Brain-derived neurotrophic factor is an anterograde survival factor in the rat visual system

Matteo Caleo, Elisabetta Menna, Sabrina Chierzi, Maria Cristina Cenni and Lamberto Maffei

Background: The neurotrophins, which include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4/5 and NT-6, are a family of proteins that play fundamental roles in the differentiation, survival and maintenance of peripheral and central neurons. Much research has focused on the role of neurotrophins as target-derived, retrogradely transported trophic molecules. Although there is recent evidence that BDNF and NT-3 can be transported in an anterograde direction along peripheral and central axons, there is as yet no conclusive evidence that these anterograde factors have direct post-synaptic actions.

Results: We report that BDNF travels in an anterograde direction along the optic nerve. The anterogradely transported BDNF had rapid effects on retinal target neurons in the superior colliculus and lateral geniculate nucleus of the brain. When endogenous BDNF within the developing superior colliculus was neutralised, the rate of programmed neuronal death increased. Conversely, provision of an afferent supply of BDNF prevented the degeneration of geniculate neurons after removal of their cortical target.

Conclusions: BDNF released from retinal ganglion cells acts as a survival factor for post-synaptic neurons in retinal target fields.

Address: Scuola Normale Superiore and Istituto di Neurofisiologia del C.N.R., Via S. Cataldo 1, 56100 Pisa, Italy.

Correspondence: Matteo Caleo
E-mail: caleo@in.pi.cnr.it

Received: **16 March 2000**
Revised: **14 August 2000**
Accepted: **15 August 2000**

Published: **11 September 2000**

Current Biology 2000, 10:1155–1161

0960-9822/00/$ – see front matter

Background

Neurons require extracellular signals in order to survive [1,2]. Target structures and afferent inputs represent the main sources of trophic support [1,2]. Several molecules that act as target-derived trophic factors have been identified. They bind to the nerve terminals of the responsive neurons, are transported in a retrograde direction and signal to the cell body [3–5]. Much less is known about the molecular identity of anterograde trophic factors. Recently, it has been demonstrated that the neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) can be transported in an anterograde direction along axons [6–10]. It has been suggested that this transport may serve to regulate the survival, phenotype, synapse formation, and dendritic growth of the target neurons [6–10]. There is, however, no direct evidence that these effects are caused by the release and post-synaptic action of the anterogradely transported neurotrophin.

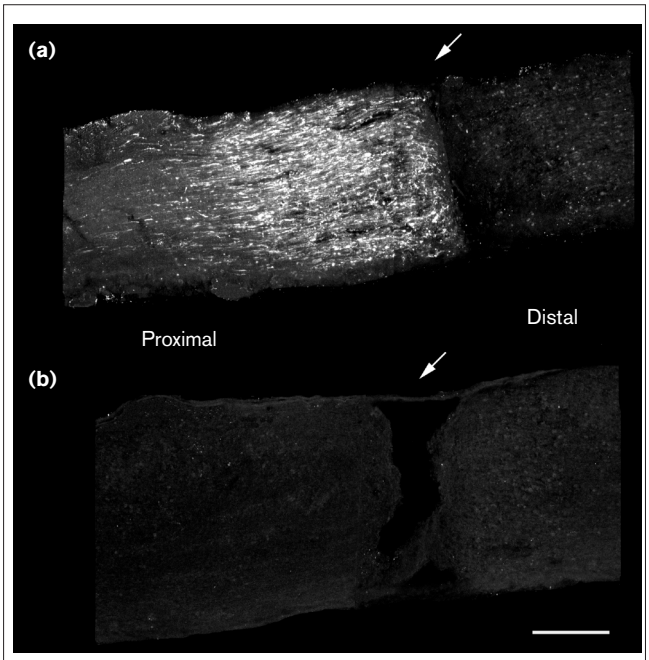
Anterograde trophic influences have been described in the visual system [11–13]. Second-order visual neurons in the lateral geniculate nucleus (dLGN) and superior colliculus (SC) in the brain are known to require afferent support from retinal ganglion cells (RGCs) during both development and adulthood [11–13]. Endogenous BDNF is a good candidate for this support because it is expressed by RGCs [14,15] and its high-affinity receptor TrkB is localised on neuronal cell bodies and dendrites in the dLGN and SC [16]. Here, we report that BDNF is transported in an anterograde direction along the optic nerve and released from RGC terminals to promote the survival of post-synaptic neurons in retinal target fields.

Results

Anterograde transport of BDNF in the optic nerve

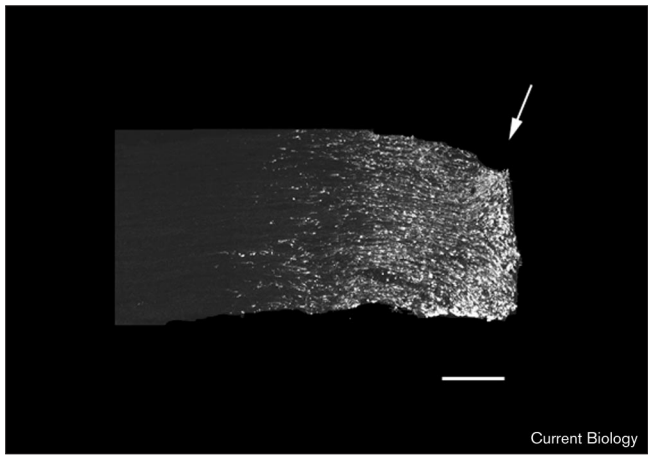
We first examined whether endogenous neurotrophins are transported in an anterograde direction by RGCs in adult rats. The optic nerve was ligated or crushed to interrupt axonal transport, and the nerves were immunostained with antibodies specific for BDNF or nerve growth factor (NGF) 20 hours after the operation (Figure 1). BDNF immunoreactivity was clearly detected in axons proximal to where the nerve was crushed, indicating that BDNF was transported in an anterograde direction towards retinorecipient structures (Figure 1a). Retrograde accumulation of BDNF was virtually undetectable (Figure 1a). No NGF immunoreactive fibres could be detected on either side of the ligation (Figure 1b). Anterograde transport of endogenous BDNF was also detected in newborn (P5) rats following an intracranial crush of the optic nerve and visualisation of BDNF accumulation 20 hours later. Numerous fibres on the proximal side, although retracted, showed robust BDNF immunostaining (data not shown).

Figure 1



Anterograde transport of endogenous BDNF in the adult rat optic nerve. Immunohistochemical staining showed **(a)** accumulation of BDNF, **(b)** but not NGF, in axons proximal to where the nerve was crushed. Staining was performed 20 h after the operation. The BDNF-labelled profiles show the typical morphology of fibre terminals oriented towards the site of transport blockade (indicated by arrows). The scale bar represents 200 μm.

Figure 2



Anterograde transport of exogenous BDNF in the adult rat. Longitudinal section through the optic nerve, showing build-up of exogenous BDNF in axons proximal to a ligation, 3 h following intraocular BDNF injection. The ligation site is indicated by an arrow. The scale bar represents 200 μm.

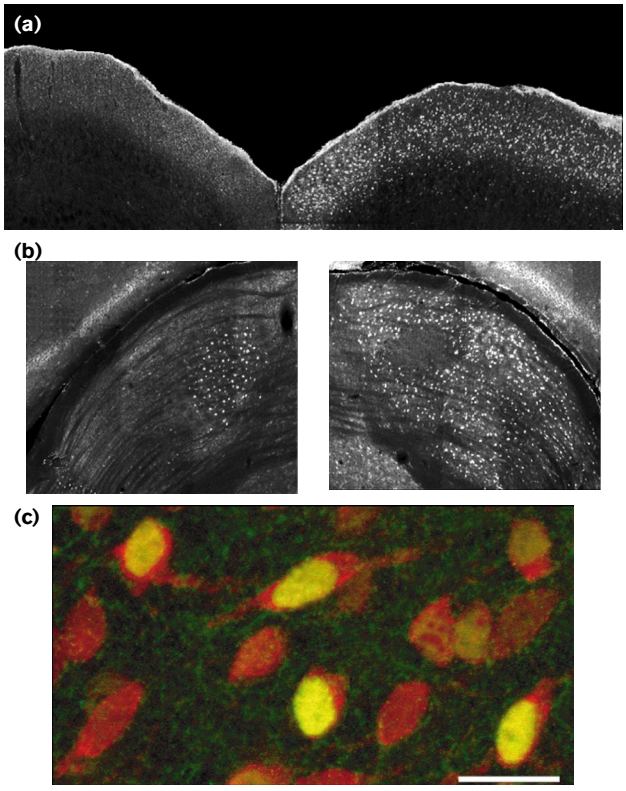
We next examined whether exogenous BDNF is also transported by RGCs. Recombinant human BDNF (5 μg) was injected intraocularly and a ligation placed in the retro-orbital portion of the optic nerve in adult rats. After 3 hours, a time at which accumulation of endogenous BDNF was still undetectable, many BDNF-positive fibres could be observed proximal to the ligation (Figure 2). Experiments performed in newborn rats yielded identical results (see also [17]). These observations indicate that exogenous BDNF injected into the vitreous humour is rapidly taken up by retinal ganglion cells and transported in an anterograde direction along the optic nerve.

Effects of BDNF on retinal target neurons

The demonstration of a specific anterograde transport of BDNF prompted us to ask whether this factor exerts functional effects on post-synaptic neurons in retinal target fields. A short delay in the target response is essential to establish a causal relationship between the anterograde factor and its post-synaptic action. We therefore analysed the expression of immediate-early genes (IEGs) that are known to be rapidly induced by neurotrophins in responsive cells [18]. BDNF was injected intraocularly and the expression of the IEG products c-Fos and Zif268 was examined in the SC and dLGN 5–7 hours after injection. This time interval takes into account the time required for fast axonal transport from RGCs to their targets (~3–4 hours) [19] as well as the time necessary for maximal IEG elevation in response to neurotrophin treatment (2 hours). We found that the intraocular injection of BDNF resulted in a clear induction of c-Fos and Zif268 in the SC and dLGN innervated by the injected eye (Figure 3a,b). Intravitreal injections of NGF failed to elicit such a response (Figure 3a,b). The c-Fos-positive cells co-expressed the neuronal marker NeuN, demonstrating that the BDNF-induced IEG expression was restricted to neurons (Figure 3c). IEG elevation in retinal targets was dependent on axonal transport as co-injections of BDNF and colchicine into the eye completely abolished IEG staining (Figure 4a).

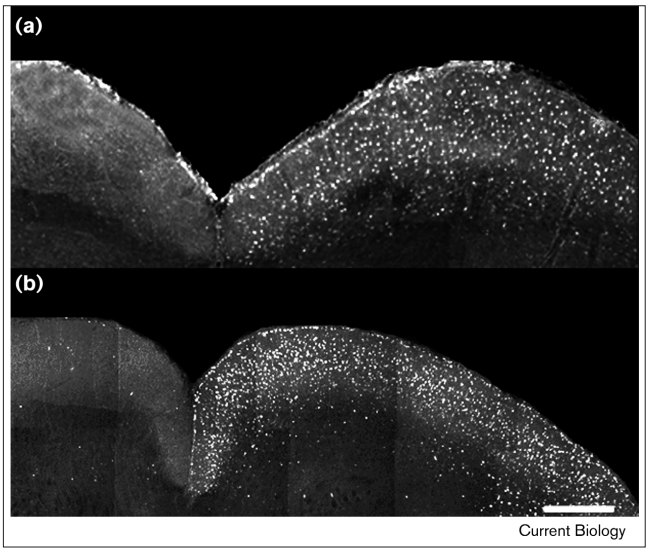
It might be argued that the intraocular injection of BDNF stimulates RGCs to produce a secondary trophic signal that, in turn, generates the target response. To rule out this possibility, we used two distinct approaches: block of BDNF signalling at the retinal level and neutralisation of BDNF within the target by infusion of specific antibodies. In a first set of experiments, we co-injected BDNF and the tyrosine kinase inhibitor K252a into the adult eye. As Trk receptors but not p75 receptors are present on RGC bodies [20], K252a blocks all effects of BDNF on RGCs. We found that K252a, when administered intraocularly, failed to abolish the BDNF-induced IEG expression in the SC and dLGN (Figure 4b). On the other hand, we found that the stereotaxic administration into the SC of antibodies that neutralise the biological activity of BDNF resulted in a complete loss of the IEG

Figure 3



Anterogradely transported BDNF modulates gene expression in retinal target structures. Adult rats received intraocular injections of BDNF (40 μg) in one eye and NGF (40 μg) in the other eye. The expression of the IEG products c-Fos and Zif268 was analysed in the SC and dLGN, 5 h after injection. **(a)** Coronal section through the SC immunostained with anti-c-Fos antibodies. A clear band of immunoreactive cells was visible within the SC contralateral to the BDNF-injected eye (right). The SC contralateral to the eye injected with NGF was not stained (left). **(b)** Coronal section through the dLGN immunostained for c-Fos. Strong c-Fos induction was restricted to the dLGN subfields receiving input from the BDNF-injected eye. In the dLGN ipsilateral to the BDNF injection (left), a narrow patch of stained cells was observed that matched the uncrossed retinal terminal field. The entire dLGN contralateral to the BDNF injection was stained (right) except for the small area innervated by the NGF-treated eye. **(c)** Coronal sections through the SC contralateral to the BDNF-injected eye were double-stained with anti-c-Fos (green) and anti-NeuN (red) antibodies. Double staining revealed that the c-Fos-positive nuclei were also NeuN immunoreactive. The scale bar represents 600 μm in (a), 300 μm in (b) and 15 μm in (c).

Figure 4



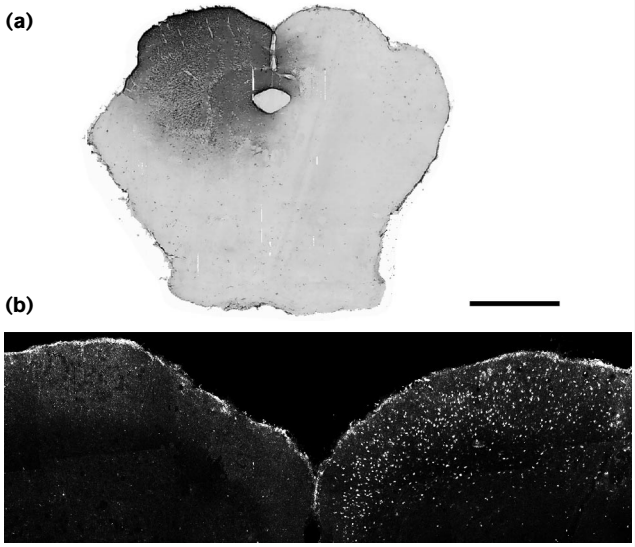
BDNF-induced IEG expression requires axonal transport but not Trk signalling within the retina. Coronal sections through the SC, immunostained for c-Fos. **(a)** This animal received BDNF in one eye and BDNF plus colchicine in the other eye, and was killed 7 h later. Strong induction of c-Fos expression was observed along the retinorecipient margin of the SC contralateral to the BDNF-injected eye (right). The SC contralateral to the eye injected with BDNF plus colchicine was not stained (left). **(b)** This animal received BDNF plus K252a in one eye and K252a alone in the other eye, and was killed 7 h later. The SC contralateral to the eye injected with BDNF plus K252a showed strong c-Fos staining (right), whereas K252a alone had no effect (left). The scale bar represents 350 μm in (a) and 400 μm in (b).

response (Figure 5a,b). Two distinct anti-BDNF antibodies (RAB from Amgen and chicken anti-BDNF antibody from Promega) whose neutralising activity has been demonstrated *in vitro* and *in vivo* [21–23] were used and gave identical results. Injection of a control anti-horseradish peroxidase (HRP) antibody (Sigma) into the SC was unable to block the BDNF-induced IEG upregulation, thus confirming the specificity of the blocking effect (data not shown). These experiments were replicated in neonates with identical results. The function-blocking anti-BDNF antibodies were injected bilaterally into the SC of P5 rats and BDNF was administered intraocularly. No IEG staining could be seen in the colliculi but a normal labelling was found in the geniculate (data not shown).

Endogenous BDNF reduces natural cell death in the superior colliculus

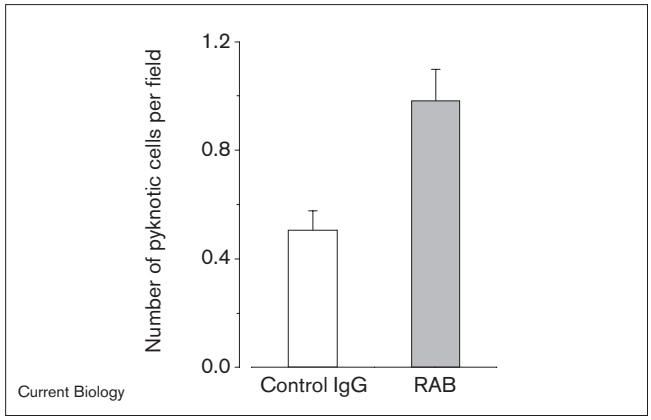
Our data demonstrate that BDNF is transported in an anterograde direction and released by RGC terminals to act directly on the post-synaptic neurons. We next tested whether regulating the availability of BDNF affects neuronal survival in retinal target fields. The SC of the rat undergoes a period of naturally occurring cell death that peaks between P5 and P8 [12]. We neutralised endogenous BDNF in the SC of P5 rats by injecting the BDNF-neutralising antibody RAB. Rabbit immunoglobulin G (IgG) was injected as a control and cell death was evaluated by stereological techniques 3 hours following the antibody infusions. The mean density of pyknotic cells was increased twofold in animals injected with the anti-BDNF antibody, compared with controls (*t*-test, $p < 0.05$;

Figure 5



(a,b) Anti-BDNF antibodies block the upregulation of IEG expression that is induced in the SC by intraocular injection of BDNF. Adult rats received unilateral stereotaxic injections of function-blocking anti-BDNF antibodies into the SC. BDNF was administered in both eyes. (a) Coronal section through the brainstem of an adult rat, immunostained to visualise the extent of diffusion of the injected chicken anti-BDNF antibodies. Only the SC on the side ipsilateral to the injection was stained. (b) Staining of c-Fos in a coronal section through the SC adjacent to that shown in (a). Labelled cells were evident in the uninjected SC (right), but absent in the side infused with anti-BDNF antibody (left). The scale bar represents 1.5 mm in (a) and 500 μm in (b).

Figure 6



Infusion of anti-BDNF antibodies increases the rate of developmental death in the SC. The graph shows the mean density of pyknotic nuclei in the SC of animals injected with control rabbit IgG ($n = 4$) or RAB ($n = 5$). The error bars indicate SEM.

Figure 6). This demonstrates a role for endogenous BDNF in promoting the survival of SC neurons.

Anterograde supply of BDNF rescues axotomised geniculate neurons

We next determined whether an anterograde supply of exogenous BDNF could be exploited to prevent the death of dLGN neurons after ablation of the visual cortex. Neonatal rats were selected as an experimental model because of the rapidity of the death process at this age. Indeed, previous studies have shown that removal of the visual cortex in newborn rats leads to the death of all dLGN cells within 96 hours, with a peak in pyknosis at 48 hours after the lesion [24,25]. Monocular injections of BDNF, or saline as control, was performed either 24 or 36 hours after ablation of the visual cortex, and cell death was evaluated at 48 hours in the dLGN ipsilateral to the lesion. For each animal, we measured the level of pyknosis in the two eye-specific subfields of the lesioned dLGN (Figure 7). The quantitative analysis showed that intraocular BDNF was remarkably effective in preventing dLGN cell death. Indeed, the density of pyknotic cells was reduced to 50% in the treated patch following BDNF administration at either 24 or 36 hours after ablation of the visual cortex (Figure 8a). In contrast, no difference between treatments could be detected in the untreated dLGN patch (analysis of variance, $p = 0.72$). This ensures that the effects of cortical injury are comparable in all animal groups. The BDNF rescue effect was dependent on axonal transport from the retina, as it was completely blocked by intraocular administration of colchicine, but not on BDNF signalling within the retina, as shown by blocking the BDNF transduction cascade at the retinal level with K252a (Figure 8b).

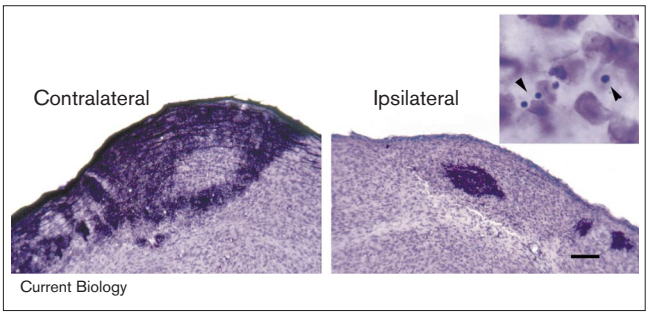
Discussion

Anterograde transport of BDNF

We have demonstrated for the first time that the endogenous BDNF synthesised by RGCs is transported in an anterograde direction along the optic nerve in both developing and adult rats. On the other hand, retrograde transport of endogenous BDNF was not detectable. Thus, despite the known retrograde effects of BDNF on RGCs [20,26], the physiological trafficking of BDNF was mainly in the anterograde direction in the optic nerve. BDNF-immunoreactive terminals have been demonstrated in both the dLGN and SC [27], suggesting that BDNF, transported in the anterograde direction, is stored in nerve terminals within the RGC innervation targets where it could be released through activity-dependent mechanisms [28].

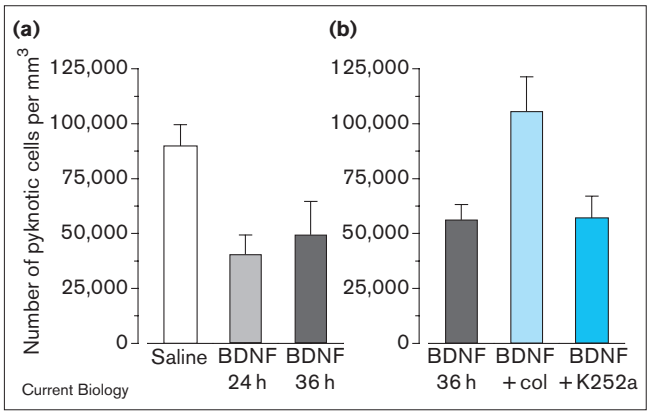
We have also demonstrated the ability of RGCs to take up, transport and release exogenous BDNF injected into the vitreous humour. It is still unresolved whether the endogenous and exogenous neurotrophins accumulate in the same cellular compartments and share the same mechanisms of transport.

Figure 7



Coronal section through the dLGN of a P9 rat that received a unilateral occipital cortex lesion 48 h earlier. Retinal projections were labelled with intravitreal HRP (black) and the section counterstained with cresyl violet. Note the clear segregation of eye-specific inputs within the dLGN and the shrunken dLGN on the side ipsilateral to the lesion. Inset, many pyknotic cells (arrowheads) were present in the lesioned dLGN 48 h after surgery. The scale bar represents 100 μm (15 μm for the inset).

Figure 8



Application of BDNF into the eye prevents the degeneration of thalamic neurons following injury. **(a)** Average density of pyknotic nuclei in the dLGN subfield receiving input from the eye injected with saline or BDNF. Stereological counting of pyknotic cells using the optical fractionator method (see Materials and methods) revealed a significant decrease in the density of pyknotic cells following BDNF treatment at either 24 or 36 h post-lesion compared with the saline control (analysis of variance; post hoc Tukey test, $p < 0.05$ for both BDNF time points). For each histogram, $n = 7–15$ rats. **(b)** Coinjection of colchicine (col), but not of K252a, blocks the BDNF effect completely (analysis of variance; post hoc Tukey test: BDNF versus BDNF plus colchicine, $p < 0.05$; BDNF versus BDNF plus K252a, $p > 0.05$). For each histogram, $n = 3$ or 4 rats. The error bars indicate SEM.

Post-synaptic effects of anterograde BDNF

Previous studies have shown convincingly anterograde transport of BDNF and NT-3 in selected peripheral and central pathways [6–9]. The anterogradely transported neurotrophins have been proposed to play a role in neuronal survival, phenotype regulation, synaptic plasticity and also as conventional neurotransmitters [29]. For example, transport of both exogenous and endogenous NT-3 has been demonstrated in the retino-tectal pathway of chick embryos [6,7]. A paper by Altar *et al.* [8] has reported transport of endogenous BDNF from cortical afferents to striatal neurons in the rat. Deletion of the BDNF gene reduces the number of parvalbumin-expressing striatal neurons, consistent with a post-synaptic role for the anterogradely transported BDNF [8]. Fawcett *et al.* [9] found localisation of BDNF in noradrenergic terminals of the adult brain. Using transgenic mice that overexpress BDNF in noradrenergic neurons, they demonstrated major effects of BDNF over-expression in noradrenergic target regions, namely a reduced cortical thickness and an increased survival of axotomised facial motoneurons [9]. In all of these studies, however, it was difficult to establish whether the observed post-synaptic effects are due to the direct action of the anterogradely transported neurotrophin. Here, using the expression of the c-Fos and Zif268 gene products as a marker, we have provided compelling evidence that the exogenous, anterogradely transported BDNF is released from RGC terminals and acts directly on the target neurons.

Physiological role of the anterogradely transported BDNF

It has long been known that second-order visual neurons in the SC and dLGN depend on their afferents from the retina for survival. Blockade of axonal transport or spike activity in the eye leads to neuronal death in retinal targets [11–13]. These experiments point to the existence of some anterograde trophic signal(s) for retinal target neurons that are released in an activity-dependent manner. Our data demonstrate that BDNF is one of these factors. Indeed, increasing or reducing the availability of BDNF regulates neuronal survival in retinal target fields. First, we have shown that infusion of function-blocking anti-BDNF antibodies into the developing SC increases the number of cells undergoing programmed cell death. This demonstrates that BDNF is an endogenous survival factor for SC neurons. To our knowledge, this is the first demonstration of the effects of endogenous BDNF on the developmental death of one population of central neurons. The effects of the anti-BDNF antibody were already apparent 3 hours after infusion. A similarly rapid increase in the rate of cell death has been observed following enucleation or intravitreal injection of tetrodotoxin (TTX) [12], suggesting that the endogenous BDNF necessary for survival is derived from retinal sources in an activity-dependent way. Nevertheless, as the antibodies remove all BDNF in the tectum, the possibility cannot be excluded that at least some of the BDNF is provided to the SC cells by paracrine or autocrine pathways, or by an additional, so far unidentified, afferent source.

Second, provision of an anterograde supply of BDNF was able to prevent the death of geniculate neurons following cortical lesions. A specific retrograde action of cortically

derived NT-4 has been shown in the ferret dLGN by Riddle *et al.* [30]. Thus, geniculate neurons appear to require two different TrkB ligands from different sources, anterograde BDNF from the retina and retrograde NT-4 from the cortex.

Supply of trophic factor to the thalamus through intraocular injections

The BDNF rescue effect on the geniculate cells can also have important clinical implications. A secondary, massive loss of thalamic neurons has been described in both animal models and man following cortical traumatic insults and cerebral ischemia [31–33]. This thalamic damage is a serious obstacle for pathway rewiring and functional recovery [34]. We have shown that BDNF delivery within the eye is a very effective strategy for promoting geniculate survival after cortical lesions. The clinical application of neurotrophins has been limited by the fact that these factors do not cross the blood–brain barrier and need to be administered directly into the brain parenchyma [35]. Our data demonstrate the feasibility of supplying functionally significant amounts of BDNF to the injured geniculate through a minimally invasive route through the eye.

Materials and methods

Animal treatment

The procedures used in this study were approved by the Italian Ministry of Public Health. For analysis of the anterograde transport of endogenous neurotrophins, 10 Long-Evans adult rats were deeply anesthetised with avertin (1 ml/100g body weight) and the optic nerve was either ligated or crushed mechanically with thin surgical forceps. In three rats, a double ligation was performed. Three P5 rats were also used. They were anesthetised by hypothermia and the optic nerve was crushed intracranially to visualise accumulation of endogenous neurotrophins. All animals were killed 20 h after surgery.

Intraocular injections of neurotrophins were performed with a glass micropipette connected to a microinjector. For the study of transport of exogenous BDNF, the optic nerve was ligated and the animals ($n = 3$) killed 3 h after the intraocular BDNF injection. For the study of IEG expression, we injected 28 adult and 16 newborn (P5–P7) rats. Animals received a total of 5–40 μg 2.5S mouse NGF or 5–40 μg recombinant human BDNF and were killed 5–7 h later. In co-injection experiments, 1 μg of the axonal transport blocker colchicine or 0.7 μg of the Trk phosphorylation blocker K252a were injected intraocularly 30–60 min before the BDNF administration. The dose of colchicine used was verified to block completely fast anterograde transport from the retina. The K252a batch used was shown to be effective in blocking Trk phosphorylation of BDNF-treated cortical neurons in culture.

Stereotaxic injections of anti-BDNF antibodies (RAB from Amgen, 0.3 mg/ml; chicken anti-BDNF antibody from Promega, 0.5 mg/ml) and control anti-HRP antibodies (mouse monoclonal, Sigma P6-38) were performed unilaterally into the SC of adult rats (volume injected, 2 μl); 5 h later, the rats were given bilateral intraocular injections of BDNF (40 μg). Animals were killed 5 h following the BDNF administration and the brains processed for c-Fos and Zif268 immunostaining. An identical protocol was followed in the P5 rats.

Immunohistochemistry

Animals were anesthetised with chloral hydrate and perfused transcardially with PBS followed by fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (for BDNF, c-Fos and Zif268 immunostaining) or 2% paraformaldehyde + 0.2% parabenzoquinone in 0.075 M phosphate buffer, pH 7.4 (for NGF immunostaining). Optic nerves and brains were dissected, rinsed in buffer and cryoprotected in 30% sucrose. Longitudinal optic nerve sections (12 μm thick) and coronal brain sections (40 μm thick) were obtained by using a cryostat and a sliding microtome, respectively. After a blocking step, sections were incubated overnight in rabbit polyclonal antibodies diluted as follows: anti-NGF antibody, 0.75 μg/ml (a kind gift of J.M. Conner); anti-BDNF antibody, 1 μg/ml (affinity-purified polyclonal antibody RAB, a kind gift of Q. Yan); anti-c-Fos antibody 1:10,000 (Oncogene Sciences); anti-Zif268 antibodies 1:1,000 (Santa Cruz Biotechnology). The anti-NGF and anti-BDNF antibodies have been characterised in previous studies [21,27,36]. Bound antibodies were detected by incubating sections with biotinylated goat anti-rabbit IgG (1:200, Vector Labs) followed by fluorescein-conjugated avidin (1:300, Vector). Selected c-Fos-stained brain sections were reacted with mouse monoclonal anti-NeuN antibody (1:500, Chemicon) followed by Alexa 568-conjugated anti-mouse IgG (1:400, Molecular Probes).

Evaluation of natural cell death in the SC

P5 rats were injected into the SC with RAB or control rabbit IgG (2 μl of a 0.3 μg/μl solution) under ether anesthesia. They were perfused 3 h later with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Coronal sections through the SC were cut with a freezing microtome and stained with cresyl violet.

The extent of diffusion of the injected antibodies was checked by staining selected coronal sections with anti-rabbit IgG biotinylated secondary antibodies, followed by ABC kit (Vector) and nickel-enhanced diaminobenzidine (DAB) reaction. Animals with limited antibody penetration were discarded. For the analysis of natural cell death, three to four coronal sections were selected for each injected SC according to a systematic random scheme [37,38]. Pyknotic nuclei were counted in three-dimensional sampling fields ($80 \times 80 \times 10$ μm) equally spaced from medial to lateral in the superficial layers of the SC (Stereo Investigator, Microbrightfield). An average of 100 fields were counted in each injected SC. To rule out that the differences in the density of pyknotic cells may result from unequal tissue shrinkage, we also estimated the density of living cells in each SC. For this analysis, three equally spaced sampling fields were counted in each coronal section (3–6 sections per animal). We found that the mean density of living cells was virtually identical (*t*-test, $p = 0.9$) in the rats treated with anti-BDNF antibody and control rats.

Lesion experiments

Unilateral lesions of the visual cortex were made by aspiration in a total of 48 P7 rats under ether anesthesia. Monocular injections of BDNF (40 μg) were performed at 24 h ($n = 7$) or 36 h ($n = 8$) post-lesion. Fifteen rats received injections of saline solution and served as controls; 1 h after BDNF or saline injection, all animals received an intravitreal injection of 3 μl 30% HRP to label the retino-geniculate projections. Another group of 12 rats with visual cortex lesion was used to determine whether the co-administration of either colchicine or K252a prevents the BDNF effects. These animals were injected intravitreally with HRP at 20 h post-lesion. BDNF, BDNF plus colchicine, or BDNF plus K252a were administered 36 h post-lesion. All rats were perfused through the heart with a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) 48 h after ablation of the visual cortex. Coronal sections, 50 μm thick, were cut on a sliding microtome and collected in serial order through the entire thalamus and SC. HRP signal was revealed using the tetramethylbenzidine (TMB) procedure and the sections were then counterstained with cresyl violet for cell somata.

Quantitative analysis of pyknosis in the dLGN

The quantitative analysis of pyknosis in the dLGN employed the principles of a modified version of the 'dissector' method called optical fractionator. The optical fractionator is a combination of performing counting with the optical dissector with fractionator sampling [37,38]. Analysis

was performed in a blind coded fashion using the Stereo Investigator (Microbrightfield) software and a Zeiss microscope. Six to eight coronal sections through the middle of the dLGN in the rostrocaudal axis were randomly selected. Boundaries of the two eye-specific subfields (identified by HRP histochemistry) within the lesioned dLGN were drawn for each section at 20× magnification. For each subfield, pyknotic nuclei were counted at 100× magnification in three-dimensional counting boxes ($40 \times 40 \times 12$ μm) selected in a systematically random manner. The mean number of pyknotic cells per counting box was then determined and the density of pyknotic cells per mm^3 of tissue derived for each dLGN subfield. This method allows comparison of the level of death in the treated and untreated portion of the lesioned dLGN so that the untreated patch provides an internal control.

Acknowledgements

We thank Q. Yan and J.M. Conner for the kind gift of anti-BDNF and anti-NGF antibodies. Regeneron Pharmaceuticals (Tarrytown, New York) kindly provided recombinant human BDNF. We are grateful to A. Tacchi and G.C. Cappagli for excellent technical assistance. This work was partly supported by the EEC through Biotech grant BIO4-CT96-0774, by Telethon project #934 and by P48/99 grant from the Institut International de Recherche en Paraplegie, Geneva.

References

1. Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD: **Programmed cell death and control of cell survival: lessons from the nervous system.** *Science* 1993, **262:**695-700.
2. Korsching S: **The neurotrophic factor concept: a re-examination.** *J Neurosci* **1993, 13:**2739-2748.
3. Lewin GR, Barde YA: **Physiology of the neurotrophins.** *Annu Rev Neurosci* 1996, **19:**289-317.
4. Watson FL, Heerssen HM, Moheban DB, Lin MZ, Sauvageot CM, Bhattacharyya A, *et al.*: **Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex.** *J Neurosci* 1999, **19:**7889-7900.
5. Riccio A, Pierchala BA, Ciarallo CL, Ginty DD: **An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons.** *Science* 1997, **277:**1097-1100.
6. von Bartheld CS, Byers MR, Williams R, Bothwell M: **Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system.** *Nature* 1996, **379:**830-833.
7. von Bartheld CS, Butowt R: **Expression of neurotrophin-3 (NT-3) and anterograde axonal transport of endogenous NT-3 by retinal ganglion cells in chick embryos.** *J Neurosci* 2000, **20:**736-748.
8. Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, *et al.* **Anterograde transport of brain-derived neurotrophic factor and its role in the brain.** *Nature* 1997, **389:**856-860.
9. Fawcett JP, Bamji SX, Causing CG, Aloyz R, Ase AR, Reader TA, *et al.*: **Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS.** *J Neurosci* 1998, **18:**2808-2821.
10. Altar CA, DiStefano PS: **Neurotrophin trafficking by anterograde transport.** *Trends Neurosci* 1998, **21:**433-437.
11. Linden R: **The survival of developing neurons: a review of afferent control.** *Neuroscience* 1994, **58:**671-682.
12. Galli-Resta L, Ensini M, Fusco E, Gravina A, Margheritti B: **Afferent spontaneous electrical activity promotes the survival of target cells in the developing retinotectal system of the rat.** *J Neurosci* 1993, **13:**243-250.
13. Catsicas M, Pequignot Y, Clarke PGH: **Rapid onset of neuronal death induced by blockade of either axoplasmic transport or action potential in afferent fibers during brain development.** *J Neurosci* 1992, **12:**4642-4650.
14. Bennett JL, Zeiler SR, Jones KR: **Patterned expression of BDNF and NT-3 in the retina and anterior segment of the developing mammalian eye.** *Invest Ophtalmol Vis Sci* 1999, **40:**2996-3005.
15. Gao H, Qiao X, Hefti F, Hollyfield JG, Knusel B: **Elevated mRNA expression of brain derived neurotrophic factor in retinal ganglion cell layer after optic nerve injury.** *Invest Ophtalmol Vis Sci* 1997, **38:**1840-1847.
16. Yan Q, Radeke MJ, Matheson CR, Talvenheimo J, Welcher AA, Feinstein SC: **Immunocytochemical localization of TrkB in the central nervous system of the adult rat.** *J Comp Neurol* 1997, **378:**135-157.
17. Spalding KL, Tan MML, Hendry IA, Harvey AR: **Cell death and the transport of neurotrophins in the developing rat visual system.** *Soc Neurosci Abstracts* 1999, **25:**767.
18. Segal RA, Greenberg ME: **Intracellular signalling pathways activated by neurotrophic factors.** *Annu Rev Neurosci* 1996, **19:**463-489.
19. Caleo M: **Different rates of horseradish peroxidase transport in the optic nerve of neonatal and adult rats.** *Neuroscience* 1996, **72:**725-730.
20. von Bartheld CS: **Neurotrophins in the developing and regenerating visual system.** *Histol Histopathol* 1998, **13:**437-459.
21. Yan Q, Rosenfeld RD, Matheson CR, Hawkins N, Lopez OT, Bennett L, *et al.*: **Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system.** *Neuroscience* 1997, **78:**431-448.
22. Giehl KM, Schütte A, Mestres P, Yan Q: **The survival-promoting effect of glial cell line-derived neurotrophic factor on axotomized corticospinal neurons *in vivo* is mediated by an endogenous brain-derived neurotrophic factor mechanism.** *J Neurosci* 1998, **18:**7351-7360.
23. Toma JG, Kaplan D: **Neutralization of TrkB tyrosine kinase receptor activity using BDNF pAb.** *Promega Neural Notes* **3:**20-21.
24. Cunningham TJ, Huddelston C, Murray M**: Modification of neuron numbers in the visual system of the rat.** *J Comp Neurol* 1979, **184:**423-434.
25. Perry VH, Cowey A**: The effects of unilateral cortical and tectal lesions on retinal ganglion cells in rats.** *Exp Brain Res* 1979, **35:**85-95.
26. Ma Y-T, Hsieh T, Forbes ME, Johnson JE, Frost DO: **BDNF injected into the superior colliculus reduces developmental retinal ganglion cell death.** *J Neurosci* 1998, **18:**2097-2107.
27. Conner JM, Lauteborn JC, Yan Q, Gall CM, Varon S: **Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport.** *J Neurosci* 1997, **17:**2295-2313.
28. Androutsellis-Theotokis A, McCormack WJ, Bradford HF, Stern GM, Pliego-Rivero FB: **The depolarisation-induced release of [^{125}I] BDNF from brain tissue.** *Brain Res* 1996, **743:**40-48.
29. Kafitz KW, Rose CR, Thoenen H, Konnerth A: **Neurotrophin-evoked rapid excitation through TrkB receptors.** *Nature* 1999, **401:**918-921.
30. Riddle DR, Lo DC, Katz LC: **NT-4 mediated rescue of lateral geniculate neurons from effects of monocular deprivation.** *Nature* 1995, **378:**189-191.
31. Al-Abdulla NA, Portera-Caillau C, Martin LJ: **Occipital cortex ablation in adult rat causes retrograde neuronal death in the lateral geniculate nucleus that resembles apoptosis.** *Neuroscience* 1998, **86:**191-209.
32. Lin CS, Polsky K, Nadler JV, Crain BJ: **Selective neocortical and thalamic cell death in the gerbil after transient ischemia.** *Neuroscience* 1990, **35:**289-299.
33. Tamura A, Tahira Y, Nagashima H, Kirino T, Gotoh O, Hojo S, *et al.*: **Thalamic atrophy following cerebral infarction in the territory of the middle cerebral artery.** *Stroke* 1991, **22:**615-618.
34. Payne BR, Cornwell P: **System-wide repercussions of damage to the immature visual cortex.** *Trends Neurosci* 1994, **17:**126-130.
35. Lindsay RM, Wiegand SJ, Altar CA, DiStefano P: **Neurotrophic factors: from molecule to man.** *Trends Neurosci* 1994, **17:**182-189.
36. Conner JM, Muir D, Varon S, Hagg T, Manthorpe M: **The localization of nerve growth factor-like immunoreactivity in the adult rat basal forebrain and hippocampal formation.** *J Comp Neurol* 1992, **319:**454-462.
37. Coggeshall RE, Lekan HA: **Methods for determining numbers of cells and synapses: a case for more uniform standards of review.** *J Comp Neurol* 1996, **364:**6-15.
38. West MJ: **Stereological methods for estimating the total number of neurons and synapses: issues of precision and bias.** *Trends Neurosci* 1999, **22:**51-61.

Because *Current Biology* operates a 'Continuous Publication System' for Research Papers, this paper has been published on the internet before being printed. The paper can be accessed from http://biomednet.com/cbiology/cub – for further information, see the explanation on the contents page.