

FIG. 4 Diagram of the superficial musculature of the neck of the barn owl (*Tyto alba*). The neck is S-shaped, contains 13 vertebrae and at least 31 distinct muscle pairs, some of which are segmentally arranged whereas others span multiple segments.

on tecto-spinal relay sites in the brainstem tegmentum¹³⁻¹⁵, suggest that the saccade generators are located in brainstem tegmental regions.

The existence of separate circuits for controlling horizontal and vertical components of movement in a complex skeleto-muscular system indicates that such an intermediate representation may be a general principle of sensorimotor organization. In the case of eye movements, the similar orthogonal character of the saccade generators¹² may be related to the essentially orthogonal arrangement of the extraocular rectus muscles. In contrast, head movements depend on the action of many pairs of muscles pulling in a variety of directions, and there is no apparent organization of muscle-pulling directions according to orthogonal planes of movement (Fig. 4). Thus, unlike in eye motor control, the independent coding of orthogonal movement components of the head is markedly different from both sensory and the muscle coordinate frames, implying that it operates as an abstract system intermediate to the sensory and motor processes that control orienting movements.

These observations, together with observations in other systems, suggest that orthogonal coordinate systems may be a general property of intermediate stages in motor control hierarchies. In cats and monkeys, electrical stimulation of several brainstem tegmental regions elicits either primarily horizontal or vertical head movements^{16,17}. Psychophysical experiments on human arm and hand movements suggest that they also may be encoded in terms of their azimuthal and elevational components^{5,18,19}. Lesion studies in the frog have demonstrated that leftward and rightward horizontal components of whole-body orienting movements are controlled by distinct circuitry, and that these circuits are independent of those controlling elevation and distance²⁰⁻²³. This evidence suggests that, despite the differences in the skeletal and muscular organization among head, arm and body movements, there may be for each an intermediate representation of movement direction encoded in orthogonal spatial coordinates.

There may be several advantages to representing movements in orthogonal directional components. The orthogonal coordinate system facilitates the integration of spatial information coming from different sensory modalities, allowing several sensory systems to access a single motor command structure^{18,24-26}. In addition, it facilitates the coordination of movements by different body parts when the goal of a movement can be accomplished in a variety of ways. For example, redirection of gaze can be carried out by movements of the eyes, head or body or by any combination of these three. To locate a desired target accurately, information about the change in gaze contributed by each kind of movement must be combined in a common form before being compared with the change in gaze that was intended. Representing orienting movements in terms of their horizontal and vertical components may provide such a common-coordinate frame for feedback regulation of movements by multiple body parts. □

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- Bernstein, N. *The Co-ordination and Regulation of Movements* (Pergamon, Oxford, 1967).
- Hollerbach, J. M. & Flash, T. *Biol. Cybern.* **44**, 67-77 (1982).
- Morasso, P. *Expl. Brain Res.* **42**, 223-237 (1981).
- Bizzi, E., Accornero, N., Chapple, W. & Hogan, N. *J. Neurosci.* **4**, 2738-2744 (1984).
- Soechting, J. F. & Flanders, M. *J. Neurophysiol.* **62**, 595-608 (1989).
- Syka, J. & Radil-Weiss, T. *Brain Res.* **28**, 567-572 (1971).
- Ewert, J.-P. *Brain Behav. Evol.* **3**, 36-56 (1970).
- Schiller, P. J. & Stryker, M. *J. Neurophysiol.* **35**, 915-924 (1972).
- Wurtz, R. H. & Goldberg, M. E. *Science* **171**, 82-84 (1971).
- Sparks, D. L. *Brain Res.* **156**, 1-16 (1978).
- du Lac, S. & Knudsen, E. I. *J. Neurophysiol.* **63**, 131-146 (1989).
- Robinson, D. *Vis. Res.* **12**, 1795-1808 (1972).
- Masino, T. & Knudsen, E. I. *Soc. Neurosci. Abstr.* **14**, 1236 (1988).
- Masino, T. & Grobstein, P. *J. comp. Neurol.* **291**, 103-127 (1990).
- Grantyn, A. & Berthoz, A. in *Control of Head Movement* (eds Peterson B. & Richmond, F.J.) 224-244 (Oxford University Press, 1988).
- Fukushima, K. *Prog. Neurobiol.* **29**, 107-192 (1987).
- Westheimer, G. & Blair, S. M. *Expl. Brain Res.* **24**, 89-95 (1975).
- Soechting, J. F. & Ross, B. *Neuroscience* **13**, 595-604 (1984).
- Lacquaniti, F. *Trends Neurosci.* **12**, 287-291 (1989).
- Kostyk, S. K. & Grobstein, P. *Neuroscience* **21**, 41-55 (1987).
- Grobstein, P. *Brain Behav. Evol.* **31**, 34-48 (1988).
- Masino, T. & Grobstein, P. *Expl. Brain Res.* **75**, 227-244 (1989).
- Masino, T. & Grobstein, P. *Expl. Brain Res.* **75**, 245-264 (1989).
- Knudsen, E. I., du Lac, S. & Esterly, S. A. *Rev. Neurosci.* **10**, 41-65 (1987).
- Simpson, J. I. & Graf, W. *Rev. Oculomotor Res.* **1**, 3-20 (1985).
- Grobstein, P. *Visuomotor Coordination* (eds Ewert, J.-P. & Arbib, M. A.) 537-568 (Plenum, New York, 1989).

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Odour-modulated collective network oscillations of olfactory interneurons in a terrestrial mollusc

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DETERMINATION of the dynamical structure of neural circuits—the general principles of how neural activity varies with time and manipulates information—is a prerequisite to understanding their computational function¹. Rhythmically active or oscillating neural circuits are particularly interesting dynamical structures, as rhythms and oscillations are a prominent feature of mammalian central nervous system electrophysiology. Coherent oscillations by networks of interneurons are observed in the vertebrate olfactory system^{2,3} and have recently been described in mammalian visual cortex⁴⁻⁶. These interneuronal networks display oscillations in local field potential (LFP) and probability of producing action potentials that are highly correlated between subcircuits sharing

the same stimulus features. Much less is known about the existence and importance of network oscillations in the higher centres of invertebrates⁷. Here we report that a network of olfactory interneurons in the cerebral ganglion of the terrestrial mollusc *Limax maximus* also displays coherent oscillations in LFP which are modified by odour input. This dynamical structure could be central to the odour recognition and odour learning ability of *Limax*^{8,9}.

The network of olfactory interneurons we studied is known as the procerebral (PC) lobe, which contains about 50% of all neurons (10^4 or 10^5 cells) in the entire *Limax* nervous system. The PC lobe receives odour information directly from the superior and inferior noses. Its anatomical features¹⁰⁻¹³ suggest substantial feedback synaptic connections. Extracellular recordings (0-3 Hz bandwidth) from the procerebral lobe of *Limax* (Fig. 1) reveal a periodic potential with a fundamental frequency of 0.69 ± 0.12 Hz ($n = 62$) (Fig. 2a). The LFP oscillation is produced by a mechanism intrinsic to the PC lobe, based on the observations that the oscillation is only recorded by an electrode in the PC lobe and that a PC lobe completely isolated from the rest of the cerebral ganglion still exhibits the oscillation (Fig. 2b). An oscillating LFP is recorded from any point within the PC lobe. Dual microelectrode recordings from within the same PC lobe demonstrate that the oscillations are always phase-locked. Apical and basal recordings within the lobe are in phase but of opposite polarity, whereas the intermediate central region shows a biphasic potential (Fig. 2c). Because the apical and basal regions of the PC lobe show LFP changes of opposite sign, current will flow between them on a cycle by cycle basis, and some of this intra-PC lobe current flow can be sensed by a surface suction electrode (Fig. 2d), directly analogous to surface EEG recordings from mammalian olfactory bulb.

Intracellular recordings from *Limax* PC lobe interneurons demonstrate periodic oscillations of membrane potential (Fig. 2e) with oscillation frequency similar to that of the LFP. During the depolarizing phase of these oscillations, cells can remain silent or can fire one or a short burst of action potentials. Thus the oscillations in *Limax* PC lobe LFP have a correlate in membrane potential oscillations. Extracellular recordings

demonstrate that all spike activity that can be recorded in the PC is phase locked to the LFP oscillation (Fig. 3a, inset). Over 90% of the action potentials occur in the 500 ms preceding the peak of the LFP oscillation (Fig. 3a). Although the ionic currents underlying the LFP and the precise relationship of the LFP to the spike and synaptic activity is at present unclear, the results suggest that the LFP is produced by synaptically-dependent events set up by the coherent collective activity of PC lobe interneurons.

Activation of olfactory afferents by odours under natural conditions will produce input to the PC lobe over several cycles of the LFP oscillation. To determine how the temporal relationship between afferent fibre activation and the LFP oscillation affects the synaptic response produced, the sizes of synaptically dependent evoked potentials were measured for olfactory nerve (ON) shock given at different phases of the LFP oscillation. ON shock produces a two-component response in the neuropil of the PC lobe (Fig. 3b, inset). The first peak is due to the action potentials in the afferent fibres entering the lobe from the ON. The second slower peak is due to synaptic events elicited by the afferent volley⁹. The size of the synaptic response to ON shock is dependent on the time of arrival of the afferent volley relative to the phase of the endogenously oscillating LFP (Fig. 3b). Thus the postsynaptic effects of afferent fibre activation by odour will vary depending upon the phase of the intrinsic PC lobe oscillation at which it arrives. ON shock also transiently alters the PC oscillation frequency and waveform for 5-15 s after the stimulus and resets the rhythm relative to its preshock phase.

The importance of the intrinsic oscillation of the PC lobe in the processing of olfactory information is suggested by experiments using a preparation which retained the olfactory epithelium of the nose connected to the PC lobe via the digitate ganglion and ON¹⁴. The receptor surface was in air while the remainder of the preparation remained immersed in saline. A two-channel puffer was used to apply either moist air or air equilibrated with a $10^{-5}\%$ solution of 2-ethyl-3-methoxypyrazine (potato odour; see ref. 15) to the olfactory surface while recording *en passant* from the olfactory nerve and from the entire PC

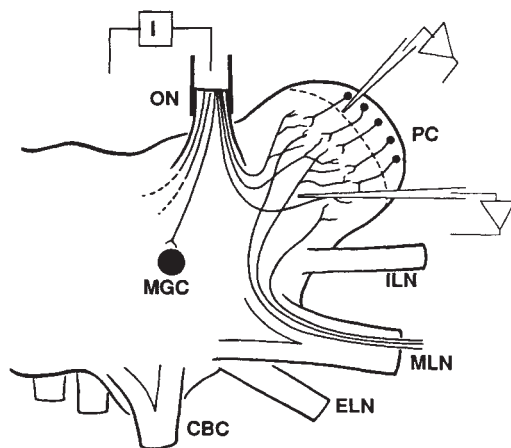


FIG. 1 Schematic diagram of the cerebral ganglion of *Limax maximus*. The olfactory nerve (ON) is in a suction electrode for stimulation. Extracellular micropipette electrodes are shown recording from apical (cell body) region and basal neuropil in the procerebral (PC) lobe. Specimens of *Limax maximus* ($n = 74$) were anaesthetized by chilling on ice for 30 min before the brain was removed into cold (4-6 °C) saline for isolation of the cerebral ganglia. Isolated PC lobe preparations were made by cutting across the base of the lobe from the insertion of the ON to the insertion of the internal lip nerve (ILN). Nose-brain preparations retained the olfactory neuroepithelium and digitate ganglion from the tip of the superior tentacle connected to the ON. The PC lobe also receives input from the inferior nose via the medial lip nerve (MLN). CBC-cerebrobuccal connective, ELM-external lip nerve, MGC-metacerebral giant cell.

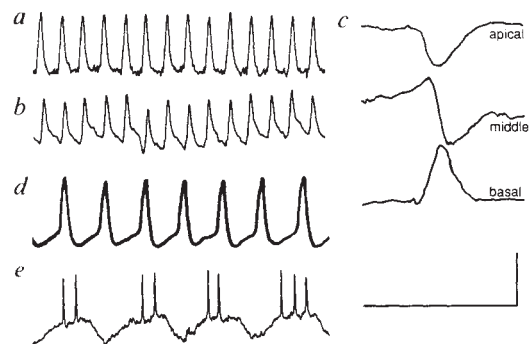


FIG. 2 Recordings of spontaneous local field potential (LFP) oscillations from a PC lobe. *a*, PC lobe attached to the cerebral ganglion as in Fig. 1. Recording site located in the neuropil. *b*, Same recording site in same lobe as (*a*) but after complete isolation from the cerebral ganglion. *c*, Simultaneous recordings from apical, central and basal recording sites in a PC lobe. *d*, Coherent PC lobe oscillation recorded from an isolated lobe lodged in a suction electrode. *e*, Intracellular recording from a neuron in the PC lobe. Calibration bars are 10 s, 1 pA (*a, b*); 700 ms, 3 pA (*c*); 10 s, 200 μ V (*d*); 2.5 sec, 50 mV (*e*). Extracellular recordings were made either with a List model EPC 5 or a Getting model 5A amplifier and were band-pass filtered between 0-3 Hz using a PAR model 113. Data were recorded on a Gould model 220 chartwriter or digitized using a MacPacq system and stored on an Apple MacII computer for later analysis. Intracellular recordings were made using beveled glass microelectrodes of 80-120 M Ω resistance and a NeuroData IR-283 amplifier.

lobe with a suction electrode. A 10 s puff of potato odour activated many small amplitude units and a few large amplitude units in the ON and dramatically altered the waveform and frequency of the PC lobe oscillation (Fig. 4a). A puff of moist air at the same velocity elicited much less activity in both the ON and in the PC lobe (Fig. 4b). The response during odour application is predominantly a transient reduction in amplitude ($15.4 \pm 2.3\%$, $n = 14$) (mean \pm s.e.m.) and reduction in frequency ($9.2 \pm 2.8\%$, $n = 15$) of the endogenous oscillation. This pattern of response was seen in 26 of 29 trials with 5 nose-brain

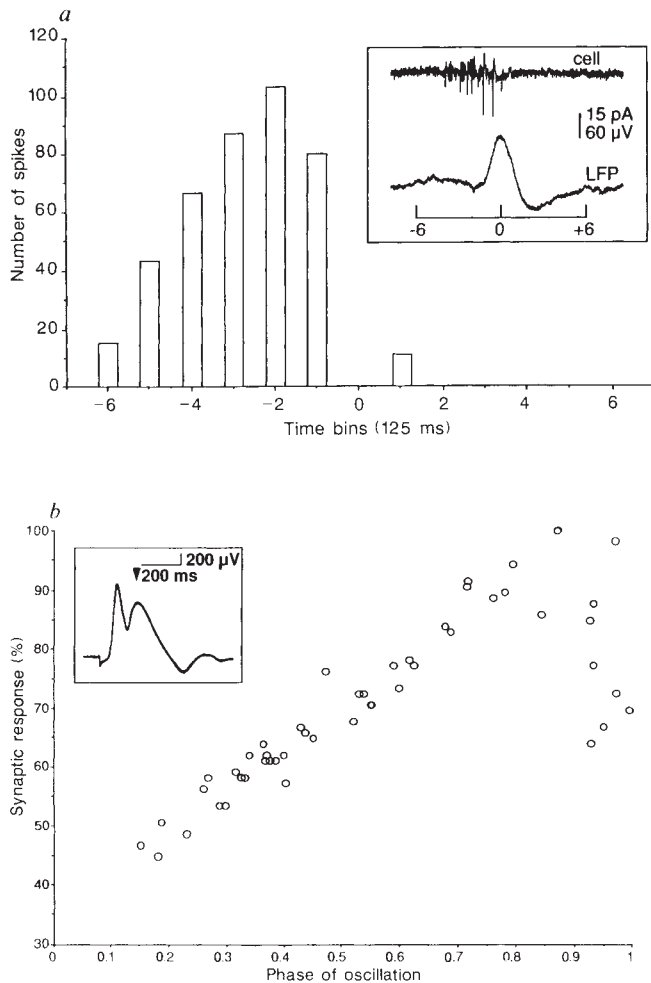


FIG. 3 Spontaneous oscillation phase data. *a*, Extracellular cell-attached patch electrode recordings ($n = 45$) showing spike activity phase-locked to the spontaneously oscillating local field potential (LFP). Inset: Typical record of multiunit extracellular recording and simultaneously recorded local field potential. Calibration bar is 15 pA for cells, 60 μ V for LFP. Histogram shows number of action potentials occurring in 125 ms time bins with zero time taken at the peak of the LFP. *b*, Amplitude of the synaptic current evoked in the PC lobe by supramaximal shock of the ON depends on the phase of the spontaneous oscillation at which it is evoked. Inset: the two-phase response to a single ON shock. The second peak of the response is the calcium-sensitive synaptically-dependent event. Calibration bars indicate 150 ms, 300 μ V. The graph shows that the evoked synaptic response amplitude depends on spontaneous oscillation phase. Data samples (5 s) were obtained every 100 s, with ON shock occurring 4 s into the sample. The time of the last complete oscillation cycle before the shock (T_1) was measured, as was the time from the last oscillation peak before the shock to the peak of the synaptic response (T_2). The phase of occurrence of the synaptic response was taken as (T_2/T_1) . The evoked synaptically-dependent event is minimal just before and after the occurrence of the peak of the spontaneous oscillation and then increases smoothly in amplitude over the middle 80% of the inter-peak interval.

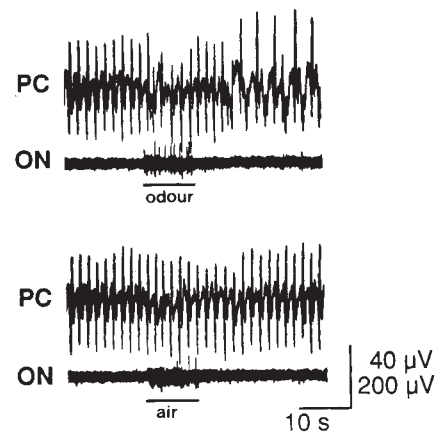


FIG. 4 Response to potato odour recorded using a nose-brain preparation. Suction electrodes recorded *en passant* from the ON and from the surface of the PC lobe. *a*, A 10-s puff of air saturated with the vapour of $10^{-5}\%$ (v/v) of 2-ethyl-3-methoxy pyrazine applied to the receptor epithelium of the nose elicited multiunit activity in the ON and dramatically altered the waveform and frequency of the PC lobe oscillation. *b*, A 10-s puff of moist air at the same velocity produced much less activity in the ON and a smaller alteration in the PC lobe LFP oscillation. The change in oscillation frequency following odour offset shown in (*a*) was not observed in every preparation.

preparations. These results indicate that activation of one of the normal sensory input pathways to the PC lobe using a behaviourally relevant stimulus at a concentration known to be attractive to the intact animal¹⁵ produces, among other effects, transient changes in the endogenously active local circuit responsible for the oscillating LFP.

The existence in *Limax* of the oscillating LFP and its responsivity to ON shock and odour-elicited input from the olfactory epithelium establishes yet another functional parallel between the molluscan and mammalian olfactory systems. A striking feature of mammalian olfactory bulb is the endogenously oscillating 40 Hz LFP that is enhanced by inspiration of odours¹⁶⁻²⁰. The amplitude of the LFP oscillation varies over the bulbar surface. The spatial pattern of oscillation amplitudes could represent an odour and can be altered by conditioning²¹⁻²³. A theoretical model has explored how the LFP oscillation by the olfactory bulb may contribute to the signal processing functions in the detection of weak odours²⁴. Different models have explored how the olfactory bulb²⁵ or olfactory (piriform) cortex²⁶⁻²⁸ could implement associative (content-addressable) memories. The possibility that endogenous oscillations in the second-order olfactory circuit of *Limax* allow detection of weaker odour inputs than would otherwise be the case has been supported by recent studies of LIMAX, a neural network model of the *Limax* olfactory processing and odour learning circuit²⁹. With the identification of an output pathway from the PC lobe to the pedal ganglia¹² it may be possible to test this idea directly. The rhythmic synchrony of PC lobe cell activity coupled throughout the entire lobe must have a critical role in the odour processing function of this structure. The striking functional analogy between such phylogenetically disparate systems indicates that the computational use of the local circuit oscillation is fundamental to the tasks of these types of olfactory processing networks. Other olfactory systems, for example the central olfactory pathways of insects^{30,31}, may display similar dynamics.

The oscillating LFP in *Limax* PC lobe is modulated in both waveform and frequency by dopamine and serotonin³² (A.G., L. Rhines, J. Flores, and D.W.T., unpublished data), both of which are present in fibres entering the lobe from sources extrinsic to the lobe^{33,34}. Both serotonin, previously implicated in mechanisms of molluscan synaptic plasticity³⁵⁻³⁹, and dopamine alter the phosphorylation state and rate of synthesis

of specific proteins in neurons of the PC lobe^{40,41}. Therefore, as in mammalian olfactory bulb⁴²⁻⁴⁴, input pathways exist that could modify PC lobe circuitry during aversive odour conditioning. □

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1. Tank, D. W. *Semin. Neurosci.* **1**, 67-79 (1989).
2. Freeman, W. J. *Mass Action in the Nervous System* (Academic, New York, 1975).
3. Shepherd, G. M. in *Taste, Olfaction and the Central Nervous System* (ed. Pfaff, D. W.) 307-332 (Rockefeller University Press, New York, 1985).
4. Gray, C. M. & Singer, W. *Proc. natn. Acad. Sci. U.S.A.* **86**, 1698-1702 (1989).
5. Gray, C. M., König, P., Engel, A. K. & Singer, W. *Nature* **338**, 334-337 (1989).
6. Eckhorn, R. et al. *Biol. Cybern.* **60**, 121-130 (1988).
7. Bullock, T. H. & Basar, E. *Brain Res. Rev.* **13**, 57-75 (1988).
8. Gelperin, A., Tank, D. W. & Tesaro, G. in *Neural Models of Plasticity* (eds Byrne, J. H. & Berry, W. O.) 133-159 (Academic, New York, 1989).
9. Sahley, C. L. in *Connectionist Modeling and Brain Function: The Developing Interface* (eds Hanson, S. & Olson, C.) 36-73 (MIT, Cambridge, 1990).
10. McCarragher, G. & Chase, R. *J. Neurobiol.* **16**, 69-74 (1985).
11. Zs.-Nagy, I. & Sakharov, D. A. *Tissue & Cell* **2**, 399-411 (1970).
12. Chase, R. & Tolloczko, B. *J. comp. Neurol.* **283**, 143-152 (1989).
13. Veratti, E. *Mem. Real. Inst. Lomb. Sci. Lett.* **18**, 163-179 (1900).
14. Egan, M. E. & Gelperin, A. *J. mollusc. Stud.* **47**, 80-88 (1981).
15. Hopfield, J. F. & Gelperin, A. *Behav. Neurosci.* **103**, 329-333 (1989).
16. Freeman, W. J. *Biol. Cybern.* **35**, 221-234 (1979).
17. Adrian, E. D. *J. Physiol.* **100**, 459-473 (1942).
18. Adrian, E. D. *Electroenceph. clin. Neurophysiol.* **2**, 377-388 (1950).
19. Adrian, E. D. *Br. med. Bull.* **6**, 330-333 (1950).
20. Freeman, W. J. & Skarda, C. A. *Brain Res. Rev.* **10**, 147-175 (1985).

21. Gray, C. M., Freeman, W. J. & Skinner, J. E. *Behav. Neurosci.* **100**, 585-596 (1986).
22. Freeman, W. J. & Viana Di Prisco, G. *Behav. Neurosci.* **100**, 753-763 (1986).
23. Baird, B. *Physica* **22D**, 150-175 (1986).
24. Li, Z. & Hopfield, J. J. *Biol. Cybern.* **61**, 379-392 (1989).
25. Freeman, W. J., Yao, Y. & Burke, B. *Neur. Net.* **1**, 277-288 (1988).
26. Wilson, M. A. & Bower, J. M. in *Neural Information Processing Systems* (ed. Anderson, D. Z.) 114-126 (American Institute of Physics, New York, 1988).
27. Lynch, G., Granger, R., Baudry, M. & Larson, J. in *Neural Connections, Mental Computation* (eds Nadel, L., Cooper, L., Culicover, P. & Harnish, R. M.) 247-289 (MIT Press, Cambridge, 1988).
28. Haberly, L. B. *Chem. Senses* **10**, 219-238 (1985).
29. Hopfield, J. J. in *Computer Simulation in Brain Science* (ed. Cotterill, R. M. J.) 405-415 (Cambridge University Press, New York, 1988).
30. Homberg, U., Christensen, T. A. & Hildebrand, J. G. A. *Rev. Ent.* **34**, 477-501 (1989).
31. Menzel, R. in *Perspectives in Neural Systems and Behavior* (eds Carew, T. & Kelley, D.) 249-266 (A. R. Liss, New York, 1989).
32. Rhines, L. thesis, Princeton Univ. (1989).
33. Osborne, N. N. & Cottrell, G. A. *Z. Zellforsch. mitrosk. Anat.* **112**, 15-30 (1971).
34. Yamane, T., Gelperin, A. & Delaney, K. *Soc. Neurosci. Abstr.* **12**, 862 (1986).
35. Kandel, E. R. & Schwartz, J. H. *Science* **218**, 433-443 (1982).
36. Sweatt, J. D. & Kandel, E. R. *Nature* **339**, 51-54 (1989).
37. Crow, T. *Trends Neurosci.* **11**, 136-142 (1988).
38. Eskin, A., Garcia, K. S. & Byrne, J. H. *Proc. natn. Acad. Sci. U.S.A.* **86**, 2458-2462 (1989).
39. Grover, L. M., Farley, J. & Auerbach, S. B. *Brain Res. Bull.* **22**, 363-372 (1989).
40. Yamane, T. & Gelperin, A. *Cell. mol. Neurobiol.* **7**, 291-301 (1987).
41. Yamane, T., Oestreicher, A. B. & Gelperin, A. *Cell. mol. Neurobiol.* **9**, 447-459 (1989).
42. Macrides, F., Davis, B. J., Young, W. M., Nadi, N. S. & Margolis, F. L. *J. comp. Neurol.* **203**, 495-514 (1981).
43. Shipley, M. T., Halloran, F. J. & de la Torre, J. *Brain Res.* **329**, 294-299 (1985).
44. McLean, J. H. & Shipley, M. T. *J. Neurosci.* **7**, 3016-3028 (1987).

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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 injury¹⁻³. 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 levels⁴. 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 neurons⁶. 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 detail⁷. 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 degeneration⁸, 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

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 previously¹⁴.

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

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 stages¹¹.

The application of CNTF to the proximal stump of the nerve in which the lesion had been made (5 µg of highly purified CNTF⁴ 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

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