B	748	>3 years	right	4 weeks	left (remote)	forskolin	1 mM	30	0.73	0.44
B					left	forskolin	1 mM	30	0.27	0.31
B	A69	>9 years	right	4 weeks	left	forskolin	1 mM	30	0.30	0.61
C	B234	29 weeks	right	1 week	left	H_2O-soluble forskolin	100 μM	47	0.21	0.58
C	B241	30 weeks	left	1 week	right	H_2O-soluble forskolin	100 μM	18	0.33	0.46
C	Cat7	>8 years	right	1 week	left	DMSO	20%	30	0.77	0.55
D	173	>2 years	right	1 week	left	dbcAMP	100 μM	30	0.23	0.31
D					right	—	—	29	0.62	0.59
D	128	>2 years	right	1 week	left	dbcAMP	100 μM	30	0.33	0.39
D					right	ascorbate saline	0.4%	30	0.70	0.61
D	508	>3 years	right	1 week	left	dbcAMP	100 μM	30	0.20	0.55
D					right	—	—	30	0.80	0.47
D	A60	>3 years	right	1 week	left	dbcAMP	100 μM	30	0.13	0.61
D	179	>2 years	right	1 week	left	dbcAMP	100 μM	28	0.39	0.68
D	S730	46 weeks	right	1 week	left	dbcAMP	10 μM	30	0.40	0.66
D					right	—	—	30	0.60	0.36
D	S731	46 weeks	right	1 week	left	dbcAMP	10 μM	30	0.37	0.34
D					right	ascorbate saline	0.4%	30	0.70	0.44
D	695	>1 year	right	1 week	left	dbcAMP	10 μM	30	0.47	0.44
D					right	—	—	30	0.83	0.47
E	S367	>1 year	—	—	left	forskolin	1 mM	60	0.82	—
E	183	>7 years	—	—	left	DMSO	20%	60	0.72	—
E	F-23	>1 year	—	—	left	dbcAMP	100 μM	30	0.57	—
E					right	ascorbate saline	0.4%	30	0.83	—
E	0667T	>4 years	—	—	left	dbcAMP	100 μM	60	0.60	—
E					right	ascorbate saline	0.4%	30	0.67	—
E	A33	>10 years	—	—	left	dbcAMP	100 μM	60	0.75	—

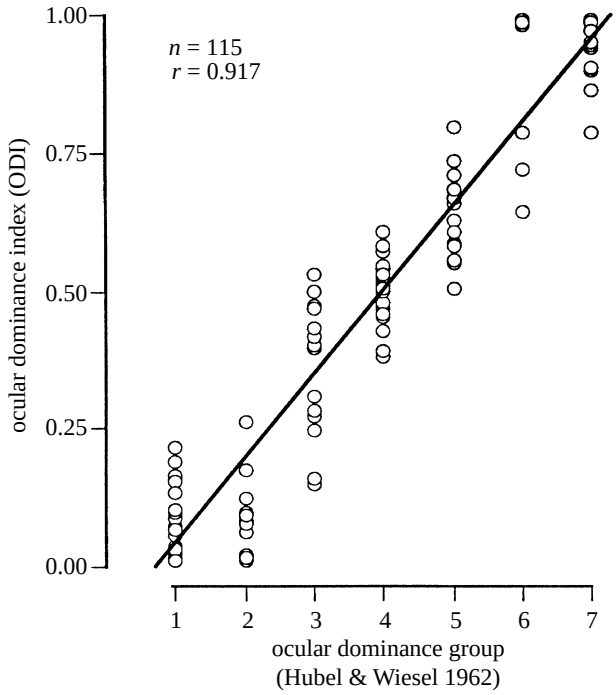

Figure 1. Measurement of ocular dominance. The ocular dominance index (ODI) was defined by the following equation: ODI = ipsilateral response/(contralateral response + ipsilateral response), where each response was calculated from the record of integrated responses for every 200 ms and its peak value was averaged over five trials. There was a significant correlation (115 neurons, $r = 0.917$ and $p < 0.0001$) between the ODI based on registered spike counts and the ocular dominance group (Hubel & Wiesel 1962) determined subjectively during recordings. See also table 1, experiment E.

These values were compared with the ocular dominance groups subjectively assigned to individual cells during recordings. The comparison clearly indicated a strong correlation between the two types of measurements (figure 1; correlation coefficient $r = 0.917$ and $p < 0.0001$), showing the appropriateness of the ocular dominance classification used in the present study.

Two indices were calculated to measure changes in ocular dominance in a given ocular dominance histogram. Binocularity (B) was the proportion of binocular cells of groups 2–6 to the total number of visually responsive cells recorded per histogram. The weighted shift (WS) was calculated as follows:

$$WS = ([D_3]+5/6[D_2]+4/6[D_1]+3/6[EQ]+2/6[ND_1]+1/6[ND_2])/([D_3]+[D_2]+[D_1]+[EQ]+[ND_1]+[ND_2]+[ND_3]),$$

where D_3 is the number of cells driven exclusively by the deprived eye, ND_3 is the number of cells driven exclusively by the non-deprived eye, EQ is the number of cells driven equally by both eyes and D_2, D_1, ND_2 and ND_1 are intermediates (Gordon *et al.* 1990). This index is equal to *zero* when all cells are driven exclusively by the non-deprived eye and *one* when all cells are driven exclusively by the deprived eye, having intermediate values for other conditions in between. In the case of recording without monocular deprivation, the WS values were calculated as if the contralateral eye had been closed. B is a conservative estimate of ocular dominance changes in general. While the WS is more sensitive, it operates under some premise such that ocular dominance shift occurs by successively changing from one ocular dominance group to the next in the given direction. Whenever necessary, changes in the two indices were statistically tested using Student's t-test.

A microlesion or a spot of dye injection was made at the end of each recording track by use of DC current of appropriate strength. The animals were injected with a lethal dose of Nembutal and perfused transcardially with saline followed by phosphate-buffered 10% formalin. Cortical tissue was frozen, sectioned at 50 µm and stained for Nissl substances. In some cases, sections were reacted for cytochrome oxidase using a modified version of the original procedure (Wong-Riley 1979). Recording tracks were reconstructed mostly on Nissl-stained sections. In cytochrome oxidase-reacted sections, layer IV was seen as a continuous, dark band differentiating itself from the supra- and infragranular layers. In Nissl-stained sections, the laminar differentiation was made based on Otsuka & Hassler's (1962) criteria.

3. RESULTS

(a) *Cholera toxin infusion*

To study the involvement of G_s-proteins in ocular dominance plasticity (ODP), an activator of G_s-proteins, ChT-A (*ca.* 28 kDa; Gill 1977), was directly infused into the visual cortex of three cats concomitantly with monocular deprivation for one week (table 1, experiment A and figure 2*a*). First, it is noteworthy that almost all visually responsive cells recorded in the present study were normal in having the usual selectivity in orientation–direction (monocular stimulation, data not shown). The incidence of visually unresponsive cells (class U in ocular dominance histograms) along a given recording track was relatively low here. The value is usually *ca.* 5% in the normal cortex (Shirokawa & Kasamatsu 1986; Imamura & Kasamatsu 1991*a*).

In a region close (< 3.5 mm) to the infusion centre of 0.01 or 0.1 mg ml^{-1} (*ca.* 0.4 or 4 µM) ChT-A, two types of changes were noted (figure 2*a*): (i) strongly reduced binocularity ($B = 0.39 \pm 0.04$), and (ii) a sign of ocular dominance shift towards the open eye ($WS = 0.44 \pm 0.15$). These values were significantly (mean ± s.d. $B = 0.75 \pm 0.10$, 95% confidence intervals $0.81 \geqslant B \geqslant 0.69$ and 5% rejection intervals $0.98 \geqslant B \geqslant 0.53$; Kasamatsu *et al.* 1985) or relatively ($WS = 0.50 \pm 0.08$; Gordon *et al.* 1990) smaller than the respective normal values. The incidence variability of cells remaining binocular was low as indicated by the small s.d. values shown at the top of the middle five columns in the ocular dominance histograms. In contrast, in the control recording from either a remote region (*ca.* 6 mm) of the same ChT-A-infused hemisphere, the opposite hemisphere infused with ChT-B (binding subunit with no biological action) or the hemisphere infused with the vehicle solution alone, many binocular cells were recorded as usual. In the resulting ocular dominance histograms, the binocularity ($B = 0.64 \pm 0.06$) and weighted shift ($WS = 0.50 \pm 0.11$) were both close to the respective measures obtained from normal cats (figure 2*b* and table 1, experiment A). In short, these results indicated that cortical infusion of ChT-A could rapidly render the adult visual cortex susceptible again to the effect of brief monocular deprivation, though to an extent far less than that usually obtained in kittens.

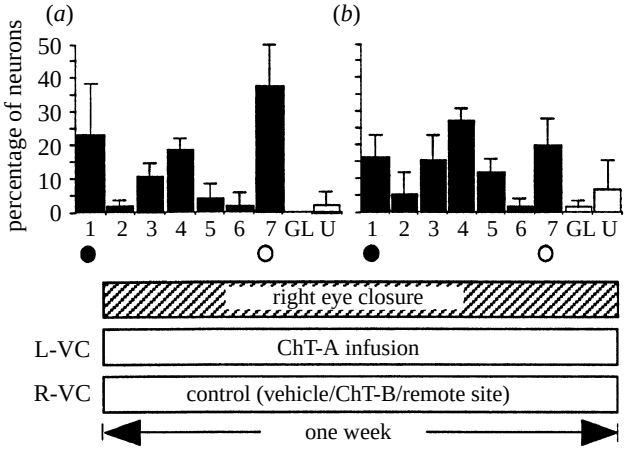

Figure 2. Effects of cortical infusion for one week of ChT-A combined with monocular deprivation on ocular dominance in the adult visual cortex. Average ocular dominance histograms were obtained from six electrode tracks at six loci in area 17 of three cats (table 1, experiment A). (*a*) This composite histogram was derived by averaging three ocular dominance histograms obtained in a region 2.5–3.5 mm from the centre of continuous infusion with 0.01 or 0.1 mg ml^{-1} ChT-A throughout one week of monocular deprivation (see bottom inset). $n=3$ cats, $N=90$ cells and $B=0.39\pm0.04$ (mean ± s.d.). (*b*) Control histogram obtained from the same three cats either *ca.* 6 mm anterior to the ChT-A infusion site (remote), *ca.* 3 mm from a ChT-B infusion site or *ca.* 3 mm from a cortical site infused with 0.4% ascorbate saline. The latter two were in the hemisphere (R-VC) opposite to the ChT-A infusion (L-VC). $n=3$ cats, $N=90$ cells and $B=0.64\pm0.06$. See table 1, experiment A. Each original histogram contained 30 visually active cells ($N=30$), which were recorded along mostly one but occasionally two recording tracks. The ocular dominance of each cell was determined based on the seven-group scheme (Hubel & Wiesel 1962). GL and U indicate unit activity of lateral geniculate axon origin and visually unresponsive units, respectively. The vertical thin bar at the top of each column refers to one standard deviation (s.d.) or the range of the mean in each ocular dominance group or GL and U classes. Note that s.d.s are generally small in the present study, with relatively large s.d.s for groups 1 and 7 monocular cells. Binocularity (B) was calculated as the ratio of the binocular cells of groups 2–6 to the total number of visually responsive cells per histogram. The eye contralateral to recording was deprived (filled circle) and the ipsilateral eye left open (open circle). The same convention for ocular dominance histograms also applies to others in figures 3–5.

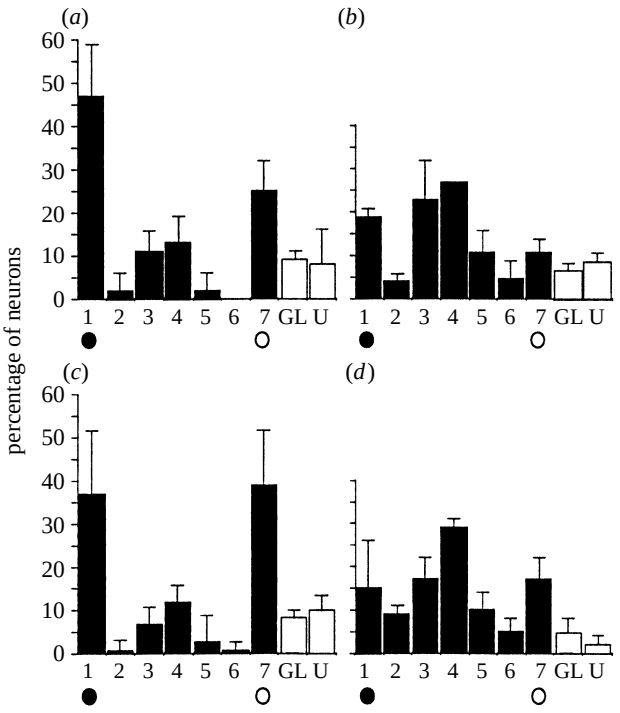

Figure 3. Effects of cortical infusion of 1 mM forskolin combined with monocular deprivation on ocular dominance in the adult visual cortex. Monocular deprivation was imposed for either (*a*, *b*) one week or (*c*, *d*) four weeks. For the latter the emptied osmotic minipump was replaced, using the same implanted cannula, with a freshly loaded one three times. (*a*, *c*) Histograms (1 mM forskolin) were obtained from data on cells recorded from a region 1.5–2.0 mm anterior to the infusion centre. (*b*, *d*) The recorded region was 3.5–5.0 mm anterior and, thus, served as the control (remote region). Note relatively large s.d.s for groups 1 and 7 monocular cells in the forskolin-affected region. In (*a*), it is also noted that the proportion of group 1 monocular cells is significantly larger than that of group 7 cells. See also table 1, experiment B. (*a*) $n=3$ cats, $N=90$ cells and $B=0.29\pm0.07$. (*b*) $n=3$ cats, $N=90$ cells and $B=0.70\pm0.05$. (*c*) $n=3$ cats, $N=90$ cells and $B=0.24\pm0.07$. (*d*) $n=2$ cats, $N=60$ cells and $B=0.68\pm0.06$.

(b) *Forskolin infusion*

Forskolin binds to the catalytic subunit of adenylate cyclase without involving G_s-proteins, thus directly activating the cyclase to enhance the production of cAMP (Seamon *et al.* 1981; Laurenza *et al.* 1989). We infused 1 mM forskolin directly into the visual cortex of three adult cats and studied how the effects of one-week-long monocular deprivation were modified (table 1, experiment B). We found that the average binocularity from a cortical region near the forskolin infusion site was significantly lower than that obtained from a distant region several millimetres from the infusion centre, which served as a control ($B=0.29$ versus $B=0.70$, $t=5.80$ and $p<0.005$; figure 3*a*,*b*). Reflecting the innate dominance of the contralateral eye (Hubel & Wiesel 1962; Blakemore & Pettigrew 1970), the proportion of group 1 monocular cells was remarkably larger than that of group 7 cells. However, there was no significant difference in the degree of shift in ocular dominance between the two distributions (WS = 0.64 versus 0.56). The extent of these changes was comparable with that obtained with ChT-A infusion.

In another three cats, the forskolin infusion and concurrent monocular deprivation were maintained for four weeks. Despite this prolongation of the combined treatment, the extent of binocularity reduction was not much enhanced ($B=0.24$ and WS = 0.49; figure 3*c*). In fact, both indices were indistinguishable from the respective values ($B=0.29$ and WS = 0.64; figure 3*a*) obtained by monocular deprivation for one week ($t=0.87$ and 1.49, and $p<0.44$ and <0.22, respectively). However, the above-mentioned dominance of group 1 cells over group 7 cells was lost, indicating the start of change in the 'right' direction by the prolongation of the combined treatment. We thus concluded that, near the site of cortical infusion with 1 mM forskolin, the initial changes in ocular dominance were attained by one week, probably followed by a long tail towards further shift.

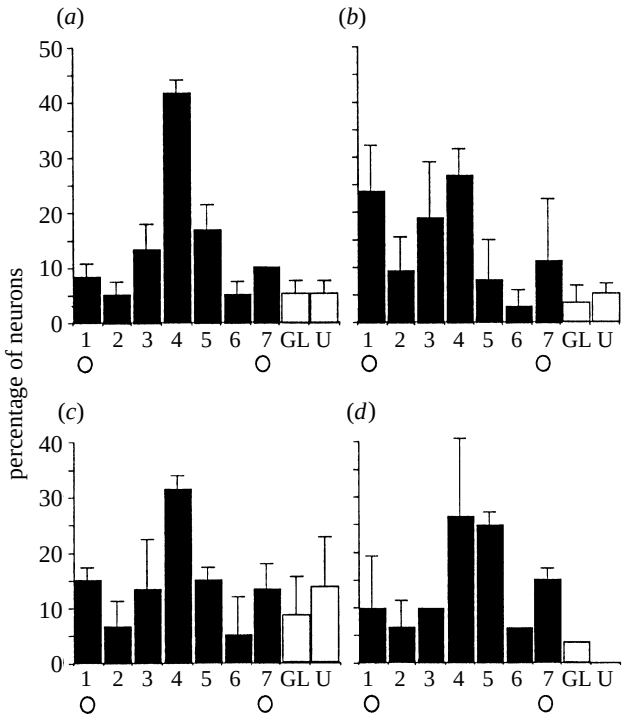

Figure 4. Four types of control experiments with forskolin, dbcAMP or the respective vehicle solutions alone infused into the normal visual cortex of adult cats, without monocular deprivation (i.e. no experimental manipulation of vision): (*a*) 1 mM forskolin in 20% DMSO, $n = 2$ tracks, $N = 60$ cells and $B = 0.82$; (*b*) 100 μM dbcAMP in 4% ascorbate saline, $n = 3$ tracks, $N = 150$ cells and $B = 0.64$; (*c*) 20% DMSO in Ringer's alone, $n = 2$ tracks, $N = 60$ cells and $B = 0.72$; (*d*) 4% ascorbate in saline alone, $n = 2$ tracks, $N = 60$ cells and $B = 0.75$. The four composite ocular dominance histograms in (*a–d*) are all full of normal binocular cells. See also table 1, experiment E.

The following control experiments were carried out on five cats. First, we infused a forskolin analogue, water-soluble forskolin (100 μM), concurrently with monocular deprivation for one week (table 1, experiment C). This compound induced a substantial reduction in binocularity ($B = 0.27$) comparable to that obtained with 1 mM forskolin ($B = 0.29$; figure 3*a*). The ocular dominance distribution became W-shaped (WS = 0.52; data not shown). Infusion of the vehicle solution alone (20% DMSO in Ringer's), concomitant with one-week-long monocular deprivation, did not affect the ocular dominance distribution at all ($B = 0.77$ and WS = 0.55; data not shown). Another experiment controlled the effects of forskolin infusion itself on the ocular dominance distribution (table 1, experiment E). When 1 mM forskolin was infused for one week into a normal adult cortex, without involving monocular deprivation, no discernible changes were found in ocular dominance ($B = 0.82$; figure 4*a*). The resulting ocular dominance distribution was quite normal as found in a cortex infused with 20% DMSO alone ($B = 0.72$; figure 4*c*). In short, when combined with brief monocular deprivation, cortical infusion of forskolin significantly reduces binocularity in the adult visual cortex without a clear shift in ocular dominance towards the open eye, though the contralateral eye dominancy is reduced.

(c) *dbcAMP infusion*

The above studies with ChT-A and forskolin both strongly indicated that the continuous activation of adenylate cyclase rapidly resulted in the partial restoration of cortical susceptibility to monocular deprivation in the adult visual cortex. We wanted to ascertain that this restoration of plasticity, albeit partial, was due to a sustained increase in cAMP in the drug-infused adult cortex. To test this likelihood, we infused dbcAMP directly into the visual cortex of eight adult cats concomitant with monocular deprivation (table 1, experiment D). Cortical infusion of dbcAMP was expected to facilitate the intracellular accumulation of cAMP in the drug-affected region. The infusion of 100 μM dbcAMP ($n = 5$) strongly reduced the binocularity ($B = 0.26$) and the ocular dominance distribution became W-shaped (WS = 0.51) when combined with monocular deprivation for one week (figure 5*a*). In contrast, normal ocular dominance histograms with high binocularity ($B = 0.71$ and WS = 0.56) were consistently found in the control hemisphere opposite the dbcAMP infusion (figure 5*b*). The difference in the two hemispheres is striking.

When the concentration of dbcAMP was reduced tenfold to 10 μM ($n = 3$), a significant decrease in binocularity ($B = 0.41$, $t = 4.00$ and $p < 0.05$) was still found in the W-shaped histogram (WS = 0.48; figure 5*c*). Again, the normal ocular dominance distribution was obtained from the hemisphere opposite the drug-infused one ($B = 0.71$ and WS = 0.42; figure 5*d*). Needless to say, 100 μM dbcAMP in the normal visual cortex produced little change in ocular dominance ($B = 0.64$; figure 4*b* and table 1, experiment E). Ascorbate saline infused into a normal cortex did not cause ocular dominance changes either ($B = 0.75$; figure 4*d* and table 1, experiment E).

(d) *Further controls*

The cAMP-related drugs used in the present study may change the excitability of the cortex (Dunwiddie *et al.* 1992; Boulton *et al.* 1993). There is a concern that the above-mentioned changes in ocular dominance might be mediated more through the increased excitability by infused drugs rather than the enhancement of ODP *per se.* However, this possibility is highly improbable since (i) even following long monocular deprivation for three months the ocular dominance distribution is no longer changeable in the mature visual cortex of one-year-old cats (Daw *et al.* 1992), (ii) the majority of adult cats used in the present study were one year old or older, and (iii) there is no known correlation between high excitability of cortical cells and their plasticity in both normal and drug-treated animals, if an inverse relation is the case (Shaw & Cynader 1984).

However, to verify the matter independently, 100 μM dbcAMP was infused in the normal cortex of an additional three cats for one week without involving monocular deprivation (table 1, experiment E). In two out of the three, we measured the spontaneous firing rate as an indicator of the general excitability of the drug-infused visual cortex. The spontaneous activity of 30 cells along recording track 1 was 4.7 ± 4.3 spikes s^{-1} (mean ± s.d.). This mean value was not different from that obtained from another 30 cells along track 2 in the opposite, control hemisphere (4.4 ± 5.4 spikes s^{-1}, $t = 0.27$ and

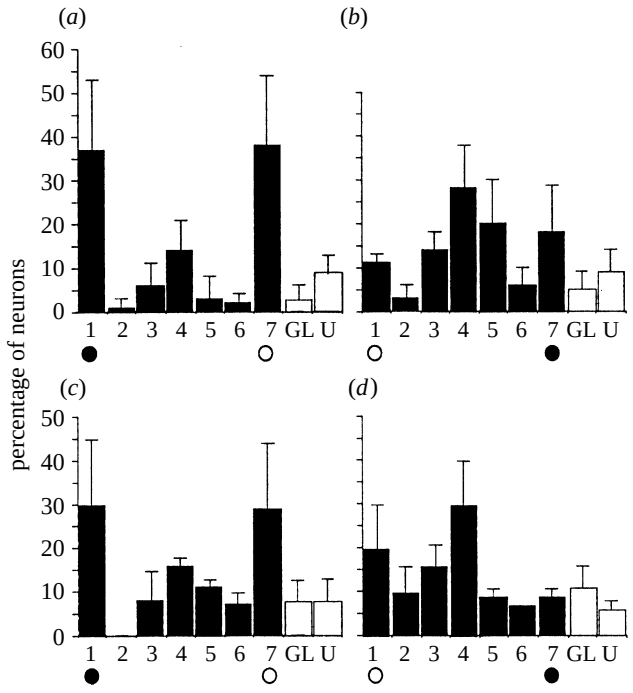

Figure 5. Effects of dbcAMP infusion combined with monocular deprivation on ocular dominance of neurons in the adult visual cortex. (*a*) Composite ocular dominance histogram from experimental hemispheres directly and continuously infused with 100 μM dbcAMP, $n = 5$ cats, $N = 148$ cells and $B = 0.26 \pm 0.10$. (*b*) Control histogram obtained by recording from the corresponding regions in the opposite hemispheres, $n = 3$ cats, $N = 89$ cells and $B = 0.71 \pm 0.09$. In (*c*) 10 μM dbcAMP was used, $n = 3$ cats, $N = 90$ cells and $B = 0.41 \pm 0.05$. (*d*) Control recording for 10 μM dbcAMP, $n = 3$ cats, $N = 90$ cells and $B = 0.71 \pm 0.12$. Note relatively large s.d.s for groups 1 and 7 monocular cells in the dbcAMP-infused hemisphere. See also table 1, experiment D.

$p < 0.80$). These neurons were recorded from layers II/III (14.7%), IV (26.5%) and V/VI (58.8%) in track 1 and layers II/III (16.7%), IV (20.0%) and V/VI (63.3%) in track 2. A similar analysis in the second cat also showed no significant difference in the spontaneous activity between the dbcAMP-infused and control hemispheres: 2.5 ± 6.1 spikes s^{-1} for dbcAMP and 1.5 ± 3.4 spikes s^{-1} for the vehicle solution. These results indicate that there was no significant change in the spontaneous firing rate immediately (1–10 h) after one week of continuous infusion of 100 μM dbcAMP. We also noted no marked changes in receptive field properties including the response vigour, suggesting that 100 μM dbcAMP infusion left little change in the excitability of cortical neurons immediately after stopping the infusion.

4. DISCUSSION

(a) *Modest changes induced in the adult cortex*

All three drugs used here, when combined with brief monocular deprivation, decreased binocularity significantly. Nevertheless, the extent of ocular dominance shift remained mostly unchanged in the drug-infused adult cortex, showing U- or W-shaped histograms. They are an initial sign of the binocular system's reaction to monocular deprivation. This interpretation is consistent with that of early physiological data obtained from the kitten visual cortex following brief monocular deprivation (Hubel & Wiesel 1970; Olson & Freeman 1975; Mioche & Singer 1989) and in the kitten visual cortex recovering from the effect of monocular deprivation, with concurrent cortical infusion of NA or 6-hydroxydopamine (6-OHDA) (Kasamatsu *et al.* 1981*b*). A similar trend was also noticeable in the visual cortex ipsilateral to the open eye of several-year-old cats following monocular deprivation for three months (left side of fig. 4 in Daw *et al.* (1992)).

The U-shaped distribution indeed emerged in a computational study on ocular dominance column formation and the ocular dominance distribution of single cells (Tanaka 1991). In this model, the U-shaped distribution appeared whenever binocular afferents impinging on cortical cells were not well correlated yet balanced in their strength, as probably occurs shortly after the onset of monocular deprivation of the innately dominant, contralateral eye (Hubel & Wiesel 1962; Blakemore & Pettigrew 1970) in kittens, after long binocular lid sutre in kittens (Mower *et al.* 1981) and after long monocular deprivation under weak adult plasticity (Daw *et al.* 1992).

Infusing ChT-A or dbcAMP at the same concentrations used here, we obtained a clear shift in ocular dominance towards the experienced eye in the kitten cortex that had been initially treated by 6-OHDA infusion (K. Imamura and T. Kasamatsu, unpublished data). A comparison suggests that the extent of the restored plasticity by the activation of cAMP-dependent protein kinase A (PKA) is more limited in the adult than kitten visual cortex, though both share the same neurochemical mechanisms (see below).

(b) *Desensitization of cAMP-mediated processes*

Besides feedback inhibition by intracellular Ca^{2+} (Cooper *et al.* 1995), adenylate cyclase is dually controlled by heterotrimeric G-proteins, stimulatory G_s-proteins and inhibitory G_i-proteins. Agonist activation of G_s-protein-coupled receptors, including β-adrenoreceptors, leads to stimulation of adenylate cyclase and an increase in intracellular cAMP. Activation of inhibitory G_i-protein-coupled receptors, such as α_2-adrenergic receptors, does the opposite. Persistent activation of adenylate cyclase inevitably invites desensitization in cAMP cascades. The desensitization is further expressed through the CREB-mediated downregulation of β_2-adrenoreceptor mRNA, for example. This downregulation may also be accompanied by an increase in both inhibitory G_i-protein expression and α_2-receptor activation, as shown in some cell lines (e.g. Hadcock & Malbon 1993).

The signal transduction initiated by cAMP increase may reach a maximum rate in the first few hours of drug infusion, followed by desensitization. Thus, the final outcome attained by one-week-long infusion of cAMP-related drugs cannot be directly correlated with an increase in the cAMP intracellular concentration. This may explain why four weeks of monocular deprivation accompanied by the continuous forskolin infusion produced a plasticity-enhancing effect only slightly stronger than that seen in one week (figure 3).

(c) *cAMP-dependent regulation of ODP*

Recently, Reid *et al.* (1996) claimed that the ontogeny of cAMP accumulated by the activation of metabotropic glutamate receptors (mGluRs) in the cat visual cortex was closely correlated with the temporal profile of the physiologically determined sensitive period.

However, there are several problems in their interpretations.

(i) The ontogeny curves obtained from two animals per age group were directly compared with the physiological time-course of ODP in brief monocular deprivation (Olson & Freeman 1975). However, this sample size was too small and no variability measurements were presented.

(ii) Then a close correlation was noted between increases in cAMP by both mGluR-agonist (ACPD, [IS,3R]-1-amino-1,3-cyclopentane-dicarboxylic acid) activation and the basal level of cAMP and physiologically determined ODP. However, the per cent increases in cAMP level by ACPD are close to each other at three ages before nine weeks of age, though the basal cAMP level peaked at five weeks of age. Then, without further evidence, it was suggested that 'before 9 weeks of age, the basal cAMP production is the prime factor' and that 'after 9 weeks of age, other factors such as the quantities of mGluRs are the main contributors for the decline of ACPD-stimulated cAMP level and the decline of plasticity' (Reid *et al*. 1996, p. 7621).

(iii) The pharmacological dissection of receptor subtypes underlying the above effects of ACPD-stimulated cAMP elevation failed, despite known potency of quisqualate, to activate group 1 type mGluRs.

(iv) A direct test of the correlation between physiologically determined ODP and ACPD-activated cAMP accumulation also failed: an antagonist of mGluRs, α-methyl-4-carboxyphenylglycine (MCPG), infused into the visual cortex did not block ODP (Hensch & Stryker 1996).

This negative finding with antagonist MCPG, however, does not necessarily exclude a possible contribution of mGluRs to ODP, since the coactivation of β-adrenoreceptors and mGluRs increases the cAMP pool *in vivo* (Gereau IV & Conn 1994). mGluR activation is reported to counteract the calcineurin-mediated dephosphorylation of NMDA receptors directly (Raman *et al*. 1996).

(d) *cAMP-dependent PKA system: common process*

In the aplastic visual cortex of adult cats, cortical infusion of either ChT-A, forskolin or dbcAMP caused significant changes in ocular dominance when combined with brief monocular deprivation. ChT-A and forskolin activate adenylate cyclase and dbcAMP increases the intracellular cAMP pool. This restoration of ODP is most probably mediated by an elevation of intracellular cAMP (Rosenberg & Li 1995). The present findings are consistent with earlier data such as partial restoration of the cortical susceptibility to monocular vision either by endogenous NA released in response to electrical stimulation of the locus coeruleus (Kasamatsu *et al*. 1985) or by exogenous NA directly infused into the adult visual cortex (Heggelund *et al*. 1987).

Taken together, they suggest that an increase in the intracellular cAMP pool in the visual cortex necessarily enhances ODP (e.g. Kasamatsu 1987, 1994). This conclusion promotes a view that the cAMP-dependent PKA system is a key step in the regulation of ODP, common to the kitten and cat visual cortex. This is also consistent with the plasticity-enhancing role of cAMP-dependent PKA proven in rapidly increasing numbers of studies in hippocampal long-term potentiation, a model of memory formation (Stanton & Sarvey 1985; Hopkins & Johnston 1988; Dunwiddie *et al*. 1992; Frey *et al*. 1993; Chavez-Noriega & Stevens 1994; Huang *et al*. 1994; Weisskopf *et al*. 1994; Wu *et al*. 1995; Huang & Kandel 1996; Thomas *et al*. 1996; Frey & Morris 1997) and in *Drosophila* learning mutants (e.g. Davis 1996).

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