NERVE GROWTH FACTOR (NGF) PREVENTS THE EFFECTS OF MONOCULAR DEPRIVATION IN THE RAT

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Hubel and Wiesel [1,2] demonstrated that the mammalian visual cortex is susceptible to manipulation of the visual experience during the first part of the postnatal development (critical period). When visual signals are available but not identical in the two eyes, as in the case of monocular deprivation, cortical neurons do not retain their binocular input and stop responding to the deprived eye [3,4,5]. In addition, the visual acuity and the contrast sensitivity of the deprived eye decrease dramatically (amblyopia)[6,7]. These functional changes correspond anatomically to an alteration of the columnar organization. The cortical territories occupied by the afferents from the non deprived laminae of the LGN increase in size at the expense of the afferents coming from the deprived laminae[8]. In addition, cell bodies in the deprived laminae of LGN shrink[9].

It is generally assumed that the phenomena occurring after a monocular deprivation (MD), are the outcome of competitive activity-dependent interactions between the geniculate afferents. Cortical synapses receiving a strong input, as it is the case for the non deprived eye, are strengthened and stabilized while those receiving a weaker input are depressed and may be removed.

Activation of the postsynaptic site is an essential prerequisite for the long-term modifications of synapses caused by MD. And indeed a number of factors acting at the postsynaptic site have been found to prevent, at least partially, the effects of MD and to have a role in synapse stabilization during visual development[10-14].

However, the crucial question, what the axons from LGN are competing for still remains to be answered.

We have formulated the hypothesis that the competition might be for a neurotrophic factor, released or produced in an electrical activity dependent manner. Activity in the deprived fibers would be inappropriate for the necessary

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production and/or uptake of neurotrophic factor and their synaptic efficacy would decrease. Cortical cells would then stop responding to the deprived eye and the visual acuity for the deprived eye would dramatically decrease. Neurons in the deprived laminae of LGN would suffer for the absence of neurotrophic factor and would shrink.

We have tested this working hypothesis by investigating the effects of intraventricular NGF injection on the visual cortex of monocularly deprived rats. NGF is a well known neurotrophic factor both in the PNS[15] and in the CNS[16,17,18].

The data are clear in indicating that when NGF is exogenously provided the effects of monocular deprivation do not take place.

These results have been previously presented in a general review on the role of neurotrophic factors in the mammalian visual cortex plasticity[19].

METHODS

Subjects and surgery

Fifty three Long Evans hooded rats were used. Seventeen rats were normal (group I). Thirty six rats were monocularly deprived for one month by means of eyelids suture starting immediately before eye opening (postnatal day 14, P14). In the rat this corresponds to a deprivation spanning the whole length of the critical period [20, 21]. In fifteen rats only monocular deprivation was performed (group II). In fifteen rats deprivation was combined with the intraventricular injection of a solution containing ß-NGF (1-1.6 µg/µl in buffered saline; group III). In six rats cytochrome C (1 µg/µl in buffered saline) was injected with the same protocol as NGF (group IV). The volume injected was 2 µl. Injections were performed every other day for one month by means of a microsyringe connected to a cannula (gauge 26) inserted through a hole 1 mm lateral and in correspondence with bregma, to reach the lateral ventricle. When a dye (Pontamine Sky Blue) was injected by this procedure it was invariably found in the ventricles. Eyelid suture and intraventricular injections were performed under ether anaestesia. The diffusion of NGF was estimated by placing a piece of fibrine (Spongestan) soaked with iodinated NGF (specific activity 64.1 µCi/µg) onto the cortical surface in correspondence with bregma (N=3 rats). The diffusion of iodinated NGF was approximately 3 mm from bregma 24 hours later.

Recording sessions

At the end of the deprivation period, single neuron responses or visual evoked potentials (VEP) were recorded in urethane anaesthetized rats (6 cc/Kg, 20% solution, Sigma) by means of a micropipette filled with NaCl (3 M), inserted in the binocular portion of the primary visual cortex (binocular area 17 or area OC1B) contralateral to the deprived eye. Both eyes were fixed by means of metal rings surrounding the external portion of the eye bulbes. Visual stimuli consisted

of light bars projected on a reflecting screen or in gratings of different orientation and spatial frequency computer generated on a display (HP 1300 A, mean luminance 12 cd/m^2) positioned 20 cm from the rat eyes and centered on the cell receptive fields, previously determined. The gratings were alternated in phase with a fixed temporal frequency, chosen in the range 1-2 Hz for extracellular unit recordings and 2-4 Hz for VEPs. The signals were filtered and amplified in a conventional manner, computer averaged and analyzed.

Extracellular unit recording

Five rats of group I (normal rats), five rats of group II (deprived rats), five rats of group III (deprived NGF treated rats) and three rats of group IV (deprived cytochrome C treated rats) were used, all aged P 45 or older. On isolating a cell, the location of the receptive field in the visual space and the optimal stimulus orientation and direction of movement were determined. Neurons were classified as orientational if the cell response was maximal for a given orientation (preferred orientation) and indistinguishable from spontaneous activity for the orthogonal stimulus orientation. The ocular dominance was then assessed with bars or gratings of optimal orientation. Neurons in ocular dominance class 1 were driven only by the stimulation of the contralateral eye; neurons in ocular dominance classes 2-3 were binocular and preferentially driven by the contralateral eye; neurons in class 4 were equally driven by the two eyes; neurons in class 5-6 were binocular and preferentaily driven by the ipsilateral eye and neurons in class 7 were driven only by the ipsilateral eye. A chi-square test, 4 degrees of freedom was used to evaluate the differences between ocular dominance distributions.

Two of the NGF treated rats were recorded during the treatment (postnatal day 42) in order to evaluate possible transient effects of NGF on neuronal excitability and on the quality of the cell visual response.

Visual evoked potentials

VEPs were recorded in five rats of group I, ten rats of group II, ten rats of group III and three control rats (deprived cytochrome C treated rats). For each condition (visual cortex, viewing eye, spatial frequency, contrast) at least 400 responses were averaged. For each record the amplitude, phase and relative power of the first twelve harmonics were measured. For the temporal frequencies employed, signals consisted mainly of the second harmonic (relative power higher than 70%). For this reason, the amplitude of the second harmonic in each record (1/2 the peak to trough amplitude) was taken as the amplitude of VEP for that condition. To assess the spatial resolution value (visual acuity) gratings of maximum (available) contrast were used (70 %); the spatial frequency was progressively increased until the signal was indistinguishable from the noise. If necessary, lenses of appropriate dioptric power were placed in front of the eyes of the rat. The visual acuity was taken as the highest spatial frequency still evoking a reliable

response. The contrast threshold at a given spatial frequency was evaluated by extrapolating to zero voltage (noise level) the linear regression through a contrast response curve (VEP amplitude vs log stimulus contrast)[22]. The contrast sensitivity is the reciprocal of the contrast threshold. The noise level for a given condition (temporal frequency of alternation, viewing eye, visual cortex) was taken as the amplitude of the second harmonic in records where the stimulus was covered with a translucent screen.

RESULTS

The functional properties of cat and monkey visual cortex are still immature at the beginning of the critical period[20,21]. To control whether this holds also in the rat we assessed the ocular dominance distribution of cortical cells and the visual acuity in four rats at postnatal day 20. We found that, as in other mammals[23] the great majority of cortical neurons are equally dominated by both eyes (ocular dominance class 4), most of the cells are not orientational and the receptive fields are large. In addition, the visual acuity measured by VEPs recording is nearly half its normal value in adults.

Effects of monocular deprivation: extracellular unit recordings

At the end of the critical period a total of 350 cells were recorded in normal rats (group I, 100 cells), monocularly deprived rats, either untreated (group II, 100 cells) or treated with cytochrome C (group IV, 50 cells) and monocularly deprived rats treated with NGF (group III, 100 cells).

In Figure 1 we report the pooled data obtained from rats of group I, II, III.

In normal rats (hatched columns) the majority of the cells are driven predominantly or exclusively by the contralateral eye (75%) and the proportion of binocular cells is 80%.

In MD rats (Fig. 1, black columns) the proportion of cells driven by the contralateral eye falls to 20% while the ipsilateral eye dominates 65% of cells. Binocularity is nearly halved (43%) with respect to normal rats.

The ocular dominance distribution in NGF treated rats (Fig. 1, white columns) is not significantly different (chi square, $p > 0.05$) from the ocular dominance distribution in normal adult rats: 66% of the cells are dominated by the contralateral deprived eye and 87% are binocular.

The treatment with cytochrome C was completely ineffective in preventing the effects of monocular deprivation: the ocular dominance distribution in cytochrome C (not reported in Figure) treated rats is indistinguishable from that of untreated monocularly deprived rats.

Thus exogenous supply of NGF prevents the shift in ocular dominance distribution induced by monocular deprivation.

A crucial point was to assess whether the treatment with NGF had altered other functional properties of visual cortical cells such as the selectivity for the stimulus orientation.

The selectivity for the stimulus orientation is reported in Fig. 2 (A) for the same cells out of which the ocular dominance histograms had been compiled. It is evident from the figure that the distribution of cells according to orientation selectivity is not altered by NGF treatment. It has to be noted that no substantial difference was found between the orientational selectivity of cells recorded in NGF treated rats before or after the end of the treatment.

It is known that pharmacological treatments altering the cells spontaneous discharge eliminate the effects of monocular deprivation on the ocular dominance distribution[11,12]. To assess whether NGF treatment affected the cells resting discharge we measured the spontaneous activity of visual cortical neurons in normal rats and in rats under NGF treatment (Fig. 2B). We found that the mean spontaneous discharge (computed from several records one-two minutes long) did not vary significantly (two tailed t test, $p>0.05$) from the cell sample in normal rats (N=15, mean=10 $\pm$ 5 spikes/sec) to the sample in NGF treated rats, either within treatment (N= 15, mean value=7 $\pm$ 4 spikes/sec) or after the end of the treatment (N=25, mean value=10 $\pm$ 8 spikes/sec).

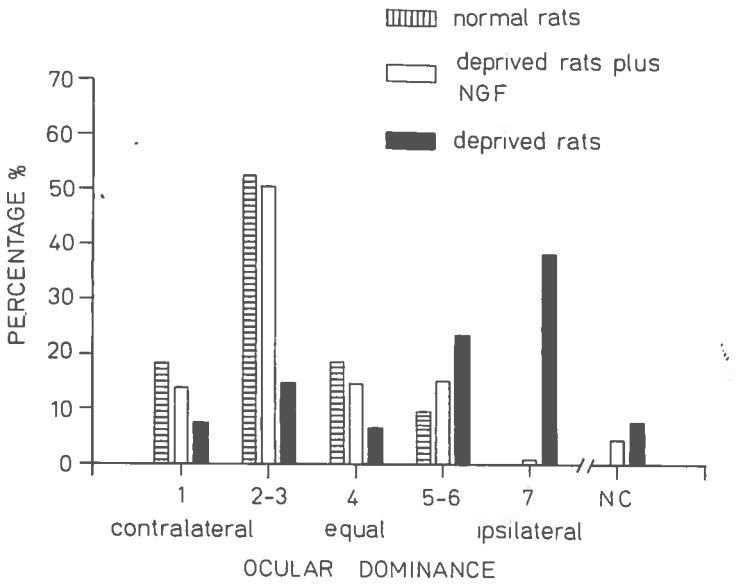

Figure 1. Ocular dominance distributions representing data from all normal rats (hatched columns), all MD rats (black columns), all MD rats treated with NGF (white columns).

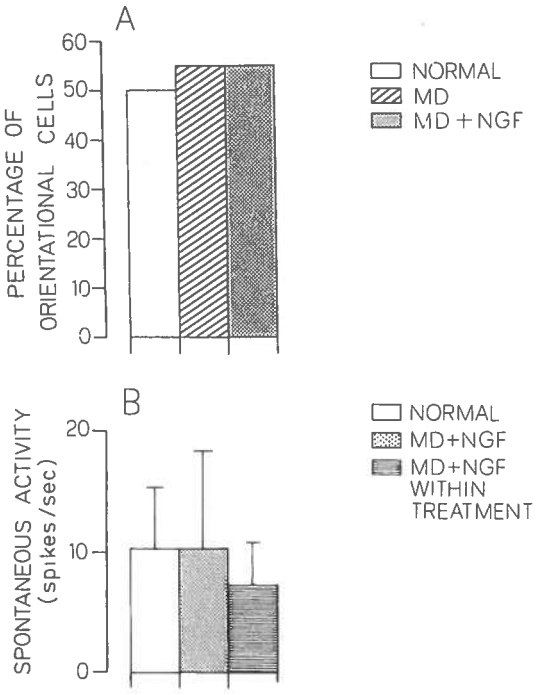

Figure 2. Histograms compiled from the neurons recorded in the primary visual cortex and classified according to their orientational selectivity (A) and their spontaneous discharge (B).
A. Histogram represents neurons recorded in the primary visual cortex of normal (open column), monocularly deprived (MD, hatched column) and monocularly deprived NGF treated (MD+NGF, dotted column) rats.
B. Histogram compiled from neurons recorded in the primary visual cortex of normal (open column), monocularly deprived NGF treated (dotted column, MD+NGF) and monocularly deprived NGF treated rats within treatment (hatched column, MD+NGF within treatment). Neurons are classified according to their spontaneous discharge, evaluated over periods lasting one minute (for each cell, data from three periods were averaged off-line).

Effects of monocular deprivation: visual evoked potentials

In adult pigmented rats, the curve relating VEP amplitude to stimulus spatial frequency (VEP spatial frequency curve) is approximately low pass shaped for spatial frequencies higher than .1 c/deg, with the estimated visual acuity being around 1.2 c/deg[24], in accordance with the behavioral visual acuity[25].

The visual acuity we found for normal rats and for the non deprived eye of MD rats (table 1) is in accordance with the data in the literature.

In figure 3 pooled data from normal rats, MD untreated rats and MD rats treated with NGF are shown separately for the ipsi and contralateral cortex. The shaded area represents the range of VEP amplitudes (mean values, inner solid line plus or minus one SD) recorded from the non deprived eyes at various spatial frequencies. The mean visual acuity was 1.1 c/deg (N= 7, SD = 0.1) for the contralateral cortex and 1 c/deg (N= 7, SD = 0.1) for the ipsilateral cortex (ipsi and contralateral to the stimulated eye).

One month of monocular deprivation strongly reduced the visual acuity of the deprived eye in all rats monocularly deprived and with no treatment. The mean visual acuity for the deprived eye was 0.4 c/deg (N= 8, SD = 0.1) in the contralateral cortex, and 0.3 c/deg (N= 8, SD = 0.1) in the ipsilateral cortex. In addition, the signal amplitude was significantly reduced (t-test $p < 0.01$) at all spatial frequencies tested in both cortices (Fig. 3 A and B; open circles).

To test whether the reduced signal amplitude was due to a loss in contrast sensitivity, we measured in two normal rats and two MD rats the contrast threshold for various spatial frequencies in the deprived and in the normal eye. Contrast thresholds for the deprived eye were increased at spatial frequencies ranging from 0.1 to 1 c/deg.

In rats with intraventricular NGF injections, one month of monocular deprivation produced a much weaker effect. Indeed, both the mean visual acuity and the mean VEP amplitude (Fig. 3 A and B; filled triangles) were not significantly (t-test $p > 0.1$) different from the corresponding values in the normal eye. In addition, the contrast sensitivity for the deprived eye recorded in two rats of the same group was within the normal range for spatial frequencies lower than 0.8 c/deg.

The injection of cytochrome C was not effective in preserving the visual acuity and the contrast sensitivity of the deprived eye. The mean visual acuity for the deprived eye in this group was 0.4 c/deg (N= 3, SD = 0.15).

Thus, intraventricular injection of NGF prevents, at least partially, loss of both visual acuity and contrast sensitivity in the deprived eye.

TABLE 1. Visual acuity for the normal rats and for the non deprived eye of MD rats.
Visual acuity assessed for both eyes in five normal rats (NOR) and for the non deprived eye (D) of ten monocularly deprived rats. VEPs were recorded in the binocular portion of both visual cortices. Ipsi and contra refer to the visual cortex where the recordings have been made (i.e. ipsilateral and contralateral to the stimulated eye). Mean values ± SD have been reported for each group.

Rat	Visual acuity (c/deg) ipsi	contra
NOR 1	0.9	1.2
NOR 2	1.2	1.0
NOR 3	0.9	1.1
NOR 4	1.0	1.0
NOR 5	1.0	1.1
Mean	1.0	1.08
SD	0.12	0.08
D 1	1.1	1.2
D 2	1.0	
D 3		1.0
D 4	1.0	1.0
D 5	1.0	1.1
D 6	1.1	
D 7	0.9	1.1
D 8	0.9	
D 9		1.0
D10		1.1
Mean	1.0	1.07
SD	0.08	0.08

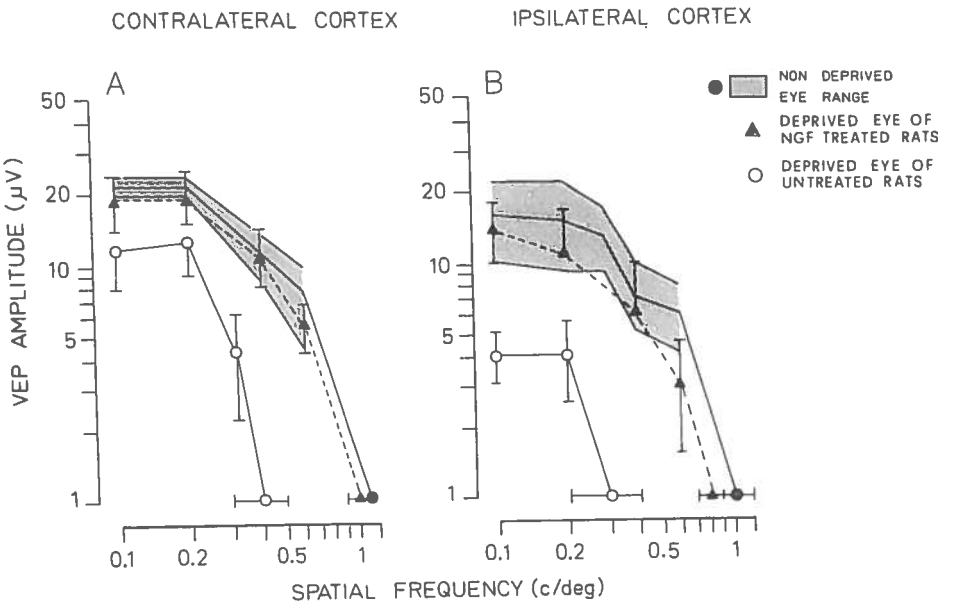

Figure 3. Effects of monocular deprivation on visual evoked potentials (VEP) recorded in untreated rats and NGF treated rats. The mean VEP amplitude is reported as a function of the stimulus spatial frequency. The contrast of the visual stimuli was 30-40% with the exception of the deprived eye of untreated rats, in which case it was 40-50%. A. VEP recorded in the cortex contralateral to the stimulated eye. B. VEP recorded in the cortex ipsilateral to the stimulated eye. The shaded area is the range found for the VEP amplitude in response to stimulation of the non deprived eye (N= 7) mean values (inner solid line) ± one standard deviation. Filled triangles: mean VEP amplitude for the deprived eyes of NGF treated rats (N=8). Open circles: mean VEP amplitude for the deprived eye of untreated rats (N=8). Vertical bars represent the standard deviation. The symbols on the abscissa correspond to the mean visual acuity, i.e. the highest spatial frequency still able to evoke a reliable signal with maximum contrast (filled circles, non deprived eye; filled triangles, deprived eye of NGF treated rats; open circles, deprived eye of untreated rats); the horizontal bars are the standard deviation. The mean noise level was 2 μv, SD= 1 μv.

DISCUSSION

Monocular deprivation in rats during the critical period[19,20] results in a loss of binocular neurons and a shift in the ocular dominance distribution toward the open eye. As in other mammals[6,7], the contrast sensitivity for the deprived eye decreases substantially, and the visual acuity is reduced by more than a factor of two.

We have found that the neurotrophic factor NGF, when exogenously supplied to monocularly deprived rats, prevents both the shift in ocular dominance distribution and the loss of visual acuity and contrast sensitivity in the deprived eye. This suggests that NGF preserves the functional input from the deprived eye to the visual cortex.

The data from control animals (cytochrome C treated) indicate that the effects of NGF are not aspecific, resulting, e.g. from animal handling or anaesthesia. A specific role for NGF in the development of the mammalian visual cortex is in accordance with the presence of both NGF[26,27] and NGF receptors[28,29,30] in the neocortex of newborn, as well as adult rats and primates. Interestingly, the content of NGF in the rat neocortex[25] and primate occipital cortex[26] is higher during the first part of the critical period, later decreasing to adult level.

The mechanisms underlying these actions of NGF in the visual system are unknown, although several possible explanations can be proposed.

For example NGF could increase the electrical activity of cortical neurons, as may occur with PC12 cells[31]. An increased electrical activity of visual cortical cells would be expected to antagonize the effects of monocular deprivation, as described by Shaw and Cynader[12] for glutamate infusion. Such an explanation seems unlikely, since single cell recordings during NGF treatment failed to detect either an increase in spontaneous discharge or an alteration in cell responses to visual stimuli. These findings also suggest that NGF does not impair the transmission of either excitatory[12] or inhibitory[11] visual information.

Another possibility is that NGF interferes with the normal development of the visual cortex . Were this to be the case, the functional properties of the visual cortex in adult NGF-treated rats should be abnormal and even resemble those found for young pups at the beginning of NGF treatment. This is not the case, since both ocular dominance distribution and visual acuity are normal in NGF treated rats.

A third hypothesis takes into account a possible effect of NGF on the cholinergic input to the visual cortex. It is well known that NGF has a neurotrophic action on the cholinergic neurons of the CNS[16,17,18], although a neurotrophic action of NGF on other CNS neurons has been reported[32]. Preliminary results (Dr. G. Vantini, Fidia Research Laboratories, Abano Terme, Italy) in the visual cortex of monocularly deprived rats show that ChAT activity is not substantially changed after treatment with NGF.

The most probable explanation for the findings presented here is that NGF preserves the functional input from the deprived eye to the visual cortex through a specific, direct action on visual neurons. Additional experiments, particularly of molecular biology, will be needed to clarify the mechanisms of this action.

Of particular interest is the result that an exogeneous supply of NGF prevents the amblyopic effects of monocular deprivation. It is well known that a number of ophthalmological pathologies, such as monocular anisometropia or strabismus during the critical period may cause amblyopia in human subjects. NGF puts itself on the stage as a factor to be tested in view of a possible therapeutic approach to treat amblyopia in man.

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