tical analysis were used. When the binomial test was applied to Backster's data, they were found to be statistically significant by our criteria.

We believe that we matched, and in several instances improved on, Backster's experimental techniques, such as controls, shielding, number of observations, methods of analysis, and number of shrimp killed per injection. We obtained no evidence of primary perception in plants. While the hypothesis will remain as an intriguing speculation one should note that only the limited published data of Backster support it.

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5. The growing medium consisted of equal parts of sterile soil, peat, and pearlite; 3 ounces of 20 percent superphosphate fertilizer was added per bushel. Illuminance was natural daylight. The maintenance program consisted of Peters 20 : 20 : 20 water-soluble fertilizer alternated weekly with KNO_3 at 1.0 pound per 100 gallons of water, Malathion sprayed weekly, and plants watered daily. The temperature was 70°F during the day, and 60°F at night.
6. *Artemia salina* were obtained in dehydrated egg form (Longlife Hatch Pack, Longlife Aquarium Products, Harrison, N.J.) and were grown to maturity in a Longlife brine shrimp hatchery according to the directions included with the unit.
7. The electrodes were constructed of polished 0.027-inch stainless steel, shaped to dimensions of 1.0 by 1.4 inches, with a soldering tab extending beyond the end at a small angle to the plane of the electrode. Leads consisted of 4 feet of No. 26 PVC type B wire and were attached to the tab with silver-based solder. The entire finished electrode was polished to eliminate sharp areas and its contact surface was covered with 12-ply gauze which had earlier been impregnated with a hot solution of 0.25*M* NaCl in 1.25 percent Difco Bacto agar and then trimmed to meet the electrode border.
8. The recording electrodes were connected to Grass model 7 polygraphs equipped with 7P5A wideband electroencephalogram preamplifiers. Routinely the system sensitivity was maintained at 5 μv/mm with bandpass filters set at 0.15 to 15.0 hertz (half-amplitude frequencies with slopes of 12 db/octave). The manufacturer's input impedance rating under push-pull operation was greater than 3.0 megohms and the common mode rejection ratio was 1 : 1600.
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Central Projection of Optic Tract from Translocated Eyes in the Leopard Frog (*Rana pipiens*)

Abstract. *In* Rana pipiens *embryos, eye anlagen were moved to the evacuated ear position, where they continued to differentiate and sent their optic nerve fibers into the hindbrain. Upon entering the medulla, the optic fibers turned caudally, penetrated the spinal cord, and traversed the dorsolateral white matter to the caudal end. We found this pattern of growth in every animal; the optic fibers did not enter the tecta. These results suggest the existence within the neural tube of a three-dimensional gradient system to which embryonic optic fibers are responsive and which may guide the normal development of the visual pathway.*

During development of the vertebrate visual system, nerve fibers from the ganglion cells in the retina achieve an orderly projection onto specific populations of cells in the central nervous system. The pathway that the fibers take in reaching the central nuclei is identical in different individuals within a given species; this suggests that the fibers are guided systematically in their growth through the central nervous system. Most previous investigators of axonal guidance in the visual system have utilized the regenerative capabilities of the optic tract in adult anurans and teleost fishes (*1*). These studies have revealed that severed or slightly deflected optic nerve fibers can often reestablish their central connections. But little is known about the mechanisms in embryonic development that are responsible for directing the initial growth of axons from the retinal ganglion cells within the eyecup to their final destination in the central nervous system. Is this initial projection an intrinsic property of the optic nerve fibers themselves or, instead, does it depend on directional guidance from the embryonic brain during its early development?

We investigated this question of axonal guidance during embryonic development by drastically altering the pathway between the retina and the cells within the central nervous system upon which the optic nerve fibers normally terminate. We present here the results of our experiments in which eye primordia of leopard frog (*Rana pipiens*) embryos were relocated in the position normally occupied by the ear anlage. In this way we have been able to cause the optic nerve from the transplanted eye to enter the hindbrain, thus completely altering the normal diencephalic route from the retina to the optic tectum in the midbrain (*2*).

The transplantation operations were performed on Shumway stage 16 to 18 *R. pipiens* embryos (*3*). The left ear capsule was removed and either the left (type 1) or the right (type 2) primordial eye was inserted in its place. A small part of the forebrain tissue adjacent to the optic stalk was routinely taken in the removal of the eye primordia. This forebrain tissue fused with the hindbrain, thus forming a tissue bridge between the medulla and the eyecup which facilitated penetration of the optic nerve from the transplanted eye into the central nervous system. The experimental animals were raised individually until late tadpole or postmetamorphic stage. A modified Holmes silver stain was used to examine the morphology of the transplanted retina and optic nerve. The optic tract from the transplanted eye was traced within the central nervous system by intraocular injection of 1 or 2 μl of tritiated L-proline [1.3 μc/μl in H_2O; New England Nuclear (6.8 c/m*M*) or Schwarz/Mann (3 c/m*M*)] 24 hours prior to killing. These specimens were then fixed in Carnoy's fluid, serially sectioned at 10-μm thickness, and examined by light microscope autoradiography.

The translocated primordial eye develops into an externally normal eye (Fig. 1A). Light microscope examination of the retinas in these transplanted eyes revealed cellular layering and cell densities that appear similar to those in the retinas of the normal (nontransplanted) eyes. Thus, translocation of the eye to a different region on the animal's head does not qualitatively alter the normal development of the organ. Furthermore, the retinas of these translocated eyes are capable of visual function. In two preparations, we have inserted gold- and platinum-coated indium micropipettes (tip diameter $\simeq$ 6 μm) into the transplanted optic nerve as it enters the cranial cavity. We recorded action potential activity in these nerves in response to small spots of light in the visual field of the transplanted eye.

Figure 1B demonstrates the altered geometry of the brain of a typical postmetamorphic animal that had a type 2 transplant operation. The optic nerve from the normal (left) eye approaches the brain ventrally. Most of the fibers in the normal tract cross the midline in the anterior region of the diencephalon and then proceed in a dorsocaudal direction along its lateral margin to enter the right optic tectum (*4*). The optic nerve from the translocated eye, however, does not enter the diencephalon; instead, it penetrates the medulla at approximately the level normally occupied by the eighth cranial nerve. The left tec-

tum, to which the transplanted optic nerve would normally have projected, exhibits a hypoplasia characteristic of tecta that have been deprived of a retinal projection during development (*5, 6*). A typical result from our autoradiographic analyses of the central projection of the optic tract from a translocated eye is presented in Fig. 2, top. The animal was killed at metamorphic climax [Taylor and Kollros (T & K) stage 20] (*7*). The apparent enlargement of the left side of the medulla is due to the addition of forebrain tissue in the transplant operation. While the optic tract appears to send small branches into this forebrain tissue, the major trunk penetrates the hindbrain and then travels in a dorsocaudal direction along the lateral border of the medulla to enter the dorsolateral white matter of the spinal cord (Fig. 1C). The tract maintains this relative position down to the most caudal extent of the cord.

By light microscopy and autoradiographic tracing techniques, we followed the central projection of the optic tract from translocated eyes in six type 1 and seven type 2 animals ranging in age from T & K stage 4 to 6 months after metamorphosis. In all of these animals, the optic tract from the translocated eye followed a remarkably similar projection. Upon penetrating the hindbrain the tract invariably turned in a caudal direction within the ipsilateral medulla, then traveled along its lateral border and entered the spinal cord dorsally. In all cases the tract was located in the same dorsolateral position within the spinal cord. This consistency is all the more remarkable since the point of entry of the transplanted optic nerve into the neural axis and its initial orientation varied considerably from animal to animal due to differences in the way the embryonic graft healed and the amount of forebrain tissue included with it. Figure 2, bottom, demonstrates an extreme form of this variation. In this animal the forebrain tissue fused with the midbrain tegmentum and hindbrain along its caudal extent but separated from the brain rostrally. The pathway of the transplanted optic tract within this forebrain tissue is markedly different from that of Fig. 2, top. Upon entering this tissue, the optic nerve fibers grew initially in a rostral direction. But at the point where the forebrain tissue began to separate from the brainstem, the optic tract underwent a complete turn. Once the fibers entered the normal neural axis, despite their close proximity to the lateral edge of the tectum, they projected caudally down the hindbrain and spinal cord to follow a pathway identical to that in Fig. 2, top.

There are no direct retinal projections beyond the midbrain from a normally located eye (*4*). We confirmed this conclusion by injecting tritiated proline into an eye of a normal postmetamorphic animal. Subsequent autoradiographic examination failed to reveal any evidence of labeled fibers in the medulla or spinal cord. We also explored the possibility that the optic tracts from the translocated eyes might be following some higher-order descending visual pathway normally present in the medulla and spinal cord. But the nerve fibers from the transplanted eye do not project along any of the visual efferent tracts from the optic tecta to the spinal cord. Two independent studies have shown that these efferent tracts run in four bilaterally symmetric bundles in the ventral half of the cord (*8*). Furthermore, there are no known fiber tracts normally found in the medulla and dorsal white matter of the spinal cord which are coextensive with the path of the translocated optic tract (*9*). We thus conclude that the optic nerve fibers from our transplanted organs do not passively follow some preexisting pathway.

During embryonic development, the optic nerve normally enters the ventral surface of the brain in the anterior region of the diencephalon. It then grows dorsocaudally along the side of the diencephalon and enters the anterior pole of the embryonic optic tectum from a dorsolateral position. The optic nerve fibers from the transplanted eyes in our study duplicate this pattern of growth. Their projection is dorsocaudal along the side of the medulla. However, since they do not encounter an optic tectum posterior to their point of entry into the central nervous system, they continue in this same dorsolateral coordinate along the entire length of the spinal cord. These comparisons indicate that the optic tract must possess a considerable degree of intrinsic information with regard to its proper cross-sectional coordinate in the embryonic brain. The central nervous system cooperates in providing the additional cue of directionality of the neural axis (*10*).

This explanation of directed growth requires the existence of a gradient system consisting of a medial-lateral x-axis, a dorsal-ventral y-axis, and a longitudinal z-axis throughout the entire central nervous system. It also requires that optic nerve fibers, during embryonic development, are sensitive to this gradient system. They can find their absolute cross-sectional position in the transverse x-y plane. The longitudinal z-axis provides directional information but the optic fibers cannot detect their absolute position along it.

Sharma (*11*) reported that the optic tract from a supernumerary eye, which had been transplanted during Shumway stage 17 to 19 to the roof of the midbrain, pene-

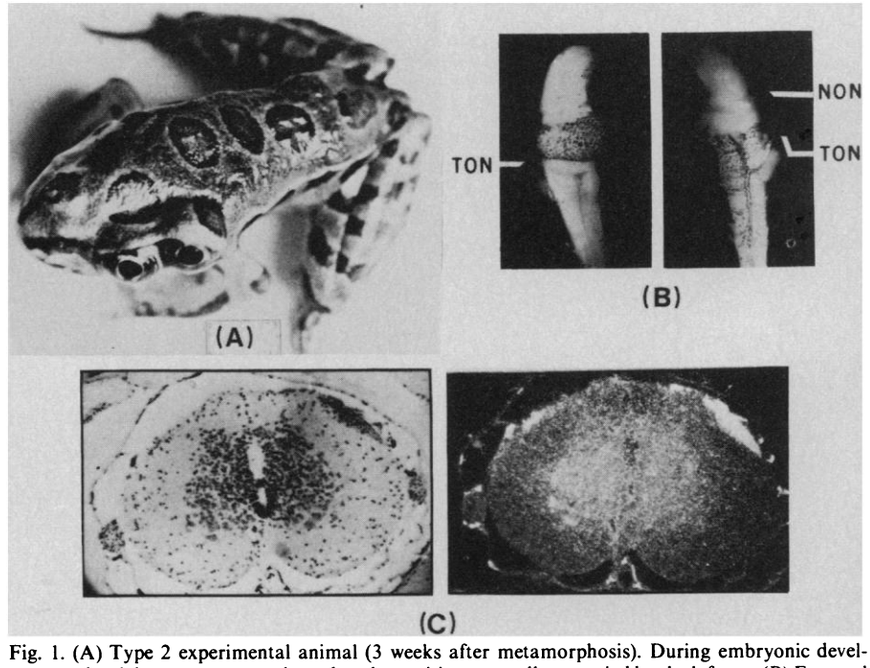

Fig. 1. (A) Type 2 experimental animal (3 weeks after metamorphosis). During embryonic development the right eye was transplanted to the position normally occupied by the left ear. (B) External morphology of the brain of a type 2 postmetamorphic frog. The dorsal view (left) demonstrates hypoplasia of the left tectum to which the transplanted optic nerve (*TON*) would normally have projected. The ventral view (right) shows the penetration of the normal optic nerve (*NON*) and the transplanted optic nerve (*TON*). The swelling adjacent to the medulla at the point of entry of the transplanted nerve is due to forebrain tissue included in the original eye graft. The olfactory bulbs have been removed. (C) Light- and dark-field autoradiographs of a representative cross section (anterior view) of the spinal cord of a type 1 experimental animal (T & K stage 20). The labeled optic tract from the translocated eye can be seen in the dorsolateral white matter on the left side of the animal's spinal cord (right side of the photographs). The section was stained through the emulsion with Harris' hematoxylin. Similar sections of the cord in type 2 animals reveal the optic tract in an identical position.

trates the dorsal diencephalon. The tract then grows caudally and eventually establishes a systematic retinal projection onto one of the optic tecta. These results are consistent with our hypothesis, namely that optic nerve fibers entering the embryonic brain at any point anterior to the midbrain should be able to establish successful connection with the optic tecta.

We emphasize that our transplantation studies have been conducted with early embryonic preparations. The results may not involve the same guidance mechanisms responsible for directional regeneration of optic fibers in more mature animals in which central pathways have already been established. In *Xenopus laevis* tadpoles (stage 47 to 48), Hibbard (*12*) cut the optic nerves from normal eyes and deflected them into the central stumps of severed oculomotor nerves. The deflected optic nerve fibers regenerated along the route of the oculomotor nerve into the central nervous system and immediately crossed the midline, and some proceeded in a rostral direction (*13*). Upon reaching the level of the normal optic chiasma, they crossed the midline once again and regenerated into the ipsilateral optic tectum. Hibbard's observation of rostral regeneration appears to conflict with our guidance hypothesis of directed growth. This may be due to a species difference. But perhaps it is due to a difference in directional guidance of sensory nerve growth during embryonic development compared to regeneration factors within an established central nervous system where preexistent tracts may play a role in guiding regrowing fibers. It is not known to what extent guided growth in embryonic development involves the same guidance factors as in neural regeneration. This is an important distinction and worthy of further study.

Our hypothesis for directed growth of optic nerve fibers does not account for the midline crossing that normally occurs at the optic chiasma. None of the optic tracts from transplanted eyes in our study project to the opposite side of the medulla and spinal cord. Thus the cues that direct the normal midline crossing do not seem to be intrinsic to the optic nerve fibers themselves. Instead, this decussation may be due to local factors within the central nervous system in the region of the optic chiasma.

We have demonstrated that axonal growth is orderly and consistently directed following a severe mismatch of receptor organ and central nervous tissue. We have not determined whether the nerve fibers from a translocated organ can form synaptic connections with cells in a foreign part of the central nervous system. Such studies could help clarify whether the guidance mechanisms responsible for axonal growth play a role in the formation of synaptic connections.

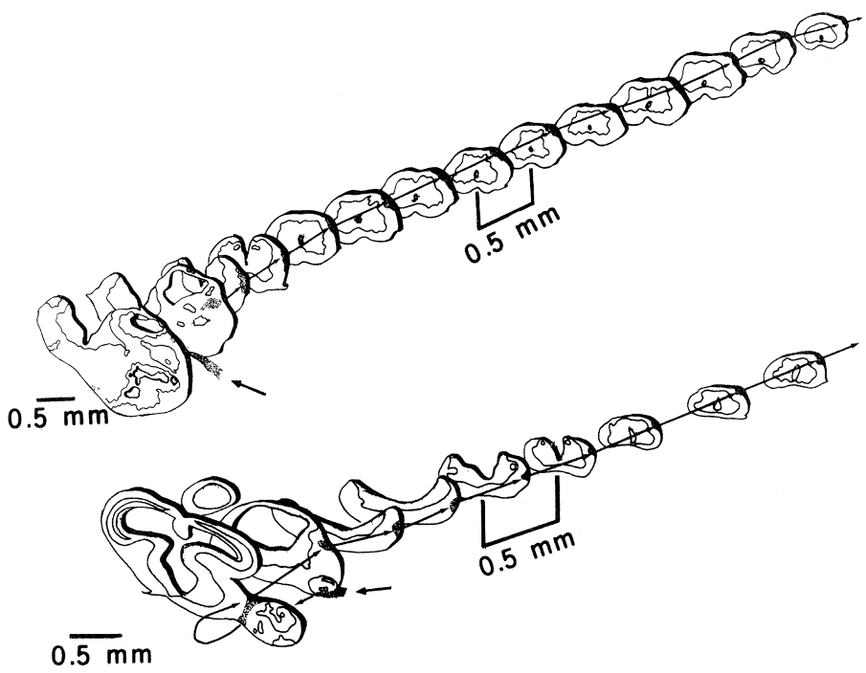

Fig. 2. Serial reconstructions of the central projection of the optic tract from the transplanted left eye in two different type 1 animals. The projections were traced by intraocular injection of [^{3}H]proline. Areas of high grain density are stippled; small arrows mark the direction of growth. The large arrows indicate the point of penetration of the optic nerve from the transplanted eye. (Top) The medulla and spinal cord of an animal killed at metamorphic climax (T & K stage 20) are shown. The enlargement on the left side of the medulla is due to forebrain tissue incorporated in the embryonic eye graft. The optic tract projects through this tissue and along the entire length of the medulla and spinal cord. (Bottom) The posterior half of the midbrain, cerebellum, medulla, and anterior portion of the spinal cord of a tadpole (T & K stage 4) are shown. This animal illustrates an extreme form of the path of an optic tract within transplanted forebrain tissue. This tissue forms a protrusion on the left side of the medulla at the point of penetration of the optic nerve. In the most anterior section, the forebrain tissue begins to diverge from the midbrain and the optic tract makes a 180° turn. Upon entering the hindbrain, the tract grows in a caudal direction down the spinal cord.

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[illegible] E. Hibbard [*Exp. Neurol.* **13**, 289 (1965)] has transplanted a supernumerary ear behind a normal ear in embryonic *Rana pipiens*. He found that the nerve fibers from the supernumerary ear apparently terminated on the same vestibular nuclei in the hindbrain as the normal eighth nerve fibers. The optic nerve fibers from our translocated eyes projected past these eighth nerve nuclei. This difference in the pattern of growth of transplanted eighth nerve fibers and transplanted optic nerve fibers within the same part of the central nervous system supports our contention that the caudal projection of the transplanted optic tract results from factors inherent within the optic nerve fibers themselves and not from some simple mechanical deflection toward the tail of the embryo.
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14. Supported by NSF grant GB-18336 and NIH grant NS09244. We thank T. Eisner and J. A. Paton for their photographic assistance, and K. Arms for use of darkroom facilities.

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