Map Formation in the Developing *Xenopus* Retinotectal System: An Examination of Ganglion Cell Terminal Arborizations¹

D. S. SAKAGUCHI² AND R. K. MURPHEY

Neurobiology Research Center, Department of Biological Sciences, State University of New York at Albany, Albany, New York 12222

Abstract

Single axonal arbors of retinal ganglion cells have been stained by injecting cobalt extracellularly into the retinae of *Xenopus* embryos and tadpoles. The axonal endings of the earliest retinal axons to arrive in the midbrain were usually simple in appearance, often ended in growth cones, and terminated in tectal regions appropriate to their location in the eye. Thus, a topographic projection exists very early in the development (stages 37 to 39) of the projection, before the elaboration of complex axonal arbors.

Retinal axons began acquiring more mature features, exemplified by the elaboration of terminal arbors, by stage 39. The arbors of most ganglion cells were elongated in the rostral-to-caudal dimension during early larval life (stages 40 to 45) and covered a large portion of tectal neuropil. During mid-larval stages (stages 46 to 50), arbors covered a relatively smaller proportion of the tectal neuropil. A quantitative analysis of this change suggests that the apparent decrease in size of the arbors, with respect to the tectum, is due to rapid growth of tectal neuropil and not due to retraction of an initially diffuse arbor. Thus, the refinement in targeting of axonal arbors during development is a phenomenon distinct from that seen during regeneration.

A striking feature of the nervous system is the high degree of precision with which its components are interconnected. This precision in connectivity is exemplified in sensory systems which preserve the topography of incoming stimuli by forming "maps" within the central nervous system. Various sensory systems have been studied in attempts to elucidate the nature of the mechanisms controlling the formation of orderly nerve patterns. Among them, none has been more aggressively exploited than the projection from the retina to the midbrain optic tectum of lower vertebrates (referred to as the retinotectal system; for reviews, see Gaze, 1970; Horder and Martin, 1978; Jacobson, 1978; Conway et al., 1980).

In the retinotectal system, the axons of retinal ganglion cells grow

to the contralateral optic tectum in an orderly manner, thereby creating a topographic map of the visual world (Gaze, 1958). Many studies have examined the regeneration of the adult retinotectal projection in an attempt to understand how such "maps" are formed (Sperry, 1943; Gaze, 1958; Gaze and Jacobson, 1963; Fujisawa et al., 1982). These studies have demonstrated that the orderly retinotectal map is re-established, after optic nerve section, in a stepwise fashion; regenerating ganglion cells first project diffusely across the tectum, and this map is subsequently refined by the elimination of aberrant terminals (Fujisawa et al., 1982; Schmidt et al., 1984). A sequence of similar events was suggested for the initial development of the *Xenopus* visual system by Gaze et al. (1974). However, recent experiments in *Xenopus* embryos have shown that the initial projection is topographically ordered (Holt and Harris, 1983; Holt, 1984). These autoradiographic (Holt, 1984) and electrophysiological (Holt and Harris, 1983) studies examined populations of cell terminals and, therefore, provided little information as to the ordering of individual terminal arbors at the cellular level.

In the present communication, injection of cobalt extracellularly into the ganglion cell layer of the *Xenopus* retina revealed the anatomy of individual axonal arborizations, thus permitting the examination of immature axonal arbors in the tectum. Utilizing this technique, we describe axonal arbors of single retinal ganglion cells throughout embryonic and larval development.

Materials and Methods

Wild-type *Xenopus laevis* embryos were obtained by injection of sexually mature adults with chorionic gonadotrophin (Sigma Chemical Co.). The embryos were maintained in dechlorinated tap water under a 12-hr light/12-hr dark cycle at 22°C. Animals were anesthetized by immersion in Steinberg's solution containing MS-222 (Sandoz) (1:10,000), and the normal table of Nieuwkoop and Faber (1967) was used as a reference for staging. Embryos and tadpoles with external maturational features between those designated by the normal table (Nieuwkoop and Faber, 1967) were assigned intermediate stages of development. Animals between stages 34 and 50 were used in these experiments.

Techniques for staining the retinal projection

Staining the whole projection. Anterograde labeling of the retinal projection was achieved by dissecting the whole brains with the optic nerves and eyes attached from anesthetized tadpoles. The brains were pinned rostrally by the olfactory nerves and caudally at the spinal cord onto the bottom of a Sylgard (Dow Chemicals)-coated Petri dish with stainless steel minuten pins. The fluid bathing the eye was isolated from that surrounding the brain by building a cup of petroleum jelly ejected from a syringe around the eye. The saline-filled pool containing the eye was then replaced with a bathing solution of aqueous hexaminocobaltic chloride (0.2 M, supplied by Pfaltz and Bauer; Brogan and Pitman, 1981). Fine forceps were then used to crush the eyeball to permit the anterograde staining of the damaged optic fibers. The cobalt-filled pool containing the eye was then sealed with additional petroleum jelly. Following a diffusion period (6 to 10 hr at 4°C) the brains were processed

Received February 14, 1985; Revised May 31, 1985;

Accepted June 4, 1985

¹ We wish to thank Drs. W. A. Harris, H. V. B. Hirsch, C. E. Holt, J. T. Schmidt, S. B. Tieman, S. Udin, and W. W. Walthall for valuable discussions and critique of the manuscript, D. Possidente and L. Eisele for excellent technical assistance, and R. Speck and L. Welch for help with preparation of the manuscript. This work was supported by National Science Foundation Grant BNS 8317929.

² To whom correspondence should be sent, at his present address: Department of Biology B-022, University of California, San Diego, La Jolla, CA 92093.

as whole mounts and the cobalt was intensified according to a modified Timm's intensification procedure (Bacon and Altman, 1977; Murphey et al., 1983). A similar staining method was also used to retrogradely label retinal ganglion cells in the eye by bathing the brains in the pool of staining solution (Sakaguchi et al., 1982). Specimens were dehydrated, cleared in methyl salicylate, mounted in Canada balsam, and viewed as whole mounts. Selected brains were subsequently embedded for sectioning in resin (Epon-Araldite; Mollenhauer, 1964). Brains were sectioned on an American Optical microtome at 10 to 20 μm and counterstained with 1% toluidine blue.

Localized dye injections and staining of single axons. For these experiments animals were anesthetized and immobilized in a Petri dish with a wax bottom that had a shallow depression just large enough to contain the animal. To secure the specimen, a small (1 cm $\times$ 1 cm) piece of surgical gauze was placed over the animal and pinned with stainless steel insect pins. In placing the gauze over the animal, care was taken to leave a clear approach to the eye. The skin overlying the eye was then gently torn with an electrolytically sharpened tungsten needle, and the sclera and lens were removed from the eye cup. Extreme caution was taken not to stress the optic nerve and to keep the eye in its normal orientation. Microelectrodes fashioned on a Kopf electrode puller were filled with a solution of 0.2 M hexaminocobaltic chloride (Brogan and Pitman, 1981). Electrode resistances were in the range between 20 and 120 megohms. The tip of a micropipette was maneuvered into position over a selected region of the vitreal surface of the retina and lowered until the electrode tip just touched the inner limiting membrane, resulting in a change in the electrode resistance. A piezoelectric microdrive was used to advance the electrode for the actual penetration of the inner limiting membrane, into the ganglion cell body layer. The cobalt complex was iontophoresed from the electrode with positive current pulses (1 Hz; 500 msec on/500msec off) of 5 to 15 nA for 30 to 60 min. In order to reference the electrode position to particular quadrants, a line was envisioned extending from the ventral fissure, through the newly forming optic disc, up to the dorsal pole of the retina (see Fig. 1D). A second line was imagined running perpendicular to this dorsal-ventral axis, along the nasal-temporal direction. The location of the micropipette tip with respect to this reference system was then noted.

Following the dye injection, the animals were removed from the dissecting chamber and placed in a Petri dish containing Steinberg's solution. The anesthetizing effects of the MS-222 wore off within minutes and the animals were allowed to swim freely for approximately 6 hr. Animals were then sacrificed, and the dorsal portion of the head was dissected from the remainder of the body just ventral to the eyes and rostral to the brainstem. Tissue overlying the brain was removed in order to permit free penetration of solutions. The cobalt was precipitated and then intensified according to the aforementioned procedures (Bacon and Altman, 1977; Murphey et al., 1983). The brains and eyes were then dissected from the remaining head tissues and mounted on slides for inspection. Selected specimens were then embedded in soft resin (Epon-Araldite; Mollenhauer, 1964) and sectioned on a rotary microtome using a steel knife.

Terminal arbor analysis

The axonal arborizations of ganglion cells were reconstructed with the aid of a drawing tube and photographed on a Zeiss photomicroscope with Kodak Technical Pan 2415 and Kodak Ektachrome 50 films. When possible, whole mounted preparations were oriented in dorsal and lateral perspectives for analysis. From these reconstructions a linear measure of the length (rostral to caudal) of the terminal arbor was obtained. The anterior edge of the arbor was taken as the site of the first branch within the tectal neuropil, and the posterior edge was taken as the most posterior process (see Fig. 1D). Based on staining of the entire retinal projection as well as on published results (Holt and Harris, 1983; Holt, 1984), we were able to determine the rostral and caudal extent of the tectal neuropil. The rostral margin was slightly anterior to the lateral curvature in the young tectum (see Figs. 1D and 2), and the caudal margin was at the border between the acellular region and the tectal cell mass which was visible in the whole mount (see Figs. 1D and 2). The relative size of the arbor was then expressed as a fraction of the length of the tectal neuropil (see Fig. 13). Reconstruction of sectioned material allowed measurement of the dorsal-to-ventral dimension of the arbor as well as the tectal neuropil.

Results

The retinal injection site

Dye was iontophoresed into localized positions within the exposed retina of embryos and tadpoles between stages 34 and 50 of

Xenopus development (Sakaguchi and Murphey, 1984). The highly organized bundles of axons within the eye (Fig. 1A; Sakaguchi et al., 1984) increases the likelihood that dye injected into a restricted portion of the eye will stain a restricted portion of the retinal projection to the brain (Fig. 1, B and C). One hundred forty-six successful injections were made into the retinae of embryos and tadpoles (Tables I and II). Examination of selected retinae following the injection confirmed the electrode position (Fig. 1, B and C) and provided information about the extent of staining. In two of the injections, single ganglion cells within the eye were stained (Fig. 1C). However, in the vast majority of injections, a small region of the retina was labeled by the dye (Fig. 1B). Individual processes and somata could be seen within the dye-injected area, but no single cells could be identified (Fig. 1B).

Eighteen percent of the injections, using this extracellular method, stained single ganglion cell axons in the brain. Although single arbors were labeled in the brain, examinations of the optic nerves sometimes revealed other labeled axons in which the dye had failed to diffuse the full distance to the brain.

The projection from eye to brain

The results from the injections will be presented in three sections: (1) the initial projection (stages 34 to 39), (2) the early larval projection (stages 40 to 45), and (3) the mid-larval projection (stages 46 to 50).

To determine the boundaries of the retinal projection, we retrogradely stained the entire optic projection with cobalt in a variety of embryonic and larval stages. One example, the optic projection seen at stage 47, is illustrated in Figure 2 and is characteristic of early and mid-larval stages (Fig. 2). This projection is very similar to that described by Steedman et al. (1979) for later stages (51 to 66).

The initial projection (stages 34 to 39)

Between 3 and 4.5 days postfertilization, the embryo hatches and the development of various features reveals that the visual system is rapidly maturing. In the eye, this is a period of synaptogenesis (Grant et al., 1980); the appearance of the first retinal response to light (stage 39) (Witkovsky et al., 1976), and the first electrical activity, was recorded from ganglion cells in the eye (Sakaguchi et al., 1984). The axons of ganglion cells first reach the brain (Holt and Harris, 1983; Holt, 1984), and electrical activity due to retinal afferents is first detected in the tectum (Holt and Harris, 1983) at stage 39.

The earliest cobalt-labeled axonal processes within the immature brain were observed in stage 34 embryos. The retinal injections in these preparations were made at dorsal and dorsotemporal locations, and the axons of the labeled ganglion cells crossed the midline at the chiasm and coursed along the ventral and lateral wall of the diencephalon (Fig. 3, A and B), but were not seen in the tectum.

Several hours later, by stages 37 to 39, some ganglion cell axons projected through the diencephalon to reach the midbrain. These axons ended in the rostral region of the ventrolateral tectum (Fig. 3, C and D). These early axonal projections often ended with few or no secondary branches (Figs. 3 and 4). Some ended in what appeared to be growth cones (Fig. 3), whereas others had no discernible terminal specialization. Neither type of ending possessed any extensive filopodial extensions (see "Discussion" for further comment on this observation). A nearly universal finding was the presence of varicosities, which varied in size, shape, and spacing along the length of the axon (Figs. 3 and 4).

During this early period of development, before the elaboration of complex terminal arbors, the axons innervated topographically appropriate regions of the tectum. For example, injections into dorsal retina resulted in labeled axons terminating in the ventrolateral aspect of the tectum (Figs. 3, C and D, and 4).

By stage 39 a few (3 of 10) retinal axons had the appearance of more mature terminal arborizations (Fig. 5). Although much smaller, these young arbors resembled those seen in adult amphibia and

Figure 1. Cobalt labeling in the retina. A, Whole mounted stage 47/48 retina viewed from the vitreal surface following retrograde cobalt labeling of retinal ganglion cells. Note the strikingly radial pattern of axonal fascicles entering the optic disc (X). In A, the cobalt-labeled ganglion cells represent only a portion of the total population of ganglion cells. B, Low power photomicrograph of a stage 50 flat mounted retina following cobalt injection and Timm's intensification. The region of highest dye concentration is marked by the arrows. The optic disc is approximately 300 μ m toward the lower right corner of the photograph (not shown). C, Single retinal ganglion cell injected with cobalt in a stage 47 retina. The axonal process was severed from the soma (S) during the histological processing. Calibration bars: A, 200 μ m; B, 60 μ m; C, 20 μ m. D, Schematic representation of the *Xenopus* visual system. Dye injections were made into the right eye. The eye was divided into four quadrants to aid in referencing retinal positions. The diencephalon and optic tectum are presented in a dorsal view with the contralateral, left tectal neuropil (TN) stippled. An example of a terminal arborization (TER. ARBOR) of a retinal ganglion cell (RGC) is illustrated. The bracket shows the determination of the rostral-to-caudal length of the arbor. D, dorsal; N, nasal; V, ventral; T, temporal; OD, optic disk; O nerve, optic nerve; OT, optic tract.

TABLE I
Retinal injections

Stages	No. of Injections	No. Successful	Percentage Success
34-36	19	4	21
37-39	78	30	38
40-45	68	34	50
46-50	112	78	70
Totals	277	146	53

fish (Potter, 1972; Merrill and Stirling, 1982; Stuermer, 1984). They consisted of a primary axonal process which branched repeatedly, either into long, filamentous, higher order branches or into relatively short side branchlets arising from the primary stem axon (Fig. 5).

These terminal arborizations were generally elongated in the rostral-to-caudal axis and were topographically organized (Fig. 5).

The early larval projection (stages 40 to 45).

By this stage, topography of the projection was easy to demonstrate and injections in a given part of the retina usually labeled axonal arbors in predictable parts of the tectum. The midbrain consists of a central cell mass surrounding the ventricle and a superficial, acellular region, referred to as the tectal neuropil (Holt, 1982), where various afferents terminated. The axonal arbors were usually restricted to the superficial two-thirds of the neuropil. Most of the terminal arbors examined were elongated in the rostral-to-caudal dimension of the tectum (Figs. 6, A and B, and 7). A given arbor usually covered 50 to 90% of the available tectal neuropil in

Figure 2. Gross anatomy of the retinal projection. Photomicrographs of sectioned material following hexaminocobalt chloride staining of the retinal projection from the contralateral, right eye. **A**, Sagittal section from a stage 47 tadpole at a point midway through the left hemibrain. *Arrowheads and letters a to e* denote approximate levels of sections illustrated in **B**. **B**, Transverse section reconstruction of a stage 47 brain. Sections *a* and *b* are 140 μm apart, and sections *b* to *e* are 80 μm apart. The first section (*a*) is at the level of the optic chiasm (*Ch*) and the last section (*e*) is in caudal tectum in the tectal cell mass (*TCM*). *R*, rostral; *D*, dorsal; *C*, caudal; *V*, ventral; *Ch*, chiasm; *OT*, optic tract; *NB*, neuropil of Bellonci; *Pt*, pretectum; *BOT*, basal optic tract; *BON*, basal optic nucleus; *Hy*, hypothalamus; *Teg*, tegmentum; *TCM*, tectal cell mass; *Cb*, cerebellum; *Med*, medulla. In **B**, the dotted lines demarcate the tectal neuropil. Calibration bar, 100 μm .

Figure 3. *A and B*, Axonal endings in the embryonic optic tract. Photomicrographs of ganglion cell axons following a retinal injection. This preparation is viewed laterally in the left optic tract at a level midway up the diencephalic wall. *A and B* are optical sections of axonal endings (arrowheads) of the same field in a stage 35/36 embryo. *r*, rostral; *d*, dorsal; *c*, caudal; *v*, ventral. *C and D*, The innervation of the optic tectum. Photomicrographs from whole mounted brains viewed laterally. *C*, Retinal projection from a dorsotemporal injection site in a stage 37/38 embryo. Several axons innervate the rostral area of the ventrolateral tectum. Note the single axon (arrowhead) that breaks caudally from the optic tract (*OT*) and innervates the basal optic nucleus (*BON*). *D*, Retinal projection from a dorsal injection site in a stage 39 embryo. In this preparation, all stained axons innervated the rostral region of the ventrolateral aspect of the tectum. The dotted lines demarcate the brain outline. Calibration bars: *A and B*, 10 μ m; *C and D*, 50 μ m.

Figure 5. Topography in the embryonic projection. *A* and *C*, Dorsal views of camera lucida-reconstructed arbors in whole mounted stage 39 brains. *B* and *D*, Transverse sections at the levels marked by the arrows in *A* and *C*, respectively. The insets are schematic representations of the contralateral right eye. In *A* and *B* a ventral retinal injection labeled a dorsomedially terminating axonal arborization. In *C* and *D* a dorsotemporal injection labeled an arbor terminating laterally in the tectal neuropil. Note the elongated nature of these young terminal arbors (rostral to caudal) and their relative restriction along the orthogonal axis. *D*, dorsal; *N*, nasal; *V*, ventral; *T*, temporal; *C*, caudal; *L*, lateral; *R*, rostral; *M*, medial. Calibration bar, 40 μ m.

Figure 4. Topography of the initial projection. Camera lucida reconstructions from whole mounted embryonic brains following dye injections into the contralateral retina. *A* and *B* are lateral views of the midbrain. The insets are schematic representations of the injection site (*) in the contralateral eye. *A*, A dorsal injection in a stage 39 embryo resulted in the majority of axons ending ventrolaterally in the tectum (from Fig. 3*D*). *B*, A ventral retinal injection resulted in labeled axons which terminated dorsomedially in the tectum. The area immediately posterior to the tectal neuropil (demarcated by the dashed outline) is the tectal cell mass. The boxed diagram (bottom) is a schematic representation showing the various regions of the embryonic brain. *T*, temporal; *D*, dorsal; *N*, nasal; *V*, ventral; *R*, rostral; *C*, caudal; *Tel*, telencephalon; *Ch*, chiasm; *Hy*, hypothalamus; *Di*, diencephalon; *TN*, tectal neuropil; *TCM*, tectal cell mass; *Cb*, cerebellum.

Figure 6. Terminal arborizations from single retinal ganglion cells labeled with cobalt. Photomicrographs from whole mounted brains from a stage 42 (A and B) and a stage 47 (C) tadpole viewed laterally. A, Low magnification view of a retinal ganglion cell axon stained following dye injection into a mid-dorsal location in the contralateral retina. The axon (small arrowheads) can be seen coursing dorsocaudally in the optic tract to innervate the ventrolateral aspect of the tectum. B, Higher magnification of the terminal arbor in A. C, Terminal arbor from a single retinal ganglion cell following dye injection into a ventral retinal location. The arbor is elaborated dorsomedially in the tectal neuropil. Note the small collaterals (arrowheads) branching from the axon. d, dorsal; c, caudal; v, ventral; r, rostral. Calibration bars: A and C, 50 μ m; B, 20 μ m.

the rostral-to-caudal dimension. In a small number of cases axonal processes from arbors were observed ending within the tectal cell mass. In the dorsal-to-ventral dimension the arbors were more restricted, covering 33 to 50% of the available neuropil.

Terminal arbor branches often emanated from a single stem axon

which branched repeatedly within the tectal neuropil (Fig. 7). However, on several occasions, what appeared in whole mount to be multiple (2 to 3) axons terminating in the same area were, in fact, from a single axon which had branched soon after the chiasm, resulting in distinct collaterals coursing along the optic tract to

Figure 7. Terminal arborization from a single retinal ganglion cell injected at a mid-dorsal retinal position in a stage 44/45 tadpole. **A**, **C**, and **E**, Low power views of three selected transverse sections. **B** and **D**, Higher magnifications of selected regions from sections **A** and **C**, respectively. **A** and **B**, Section at the level of the optic chiasm. The single stained axon crosses the midline and branches in the optic tract. **C** and **D**, Section at the level of the optic tract: the more medially placed process branches, resulting in three branches from the same axon innervating the tectum. **E**, Section at the level of mid-ectum. The terminal arbor from this ganglion cell elaborates within a very restricted region of the optic tectal neuropil. *Calibration bars*: **A**, **C**, and **E**, 50 μm ; **B** and **D**, 20 μm . *Inset*, Camera lucida reconstruction of the terminal arborization illustrated in **A** to **E**. The arrows and letters indicate the approximate site for each respective section.

Figure 8

innervate the tectum (Fig. 7). Misinterpretation as to whether one or more axons were labeled was avoided by reconstruction of the whole mounts and subsequent sectioned material (Fig. 7).

Ipsilateral projections. The anterograde labeling of ganglion cells also revealed ipsilateral projecting axons during these stages of early larval development (Fig. 8). In some preparations axons coursed ipsilaterally before reaching the midline (Fig. 8*B*). Axons were also observed crossing the midline, only to turn sharply back toward the ipsilateral hemibrain (Fig. 8, *B*, *C*, *E*, and *F*). A few retinal axons were observed during early stage 40's that innervated diencephalic visual nuclei (Fig. 9) as well as the tectum (not shown). Figure 9 also illustrates examples of single axons which arborized in the neuropil of Bellonci as well as within the pretectum.

The mid-larval projection (stages 46 to 50)

Most of the stained terminal arbors in the midlarval brain were restricted to regions of the tectum correlated with the site of the retinal injection, thus creating a highly ordered projection (Table II, Fig. 10). For example, dye injections into the dorsal retina labeled axons projecting to the ventrolateral aspect of the tectal neuropil (Fig. 10). However, about 6% (Table II; 5 of 78) of the arbors projected to inappropriate tectal locations. These "mistakes" may reflect axons with "unusual" trajectories within the retina (Cook, 1982; Sakaguchi et al., 1984). Figure 10 also reveals an interesting relationship that was observed only after these middle stages of larval growth. There was a tendency for cells terminating rostrally to possess small arbors relative to those axons terminating caudally (cf. Fig. 10, *B* and *D*, see below).

Figure 11 illustrates the range of terminal arbor morphologies observed during these mid-larval stages in the central region of the tectum. Arbors characterized by a relatively small (~50 to 80 μm in length) and profusely branched arborization (Fig. 11, *A* and *B*) composed only 5% of the sample at these stages. The majority (70%) of terminal arbors were similar to those in Figure 11, *C* and *D*, in morphology. These arbors were relatively large (80 to 150 μm

TABLE II
Topography of the larval projection^a

Stages	Total No. of Successful Injections	No. and Positions of Retinal Injections		Tectal Location of Labeled Arbors			
		Position	No.	V ^b	R	D	C
40–45	34	D ^c	10	10			
		T	8		8		
		V	11	(1) ^d	10		
		N	5	(1)			4
46–50	78	D	23	21	(1)	(1)	
		T	20	(1)	18	(1)	
		V	20	(1)	(1)	19	
		N	15	(1)			14

^a Axons from dorsal retina terminate ventrally in the tectum, and those from temporal, ventral, and nasal retina terminate in rostral, dorsal, and caudal tectum, respectively.

^b V, ventral; R, rostral; D, dorsal; C, caudal tectum.

^c D, dorsal; T, temporal; V, ventral; N, nasal retina.

^d Numbers in parentheses, examples in which afferents were observed terminating within inappropriate tectal locations for the position of dye injection within the retina.

in length), highly branched, and elongated rostrocaudally in the tectal neuropil. The remaining 25% of the sample was characterized by long filamentous processes with little higher order branching (Fig. 11, *E* and *F*), and were the largest encountered (150 to 200 μm in length).

Ipsilateral projections. Ipsilaterally directed axons were also observed during these mid-larval stages of development (Figs. 9 and 12). The axon projecting to the ipsilateral tectum, illustrated in Figure 12, terminated in the topographically appropriate location for the site

Figure 8. Axonal "disorganization" in the chiasm and optic tract. Photomicrographs from a transverse-sectioned stage 44/45 tadpole brain. *A*, Low magnification view of the chiasm and rostral optic tract. *B* and *C*, Higher magnifications at different focal planes from the same section illustrated in *A*. Note the midline (dashed line) and the axonal branches and their intertwining trajectories. In *B*, an axonal branch makes a "hairpin" turn (small arrowheads) and is directed back toward the midline. The arrowhead in *C* points at the growth cone of an axon directed back toward the midline and can be viewed at higher magnification in *F*. *D*, Low power view of the section immediately caudal to *A*, at the level of the optic tract. Note the axonal branches directed ipsilaterally. *E*, Higher magnification of *D*. Calibration bars: *A* and *D*, 200 μm ; *B*, *C*, and *E*, 75 μm ; *F*, 25 μm .

Figure 9. Diencephalic retinal projections. Camera lucida reconstructions of whole mounted brains viewed dorsally. *A*, Ganglion cell axon terminating contralaterally in the NB and Pt in a stage 45 tadpole brain. *B* and *C*, Ipsilaterally directed axons. *B*, Axon with arbors in the neuropil of Bellonci and the pretectum in a stage 47 brain. *C*, Ipsilateral axon which terminated only in the neuropil of Bellonci in a stage 42 brain. The arrows point to the axons. R, rostral; M, medial; C, caudal; L, lateral.

Figure 10. Topography of the retinal projection. Semischematic representation of the retinal projection. The *top panel* schematizes the retinal projection to the contralateral tectum. In *A* to *E* the terminal arborizations of individual retinal ganglion cells were reconstructed in dorsal (*A*₁ to *E*₁) and lateral (*A*₂ to *E*₂) views from whole mounts following cobalt injections at various positions around the contralateral eye (*circular insets*). *A*, Dorsal (*D*) retinal injection labeled ventrolaterally projecting axons in a stage 47 tadpole. *B*, Temporal (*T*) injection labeled a rostrally terminating axonal arbor in a stage 47 tadpole. *C*, Ventral (*V*) retinal injection labeled cells which terminated dorsomedially in the tectum of a stage 48 tadpole. *D*, Nasal (*N*) injection labeled a cell which terminated caudally in a stage 47 tadpole. *E*₁ and *E*₂ are composite representations made by the superimposition of the various arbors (*A* to *D*).

Figure 11. Camera lucida reconstructions of six retinal ganglion cell terminal arborizations. Each arbor is viewed dorsally in the tectum of stage 47 tadpoles. These arborizations represent the range of variability in the morphologies of terminal arborizations. Calibration bar, 50 μ m.

Figure 12. Photomontage of transverse sections from a stage 46 tadpole brain. The distance between sections a and b is 120 μm . Sections b to e are each separated by 20 μm . Dye was injected dorsally at the rim of the right eye. The majority of stained axons project contralaterally, innervating the ventrolateral aspect of the tectal neuropil. However, a single, darkly stained axon (arrowheads in a) was observed projecting ipsilaterally. This cell also terminated ventrolaterally, in the location analogous to the contralateral projection, but in the ipsilateral hemitectal neuropil (arrowheads in b to e). f, Higher magnification of the terminal arbor from section c. Calibration bars: a to e, 50 μm ; f, 20 μm .

Figure 13. *A*, Graphic representation of the relationship between terminal arbor size (rostral to caudal) with respect to the tectal neuropil expressed as a percentage (*ordinate*) versus stage of development (*abscissa*). Each datum point (●) represents a single terminal arborization. The open diamonds are the mean percentage values at particular stages. Points plotted above the 100% value for the tectal coverage were characterized by terminal arbors

of the retinal injection. Although we cannot be very accurate in our assessment of the number of ipsilaterally projecting cells, data like those of Figure 12 indicated that it is a small but potentially important number; we estimate between 0.1 and 1% at this stage.

Quantitative analysis of retinotectal mapping

It was clear from a qualitative examination that the majority of the axonal arbors were elliptical, with the long axis parallel to the rostral-to-caudal dimension (Figs. 6, 7, 10, and 11). In order to compare the retinotectal projection from various stages of development, the length and width of individual arbors were measured and compared to the size of the tectal neuropil. We examined 51 single axons between stages 42 and 50. Ninety-three percent were elongated in the rostral-to-caudal dimension of the tectum and therefore compressed with respect to the medial-to-lateral axis. As described under "Materials and Methods," a linear measure of the rostral-to-caudal length of the terminal arbor and of the tectal neuropil could be determined from whole mounted brains. These values were used to calculate the percentage of the tectal neuropil covered, along the rostral-to-caudal axis, by a given axonal arbor (termed the "percentage of coverage"). The percentage of coverage of terminal arbors at various stages of development was plotted as a function of the developmental stage of the tadpoles (Fig. 13*A*). Clearly the percentage of coverage decreased with maturation.

To examine the mechanism responsible for this change, the lengths of the terminal arbors and the tectal neuropil were plotted as a function of the stage of development (Fig. 13*B*). Between stages 42 and 50, the rostral-to-caudal length of the tectal neuropil increases over three times, from approximately 110 μm at stage 42 to more than 350 μm in a stage 50 tadpole (Fig. 13*B*). The range of sizes of the terminal arbors also increased but not nearly as rapidly as tectal size (see Fig. 13*B*). Thus, during development the terminal arbors occupy a continuously decreasing percentage of the available tectal neuropil. This appears to be caused primarily by the increase in tectal area and not by a decrease in terminal arbor size. In fact, there is no evidence in our data for any decrease in arbor size; they seem to grow throughout the period of development examined in this study (stages 39 to 50).

Due to the curvature of the brain, the dorsal-to-ventral dimension was reliably obtained only from transversely sectioned material. Although 51 single terminal arbors were examined in 41 whole mounts, only 12 (29%) whole mounts were successfully embedded and sectioned for further analysis. The small size and fragility of whole mounted brains made it difficult to recover, embed, and section the brains. In general, the size of the terminal arbors was restricted to less than 50% of the tectum, but the low percentage of recovery prevented any thorough analysis of the dorsal-to-ventral dimension of terminal arbor and tectal growth.

Discussion

To summarize briefly, the present results demonstrate the following. (1) The earliest ganglion cell axons to reach the tectum generally took direct routes, through the optic tract, and ended as simple axonal endings in appropriate regions of the tectum. Thus, a topographic map exists very early in the development of the projection, even before the axons have elaborated axonal arborizations. These results support the observations of Holt and Harris (1983) and Holt (1984). (2) The projection was characterized by axonal arbors which were large with respect to the tectal neuropil, and with subsequent

that covered the length of the tectal neuropil and, in addition, had processes extending caudally into the tectal cell mass. *B*, The relationship between stage of development (*abscissa*) and the rostral-to-caudal length of the tectal neuropil (○) and terminal arbors (●). The lines connect mean values for particular stages of development (*dashed line* for tectal neuropil; *solid line* for terminal arbors). Note that the actual time of development between stages 42 and 44 is approximately 18 hr; time between stages 48 and 50 is approximately 8 days (from Nieuwkoop and Faber, 1967).

growth, the arbors were seen to cover a relatively smaller proportion of the tectal neuropil. (3) Measurements of the size of terminal arbors with respect to the tectal neuropil provide evidence that the change in coverage was due primarily to tectal growth and not to any dramatic shrinkage of arbors.

The dye injection technique. A variety of anatomical techniques have been applied to the visual system of lower vertebrates to examine the projection of the retina onto the brain. The present study has adapted an extracellular dye injection technique first used in insects (modified from Rehbein et al., 1974) to the study of the development of the retinotectal projection in *Xenopus laevis*. In spite of the extracellular application of dye, 18% of all successful injections revealed the axonal arborizations of single retinal ganglion cells (Figs. 6 and 7). The retinal site of injection has been examined and it is clear that many neurons near the electrode tip were stained by this method (Fig. 1B). Although the tip of the microelectrode is in the micrometer range, the region of the retina showing the presence of dye is considerably larger. Nevertheless, the method is useful since the dye is presumably taken up only by those cells in which the soma, axon, or dendrites are damaged by the impalement. In addition, the radial pattern of axons within the retina (Fig. 1A) restricts staining to cells from a limited sector of the eye having axons that pass through the injection site (Fig. 1B). Thus, it is possible to map the afferent projection with considerable reliability at the cellular level.

The growth cones. Since the seminal work of Ramón y Cajal (1890) and Harrison (1910), it has been recognized that specialized structures called growth cones exist at the terminal portion of growing neurites. In *Xenopus* embryos, we have observed growth cones at the tips to the first group of ganglion cells to pioneer the pathway to the brain (Figs. 3 and 4). These specialized endings on the axons were devoid of the fine filopodia that have been associated with some growth cones seen within the eye using intracellular injection techniques (Sakaguchi et al., 1984). A second type of axonal ending was also observed in this study in which no terminal enlargement or apparent specialization appeared at the tip of the axon (Figs. 3 and 4).

Recently, Harris et al. (1985) have utilized the localized application of horseradish peroxidase (HRP) crystals to examine the growth cones of ganglion cells in the embryonic optic tract of *Xenopus* (Harris et al., 1985). Their results clearly show the presence of fine filopodia emanating from the growth cones. Similar observations in the tectum have also been reported in *Rana* (Reh and Constantine-Paton, 1984) and in the chick (Thanos and Bonhoeffer, 1983) visual systems.

There may be several reasons why extensive filopodia were not observed on the growth cones in the present study. Cobalt is known to be detrimental to a cell's health, and this may in some way affect the filopodia and therefore inhibit their staining or cause their retraction.

Mature axonal arborizations. During late embryonic and early larval stages, a number of distinct types of arborizations were detected throughout the tectal neuropil. Some were characterized by a highly branched, dense arborization with small terminal fields (Fig. 11, A and B). Others had relatively long processes with less higher order branching and larger terminal fields than the first variety (Fig. 11, C and D). Arbors in a third group possessed long, sparsely branched processes, often covering most of the tectal neuropil (Fig. 11, E and F).

In the mature bullfrog (*Rana catesbeiana*), Potter (1969, 1972) has described three main types of retinal afferent arborizations. He based his characterization on a combination of size, branching pattern, and layering of the terminals within the tectum. The smaller, more densely branched arbors (termed DB) terminated superficially, and he equated them with the sustained and convexity detectors of Maturana et al. (1960). The larger arbors with less profuse branching (termed WB and TB) terminated deeper, and these were equated with the changing contrast and dimming detectors of Maturana et al. (1960). In accord with Potter's (1969, 1972) results, Merrill and

Stirling (1982) recorded and injected HRP into a dimming unit and showed that its terminals arborized in the deeper layers of tectal neuropil in adult *Rana*. Similarly, in mature goldfish, Stuermer (1984) has demonstrated a morphological layering of optic afferents, with the smallest arbor types terminating superficially, and the larger arbor types terminating more deeply. Our results on embryonic and early larval tissue suggest a similar morphological grouping of terminals. Our small, highly branched arbors are similar to Potter's "densely branched" arbors. Our intermediate group is similar to Potter's (1969, 1972) "widely branched" category and our third group is similar to his large "thinly branched" arbors.

Potter (1972) used the layering of the tectum to aid his categorization, and both he and Stuermer (1984) showed that the small arbors were located in superficial layers and the largest in the deeper layers. Our results prior to stage 43 indicate very little evidence for layering of single arbors; thus, this feature must emerge as both the arbors and tectum mature.

Retinotopic mapping during early development. An issue we have tried to address in this work is, what is the nature of the initial retinal projection and how does it mature? It has been suggested that initial afferent projections may be diffuse early in embryonic time and become more precise with maturation (Gaze et al., 1974; Schneider et al., 1981; McLoon, 1982; Sretavan and Shatz, 1984). For example, the retinotectal projection of larval *Xenopus* was first thought to be lacking in retinotopic organization (Gaze et al., 1974). However, Holt and Harris (1983) showed that there was in fact a retinotopic order to the initial projection in *Xenopus*.

Our results reveal that by early stage 40's the axonal arbors are fairly elaborate and the topography of the projection is similar to that seen at more mature stages. However, the arbors are large relative to the size of the tectum. For example, at stages 40 to 42, terminal arbors span most of the tectal neuropil in the rostral-to-caudal dimension. During subsequent larval growth, arbors increase in size slowly, but the tectum grows rapidly, and thus the "precision" of the mapping appears to improve such that each arbor covers a relatively smaller percentage of the tectal neuropil (Fig. 13). In functional terms this increase in the mapping precision is manifest when considering the changes in the relative amounts of tectum representing a given portion of visual field (Jacobson, 1962; Sachs and Schneider, 1984). For example a 100- μ m step (rostral to caudal) across the stage 40 tectum (total rostral-to-caudal length about 100 μ m) corresponds to the entire temporal-to-nasal visual field (180°). A similar increment across the stage 50 to 51 tectum (total length about 600 μ m) corresponds to approximately 30° of the visual field. Thus, the apparent increasing precision is not necessarily brought about by pruning a diffuse arbor. The axonal arbors continue to cover the same approximate area, and some of them presumably grow larger; the apparent increase in the precision of mapping is due to a relatively rapid growth of the tectal neuropil compared to the growth of the axonal arbors.

The precision of mapping appears to be different in the vertical and horizontal axes of visual space. At stages 42 to 44, axonal arbors typically cover a majority of the tectum in the rostral-to-caudal dimension but relatively less of the tectum in the medial-to-lateral dimension. This suggests, but cannot prove, that the medial-to-lateral axis is more precisely ordered early in development than the rostral-to-caudal axis.

There is an additional nonuniformity in the developing projection because some of the axonal arbors from temporal retina are more compact in the rostral-to-caudal dimension than others (Fig. 10). In fact, the anatomical results shown in Figure 10 suggest that at these early stages some of the lack of precision in mapping may be attributed to the relative maturation rates of the various areas of tectum (Straznicki and Gaze, 1972; Raymond and Easter, 1983). The precision of mapping in relation to the tectal neurons receiving the input may well be constant across the tectum. If the dendritic fields of tectal neurons are elongated in a manner similar to that of the axonal arbors, then the resolution of the tectum may be constant

across its surface. Until we know the dendritic shapes of tectal interneurons, rather little can be said about the functional resolution of the retinal projection.

An alternative explanation of this nonuniformity suggests that the more elongated arbors may be expanding caudally toward the tectal cell mass, where new tectum is continuously generated (Raymond and Easter, 1983). This idea is consistent with Stuermer's (1984) description in goldfish.

An early ipsilateral retinal projection. In addition to the crossed retinofugal projection in anuran amphibians, there also exists a robust ipsilateral projection (Knapp et al., 1965; Scalia et al., 1968; Currie and Cowan, 1975; Glastonbury and Straznicki, 1978; Levine, 1978, 1980; Straznicki et al., 1980; Toth et al., 1980; Kennard, 1981; Hoskins and Grobstein, 1984). The ipsilateral projection has been shown in almost all studies to innervate primarily the ipsilateral diencephalic visual regions (Kennard, 1981). Studies using anatomical techniques found that the ipsilateral projection developed during metamorphosis (Khalil and Szekely, 1978; Kennard, 1981; Hoskins and Grobstein, 1984, 1985a, b), and its formation was dependent on the levels of thyroxine (Hoskins and Grobstein, 1985b).

In contrast to these studies, we have observed a direct ipsilateral pathway which exists early in development (as early as stage 42; Figs. 8, 9, and 12). Furthermore, this early projection terminated not only in the diencephalon but in the tectum as well. Within the tectum the ipsilateral fibers arborized in regions appropriate to the retinal injection (Fig. 12). Thus, the present results argue against the idea that the ipsilateral projection forms *de novo* at metamorphosis. A more likely scenario is that an ipsilateral projection exists throughout larval development, and it is enhanced and, thus, becomes detectable, at metamorphosis. The less likely alternative is that the larval ipsilateral projection disappears in mid-larval development and a new one is formed at metamorphosis.

The presence of a naturally occurring, ipsilateral retinotectal projection may be very important in studies where one eye is manipulated early in development and the other left undisturbed. The present results suggest that an ordered afferent projection from the intact eye, although less dense, would always be present in the "experimental" half-tectum. For example, attempts to create a virgin tectum by unilateral eye removal can never create totally virgin tectum.

Does regeneration recapitulate ontogeny? Many investigators have used regeneration of crushed or severed axons in adult animals as a model for the formation of nerve connections (e.g., Sperry, 1943, 1963; Gaze and Jacobson, 1963; Gaze and Keating, 1970; Fujisawa et al., 1982). It is often argued that regeneration studies can tell us the rules for normal development. One of our concerns here was to assess this idea by showing directly how the retinotectal map develops under normal conditions and compare it with data on the early stages of regeneration.

Various anatomical and physiological studies have shown that, after nerve crush or section, the retinal projection regenerates in a stepwise fashion, initially forming a diffuse projection which is then molded into the more precise one (Gaze and Jacobson, 1963; Westerman, 1965; Gaze and Keating, 1970; Meyer, 1980; Fujisawa et al., 1982; Humphrey and Beazley, 1982; Murray and Edwards, 1982; Schmidt et al., 1984; Stuermer and Easter, 1984). The studies of Fujisawa and co-workers (Fujisawa, 1981; Fujisawa et al., 1982) on the newt retinotectal system confirmed the diffuse nature of the regenerating projection anatomically. At 4 weeks after transection of the optic nerve, the axons of ganglion cells often took unusual and circuitous routes to their target regions. The terminal arbors contained numerous sprouts projecting to ectopic locations on the tectum, suggesting a form of trial and error search for the appropriate target area. Finally, at 10 weeks of regeneration they observed terminal arbors covering smaller areas with fewer ectopically located sprouts, implying that these branches were lost or retracted. Similar observations have since been made in a variety of other regenerating retinotectal systems as well (fish: Meyer, 1980; Murray and Edwards,

Figure 14. Initial development versus regeneration. Schematic representation of the mode of initial development of the retinotectal projection and of regeneration in mature animals. In both models A and B, the terminal arbors originate from a ganglion cell in dorsal retina. The axon is seen to innervate the left tectum, viewed laterally at successive stages of development. The stippling represents new tectum generated caudomedially from the tectal cell mass during the intervening time periods (Raymond and Easter, 1983). **A**, Initial development. A retinal ganglion cell axon is represented at successive stages of development starting from stage 38 with the initial innervation, to stage 50, when the terminal arbor has reached a relatively mature state. **B**, Regeneration. During the early and middle phases of regeneration the axon sprouts several branches. (This accounts for the diffuse projection.) These ectopic collaterals are subsequently retracted or degenerate (indicated by dashed lines), resulting in the topographically ordered map. This schematic model of regeneration is based primarily on the results of Fujisawa et al. (1982). Note the difference in calibration bars for the two models.

1982; Schmidt et al., 1984; Stuermer and Easter, 1984; frog: Adelman and Grobstein, 1982; Humphrey and Beazley, 1982).

We have shown here that, during the early stages of development, the terminal arbors are large with respect to the tectal neuropil. In relative terms, this coverage gradually decreases with development (Fig. 14), and this decrease superficially resembles the refining of a diffuse projection seen during regeneration. However, this process of refinement could occur in one of at least three ways: (1) pruning by retraction or loss of aberrant collaterals and terminals, (2) growth of the brain structure, or (3) a mechanism involving a combination of both method 1 and method 2. During regeneration it is clear that the refining process is the result of pruning (loss or retraction of ectopic collaterals) (Fujisawa et al., 1982; Schmidt et al., 1984). In contrast, during early development the refining process appears to be due primarily to the growth of the brain. If any drastic pruning of terminal arbors occurred, as suggested by Lazar (1973) in stage 50 and older *Xenopus*, one would expect to see an overall decrease in the size of the terminal arbors with development. On the contrary, the sizes of the arbors sampled at stages 48 to 50 compared with the earlier stages (42 to 45) are slightly larger (Fig. 13). In addition to the refinement discussed above, another form of sculpting is also

known to occur. With maturation there is a shifting of connections which is required to maintain the orderly retinotectal map (Gaze et al., 1974; Fraser, 1983; Easter and Stuermer, 1984; Reh and Constantine-Paton, 1984). It is likely that this modification may play some subtle role in the refining process as well. The most parsimonious interpretation of the present findings is that the refinement process seen during initial map development (between stages 37 and 50) is primarily the result of the differential growth of the tectal neuropil with respect to the terminal arbors, whereas during regeneration it is due primarily to pruning of aberrant collaterals (Fig. 14).

References

- Adamson, J. R., and P. Grobstein (1982) Reestablishment of the ipsilateral oculotectal projection after optic nerve crush in the frog: Evidence for synaptic remodeling during regeneration. *Soc. Neurosci. Abstr.* 8: 514.
- Bacon, J. P., and J. S. Altman (1977) A silver intensification method for cobalt-filled neurones in wholemount preparations. *Brain Res.* 138: 359–363.
- Brogan, R. T., and R. M. Pitman (1981) Axonal regeneration in an identified insect motoneuron. *J. Physiol. (Lond.)* 319: 34P.
- Conway, K., K. Feiock, and R. K. Hunt (1980) Polyclones and patterns in growing *Xenopus* eye. *Curr. Top. Dev. Biol.* 15: 217–317.
- Cook, J. E. (1982) Errant optic axons in the normal goldfish retina reach retinotopic tectal sites. *Brain Res.* 250: 154–158.
- Currie, J., and W. M. Cowan (1975) The development of the retino-tectal projection in *Rana pipiens*. *Dev. Biol.* 46: 103–119.
- Easter, S. S., and C. A. O. Stuermer (1984) An evaluation of the hypothesis of shifting terminals in goldfish optic tectum. *J. Neurosci.* 4: 1052–1063.
- Fraser, S. E. (1983) Fiber optic mapping of the *Xenopus* visual system shift in the retinotectal projection during development. *Dev. Biol.* 95: 505–511.
- Fujisawa, H. (1981) Persistence of disorganized pathways and tortuous trajectories of regenerating retinal fibers in the adult newt *Cynops pyrrhogaster*. *Dev. Growth Differentiation* 23: 215–219.
- Fujisawa, H., N. Tani, K. Watanabe, and Y. Iwata (1982) Branching of regenerating retinal axons and preferential selection of appropriate branches for specific neuronal connections in the newt. *Dev. Biol.* 90: 43–57.
- Gaze, R. M. (1958) Regeneration of the optic nerve in *Xenopus laevis*. *Q. J. Exp. Physiol.* 44: 290–308.
- Gaze, R. M. (1970) *Formation of Nerve Connections*, Academic Press, Inc., New York.
- Gaze, R. M., and M. Jacobson (1963) A study of the retinotectal projection during regeneration of the optic nerve in the frog. *Proc. R. Soc. Lond. (Biol.)* 157: 420–448.
- Gaze, R. M., and M. J. Keating (1970) Further studies on the restoration of the contralateral retinotectal projection following regeneration of the optic nerve in the frog. *Brain Res.* 21: 183–207.
- Gaze, R. M., M. J. Keating, and S. H. Chung (1974) The evolution of the retinotectal map during development in *Xenopus*. *Proc. R. Soc. Lond. (Biol.)* 185: 301–330.
- Glastonbury, J., and K. Straznicky (1978) Aberrant ipsilateral retinotectal projection following optic nerve section in *Xenopus*. *Neurosci. Lett.* 7: 67–72.
- Grant, P., E. Rubin, and C. Cima (1980) Ontogeny of the retina and optic nerve in *Xenopus laevis*. I. Stages in the early development of the retina. *J. Comp. Neurol.* 189: 593–613.
- Harris, W. A., C. E. Holt, T. Smith, and N. Gallenson (1985) Growth cones of developing retinal cells *in vivo*, on culture surfaces and in collagen matrices. *J. Neurosci. Res.* 13: 101–122.
- Harrison, R. G. (1910) The outgrowth of the nerve fiber as a mode of protoplasmic movement. *J. Exp. Zool.* 9: 787–846.
- Holt, C. E. (1982) The development of the eye and its central connections in *Xenopus*. Ph.D. thesis, University of London.
- Holt, C. E. (1984) Does timing of axon outgrowth influence retinotectal topography in *Xenopus*? *J. Neurosci.* 4: 1130–1152.
- Holt, C. E., and W. A. Harris (1983) Order in the initial retinotectal map in *Xenopus*: A new technique for labelling growing nerve fibres. *Nature* 301: 150–152.
- Horner, T. J., and K. A. C. Martin (1978) Morphogenetics as an alternative to chemospecificity in the formation of nerve connections. *Symp. Soc. Exp. Biol.* 32: 275–358.
- Hoskins, S. G., and P. Grobstein (1984) Induction of the ipsilateral retinothalamic projection in *Xenopus laevis* by thyroxine. *Nature* 307: 730–733.
- Hoskins, S. G., and P. Grobstein (1985a) Development of the ipsilateral retinothalamic projection in the frog *Xenopus laevis*. II. Ingrowth of optic nerve fibers and production of ipsilaterally projecting retinal ganglion cells. *J. Neurosci.* 5: 920–929.
- Hoskins, S. G., and P. Grobstein (1985b) Development of the ipsilateral retinothalamic projection in the frog *Xenopus laevis*. III. The role of thyroxine. *J. Neurosci.* 5: 930–940.
- Humphrey, M. F., and L. D. Beazley (1982) An electrophysiological study of early retinotectal projection patterns during optic nerve regeneration in *Hyla moorei*. *Brain Res.* 239: 595–602.
- Jacobson, M. (1962) The representation of the retina on the optic tectum of the frog. Correlation between retinotectal magnification factor and retinal ganglion cell count. *Q. J. Exp. Physiol.* 47: 170–178.
- Jacobson, M. (1978) *Developmental Neurobiology*, Holt, Rinehart and Winston, Inc., New York.
- Kennard, C. (1981) Factors involved in the development of ipsilateral retinothalamic projections in *Xenopus laevis*. *J. Embryol. Exp. Morphol.* 65: 199–217.
- Khalil, S. H., and G. Szekely (1978) The development of the ipsilateral retinothalamic projections in the *Xenopus* toad. *Acta Biol. Acad. Sci. Hung.* 27: 253–260.
- Knapp, H., F. Scalia, and W. Riss (1965) The optic tracts of *Rana pipiens*. *Acta Neurol. Scand.* 41: 325–355.
- Lazar, G. (1973) The development of the optic tectum in *Xenopus laevis*: A Golgi study. *J. Anat.* 116: 347–355.
- Levine, R. L. (1978) An autoradiographic analysis of the retinal projection in the frog *Xenopus laevis*: New observations in an anuran visual projection. *Brain Res.* 148: 202–206.
- Levine, R. L. (1980) An autoradiographic study of the retinal projection in *Xenopus laevis* with comparisons to *Rana*. *J. Comp. Neurol.* 189: 1–29.
- Maturana, J. R., J. Y. Lettvin, W. S. McCulloch, and W. T. Pitts (1960) Anatomy and physiology of vision in the frog (*Rana pipiens*). *J. Gen. Physiol.* 43 (Suppl. 2): 129–175.
- McLoon, S. C. (1982) Alterations in precision of the crossed retinotectal projection during chick development. *Science* 215: 1418–1420.
- Merrill, E. G., and R. V. Stirling (1982) HRP intracellular recordings from single frog optic axons: Preliminary observations on the functional morphology of ganglion cells, their axons and central arbors. *J. Physiol. (Lond.)* 310: 25P.
- Meyer, R. L. (1980) Mapping the normal and regenerating retinotectal projection of goldfish with autoradiographic methods. *J. Comp. Neurol.* 189: 273–289.
- Mollenhauer, H. H. (1964) Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* 39: 111–114.
- Murphey, R. K., S. E. Johnson, and D. S. Sakaguchi (1983) Anatomy and physiology of supernumerary cercal afferents in crickets: Implication for pattern formation. *J. Neurosci.* 3: 312–325.
- Murray, M., and M. A. Edwards (1982) A quantitative study of the reinnervation of the goldfish optic tectum following optic nerve crush. *J. Comp. Neurol.* 209: 363–373.
- Nieuwkoop, P. D., and J. Faber (1967) *A Normal Table of Xenopus laevis (Daudin)*, Ed. 2, pp. 227, 607–617, Elsevier/North Holland Publishing Co., Amsterdam.
- Potter, H. D. (1969) Structural characteristics of cell and fiber populations in the optic tectum of the frog (*Rana catesbeiana*). *J. Comp. Neurol.* 136: 203–232.
- Potter, H. D. (1972) Terminal arborizations or retinotectal axons in the bullfrog. *J. Comp. Neurol.* 144: 267–283.
- Ramón y Cajal, S. (1890) Sur l'origine et les ramifications des fibres nerveuses de la moelle embryonnaire. *Anat. Anz.* 5: 609–631.
- Raymond, P. A., and S. S. Easter, Jr. (1983) Postembryonic growth of the optic tectum in goldfish. I. Location of germinal cells and numbers of neurons produced. *J. Neurosci.* 3: 1077–1091.
- Reh, T. A., and M. Constantine-Paton (1984) Retinal ganglion cell terminals change their projection sites during larval development of *Rana pipiens*. *J. Neurosci.* 4: 442–457.
- Rehbein, H., K. Kalmring, and H. Romer (1974) Structure and function of acoustic neurons in the thoracic ventral nerve cord of *Locusta migratoria* (Acrididae). *J. Comp. Physiol.* 95: 263–280.
- Sachs, G. M., and G. E. Schneider (1984) The morphology of optic tract axons arborizing in the superior colliculus of the hamster. *J. Comp. Neurol.* 230: 155–167.
- Sakaguchi, D. S., and R. K. Murphey (1984) Initial development of the retinotectal projection in *Xenopus*: An examination of retinal ganglion cell terminal arborizations. *Soc. Neurosci. Abstr.* 10: 669.

- Sakaguchi, D. S., R. Tompkins, R. K. Hunt, and R. K. Murphey (1982) Single cell techniques for the developing *Xenopus* retinotectal system. Soc. Neurosci. Abstr. 8: 515.
- Sakaguchi, D. S., R. K. Murphey, R. K. Hunt, and R. Tompkins (1984) The development of retinal ganglion cells in a tetraploid strain of *Xenopus laevis*: A morphological study utilizing intracellular dye injection. J. Comp. Neurol. 224: 231–251.
- Scalia, F., H. Knapp, M. Halpern, and W. Riss (1968) New observations on the retinal projection in the frog. Brain Behav. Evol. 1: 324–353.
- Schmidt, J. T., M. J. Buzzard, and J. Turcotte (1984) Morphology of regenerated optic arbors in goldfish tectum. Soc. Neurosci. Abstr. 10: 667.
- Schneider, G. E., L. Rava, G. M. Sachs, and S. Jhaveri (1981) Widespread branching of retinotectal axons: Transient in normal development and anomalous in adults with neonatal lesions. Soc. Neurosci. Abstr. 7: 732.
- Sperry, R. W. (1943) Visuomotor coordination in the newt (*Triturus viridescens*) after regeneration of the optic nerves. J. Comp. Neurol. 79: 33–55.
- Sperry, R. W. (1963) Chemoaffinity in the orderly growth of nerve fiber patterns and connections. Proc. Natl. Acad. Sci. U. S. A. 50: 703–710.
- Sretavan, D., and C. J. Shatz (1984) Prenatal development of individual axons during the period of segregation. Nature 308: 845–848.
- Steedman, J. G., R. V. Stirling, and R. M. Gaze (1979) The central pathways of optic fibres in *Xenopus laevis*. J. Embryol. Exp. Morphol. 50: 199–215.
- Straznicky, K., and R. M. Gaze (1972) The development of the tectum in *Xenopus laevis*: An autoradiographic study. J. Embryol. Exp. Morphol. 28: 87–115.
- Straznicky, C., D. Tay, and J. Glastonbury (1980) Regeneration of an abnormal ipsilateral visuotectal projection in *Xenopus* is delayed by the presence of optic fibres from the other eye. J. Embryol. Exp. Morphol. 57: 129–141.
- Stuermer, C. A. O. (1984) Rules for retinotectal terminal arborizations in the goldfish optic tectum: A wholemount study. J. Comp. Neurol. 229: 214–232.
- Stuermer, C. A. O., and S. S. Easter, Jr. (1984) A comparison of the normal and regenerated retinotectal pathways of goldfish. J. Comp. Neurol. 223: 57–76.
- Thanos, S., and R. Bonhoeffer (1983) Investigations on the development and topographic order of retinotectal axons: Anterograde and retrograde staining of axons and perikarya with rhodamine *in vivo*. J. Comp. Neurol. 219: 420–430.
- Toth, P., G. Lazer, and T. Gorcs (1980) Retinal projections in the adult *Xenopus laevis*: A study with cobalt filling technique. Acta Morphol. Acad. Sci. Hung. 28: 365–374.
- Westerman, R. A. (1965) Specificity of optic and olfactory pathways in teleost fish. In *Studies in Physiology*, D. R. Curtis and A. K. McIntyre, eds., pp. 263–269, Springer-Verlag, Berlin.
- Witkovsky, P., E. Gallin, J. G. Hollyfield, H. Ripps, and C. D. B. Bridges (1976) Photoreceptor thresholds and visual pigment levels in normal and vitamin A-deprived *Xenopus* tadpoles. J. Neurophysiol. 39: 1272–1287.