

Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section

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MOTONEURONS innervating the skeletal musculature were among the first neurons shown to require the presence of their target cells to develop appropriately^{1,2}. But the characterization of molecules allowing motoneuron survival has been difficult. Ciliary neurotrophic factor prevents the death of motoneurons³⁻⁶, but its gene is not expressed during development⁷. Although the presence of a neurotrophin receptor on developing motoneurons⁸⁻¹⁰ has suggested a role for neurotrophins, none could be shown to promote motoneuron survival *in vitro*³. We report here that brain-derived neurotrophic factor can prevent the death of axotomized motoneurons in newborn rats, suggesting a role for this neurotrophin for motoneuron survival *in vivo*.

The facial nerve of newborn rats was sectioned unilaterally at birth and the effects of three neurotrophins (nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3)) assessed 7 days later. This nerve was chosen for lesion and local application of neurotrophins as it contains only motor nerve fibres at the lesion site. Axons of proprioceptive sensory neurons (putative *in vivo* targets of BDNF and NT-3 (ref. 11)) were not lesioned (they are anatomically separated from this nerve) so that indirect effects through these cells are unlikely. Motoneurons that constitute the facial nucleus were counted on both sides. More than 80% of the axotomized motoneurons are lost when axotomy is done at birth^{5,12,13}. NGF, which has been shown to be ineffective in

supporting motoneuron survival *in vivo*⁹, was used as a control, in addition to BSA. The application of NGF to the cut end of the facial nerve did not prevent the loss of motoneurons. In fact, fewer motoneurons were observed on the NGF-treated side as compared to BSA-treated animals ($P < 0.05$, Table 1).

In contrast to the animals treated with NGF, significant survival of axotomized motoneurons was observed when BDNF was applied to the transected facial nerve. On average, about 50% of the lesioned motoneurons were still alive 1 week after lesion (Table 1) which represents a surplus of 40% in comparison to NGF or 31% to BSA.

The survival of motoneurons was also enhanced following NT-3 treatment ($P < 0.02$ versus BSA-treated controls), though the effects were substantially smaller than those observed with BDNF (Table 1). In both BDNF- and NT-3-treated animals, the lesioned motoneuron cell bodies were smaller compared to unlesioned controls and showed typical reactive changes (Fig. 1). Although the lesioned motoneurons still remained clearly identifiable (see Fig. 1), the nuclei were displaced and chromatinolysis was pronounced both in BDNF- and in NT-3-treated animals. But compared to control animals treated with BSA-gel foam, the nuclei appeared larger (Fig. 1).

These data show that molecules of the neurotrophin gene family can support motoneuron survival *in vivo*. In previous studies using purified motoneurons isolated from the chick spinal cord at 6 days of embryonic age, no survival effects could be observed with either BDNF or NT-3. This was not due to inadequate culture conditions, because the combination of ciliary neurotrophic factor (CNTF) and basic fibroblast growth factor (FGF) added to parallel cultures could rescue essentially all neurons³. Among the various explanations for the discrepancy with the present results are the possibility that some crucial cofactor, absent in culture, is required for the response of the neurotrophins to be seen. This cofactor might be another growth factor present *in vivo* or might be related to the presynaptic input of the motoneurons in the spinal cord. Alternatively, it is possible that motoneurons isolated from the chick embryo are not yet responsive to the neurotrophins or that they lose their responsiveness as a consequence of the isolation procedure. In any event, an important difference exists between motoneurons and sensory neurons cultured in isolation, in that all neurotrophins known so far support (to various degrees) the survival of neurons isolated from dorsal root ganglia.

In contrast to the CNTF gene⁷, both the NT-3 and BDNF genes are expressed during embryonic development¹⁴⁻¹⁶. For example, NT-3 messenger RNA is expressed at relatively high levels in the rat spinal cord between embryonic days 13 and 16, its expression decreasing gradually to low levels at birth¹⁵. Also, *in situ* hybridization studies have revealed that the spinal motoneurons themselves express the NT-3 gene^{15,16} and that BDNF expression is seen in neurons of the dorsal root ganglia in embryonic mice¹⁶. In addition to the central nervous system, measurable levels of BDNF mRNA have been detected in skeletal muscle¹⁴, as well as a variety of tissues in developing chick embryos including the skeletal musculature (K.-H. Herzog and Y.-A.B., unpublished results).

In the context of our results, it is worth noting that in adult rats there is substantial transport of radiolabelled BDNF, and to a lesser extent of NT-3, by spinal motoneurons¹⁷. But retrograde transport of neurotrophins is not always a reliable indicator of a biological response. In particular, retrograde transport of labelled NGF can be demonstrated in newborn rats (unlike in adults), but NGF does not rescue axotomized motoneurons (see ref. 9 and this study).

Finally, the expression of the BDNF gene is dramatically upregulated in the sciatic nerve of the adult rat with a delay of at least 3 days after lesion, suggesting that BDNF might play a role in motor axon regeneration¹⁸. In contrast, CNTF is constitutively expressed in high amounts in myelinating Schwann cells

TABLE 1 Motoneuron survival after lesion of the facial nerve in newborn rat: effects of neurotrophins

Treatment	Number of surviving neurons \pm s.e.m.	
	Lesioned side	Control side
BSA ($n=8$)	844 \pm 115	4,597 \pm 222
NGF ($n=7$)	407 \pm 127**	4,294 \pm 227
BDNF ($n=9$)	2,222 \pm 446*	4,534 \pm 129
NT-3 ($n=10$)	1,221 \pm 71*	4,510 \pm 271

Transection of the facial nerve was done as described previously⁵. Newborn Wistar rats were anaesthetized by hypothermia, the right facial nerve exposed at the foramen stylomastoideum and transected about 1 mm distal from this position. Gel foam (Spongostan, gift of K. Unsicker, Marburg, Germany) soaked in 30 μ l of PBS containing either 5 μ g of BSA (Cohn Fraction V, Sigma) or trophic factor was inserted at the site of lesion. Nerve growth factor (2.5S) was isolated from submaxillary glands of adult male mice, as described²². Both recombinant mouse BDNF and NT-3 were produced in rabbit kidney cells infected with recombinant vaccinia viruses and purified as described²³. The skin was sutured by silk (Ethikon 3-0) and the animals returned to their mothers. On postnatal day 7 the animals were killed by ether overdose and perfused transcardially with 50 ml 4% formaldehyde. Brainstems were dissected, postfixed for 1 hour, rinsed with water, and dehydrated with increasing concentrations of ethanol (70-100%). After embedding with paraffin, serial sections (7 μ m) were made from the whole brain stem with a serial section microtome (Reichert-Jung 2050 supercut). The sections were stained with cresyl violet (Sigma), and facial motoneurons with clearly identifiable nuclei and nucleoli were counted on both sides at a magnification \times 125 in every fifth section as previously described⁵. The counts were not corrected for split nucleoli.

Different from BSA-treated animals at $P < 0.02^*$ or $P < 0.05^{**}$ (Student's *t*-test, 2-tailed).

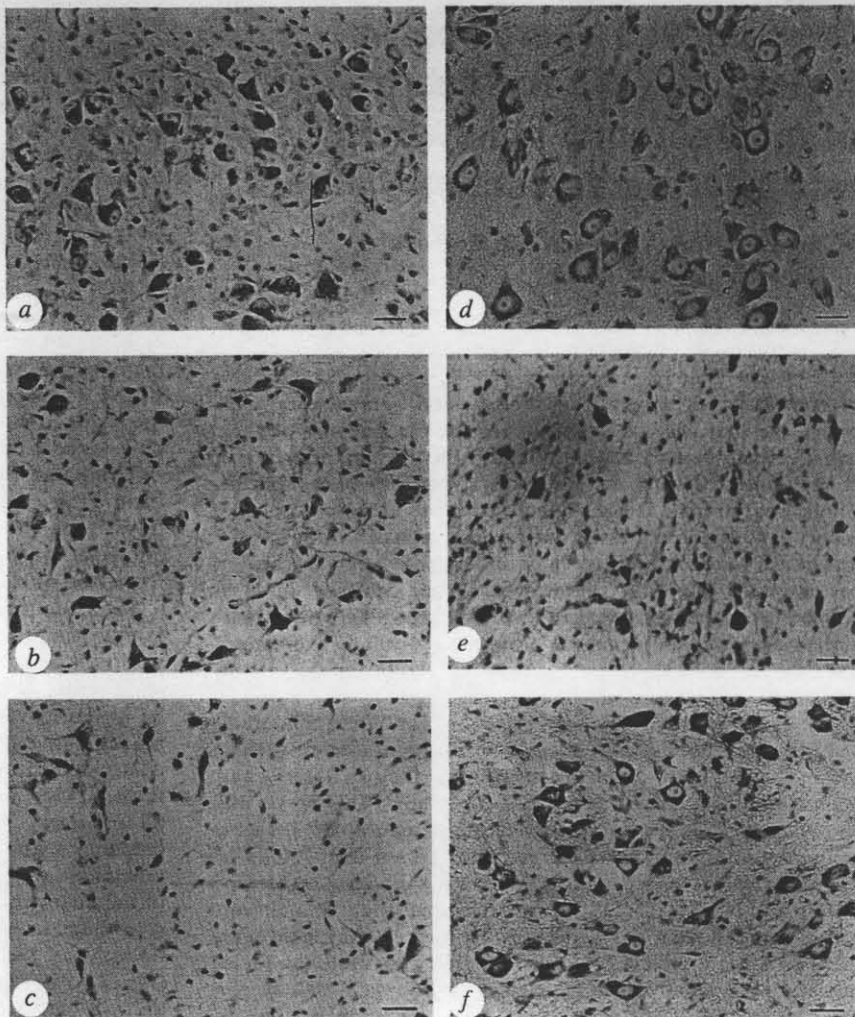


FIG. 1 Morphology of facial motoneurons in the brainstem of 7-day-old rats after unilateral facial nerve section at birth. *a*, Lesioned side after BDNF treatment. *b*, Lesioned side after NT-3 treatment. *c*, Lesioned side after NGF treatment. *d*, Unlesioned contralateral side of the same animal as in *a*. *e*, Lesioned side after BSA treatment. *f*, Lesioned side after CNTF (5 µg) treatment as a positive control. Scale bar, 50 µm.

of peripheral nerves of the adult rat¹⁹. After lesion, significant quantities of CNTF protein seem to persist for at least 1 week in the lesioned nerve and could be available to regenerating neurons¹⁹. Thus, a plausible scenario after peripheral nerve lesion would be that motoneurons are supported first by CNTF released from injured Schwann cells and, subsequently by BDNF, the latter supporting the regeneration of motor axons to the periphery.

Our findings together with those reported by Oppenheim *et al.*²⁰ and Yan *et al.*²¹ indicate that motoneurons are responsive to BDNF *in vivo*. The observation that BDNF can support the survival of lesioned facial motoneurons that would die otherwise in the absence of survival factors indicates that this neurotrophin could have important functions on motoneurons, and mediate the regeneration of motor axons to the periphery after neuronal lesion. □

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