Nerve Growth Factor (NGF) Prevents the Shift in Ocular Dominance Distribution of Visual Cortical Neurons in Monocularly Deprived Rats

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The hypothesis that NGF could play a role in the plasticity of the developing mammalian visual cortex was tested in monocularly deprived (MD) rats. In particular, we have asked whether an exogenous supply of NGF could prevent the changes in ocular dominance distribution induced by monocular deprivation.

Hooded rats were monocularly deprived for 1 month, starting at postnatal day 14 (P14), immediately before eye opening, by means of eyelid suture. In eight rats, only monocular deprivation was performed; in eight rats, monocular deprivation was combined with intraventricular injections of β-NGF, and in three rats, with intraventricular injections of cytochrome C. Injections (2 μl) were given every other day for a period of 1 month.

Single neuron activity was recorded in the primary visual cortex of MD rats, MD rats treated with NGF, and MD rats treated with cytochrome C at the end of the deprivation period, and in normal rats of the same age.

We found that monocular deprivation caused a striking change in the ocular dominance distribution of untreated MD rats, reducing binocular cells by a factor of two and increasing by a factor of eight the number of cells dominated by the nondeprived eye. In MD NGF-treated rats, the ocular dominance distribution was indistinguishable from the normal. Cytochrome C treatment was completely ineffective in preventing the ocular dominance shift induced by monocular deprivation.

To test whether NGF affected cortical physiology or interfered with transmission of visual information, we evaluated in NGF-treated rats the spontaneous discharge and the orientation selectivity. We found these functional properties to be in the normal range.

We conclude that NGF is effective in preventing the effects of monocular deprivation in the rat visual cortex and suggest that NGF is a crucial factor in the competitive processes leading to the stabilization of functional geniculocortical connections during the critical period.

Depriving one eye of patterned vision during early postnatal development (critical period) modifies the balance of input from the two eyes onto individual neurons in the mammalian visual cortex. Most visual cortical neurons loose their functional input from the deprived eye, and the distribution of cell ocular dominance changes in favor of the nondeprived eye (Wiesel and Hubel, 1963; Baker et al., 1974; Dräger, 1978; Boothe et al., 1985; Berardi et al., 1991). The deprived eye becomes amblyopic: its visual acuity is strongly diminished and its contrast sensitivity depressed (Giffin and Mitchell, 1978; Harwerth et al., 1989; Domenici et al., 1991a,c).

Anatomically, monocular deprivation performed during the critical period results in the reduction of the territories occupied in the primary visual cortex by the terminals from the deprived laminae of the LGN and the complementary expansion of the territories occupied by the inputs from the nondeprived laminae (Shatz and Stryker, 1978; LeVay et al., 1980). In addition, there is a substantial degree of shrinkage for LGN projection cells in the deprived laminae, but only in the portion corresponding to the representation of the binocular visual field (Guillery and Stelzner, 1970; Sherman et al., 1974).

The effects of monocular deprivation are thought to be the outcome of competition between the inputs from the ipsilateral and contralateral laminae of the LGN in synapse formation onto binocular cortical neurons, competition that normally leads to their segregation into separate territories.

As a consequence of monocular deprivation, the eye with normal vision dominates the cortical input. Binocular neurons are strongly diminished, and most cortical cells stop responding to the deprived eye.

The presumed underlying mechanism is that cortical synapses receiving stronger electrical messages become functionally and structurally strengthened. As postulated by Hebb (1949), the correlated activity between the pre- and postsynaptic neurons represents an essential prerequisite for synaptic strengthening also in the visual cortex (Kasamatsu and Pettigrew, 1976; Shaw and Cynader, 1984; Stryker and Strickland, 1984; Bear and Singer, 1986; Reiter and Stryker, 1987; Ramoa et al., 1989; Bear et al., 1990; Shatz, 1990).

Two crucial questions still remain to be answered: what the axons from the LGN are competing for, and what is the process by which synapses receiving stronger or better organized electrical messages become functionally and structurally strengthened?

We have formulated the hypothesis that the strengthening of synaptic contacts could be based on the acquisition of a trophic factor, present in limited amount in the target structure. The production and release of this factor, and possibly its uptake, are supposed to be dependent on electrical activity (thus cor-
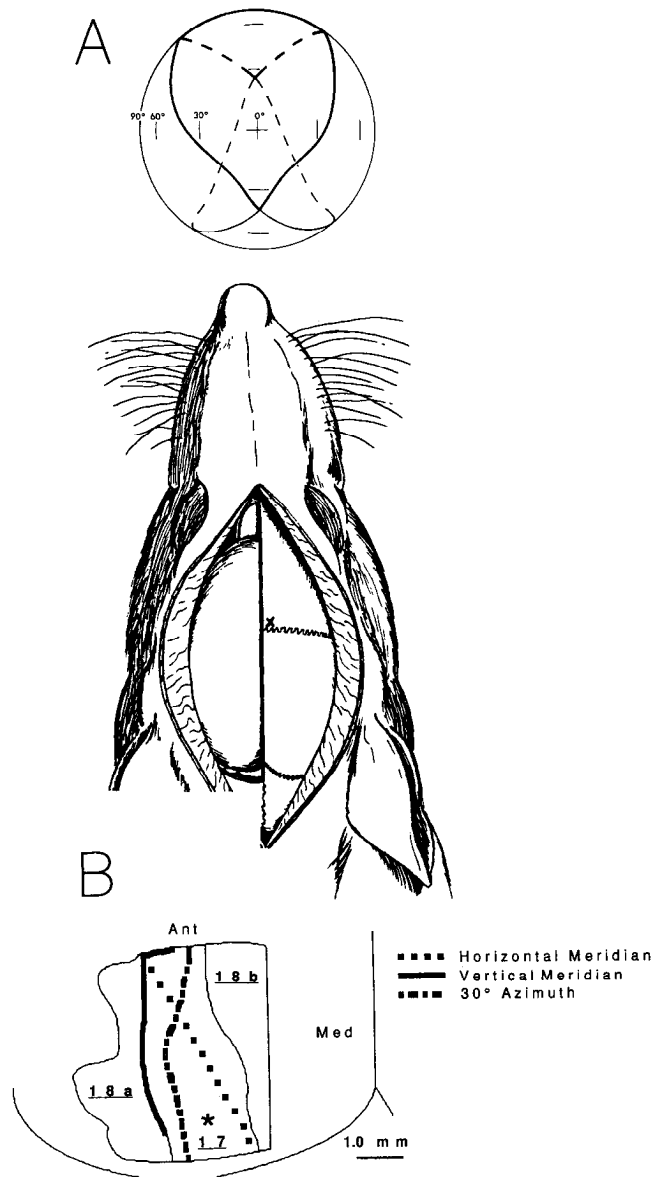
Figure 1. *A*, Rat visual field; the *heavier lines* indicate the binocular part. *Numbers* refer to eccentricities along the horizontal meridian. The *broken lines* define the limit of each monocular visual field behind the rat (adapted from Hughes, 1979). *B*, Representation of the left visual cortex with the landmarks of the contralateral visual field (adapted from Sefton and Dreher, 1985). Our sample of cells was collected between the projection of the vertical meridian and 30° azimuth. The *cross on bregma* in the *drawing* indicates the site for intraventricular injections.

responding to the activity-dependent reward postulated by Hebb). The activity in the LGN fibers driven by the deprived eye would be insufficient or, more likely, inappropriate for the necessary production and/or uptake of the neurotrophic factor. Loss in competition would therefore be equivalent to lack of neurotrophic factor for the axons driven by the deprived eye; the synapses would loose their strength and eventually become ineffective in driving the postsynaptic target. Correspondingly, cell bodies in the LGN deprived laminae would shrink.

We have tested this hypothesis by exogenously supplying a neurotrophic factor to monocularly deprived (MD) rats during the critical period and investigating whether the availability of trophic substance would eliminate competition between the two

visual inputs, thus preventing the effects of monocular deprivation. The neurotrophic factor we have tested has been NGF (see Levi-Montalcini, 1987). The choice of NGF has been prompted by the fact that NGF is widely expressed in the visual pathways and in particular in the visual cortex of mammals (rat and monkey), with a characteristic pattern of variation through the postnatal development (Large et al., 1986; Hayashi et al., 1990; Cremisi et al., 1991), and that the action of NGF is well characterized both in the PNS and in the CNS (Hendry, 1989; Carmignoto et al., 1989; Araujo et al., 1990; Thoenen, 1991).

The results we have obtained are clear in indicating that when NGF is exogenously provided, the effects of monocular deprivation on the ocular dominance distribution do not take place.

In a previous work (Berardi et al., 1990; Domenici et al., 1991a,c), we have reported that in NGF-treated rats, visual acuity and contrast sensitivity, evaluated electrophysiologically with visual evoked potentials, are also unaffected by monocular deprivation.

Materials and Methods

Animal treatment. A total of 37 Long-Evans hooded rats were used. Fourteen rats were normal. Nineteen rats were monocularly deprived for 1 month by means of eyelids suture starting immediately before eye opening [postnatal day 14 (P14)]. In eight rats, only monocular deprivation was performed. In eight rats, deprivation was combined with the intraventricular injection of a solution containing β-NGF (FIDIA Research Laboratories, Abano Terme, Italy; 1–1.6 μg/μl in buffered saline). In three rats, cytochrome C (1 μg/μl in buffered saline) was injected with the same protocol as for NGF, to control for possible aspecific effects of NGF treatment (e.g., animal handling, anesthesia, i.v. injections of NGF). Cytochrome C is a molecule generally used to control for NGF effects (Kromer, 1987; Hendry, 1989). Four rats were left undeprived and were treated with NGF following the same protocol.

Injections (volume injected, 2 μl) were repeated every 2 d for a period of 1 month by means of a microsyringe connected to a cannula (27 gauge) acutely inserted through a hole 1 mm lateral and in correspondence with bregma to reach the right lateral ventricle (Vantini et al., 1989). To check that the injections effectively reached the ventricle, a dye (Pontamine sky blue) was injected with the same procedure and was invariably found in the ventricles. According to previous controls, the diffusion of NGF within the cerebral tissue is at least 3–4 mm (Domenici et al., 1991a). Injections were well tolerated and no gross behavioral effect was found following intraventricular NGF; rats were normally active and both feeding behavior and body weight were in the normal range, such that NGF-treated animals were indistinguishable from untreated and cytochrome C–treated animals.

Eyelids suture and intraventricular injections were performed under ether anesthesia.

Single-cell recording. Six of the normal rats were recorded during the critical period (P19, $N = 3$; P27, $N = 3$); the remaining eight normal rats and all monocularly deprived rats were recorded at the end of the presumed critical period ($>$P45). Two of the monocularly deprived NGF-treated rats were recorded during the treatment (P42), and four undeprived NGF-treated rats were recorded during the treatment and within the presumed critical period (P19, $N = 2$; P27, $N = 2$) in order to evaluate possible transient effects of NGF on neuronal excitability and on the quality of the cell visual response.

To record single cortical unit activity, the animals were anesthetized in urethane (6 cc/kg, 20% solution; Sigma) by intraperitoneal injection. A hole was drilled in the skull in correspondence with the binocular portion of the primary visual cortex (binocular area 17 or area OC1B; Fig. 1). In the rat, OC1B corresponds to the lateral portion of the primary visual cortex (stereotaxic coordinates $>$4 mm from the central fissure) mapping the upper nasal visual field (Fig. 1). It is well characterized by the use of several anatomical markers (Zilles et al., 1984), and according to 14-deoxyglucose autoradiographies (Thurlow and Cooper, 1988), it is divided into a large, more medial portion where alternate patches of stronger and weaker contralateral input are present, and a small, more lateral zone predominantly activated by contralateral input. After exposure of the brain surface, the dura was removed and a micropipette filled with NaCl (3 M) was inserted into the cortex. The cortical surface
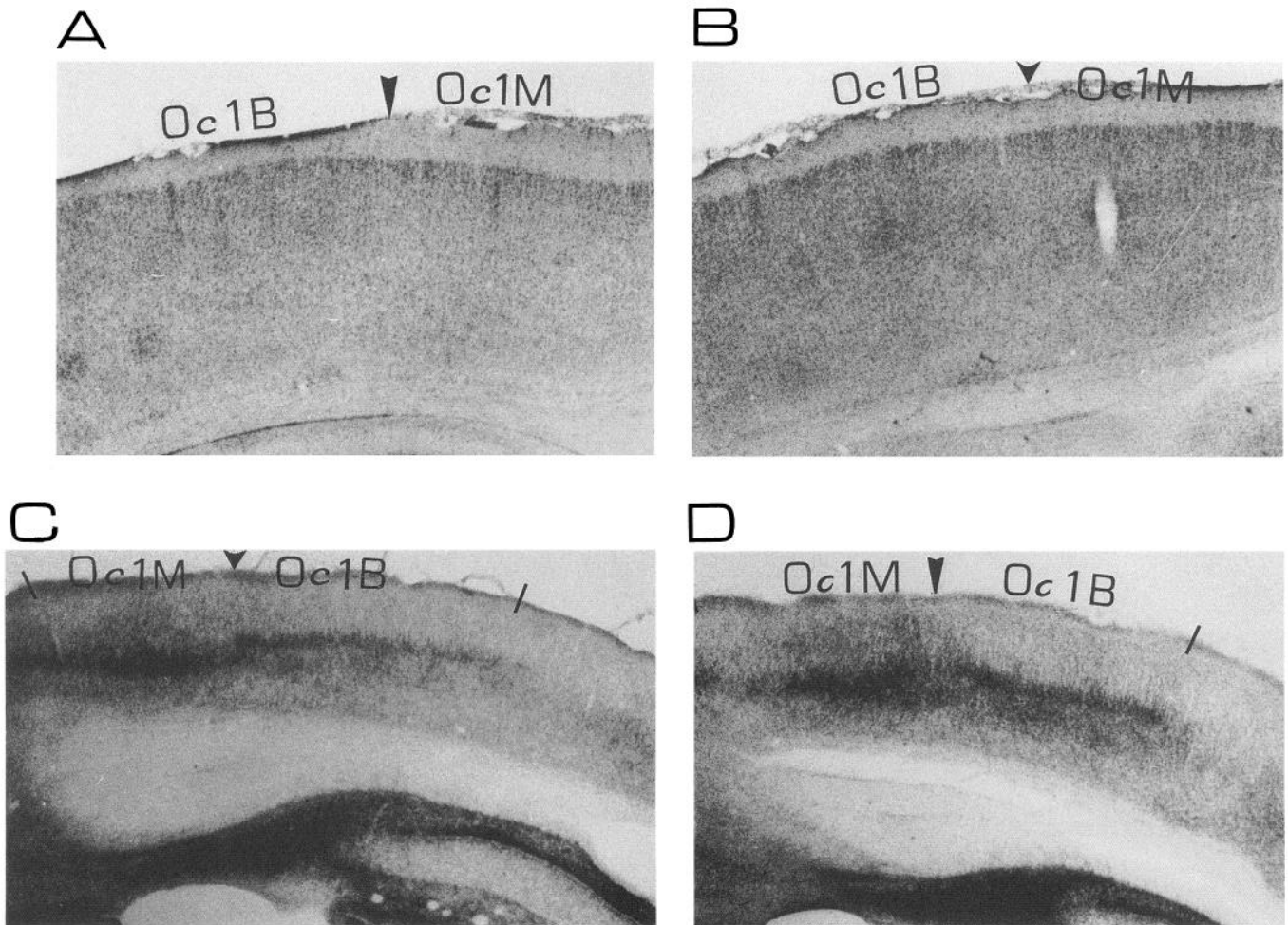
Figure 2. Nissl- (*A* and *B*) and AChE- (*C* and *D*) stained coronal sections cut at the level of the visual cortex of normals rats (*A* and *C*) and of NGF-treated rats (*B* and *D*). In Nissl- and AChE-stained sections, the *arrowheads* delimitate subfields OC1M and OC1B according to the atlas of rat brain (Paxinos and Watson, 1986). In AChE-stained sections, the primary visual cortex OC1 is intensely stained. In the subfield OC1M the stain is higher in the middle-deep layers, while in area OC1B AChE reaction is more evident in middle-superficial layers. Scale: *A* and *B*, 1 cm = 360 μm; *C* and *D*, 1 cm = 570 μm.

was then protected from drying with agar (1.5 gm in saline, 60 ml). To prevent sampling biases, due to the organization of area OC1B with respect to the ocular dominance, our penetrations were angled (i.e., nonperpendicular to the cortical surface) and for each animal at least two well-spaced penetrations were performed. The last penetration was marked by lesions made by passing a small current (10 μA, 10 sec) every 250 μm during electrode withdrawal for subsequent track reconstruction.

Both eyes were fixed and kept open by means of adjustable metal rings (Parnavelas et al., 1981) surrounding the external portion of the eye bulbs, and the cornea was protected with artificial tears (Lacrinorm, Farmigea, Pisa, Italy). With this restraint, the stability of the eyes is good. Pupils were left undilated since it proved extremely difficult to provide artificial pupils that would not cause vignetting, as observed by Lennie and Perry (1981). With dilated pupils and without artificial pupils, the quality of the eye optics would be extremely poor, and in addition, glare would probably occur. Given the impossibility of back-projecting the optic disk with natural pupils (0.5–1 mm in diameter with the luminances we used), we dilated the pupil (atropine sulfate, 0.1%) at the end of the experiment. The position of the optic disk was marked onto a tangent screen where all the cell receptive fields position had been plotted (Lennie and Perry, 1981).

Body temperature was continuously monitored and maintained around 38°C by means of a thermostatted electric blanket. Electrocardiogram was also continuously monitored.

The visual stimuli were hand-moved light bars projected on a reflecting tangent screen or gratings or bars computer generated on a display

(HP1300A, 28 × 22 cm; mean luminance, 12 cd/m²). Both the screen and the display were 20 cm from the rat eyes.

On isolating a cell, the following experimental protocol was followed.

(1) The location of the receptive field in visual space, and the optimal stimulus orientation and direction of movement were determined with hand-held stimuli. Only cells with receptive fields farther than 30° nasal from the optic disk and in the upper visual field were included in our sample. We took care that cells were sampled at comparable eccentricities in the different experimental groups. In the rat, the vertical meridian is estimated to be around 55–58° from the projection of the optic disk (Montero et al., 1968; Hughes, 1979; Reese and Jeffery, 1983; Reese, 1988). The estimated mean receptive fields eccentricities from the vertical meridian in the different experimental groups were 13 ± 9° in normal rats (*N* = 164), 11 ± 8° in MD rats (*N* = 112), 12 ± 9° in MD+NGF rats (*N* = 119), and 14 ± 8° in MD cytochrome C–treated rats (*N* = 50).

(2) Neurons in ocular dominance class 1 were defined as being driven only by the stimulation of the contralateral eye; neurons in ocular dominance classes 2 and 3 were binocular and preferentially driven by the contralateral eye; neurons in ocular dominance class 4 were equally driven by the two eyes; neurons in ocular dominance classes 5 and 6 were binocular and preferentially driven by the ipsilateral eye; and neurons in ocular dominance class 7 were driven only by the ipsilateral eye. The category labeled NC contains those neurons that could not be classified using visual stimuli.

(3) Receptive field type and cell responsiveness were assessed with bars or gratings of optimal orientation according to standard criteria

Table 1. Development of the functional properties of visual cortical neurons

	P19	P27	P45 to adult
RF size (degrees)	35 ± 6	16 ± 7	6 ± 1
NO cells	98%	35%	25%
Binocular cells	92%	79%	80%
Class 4 cells	50%	20%	23%
Class 1–3 cells	46%	65%	66%

Data show receptive field (RF) size, percentages of nonorientational (NO) cells, and percentages of binocular, ocular dominance class 4, and ocular dominance classes 1, 2, or 3 cells in rats of different ages (P19, P27, and older than P45). Cell sample size: $N = 15$ for RF at all ages; NO cells, $N = 50$ at P19, $N = 57$ at P27, and $N = 168$ at P45 to adult; ocular dominance, $N = 50$ at P19, $N = 50$ at P27, and $N = 164$ at P45 to adult. Significance of differences between groups: RF size (two-tailed t test): P19–P27, $p \ll 0.001$; P19–adult, $p \ll 0.001$; P27–adult, $p \ll 0.001$. NO cells (test for binomial distribution): P19–P27, $p \ll 0.00003$; P19–adult, $p \ll 0.00003$; P27–adult, NS. Ocular dominance distribution (x^2, 4 df): P19–P27, $p < 0.005$; P19–adult, $p < 0.02$; P27–adult, NS. Binocular cells (test for binomial distribution): P19–adult, $p < 0.02$; P19–P27, $p < 0.02$. Class 4 cells: P19–adult, $p < 0.0001$. Class 1–3 cells: P19–adult, $p < 0.005$.

(Hubel and Wiesel, 1962; Burne et al., 1984). Orientation selectivity was determined with bars or gratings (contrast, 30–40%). Neurons were classified as *orientational* if the cell response was maximal for a given stimulus orientation (preferred orientation) and indistinguishable from spontaneous activity for at least the orthogonal stimulus orientation; cells were classified as *biased* if the response was present at all orientations but clearly greater ($>2\times$) for certain orientations than for others; cells were classified as *nonorientational* if the response was of comparable strength on six orientations (vertical, horizontal, $+30°$, and $+60°$). Examples of orientational tuning curves for each category are reported in Figure 6A. For each orientation, the cell response was computer averaged over at least 20 stimulus cycles. The response amplitude was taken as the peak amplitude (response to a bar) or as the amplitude of modulation (response to a grating).

The average spontaneous discharge was determined by recording the cell firing rate for 1–2 min in the absence of any patterned visual stimulus.

At the end of the experiment, animals were perfused with normal saline followed by 4% paraformaldehyde (Riedle, D) in 0.1 M phosphate buffer. The brains were coronally sectioned (Vibratome, series 1000) and stained with cresyl violet. The site of injection was examined for possible damage. We found that the injected ventricles were not dilated with respect to the other side or to controls. We did not find alterations of cerebral structures in NGF-treated animals. In particular, no difference was noted in the morphology of the LGN (not shown) and of the visual cortices (Fig. 2A,B) between NGF-treated and control animals. The pattern of acetylcholinesterase (histochemical reaction protocol according to Bear et al., 1985), which is a typical marker of the architecture of the rat visual cortex (Zilles et al., 1984), was also normal in NGF-treated rats (Fig. 2C,D).

Statistical analysis. The following types of statistical analysis have been performed to evaluate the significance of differences between data obtained for different groups: x^2 test (4 df) for the difference between ocular dominance distributions, analysis of variance and two-tailed t test for the difference between mean values, and the variance of the binomial distribution for differences between percentages. A difference was considered significant if its probability p was less than 0.05. To test whether the data obtained within the same group were consistent with having been sampled from a common parent distribution, the Kolmogorov–Smirnov (K-S) statistic has been applied (Lindgren, 1960; Edwards, 1961).

Results

Ocular dominance distribution and receptive field properties in the binocular portion of the rat primary visual cortex

In the pigmented rat, the binocular portion of each visual hemifield extends approximately 40° from the vertical meridian, in the upper visual field (Hughes, 1979). The corresponding bin-
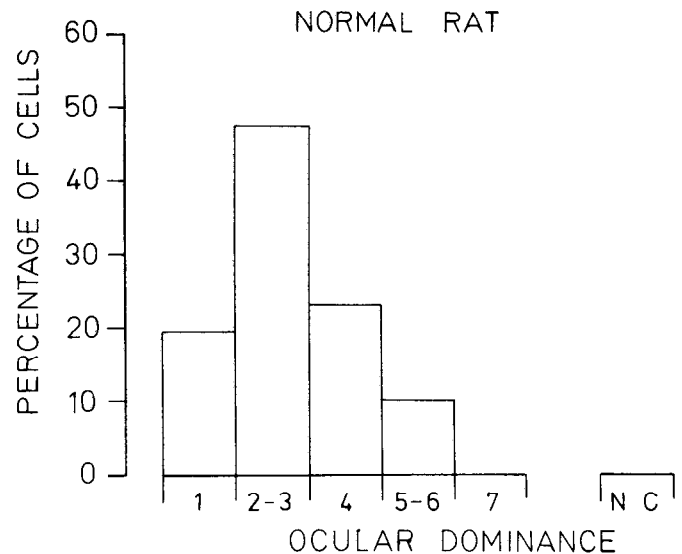
Figure 3. Ocular dominance distribution for visual cortical neurons (area 17) in normal rats (164 cells). Neurons in ocular dominance class 1 were driven only by the stimulation of the contralateral eye; neurons in ocular dominance classes 2 and 3 were binocular and preferentially driven by the contralateral eye; neurons in ocular dominance class 4 were equally driven by the two eyes; neurons in ocular dominance classes 5 and 6 were binocular and preferentially driven by the ipsilateral eye; and neurons in ocular dominance class 7 were driven only by the ipsilateral eye. The category labeled NC contains those neurons that could not be classified using visual stimuli.

ocular portion of the primary visual cortex is binocular area 17 or OC1B (see Fig. 1).

We have recorded 170 cells from area OC1B in normal adult rats within 30° from the vertical meridian (mean eccentricity of the receptive fields centers $= 13 \pm 9°$). For each cell, the receptive field properties and the ocular dominance were assessed. As demonstrated by Burne et al. (1984) and Parnavelas et al. (1981), the properties of neurons in rat primary visual cortex are comparable to those found in higher mammals' visual cortex (cats and monkeys). Receptive fields are well defined and structured, cells can be classified as simple-like or complex-like, and the great majority of the cells are orientation selective. We have confirmed these observations (Table 1; see also Fig. 6).

Ocular dominance was expressed according to the Hubel and Wiesel classification. Cells in ocular dominance class 1 and 7 are exclusively responsive to the contralateral or ipsilateral eye, respectively. Cells in ocular dominance class 4 are equally dominated by both eyes, cells in ocular dominance classes 2 and 3 are dominated by the contralateral eye, and cells in class 5 and 6 are dominated by the ipsilateral eye.

The ocular dominance distribution normally found in adult rats (older than P45) is illustrated in Figure 3. This is the cumulative distribution obtained from recordings in seven rats. A few remarks have to be made.

(1) There was a clear dominance of the input from the contralateral eye (contralateral bias): 47% of the cells fell in class 2 or 3 and 19% in class 1. This contralateral bias (also present in cat visual cortex but less pronounced) reflects the predominance of the crossed versus the uncrossed visual input to each hemisphere, which in the rat is quite strong (Polyak, 1957).

(2) In spite of the small size of the contingent of uncrossed optic fibers, the percentage of binocular cells (classes 2–6) is quite high (80% in the total distribution) and comparable to

Table 2. Index of binocularity (binocular index) and index of ipsilateral eye dominance (ipsilateral index) in the four groups of experimental rats
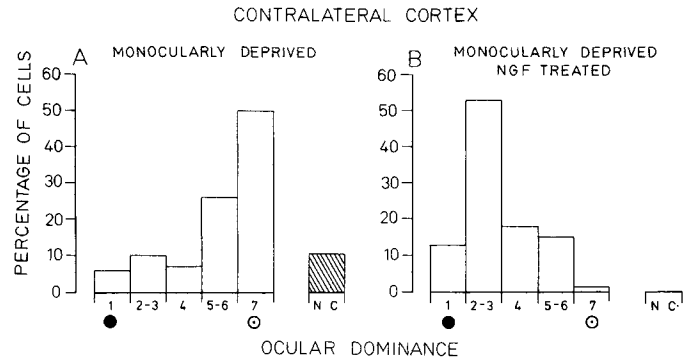
Treatment	Rat	Ipsilateral index	Binocular index	Cells
None	NOR1	0.06	0.69	16
	NOR2	0.05	0.84	37
	NOR3	0.11	0.83	18
	NOR4	0.16	0.89	19
	NOR5	0.17	0.83	23
	NOR6	0.10	0.70	20
	NOR7	0.13	0.77	31
MD	MD1	0.97	0.13	31
	MD2	0.93	0.28	14
	MD3	0.56	0.28	18
	MD4	0.77	0.53	17
	MD7	0.89	0.44	18
MD+NGF	NGF1	0.0	0.96	25
	NGF2	0.0	0.75	12
	NGF3	0.16	0.91	32
	NGF5	0.35	0.95	20
	NGF6	0.31	0.92	13
	NGF8	0.13	0.80	15
MD+CIT	CIT1	0.83	0.42	12
	CIT2	0.68	0.36	22
	CIT3	0.69	0.37	16

Data for the MD, NGF, and MD+CIT (monocular deprivation combined with intraventricular cytochrome C injection) animals come from the cortex contralateral to the deprived eye. Ipsilateral index = (cells in classes 5–7)/(total number of responsive cells); binocular index = (cells in classes 2–6)/(total number of responsive cells). The last column shows the number of responsive cells recorded; only data for those animals where more than 10 responsive cells had been recorded are reported in this table. Significance of differences between groups (two-tailed t test) were, for ipsilateral index: None–MD, $p \ll 0.001$; MD–NGF, $p \ll 0.001$; None–NGF, NS; None–CIT, $p \ll 0.001$; CIT–MD, NS; CIT–NGF, $p \ll 0.001$; for binocular index: None–MD, $p \ll 0.001$; MD–NGF, $p \ll 0.001$; None–NGF, NS; None–CIT, $p \ll 0.001$; CIT–MD, NS; CIT–NGF, $p \ll 0.001$.

CONTRALATERAL CORTEX

Figure 4. Ocular dominance distribution of visual cortical neurons recorded in area 17 contralateral to the deprived eye. *A*, Data from eight untreated MD rats (125 cells, 13 NC). *B*, Data from seven MD rats treated with NGF (119 cells, all responsive). ●, deprived eye; ⊙, nondeprived eye. The ocular dominance distribution for untreated MD rats is significantly different both from the distribution in normal rats ($p \ll 0.001$) and from the distribution in MD rats treated with NGF ($p \ll 0.001$). The ocular dominance distribution for MD NGF-treated rats does not significantly differ from the distribution in normal rats.

significantly different from the adult. Within the same period, other properties of visual cortical cells mature. At P19, the vast majority of cortical cells (50 of 51) were already visually responsive, and the quality of the visual response (vigor, reliability, briskness) progressed rapidly from P19 to P27. It has to be noted that at P19 cells showed clear adaptation. Receptive field size, which was nearly as large as the entire binocular hemifield ($35 \pm 6°$) at P19 (Table 1) was down to $16 \pm 7°$ by P27, a value still significantly larger than in the adult ($6 \pm 1°$). Nonorientational cells, which were the overwhelming majority at P19 (98%), decreased to 35% at P27, a fraction very close to the adult value (25%; Table 1).

Ocular dominance distribution in MD rats and effects of NGF treatment

In all our MD rats, the duration of the deprivation was 1 month or more. Judging from the existing anatomical and behavioral data (Rothblat et al., 1978; Rothblat and Schwartz, 1979; Stafford, 1984), the presumed critical period for the effects of monocular deprivation in the rat does not extend beyond P40. We have confirmed this observation: monocular deprivations starting after P40 did not affect the visual acuity of the deprived eye (L. Maffei, N. Berardi, L. Domenici, V. Parisi, and T. Pizzorusso, unpublished observations). One month of monocular deprivation should therefore span most of the critical period. It has to be noted that the relatively brief duration of the critical period in the rat is an advantage in pharmacological studies, reducing the period of treatment.

The effects of monocular deprivation on the ocular dominance distribution were first assessed in the visual cortex contralateral to the deprived eye. As shown in Figure 4*A*, the ocular dominance distribution in area 17 is dramatically altered by monocular deprivation [cumulative data from all eight MD rats: 125 cells recorded, 13 nonresponsive (10%), 112 responsive; mean eccentricity of the receptive field centers = $11 \pm 8°$]. The percentage of binocular cells is reduced from 80% to 40%. The contralateral, deprived eye dominates only 16% of cortical cells, and the ipsilateral, nondeprived eye now dominates, exclusively or predominantly, 75% of the cells. The difference with the normal ocular dominance distribution is highly significant ($p <$

that found in cats (Hubel and Wiesel, 1962) and monkeys (Baker et al., 1974).

Results from recordings in single animals are reported in Table 2. Here, two indexes summarizing the ocular dominance distribution are reported, the binocular index (number of cells in classes 2–6 ∥ total number of cells) and the ipsilateral input index (number of cells in classes 5–7 ∥ total number of cells). There is a certain degree of interindividual variability, but for each index the data are consistent with the hypothesis that they were all sampled from the same parent distribution (K-S test, 6 df, $p > 0.20$).

To test whether the ocular dominance distribution found in adult rats emerges gradually during the first weeks of postnatal development, as it does in other mammals (Hubel and Wiesel, 1963; Blakemore and Van Sluyters, 1975; Albus and Wolf, 1984), we have recorded six normal rats at two stages of postnatal development, P19 ($N = 3$) and P27 ($N = 3$) (Table 1). In both cases, the centers of the receptive fields (although of difficult determination in the youngest animals given their large dimensions) were of comparable eccentricities with those recorded in the adult. At P19 (Table 1, first column), most cortical cells were binocular (92%) and 50% were equally dominated by both eyes (class 4). By P27, class 4 cells amounted to 26% (Table 1, second column) and the ocular dominance distribution was not
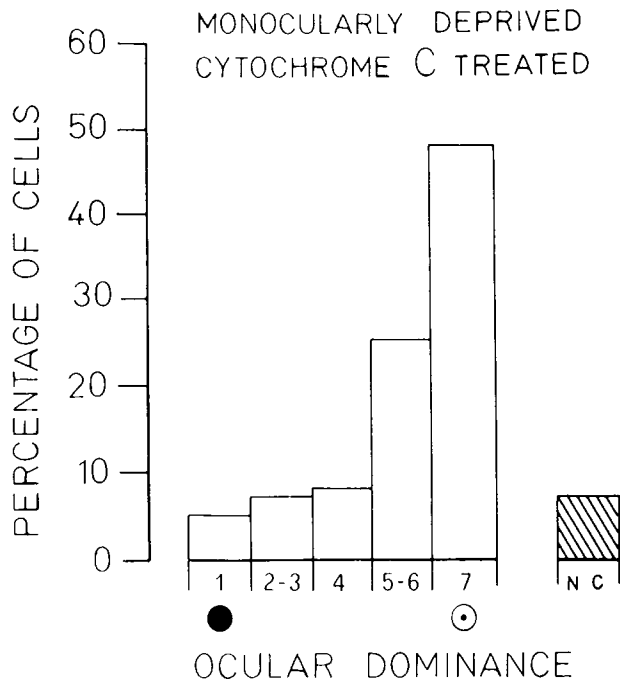
Figure 5. Ocular dominance distribution for visual cortical cells recorded in area 17 contralateral to the deprived eye in MD rats treated with cytochrome C (54 cells, 4 NC). Other conventions are as in Figure 3. The difference between the ocular dominance distributions in normal and in MD NGF-treated rats is highly significant ($p \ll 0.001$). The difference in the distribution found in untreated MD rats is not significant.

0.001, χ^2). Data from recordings in single MD rats are reported in Table 2. There are interindividual variations, but the shift toward the dominance of the ipsilateral, nondeprived eye and the strong reduction in binocular cells are present in all animals. For each index, the data from single animals are consistent with the hypothesis that they had all been sampled from the same parent distribution (K-S test, 4 df, $p > 0.20$). The two-tailed t test (ANOVA for the difference between means for two groups) for the MD group and the normal group shows that the interindividual variability within groups is much smaller than the intergroup variability and that the mean values for the ipsilateral and binocular indexes for MD rats are significantly different from the corresponding values in normal rats ($p \ll 0.001$).

The results obtained in those MD rats that received NGF throughout the deprivation period are reported in Figure 4*B* (119 cells recorded in 7 animals, all 119 responsive; mean eccentricity of receptive fields centers = 12 ± 9°). It is evident from the figure that in this case monocular deprivation caused no change in the ocular dominance distribution. Indeed, the distribution obtained in MD NGF-treated rats was not significantly different from the distribution in normal rats ($p > 0.05$), while being significantly different from the distribution in MD rats ($p < 0.001$). The percentage of binocular cells is 86%, and the contralateral deprived eye dominates 66% of the cells. Note also that no unresponsive cell was found (unlike untreated MD rats). Data from recordings in single MD NGF-treated rats are reported in Table 2. There is some interindividual variability, but it is evident that NGF was effective in counteracting the effects of monocular deprivation in all animals. The data for single animals are consistent with the hypothesis that they had all been sampled from a common parent distribution for the

binocular index but not for the ipsilateral index, (K-S test, 5 df; $0.05 > p > 0.01$ for the ipsilateral index; $p > 0.20$ for the binocular index). The two-tailed t test for the MD+NGF, the MD, and the normal rats group shows that the mean binocular and ipsilateral indexes for the MD+NGF group are not significantly different from the corresponding values in normal rats, while being so with respect to those obtained in untreated MD rats ($p \ll 0.001$). An ANOVA across the three groups (normal, MD, MD+NGF) confirms that the means are not homogeneous and shows that the intergroup variability is larger than the variability within groups [$F(2,15) = 5.3$ for the ipsilateral index and 5.44 for the binocular index; $p < 0.05$ in both cases]. We then applied Tukey's procedure (Edwards, 1961) to classify the means into groups that are alike among themselves but differ from each other. The result of this test is that, for both indexes, normal and MD+NGF rats form one group and MD rats form a different group.

We conclude that MD causes a dramatic shift in the ocular dominance distribution and that NGF treatment is very effective in counteracting MD effects.

To control for possible aspecific effects of NGF treatment, we determined the ocular dominance distribution in three MD rats treated with cytochrome C, a molecule that is generally used to control for NGF treatment (Hendry, 1989). The results from these rats, shown in Figure 5 (54 cells, 50 responsive; mean eccentricity of receptive fields centers = 14 ± 8°) and Table 2, clearly indicate that cytochrome C treatment was completely ineffective in preventing the effects of monocular deprivation. The ocular dominance distribution from cytochrome C–treated rats is indistinguishable from that obtained in MD rats.

The ANOVA for all four groups shows again that there are different populations and that the intergroup variability is greater than the variability within groups [F(3,17) = 8.15 for the ipsilateral index and 37.95 for the binocular index; $p < 0.01$ in both cases]. Normal rats and MD+NGF rats form one group; MD rats and cytochrome C rats form a different group.

Ipsilateral cortex

Recordings have also been made in the cortex ipsilateral to the deprived eye in MD rats untreated (52 cells, 3 rats) and NGF treated (50 cells, 3 rats). The results indicate that monocular deprivation effects are stronger in the ipsilateral than in the contralateral cortex. Only 15% of the cells are binocular, and all of them are dominated by the nondeprived eye (both values are significantly different from the corresponding ones in normal and NGF-treated rats. $p \ll 0.0001$). In NGF-treated rats, monocular deprivation was much less effective: 75% of the cells are binocular, a value not significantly different from normal, although the dominance of the contralateral nondeprived eye is significantly stronger than normal (86% cells in classes 1–3 vs 66%, $p < 0.004$).

Functional properties of cortical cells in NGF-treated rats

An important point was to control whether NGF treatment affected the functional properties of visual cortical cells.

Spontaneous discharge

First of all, we tried to assess possible effects of NGF on the spontaneous activity of cortical cells. In NGF-treated rats, recorded 3 or more days after the end of the treatment, the mean spontaneous activity resulted 9 ± 6 spikes/sec (three animals, $N = 22$). This value is not significantly different from the mean
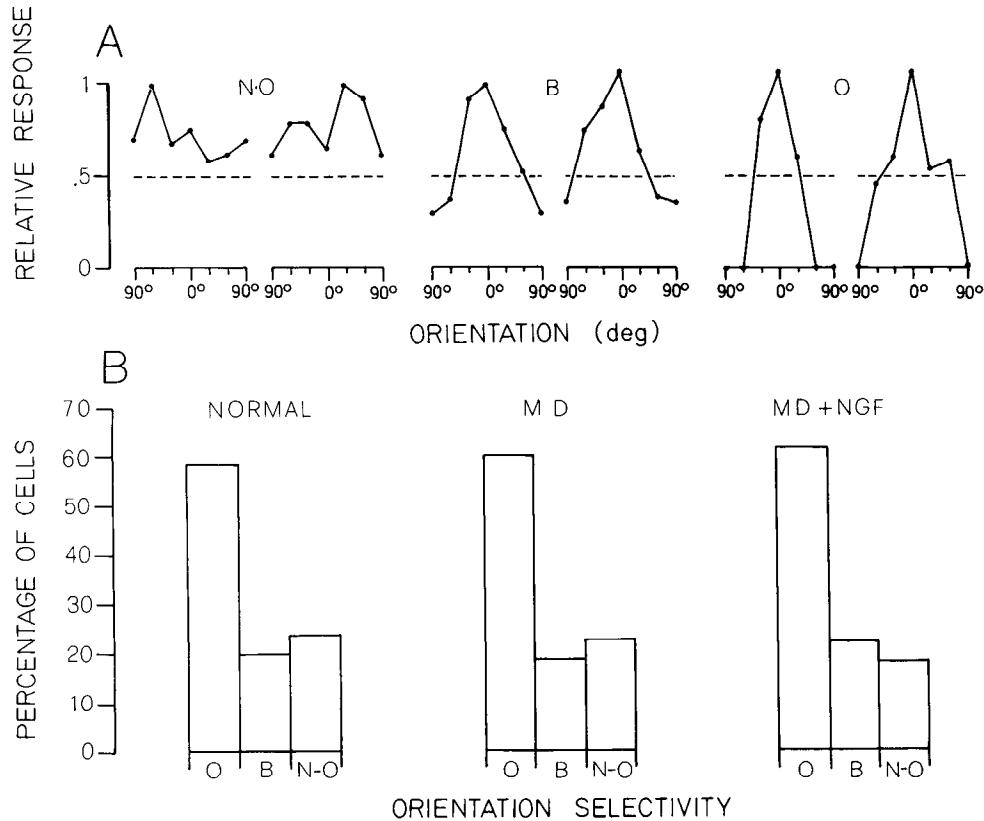
Figure 6. Orientation selectivity of cells in the rat primary visual cortex. Neurons were classified as orientational (O) if the cell response was maximal for a given stimulus orientation (preferred orientation) and indistinguishable from spontaneous activity for at least the orthogonal stimulus orientation; cells were classified as biased (B) if the response was present at all orientations but clearly greater (>2×) for certain orientations than for others; cells were classified as nonorientational (N-O) if the response was of comparable strength on six orientations (vertical and horizontal, +30° and +60°). A, Examples of orientational tuning curves for each category. The cell relative response amplitude (1 = strongest response) is plotted as a function of the stimulus orientation. Points on the abscissa correspond to responses indistinguishable from the spontaneous activity. 0° is the preferred orientation for O and B cells, and is the vertical orientation for N-O cells. Clockwise and anticlockwise rotations from 0° are represented on the right and left abscissas, respectively, in steps of 30°. For each orientation, the cell response was computer averaged over at least 20 stimulus cycles. B, Histograms compiled from neurons recorded in the primary visual cortex of normal, MD, and MD+NGF rats and classified according to their orientation selectivity. The total number of cells recorded is N = 164 for normal rats, N = 184 for MD rats, and N = 186 for MD+NGF rats (data for the ipsilateral and contralateral cortex are pooled together). The differences between the distributions in normal, MD, and MD+NGF rats are not significant.

spontaneous discharge in normal rats (three animals, N = 24; mean = 10 ± 5 spikes/sec), suggesting that there are no long-term effects of NGF on spontaneous activity. To control for possible transient effects of NGF on spontaneous activity, the spontaneous discharge of H20 cells was recorded in two rats (P42) 4 hr after the last NGF injection in the treatment. The mean spontaneous activity resulted in 8 ± 6 spikes/sec. This value is also not significantly different from the normal spontaneous activity, suggesting that there are no short-term effects of NGF on this parameter.

Orientation selectivity

We then controlled for whether NGF treatment had affected the orientation selectivity of visual cortical cells. Figure 6A reports examples of orientational tuning curves for two orientational (O), two biased (B), and two nonorientational (N-O) cells. For the orientational cells, one of the most selective (bandwidth, ~70°) and one of the least selective (bandwidth, ~100–110°) are reported. On average, the mean tuning for orientational cells in our sample was 80–90°. In Figure 6B, we have reported the percentage of cells recorded in normal, MD, and MD+NGF rats classified, according to their orientation selectivity, as or-

ientational (O), biased (B), and nonorientational (N-O) (see Materials and Methods). Two points should be made. First, the orientation selectivity for cells recorded in NGF-treated rats was significantly higher than in P19 rats (Table 1), suggesting that NGF did not block the development of the functional properties of visual cortical cells. Second, the orientation selectivity in NGF-treated rats was not significantly different from normal rats, suggesting not only that the visual cortex has developed but that it has developed normally. This is well in accordance with the fact that no gross differences in cell responsiveness and receptive field size and organization were noted in NGF-treated rats compared with normal rats.

An interesting result emerged when the orientation selectivity distributions for untreated MD rats and NGF-treated MD rats were examined separately for cells dominated by the deprived eye and the nondeprived eye. This was done only for the contralateral cortex, since class 5–7 cells are missing in the ipsilateral cortex of untreated MD rats. The result of this analysis is shown in Figure 7. In untreated MD rats, the few cells dominated by the deprived eye (N = 20) are less orientational than those dominated by the nondeprived eye (N = 84). In the first case, cells classified as orientational or biased were 13 of 20 (65%);
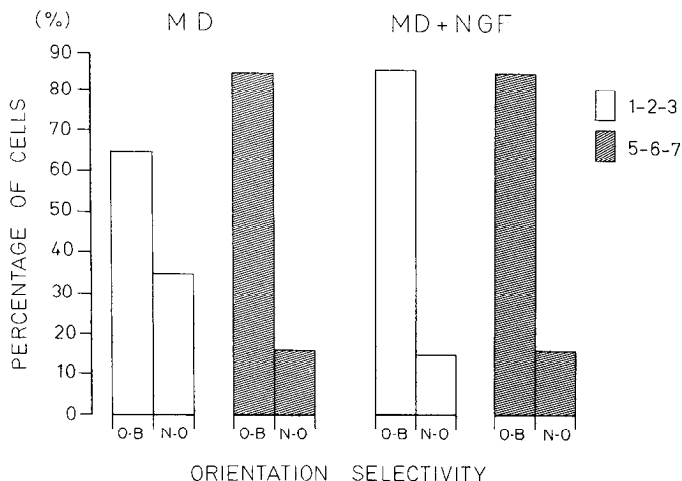
Figure 7. Histograms compiled from neurons recorded in the primary visual cortex contralateral to the deprived eye for untreated MD rats and MD+NGF rats. Cells were divided into two groups, contralaterally dominated (classes 1-2-3, open columns) and ipsilaterally dominated (classes 5-6-7, shaded columns) and then classified according to their orientation selectivity. Orientational (O) and biased (B) cells have been grouped together. The difference between the percentage of orientational-biased cells dominated by the deprived and nondeprived eye, respectively, is significant for the MD group ($p < 0.05$).

in the second case, they were 71 of 84 (84.5%). The difference is significant ($p < 0.05$). This is not the case for NGF-treated MD rats, where the two distributions are almost identical.

Functional properties of visual cortical cells in NGF-treated rats recorded during the critical period

It might be argued that NGF could have transiently affected the functional properties of visual cortical cells during the critical period, due to their possible higher sensitivity to the trophic factor. To control for this, we have recorded four rats under NGF treatment at two stages of the critical period, P19 ($N = 2$) and P27 ($N = 2$) and compared the results with those obtained in normal rats of the same age. Recordings began 1 hr after the last NGF injection.

At P19, all cells were visually responsive in NGF-treated rats, and their response quality was comparable to that in normal rats. The mean spontaneous discharge was 4 ± 5 spikes/sec ($N = 56$; median, 2 spikes/sec; interquartile range, 0.5–7 spikes/sec) in normal rats and 4 ± 5 spikes/sec ($N = 51$) in NGF-treated rats (median, 2.5 spikes/sec; interquartile range, 0.50–7 spikes/sec). The distributions being skewed (due to the zero inferior limit), we have performed, in addition to the t test for the difference between the mean values, a t test on the difference between the mean values of the logarithmic transform of the data and a χ^2 test for the difference between the distributions. All differences were nonsignificant. Orientation selectivity is a property not yet well developed in our sample of normal P19 rats (Table 1), and this was the case also for P19 NGF-treated rats.

At P27, all cells were visually responsive in the NGF-treated rats, and their response quality was comparable to that in normal rats of the same age. The spontaneous discharge was 4 ± 6 spikes/sec ($N = 61$) in normal rats (median, 2 spikes/sec; interquartile range, 0.5–5 spikes/sec) and 5 ± 6 spikes/sec ($N = 42$) in NGF-treated rats (median, 3 spikes/sec; interquartile range, 0.5–6 spikes/sec). Also in this case, the distributions are skewed,

and in addition to the t test for the difference between the mean values, a t test on the difference between the mean values of the logarithmic transform of the data and a χ^2 test for the difference between the distributions have been performed. All differences are nonsignificant. In normal P27 rats, only 37% of visual cortical cells are nonorientational (Table 1). The percentage of nonorientational cells in NGF-treated rats (17 of 42, 40%) is not significantly different from the normal value. We conclude that NGF treatment did not affect the functional properties of visual cortical cells tested during the critical period.

Discussion

The visual cortex of the rat: a suitable experimental model for neural plasticity studies

The modifications induced by early sensory deprivations in the functional organization of the sensory cortices are a good model to study the activity-dependent strengthening of synapses in mammals. In the rat, this has been done for the barrel field in the somatosensory cortex (Simons and Land, 1987) and in the acoustic system (Sanes and Constantine-Paton, 1983).

Our results extend those already present in the literature (Fifkova, 1968; Rothblat et al., 1978; Rothblat and Schwartz, 1979; Stafford, 1984; Dean, 1990) in indicating that the rat visual cortex is a suitable model to study neural plasticity and that the paradigm of monocular deprivation, widely exploited to study visual cortical plasticity in other mammals, can be successfully used. Indeed, neurons in the rat primary visual cortex have well-defined functional properties and are distributed in different classes of ocular dominance with a high proportion of binocular cells, comparable to that in cats and monkeys (Hubel and Wiesel, 1962; Baker et al., 1974). Both the ocular dominance distribution and the functional properties of cortical cells develop gradually during the first month of postnatal development. In the rat, there is no clear anatomical demonstration of ocular dominance column. However, Thurlow and Cooper (1988) have reported alternate patches of stronger and weaker contralateral input to OC1B using ^{14}C deoxyglucose.

Our results show that the effects of monocular deprivation on the ocular dominance distribution are robust. Binocularity is decreased and the percentage of cells dominated by the deprived eye falls dramatically, even when the deprived eye is the normally dominant contralateral eye. Similar results had been obtained by Dräger (1978) in the mouse. The anatomical correlate of monocular deprivation at cortical level is the change in the number of cortical dendritic spines observed by Rothblat and Schwartz (1979). We have also reported that monocular deprivation, as it is the case for other mammals, induces in the rat shrinkage of cell bodies in the deprived laminae of the LGN (Domenici et al., 1991b).

Specificity of NGF effects

The main result of this work is that NGF treatment prevents the shift in ocular dominance induced by monocular deprivation spanning the whole presumed rat critical period.

The following observations seem to suggest that the effects of NGF are neither pathological in nature nor unspecific.

(1) The absence of any gross behavioral effects in experimental animals following intraventricular injections of NGF seems to exclude general pathological alterations. This is further supported by behavioral experiments in MD NGF-treated kittens: in these animals, the behavior was normal, the performance in

the jumping stand was undistinguishable from that of control kittens, and the visual acuity of the deprived eye was normal (Maffei et al., in press).

(2) The results obtained in control animals (cytochrome C treated) indicate that NGF effects are not due to the treatment per se (resulting, e.g., from repeated anesthesia). Unspecific NGF effects are also excluded by our previous findings that local applications of NGF onto one visual cortex prevents the amblyopic effects of monocular deprivation only in the treated hemisphere (Domenici et al., 1991a). This last finding suggests that the action of NGF occurs within the visual cortex and/or its afferent systems.

(3) Intraventricular transplant of hybridoma cells producing anti-NGF antibodies causes a decrease in the number of cortical binocular neurons and the shrinkage of cell bodies in the LGN (Berardi et al., in press). By contrast, intraventricular injections of NGF prevent the LGN cell body shrinkage induced by monocular deprivation (Domenici et al., 1991b).

(4) Responsiveness, spontaneous activity, and orientation selectivity of visual cortical cells were normal in NGF-treated animals, when measured both at the end and at different ages during the critical period. This suggests that NGF did not cause irritation in visual cortical neurons, nor did it interfere with the transmission of visual information, either excitatory or inhibitory. It should be noted that pharmacological treatments that interfere with normal neural activity, such as glutamate, bicuculline, and aminophosphonovalerate (APV), result in abnormal responsiveness and abnormal orientation selectivity of visual cortical neurons.

NGF in the visual system

In the introductory remarks, we advanced the hypothesis that geniculate afferents compete at cortical level for a neurotrophic factor, the production of which is activity dependent. For NGF to be the neurotrophic factor in question, it is required that (1) NGF is present in the visual cortex during the critical period, (2) NGF production and/or uptake is dependent on electrical activity, and (3) NGF-specific receptors are present in the visual cortex and in particular on the afferent fibers from the LGN during the critical period.

As to the presence of NGF in the visual cortex, it is known that the content of NGF in the rat neocortex (Large et al., 1986) and visual cortex (Cremisi et al., 1991) and in the primate occipital cortex (Hayashi et al., 1990) is higher during the first part of the critical period, later decreasing to adult values. Whether the source of NGF is neuronal or glial is not yet clear. Astrocytes are known to produce NGF *in vitro* (Lu et al., 1991b). On the other hand, in the hippocampus, kainic acid lesions that spare glial cells abolish the content of mRNA for NGF (Ayer-LeLièvre et al., 1988; but see Bakhit et al., 1990).

There is already evidence that the production of neurotrophic factors of the NGF family can be dependent on electrical activity. It has been shown in the hippocampus that the regulation of mRNA levels for NGF and for another neurotrophic factor of the same family, brain-derived neurotrophic factor (BDNF), depends on neuronal activity, their upregulation being mediated via the glutamate receptors and their downregulation via the GABA system (Zafra et al., 1990; Ernfors et al., 1991; Lu et al., 1991b).

As far as the NGF receptor is concerned, controversy has recently arisen about the encoding gene and the associated protein that mediates the biological activity of NGF, that is, its high-affinity receptor (Hempsted et al., 1991; Klein et al., 1991; Weskamp and Reichardt, 1991).

Another consideration to be made is that neither the presence of NGF nor even the presence of the NGF-specific receptor in the visual cortex would be per se evidence that the only neurotrophic factor possibly involved in cortical plasticity is NGF. Indeed, other neurotrophic factors bearing strong similarities with NGF, namely, BDNF (Barde et al., 1982) and neurotrophin-3 (NT3; Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990; Kaisho et al., 1990; Maisonpierre et al., 1990a; Rosenthal et al., 1990), have been found in the rat cortex during development, although with different time windows and levels of expression. In particular, NT3 seems to be highly expressed only in earlier stages of development, in immature regions of the CNS where proliferation, migration, and differentiation of neuronal precursors are still ongoing (Maisonpierre et al., 1990b). At high concentrations (1000-fold excess of heterologous ligand), both BDNF and NT3 interact with NGF high-affinity receptors and vice versa (Rodriguez-Tebar et al., 1990). The amounts of NGF we have used (order of 1 μg) are very large if compared with the endogenous NGF level in the visual cortex, which is of the order of nanograms per gram of tissue (Large et al., 1986; Hayashi et al., 1990). We cannot rule out the possibility that what we observed is a cross-reaction, in that NGF has mimicked the action of other neurotrophic factors of the same family. It has to be noted that our hypothesis, namely, a role for neurotrophic factors target released in the formation of neural connections and, in particular, in visual cortical plasticity, does not depend on the exact nature of the trophic substance(s).

Our proposed hypothesis implies that the effects of monocular deprivation are due to lack of NGF that, in our experiment, is compensated by exogenous supply. There are, however, other possible explanations for the effects of NGF on monocular deprivation, which although possible on the basis of the current literature, turn out to be rather unlikely in view of our experimental findings.

(1) The effects of NGF could be indirect and mediated via cholinergic neurons. It is well known that NGF has a neurotrophic action on the cholinergic neurons of the CNS.

The visual cortex receives a substantial cholinergic input from the basal forebrain (Carey and Rieck, 1987), and according to Pioro and Cuello (1990), NGF receptor immunoreactivity (192 IgG) in adult rat visual cortex is consistent with the pattern of cholinergic afferents. NGF could activate the cholinergic system and thus increase the cholinergic input onto visual neurons, which could in turn affect cortical plasticity. An increase in the cholinergic input onto visual neurons, however, is not necessarily expected to *decrease* cortical plasticity and thus prevent an ocular dominance shift. Indeed, the contrary has been reported in the literature; that is, a lesion to the cholinergic afferents decreases visual cortical plasticity (Bear and Singer, 1986). In addition, a variation in the cholinergic input to the visual cortex, in either direction, is expected to affect the spontaneous discharge and the cell responsiveness and stimulus selectivity (Sillito and Kemp, 1983; Sato et al., 1987), a finding confirmed also in the somatosensory cortex of awake undrugged rats (Delacour et al., 1990). We have failed to observe these effects. In addition, measurements of CHat activity (Domenici et al., 1991a) in the visual cortex of NGF-treated rats have shown that this parameter, which is an indicator of cholinergic activation, was not changed substantially by NGF treatment.

(2) NGF could interfere with the normal development of the visual cortex that takes place within the first postnatal month, freezing the cortex in its immature state, with a high proportion of binocular cells. In this case, the functional properties of visual cortical cells in NGF-treated rats should be different from those found in normal rats of the same age. None of the functional properties tested (ocular dominance distribution, orientation selectivity) showed difference between normal and NGF-treated rats of any age. This suggests that the process of cortical development has not been affected by NGF treatment, in accordance with the observation that also visual acuity develops normally in NGF-treated rats (Domenici et al., 1991a,c) and kittens (Maffei et al., in press). It may be noted that dark rearing, which is known to delay the development of the visual cortex, affects visual acuity and orientation selectivity of visual cortical cells (for cats, Timney et al., 1978; for rats, Maffei, Berardi, Domenici, Parisi, and Pizzorusso, unpublished observations).

A recent report by Ghosh and Shatz (1992) shows that subplate cells may be vital for normal ocular dominance development. Low-affinity NGF immunoreactivity has been transiently detected in the subplate region during the prenatal period in the cat (Allendorfer et al., 1990) and during prenatal and postnatal periods in the rat (Koh and Loy, 1989; Koh and Higgins, 1991). In the rat, the expression of low-affinity NGF receptors in the subplate region becomes undetectable by the end of the second postnatal week (Koh and Higgins, 1991). By this period, subplate cells seems also to disappear. In the experiments reported here, NGF treatment was started at P14, which is probably late to interfere with the function or survival of subplate cells, although we cannot exclude it.

In conclusion, the findings presented here together with similar results reported in kittens (Carmignoto et al., 1991) are consistent with the hypothesis that exogenous NGF compensates for a shortage of endogenous NGF due to sensory deprivation and that NGF action is exerted primarily on visual neurons. The recent finding obtained in this laboratory that the shrinkage induced by monocular deprivation in rat LGN cells is prevented by NGF treatment (Domenici et al., 1991b; Maffei et al., in press) is not contrary to this interpretation.

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