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TrkA activation in the rat visual cortex by antirat trkA IgG prevents the effect of monocular deprivation

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Abstract

It has been recently shown that intraventricular injections of nerve growth factor (NGF) prevent the effects of monocular deprivation in the rat. We have tested the localization and the molecular nature of the NGF receptor(s) responsible for this effect by activating cortical trkA receptors in monocularly deprived rats by cortical infusion of a specific agonist of NGF on trkA, the bivalent antirat trkA IgG (RTA-IgG). TrkA protein was detected by immunoblot in the rat visual cortex during the critical period. Rats were monocularly deprived for 1 week (P21–28) and RTA-IgG or control rabbit IgG were delivered by osmotic minipumps. The effects of monocular deprivation on the ocular dominance of visual cortical neurons were assessed by extracellular single cell recordings. We found that the shift towards the ipsilateral, non-deprived eye was largely prevented by RTA-IgG. Infusion of RTA-IgG combined with antibody that blocks p75^{NTR} (REX), slightly reduced RTA-IgG effectiveness in preventing monocular deprivation effects. These results suggest that NGF action in visual cortical plasticity is mediated by cortical TrkA receptors with p75^{NTR} exerting a facilitatory role.

Keywords

critical period; neurotrophin; NGF; plasticity; trk

Introduction

Neurotrophins have been shown to regulate plasticity of the mammalian visual cortex during development (see references in Thoenen, 1995; Bonhoeffer, 1996; Cellerino & Maffei, 1996). Among possible mechanisms, neurotrophins have been shown to regulate synaptic efficacy at the neuromuscular junction and in various brain areas including the visual cortex, and expression levels of neurotransmitters, calcium-binding proteins, and other proteins essential for normal neuronal function (Lohof *et al.*, 1993; Jones *et al.*, 1994; Kang & Schuman, 1995; Akaneya *et al.*, 1997; Carmignoto *et al.*, 1997; Sala *et al.*, 1998).

The characterization of neurotrophin receptors and their expression patterns have been central to understanding their mechanisms of action and potential targets. Each of the neurotrophins is capable of binding to p75^{NTR}, also known as the low-affinity nerve growth factor (NGF) receptor. NGF binding to this receptor increases sphingomyelin hydrolysis and induces translocation of nuclear factor-kappaB (NF-κB) to the nucleus (e.g. Casaccia-Bonneli *et al.*, 1996; Frade *et al.*, 1996; van der Zee *et al.*, 1996). Intriguingly, even though all members of the neurotrophin family are able to bind to this receptor, only NGF has been shown to induce

these signalling events. Each neurotrophin also binds to one or more members of the Trk receptor tyrosine kinase family and much of the specificity of neurotrophin action appears to be regulated by specificity in interactions with these signalling molecules. NGF appears to bind exclusively to TrkA; BDNF and NT4 to TrkB and NT3 to TrkC. NT3 can also activate TrkA and TrkB receptors in some cellular contexts (e.g. Ip *et al.*, 1993).

Of particular importance for the present paper, NGF has been shown to regulate developmental plasticity in the rodent visual cortex. Injection of function inhibiting monoclonal antibodies to NGF affects development of the geniculocortical system (Berardi *et al.*, 1994) and delays the end of the critical period for monocular deprivation (Domenici *et al.*, 1994a). In addition, intraventricular injections of NGF prevent the effects of monocular deprivation during the critical period (Maffei *et al.*, 1992; Fagiolini & Stryker, 1996). Potentially, these actions of NGF could be mediated through TrkA receptor tyrosine kinase, the low-affinity p75^{NTR}, a combination of both receptors, or an unidentified signalling molecule. The low-affinity neurotrophin receptor p75^{NTR} has been shown to be present in the visual cortex with a fibre-like pattern of labelling corresponding to the cholinergic input (Pioro & Cuellar, 1990). The presence of TrkA receptors in the visual cortex is less well characterized, although TrkA is expressed by basal forebrain cholinergic neurons and therefore seems likely to be localized also on their fibres which innervate the visual cortex, other cortical regions and hippocampus (e.g. Sobreviela *et al.*, 1994). Some, but not all laboratories have also reported low levels of TrkA in intrinsic cortical cells (see Bonhoeffer, 1996; Cellerino & Maffei, 1996).

The major objective of the present work has been to determine whether activation of the receptor tyrosine kinase trkA in the visual cortex mediates the previously observed effects of NGF in monocularly deprived (MD) rats. To do this we have utilized IgG polyclonal antibodies directed against the extracellular domain of rat TrkA (RTA antibodies). IgG fractions of RTA bind, dimerize and activate TrkA but not other Trk receptor family members *in vitro* (Clary *et al.*, 1994). Injection of these agonistic antibodies also mimics NGF actions *in vivo*, rescuing axotomized basal forebrain cholinergic neurons (Lucidi-Philippi *et al.*, 1996). In this report we demonstrate that TrkA is present in the visual cortex as detected by antigen blot using RTA antibodies and that infusion of RTA IgG into the visual cortex by means of osmotic minipumps prevents the effects of monocular deprivation during the critical period; RTA effectiveness is slightly reduced by simultaneous treatment with blocking antibodies to p75^{NTR}.

Materials and methods

Immunoblot analysis

Protein extracts were prepared from the visual cortex, striatum and cerebellum of rats at postnatal age 23 (P23), which corresponds to the peak of the critical period for monocular deprivation in rats (Fagiolini *et al.*, 1994). PC12 cells and fibroblasts were used as positive and negative controls, respectively. Proteins were extracted according to Knüsel *et al.* (1994); the rats were deeply anaesthetized with an overdose of Avertin i.p. prior to decapitation. Protein content was determined with a BioRad protein assay kit (BioRad, Milan, Italy). Samples were boiled in sample buffer, electrophoresed on 7.5% SDS–polyacrylamide (PAGE) minigels and transferred to nitrocellulose (Amersham, Bucks, UK). Protein blots were probed overnight at 4 °C with RTA IgG (1 mg/mL in Tris-buffered saline with 2% non-fat dry milk BioRad, 0.2% Tween-20). Blots were then incubated with horseradish peroxidase labelled secondary antibody for 2 h at room temperature and analysed using ECL chemiluminescence system (Amersham, UK).

Animals and surgical procedures

We have used 31 Long Evans hooded rats. Eight animals have been left untreated (normal rats). Minipump implantation and lid sutures were done under Avertin anaesthesia (1.5 ml/kg i.p.). The Italian Ministry of Public Health approved the study, which followed the ARVO guidelines for the use of animals in ophthalmic and vision research. Twenty-three rats were implanted with Alzet minipumps (rate 0.5 μ L/h, volume 95–99 μ L, duration 1 week) at postnatal day 21 (P21) and monocularly deprived for 1 week by eyelid suture. Minipumps were connected via PE tubing to a cannula (gauge 30) implanted in the primary visual cortex contralateral to the deprived eye [1 mm mediolateral (1 mm M–L), 0 mm anteroposterior (AP) from the lambda]. In nine animals minipumps and attached cannulae were filled with RTA antibody (1.4 mg/mL in sterile saline); in four rats they were filled with RTA IgG at a lower concentration (0.3 mg/mL); in five rats (control rats) they were filled with either sterile saline (two rats) or control IgG (1.4 mg/mL, three rats); in five rats (RTA + REX) they were filled with RTA IgG (1.4 mg/mL) plus antip75^{NTR} IgG, (REX, 1 mg/mL). In four RTA (three with concentration 1.4 mg/mL and one with concentration 0.3 mg/mL) and in two control animals the experiment was run double blind: animals were letter coded and the code was broken only after electrophysiological and anatomical experiments had been completed. IgG concentrations were chosen on the basis of published work both *in vivo* and *in vitro* (Clary *et al.*, 1994; Lucidi-Philippi *et al.*, 1996). No alterations in social and feeding behaviour or in body weight were observed in treated animals as compared with controls.

Electrophysiological and anatomical experiments

All animals have been recorded at P28. To record single unit activity, animals were anaesthetized in urethane (6 mL/kg, 20% solution in saline; Sigma) by intraperitoneal injection. Body temperature was continuously monitored and maintained at about 37 °C by a thermostated electric blanket during the experiment. The electrocardiogram was also monitored. A hole was drilled in the skull in correspondence with the binocular portion of the primary visual cortex (binocular area 17 or area Oc1b) contralateral to the deprived eye (treated cortex in RTA, control and RTA + REX animals). In the rat, Oc1b corresponds to the lateral portion of the primary visual cortex mapping the upper nasal visual field. Recordings were performed at least 3 mm. away from the minipump cannula. After exposure of the brain surface, the dura was removed and a micropipette (2 M Ω) filled with NaCl (3 M) was inserted into the cortex (> 4 mm. M–L, between 0 and 1 mm AP from the lambda). To prevent sampling bias, due to the organization of area Oc1b with respect to the ocular dominance, our penetrations were angled and for each animal at least four well-spaced penetrations were performed.

Both eyes were fixed and kept open by means of adjustable metal rings surrounding the external portion of the eye bulb, 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 as it proved extremely difficult to provide artificial pupils that would not cause vignetting, as observed by Lennie & Perry (1981). Given the impossibility of back-projecting the optic disk with natural pupils (about 0.5 mm in diameter with the luminance used), we dilated the pupil (atropine sulphate, 0.1%) at the end of the experiment. The position of the optic disk was marked on to a tangent screen where the positions of all the cells receptive fields had been plotted.

Visual stimuli were hand-moved light bars projected on a reflecting tangent screen or gratings and bars generated on a computer display (Daewoo, 28 $\times$ 22 cm; mean luminance, 15 cd/m²). Both the screen and the display were 20 cm from the rat eyes. Upon isolating a cell, the following experimental protocol was followed.

The location of the monocular receptive fields in visual space and the orientation properties were determined with hand held stimuli. In the pigmented rat, the binocular portion of each visual hemifield extends $\approx 40^\circ$ from the vertical meridian in the upper visual fields and the vertical meridian is $55\text{--}58^\circ$ from the optic disk (Reese & Jeffery, 1983). Only cells with receptive field within 20° from the vertical meridian were included in our sample. Care was taken that cells were sampled at comparable eccentricities in the different groups.

Quantitative measures of the main cell response properties

Cell responsiveness was assessed with bars or gratings of optimal orientation according to standard criteria. Spontaneous discharge was evaluated over a period of 1 min during which the cell discharge was measured while the screen was kept at constant luminance. Signal to noise ratio was evaluated in terms of amplitude of modulation of cell discharge in response to an optimal stimulus (peak response divided spontaneous discharge). Cell orientation selectivity and ocular dominance were determined on the basis of the cell firing rate (spikes/s), read from a counter.

Ocular dominance—Neurons in ocular dominance class 1 were neurons driven only by the stimulation of the contralateral eye; neurons in ocular dominance 2–3 were binocular and preferentially driven by the contralateral eye (ratio of contra to ipsi peak response > 1.5); neurons in ocular dominance 4 were equally driven by the two eyes; neurons in ocular dominance 5–6 were binocular and preferentially driven by the ipsilateral eye (ratio of ipsi to contra peak response > 1.5) and neurons in ocular dominance class 7 were driven only by the ipsilateral eye. To evaluate the binocularity and the eye dominance of each individual animal we calculated a binocular index and an Ipsilateral index.

The binocular index is:

$$[\frac{1}{2}N(2-3)+N(4)+\frac{1}{2}N(5-6)]/N_{\text{tot}};$$

where $N(i-j)$ = number of cells in classes $(i-j)$ and N_{tot} = total number of cells recorded.

Ipsilateral index is:

$$[N(7)+\frac{1}{2}N(5-6)]/N_{(\text{Tot})},$$

with $N(i-j)$ = number of cells in ocular dominance class $(i-j)$ and $N_{(\text{Tot})}$ is the total number of cells recorded.

Orientation selectivity. Neurons were classified as orientation-selective (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 orientation-biased (B) if the response was present at all orientations but was clearly greater ($> 2\times$) for certain orientations than for others; cells were classified as non-orientation-selective (N) if the response was of comparable strength at all six orientations (0° , 30° , 60° , 90° , 120° , 150°).

At the end of the recording session the position of the last electrode penetration was marked by withdrawing the electrode, gently brushing its tip with a saturated solution of DiI (Molecular Probes, Portland, OR, USA) in absolute ethanol and, after drying of the DiI solution, slowly reinserting it into the cortex for 1.5 mm. The animal was then perfused transcardially (50 mL.

saline followed by 150 mL paraformaldehyde 4%), the brain removed, immersed overnight in sucrose 30% and sectioned coronally at 30 μ m with a freezing microtome.

To reveal the diffusion of infused antibodies, sections were blocked with a solution containing 10% horse serum, 0.1% Triton-X100 and then reacted with biotinylated goat antirabbit IgG (Vector, 1: 200). Staining was developed by the avidin–biotin method (Vector, UK) with nickel intensification using 3,3' diaminobenzidine (Sigma Italy, Milan) as a chromogen.

Statistical analyses

One way ANOVA with Tukey's *post-hoc* test, Mann–Whitney test or Student's *t*-test were performed to test the significance of differences between groups. Differences in ocular dominance distribution were assessed using a chi-squared test (4 d.f.). Level of significance $P = 0.05$.

Results

Role of TrkA activation in preventing MD effects

Detection of trkA protein in the rat visual cortex—Before undertaking experiments assessing the function of TrkA it was important to demonstrate its presence in the visual cortex during the critical period for cortical plasticity. Protein extracts were prepared from various brain regions at P23, at the peak of the critical period. The presence of TrkA in these extracts was investigated by antigen blot using RTA antibody. PC12 cells and fibroblasts were used as positive and negative controls, respectively (data not shown). Results in Fig. 1 show the presence of an antigen of the expected molecular weight of TrkA (140 kDa) in striatum and visual cortex. TrkA expression was less abundant in the visual cortex with respect to the striatum, indeed it could be only detected loading 200 μ g of total proteins. By contrast, trkA signal in the striatum was already clearly visible loading 100 μ g of total proteins. As expected from previous reports (e.g. Sobreviela *et al.*, 1994), a band at 110 kDa attributed to a partially glycosylated form of TrkA (Clary *et al.*, 1994) is also visible in the extract of striatum.

Anti-TrkA IgG infusion into the visual cortex—As TrkA is present in the visual cortex during the critical period, it was important to determine whether TrkA activation by a divalent antirat trkA IgG (RTA) was sufficient to prevent the effects of monocular deprivation. Twenty-three rats have been implanted with Alzet minipumps (rate 0.5 μ L/h, volume 95 μ L, duration 1 week) at postnatal day 21 (P21) and monocularly deprived for 1 week by eyelid suture. This period corresponds to the peak of the critical period for monocular deprivation in rats (Fagiolini *et al.*, 1994). Eight animals were left non-deprived (normal animals) and recorded at the same age as MD animals (P28). At the end of the recording session the last electrode penetration was marked by gently brushing the electrode tip with DiI (Fig. 2E). Animals were then killed, perfused and coronal sections of the brain were immunostained to visualize the extent of diffusion of the infused antibodies (RTA or control IgG). Anti-rabbit IgG antibodies were used to show the distribution of both RTA or control IgG in the brain the after 1 week of continuous infusion.

As shown in Fig. 2, the infused visual cortex, and in particular area Oc1b, the site of recordings, were strongly immunostained after infusion, for either RTA (Fig. 2A) or control IgG (Fig. 2B). There was a clear gradient of staining from the infusion site; the presence of infused antibodies became undetectable in the cortex at distances greater than 5 mm from the cannula. Staining was not detected in the visual cortex contralateral to the infusion site, indicating that the diffusion of IgG was limited primarily to the ipsilateral cortex. No labelling was found in subcortical structures. Occasionally, a weak staining limited to their dorsal-most part was found

in the superior colliculus and in the hippocampus. Immunostaining was completely absent in control rats infused with saline (Fig. 2D).

Although diffusion of the antibodies appeared limited to cortical structures, trkA expressing cells in the basal forebrain cholinergic nuclei (e.g. Sobreviela *et al.*, 1994) projecting to the visual cortex were clearly immunostained for RTA IgG (Fig. 3). In Fig. 3 this is shown for the horizontal limb of the diagonal band (Mesulam *et al.*, 1983; Saper, 1984). While the mechanism of this IgG labelling is not certain, it is likely to reflect retrograde transport of TrkA-RTA IgG complexes from axon terminals in the visual cortex; this is because many nearby cell populations, known to also express TrkA, which do not project to the visual cortex, including septal cholinergic cells projecting to the hippocampus and cholinergic interneurons in the striatum, were not stained (Fig. 3). As expected, no staining of neurons in the diagonal band was observed in animals treated with control IgG. Cells in the lateral geniculate nucleus, whose axons form a major projection to the visual cortex, were not stained. As these cells express TrkB, but do not express detectable levels of TrkA, this result provides additional strong evidence for the specificity of RTA IgG for TrkA expressing cells *in vivo*.

Intriguingly, after prolonged perfusion of anti-TrkA IgG (Fig. 2C) but not control IgG, a number of cells intrinsic to the cortex were observed to be immunostained. The number of labelled cells and the intensity of the labelling was variable. One possible explanation for this observation is the existence of a population of cells within the cortex which express low level of endogenous TrkA and are therefore able to concentrate RTA IgG by endocytosis. Alternatively, prolonged activation of trkA may result in increased TrkA gene expression and protein accumulation as has been observed in basal forebrain cholinergic neurons (e.g. Li *et al.*, 1995).

TrkA activation in the visual cortex is sufficient to prevent the effects of monocular deprivation—We have recorded 293 cells in anti-trkA IgG (1.4 mg/mL) treated animals, 199 in normals and 144 in MD controls. For each cell, we assessed receptive field location in the visual field, ocular dominance class (according to the Hubel and Wiesel criteria), orientation selectivity and cell responsiveness. Only cells with receptive fields located within 20° from the vertical meridian have been included in our sample. Spontaneous discharge, signal to noise ratio and orientation selectivity of visual cortical neurons were normal in RTA-treated rats (Table 1), suggesting that RTA IgG did not have detrimental effects on the functional properties of visual cortical cells.

The ocular dominance distributions of neurons recorded in normal, RTA IgG treated and MD control rats are depicted in Fig. 4. It is evident that 1 week of monocular deprivation is sufficient to induce a strong shift in the ocular dominance distribution towards the non-deprived (ipsilateral) eye. In MD control rats more than 90% of recorded cells can be driven exclusively or predominantly by the non-deprived eye (classes 7 and 5–6) compared with 17% in normal animals not subjected to monocular deprivation. Strikingly, the shift is totally prevented by application of RTA IgG during the period of monocular deprivation.

For each animal, the shift towards the ipsilateral eye dominance can be summarized with the ipsilateral index, which is 1 when all cells are monocular and exclusively dominated by the ipsilateral, non-deprived eye (class 7 cells) and 0 when no cell is dominated by the ipsilateral eye (no cells in class 7 or 5–6). The ipsilateral index is calculated as follows:

$$N(7) + \frac{1}{2}N(5-6)/N_{(\text{Tot})},$$

with $N(i-j)$ = number of cells in ocular dominance class $(i-j)$ and $N_{\text{(Tot)}}$ is the total number of cells recorded. As shown in Fig. 5A, the ipsilateral indexes of all nine RTA IgG treated animals are in the normal range (0.05–0.25) while the ipsilateral indexes of MD animals range from 0.65 to 0.82. In Fig. 5B, the binocular indexes for the same animals are reported; the binocular index is a measure of binocularity, and is 1 when all cells are binocular (classes 2–3, 4 and 5–6) and 0 when all cells are monocular (classes 1 and 7). The binocular index is calculated as follows:

$$N(4) + \frac{1}{2} [N(5-6) + N(2-3)] / N_{\text{(Tot)}}.$$

Monocular deprivation clearly reduces the binocular index in all MD control rats (binocular index about 0.25). It is evident that a normal binocularity (binocular index about 0.6) is preserved in all RTA IgG treated animals.

To investigate whether the dose of anti-trkA IgG employed was saturating, we recorded four animals treated with a fivefold lower dose of RTA IgG. The results, reported in Fig. 6 clearly indicate that this dose is much less effective in preventing the shift of ocular dominance distribution (ipsilateral indexes about 0.4).

We conclude that activation of TrkA by anti-trkA IgG in the visual cortex is sufficient to prevent the effects of monocular deprivation. The extent of RTA preventive action is dose dependent.

Role of p75^{NTR}

We have tested whether p75^{NTR} binding by endogenous ligands is necessary for RTA prevention of MD effects. We have recorded 221 cells in five monocularly deprived rats (MD + RTA + REX rats) treated with RTA (1.4 mg/mL) along with anti-p75^{NTR} IgG (REX, 1 mg/mL). REX binds p75^{NTR} but does not induce ceramide production. Instead, it inhibits NGF-induced ceramide production by preventing p75^{NTR} signalling (Casaccia-Bonnel *et al.*, 1996); therefore, REX functions to inhibit neurotrophin binding to p75^{NTR} and thereby inhibits p75^{NTR}-mediated signal transduction. REX does not inhibit RTA binding to trkA in PC12 cells (Clary *et al.*, 1994). In addition, REX did not affect the effectiveness of RTA in rescuing axotomized basal forebrain neurons (Lucidi-Philippi *et al.*, 1996).

The results obtained in MD + RTA + REX animals are shown in Fig. 7A–C. The ocular dominance distribution (Fig. 7A) shows a proportion of binocular cells close to normal; however, the shift towards the ipsilateral eye is not as effectively counteracted as in animals treated with RTA IgG alone. This is further illustrated in Fig. 7B,C, where the mean ipsilateral and binocular indexes for MD + RTA + REX animals are reported together with those for normal, RTA and control MD animals for comparison. Only the mean ipsilateral index (Fig. 7B) is slightly but significantly different from the results obtained with RTA alone. The block of p75^{NTR} seems therefore to reduce the effectiveness of trkA activation.

In summary, the similarity of the results obtained with the infusion of RTA alone to the previously reported effects of exogenous NGF indicate that TrkA plays the major part in accounting for the effects of NGF on plasticity during the critical period in the binocular region of the rodent visual cortex. The results obtained with infusion of RTA + REX suggest that endogenous neurotrophins acting through p75^{NTR} modulate the effect of trkA activation by either anti-trkA IgG or NGF.

Discussion

The major conclusion of this paper is that selective activation within the visual cortex of a receptor tyrosine kinase, *trkA*, can regulate plasticity in the rodent visual cortex. In our experiments, cortical *trkA* was activated by continuous infusion into the rat visual cortex of IgG specific for the extracellular domain of *TrkA* (RTA) which has been shown previously to dimerize and activate this receptor (Clary *et al.*, 1994). Continuous infusion of RTA prevented the effects of monocular deprivation. At higher doses of RTA (1.4 mg/mL), the prevention of MD effects was complete in all treated animals. Normal binocularity was maintained; the shift of ocular dominance distribution towards the non-deprived eye was completely suppressed. RTA treatment did not interfere with the transmission of visual information in cortical neurons as their functional properties, such as orientation selectivity, responsiveness and spontaneous activity, were indistinguishable from those in untreated littermates of the same age.

NGF is almost certainly the physiological ligand which regulates the activity of *trkA* in physiological conditions within the cortex. Previous work has provided clear documentation that NGF is synthesized within the cortex and transported retrogradely to cholinergic nuclei whose axons innervate the cortical regions (e.g. Seiler & Schwab, 1984; Large *et al.*, 1986). In addition, a continuous supply of NGF either into the ventricles (Domenici *et al.*, 1991; Maffei *et al.*, 1992; Pizzorusso & Maffei, 1996) or directly into the visual cortex (Berardi *et al.*, 1998), prevents the effects of MD in the rat much to the same extent as shown here using RTA IgG as an activating agent of *trkA*. These results suggest that NGF regulation and *trkA* expression within neurons and axons in the cortex may play a crucial part in regulating the plasticity within the rat visual cortex during normal postnatal development.

NGF action in rodents and cats

The effectiveness of NGF in preventing MD effects in the rat has been recently confirmed in mice (Fagiolini & Stryker, 1996). Thus, there is agreement that NGF is effective in rodents independently from the intracortical or intraventricular site of delivery. Results are not as clear in the cat where different sites of delivery produce different results. Indeed, intracortical infusion of NGF does not prevent the effects of monocular deprivation (Galuske *et al.*, 1996), although it seems able to restore ocular dominance plasticity within the adult visual cortex (Gu *et al.*, 1994). In addition, intracortical supply of NGF does not affect the formation of ocular dominance columns (Cabelli *et al.*, 1995). By contrast, intraventricular administration of NGF to monocularly deprived kittens attenuates the ocular dominance shift induced by MD (Carmignoto *et al.*, 1993) and prevents the reduction in visual acuity of the deprived eye measured behaviourally (Fiorentini *et al.*, 1995). Thus, as opposed to rats, the site of NGF delivery seems to be crucial for the effectiveness of the treatment in kittens. This could stem from a species difference either in density and/or distribution of *trkA* bearing afferents to the visual cortex and/or in intracortical mechanisms. Intraventricular delivery is likely to activate, massively, in both species subcortical structures bearing NGF receptors and relevant for cortical plasticity such as the basal forebrain cholinergic system.

Roles of *TrkA* and *p75^{NTR}*

While NGF has been shown to activate two receptors, *trkA* and *p75^{NTR}*, RTA IgG has been shown to be a specific and selective agonist of NGF high-affinity receptor *TrkA* (Clary *et al.*, 1994). Application of saturating doses of RTA IgG or Fab fragments eliminates all high-affinity NGF binding sites present on PC12 cells in contrast to application of anti-*p75^{NTR}* IgG which eliminates low-affinity NGF binding sites (Clary *et al.*, 1994). RTA IgG has been shown to bind and activate *trkA*, but not *trkB* nor *trkC*. *In vitro* binding of RTA IgG, but not Fab fragments to sympathetic neurons or PC12 cells has been shown to stimulate autophosphorylation of *trkA* receptors, phosphorylation of MAP kinases and other substrates.

RTA IgG also promotes cell survival and neurite outgrowth, i.e. it mimics both early and late actions of NGF. In addition, RTA mimics NGF actions on neurotransmitter release from visual cortex synaptosomes (Sala *et al.*, 1998) and neurotrophin release from PC12 cells (Krüttgen *et al.*, 1998). *In vivo*, TrkA activation by RTA has also been shown to mimic NGF effects on survival of axotomized basal forebrain cholinergic neurons (Lucidi-Philippi *et al.*, 1996). We show here that RTA IgG infused within the visual cortex binds cortical trkA receptors, is retrogradely transported to the soma of trkA bearing neurons and mimics the effects of NGF in monocularly deprived animals. It has been suggested that NGF mechanism of action in monocular deprivation induced ocular dominance plasticity could involve the activation of other neurotrophin receptors. The fact that RTA mimics NGF effects in monocularly deprived animals, suggests that selective TrkA activation *in vivo* is sufficient to account for the action of NGF in cortical plasticity during the critical period.

Is this TrkA action modulated by p75^{NTR}? The most recent data (see Carter & Lewin, 1997 for a review) suggest that, in different cellular contexts, p75^{NTR} can modulate Trk functions. For example, neurotransmitter release from visual cortex synaptosomes is potentiated by NGF and BDNF via trk receptors with p75^{NTR} playing a modulatory role on NGF action (Sala *et al.*, 1998). Our results suggest that this is the case also in visual cortical plasticity: indeed, RTA prevention of MD effects is diminished when p75^{NTR} is blocked. This suggests that activation of p75^{NTR} by endogenous neurotrophins helps modulate the effectiveness of trkA-regulated signalling.

Localization of NGF receptors in the visual cortex

p75—The low-affinity receptor p75^{NTR} has been previously detected in the visual cortex on fibres which probably originate from cholinergic neurons in the basal forebrain (e.g. Pioro & Cuello, 1990). This suggests that the modulatory effects of p75^{NTR} inhibition are related to the cholinergic input. We do not know, however, whether after prolonged treatment with RTA, other cells or fibres in the visual cortex express detectable levels of p75^{NTR} or whether prolonged activation of trkA by RTA induces p75^{NTR} in cells that otherwise would not express it.

TrkA—In the present paper, we show by histological analyses that diffusion of RTA from the infusion cannula was limited to the cortex. Accumulation of RTA was also seen within cholinergic neurons in the basal forebrain which are known to express trkA and to develop axonal projections to the cortex. This suggests that the TrkA receptors involved in RTA effects are present within the cortex. In agreement with this conclusion, results in the present paper have demonstrated that trkA protein is present in the rat visual cortex during the critical period. Similar results have been recently obtained in mice (Fagiolini, M., Stryker, M.P., Mobley, W.C., personal communication). These observations do not rule out the possibility that continued activation of these receptors after retrograde transport to non-cortical regions is also important.

Which structures in the visual cortex bear the TrkA receptors activated by RTA? Cholinergic neurons in the basal forebrain express TrkA, project to the visual cortex and are known to transport, retrogradely, radioactively labelled NGF from their cortical terminals (Seiler & Schwab, 1984; Domenici *et al.*, 1994b; Sobreviela *et al.*, 1994). We have found that RTA is also retrogradely transported from the cortex to the cell bodies of those cholinergic neurons in the basal forebrain projecting to the visual cortex. Two observations indicate that this is specific transport: first, septal neurons and striatal interneurons, cholinergic neurons that express trkA, but do not project to the visual cortex, did not accumulate RTA; secondly, thalamic neurons which form major projections to the visual cortex, but do not express detectable trkA, were not labelled. Thus the only neurons labelled outside of the cortical perfusion site were neurons

which are known to express trkA and to have a major projection to this region. The results argue that at least a portion of the trkA protein detected by immunoblot in visual cortex is localized to the terminals of the cholinergic afferents.

Results in the present paper also suggest that some neurons intrinsic to the cortex express at least low levels of trkA. In previous work, using RTA as primary antibody, it has not been possible to detect intrinsic neurons clearly expressing trkA within the cortex (Sobreviela *et al.*, 1994; Holtzman *et al.*, 1995). We have repeated these experiments in normal untreated rats and confirm that no clear TrkA positive profiles are found in the visual cortex (data not shown). By contrast, after prolonged perfusion with RTA in the visual cortex, we have observed the presence of RTA immunopositive cells. No such labelling was found after a comparable period of perfusion with control IgG. This suggests the presence of a population of neurons in the visual cortex that express very low levels of TrkA, which cannot be detected using conventional immunocytochemical procedures. Visualization of these neurons after prolonged RTA exposure may be possible because they are able to accumulate RTA and thus become detectable. Alternatively, prolonged exposure to NGF has been shown to induce trkA mRNA and protein in PC12 cells and basal cholinergic forebrain neurons (e.g. Holtzman *et al.*, 1995). Prolonged exposure to RTA IgG may have resulted in induction of trkA within neurons, which normally express only very low levels of this receptor. Results in the present paper do not exclude the possibility that trkA is normally not expressed within neurons intrinsic to the cortex, but is induced within these neurons indirectly as a result of actions on the cholinergic projection from the basal forebrain. In summary, there are persuasive arguments that trkA is present in the cholinergic projection from the basal forebrain because specific retrograde transport of RTA IgG is observed; it is also clear that some intrinsic cortical neurons are capable of expressing this neurotrophin receptor; it is not certain whether these neurons normally express this protein.

In conclusion, the results presented here show that activation of TrkA in the visual cortex, either on basal forebrain cholinergic afferents or, possibly, on cortical neurons, prevents the effects of monocular deprivation in the rat. Comparison of these results to those previously obtained by exogenous NGF leads to the conclusion that NGF action in visual cortical plasticity is mediated by cortical TrkA receptors, with p75^{NTR} exerting a facilitatory role.

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Abbreviations

MD	monocularly deprived
NGF	nerve growth factor
NF-κB	nuclear factor-kappaB

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Fig. 1.

Detection of TrkA in rat brain regions. Protein extracts were prepared from the indicated brain regions. One hundred and 200 μ g of total proteins were fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and analysed by immunoblotting using rat TrkA (RTA) IgG. Positions corresponding to molecular weight marker proteins are indicated on the left. Samples from striatum (STR), visual cortex (VC) and cerebellum (CER) were analysed. Note the presence of a band of MW 140 kDa in extracts of striatum and visual cortex. The lower band at 110 kDa, present in striatal extracts, has been shown to correspond to an intracellular pool of incompletely glycosylated TrkA (Clary *et al.*, 1994).

Fig. 2.

Coronal sections through the primary visual cortex immunostained to visualize the extent of diffusion of RTA or control IgG after 1 week of infusion. (A) RTA IgG (1.4 mg/mL) infused animal, (B) control rabbit IgG (R-IgG) infused animal, (D) saline infused animal. Arrowheads in (A,B,D) mark the binocular portion of the primary visual cortex, area Oc1b. No immunoreactivity is detectable in the saline infused animal after 1 week of infusion. It is evident that antibody penetration (RTA or rabbit IgG) was sufficient to reach the recording area in Oc1b. (C) Magnification of an area within the RTA perfused cortex. Several RTA positive cells are present, and their apical dendrite is often visible. (E) A portion of an electrode penetration traced using DiI. Receptive fields location of cells recorded within this track were at about 9° from the vertical meridian, within the binocular subfield of the visual field. Calibration bar: A,B,D = 900 μm ; C = 50 μm ; E = 250 μm

Fig. 3. Immunolabelling in the basal forebrain ipsilateral to the cortex treated for 1 week with RTA (1.4 mg/mL). RTA was revealed using an anti-rabbit IgG antibody. Stained cells are present only in the horizontal limb of the diagonal band of Broca (HDBB) (region outlined with a black rectangle). This area is shown at higher magnification in (B). Calibration bar: A = 620 μ m; B = 100 μ m. CPu: caudate-putamen, LV: lateral ventricle, MS: medial septum.

Fig. 4.

Ocular dominance distributions in normal rats ($n = 8$, 199 cells), monocularly deprived rats treated with RTA (1.4 mg/mL, 17 μ g/day) (MD + RTA, $n = 9$, 293 cells) and MD rats treated with control solutions (MD + CON, $n = 5$, 144 cells). Cells in ocular dominance class 1 and 7 are monocular and exclusively driven by the contralateral eye (deprived eye in MD rats) or the ipsilateral eye, respectively. Cells in class 2–3 and 5–6 are binocular and preferentially driven by the contralateral or the ipsilateral eye, respectively, and cells in class 4 are equally driven by either eye. The distributions for normals and MD + RTA do not differ significantly while both differ from that in MD + CON (χ^2 test, 4 d.f.).

Fig. 5.

Ipsilateral (A) and binocular (B) indexes for all animals recorded [normals (NOR)], MD treated with RTA (MD + RTA), MD treated with control solution (MD + CON). RTA dose 17 $\mu\text{g}/\text{day}$. The groups NOR and MD + RTA do not differ (one way ANOVA and *post-hoc* Tukey's test) while the group MD + CON differs from both.

Fig. 6.

(A) Mean ipsilateral index for normal ($n = 8$), MD + RTA (17 µg/day) ($n = 8$), MD + CON ($n = 5$) and MD + RTA at a lower dose (3.4 µg/day) ($n = 4$). Vertical bars = SD. The groups of normals and MD + RTA (17 µg/day) are not significantly different, while they differ from the group MD + RTA (3.4 µg/day) and MD + CON. The latter two are also significantly different (one-way ANOVA and *post-hoc* Tukey's test). (B) Mean binocular index for normal ($n = 8$), MD + RTA (17 µg/day) ($n = 8$), MD + CON ($n = 5$) and MD + RTA at a lower dose (3.4 µg/day) ($n = 5$). Vertical bars = SD. The groups of normals and MD + RTA differ from the group of MD + CON (one-way ANOVA and *post-hoc* Tukey's test).

Fig. 7.

(A) Ocular dominance distribution of MD rats treated with RTA ($\mu\text{g/day}$) and REX ($12 \mu\text{g/day}$). Ipsilateral (B) and binocular (C) index of normal (NOR), control MD (MD + CON) and MD rats treated with RTA alone (MD + RTA) or with RTA and REX (MD + RTA + REX). REX significantly attenuated the effect of RTA on ipsilaterality but not on binocularity (Mann–Whitney test).

Table 1

Functional properties of visual cortical cells in normal and RTA treated rats. Normally distributed data (Orientation selectivity index) are described with their mean $\pm$ SD and were compared using Student's *t*-test. Data not distributed normally (spontaneous discharge and signal to noise ratio) are described with their median and 25th and 75th percentile (in parenthesis in the table) and were compared using the Mann–Whitney test. None of the parameters was significantly different between normal and RTA-treated animals. Spontaneous discharge was calculated over 1 min of acquisition in normal rats (82 cells) and in MD + RTA rats after 7 days of continuous intracortical infusion (51 cells); signal to noise ratio was calculated for each cell (same cell numbers as for spontaneous activity) as the ratio between the peak of the cell response to the optimal visual stimulus (spikes/s) and the spontaneous discharge (spikes/s); orientation selectivity index was calculated as

$$\{N(o)+N(b)/2\} / N_{TOT},$$

where $N(o)$ is the number of orientation selective cells and $N(b)$ is the number of orientation biased cells and N_{TOT} is the total number of cells. RTA dose 17 μ g/day

	Normal rats	RTA-treated rats
Spontaneous discharge (spike/s)	3.1 (0.5–7)	3 (1.3–4.5)
Signal to noise ratio	4.4 (2.6–6)	4 (3–5.8)
Orientation selectivity index	0.39 $\pm$ 0.16	0.37 $\pm$ 0.09