EXOGONOUS SUPPLY OF NERVE GROWTH FACTOR PREVENTS THE EFFECTS OF STRABISMUS IN THE RAT

L. DOMENICI, †† V. PARISI†† and L. MAFFEI††
†Istituto di Neurofisiologia dei CNR, Via S. Zeno 51, 56127 Pisa, Italy
‡Scuola Normale Superiore, Pisa; Fidia Research Laboratories, Abano Terme, Italy

Abstract—It has recently been reported that exogenous supply of nerve growth factor prevents the effects of monocular deprivation both in rats and in cats. Here we have extended these experiments to the case of strabismus. Repeated intravitreal injections of nerve growth factor were performed in rats made surgically strabismic early in the critical period. At the end of the critical period the ocular dominance distribution of visual cortical neurons was assessed in strabismic untreated, strabismic nerve growth factor-treated and strabismic Cytochrome C-treated (control) rats by means of extracellular recordings. We found that in rats surgical strabismus causes a consistent loss of binocular neurons. By contrast the treatment with nerve growth factor maintains the normal ocular dominance distribution of neurons in the primary visual cortex.

We conclude that nerve growth factor exogenously supplied prevents the effects induced by surgical strabismus in rats and suggest that nerve growth factor has a role in visual cortical plasticity.

The importance of surgically induced squint experiments in animals stems from the likely relevance of these experiments to human strabismus, to help understand its etiology and possibly suggest therapeutic aids. In mammals with binocular vision a misalignment of the optical axes of the eyes (usually induced by severing a tendinous insertion of an extra ocular muscle) during the critical period causes dramatic changes in cortical physiology. Hubel and Wiesel were the first to report that in neonatal kittens reared for several months with a surgical strabismus the proportion of binocular cells falls from a normal level of around 80% to about 20%. These data have been successively replicated both in kittens and in monkeys.

Since the experiments of Hubel and Wiesel the interpretation of the effects of strabismus has been based on the lack of congruence between the two visual inputs. Synchronous activity in the two optic nerves is necessary to maintain binocularity. With an artificial squint, corresponding retinal points see different parts of the visual scene and therefore cortical neurons that would be activated more or less simultaneously by inputs from the two eyes are activated asynchronously. In kittens with an esotropic strabismus the latencies of visual cortical responses to visual stimulation or electrical stimulation of the two optic nerves are different.

The mechanism underlying the modifiability of visual cortical connections during the critical period are as yet unclear, although several theories have been proposed (for a recent review see Ref. 27).

Suppression of the input from one or the other eye may occur at the level of geniculocortical interactions or within the cortical net, for example at the level of inhibitory circuitry. Whatever the site of action, the final result is that many visual cortical synapses are depressed or lost leading to a reduction of binocularly innervated neurons.

The experiments to be reported here originate from previous observations from our laboratory on monocularly deprived rats and cats (20) (monocular deprivation is a useful animal model to study activity-dependent changes in connectivity of the visual cortex). We have shown that exogenous supply of nerve growth factor (NGF) (a well characterized trophic factor at both PNS and CNS levels) prevents the effects of monocular deprivation. It is well known that the visual cortex contains NGF (21) and that its expression is higher during the early stages of postnatal development, later decreasing to adult levels. The hypothesis has been raised that during the critical period the strengthening and stabilization of geniculocortical synapses could depend on the physiological level of a target-derived neurotrophic factor, namely NGF, whose production and/or uptake would be activity dependent.

The question we addressed in this paper was whether exogenous supply of NGF could prevent alterations in the visual cortical physiology of rats made surgically strabismic?

EXPERIMENTAL PROCEDURES

Animals and injection procedure

Eighteen Long-Evans (Charles River, Italy) hooded rats were used in this study. Five rats were normal. In 13 rats an esotropic strabismus was surgically induced by cutting the tendinous insertion of the lateral rectus muscle at the scleral

*To whom correspondence should be addressed.

Abbreviation: BDNF, brain derived neurotrophic factor; NGF, nerve growth factor; P, postnatal day.
insertion, 2 mm from the limbus. In all rats the tenotomy was performed immediately after eye opening (postnatal day 14, P14). The degree of strabismus was of the order of 10-14 prism-dioptries, as measured by pupil reflexes.

Five of the strabismic rats were left untreated; five of them were treated with intraventricular injections of β-NGF, and three rats (control rats) were treated with a solution of Cytochrome C, a molecule with the same isoelectric point as NGF, normally used as a control for NGF. 6 β-NGF (Fidia Research Laboratories, Abano Terme, Italy) and Cytochrome C were prepared in a saline solution at a concentration of 1-1.6 μg/μl. The NGF or Cytochrome C treatment was started at P14 and was repeated every two days for a period of one month (P45). In the rat this corresponds to the whole length of the critical period. 22,23 Injections were made into the lateral ventricle by means of a micropipette connected to a cannula (gauge 27) inserted 1 mm laterally to the median line and 1 mm behind bregma; the volume injected was 2 μl. To check that injections actually reached the lateral ventricle, Pontamine Sky Blue was injected at the same coordinates and was invariably found in the ventricle. Surgery and injections were performed under brief general anaesthesia induced by inhalation of ethane. To control for possible structural alterations caused by repeated injections histology was performed in treated rats using classical staining techniques (Cresyl Violet).

**Extracelluar unit recordings**

At the end of the critical period (P45) the effects of the strabismus in treated and untreated rats were assessed by means of extracellular recordings of single neurons at the level of the binocular region of the primary visual cortex (binocular area 17 or area OC1B). Each rat was anaesthetized with urethane by means of intraperitoneal injections (7 ml/kg, 20% solution, Sigma) and mounted into a stereotaxic apparatus. The angle of strabismus was eliminated by rotating and fixing both eyes by means of metal rings surrounding the bulb and attached to the stereotaxic apparatus. Pupils were left undilated since it proved extremely difficult to provide artificial pupils that would not cause disturbances as observed by Lennie and Perry. 16 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 to backproject the optic disc with natural pupils (0.5-1 mm in diameter with the luminance we used) at the beginning of each experimental session pupil reflexes and superimposition of binocular receptive fields were assessed to control for correct alignment of the optic axes. At the end of each experimental session the eye alignment and the position of receptive fields within the visual field were controlled by projecting the optic disc of both eyes on a tangential screen (pupils were previously dilated by eye drops of 1% atropina, Allergan, Italy). Animals were electrocardiographically monitored and their body temperature was maintained around 38°C throughout the experiment. A micropipette filled with NaCl (3 M) was inserted into the binocular portion of the primary visual cortex. Recordings were carried out in the cortex contralateral to the strabismic eye. In three strabismic untreated and three strabismic NGF-treated rats of our sample, recordings were also performed in the cortex ipsilateral to the strabismic eye. To avoid sample biases due to the organization of ocular dominance in area OC1B, the electrode penetrations were angled 35-60° with respect to the cortical surface and at least two well-spaced penetrations were performed for each hemisphere in each rat. Cells were recorded throughout all primary visual cortical layers from a depth of 100 to 1200 μm below the cortical surface. The depth within the cortex was directly read off the micromanipulator. No gross changes in ocular dominance were found with cortical depth.

Visual stimuli were either light bars projected on a reflecting screen or gratings generated by computer on a display (HP 1300A). Both the screen and the display distance from the rat eyes were 20 cm. For each cell the location of the receptive field in visual space, the ocular dominance and the optimal stimulus orientation were evaluated. Only penetrations with all neuronal receptive fields within 30° from the vertical meridian and in the upper visual field (binocular visual field) 24 were considered. The mean eccentricity of receptive fields in our cell sample was 18° from the vertical meridian, without gross differences between normal (mean eccentricity = 17°), strabismic (mean eccentricity = 18°) and strabismic NGF-treated rats (mean eccentricity = 19°). Ocular dominance was assessed with bars of optimal orientation, following the criteria of Hubel and Wiesel: neurons in ocular dominance class 1 were driven only by the contralateral eye; neurons in ocular dominance classes 2 and 3 were binocular and preferentially driven by the contralateral eye; neurons in class 4 were equally driven by the two eyes; neurons in classes 5 and 6 were binocular and preferentially driven by the ipsilateral eye; neurons in class 7 were driven only by the ipsilateral eye. Neurons were classified as oriented if (1) the cell response was maximal for a given orientation (preferred orientation) and (2) it was indistinguishable from the spontaneous activity for stimuli orthogonal to the preferred orientation. Neurons were classified as biased if the cells responded to all tested orientations (vertical, horizontal, ±45°) but with a clear preference for one of them and nomenclatural if no preference. For the responses for the different orientations tested were indistinguishable. Cells unresponsive to visual stimulation were considered as non-classifiable. A blind procedure was adopted in strabismic rats treated either with NGF or Cytochrome C.

In some cells of normal, strabismic and strabismic NGF-treated rats response curves as a function of the stimulus spatial frequency were determined. Eye refraction was optimized by recording visual evoked potentials in response to alternating gratings of various spatial frequencies, Optic lenses of different power were positioned in front of the stimulated eye. The lens that imparted highest visual acuity was considered optimal. Sinusoidal gratings of 40-50° contrast, alternating in contrast at a temporal frequency of 0.5-1 Hz, were generated on a display positioned on the cell's receptive field. The responses (spikes/s) of single cells were recorded and fed into a computer for on-line averaging and Fourier analysis. The cell response evoked by the stimulus could either be at the same temporal frequency as the stimulus (first harmonic) or at twice that frequency (second harmonic) according to the cell's receptive field type. For each spatial frequency the response amplitude was taken as the amplitude of the main harmonic in the Fourier spectrum and the ratio between the amplitude of the response (main harmonic) and the amplitude of the noise was assessed. The noise was estimated by measuring the response when the stimulus was a blank field.

**Statistical analysis**

The distributions in ocular dominance classes observed in normal, strabismic and strabismic NGF-treated rats were statistically evaluated by a chi-square test (four degrees of freedom). The test of significance for binomial distribution was used to evaluate the differences between percentages for orientational selective cells.

**RESULTS**

The normal ocular dominance distribution for single neurons recorded in the binocular portion of the adult rat primary visual cortex contralateral to the stimulated eye is illustrated in Fig. 1A. The proportion of binocular cells is 77%. The bias in favour of the contralateral eye (classes 1 and 2-3) is evident and reflects the predominance of crossed
optic nerve fibres. Only 11% of the cells in our sample are dominated by the ipsilateral eye.

Surgical strabismus causes clear changes in the ocular dominance distribution (Fig. 1B). Binocularity is strongly affected, with the proportion of binocular cells decreasing from 77% to 30% of the total.

By contrast the ocular dominance distribution in NGF-treated rats (Fig. 1C) is very similar to normal: 67% of the cells are dominated by the contralateral strabismic eye and 73% are binocular (normal vs strabismic NGF-treated rats, $P > 0.5$).

To control for some non-specific effects of NGF treatment due, for example, to animal handling or anaesthesia, we also recorded three strabismic rats treated with Cytochrome C. The treatment with Cytochrome C was completely ineffective in preventing the effects caused by strabismus (Fig. 1D). The ocular dominance distribution of strabismic Cytochrome C-treated rats is indistinguishable from that of untreated strabismic rats ($P > 0.5$).

In six strabismic rats of our sample we also recorded from the primary visual cortex ipsilateral to the deviated eye (Fig. 2A). The treatment with NGF (three rats) completely prevented this loss of binocularity (Fig. 2B).

Thus, an exogenous supply of NGF prevents the reduction of binocular neurons induced by the strabismus in both cortices, contralateral and ipsilateral to the squinting eye.

An important 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 and spatial frequency.

The selectivity for the stimulus orientation is reported in Table 1 for the same cells for which the ocular dominance had been assessed. The table shows the proportion of non-oriented cells in the total population; no significant differences were found between normal, strabismic untreated and strabismic NGF-treated rats, indicating that at least this gross measure of orientation selectivity is not affected by both strabismus and NGF treatment.

Spatial frequency selectivity of visual cortical cells was assessed in normal (two animals, recorded cells, $N = 10$) and strabismic NGF-treated rats (two animals, recorded cells, $N = 8$). In our limited sample no gross differences between control and NGF-treated rats were observed.
Using an electrophysiological approach we found that, in hooded rats, strabismus surgically induced during the critical period results in a significant loss of binocular neurons of the primary visual cortex. First of all it is necessary to exclude the possibility that this loss of binocular neurons might be caused by a sampling mistake, namely by cell recording in different parts of the visual cortex in normal vs strabismic animals. This possibility is really unlikely since great care was taken in recording neurons with the receptive field in the same part of the binocular visual field.

As far as the ocular dominance distribution is concerned, the binocular region of the rat visual cortex differs from that of cat, dog,13,14,15 monkey15 and in that there is clear predominance of the contralateral eye. In surgically strabismic rats, the ocular dominance distribution histogram shows that most cells remain dominated by the contralateral eye.

**Ocular dominance in strabismic nerve growth factor-treated rats**

In strabismic NGF-treated rats both the binocularity and the distribution of visual neurons in ocular dominance classes is the same as that observed in normal rats; this is true both in the cortex contralateral and ipsilateral to the strabismic eye. This effect cannot be attributed to non-specific effects such as those produced by surgery or anaesthesia. In rats used as a control, treatment with Cytochrome C does not prevent the effects produced by surgical strabismus.

Another point to be considered is the possibility that NGF treatment could cause direct or indirect pathological effects in the visual system. The fact that the responses of visual cortical neurons both in terms of orientational and spatial frequency selectivity and spontaneous discharges16 remained relatively normal in NGF-treated rats seems to make this possibility rather unlikely.

The effects observed of exogenous supply of NGF in strabismic rats are not surprising as NGF is a neurotrophic factor physiologically present at the level of CNS (for a review see Ref. 36). In rat neocortex, NGF is expressed in the adult15 as well as during development.22 It has recently been reported that the content of NGF in the rat14 and primate16 occipital cortex is higher during the first part of postnatal development, later decreasing to adult level.

As far as the NGF receptors are concerned, several papers have described the presence and the distribution of the low affinity form of NGF receptor in many regions of the CNS,12,23,24,27 the visual system included. The high affinity form of NGF receptor (trk)16 mediating the biochemical activity of NGF is also represented at the level of CNS.17 However, data on the distribution of trk in the visual system are lacking.

At the moment we cannot even be sure that NGF is the neurotrophic factor in question or the only one. NGF is present in the visual cortex but other neurotrophic factors of the NGF family like brain derived neurotrophic factor (BDNF) and neurotrophin-3 are also present.23 It has been shown that BDNF at high concentration can interact with high affinity NGF receptors and vice versa.29 The amount of NGF we
have injected is considerable and thus it may well be that it cross-reacts with the receptors of other neurotrophic factors, such as BDNF, mimicking their action.

**Nerve growth factor and strabismus**

The visual system of mammals is very sensitive during the critical period to manipulations of the visual environment. In particular, binocularity of visual cortical neurons is easily lost if the two visual inputs become imbalanced. The imbalance may consist of alteration of the relative strength of the discharge pattern of one of the two inputs (as in the case of monocular deprivation) or of alteration of temporal simultaneity or correlation of the two inputs (as in the case of natural or artificial strabismus).

Recently we have found that NGF treatment prevents the amblyopic effects of monocular deprivation during the critical period both in rats\(^1\) and cats.\(^2\) On the basis of these results we have proposed that during development the two monocular inputs compete for the acquisition of a neurotrophic factor whose production, release or uptake are activity dependent. When the inputs from the two eyes are synchronous the response of the cell is likely to be stronger and the release of neurotrophic factor sufficient for strengthening synaptic connections of both visual inputs. When synchronization between the two visual inputs is lost, the production, release or uptake of neurotrophic factor is reduced, competition emerges and as a consequence only the stronger of the two visual inputs is able to maintain or establish its synaptic connections. According to this hypothesis the action of NGF would take place at the level of geniculate afferent–cortical cell connections.

However, reciprocal inhibition between the two eyes has been described and the inhibition exerted by the normal onto the squinting eye is reported in cat to be much stronger than the reciprocal effect.\(^3\) In addition, a pharmacological treatment (bicuculline) blocking inhibitory receptors restores binocular responses in strabismic cats.\(^4\) Thus the hypothesis cannot be excluded that the action of the neurotrophic factor is not, or not only, at the level of geniculo-cortical connections. It could be primarily within the cortical net at the level of inhibitory interneurons which mediate interocular inhibition.

**Acknowledgements**—We thank Dr D. C. Burr and Dr N. Berardi for critical reading of the manuscript, Mr G. Cappagli, Mr V. Alpigiani and Mr P. Tacchini for technical assistance. Dr M. F. G. Fapinoli took part in some experiments. Dr D. C. Burr and Dr C. Morrone made the computer software for the signal acquisition of analysis.

**REFERENCES**


(Accepted 17 June 1992)