Eyes Transplanted to Tadpole Tails Send Axons Rostrally in Two Spinal-Cord Tracts

Abstract. *Axons from eyes transplanted to the tail in* Xenopus *larvae enter the caudal spinal cord and follow two adjacent tracts rostrally to the level of the cerebellum. When eyes are transplanted to the ear area, optic axons enter the hindbrain and follow the same tracts rostrally and caudally. These sensory pathways normally contain the embryonic sensory system of the Rohon-Beard axons and the descending and ascending tracts of nerve V. We propose that the transplanted optic axons have followed a continuous substrate sensory pathway normally shared by a number of different sensory tracts.*

During development, two steps are usually necessary for the formation of a neural circuit. (i) An axon must grow from its cell body to the vicinity of its target population. (ii) The axon must find the appropriate target cell on which to synapse (*1*). Much is known about the way axons apportion themselves among their target cell populations (*2*), but little is known about how an axon finds its way to the vicinity of its target cell population (*3*).

Constantine-Paton and Capranica (*4*) recently introduced an important technique for exploring in vivo long-distance axon guidance. They transplanted eye primordia to presumptive ear areas and used autoradiographic axon tracing to follow optic axons that grew into the nervous system. They reported that transplanted optic axons traveled dorsocaudally in a single well-defined tract from the hindbrain to the end of the spinal cord.

Will optic axons travel dorsocaudally in the nervous system regardless of their level of entry, as Constantine-Paton and Capranica have proposed? To answer this question we have transplanted eye primordia to the caudal end of the developing spinal cord and autoradiographically traced those optic axons which grew into the cord. We have also transplanted eye primordia to the presumptive ear area. We have found that optic axons will grow both caudally and rostrally in a specific set of pathways normally shared by three general somatic afferent systems: the trigeminal tracts, the tract of Lissauer, and the Rohon-Beard cell tracts. We call these pathways the substrate sensory pathways. (We use the term *pathway* to mean embryonic route.)

We transplanted a third eye primordium to host *Xenopus* embryos, Nieuwkoop and Faber (*5*) stages 21–22 (after the neural tube has closed), from donor embryos of stages 24–26 (early tailbud). Operations were done in sterile full-strength Holtfreter's solution (*p*H 7.5 to 7.6), which was supplemented with $2 \times 10^{-4}M$ $MgCl_2$ and antibiotics (*6*). The animals were allowed to grow (at 22° to 23°C) into tadpoles, and after about 3 weeks (stages 48–50), the extra eyes were injected with approximately 0.05 μl of 20 to 30 μc/μl L-[2,3-^{3}H(N)]proline (New England Nuclear). Three days after the injections, the animals were fixed (*7*), embedded in paraffin, serially sectioned at a thickness of 10 μm, and processed for autoradiographic axon tracing (*8*) with nuclear track emulsion (Kodak NTB-3). Slides were exposed for 6 days at 4°C, developed with Dektol developer (Kodak), and stained with gentian violet.

Identical processing of five control tadpoles, in which one normal eye had been injected, showed uniform labeling of the optic nerve and the complete contralateral optic tract. No ipsilateral optic projections were evident at this age (*9*). Other parts of the nervous system showed only background labeling, which, in the white matter of the rostral spinal cord, was about four grains per 100 μm^2.

The results include three animals in which optic fibers entered the caudal spi-

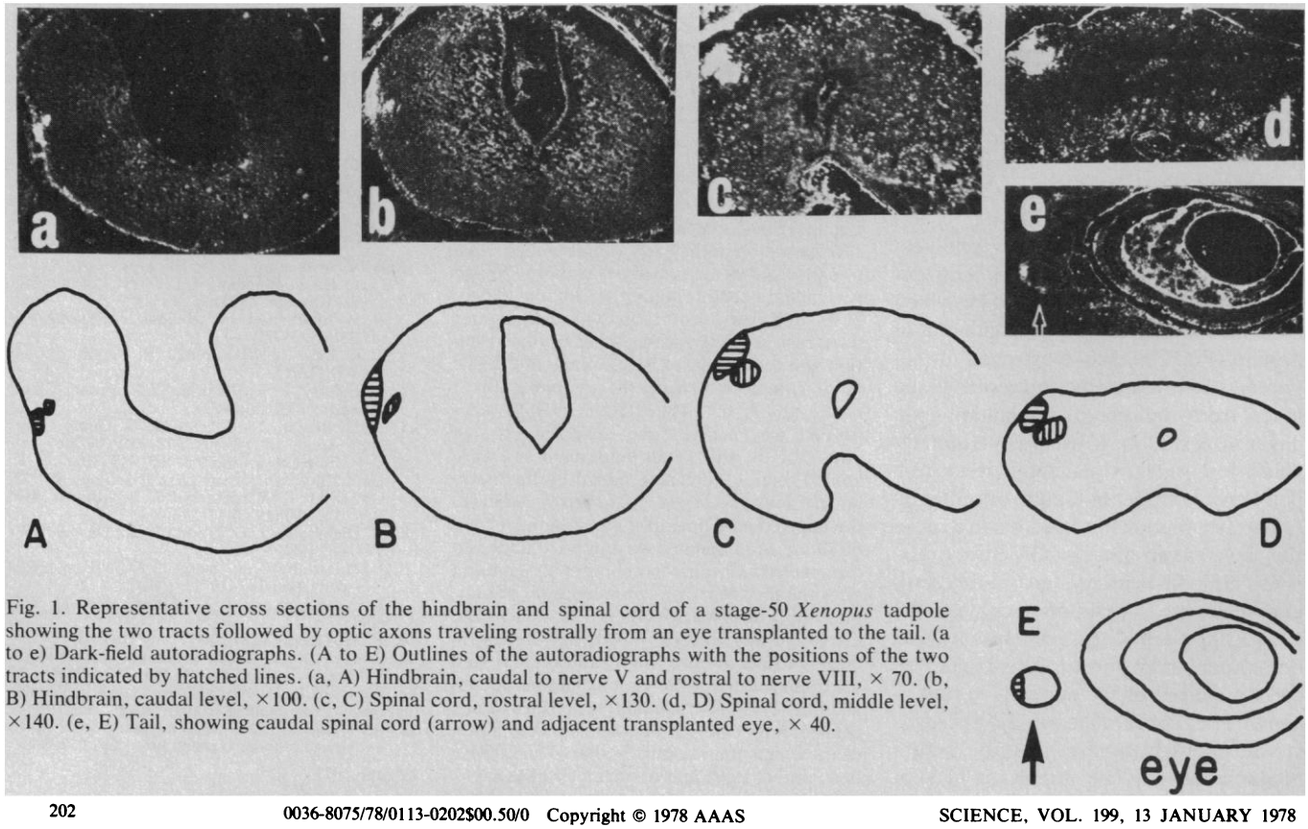

Fig. 1. Representative cross sections of the hindbrain and spinal cord of a stage-50 *Xenopus* tadpole showing the two tracts followed by optic axons traveling rostrally from an eye transplanted to the tail. (a to e) Dark-field autoradiographs. (A to E) Outlines of the autoradiographs with the positions of the two tracts indicated by hatched lines. (a, A) Hindbrain, caudal to nerve V and rostral to nerve VIII, × 70. (b, B) Hindbrain, caudal level, ×100. (c, C) Spinal cord, rostral level, ×130. (d, D) Spinal cord, middle level, ×140. (e, E) Tail, showing caudal spinal cord (arrow) and adjacent transplanted eye, × 40.

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nal cord and four in which optic fibers entered the hindbrain. Of six tadpoles in which eyes developed on the tail, three showed only background levels of radioactive labeling throughout their nervous systems. The three remaining tadpoles had heavy radioactive labeling of two adjacent, well-defined tracts running from the spinal cord of the tail through the hindbrain to the level of the cerebellum (Figs. 1 and 2). In each animal, the tracts were present on only one side of the nervous system and never crossed the midline. One tract, the external sensory tract, was immediately adjacent to the pial surface. The other, the internal sensory tract, was more medial. In the spinal cord both tracts were dorsolateral, but near the spino-bulbar junction they began to take a more ventral position. Through the hindbrain the external tract remained immediately adjacent and slightly ventral to the entry zones of most of the cranial nerves (notably nerves X, VIII, and V). The internal sensory tract ran about 60 μm medial to the external sensory tract; however, in the region of the entry zone of nerve V, the internal sensory tract moved laterally to merge with or run immediately adjacent to the external sensory tract. Both tracts seemed to disperse in the region of the midcerebellum. Preliminary grain counts could not detect labeled optic fibers in the stratum opticum of the tectum. Most labeled fibers probably ended in the ipsilateral cerebellum. There was a suggestion that some of the dispersing fibers ran along the ventral rim of the brain; however, the amount of label in that area was not much above background levels. Likewise, it is possible that there were other minor fiber paths that were too small or too dispersed to be distinguished from the background label. We are still examining the question of where transplanted optic fibers terminate.

In another set of tadpoles in which eyes were transplanted to the presumptive ear area, four clearly showed optic axons entering the caudal hindbrain along nearby cranial nerves (notably nerve X). (One of these tadpoles was fixed at stage 46.) In each case the optic axons followed two ipsilateral tracts identical to those illustrated in Figs. 1 and 2. Here the axons traveled both rostrally and caudally.

Like Constantine-Paton and Capranica (*4*), we have found that transplanted optic axons chose specific, consistent, and circumscribed hindbrain and spinal-cord pathways in which to travel. These same pathways were followed regardless of the level of entry of the optic axons, and the position of these pathways in the *Xenopus* nervous system was identical to their position in the *Rana* nervous system.

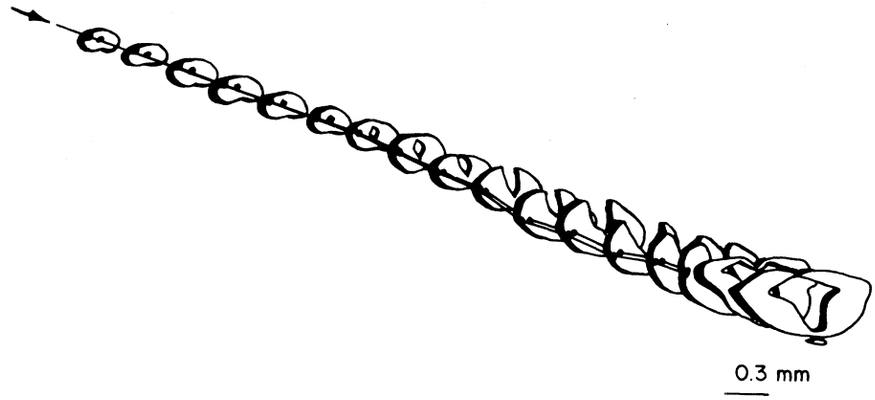

Fig. 2. Camera lucida tracings of serial cross sections of the hindbrain and spinal cord of a stage-50 *Xenopus* tadpole (the same animal as in Fig. 1). Lines illustrate the relative positions of the two tracts taken by optic axons from an eye transplanted to the tail (beyond the drawing). Arrow indicates the direction of travel of optic axons. The axons disperse suddenly upon reaching the level of the midcerebellum rostral to the entry of nerve V between the second and third serial cross sections of the figure. Labeled optic axons cannot be traced to the superficial white matter of the tectum (space between serial cross sections, 200 μm).

However, unlike Constantine-Paton and Capranica (*4*) we found that optic axons followed two adjacent pathways. Another difference is our finding that optic axons traveled both rostrally and caudally in these pathways (*10*). This indicates either that the two pathways do not contain rostro-caudal directional cues or that optic axons cannot read those cues. Clearly, the answer to the question posed at the outset is that optic axons do not travel dorsocaudally in the nervous system regardless of the level at which they enter.

Optic axons that enter the hindbrain and spinal cord appear to grow longitudinally (both rostrally and caudally) in only two ipsilateral pathways, the external and internal sensory tracts. Are these optic axons following preexisting pathways? In the hindbrain, the external sensory tract coincides with the descending tract of nerve V (*11*), and in the spinal cord, the two pathways follow the future tract of Lissauer (*12*). However, at the time that these extra optic fibers enter and grow within the central nervous system (prior to stage 47) there are no dorsal-root ganglion fibers to form the tract of Lissauer (*13*). Instead, the only dorsolateral fiber tracts normally found in the spinal cord are the longitudinal axons of the Rohon-Beard cells (*13–15*). Rohon-Beard cells, the embryonic sensory system in many vertebrates, compose the entire spinal sensory system in *Xenopus* tadpoles until stage 47 (*13*). Rostrally the Rohon-Beard tracts extend into the descending tracts of nerve V (*14, 15*). There is some evidence that before nerve V has entered the central nervous system, Rohon-Beard cells and axons extend through the hindbrain along the future descending tracts of nerve V (*14*).

Thus, optic axons growing in the hindbrain and spinal cord follow the preexisting substrate sensory pathways of the Rohon-Beard cells (*16*). These optic axons show that the substrate sensory pathways are continuous from the rostral hindbrain to the caudal spinal cord. Two important features of the substrate sensory pathways are: (i) they are normally shared by at least three different sensory systems—the Rohon-Beard longitudinal tracts, the descending tracts of nerve V, and the tracts of Lissauer—and (ii) they permit both rostral and caudal axon growth as evidenced by the fact that transplanted optic axons (*17*), Rohon-Beard axons (*13*), axons of nerve V (*18*), and axons of the tract of Lissauer (*12*) can be found traveling in either direction. The substrate sensory pathways described here provide the organization for a number of sensory tracts during development. We suggest that substrate pathways are a device for organizing the long ascending and descending axon tracts during development, and we predict the existence of other substrate pathways such as substrate motor pathways.

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References and Notes

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target cell population has been restructured [S.-H. Chung and J. Cooke, *Nature (London)* **258**, 126 (1975)].
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3. One of the few experimental observations about in vivo axonal guidance is that axons destined to grow in one direction can often properly reorient themselves if their initial orientation has been reversed [H. van der Loos, *Bull. Johns Hopkins Hosp.* **117**, 228 (1965)]. Thus Mauthner axons growing in blocks of tissue that have been reversed (anterior for posterior) will often turn and grow in the correct (caudal) direction [C.-O. Jacobson, *Zool. Bidr. Uppsala* **36**, 73 (1964); E. Hibbard, *Exp. Neurol.* **13**, 289 (1965)].
4. M. Constantine-Paton and R. P. Capranica, *Science* **189**, 480 (1975); *J. Comp. Neurol.* **170**, (1976).
5. P. D. Nieuwkoop and J. Faber, *Normal Table of* Xenopus laevis (North-Holland, Amsterdam, 1956). In this report, all *Xenopus* stagings follow the Nieuwkoop and Faber criteria.
6. Potassium penicillin G, 1500 to 2000 unit/liter; streptomycin sulfate, 1 to 2 mg/liter; and sulfathiazol (sodium salts), 1 to 2 mg/liter.
7. The animals were fixed with a mixture of 90 ml of 80 percent ethanol, 5 ml of 40 percent formaldehyde, and 5 ml of glacial acetic acid.
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9. A similiar result was reported by J. Currie and W. M. Cowan, *Brain Res.* **71**, 133 (1974).
10. Differences between our procedures and those of Constantine-Paton and Capranica (*4*) which may relate to the differences in our findings are (i) our use of *Xenopus* as opposed to *Rana*, (ii) our examination of the axon tracts in larval animals as opposed to older animals, (iii) the lack of transplanted brain bridges conducting axons from eye to nervous system in our experiments, and (iv) the apparent lower background labeling in our slides.
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12. R. J. Lasek, B. S. Joseph, D. G. Whitlock, *Brain Res.* **8**, 319 (1968); B. S. Joseph and D. G. Whitlock, *Anat. Rec.* **160**, 279 (1968).
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14. G. E. Coghill, *J. Comp. Neurol.* **26**, 247 (1916).
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16. Do the optic axons follow the substrate sensory pathways solely because of temporal and physical factors, or is there recognition of chemical cues along the pathways? That optic axons recognize entries and exits from chemical cues is suggested by two facts. (i) The axons chose to enter sensory pathways and not motor pathways, and (ii) at least some of the axons chose not to exit anywhere along the substrate sensory pathways.
17. We find evidence that optic axons entering the caudal spinal cord travel caudally as well as rostrally.
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19. We thank J. D. Caston for advice on embryologic techniques. Supported by grant NS09299 from the National Institutes of Health and a Career Development Award to R.J.L. and also by a Sloan Foundation training grant.

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Intracellular Analysis of Trigeminal Motoneuron Activity During Sleep in the Cat

Abstract. *Intracellular recordings were made from trigeminal motoneurons of normally respiring, unanesthetized cats during naturally occurring sleep. The transition from quiet to active sleep was accompanied by tonic motoneuron hyperpolarization. Stimulation of the reticular formation induced a depolarizing potential in trigeminal motoneurons during quiet sleep and a hyperpolarizing potential during active sleep. The results provide a synaptic explanation for the phenomenon of reticular response reversal and insights into the basic mechanisms controlling motor activity during the sleep states.*

Considerable effort has been made over the last 20 years in obtaining indirect indications of the synaptic processes that occur during the sleep states (*1*). However, the mechanisms controlling neuronal activity can be determined only by direct intracellular monitoring of neuronal processes. Unfortunately, there has been, to our knowledge, no intracellular recording in any neuron during sleep (*1*).

We have developed a method of recording intracellularly during sleep, and we now report the results obtained by recording the membrane potential of trigeminal motoneurons in cats during natural sleep. Since the animals were unanesthetized, completely undrugged, and unrestrained (with the exception of head fixation), we were able to study the spontaneous synaptic influences exerted upon these motoneurons during quiet [that is, non–rapid eye movement (NREM)] and active (REM) sleep. In addition, we will present evidence concerning the synaptic mechanisms underlying the state-dependent control of the trigeminal myotatic reflex by the reticular formation during sleep and wakefulness (*2*). This phenomenon of reticular response reversal is well suited for study by intracellular methods during the sleep states, for the pattern of reticular modulation of the masseteric reflex that occurs during quiet sleep and wakefulness (reflex facilitation) is diametrically opposite that induced during active sleep (reflex inhibition) (*2*). Thus, with the reticular site and level of stimulation remaining constant, the state of the animal determines the direction of effect. A resolution of the mechanisms underlying this state-dependent response and the importance of this reticular site in the control of motor activity during sleep must reside in an intracellular analysis of the synaptic events that result in motor facilitation and inhibition.

Seven animals were anesthetized with sodium pentobarbital (35 mg per kilogram of body weight), and standard electrodes were implanted for recording the electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG) (*2*). Stimulating bipolar strut electrodes were permanently placed in the mesencephalic nucleus of the fifth nerve and in the nucleus reticularis pontis oralis (*2*). A small circle (diameter, 5 mm) of occipital bone was removed by trephination in order to permit the subsequent penetration by a microelectrode through the cerebellum to trigeminal motoneurons. The trephined hole was filled with bone wax, which was removed during experimental sessions. The animals were allowed at least 1 week to recover from surgery (*3*).

During experimental sessions, after the penetration and identification of a trigeminal jaw-closer motoneuron with a micropipette filled with 3*M* KCl (tip resistance, 8 to 20 megohms), the spontaneous membrane potential of the motoneuron was correlated with polygraphic data indicating the state of the animal as one of quiet or active sleep. For certain cells, a short train of one to three pulses (interpulse interval, 2 msec) was delivered to the nucleus reticularis pon-

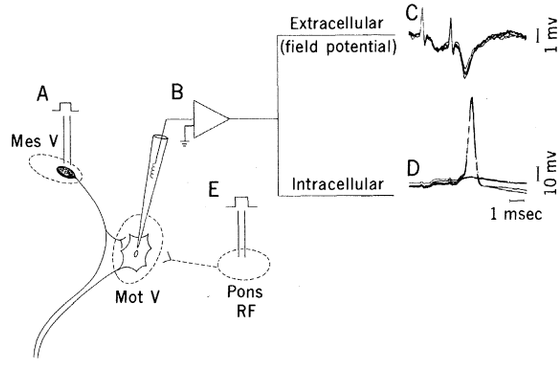

Fig. 1. Diagram of the basic stimulation and recording paradigm. The trigeminal motor nucleus (*Mot V*) was identified by monitoring its extracellular field potential (C) induced by stimulation (A) of the mesencephalic nucleus of the fifth nerve (*Mes V*). Jaw-closer motoneurons were identified by intracellular recording of the monosynaptic EPSP's and spike potentials of mesencephalic V origin (A, B, D). (E) Stimulation of the nucleus pontis oralis (*Pons RF*) (*4*).

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