of specific proteins in neurons of the PC lobe[40,41]. Therefore, as in mammalian olfactory bulb[42–44], input pathways exist that could modify PC lobe circuitry during aversive odour conditioning. □

Received 20 November, 1989; accepted 13 March, 1990.

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ACKNOWLEDGEMENTS. We thank Jorge Flores for help with data collection and John Hopfield, Alan Kay, Wade Regehr and Kerry Delaney for comments on the manuscript.

Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy

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THE period of natural cell death in the development of rodent motor neurons is followed by a period of sensitivity to axonal injury[1–3]. In the rat this early postnatal period of vulnerability coincides with that of very low ciliary neurotrophic factor (CNTF) levels in the sciatic nerve before CNTF increases to the high, adult levels[4]. The developmental time course of CNTF expression, its regional tissue distribution and its cytosolic localization (as suggested by its primary structure)[4,5] favour a role for CNTF as a lesion factor rather than a target-derived neurotrophic molecule like nerve growth factor. Nevertheless CNTF exhibits neurotrophic activity *in vitro* on different populations of embryonic neurons[6]. To determine whether the vulnerability of motor neurons to axotomy in the early postnatal phase is due to insufficient availability of CNTF, we transected the axons of newborn rat motor neurons and demonstrated that local application of CNTF prevents the degeneration of the corresponding cell bodies.

We have chosen the rat facial nerve as an experimental system as it exclusively contains motor axons distal to the stylomastoid foramen. The corresponding cell bodies comprise a homogeneous, well-delineated nucleus in the ventral-lateral region of the brain stem (Fig. 1). Moreover, the retrograde and degenerative changes occurring after facial-nerve lesion have been investigated in great detail[7]. The facial nerves of newborn rats were unilaterally transected on the first postnatal day (see Table 1) and morphology of the facial nuclei was analysed 1 week after lesion. More than 80% of the cell bodies on the side having the lesion were completely lost after 1 week (Table 1). The remaining motor neurons showed severe signs of degeneration[8], that is, the nuclei were located eccentrically and the Nissl bodies showed the characteristic dispersion that occurs in adult motor neurons after axotomy (Fig. 1). In adult animals astrocytes react characteristically to peripheral axonal lesion of motor neurons by increasing their glial fibrillary acidic protein (GFAP) production[9,10]. In agreement with this there was enhanced GFAP staining in the astrocytes surrounding the motor neurons on the side having the lesion (data not shown). This is noteworthy because, at the end of the first postnatal week there is normally little GFAP reactivity in the brain stem; it only becomes weakly apparent at later developmental stages[11].

The application of CNTF to the proximal stump of the nerve in which the lesion had been made (5 μg of highly purified CNTF[4] corresponding to 50,000 TU of biological activity) resulted in the survival of most of the motor neurons up to 1 week after lesion (Table 1). Morphological degenerative changes in

TABLE 1 Motor neuron survival after lesion of the facial nerve in newborn rats

	Number of surviving neurons ± s.e.m.	
	Side with lesion	Side without lesion
Untreated animals ($n=1$)	685	2,985
Animals treated with BSA gel foam ($n=3$)	620 ± 98*	3,271 ± 61
Animals treated with gel foam containing 5 μg of CNTF ($n=4$)	2,503 ± 487*	3,281 ± 112

Newborn Wistar rats were anaesthetized by hypothermia and the right facial nerves cut ~1 mm distal to the stylomastoid foramen. Gel foam (Spongostan, gift of K. Unsicker, Marburg) was cut into small pieces (3 mm long) and soaked in PBS buffer (20 μl) containing 5 μg purified adult rat sciatic nerve CNTF[4,13]. The soaked foam pieces were inserted at the sites of lesion. Control animals were treated with gel foam soaked in PBS buffer containing 5 μg BSA (Fraction V, Sigma) (20 μl). The skin was sutured with silk (Ethicon 3–0) and the animals returned to their mother. Operated rats were inspected daily, lesion of the right facial nerve was detected in the animals by lack of movement of the right whisker and the right corner of the mouth. On postnatal day 7 the animals were anesthetized with ether and killed by transcardial perfusion with 4% formaldehyde. The brainstem was dissected, postfixed (1 h), rinsed in water, dehydrated with increasing concentrations of ethanol (70–100%) and embedded in paraffin. Serial sections 7 μm were made from the whole brainstem. After Nissl staining the motorneurons of both facial nuclei were clearly detectable. Only cells containing a clearly-visible nucleolus were counted, in every fifth section, as described previously[14].

* $P<0.05$, *t*-test

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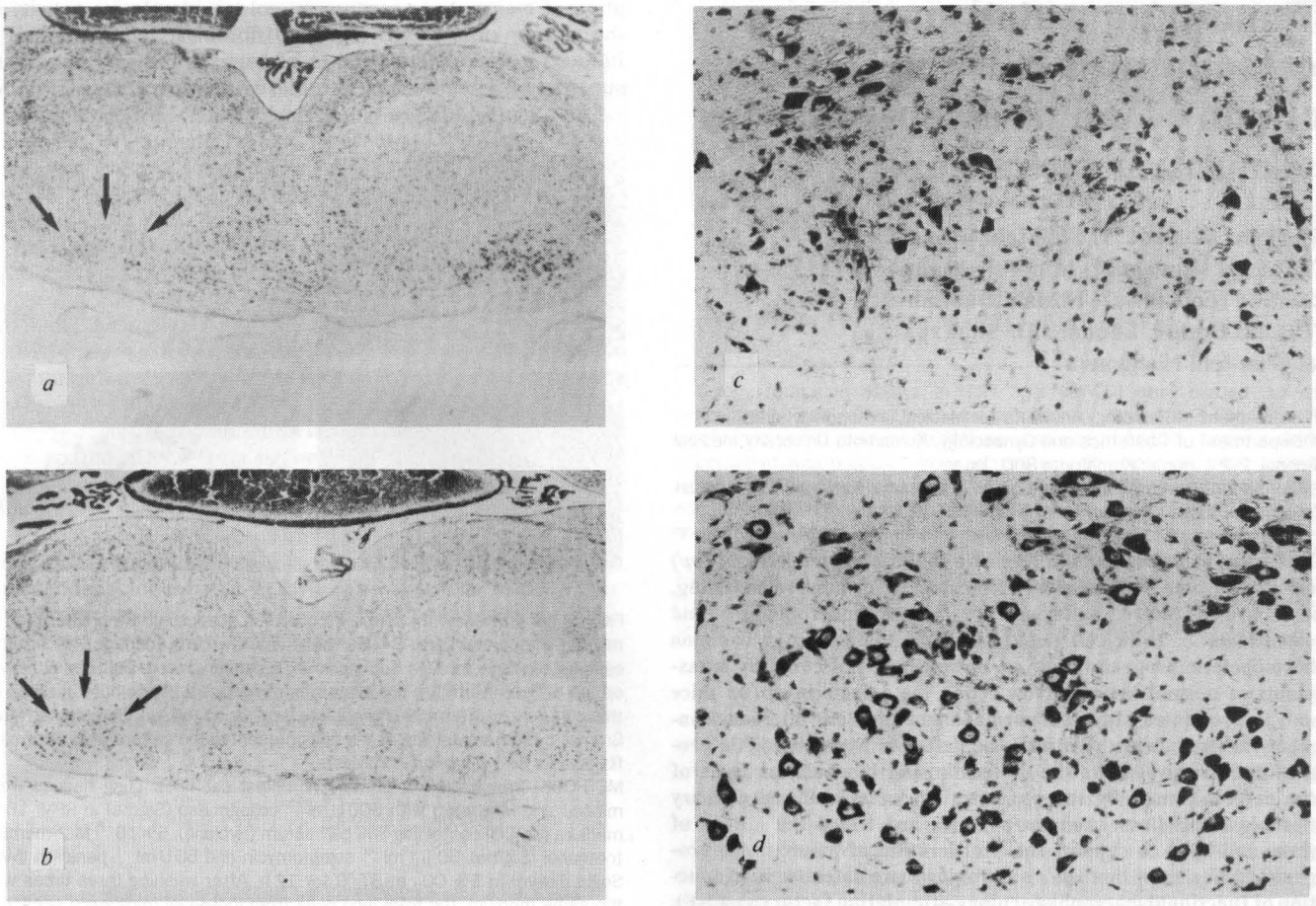

FIG. 1 *a, b,* Sections of the brainstem of 7-day-old rats: The facial nucleus of the side without a lesion is located on the righthand side. The facial nucleus containing the cell bodies of the neurons in which lesions have been made are located on the lefthand side (arrows). *a,* Control animal after insertion of gel foam containing 5 μg of BSA. *b,* CNTF-treated animal. (Magnification ×25). c, d, Cell bodies of the facial nucleus seven days after lesion. *c,* Control animal treated with BSA. *d,* CNTF-treated animal. (Magnification ×250). The nuclei of the motor neurons in which a lesion has been made in BSA-treated animals (*c*) are deformed and located peripherally (a typical sign of chromatolysis), whereas in CNTF-treated animals most nuclei appear round in shape and are located in the centre of the cell (*d*).

facial neurons in which a lesion had been made were also markedly reduced compared to those observed in the small number of surviving neurons where the lesion had been made and the animals BSA-treated (Fig. 1*c,d*). Despite the survival of most motor neurons in which a lesion had been made, there was still an enhanced GFAP reactivity in the facial nucleus of CNTF-treated nerves (data not shown), showing that CNTF can prevent the degenerative changes in motor neurons (although the reactive changes in astrocytes were still clearly visible). Immunohistochemical procedures[12] did not show whether there was a quantitative difference in the GFAP staining between the CNTF- and BSA-treated animals.

These experiments show that the local application of purified CNTF prevents the lesion-induced death of motor neurons in the rat facial brain stem nucleus. This observation is consistent with our hypothesis that in adult animals the CNTF—present in large quantities in non-neuronal cells of the peripheral nerves—is released after lesion in quantities sufficient to prevent the degeneration of motor neurons in which a lesion has been made. In contrast, in the early postnatal period the quantities of CNTF available are normally insufficient to prevent this, unless exogenous CNTF is applied locally. Although our experiments do not indicate whether the protective effects of locally applied CNTF are due to direct or indirect action on motor neurons, CNTF does support the survival of motor neurons during the naturally occurring period of cell death (Arakawa and M.S., unpublished observation). Thus, CNTF may act as a general neuronal-survival factor unrestricted in its time of action.

The observation that motor neurons' death can be prevented after lesion in the early postnatal period could introduce novel therapeutic approaches to the treatment of motor neuron degeneration. For example, it will be of interest to investigate whether CNTF also protects against toxic or genetic degenerative changes in motor neurons, and possibly also in those motor neuron diseases of unknown aetiology. □

Received 18 January; accepted 28 March 1990.

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ACKNOWLEDGEMENTS. We thank David Edgar for critical reading of the manuscript, Waltraud Komp and Dietmute Bueringer for technical assistance, and Elfi Grossmann for typing the manuscript.