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Development of the Ipsilateral Retinothalamic Projection in the Frog *Xenopus laevis*

I. Retinal Distribution of Ipsilaterally Projecting Cells in Normal and Experimentally Manipulated Frogs[1]

SALLY G. HOSKINS[2] AND PAUL GROBSTEIN

Departments of Biology and of Pharmacological and Physiological Sciences, University of Chicago, Chicago, Illinois 60637

Abstract

The distribution of ipsilaterally and contralaterally projecting cells within the retina in *Xenopus laevis* was studied by injection of horseradish peroxidase into the thalamus on one side of the brain and subsequent determination of the locations of retrogradely labeled cells in both retinas. In normal animals, contralaterally projecting cells were found throughout the retina. Ipsilaterally projecting cells, in contrast, were most frequent in temporoventral retina and largely absent from dorsonasal retina as well as from a region surrounding the nerve head. A similarly restricted distribution of ipsilaterally projecting cells was observed in retinas of animals after regeneration of one optic nerve as well as in animals from which one eye was removed prior to the time when the ipsilateral projection first develops.

The restricted distribution of ipsilaterally projecting cells in normal animals raises the possibility that these cells may be produced relatively late in development. This hypothesis is explored in the following paper (Hoskins, S. G., and P. Grobstein (1985) J. Neurosci. *5:* 920-929). The fact that similar distributions were seen in normal and experimental animals implies that organization of the ipsilateral retinothalamic projection in *X. laevis* is not critically dependent either on particular patterns of axonal organization which may be present during normal development or on interactions among fibers from the two eyes.

The ipsilateral retinothalamic projection of *Xenopus* is the major direct retinal projection to the ipsilateral side of the brain (Levine, 1980). It is also distinctive in that it develops postembryonically. Projections from the retina to the contralateral thalamus and tectum begin to form early in embryogenesis (Gaze et al., 1974; Grant and Ma, 1983; Holt and Harris, 1983) and are well developed in the tadpole. Projections to the ipsilateral thalamus, in contrast, are not formed until late in development, near the time of metamorphosis (Currie and Cowan, 1974; Khalil and Szekely, 1976; Hoskins and Grobstein, 1981; Kennard, 1981). Little is known regarding the events which influence optic nerve development at these late stages and bring about the development of an uncrossed projection to the thalamus. In this and the following two papers (Hoskins and Grobstein, 1985a, b), we report studies on the development of the ipsilateral retinothalamic projection. In the present paper we define the retinal regions which give rise to ipsilaterally projecting axons in normal frogs and show that the projection arises from the same portion of the retina in frogs with regenerated optic nerves and in frogs with only one eye. The second paper (Hoskins and Grobstein, 1985a) examines the morphogenesis of ipsilateral terminal fields and shows that the vast majority of neurons whose axons project ipsilaterally are themselves born late in development. In the third paper (Hoskins and Grobstein, 1985b) we show that a hormone, thyroxine, is required for the development of an ipsilateral retinothalamic projection and that it can induce the development of the projection in the absence of other usually concurrent metamorphic changes.

The factors which influence the pattern formed by retinal axons at their targets in the CNS have been investigated extensively (reviewed by Hunt and Jacobson, 1974; Horder and Martin, 1978; Fraser and Hunt, 1980; Hollyday and Grobstein 1981). Such investigations have characterized a variety of mechanisms which may be involved in ensuring the topographic ordering of retinal projections once the axons have reached their targets in the CNS. Less attention has been given to the antecedent question of why some fibers cross and others run ipsilaterally in the optic chiasm. The possibility that axons of retinal ganglion cells might be chemically "labeled" so as to project only to the right or left side of the brain was tested in the amphibian retinotectal system by transplanting an eye to the opposite side of the head (Sperry, 1945b; Beazley, 1975b). The regenerated axons still projected contralaterally at the optic chiasm, to the tectal lobe on the opposite side of the brain, suggesting that their trajectories were not controlled by right/left "labels." The timing of axon outgrowth and the spatial relationships of axons to their neighboring fibers have been shown to influence patterns of projection in some neural systems (Gottlieb and Cowan, 1972; Macagno, 1978). In the frog, the observation that regenerating optic nerves sometimes make aberrant ipsilateral projections (Gaze and Jacobson, 1963; Beazley, 1975a; Bohn and Stelzner, 1981) is consistent with this possibility, since cutting an optic nerve causes both a delay in development and the disruption of axonal order within the nerve (Attardi and Sperry, 1963). Besides fiber relationships within one optic nerve, fiber interactions between axons of the two optic nerves

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This series is dedicated by S.G.H. to the memory of Dr. Ray L. Watterson, mentor and friend.

[2] To whom correspondence should be sent, at her present address: Department of Biological Sciences, Columbia University, 901 Fairchild Center, New York, NY 10027.

have been proposed to affect the patterns of projection made by axons in the optic chiasm (Lund, 1975). In a variety of neonate mammals, removal of one eye is followed by an increase in size of the ipsilateral retinocollicular projections from the remaining eye (Lund, 1975, 1978). In *Xenopus*, removal of one eye at late tadpole stages can result in the formation of an unusual projection to the ipsilateral tectal lobe (Fraser, 1978).

As a first step in an analysis of the development of the ipsilateral retinothalamic projection in *X. laevis*, we used retrograde transport of horseradish peroxidase (HRP) to define the retinal regions which contain substantial numbers of ipsilaterally projecting retinal ganglion cells in adult frogs. To investigate whether interrelationships of axons within an optic nerve or interactions between fibers from the two optic nerves were influential in establishing the normal pattern of projection to the ipsilateral thalamus, we also examined the retinal locations of ipsilaterally projecting ganglion cells in frogs with regenerated optic nerves and in one-eyed frogs. A preliminary report of some of these observations has appeared (Hoskins and Grobstein, 1980).

Materials and Methods

In most of the frogs described in this paper, retrograde transport of HRP was used to define the distribution of ipsilaterally projecting retinal ganglion cells. In a few animals, anterograde transport of HRP was used to reveal the ipsilateral and contralateral projections made by regenerated optic nerves or by nerves which developed in one-eyed animals.

Anterograde transport of HRP. Frogs were anesthetized in 10^{-4} to 5×10^{-5} M tricaine methanesulfonate (MS-222) and placed ventral side up in a dish coated with Sylgard. The lower jaw was propped open, and the optic nerve was exposed by cutting through the soft palate with a scalpel. The intact nerve was blotted and then cut with iridectomy scissors or with a fresh scalpel blade. Crystals of HRP (type VI, Sigma) were immediately applied to the surface of the cut nerve and held in place with a small piece of Gelfoam. The frog was left in this position for 15 to 30 min and then returned to a plastic tank containing 10% Holtfreter's solution. Two to 4 days later the animal was anesthetized with MS-222 and killed by perfusion, first with anesthetic and then with fixative (1% paraformaldehyde/2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4). The brain was removed and embedded in gelatin/albumen, and the gel block was left overnight in the refrigerator in a solution of 30% sucrose and 10% formalin in 0.1 M phosphate buffer, pH 7.4. The following day, frozen sections were cut at 40 μm in the transverse plane using a sliding microtome. Sections were collected sequentially in 0.1 M Tris buffer (pH 7.6) and processed through solutions of 1 mg/ml of diaminobenzidene (DAB) and 0.01% hydrogen peroxide in 0.1 M phosphate buffer after pretreatment with 0.5% cobalt chloride in a pH 7.6 Tris buffer (Adams, 1977). Sections were mounted on gelatinized slides, allowed to dry overnight on a slide warmer, and stained with cresyl violet.

Retrograde transport of HRP. For injections of HRP, frogs were anesthetized as above. A skull flap was removed at the level of the thalamus, the meninges were slit, and a glass pipette filled with a concentrated solution of HRP (about 20%) in distilled water was inserted. Several small pressure injections (50 to 100 nl each) were made at a range of depths throughout the rostral thalamus on one side of the brain. The skull flap was then replaced and the skin was sutured. Two to 4 days later the animals were anesthetized by immersion in 10^{-4} M MS-222 and then perfused intracardially with anesthetic. The eyes were then removed, and the neural retinas were dissected away from the pigment layer. Lenses were discarded and the retinas were fixed for 2 to 4 min in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. During the dissection, small cuts were made in the retinas to facilitate later identification of dorsal, ventral, nasal, and temporal quadrants. The retinas were subsequently reacted intact in DAB and 0.01% hydrogen peroxide for 30 min, flattened onto slides coated with 10% gelatin, cleared in alcohols and xylene, stained briefly with cresyl violet, and coverslipped (Peterson and Ulinski, 1979). After the eyes were removed the frogs were perfused with paraformaldehyde/glutaraldehyde fixative and the brains were handled as described above. Transverse frozen sections were cut at 40 μm and reacted in DAB for visualization of the injection sites. These were sketched using a camera lucida and a Zeiss drawing tube at × 4 to 10 magnification. The distribution of retrogradely labeled cells in ipsilateral and contralateral retinas was determined by counting the numbers of HRP-labeled ganglion cells in adjacent microscope fields viewed at × 40 (objective magnification) and distributed across the entire retinal surface. Total numbers of labeled cells per microscope field (350 μm in diameter) were plotted on an outline drawing of the retina (see Fig. 3, for example). Controls included DAB-processed retinas from frogs not injected with HRP (reaction product was seen in photoreceptors and in a few epithelial cells of blood vessels; not in ganglion cells) and retinas processed from animals which had received large doses of HRP directly into the third ventricle (occasional faint granular label in large cells).

For the regeneration study, young frogs were anesthetized as above. One optic nerve was exposed and either cut with sharpened forceps or crushed between flattened forceps blades until a cleared area was visible within the nerve sheath. Injections of HRP were made into the thalamus ipsilateral to the cut or crushed nerve, 4 to 32 weeks later.

One-eyed frogs were produced by removing one eye from tadpoles staged according to the atlas of Nieuwkoop and Faber (1967). The animals were anesthetized and the extraocular muscles and connective tissue surrounding the eye were cut with iridectomy scissors. The optic nerve was then severed and the eyeball was removed. Healing after such operations was rapid and uneventful. After these tadpoles completed metamorphosis, the distribution of ipsilaterally or contralaterally projecting cells in their remaining retina was assessed using the methods described above.

Results

Characteristics of normal retrograde labeling. Figure 1 shows an injection site in the rostral thalamus typical of those used to determine the retinal distribution of ipsilaterally projecting cells. HRP reaction product is dense in the region of the nucleus of Bellonci (*NB*), the largest of the three rostral thalamic terminal zones in adult *Xenopus* (Levine, 1980). In adjacent sections, HRP was evident in the other two terminal zones, the corpus geniculatum thalamicum and rostral visual nucleus, as well. Figure 2*A* shows a retinal whole mount from the eye ipsilateral to such an injection site. Fibers filled with HRP can be seen running from the retinal periphery to the nerve head (Fig. 2*B*), and numerous solidly filled cells are evident in the periphery (Fig. 2*C*). In some labeled retinal ganglion cells the dendrites were also filled with HRP reaction product, whereas in others the reaction product was confined to perikarya. Additional retinal ganglion cells contained granular deposits of reaction product. The *numbers* on the retinal profiles in the following figures represent the total number of solidly and granularly labeled cells per microscope field. These numbers, particularly in the ipsilateral retinas, are small relative to the total number of ganglion cells in a field, on the order of 10% or

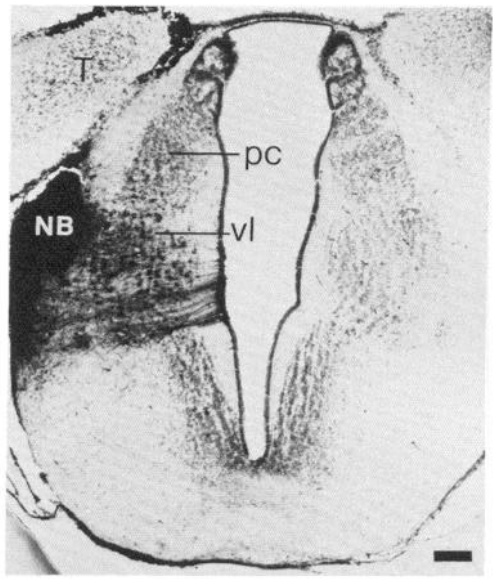

Figure 1. HRP injection site in the rostral thalamus of an adult *X. laevis*: a transverse section taken at the level of the caudal pole of the telencephalon (*T*). Dorsal is *up*; ventral is *down*. The nucleus posterocentralis (*pc*) and nucleus ventrolateralis (*vl*) are indicated. Dense deposits of reaction product mark the area into which HRP was injected. The nucleus of Bellonci (*NB*) is heavily labeled. Reaction product was also found in the corpus geniculatum thalamicum and in the rostral visual nucleus in other sections. *Scale bar*, 400 μm.

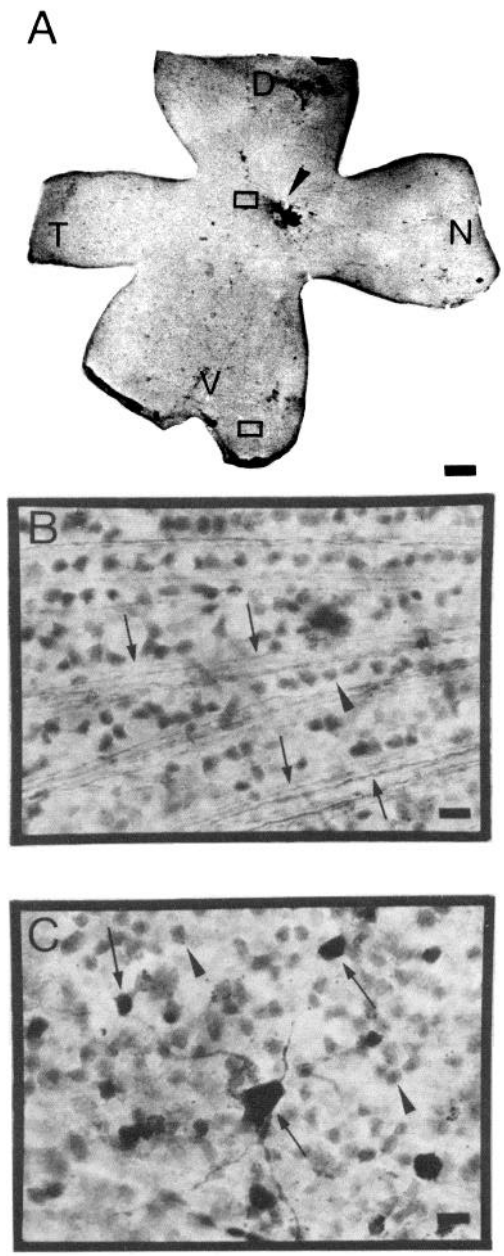

Figure 2. *A*, A retinal whole mount from an adult eye ipsilateral to an injection site like the one in Figure 1. The retina has been flattened onto a slide, and the ganglion cell layer has been stained with cresyl violet. The optic nerve head is indicated (*arrowhead*). *D*, dorsal; *V*, ventral; *N*, nasal; *T*, temporal. The *boxed regions* in *A* are enlarged in *B* and *C*. At the level of the *upper boxed region* (*B*), near the optic nerve head, HRP-stained fibers are apparent (*arrows*), but retinal ganglion cells (*arrowhead*) are not stained with HRP. In the retinal periphery (*C*), numerous HRP-stained retinal ganglion cells are apparent (*arrows*), intermingled with cells which do not contain HRP (*arrowheads*). *Scale bars*: *A*, 900 μm; *B* and *C*, 25 μm.

less. In a few cases, a significant amount of pale granular label was seen in ganglion cells throughout the retina. This usually occurred in frogs with very large injections of HRP. Injection sites in these animals showed large deposits of reaction product at the ventricle. The diffuse labeling was attributed to generalized uptake of HRP from the ventricle, since the palely labeled cells seen resembled those observed after deliberate injection of HRP into the ventricle. The granular label in these cases was considered background, and the retinas of these few animals were scored only for solidly filled cells.

The retinal origin of the ipsilateral retinothalamic projection in normal Xenopus. After injecting HRP into the rostral thalamus on one side of the brain, we determined the distributions of HRP-labeled cells in both retinas of 18 animals. Three examples are presented in Figure 3. The most extensive distribution of labeled cells was observed in cases in which histological examination of the injection sites showed heavy deposits of reaction product centered in the area of the rostral thalamic terminal zones. The *top pair* of retinas in Figure 3 is representative of eight cases of this kind. The retinas contralateral to the injection site contained labeled ganglion cells in all regions. In retinas ipsilateral to the injection site, large numbers of HRP-filled cells were seen in ventral and dorsotemporal retina, but labeled cells were sparse or absent from most of nasodorsal retina, as well as from a central region surrounding the optic nerve head.

The *middle* and *lower pairs* of retinas in Figure 3 exemplify labeling patterns obtained in cases in which histological examination of injection sites showed them to be slightly medial (the middle pair and five similar cases) or ventral (the lower pair and three similar cases) to the rostral thalamic terminal zones. The labeling patterns were in general quite similar to those of the uppermost retinal pair. Again, the contralateral retinas contained larger numbers of labeled cells. In general, labeling was found throughout the contralateral retina, although it was sometimes sparse or absent at particular locations in individual cases. Ipsilateral retinas contained fewer HRP-labeled neurons, and these were always found in subregions of the areas labeled by the more centrally located injections. In no case did an injection produce labeling in substantial number of cells of the ipsilateral nasodorsal retina.

Since the major ipsilateral terminal zones are clustered in the rostral thalamus (Levine, 1980), injections of HRP were directed at this area in the majority of cases. In addition, we made small injections of HRP into the caudal thalamus of several frogs, to determine the distributions of cells which project to the ipsilateral uncinate and thalamopretectal fields, a second group of terminal zones which receive a minor ipsilateral projection (Levine, 1980). The small number of labeled ganglion cells found in retinas ipsilateral to these injection sites always lay within the region of the ipsilateral retina that contained labeled cells after an injection of HRP into the rostral thalamus.

Our results indicate that the ipsilateral retinothalamic projection derives from a large part but not from the entirety of the retina. Regardless of the locations of injections, we did not see significant numbers of labeled cells in the nasodorsal quadrant of the ipsilateral retina or in a region surrounding the nerve head. In contrast, labeled cells were found in all parts of the contralateral retinas. Our results indicate that the retina of the adult frog consists of a nasodorsal and central region, almost no cells of which project ipsilaterally, and a remaining region which contains both contralaterally and ipsilaterally projecting ganglion cells. Given the locations of our injections, and the fact that there is at most a sparse direct retinal projection to the ipsilateral tectal lobe (Levine, 1980), we are confident that the observed distribution of labeled cells in the ipsilateral retina indicates the source of the uncrossed retinothalamic projection. We suspect that the distribution of labeled cells in the contralateral retina similarly reflects the source of the crossed retinothalamic projection, but cannot wholly exclude the possibility of some labeling in the contralateral retina resulting from damage to crossed retinotectal fibers passing near the injection site.

The retinal origin of the ipsilateral projection in frogs with regenerated optic nerves. The re-establishment of the normal crossed optic nerve projection to the tectum following optic nerve regeneration has been documented extensively (Gaze, 1960; Gaze and Jacobson, 1963). However, the organization of the thalamic components of the optic nerve projection following regeneration has not been examined systematically. Figure 4, *top*, shows a cross-section through the rostral thalamus in a young frog whose optic nerve was cut 6 months earlier. Anterograde transport of HRP in the regenerated optic nerve labeled the usual ipsilateral and contralateral thalamic terminal zones, both in the section illustrated and throughout

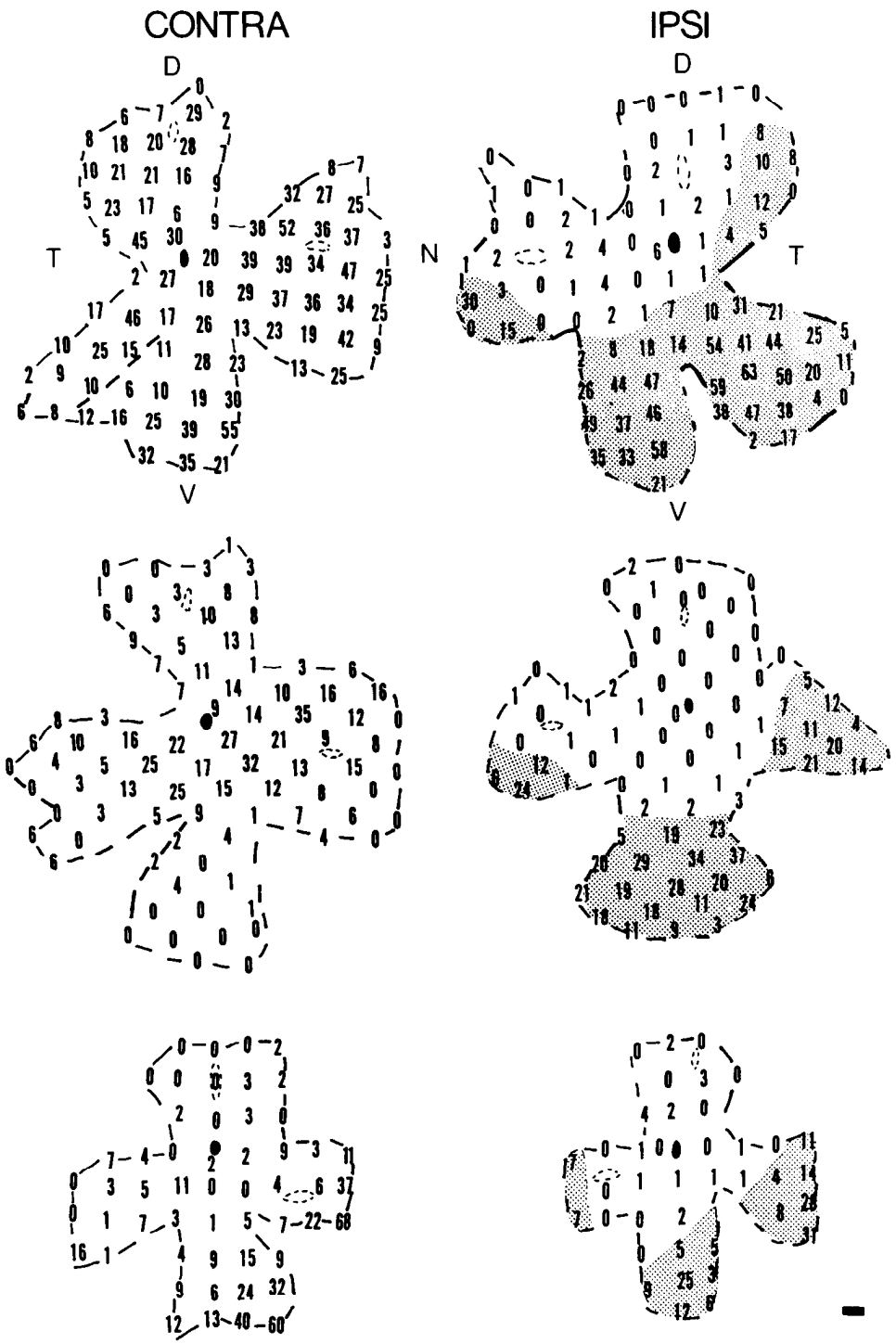

Figure 3. Distribution of HRP-labeled ganglion cells in retinas of three normal adult *X. laevis* after injection of HRP into rostral thalamus on one side of the brain. Each *pair* of *retinas* is oriented as if the frog were facing the reader. *D*, dorsal; *V*, ventral; *N*, nasal; *T*, temporal, as indicated for the top pair; the other two paris are oriented similarly. *IPSI*, ipsilateral to the injection site; *CONTRA*, contralateral to the injection site. The centrally located *black oval* in each *profile* represents the position of the optic nerve head. *Dashed ovals* seen in some retinas represent the edges of small slits which were cut for purposes of orientation. Each *number* represents the number of HRP-labeled cells seen in a × 40 field at that point on the retinal surface; in all cases this was substantially fewer cells than were present in the entire field. The *shaded regions* contained the largest numbers of ipsilaterally projecting cells. The border of the *shaded region* is somewhat arbitrary and is meant only to draw the reader's attention to areas of the retina which contain substantial numbers of ipsilaterally projecting retinal ganglion cells. The *uppermost pair* of retinas represents the cases in which all of the rostral thalamic terminal fields were exposed to HRP, as determined by an assessment of the injection sites. The *middle pair* represents cases in which the injection site was slightly medial to the nucleus of Bellonci and *corpus geniculatum thalamicum* terminal fields. The *lower pair* represents cases in which the injection site was slightly ventral. Retinas came from animals of different ages and hence vary in size. *Scale bar*, 300 μm.

the brain. Thus, the normal bilateral projection to the thalamus, like the crossed retinotectal projection, is re-established during optic nerve regeneration.

To determine the retinal source of the regenerated ipsilateral retinothalamic projection, we used retrograde transport of HRP to study 21 animals. One to 8 months after one optic nerve had been cut or crushed, HRP was injected into the rostral thalamus ipsilateral to that nerve. Retinas and brains were processed as described previously, for histochemical demonstration of the HRP reaction product.

The results of this group of experiments fell into two classes. In one group of frogs, no labeled cells were found in ipsilateral retinas although the contralateral retinas were, as usual, labeled in all regions. This result was most often obtained in frogs whose optic nerves had been cut (7 of 15 cases) and was rarely seen after optic nerve crush (1 of 6 cases). Ipsilateral retinas in such cases typically appeared cell sparse and had unusually small optic nerve heads. In some cases only one optic nerve (the contralateral one, which had not been cut) was seen clearly at the optic chiasm when the brains were dissected. It seems likely that these were cases in which regeneration failed to occur, possibly due to disruption of retinal circulation at the time of optic nerve section. Another possibility is that severing the optic nerve in some cases causes degeneration of substantial numbers of retinal ganglion cells (Beazley, 1981). The higher incidence of nonregeneration after optic nerve cuts as opposed to crushes may indicate that there is greater trauma resulting

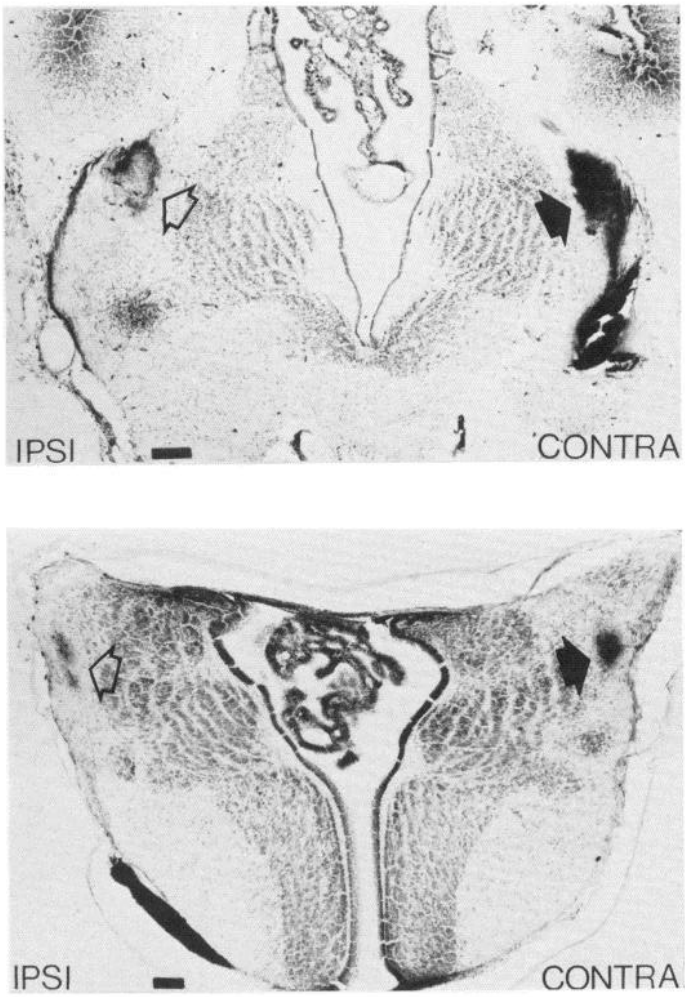

Figure 4. Top, Thalamic projections of a regenerated optic nerve, as revealed by anterograde transport of HRP: transverse section, oriented as in Figure 1. Reaction product is evident in both the ipsilateral (*open arrow*) and contralateral (*solid arrow*) nucleus of Bellonci terminal fields. *Scale bar* = 200 μm. *Bottom*, Thalamic projections of the optic nerve in a one-eyed frog, as revealed by anterograde transport of HRP. Conventions are the same as in the *top panel*. Reaction product is apparent in the nucleus of Bellonci on both sides of the brain. This animal was enucleated unilaterally at stage 48. *Scale bar* = 200 μm.

from a nerve cut, and a consequent greater interference with the process of regeneration.

In the remaining 13 frogs, labeled neurons were found in the ipsilateral retina following nerve regeneration and injection of HRP into the thalamus. In the majority of cases, the pattern of labeling in contralateral and ipsilateral regions of retina was the same as that seen in normal animals. An example is shown in Figure 5. In the remaining cases, there was as usual no substantial number of labeled cells in nasodorsal retina, but the total extent of labeled retina was smaller than usual, and the labeled cells tended to be clustered at the retinal periphery. Neither the time course of optic nerve regeneration to the thalamus nor the possibility that different retinal regions regenerate at different rates was examined systematically in this study. However, at no time (ranging from 4.5 to 32 weeks) after optic nerve section did we find significant numbers of ganglion cells labeled in nasodorsal retina after HRP was injected into the ipsilateral thalamus. Whether the cases of diminished retinal labeling represent animals analyzed before the regeneration process was complete or are a true abnormality caused by optic nerve section remains to be determined. Regardless, these results indicate that the disruption of the normal timing of ingrowth and normal neighbor relationships of axons effected by the optic nerve section (Attardi and Sperry, 1963; Fawcett and Gaze, 1981) did not alter the laterality of projections made by fibers from different retinal regions at the optic chiasm.

The retinal origin of the ipsilateral retinothalamic projection in one-eyed frogs. To test whether interactions between fibers from the two eyes are necessary for accurate routing of axons from different retinal regions in the optic chiasm, we removed one eye from tadpoles at stages 39 to 48 (eight cases), well before the ipsilateral projection normally appears, or at stages 54 to 57 (four cases), when the projection is just beginning to develop, and reared the animals through metamorphosis. In a few such one-eyed frogs the optic nerve was cut and exposed to crystals of HRP as described previously, to check whether the usual bilateral thalamic projection was established from the remaining eye. In these cases HRP was transported anterogradely to terminal zones in both ipsilateral and contralateral thalamus. Thus, like those of normal frogs, the retinothalamic projections of one-eyed frogs are bilateral. An example is illustrated in Figure 4, *bottom*.

Retrograde transport of HRP was used to determine the distribution of ipsilaterally projecting retinal ganglion cells in the remaining one-eyed frogs. The labeling patterns in seven of the eight animals of the early-enucleation group were like those of normal frogs. A representative example from this group is diagrammed in the *upper part* of Figure 6. Labeled cells are present in temporal and ventral regions, and at the nasoventral and dorsotemporal peripheries. Few labeled cells were found centrally or in nasodorsal retina. The labeling was not this extensive in all cases, displaying the same range of variability of retinal distribution with injection site as did the normal cases. In none of these seven animals were significant numbers of labeled cells found at unusual locations. In the eighth animal, however, nasodorsal retina did have significant numbers of labeled cells in a few microscope fields. We are uncertain why the projections in this animal were different from those of the other seven. Unusual projections are occasionally found in ostensibly "normal" frogs; for example, axons are sometimes seen running from one optic nerve into the other at the optic chiasm (Bohn and Stelzner, 1981), or making atypical direct ipsilateral retinotectal projections (Levine, 1980). The retinofugal organization in this case may reflect the normal variability in a population of frogs. Regardless, the results in the other seven cases make it clear that the normal distribution of ipsilaterally and contralaterally projecting retinal regions can be established in the absence of interactions among axons from the two eyes.

In the case of the retinotectal projection of *Xenopus*, aberrant ipsilateral projections were not found if one eye was removed at early stages, but were seen after eye removals beginning at stage 56 (Fraser, 1978). To see whether a similar effect could be demonstrated in the case of the retinothalamic projection, we repeated the eye removal experiment in four frogs unilaterally enucleated at stages 54 to 57. Stage 54 marks the beginning of the period during which the ipsilateral retinothalamic projection develops (Hoskins and Grobstein, 1985a); thus, axons approaching the optic chiasm at or after stage 54 may encounter different environmental conditions than did axons which arrived earlier. A retina from a frog unilaterally enucleated at stage 54 and analyzed 5.5 months later is shown in the *lower part* of Figure 6. Again, temporoventral peripheral retina is the primary source of ipsilaterally projecting fibers. Similar distributions of ipsilaterally projecting retinal ganglion cells were obtained in the other three cases. Ganglion cells of the nasodorsal and central retinal regions showed no alterations in patterns of projection as a result of eye removal. This evidence indicates that the same retinal regions project ipsilaterally whether the frog has one or two eyes.

In several one-eyed frogs, we injected HRP into the thalamus *contralateral* to the remaining eye. In such cases, labeled cells were found throughout the contralateral retina, including the nasodorsal and central regions. Thus, both the ipsilateral and contralateral thalamic projections made by retinas of one-eyed frogs are like those

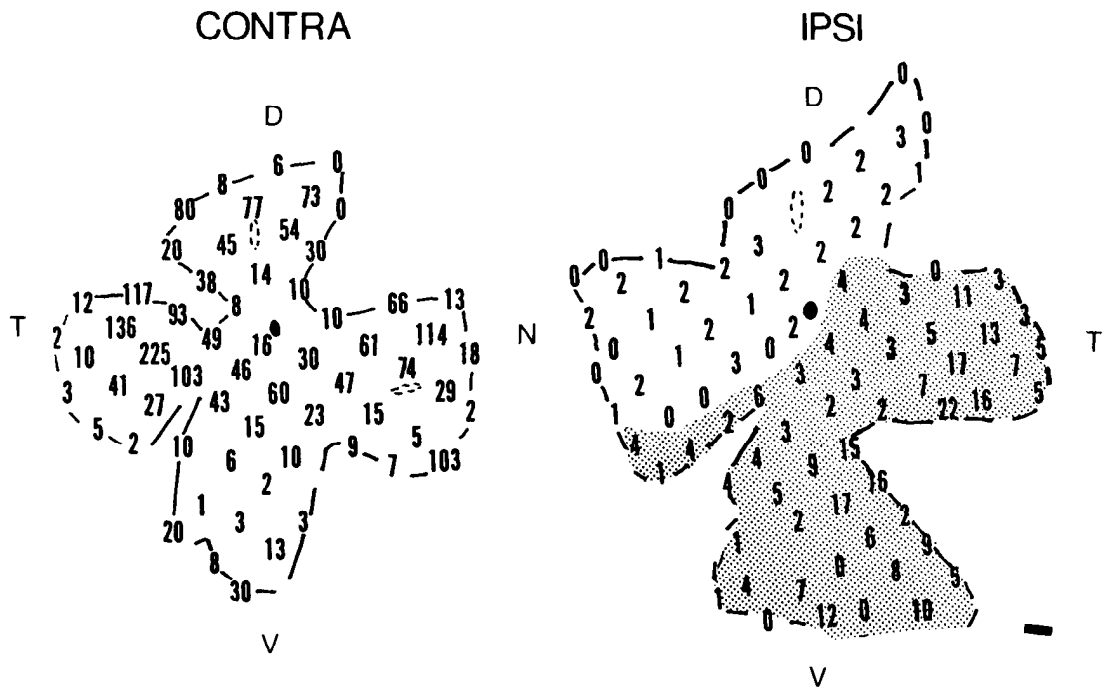

Figure 5. Distribution of HRP-labeled ganglion cells in a frog in which one optic nerve was cut 14 weeks earlier. The injection site was in the thalamus ipsilateral to the regenerated nerve. Conventions are as in Figure 3. *Scale bar* = 300 μm.

of normal two-eyed frogs. These results make it unlikely that any normally occurring interactions between fibers from the two eyes at the optic chiasm are of major importance in determining the patterns of projection made by ganglion cells from different retinal regions.

Discussion

Adult organization. Our results show that the ipsilaterally projecting portion of the retina is probably larger in *X. laevis* than in the ranid frogs which have been studied previously. In *Rana pipiens*, degeneration methods indicated that the projection originates primarily from temporal retina (Scalia and Fite, 1974). In *Rana esculenta*, Lazar (1971) used similar methods and concluded that the ipsilateral projection originated in peripheral temporal retina. The retrogradely labeled retinas presented here show that temporal retina is also a source of ipsilateral fibers in *X. laevis*, but ventral retina commonly is heavily labeled as well, and label at the retinal periphery often extends into dorsotemporal and nasoventral regions.

That these differences in the amount of ipsilaterally projecting retina of *R. pipiens* and *X. laevis* are not an artifact due to differences in experimental method is suggested by a recent report (Kennard, 1981) in which degeneration techniques were used to map regions of ipsilaterally projecting peripheral retina in *X. laevis*. Only nasal, nasodorsal, and dorsal peripheral lesions failed to produce degeneration in ipsilateral thalamic terminal fields. Lesions at all locations in peripheral retina produced degeneration in the contralateral thalamus. Our results extend those of Kennard (1981) by indicating the distribution of ipsilaterally projecting cells not only at the retinal periphery but throughout the retina. In our normal cases, the region of retina surrounding the nerve head contains few ipsilaterally projecting cells, and the largest numbers of ipsilaterally projecting cells are found at the retinal periphery. This distribution, coupled with the fact that the retina grows by adding new cells at the periphery (Hollyfield, 1971; Straznicky and Gaze, 1971; Jacobson, 1976; Beach and Jacobson, 1979), suggests that the cells which give rise to the late-developing ipsilateral projection may be members of a population born relatively late in development; an examination of this possibility is presented in the following paper (Hoskins and Grobstein, 1985a).

The difference between *Xenopus* and *Rana* in the extent of ipsilaterally projecting retina may be related to differences between the two species in the position of the eyes, and a consequent difference in the extent of binocular field (Grobstein and Comer, 1977), as also suggested by Kennard (1981). *X. laevis* has more dorsally positioned eyes and, consequently, a more extensive binocular visual field than does *R. pipiens*. The region of retina in *X. laevis* which contains ipsilaterally projecting cells is approximately situated so as to receive input from the binocular visual field, whereas dorsonasal retina is located so as to receive input from the monocular visual field. It may be, then, that the line which separates bilaterally from contralaterally projecting retina also divides binocular from monocular retina. The smaller binocular field of *R. pipiens* similarly would correlate with the less extensive ipsilateral projections in this animal.

Whether this is the case or not, the retina of *X. laevis* clearly consists of a dorsonasal and central region within which almost all ganglion cells project contralaterally, and a larger area comprising the rest of the retina and containing both ipsilaterally projecting and contralaterally projecting neurons. This pattern of organization differs from that classically thought of in connection with bilateral optic nerve projections—a line of decussation running through the retina and dividing purely ipsilaterally projecting from purely contralaterally projecting regions. Recent evidence in mammals suggests that a pattern like that of the frog, in which the entire retina projects contralaterally and a subregion projects ipsilaterally as well, may be more common than previously thought (Guillery, 1982).

The fact that the ipsilaterally projecting region of retina in *Xenopus* also gives rise to a contralateral projection raises the question of whether individual ganglion cells project bilaterally or whether, instead, there are distinct ipsilaterally projecting and contralaterally projecting populations. In mammalian visual systems in which retrograde double-labeling techniques have been employed, few bilaterally projecting neurons have been found (Jefferey and Perry, 1982; Hsiao et al., 1984), indicating that these retinas are probably composed primarily of separate populations of ipsilaterally projecting and contralaterally projecting neurons. Ipsilaterally projecting retinal ganglion cells in *X. laevis*, although they are distributed over a large portion of the retina, are relatively few in number. Clearly, not *all* cells of non-nasodorsal retina project ipsilaterally, since many cells

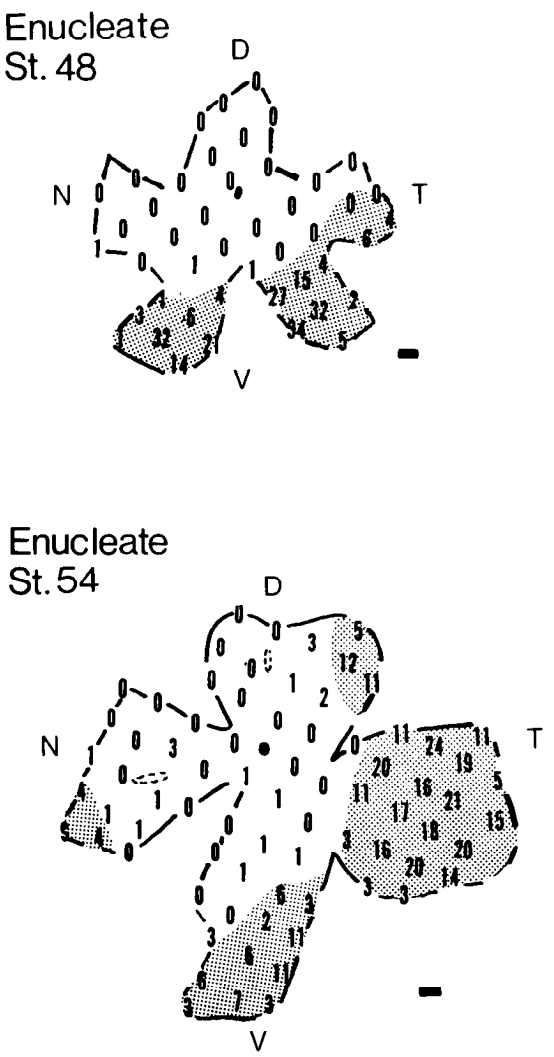

Figure 6. Distribution of HRP-labeled ganglion cells in the retinas of two one-eyed frogs. The *upper retina* is from an animal enucleated unilaterally at stage 48 and analyzed 22 weeks after the completion of metamorphosis. The *lower retina* is from an animal enucleated unilaterally at stage 54 and analyzed 14 weeks after the completion of metamorphosis. Conventions are as in Figure 3. *Scale bar* = 300 μm.

in this area remain unlabeled even after large injections of HRP to the ipsilateral thalamus. Most of these unlabeled cells presumably project contralaterally only. Whether the labeled cells of this region project ipsilaterally only or bilaterally remains to be determined. Regardless, our evidence indicates that, as in many mammals, the bilaterally projecting region of retina contains intermingled cells of two kinds: one projecting contralaterally only and the other ipsilaterally and/or bilaterally. Since ganglion cells whose axons behave dissimilarly at the optic chiasm are intermingled in the retina, it will not be possible to account for the control of axonal trajectories at the chiasm simply in terms of mechanisms which differentially affect cells at different locations in the retina. An explanation must also be provided for the divergent trajectories taken by axons of neighboring retinal ganglion cells.

Regenerated ipsilateral projection. For the retinotectal system, the existence of unique cytochemical tags which bring about accurate matching between axon tip and target locus was inferred originally from behavioral studies of frogs with regenerated optic nerves (Sperry, 1944, 1963). Since sectioning the optic nerve disrupts both the normal timing of ingrowth and the normal neighbor relationships among the optic axons (Sperry, 1945b), it was proposed that the return of properly organized prey-catching behavior after regeneration was achieved by an interaction of unique identifying "labels" on the retinal ganglion cell bodies with their corresponding cues in the growth path (Attardi and Sperry, 1963) and at the optic tectum (Sperry, 1963). In a formally similar way, our data for frogs with regenerated ipsilateral retinothalamic projections can be interpreted as indicating that some sort of label is involved in determining whether an ipsilateral, contralateral, or possibly bilateral trajectory will be taken by an axon or axon branch at the optic chiasm. The ipsilateral retinothalamic projection normally begins to form at about stage 54 in *Xenopus* tadpoles (Hoskins and Grobstein, 1981; Kennard, 1981), between 3 and 4 weeks of development. Regenerating optic axons in an adult frog approach the chiasm at an unusual time (many weeks after their initial development), and after growing through the site of cut or crush and diverging from their usual neighboring fibers (Attardi and Sperry, 1963; Fawcett and Gaze, 1981). Despite these disruptions of potentially relevant parameters, the projections made by axons of regenerated projections seem to be organized in the usual way with regard to the thalamus. The vast majority of fibers from nasodorsal retina cross in the optic chiasm, whereas fibers from other retinal regions project to both sides of the brain. This suggests that the axons and/or their ganglion cell bodies possess distinguishing information which determines their normal patterns of projection in the optic chiasm and enables them to re-establish these patterns during regeneration. Interestingly, the high incidence of anomalous bilateral projections to the optic tectum after regeneration (Glastonbury and Straznicky, 1978; Bohn and Stelzner, 1981) suggests that the factors involved in determining laterality of projection may be different for different groups of axons. Axons of the contralateral retinotectal projection may be more sensitive to environmental alterations brought about by sectioning the optic nerve. For the ipsilateral retinothalamic projection, however, the same pattern of projection is re-formed during regeneration, as was established previously, during development.

The ipsilateral retinothalamic projection in one-eyed frogs. Removal of one eye either before (stages 39 to 48) or during (stages 54 to 57) the period of initial development of the ipsilateral retinothalamic projection did not result in an expansion of the area of the remaining retina which projected ipsilaterally at the optic chiasm. Instead, ipsilaterally projecting cells again were localized primarily in peripheral and non-nasodorsal retina. A similar observation on frogs enucleated during embryonic or early tadpole stages, but based on an analysis of the peripheral distribution of ipsilaterally projecting cells, was made by Kennard (1981). Our results in one-eyed frogs show that an essentially normal pattern of ipsilateral and contralateral projection can be established by fibers from one eye in the absence of *any* developing fibers from the other eye. This conclusion holds whether the eye removal is done before or during stages of initial development of ipsilaterally projecting fibers. It is noteworthy that the results in the regeneration experiment demonstrate the complementary effect; the bilateral projection from one eye to the thalamus can be re-established normally in the constant *presence* of fibers from the other eye. This suggests that interactions between fibers from the two eyes do not determine which regions of one eye project ipsilaterally.

The generality of this finding remains to be ascertained. A number of studies in neonate or fetal mammals have been interpreted as indicating that interactions between fibers from the two optic nerves in the optic chiasm are of substantial importance for the subsequent routing of axons from different retinal regions (reviewed in Lund, 1978). The remaining eye in unilaterally enucleated animals often projects to unusually large areas of ipsilateral target structures (Lund, 1978). Such enlarged terminal fields could result from misrouted axons but could also be formed by sprouting or by failure of retraction of processes by the usual ipsilaterally projecting population of axons. In several studies where the retinal source of the expanded ipsilateral projections has been examined directly, the enlarged projections have been found, in fact, to originate from the portion of the retina which normally projects ipsilaterally (Thompson, 1979;

Lent and Mendez-Otero, 1980; Hsiao, 1984). In other cases, however, some evidence for abnormally located ipsilaterally projecting cells has been reported (Lund et al., 1980).

The situation in mammals has become further complicated with the recognition that removal of one eye from neonates can cause an increase in survival of ganglion cells of the remaining eye (Sengelaub and Finlay, 1981; Jefferey and Perry, 1982). It has been suggested that the entire retina may initially project bilaterally and that subsequent interactions between axons of the ipsilateral and contralateral projections in the target structures rather than at the chiasm may influence cell survival and thus shape the adult projections (Land and Lund, 1979; Sengelaub and Finlay, 1981). The results presented here do not exclude the possibility that cell death may be important in the shaping of connection patterns in the visual system of *X. laevis*. These results do, however, indicate that the normal restricted distribution of ipsilaterally projecting cells does not depend critically on interactions among fibers from the two eyes either in the chiasm or at the target.

"Labeling" in the retinothalamic projection. We have determined the retinal distribution of the ganglion cells whose axons form the ipsilateral retinothalamic projection of adult *X. laevis*. We find that the retina contains a nasodorsal and central subregion which contains almost no ipsilaterally projecting cells, and a large peripheral area within which substantial numbers of ipsilaterally projecting ganglion cells are found. In addition, we have shown that essentially the same distribution of ipsilaterally projecting cells is found in frogs with regenerated optic nerves and in frogs with only one eye. Thus, the factors which permit substantial numbers of neurons in a large part of the retinal periphery to project ipsilaterally, while virtually none of their counterparts in the nasodorsal periphery do so, must be of a sort which are not affected significantly by alterations in developmental timing or by changes in the disposition of fibers in the optic nerves. Rather, the ipsilaterally projecting population of neurons projects as if its members differ from other neurons in a way which is unaffected by various experimental perturbations. The ipsilaterally projecting population, then, seems to be "labeled" distinctly from other populations of retinal ganglion cells, which project to targets on the other side of the brain. Given prior work on the retino*tectal* projection (Sperry, 1945b; Beazley, 1975b), it seems likely that the labeling is such that it influences whether axons project contralaterally or ipsilaterally rather than causing them to distinguish between left and right sides of the brains. However, this remains to be proven for the retino*thalamic* system. Whether the ipsilaterally projecting retinal ganglion cells are in fact cytochemically distinguishable from other retinal ganglion cells, as would be predicted by the chemoaffinity hypothesis of Sperry (1945a, 1963), is a question for future investigation. An initial approach, given the differential distribution of ipsilaterally projecting neurons in the retina, would be to look for factors which specifically affect the region of retina which contains ipsilaterally projecting cells. In the following papers (Hoskins and Grobstein, 1985a, b) we report some additional distinctive characteristics of this retinal region.

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