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EFFECTS OF NERVE GROWTH FACTOR ON NEURONAL PLASTICITY OF THE KITTEN VISUAL CORTEX

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SUMMARY

1. The effect of intraventricular administration of nerve growth factor (NGF) by means of a cannula–minipump system was studied in kittens monocularly deprived during the critical period. The ocular dominance of area 17 neurones of NGF-treated and control kittens was determined by conventional extracellular recordings. The soma size of cells in A and A1 laminae of the lateral geniculate nucleus (LGN) was also evaluated in Cresyl Violet preparations.

2. Binocularly responsive neurones were found to be significantly more numerous in NGF-treated than in control kittens. The shrinkage of cells from the deprived LGN laminae normally observed in control kittens was prevented by NGF administration.

3. Following an initial period of monocular deprivation (MD) kittens subsequently treated with NGF showed a substantial recovery of functional binocular connections.

4. These findings indicate that the administration of NGF during the period of deprivation reduces the amblyopic effects of MD, while its administration to kittens with both eyes open following the initial deprivation promotes recovery of the deprived eye.

5. Neurotrophic factors may contribute to the regulation of experience-dependent modifications of synaptic connectivity in the visual cortex.

INTRODUCTION

Monocular visual experience in early postnatal life, the so-called critical period, leads to profound functional reorganization of visual cortical areas. One of the most striking examples of these cortical modifications is the shift of ocular dominance of area 17 neurones in favour of the normal eye, accompanied by an almost total loss of excitability of cortical cells with stimulation of the deprived eye and by a shrinkage of cells in the deprived laminae of the lateral geniculate nucleus (LGN) (Wiesel & Hubel, 1963*a*, *b*; Sherman & Spear, 1982).

A number of recent studies have demonstrated the fundamental role of neural activity in the regulation of developmental connections in the visual cortex (Shatz,

1990). These experiments were based in part on the concept of the Hebbian synapse. According to this hypothesis (Hebb, 1949), it has been suggested that modifications of neural activity related to the deprived eye (DE) might cause a lack of correlation between pre- and postsynaptic activation. This would lead, in turn, to a diminished synaptic efficiency and, eventually, complete loss of function. In contrast, a co-activation of the pre- and postsynaptic elements would lead to the strengthening of synapses (Stent, 1973). The manner in which neural activity regulates synaptic connections is, however, not known. Activation of the *N*-methyl-D-aspartate (NMDA) receptor seems to be one of the steps necessary for experience-dependent modifications in the visual system (Artola & Singer, 1987).

A potential additional factor to be considered is the interaction between neural and neurotrophic activities. It is well known that target cells provide limited amounts of specific neurotrophic molecules to innervating neurones; each axonal terminal must acquire sufficient neurotrophic factor for its maintenance, otherwise it is eliminated (Purves, 1988). It can, therefore, be hypothesized that geniculo-cortical afferents from the two eyes are in competition (Wiesel & Hubel, 1963*a*, *b*: Guillery & Stelzner, 1970; Guillery, 1972), perhaps for a neurotrophic factor produced in limited amounts by cortical cells. We recently tested this hypothesis in the rat visual system and showed that repetitive administrations of nerve growth factor (NGF) (Levi-Montalcini, 1987) prevented the effects of monocular deprivation (MD) in the visual cortex (Domenici, Berardi, Carmignoto, Vantini & Maffei, 1991).

The present study was designed to test if the amblyopic effects of MD could also be prevented by NGF treatment in the cat, which represents a far better characterized model for studies of visual cortical plasticity. We also addressed the question of whether, following the period of MD, subsequent administration of NGF would restore the normal functionality of afferents from the DE. To investigate this latter point, kittens monocularly deprived for 3 and 5 weeks were allowed a period of normal binocular vision and NGF treatment.

We report here the following results: (1) the administration of NGF during the period of MD reduces the amblyopic effects of MD and (2) following the initial period of MD the subsequent administration of NGF promotes the functional recovery of the deprived eye. Some of these results have been presented in abstract form (Carmignoto, Camella, Candeo & Comelli, 1991).

METHODS

Subjects and animal preparation

Experiments were performed in thirty-three kittens bred in our colony. Kittens (28–37 days old; see Table 1) were monocularly deprived by lid suture (7–0 Ethilon suture, SSC, Switzerland) under aseptic conditions and deep anaesthesia (halothane 4% in a mixture of nitrous oxide 70% and oxygen 30%). Drops of local anaesthetic (procaine 0·4%, Novesina, Sandoz, Italy) were given before and immediately after surgery. In the same surgical session a cannula–minipump perfusion system was implanted as follows. A small hole was made in the skull (AP+6 mm, L 1 mm; see Snider & Niemer, 1961), and a cannula connected to an osmotic minipump (Alzet 2002, Alza, USA), positioned subcutaneously under the neck, was lowered through it 6 mm below the dura to reach the ventricle. The cannula was then secured to the skull with acrylic cement (methacrylic resin swebond compact, Swedia, Sweden). The minipump was filled with either NGF or cytochrome c (cyt c), each at 0·5 μg μl^{-1}. The rate of drug delivery was 0·5 μl h^{-1}. To ensure a double-blind procedure in NGF-treated kittens, a black silk wire was ligated to the cannula segment inserted in

the minipump. During recording the minipump was left in place and only at the end of the recording session was the history of the animal revealed by inspection of the minipump. The sutured eye of deprived animals was carefully inspected every day for any small openings. Kittens included in this study never showed openings of the DE at any time.

For recording, animals were deeply anaesthetized with halothane (4%) in a mixture of nitrous oxide (70%) and oxygen (30%), an endotracheal tube was inserted, the cannula (but not the minipump) removed and a small hole in the skull made at the stereotaxic coordinates of the area centralis. After cutting the dura, the animal was paralysed with an intravenous injection of Pavulon (pancuronium bromide, Organon, Holland) and artificially ventilated. Pavulon was then infused at the rate of 0·2–0·3 ml kg^{-1} h^{-1}. P_{CO_2} (3·8–4·2%), EEG and heart rate (ECG, Biotach, Gould Inc., Cleveland, OH, USA) were continuously monitored. Throughout recording sessions an adequate level of anaesthesia was obtained with halothane (0·5–1·0%) in nitrous oxide (70%) and oxygen (30%). Cardiac accelerations in response to a noxious stimulus were not observed during experiments. Body temperature was maintained at 37 °C with a heating pad. At the end of the surgical procedure the operated areas were infiltrated with local anaesthetic (Neolidocaton, lidocaine 2%, Pharmaton, Switzerland). After insertion of the electrode a solution of agar–agar in saline was used to maintain cortical temperature and prevent drying of the cortex.

The DE was reopened at the beginning of the recording session. Pupils were dilated with atropine sulphate (Visumidriatic 1%, MSD, USA) and nictitating membranes retracted with phenilephrine (Isonefrine, Allergan, Italy). Optically neutral lenses with artificial pupils of 3 mm diameter (Galileo, Italy) were applied to protect corneas, and refraction corrected with additional lenses placed in front of the eye, as necessary. At the beginning of the experiment, positions of the papillae and areae centrales were determined using the technique described by Fernald & Chase (1971).

Preparation of NGF

The β-subunit of NGF was prepared from adult male mouse submandibular glands according to the method of Bocchini & Angeletti (1969). The biological activity of the purified NGF, evaluated using dissociated embryonic chicken dorsal root ganglion neurones *in vitro* (Skaper & Varon, 1982), was in the range of 1–2 ng protein per trophic unit.

Recording procedures

Extracellular action potentials were recorded from single units with tungsten microelectrodes (Digitimer, England). Potentials were conventionally filtered, amplified and audiomonitored. Penetrations were made down the medial bank of the postlateral gyrus, so that the microelectrode penetrated tangentially to the surface of the cortex, passing across the ocular dominance columns. A window discriminator was used to isolate single unit activity, as necessary. The electrode was often angled at 10–15 deg along the vertical meridian. Cells were sampled every 100–150 μm. Each cell was carefully classified for orientation selectivity and ocular dominance. Once the cell was isolated the optimal orientation, direction and drifting velocity of the stimulus that elicited the maximal response was presented alternately to each eye and the relative response determined from the audiomonitor. The electrode was then advanced at least 100 μm from the previously classified cell, the preferred orientation of the background activity determined and a new cell isolated by moving the electrode further. Each cell was assigned to one group of the seven-point scale following Hubel & Wiesel (1962).

Visual evoked potentials (VEPs) in response to sinusoidal gratings of various spatial frequency and contrast (Campbell, Maffei & Piccolino, 1973) were recorded in one monocularly deprived kitten treated with NGF by means of a 3 M NaCl-filled glass pipette inserted into the hemisphere ipsilateral to the DE. The visual acuity of each eye was obtained with square-wave gratings of maximum contrast. A detailed description of such recording procedures was previously reported (Bisti & Carmignoto, 1985).

Histology

At the end of the experiment the penetration was marked for its construction. Two electrolytic lesions (10–20 μA for 5 s) were made along the track during the retraction of the electrode. The animal was killed by an overdose of sodium pentobarbitone, the skull was opened and two blocks

of both left and right occipital cortices were dissected out and fixed by immersion in a Bouin's solution for 12–24 h at room temperature. After dehydration in a graded series of ethanols, tissue blocks were embedded in paraffin (Paraplast, Monoject Scientific Inc, Athy, Ireland). Coronal sections (10 μm) were collected and stained with Cresyl Violet. To analyse the effect of MD on the

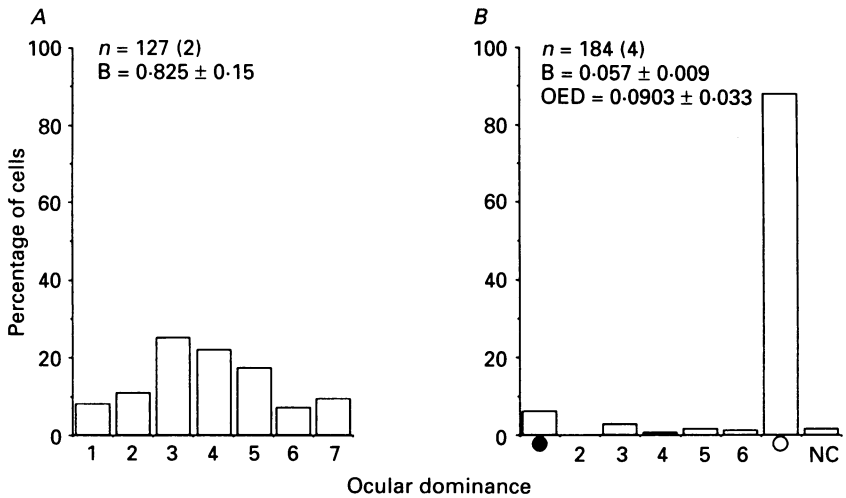

Fig. 1. Normalized ocular dominance (OD) histograms of area 17 neurones recorded in two normal three-month-old kittens (*A*) and in four kittens monocularly deprived for 2 weeks at about 5 weeks of age (*B*). Neurones in OD class 1 and 7 are monocularly responsive; neurones in classes 2–6 are responsive to stimulation of either eye. The class labelled NC indicates neurones that are unresponsive to visual stimuli. Also indicated are the number of neurones and, in parentheses, the number of kittens recorded. Mean values ± S.E.M. of the index of binocularity (B) and of the index indicating the degree of dominance by the normal open eye on cortical neurones (OED) in monocularly deprived kittens are also reported. An OED value of one means that all cells recorded are monocularly responsive to stimulation of the open eye. See Methods for the detailed definition of these indices. ●, deprived eye; ○, normal eye.

soma size of cells of the LGN, four additional kittens were subjected to MD at 33 days after birth and treated with either NGF ($n = 2$) or cyt c ($n = 2$). Following two weeks of MD kittens were killed by an overdose of sodium pentobarbitone and both right and left LGN were dissected out, fixed in Bouin's solution and then paraffin embedded. Coronal sections (10 μm) were serially collected and Cresyl Violet stained. Measurements of soma diameter of cells from the A and A1 laminae were made using camera lucida drawings of cell profiles (final magnification 1460 ×) and an Ibas-1 image analysis system. Three different levels corresponding to 15, 45 and 70 % of the LGN extension were analysed. Only cells with a clearly visible nucleus and nucleolus were drawn.

Data analysis

Binocularity which provides a measure of binocular connectivity, is defined as the number of cells in ocular dominance groups 2, 3, 4, 5 and 6 divided by the total number of visually responding cells. We also calculated the open eye dominance (OED) in the hemisphere contralateral to the open eye as follows: OED = (number of group 1 cells) + 2/3 (number of group 2 cells) + 1/3 (number of group 3 cells) divided by the total number of visually responding cells. The statistical significance of the differences between groups was evaluated with Student's t test. Differences were considered significant at $P < 0{\cdot}05$.

RESULTS

Effects of NGF on monocularly deprived kittens

Figure 1 shows the normalized ocular dominance (OD) histograms of area 17 neurones recorded in two normal kittens (*A*) and in four kittens subjected to MD by lid suture (*B*). Two weeks of MD led to the expected OD shift; most neurones were

TABLE 1. Rearing conditions and physiological findings for each monocularly deprived kitten used in this study

Kitten	DE	Age at deprivation (days)	Duration of MD (days)	Responsive cells (no.)		Binocularity		OED	
				Ipsi	Contra	Ipsi	Contra	Ipsi	Contra
N1	—	—	—	34	35	0·765	0·914	—	—
N2	—	—	—	31	27	0·839	0·777	—	—
C1	R	34	15	29	28	0·0	0·143	0·965	0·786
C2	R	28	26	20	36	0·0	0·111	1·0	0·741
C3	R	31	26	—	33	—	0·030	—	0·989
C4	L	35	15	—	35	—	0·057	—	0·914
T1	R	28	13	33	33	0·545	0·333	0·515	0·963
T2	R	28	16	26	38	0·923	0·737	0·333	0·281
T3	R	30	17	41	37	0·415	0·513	0·691	0·595
T4	R	34	19	—	41	—	0·463	—	0·545
T5	L	35	14	27	36	0·296	0·528	0·691	0·435
CT1	R	37	14	—	32	—	0·125	—	0·812
CT2	R	28	17	34	32	0·029	0·156	0·980	0·844
CT3	R	31	20	24	31	0·125	0·097	0·930	0·849
CT4	R	34	16	26	23	0·115	0·043	0·910	0·970

Values of binocularity and open eye dominance (OED) are reported for cells recorded in the hemicortex ipsi- and contralateral to the DE. Animal code: N, non-deprived untreated; C, MD untreated; T, MD NGF-treated; CT, MD cyt c-treated. R, right eye; L, left eye.

responsive only to stimulation of the normal eye (NE) with very few neurones remaining binocularly responsive. History and OD of individual animals are reported in Table 1.

In the first series of experiments, the effect of a continuous infusion of NGF on the OD shift that occurs after 2 weeks of MD was studied. NGF was intraventricularly administered through a cannula connected to an osmotic minipump. This procedure was chosen because in the rat repetitive intraventricular injections were as effective as local applications (Domenici *et al.* 1991) and, with respect to intracortical infusion, it avoids damage to cortical areas near the visual cortex (Paradiso, Bear & Daniels, 1983). Figure 2 shows the normalized OD values for monocularly deprived kittens injected with NGF, compared with cyt c-injected littermates. In the NGF-treated monocularly deprived kittens the OD shift was greatly attenuated, with more than 50% of visually responsive neurones being binocularly responsive. As no differences were found between recordings from the hemispheres ipsilateral and contralateral to the DE (Table 1), results obtained from the two hemispheres were pooled. Although a certain degree of variability was present, the effect of NGF was observed in all animals tested (Fig. 3*A*; Table 1). In contrast, cyt c was completely ineffective (Figs

2*B* and 3*B*; Table 1): control cyt c-treated kittens displayed a large OD shift towards the open eye which was indistinguishable from that of untreated monocularly deprived kittens (Fig. 1). When the mean percentage of binocularly responsive cells and the mean value of the open eye dominance (OED) (see Methods) for NGF- and

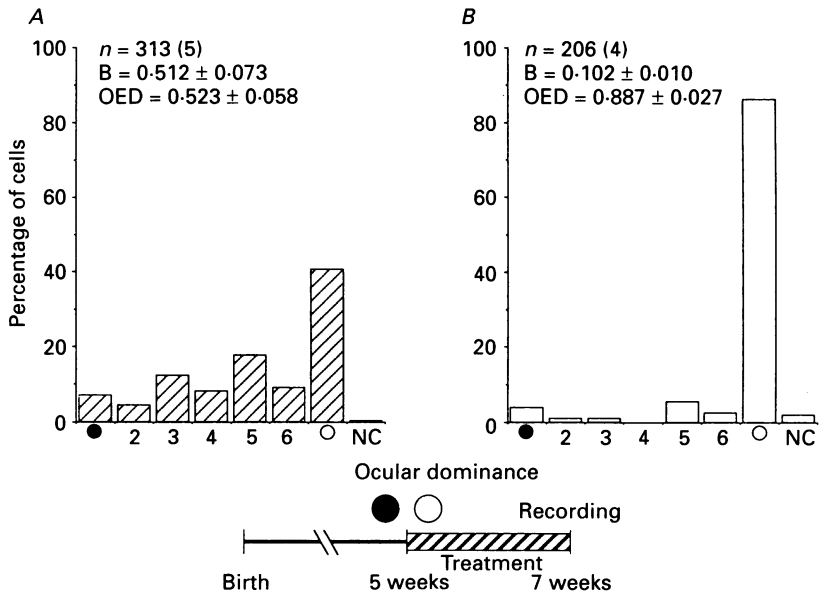

Fig. 2. Normalized OD histograms of neurones recorded from area 17 of monocularly deprived kittens that received intraventricular infusion of either NGF (*A*) or cyt c (*B*) at a dose of 0·5 μg h^{-1}. The history of the kittens is displayed in schematic form beneath the histograms. Conventions and symbols as in Fig. 1.

cyt c-treated monocularly deprived kittens were compared large differences were evident (Fig. 4). It is clear from these data that intraventricular administration of NGF partially prevents the OD shift in monocularly deprived kittens. In order to evaluate the possibility that this effect of NGF was due to an aspecific alteration of cell responsiveness, the following experiments were performed. In two kittens (T1 and T5) the infusion cannula was not removed prior to onset of recording. Recording from these kittens was, therefore, performed during the infusion of NGF. Responsiveness of area 17 neurones was not found abnormal, either in terms of orientation selectivity or spontaneous activity. In addition, visually evoked potentials (VEPs) in response to gratings of different spatial frequencies and contrast (Cambell *et al.* 1973) were evaluated in one monocularly deprived kitten treated with NGF (kitten T4). As Fig. 5 shows, VEP amplitude from the DE does not differ from that of the normal eye at all spatial frequencies tested. Values of the visual acuity for the two eyes are also similar (3·4 and 3·6 cycles deg^{-1} for the DE and NE, respectively). These results indicate that the spatial frequency tuning curve obtained from this monocularly deprived kitten treated with NGF is indistinguishable from that observed in normal untreated cats (Cambell *et al.* 1973).

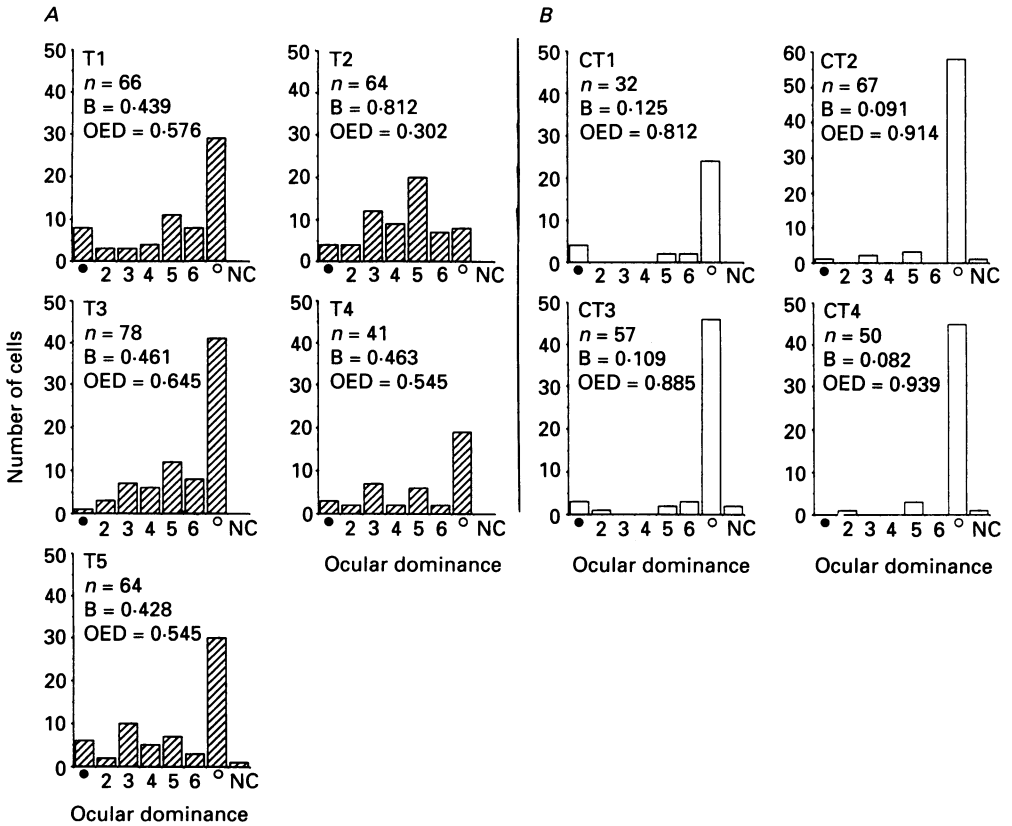

Fig. 3. Ocular dominance histograms from individual kittens in NGF- (*A*) and cyt c- (*B*) treated groups. Neurones recorded from the hemispheres ipsi- and contralateral to the DE are pooled, values from each hemisphere being reported in Table 1. Animal code, B and OED values are indicated above each histogram. Conventions and symbols as in Fig. 1.

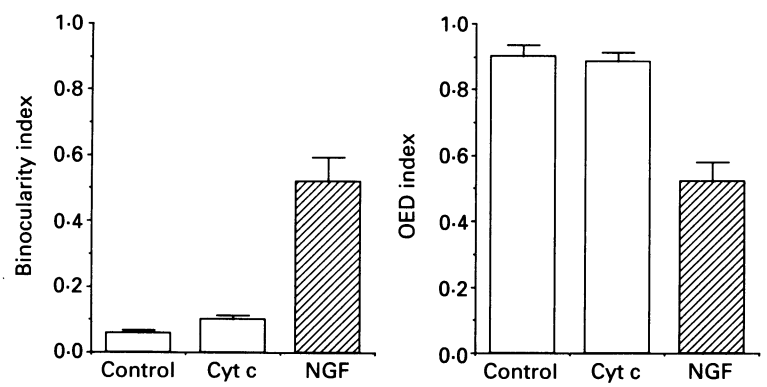

Fig. 4. Means ± S.E.M. of binocularity and OED indices calculated from control untreated, cyt c- and NGF-treated kittens. All kittens were monocularly deprived at about 5 weeks for 2 weeks (see Table 1). Mean values of both binocularity and OED in NGF-treated kittens differ significantly from those in control and cyt c-treated kittens ($P < 0·005$).

The effect of NGF at the level of LGN cells was also evaluated. Following MD cells receiving inputs from the DE are smaller than those receiving inputs from the NE (Wiesel & Hubel, 1963*c*; Guillery & Stelzner, 1970; Guillery, 1972). The soma size of cells from A and A1 laminae of two cyt c- and two NGF-treated monocularly

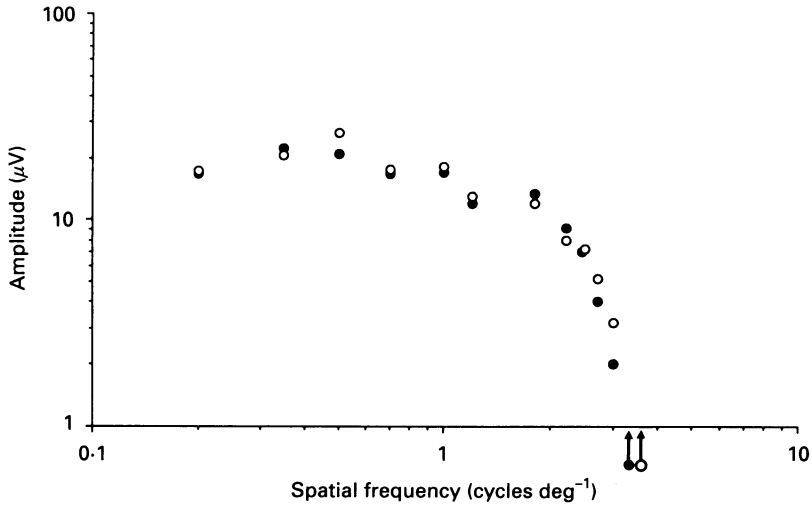

Fig. 5. Visually evoked potentials from one kitten (T3) monocularly deprived at 5 weeks and treated with an intraventricular infusion of NGF. Records were obtained from the hemisphere ipsilateral to the DE in response to the stimulation of the normal (○) and deprived (●) eye. The second harmonic amplitude of the response is plotted as a function of spatial frequency of the stimulus. Arrows indicate the visual acuity of the two eyes. The stimulus consists of vertical sinusoidal gratings of various spatial frequencies reversed in contrast at 6 Hz; contrast 0·18; mean luminance 10 cd m^{-2}. Noise level 0·7 μV.

deprived kittens was analysed in Cresyl Violet preparations. A representative example of the LGN contralateral to the DE from one cyt c- (Fig. 6*A*) and one NGF- (Fig. 6*B*) treated kitten is shown. In the cyt c- but not in the NGF-treated kitten cells from the deprived A lamina are smaller than cells from the non-deprived A1 lamina. Quantitative results are summarized in Fig. 7. By comparing the histograms from the deprived lamina with that of the non-deprived A lamina of the two cyt c-treated monocularly deprived kittens, it appears that cells within the deprived laminae are significantly smaller (mean soma diameter $\pm$ S.E.M. $14{\cdot}0 \pm 0{\cdot}04$ *vs.* $16{\cdot}3 \pm 0{\cdot}07$ μm; $P < 0{\cdot}05$; Fig. 7). The mean soma diameter of cells from the A1 deprived lamina is also significantly reduced compared with that of cells from the A1 non-deprived lamina ($14{\cdot}9 \pm 0{\cdot}18$ *vs.* $16{\cdot}7 \pm 0{\cdot}05$ μm; $P < 0{\cdot}05$; Fig. 7). In contrast, following NGF treatment the size of cells receiving projections from the DE is not significantly different from that receiving projections from the NE (mean soma diameter of cells from deprived and non-deprived A laminae: $15{\cdot}42 \pm 0{\cdot}05$ and $15{\cdot}5 \pm 0{\cdot}22$ μm, respectively; values from deprived and non-deprived A1 laminae: $15{\cdot}65 \pm 0{\cdot}08$ and $15{\cdot}83 \pm 0{\cdot}25$ μm, respectively).

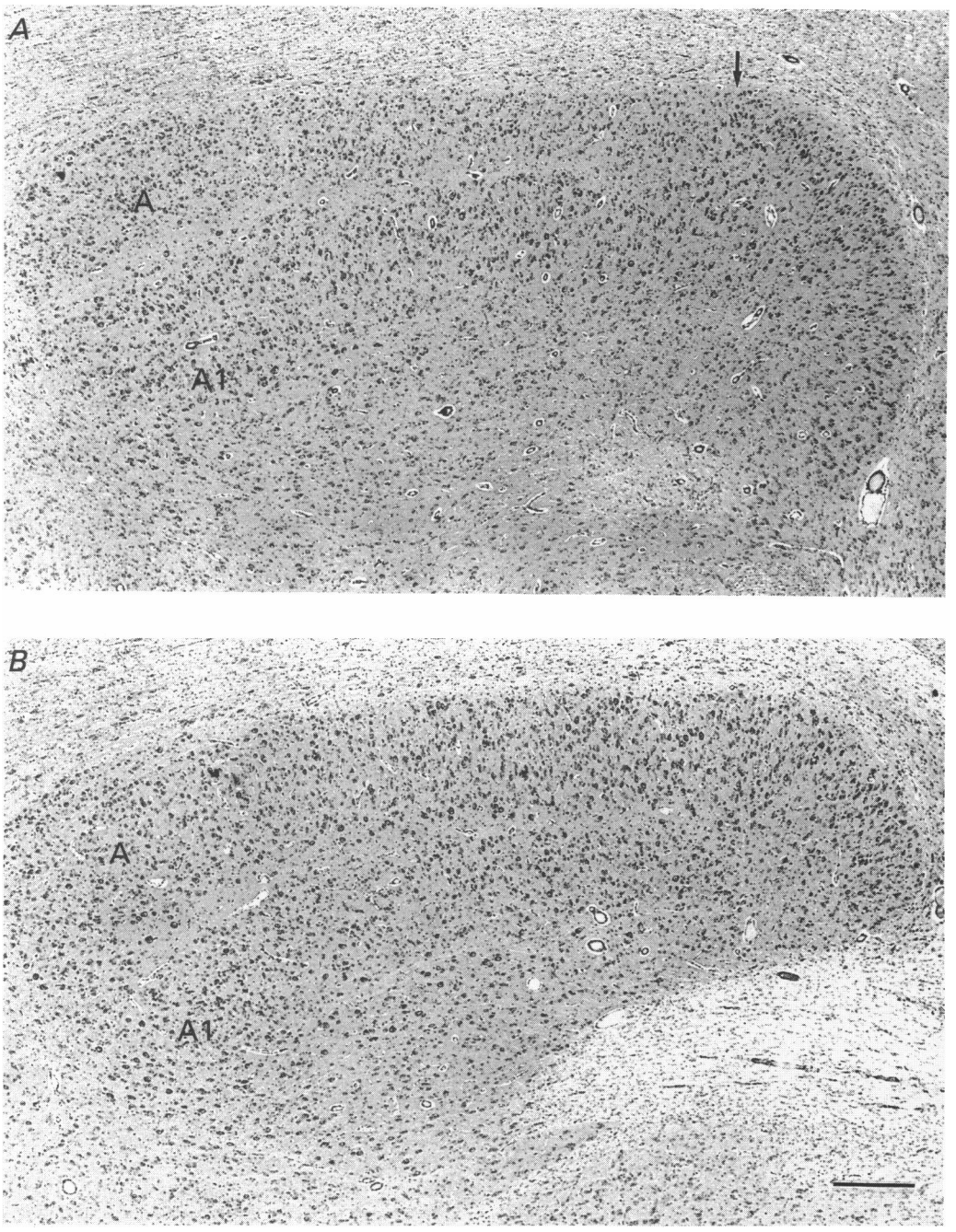

Fig. 6. Coronal sections of the dorsal LGN contralateral to the DE from one cyt c- (*A*) and one NGF- (*B*) treated kitten stained with Cresyl Violet. Cells from deprived lamina in cyt c- but not in NGF-treated kitten are clearly smaller than cells from non-deprived A1 lamina. Arrow in (*A*) indicates the border of the monocular segment. Scale bar represents 0·4 mm.

Effect of NGF on the recovery after MD

The effects of MD are reversible, provided that the DE is reopened and the originally NE is occluded within the critical period (Hubel & Wiesel, 1970; Blakemore & Van Sluyters, 1974). The second series of experiments was, therefore,

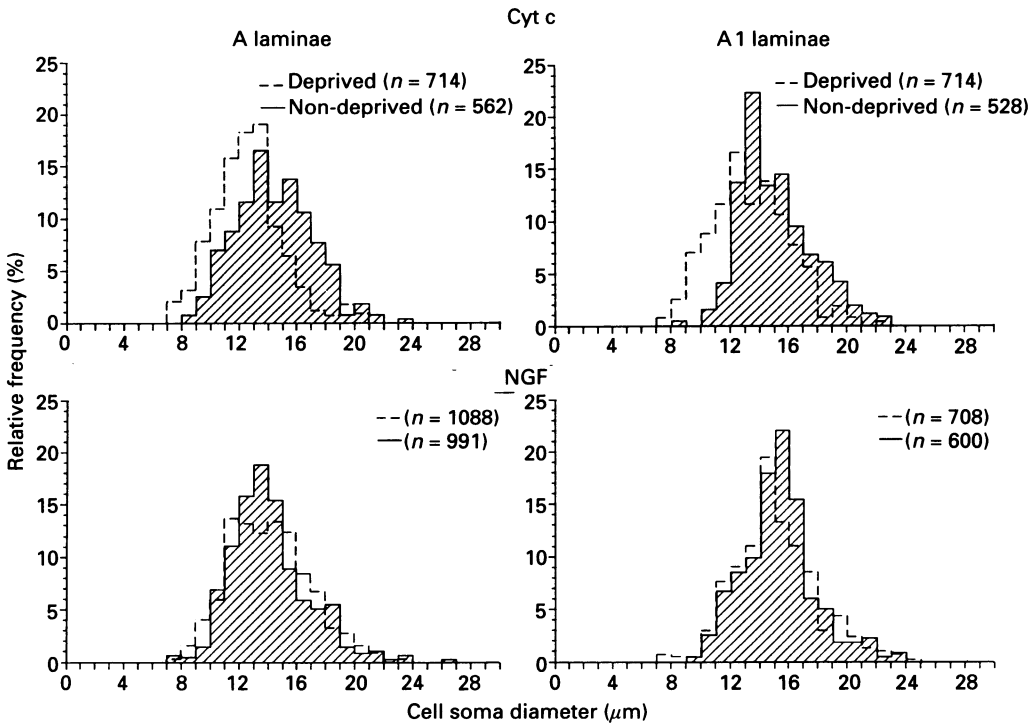

Fig. 7. Frequency histograms of soma diameter of cells from deprived (interrupted line) and non-deprived laminae (continuous line) of the LGN in two cyt c- and two NGF-treated monocularly deprived kittens. The total number of cells recorded in each group is reported above each histogram.

designed to investigate the ability of NGF to promote functional recovery of the DE without performing the reverse suturing. After 3 and 5 weeks of MD the DE was reopened and, at the same time, a cannula–minipump infusion system delivering either cyt c or NGF implanted. Kittens were then allowed 2 weeks of normal binocular vision before recording.

Following 3 weeks of MD, cyt c-treated kittens show a partial recovery of functional binocular connections (Figs 8*A* and 9*B*; Table 2). The potentiality for this recovery was dependent on the duration of the previous monocular visual experience, since after a more prolonged MD (5 weeks) the period of binocular vision did not result in any recovery: only 9·1% of visually responsive cells were binocularly responsive and the value of OED was high (Figs 8*B* and 9*D*; Table 2). These values were similar to those obtained in kittens recorded immediately after the period of MD (see Figs 1 and 2 for comparison).

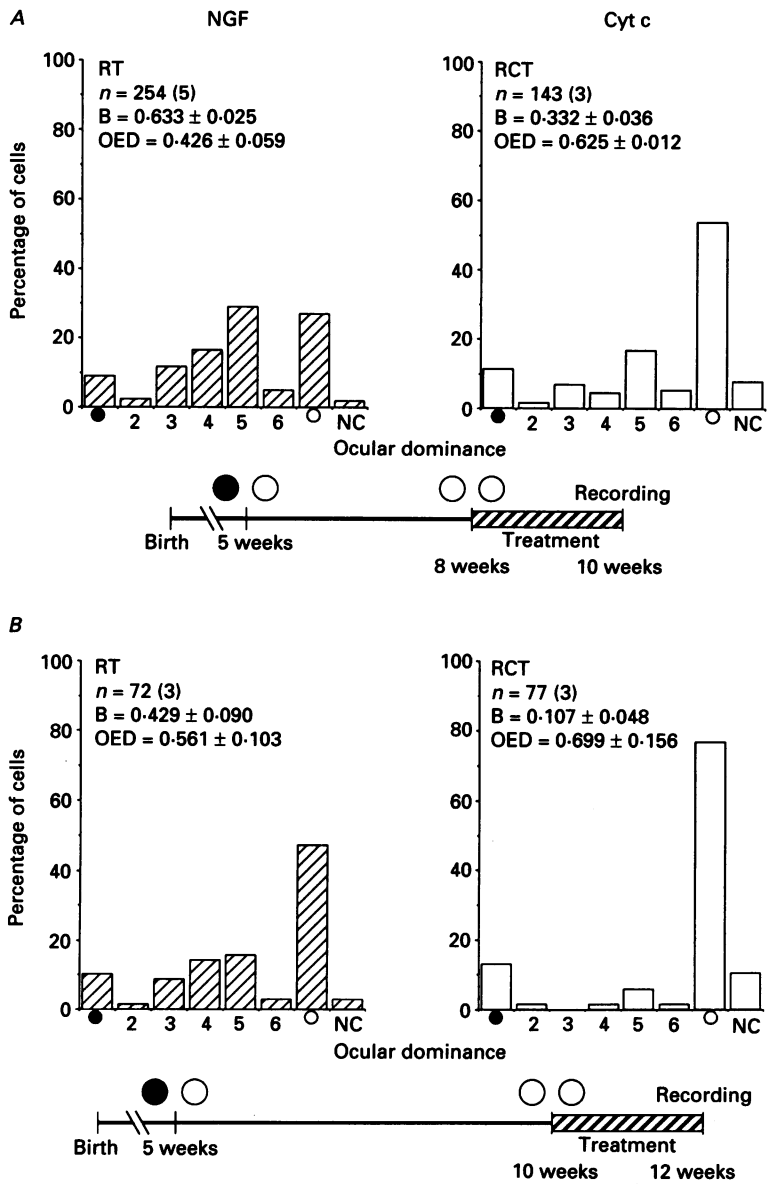

Fig. 8. Normalized ocular dominance histograms of area 17 neurons recorded from kittens that were monocularly deprived at about 5 weeks of age for 3 (*A*) or 5 (*B*) weeks and then allowed a period of normal binocular vision before assessing the changes in OD of area 17 neurones (see Table 2 for details). Treatment with either NGF or cyt c started at the beginning of the period of binocular vision. The mean value of binocularity in NGF-treated kittens differs significantly from that in cyt-c treated kittens both at 3 ($P < 0·001$) and 5 weeks ($P < 0·05$). The mean value of OED differs significantly only at 3 weeks ($P < 0·05$). Conventions and symbols as in Fig. 1.

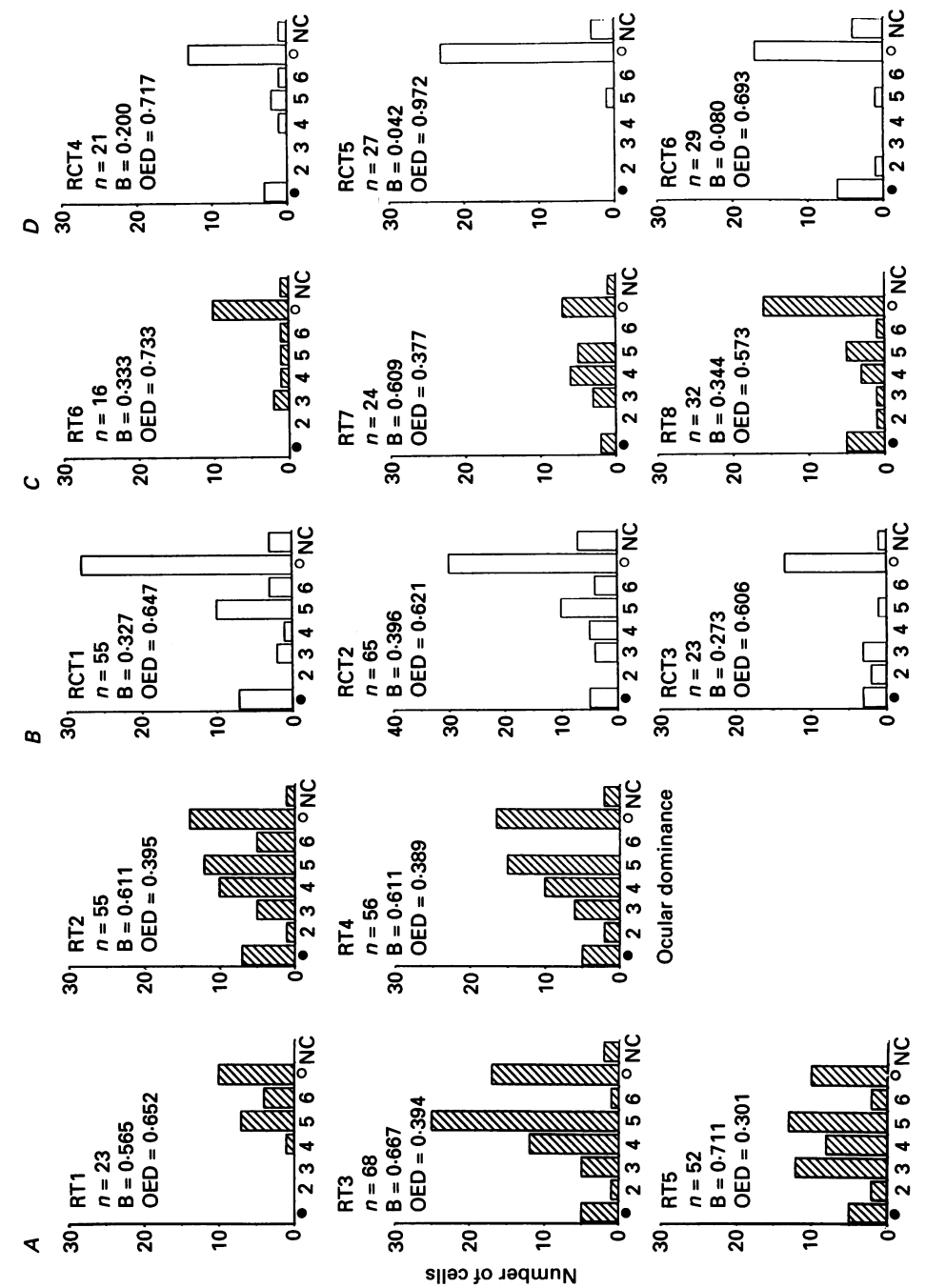

Fig. 9. Histograms of OD distribution of area 17 neurones from individual NGF- (*A* and *C*) and cyt c- (*B* and *D*) treated kittens that were raised with an initial period of MD and a subsequent period of normal binocular visual experience of 3 (*A* and *B*) and 5 (*C* and *D*) weeks. Neurones recorded from the hemisphere ipsi- and contralateral to the DE are pooled, values from each hemisphere being reported in Table 2. Conventions and symbols as in Fig. 1.

Administration of NGF allowed for the recovery of the DE following either 3 or 5 weeks of MD (Figs 8 and 9). Following 3 weeks of MD, NGF significantly enhanced the recovery of the DE (Figs 8*A* and 9*A*); furthermore, after 5 weeks of MD, NGF promoted a substantial recovery of binocular connections (Figs 8*B* and 9*C*), which was completely absent in monocularly deprived kittens treated with cyt c (Figs 8*B* and 9*D*).

TABLE 2. Rearing conditions and physiological findings for each of the kittens in the recovery group

Kitten	DE	Age at deprivation (days)	Duration of MD (days)	Duration of recovery (days)	Responsive cells (no.)		Binocularity		OED	
					Ipsi	Contra	Ipsi	Contra	Ipsi	Contra
RT1	R	37	21	16	—	23	—	0·565	—	0·652
RT2	R	36	22	15	26	29	0·731	0·500	0·346	0·440
RT3	R	36	22	16	35	31	0·686	0·645	0·409	0·376
RT4	R	36	23	20	19	35	0·526	0·657	0·421	0·371
RT5	R	36	23	22	22	30	0·682	0·733	0·257	0·333
RCT1	R	37	22	19	28	24	0·357	0·292	0·654	0·639
RCT2	R	28	26	18	27	31	0·370	0·419	0·580	0·656
RCT3	R	37	21	17	—	22	—	0·273	—	0·606
RT6	L	35	36	15	—	15	—	0·333	—	0·733
RT7	L	33	35	15	23	—	0·609	—	0·377	—
RT8	L	33	35	19	—	32	—	0·344	—	0·573
RCT4	L	33	35	16	—	20	—	0·200	—	0·717
RCT5	L	33	35	17	24	—	—	0·042	—	0·972
RCT6	L	33	35	18	—	25	0·080	—	0·693	—

The duration of recovery indicates the period (days) of normal binocular vision subsequent to the period of MD. Values of binocularity and open eye dominance (OED) are reported for cells recorded in the hemicortex ipsi- and contralateral to the DE. The treatment with either NGF (RT) or cyt c (RCT) was performed only during the period of binocular vision. R, right eye; L, left eye.

DISCUSSION

Effects of NGF on monocularly deprived kittens

The administration of NGF in kittens significantly reduced the effects of MD, i.e. the OD shift of area 17 neurones and the shrinkage of LGN cells in the deprived laminae. Although the cellular mechanism of the NGF effect remains to be elucidated, a plausible interpretation of these data is that NGF preserves the functional input from the DE to the primary visual cortex. A possible direct or indirect pathological effect of NGF on cortical neurones was investigated by analysing their response to visual stimuli. Specific properties of cortical neurones from monocularly deprived kittens during treatment with NGF, such as orientation selectively and spatial frequency tuning curve, either in kittens (this study) or in rats (Domenici *et al.* 1991), were indistinguishable from those observed in normal animals (Cambell *et al.* 1973; Maffei, 1978).

Two additional sets of observations strongly suggest that NGF did not induce pathological phenomena. First, experimental animals treated with NGF behaved quite normally. Second, recent results have shown that intraventricular administration of NGF in monocularly deprived kittens largely prevents loss of visual acuity of the deprived eye, as tested behaviourally (Maffei *et al.* 1991).

NGF and recovery of functional binocular connections

Monocular visual experience during the critical period causes a profound visual impairment of the DE. Although restoration of patterned visual stimuli to the DE results in a limited recovery of influences by the DE in the kitten visual cortex (Mitchell, Cyander & Movshon, 1977), far greater recovery can be obtained by the reverse suturing procedure which depresses activity of the originally non-deprived eye (Blakemore & Van Sluyters, 1974). In the present experiments the re-establishment of DE functional synapses suppressed by the effects of visual deprivation was also possible under normal binocular vision, provided that NGF was exogenously supplied. The combination of normal visual activity and the availability of neutrophic factor is likely to be necessary for recovery of functional binocular connections. Concerning the properties of electrical activity, we cannot indicate whether specific activity driven by patterned visual stimuli is necessary or whether spontaneous activity *per se*, such as that occurring in binocularly deprived animals, is sufficient to achieve the same degree of recovery. It is also possible that electrical activity is not necessary at all, with recovery being due, in this case, only to action of the neurotrophic factor. In this instance, recovery of vision should still take place even when the electrical activity of cortical afferents is blocked by tetrodotoxin administration. The question of interactions between electrical activity and neurotrophic factors is currently under investigation.

The potential use of NGF as a therapeutic drug has recently been proposed for several central nervous system degenerative pathologies (Hefti, Hartikka & Knusel, 1989). First, however, a series of problems related to the specific characteristics of the molecule need to be addressed. Once resolved, the observation that exogenous NGF favours the recovery of visual function following MD could be of potential clinical interest.

Possible role of NGF on visual cortical plasticity

The effect of NGF on visual cortical plasticity might be indirect, through the cholinergic neurones of the basal forebrain, a known NGF-sensitive population. These cholinergic neurones project to the visual cortex (Dinopoulos, Eadie, Dori & Parnavelas, 1989) and exert a modulatory effect on visual cortical plasticity, in that a reduction of their activity reduces the ocular dominance shift following MD (Bear & Singer, 1986). Since NGF is known to increase the cholinergic activity of this system (Gnahn, Hefti, Heuman, Schwab & Thoenen, 1983), the hypothesis of an indirect action of NGF through cholinergic neurones appears unlikely.

Another possibility could be that NGF decreases neuronal plasticity through some aspecific, as yet unknown, mechanism. The observation that NGF favours the recovery of the DE, a process which certainly implies a high degree of plasticity, seems to contradict this idea.

A third possibility is that NGF interacts directly with a specific neuronal population of the visual system. The hypothesis that the OD shift of area 17 neurones towards the open eye in monocularly deprived animals is due to competition between the afferents from the two eyes for NGF implies that geniculo-cortical axonal terminals express the NGF receptor. Recent evidence indicate that NGF receptor

mRNA encoding for the low-affinity NGF receptor, and related protein are indeed expressed in many visual system related nuclei of the rat, including the visual cortex (Yan & Johnson, 1989; Pioro & Cuello 1990; Carmignoto *et al.* 1991). We recently obtained evidence that in the lateral geniculate nucleus of the rat the low-affinity NGF receptor is exclusively related to retinal ganglion cell axonal terminals and not to intrinsic projecting neurones (Carmignoto, Candeo, Comelli, Calderini & Maffei, 1990). These immunocytochemical studies, however, give no information on expression of the high-affinity NGF receptor, which is essential for the biological action of NGF (Meakin & Shooter, 1991). Because NGF binding specificity is probably conveyed by the *trk* proto-oncogene product $p140^{trk}$, which is likely to correspond to the high-affinity NGF receptor (Klein, Jing, Nanduri, O'Rourke & Barbacid, 1991; Kaplan, Hempstead, Martin-Zanca, Chao & Parada, 1991), additional studies on the specificity of the NGF effects on different neuronal population using monoclonal antibodies raised against *trk* are needed.

Other NGF-like neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), at high concentrations can bind to the high-affinity NGF receptor and *vice versa* (Rodriguez-Tebar, Dechant & Barde, 1990; Ernfors, Ibanez, Ebendal, Olson & Persson, 1990). It cannot, therefore, be excluded that the amounts of NGF used here are sufficient to induce an interaction with the high-affinity form of another neurotrophic factor receptors with similar molecular characteristics, thereby mimicking the latter's action. Experiments using BDNF and NT-3 are necessary to clarify this point.

The notion that the *N*-methyl-D-aspartate (NMDA) receptor is involved in processes related to experience-dependent modifications of synaptic strengthening during development has gained considerable attention (Carmignoto & Vicini, 1993; Artola & Singer, 1987; for review see Rauschencker, 1991). The possibility that NGF affects visual cortical plasticity by interfering with NMDA receptor activation or with events which follow it should be considered. It has been reported that basic fibroblast growth factor (bFGF) is able to protect hippocampal neurones *in vitro* from the excitotoxic action of glutamate (Freese, Finkelstein & Di Figlia, 1991). Furthermore, bFGF, but not NGF, induced a marked reduction in the level of a 71 kDa subunit of the NMDA receptor expressed by these neurones in culture (Michaelis, Wang & Mattson, 1991). This suggests that neurotrophic factors could modulate synaptic efficiency by regulating the expression of neurotransmitter receptors.

The possibility of a reciprocal influence between neurotrophic factors and neuronal activity is also supported by recent results indicating that the expression of NGF mRNA and protein by neurones of the hippocampus and Purkinje cells of the cerebellum are elevated by limbic seizures, depolarizing pharmacological agents and excitatory neurotransmitters (Gall & Isackson, 1989; Zafra, Hengerer, Leibrock, Thoenen & Lindholm, 1990; Cohen-Cory, Dreyfus & Black, 1991; Lu, Yokoyama, Dreyfus & Black, 1991). It can be hypothesized that trophic interactions may be influenced by the level and/or pattern of impulse activity among neurons, as originally proposed by Purves (1988). In particular, acquisition of trophic support might represent a feedback mechanism triggered by the simultaneous activity of the pre- and postsynaptic elements. Modifications in the level of impulse activity related

to the DE or the lack of temporal correlation between pre- and postsynaptic activation could lead to a reduced availability of the trophic factor and, finally to a diminished synaptic efficiency.

In conclusion, although the cellular mechanism of NGF effects on visual cortical plasticity remains largely to be established, the possibility that NGF, or a NGF-like molecule, contributes to the functional modification of cortical connections gives new perspectives to future studies on neuronal cortical plasticity.

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