X. NEUROPHYSIOLOGY

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A. AUTORADIOGRAPHIC STUDY OF CHANGES IN THE FROG TECTUM AFTER CUTTING THE OPTIC NERVE

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In adult amphibia, cut optic nerves will grow back to reform precise retino-topic maps onto the tectum.[1-3] Most theories of mechanisms for the remapping have focused on the optic fibers playing the active role in reorganizing. The brain has generally been assumed to remain a passive tissue onto which the optic fibers go about the reconnecting process. By default, we had shared this view and we were about to commence on a set of frog tectum manipulations and reversals to test some hypotheses about the manner of regrowth of optic fibers. But we were aware that our forthcoming experiments were predicated on the assumption that the brain was well-behaved, i. e., it would not reorganize itself in the course of the experiments. There was one notable report by Gaze and Watson[4] describing changes in the tectum of the newt when the optic nerve was cut. At a variety of intervals post-operatively, they had injected animals with ^{3}H-thymidine, and the brains were subsequently processed using autoradiography. They found labeled cells in the tectal neuropil and in the ependymal cell layer. Salamanders, even compared with frogs (let alone higher vertebrates), are, of course, exemplary for their regenerative ability. But as a preliminary control, we thought we would repeat and, if necessary, extend the experiments of Gaze and Watson in the frog.

Four frogs (Rana pipiens), from 2 in. to 2 1/2 in. long and weighing 20-30 grams, were anesthetized with Finquel (Ayerst). By cutting the appropriate extraocular muscles of the left eye, the optic nerve was exposed and cut. The animals were subsequently kept in a constant-temperature room (20-21°C) and injected subcutaneously in the foreleg with 20 μC ^{3}H-thymidine (New England Nuclear Corporation) after 8, 11, 15, or 20 days. The day after injection the brains were removed after decapitation and fixed overnight in 4% buffered neutral formalin. The brains were then dehydrated through a set of graded alcohols and cedarwood oil, embedded in paraffin, serially sectioned at 7 μm and mounted on albumenized slides. The sections were deparaffinized in

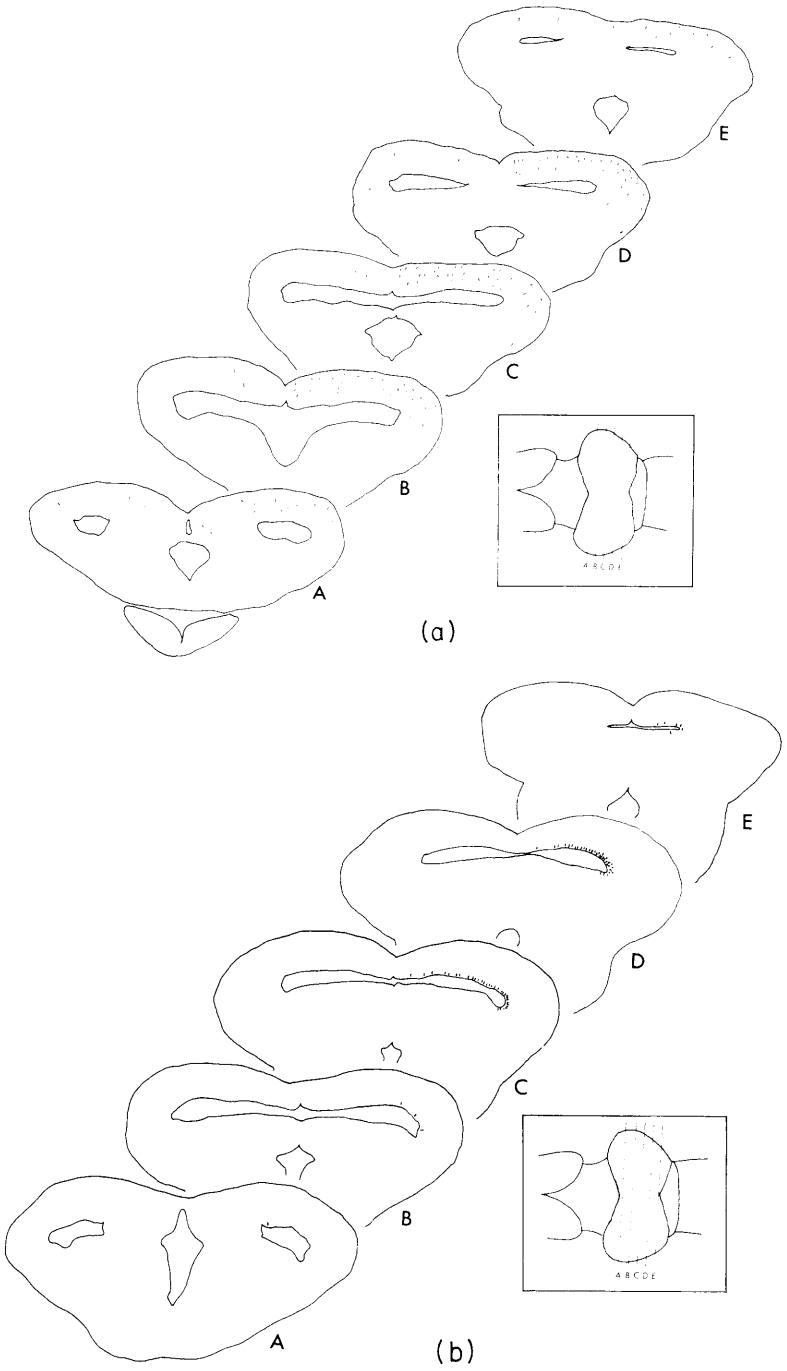

Fig. X-1. Camera lucida transverse sections of the midbrain of a frog showing the distribution of labeled cells (oblong marks). Inset: locations of the sections.

(a) After injecting 20 μC ^{3}H-thymidine 8 days after left optic nerve section. Brain excised on the following day. Each mark corresponds to one labeled cell.

(b) After injecting 20 μC ^{3}H-thymidine 14 days after left optic nerve section near parasphenoid bond. Brain excised on the following day. Each mark corresponds to 3 labeled cells.

xylene, then hydrated. In total darkness the slides were dipped in Kodak NTB-2 nuclear-track emulsion diluted 1:1 with water warmed to 40°C, dried for 1 hour, and placed in light-tight slide boxes in a refrigerator (4°C) for 1 week. The slides were developed in total darkness for 2 min in full strength D-19 (Kodak), rinsed briefly in acid stop, and fixed (Kodak fixer) for 6 min. The slides were washed in running water for 20 min, stained for 10 min in 0.1% cresyl violet, dehydrated, and mounted with coverslips.

We found that after 8 and 11 days, labeled cells were distributed homogeneously in optic fiber layers of the right (contralateral) tectum. The left tectal lobe had only a few labeled cells, but they were distributed in the retinal fiber layers. Eight and 11 days after cutting the optic nerve significant degeneration is seen in the tectal retinal fiber layers (Fig. X-1a). It suggests that the labeled cells could be newly formed macrophages concerned with clearing away the debris of optic fiber degeneration.

After 15 days, labeled cells are not seen in retinal fiber layers of the contralateral tectum, but the ependymal cell layer is replete with them (Fig. X-1b). The labeled cells are not distributed uniformly in the ependymal layer but are found for the most part in the postero-lateral area. No labeled cells are seen in the ipsilateral tectum. In development, the cells of this layer generate all the cells of the tectum, which then migrate out to their final locations. No labeled cells were found after 20 days.

The distribution and times of formation of the labeled ependymal cells were studied further. In a set of animals, the optic nerve was cut and, using the same procedure, we injected with 20 μC ^{3}H-thymidine after 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 days. Only at 15 and 16 days did we find labeled ependymal cells. The distribution was identical with that described.

In the next set of animals, a slit was made in the roof of the mouth, and a small length of the optic nerve was cut just outside its entry into the parasphenoid bone. Animals were injected with 20 μC ^{3}H-thymidine after 4, 6, 8, 10, 12, 14, 15, 16, 17, or 18 days. The eyes and brain were removed the next day. Since the nerve was cut closer to the brain, ependymal cells were labeled earlier, but only on days 13 and 14 (Fig. X-2). The distribution was the same as described above. Except for the ciliary margin, few labeled cells were seen in the retina at any time, but many labeled cells were found in the optic disc on days 12, 13, and 14 (Fig. X-3a, 3b).

We next looked at the long-term fate of the labeled ependymal cells: Would they migrate out to neural layers, and would that depend upon the reacquisition of vision of the eye whose optic nerve had been cut? Each animal was injected with 20 μC ^{3}H-thymidine 13, 13 1/2, and 14 days after the left optic nerve was cut. They were then transferred to a warm, sunny room (average temperature approximately 23-24°C) and fed 6-10 crickets a week. Animals were tested behaviorally for vision by a sweeping motion with the hand from the left or right side. After the optic nerve was cut, the

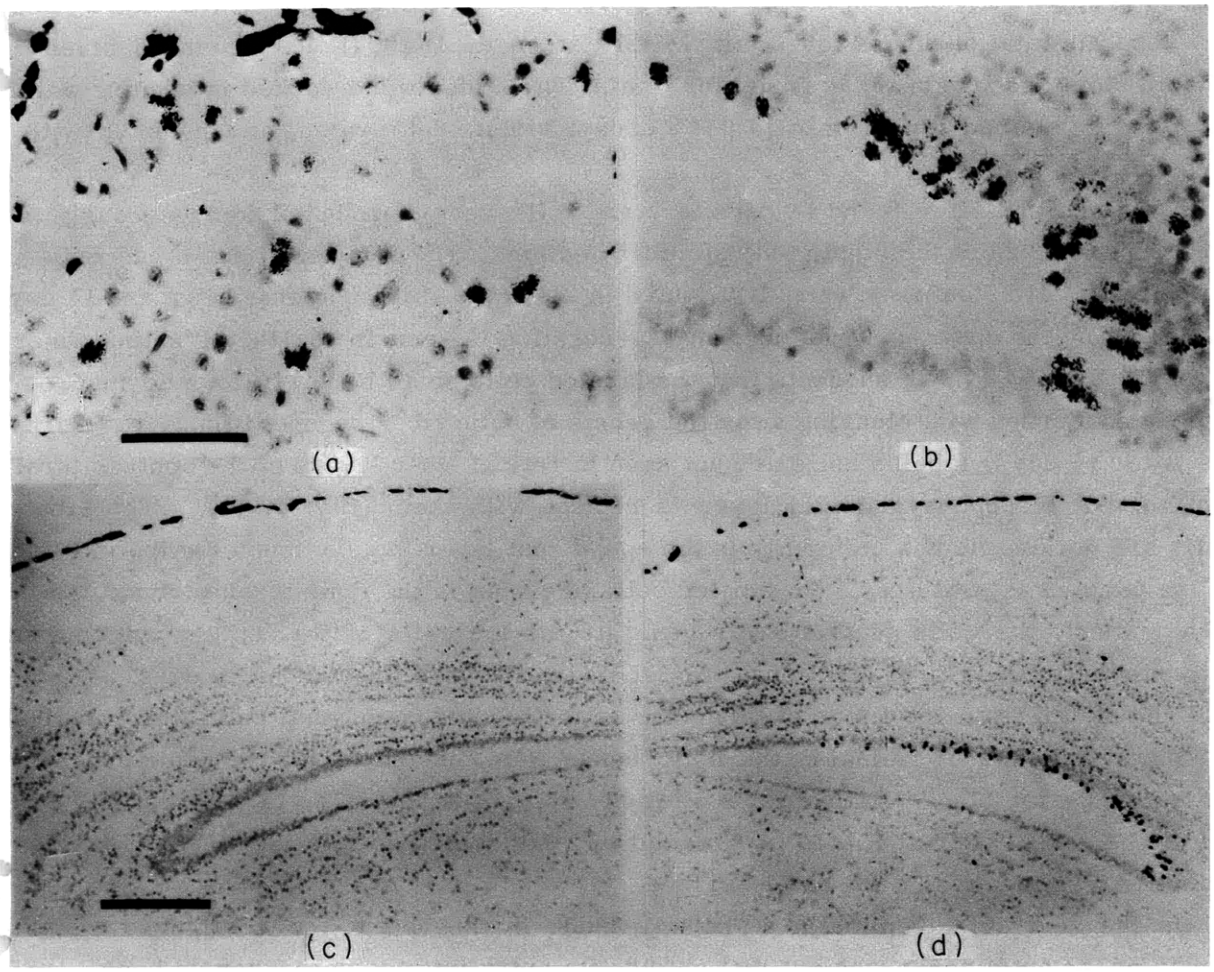

Fig. X-2. (a) Superficial tectum showing distribution of labeled cells. ^{3}H-thymidine injected 8 days after optic nerve section. Brain excised on the following day. Scale: 50 μm magnification.

(b) Periventricular area showing distribution of labeled cells. Thymidine injected 14 days after left optic nerve section. Brain excised on the following day. Scale: same as in (a).

(c) and (d) Refer to (D) in Fig. X-1b. Same section as in (a) showing ipsilateral (c) and contralateral (d) tectum. Black mark in the middle of the right area of the tectum (d) is a pigment granule. Scale: 200 μm.

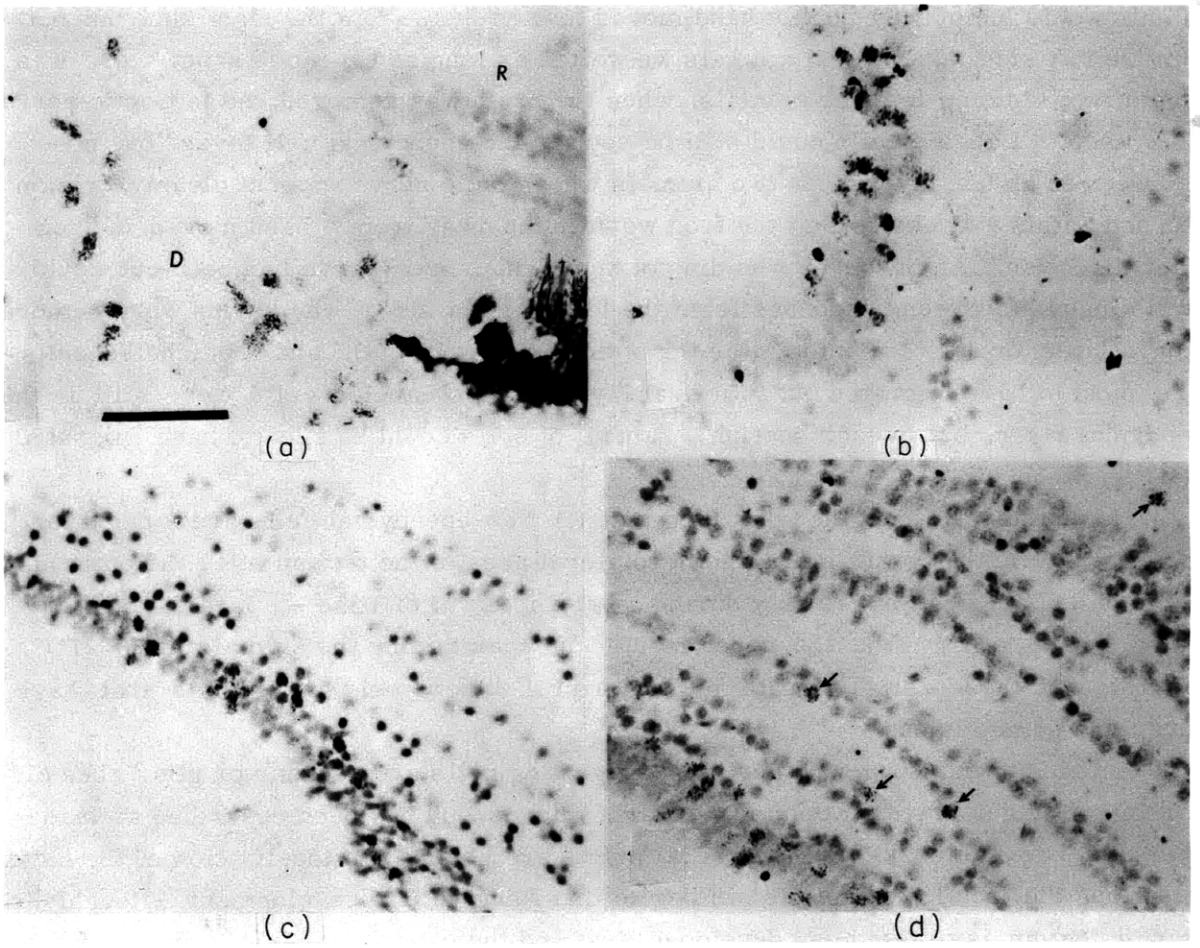

Fig. X-3. (a) Section of eye through the optic disc (D) and adjacent retina (R) showing accumulation of labeled cells in the disc. ^{3}H-thymidine injected 14 days after optic nerve section. Eye and brain excised on the following day. Scale: 50 μm.

(b) Pretectal area of ventricle showing some labeled cells. Section from same brain as in (a). Dorsal is down, ventral is up. Scale: same as in (a).

(c) and (d) Comparable sections of two animals injected with ^{3}H-thymidine 13, 13 1/2, and 14 days after optic nerve cut in which the animals were maintained for 3 additional months. At the time of brain excision, animal (c) did not recover vision in the blind eye, no optic nerve was seen, and labeled cells were observed only in ependymal layer. Animal (d) had recovered vision 2 1/2 weeks earlier, and some labeled cells have migrated out (arrows). Scale: 50 μm.

animals would jump away from a hand moved toward them from the right side but not from the left side. In several animals we waited as long as six months but there was no return of vision. In these animals, when the brain was removed, no left optic nerve was present. Labeled cells could still be seen in the ependymal cell layer, but none were seen in higher layers. In two animals whose optic nerve was cut cleanly, vision returned within 9 weeks so that the frog would jump away from a hand moved in from either direction. An observer who did not know which optic nerve had been cut could not distinguish between responses from the left or right side. The brains were removed 17 days later (or 3 1/2 months after the optic nerve was cut), and the labeled cells were found to have migrated out to neural layers. Most labeled cells were still in the ependymal layer, but in each section a handful of cells could be seen to have migrated out (Fig. X-3c, 3d).

We have thus shown in the adult frog that (i) New ependymal cells are formed for the most part in the contralateral-posterolateral area of the tectum after the optic nerve is cut. (ii) They are formed only during a brief interval of time – 13-14 days after cutting the optic nerve via the upper mouth and maintaining the animals at 20-21°C. (iii) Some of these cells migrate out to neural layers only in animals that have reacquired some vision.

It remains to be shown whether these migrating cells are neurons or glia. The criteria for distinguishing between them in light microscopy has been described as inadequate by several authors.[5,6] But several points are clear. The localization of the labeled cells along the tectal ventricle is similar to that found in late development.[7] Roughly, the anterior tectum is created and developed first and the caudal part last.[8] In the absence of optic fiber input, it appears that the tectal lobe has regressed to a late embryonic state. This would suggest that at least some of the migrated cells are neurons. But the answer to this will await future results of experiments in progress.

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B. PRESENCE OF MONOAMINES IN THE SALAMANDER TECTUM AND THEIR RELATION TO THE SPINAL LEMNISCUS

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The salamander tectum is a cylindrical structure monopolizing the dorsal mesencephalon. There is a ventricle in the center (the "aqueduct"), several rows of cells periventricularly, and an outer neuropil free of cell bodies but composed of a dense network of dendrites from the periventricular cells and incoming axons from a variety of

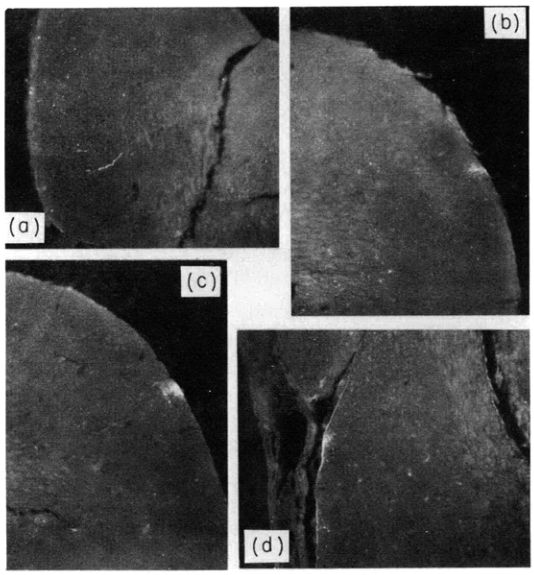

Fig. X-4. (a) Section through thoracic spinal cord showing scattered fluorescence.
(b) Section near rostral border of spinal cord where the discrete lateral fluorescent tract is first encountered.
(c) Same tract as (b) in vagal region.
(d) Same tract as (b) in rostral medulla. Upper right: nerve V.

sources. The outer half of the neuropil is replete with optic nerve fibers forming a retino-topic projection. Using histochemical techniques, we have shown previously that these fibers contain acetylcholinesterase. The boundary of the enzyme is sharp, and the inner half of the neuropil shows no acetylcholinesterase activity. This inner area contains numerous somesthetic fibers and they are organized to form a coarse but recognizable map of the contralateral body that is in register with the retino-tectal map. It was of interest to see if there are potential neurotransmitters that could be correlated with the somesthetic input. We knew that SSADH, the final enzyme in the degradative pathway of GABA, was not localized to this region, so another set of possibllities were the monoamines. We used the standard histo-fluorescent method to show the distribution of monoamines in the brain of the tiger salamander.

Brains were removed rapidly from the salamanders after decapitation and frozen quickly in 2-methylbutane cooled by liquid nitrogen. The brains were transferred to a freeze drier kept at -40°C and dried until the pressure was reduced to 0.01 Torr (approximately 1-3 days). The brains were then fixed at 80°C for 1 hour over paraformaldehyde that had in turn been dried overnight over concentrated sulfuric acid. The fixed brains were vacuum-embedded in paraffin in 2 minutes. Transverse sections (10 μm) were cut just prior to viewing in a dark-field fluorescence microscope. Fluorescent tissue stood out as bright green-yellow on a dark blue-green background. Different monoamines fluoresce at different wavelengths, but without a microspectrophotometer subjective judgments about the wavelengths are erratic. Therefore, for purposes of this description, no distinction between monoamines is made. Since the half-life of the fluorescence under the ultraviolet illumination used in our microscope was less than 1 min, sections were scanned rapidly and photographed immediately on Type 47 Polaroid film (3000 speed). Exposure times were 20-40 seconds.

In the upper spinal cord, fluorescence is scattered through most of the neuropil. Only at the border of the medulla is a well-defined tract seen on the lateral wall. This tract can be followed through the whole length of the medulla to the level of the cerebellum (Fig. X-4). The tract splays out through the caudo-dorsal tegmentum into the inner neuropil of the tectum. The whole course of the tract is coincident with Herrick's description of the spinal lemniscus[2] (this was also checked by using degeneration staining). The fluorescence does not extend into the outer neuropil, and in favorable sections the optic fiber area is a dark wedge silhouetted by surrounding fluorescence (Fig. X-5). With notable exceptions, very few cell bodies fluoresce. Some scattered fluorescing cells are seen in the spinal cord. The medial cells of the nucleus interpeduncularis are moderately fluorescent. The most vividly fluorescent nucleus is located in the region between the dorsal and ventral hypothalamus. In the anterior preoptic nucleus some cells lining the preoptic recess also show vivid fluorescence (Figs. X-6 and X-7).

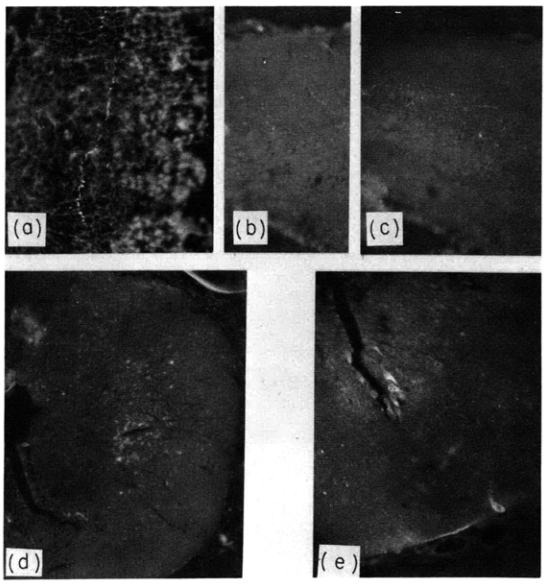

Fig. X-5. (a), (b), and (c) Section of tectum showing fluorescent fibers in inner neuropil with no fluorescence in outer neuropil.
(d) Fluorescence in dorsal tegmentum extending up to tectum.
(e) Cellular fluorescence in nucleus interpeduncularis.

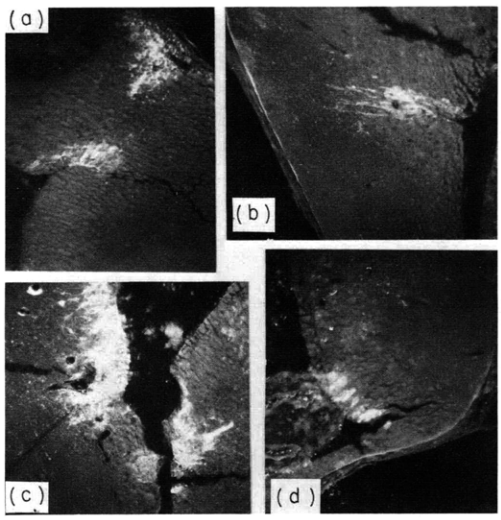

Fig. X-6. (a) and (b) Fluorescence in a lamina of the hypothalamus.
(c) Fluorescence in a ventral thalamic nucleus.
(d) Fluorescence in cells of the anterior part of the preoptic nucleus.

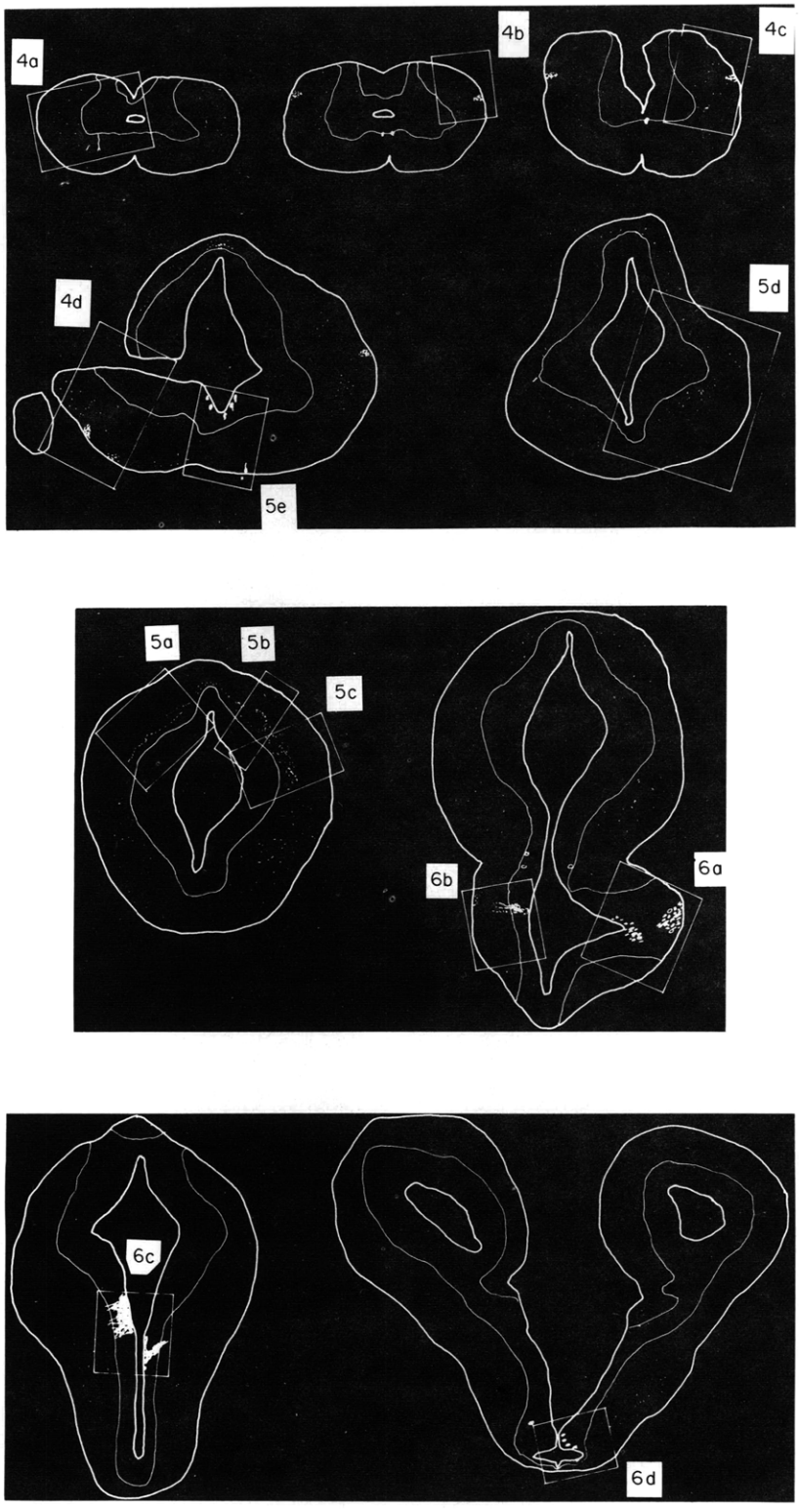

Fig. X-7. Sketches of transverse spinal cord and brain sections with numbered boxes showing approximate locations of the photomicrographs in Figs. X-4 through X-6.

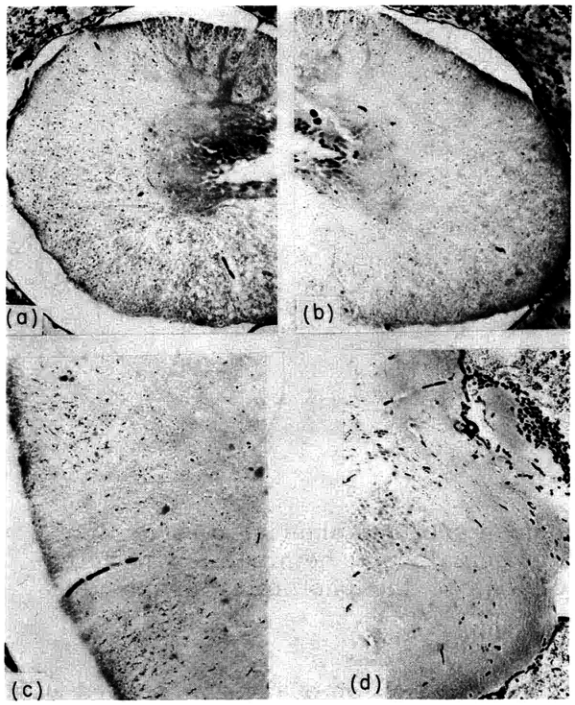

Fig. X-8.

(a) Rostral spinal cord showing degeneration ipsilateral to more caudal hemisection. 10-day degeneration.
(b) Same as (a) showing contralateral area.
(c) Two branches of spinal lemniscus. Lower center branch referred to by Herrick[2] as bulbar lemniscus. Same animal as (a).
(d) Ipsilateral to cord hemisection of another animal showing degeneration in dorsal tegmentum and extending into tectum. 12-day degeneration.

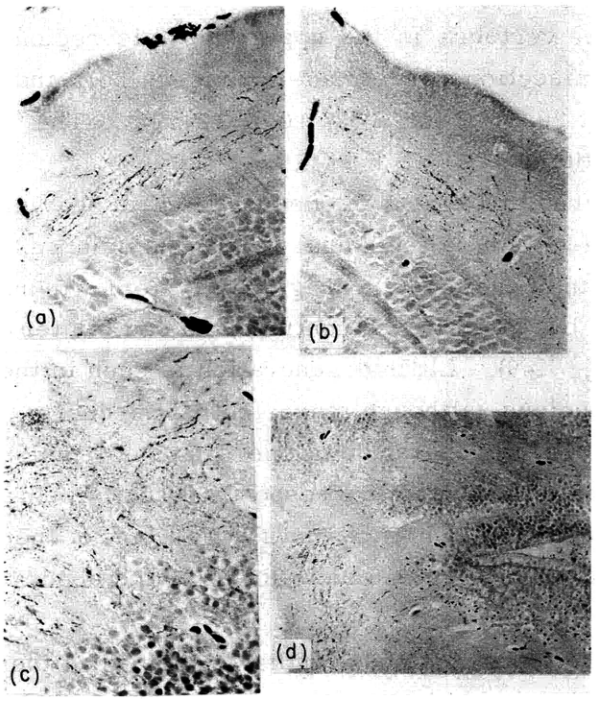

Fig. X-9.

Tectum caudal to oculomotor nucleus showing degeneration restricted to inner neuropil. (a) Ipsilateral to cord hemisection and (b) contralateral. 10-day degeneration. (c) and (d) Sections in ipsilateral cerebellar areas and rostral medulla showing pattern of 10-day degeneration.

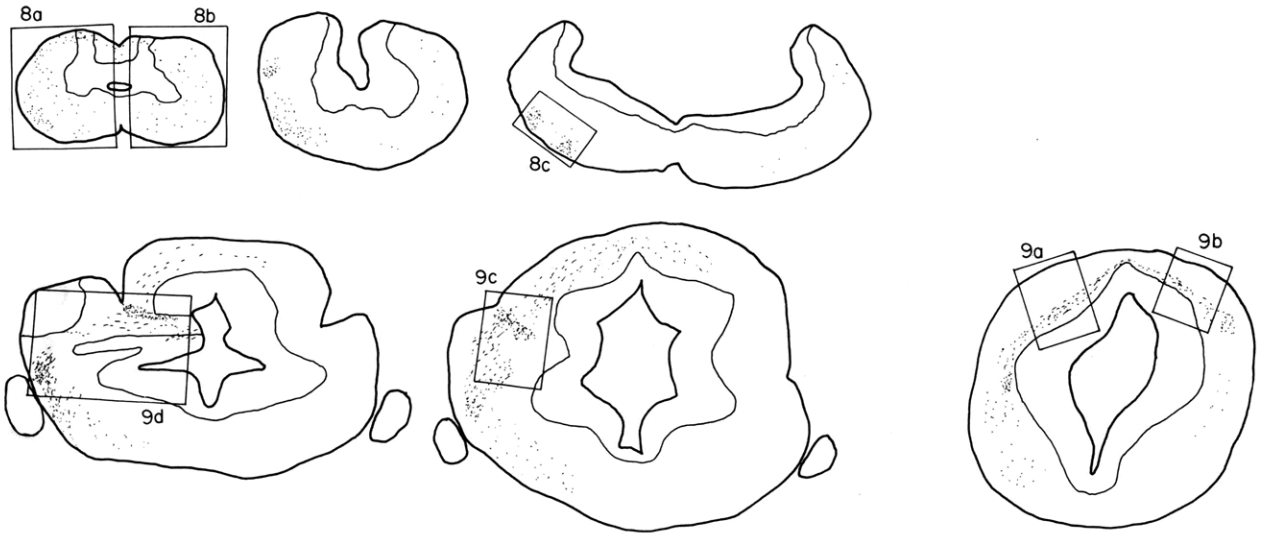

Fig. X-10. Camera lucida drawings of transverse sections showing distribution of 10-day degeneration. Thoracic spinal cord hemisection of left side. Numbered boxes show locations of the photomicrographs in Figs. X-8 and X-9.

To check for the course of the spinal lemniscus, hemisection of the spinal cord was made in several salamanders (all approximately 4 in. snout to vent). Then by a modification of the Fink-Heimer stain for degenerating nerve fibers, the lemniscal pathway was traced. Under Finquel (Ayerst) anesthetic, one vertebra in the upper thoracic region was removed in exposing the spinal cord. Hemisections were made with a scalpel, and the animals were maintained postoperatively at 20-21°C for 6, 8, 10 or 12 days. The tissue was then processed for degeneration staining.

Degeneration rostral to the lesion is scattered throughout the neuropil. It is heavier ipsilaterally than contralaterally. By the caudal medulla the spinal lemniscus emerges as a distinct tract and can be followed the length of the medulla (Fig. X-8). Some fibers terminate in a narrow band in the cerebellum, but many fibers continue and end bilaterally in the inner half of the tectal neuropil (Fig. X-9). Little degeneration is seen in the tectum rostral to the level of the oculomotor nucleus, although more ventral degeneration can be traced up to the ventral thalamus. The degeneration of one branch of the spinal lemniscus through its whole length and into the tectum corresponds directly with the fluorescent tract seen above. A second more medial branch (not fluorescent) of the lemniscus, which Herrick[2] assumed was of bulbar origin, can be followed to the upper medulla (Fig. X-10).

The fluorescence in the inner neuropil of the tectum extends farther rostral than the lemniscal degeneration. From electrical recordings it is known that this area has somesthetic input from the head region. Thus contributions to the rostral tectal fluorescence must be made from cranial sources.

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