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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[1]

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Abstract

We have studied the development of the ipsilateral retinothalamic projection in the frog *Xenopus laevis* by analyzing patterns of histochemical reaction product resulting from anterograde transport of horseradish peroxidase (HRP) applied to cut optic nerves in animals of various ages. We have also determined the stages during which ipsilaterally projecting ganglion cells are born using a combination of [^{3}H] thymidine autoradiography and retrograde marking of ganglion cells following injection of HRP into the thalamus.

Projections to ipsilateral thalamic terminal zones were first detectable beginning at about larval stage 54. There was a clear asynchrony in innervation, with projections to some terminal zones appearing before projections to others; projections to all terminal zones were present by late metamorphic stages. Within individual terminal zones there were progressive increases in the density of the projections as well as changes in their distribution. By these criteria, development of the ipsilateral projection was not complete at the end of metamorphosis but continued for some months thereafter.

Our birth dating studies show that ipsilaterally projecting cells are produced relatively late in development and that, like the development of the projection, the production of ipsilaterally projecting ganglion cells continues postmetamorphically. The vast majority of ipsilaterally projecting cells are born over a period beginning at stage 54/55, when the projection first appears. This stage is significant, since it is at approximately this time that thyroxine-dependent metamorphic events begin. In the following paper (Hoskins, S. G., and P. Grobstein (1985) J. Neurosci. *5:* 930-940) we report studies on the involvement of thyroxine in the development of the ipsilateral retinothalamic projection.

In the preceding paper (Hoskins and Grobstein, 1985a), we reported studies in which retrograde transport of horseradish peroxidase (HRP) was used to determine the retinal distribution of ganglion cells whose axons form the ipsilateral retinothalamic projection in the frog *Xenopus laevis*. Ipsilaterally projecting cells are not evenly distributed across the entire retina, being, instead, sparse or absent in central and nasodorsal regions and abundant in peripheral and temporoventral regions. This pattern is interesting in light of available information on the development of both the retina and the ipsilateral projection in *X. laevis*. The retina grows by adding rings of cells to the periphery, so that older neurons are found more centrally and younger ones more peripherally. During early tadpole stages, the addition is more or less symmetrical (Straznicky and Gaze, 1971). Beginning at early metamorphic stages, the addition becomes markedly asymmetric, with substantially more addition ventrally than dorsally (Hollyfield, 1971; Jacobson, 1976; Beach and Jacobson, 1979a; Tay et al., 1982). Although previous reports (Khalil and Szekely, 1976; Kennard, 1981) differ somewhat as to the exact stage at which ipsilateral terminal fields are first seen, there is agreement that the pathway develops late, primarily during metamorphic stages. If the cells giving rise to the projection were themselves born at late stages, one would expect to find the majority of them located peripherally and ventrally in the retina, a pattern like the one we have described for adult frogs.

In this paper, we report studies on the relation between the development of the optic terminal fields in the ipsilateral thalamus and the production of ipsilaterally projecting ganglion cells in the retina. We have reinvestigated the development of the projection using anterograde transport of HRP, a method potentially more sensitive than the autoradiographic and degeneration techniques used by prior investigators. Our findings reveal a slightly earlier initial appearance of the projection than described previously, provide new information about the morphological maturation of terminal fields, and show that this maturation continues postmetamorphically. To relate the development of the projection to the production of retinal ganglion cells, we have used a combination of [^{3}H]thymidine labeling and retrograde transport of HRP to determine the stages at which ipsilaterally projecting cells are born. Our results indicate that the vast majority of ipsilaterally projecting cells are born beginning at the time when the ipsilateral projection is first detected. They also show that ganglion cells continue to be produced and continue to make ipsilateral projections after the conclusion of metamorphosis. Preliminary reports of some of these observations have appeared (Hoskins and Grobstein, 1981, 1982).

Materials and Methods

All tadpoles and young postmetamorphic frogs used in these studies were bred and raised in the laboratory. A few of the adult frogs were purchased commercially (Nasco, Inc.).

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Anterograde transport of HRP. Tadpoles of various stages (Nieuwkoop and Faber, 1967) were anesthetized in 5×10^{-5} M tricaine methanesulfonate (MS-222), and one optic nerve was cut with a scalpel. Crystals of HRP (type VI, Sigma) were applied directly to the central stump of the cut nerve. A small piece of Gelfoam was then inserted at the cut site. Animals were kept at room temperature. Various survival times were tested, and 24 hr was chosen as optimal for tadpoles of stages 49 to 63. Transport of HRP after shorter survival times (8 to 12 hr) was often incomplete, and longer (48 hr) survival times produced broken and "beaded," possibly degenerating, fibers. In these cases it was more difficult to distinguish between fibers and terminal fields. For older animals near completion of metamorphosis (stages 64 to 66 and older), survival times of 48 hr were used. After the appropriate survival time, animals were killed and their brains were processed as described previously (Hoskins and Grobstein, 1985a). The ipsilateral projections to thalamic terminal zones were reconstructed using a Zeiss drawing tube at ×25 magnification.

The results presented here are based on anterograde transport of HRP in optic nerves of animals ranging from stage 49 (1½ to 2 weeks after fertilization; Nieuwkoop and Faber, 1967) to adult (approximately 1 year or more postmetamorphic). Tadpoles midway through the metamorphic period (stages 59 to 62) seemed to be unusually sensitive to the anesthetic used and frequently did not revive even after very low doses of anesthesia. As a result, some gaps exist in the developmental series presented. These do not affect the general descriptions of changes in terminal zone morphology across metamorphosis which are given under "Results" but make it possible that additional significant changes might be revealed by detailed studies of intermediate stages.

Retrograde labeling and autoradiography. For the birth dating study, 5-μl injections of [^{3}H]thymidine (New England Nuclear; specific activity, 81 to 86 Ci/mmol, 1 mCi/ml) were made intra-abdominally in anesthetized tadpoles, using a sharpened Hamilton syringe. The tadpoles were revived, housed in 10% Holtfreter's solution in plastic boxes, and fed yeast. After they metamorphosed, the frogs were fed small pieces of beef heart every 2 to 3 days. One to 16 weeks after metamorphosis, we made injections of HRP into thalamic terminal zones, following procedures described previously (Hoskins and Grobstein, 1985a). After a 48- to 72-hr survival time, the animals were perfused with anesthetic, their eyes were removed, and the brains were processed as described previously for histochemical demonstration of the areas of the thalamus exposed to HRP. Eyes were delensed, fixed in 2% glutaraldehyde for 4 min, and stored overnight in 0.1 M phosphate buffer, pH 7.4. The following day they were pretreated for 30 min in 1 mg/ml of diaminobenzidine (DAB) in 0.1 M phosphate buffer, pH 7.4, and then reacted in 1 mg/ml of DAB + 0.01% hydrogen peroxide for 30 min, for histochemical demonstration of retrogradely transported HRP. The eyes were then rinsed in six changes of phosphate buffer, dehydrated through alcohols, and embedded in known orientation in blocks of paraffin. Blocks were sectioned parasagittally at 10 μm and the sections were mounted onto slides. The slides were coated with Kodak NTB-2 emulsion diluted 1:1 with distilled water and exposed at 4°C for 6 to 24 weeks. The slides were then developed in Kodak D-19 for 2 min, rinsed briefly in ice water, fixed in Kodak Rapidfix (diluted 1:3 in water) for 5 min, washed 5 min in running water, and stained with cresyl violet. The HRP reaction product survives these procedures and is visible as a brown or an orange-brown granular deposit. Thymidine labeling appeared as black grains clustered above nuclei.

Results

Ipsilateral terminal fields in adults. A description of the optic nerve projection in adult *Xenopus* as revealed by anterograde transport of

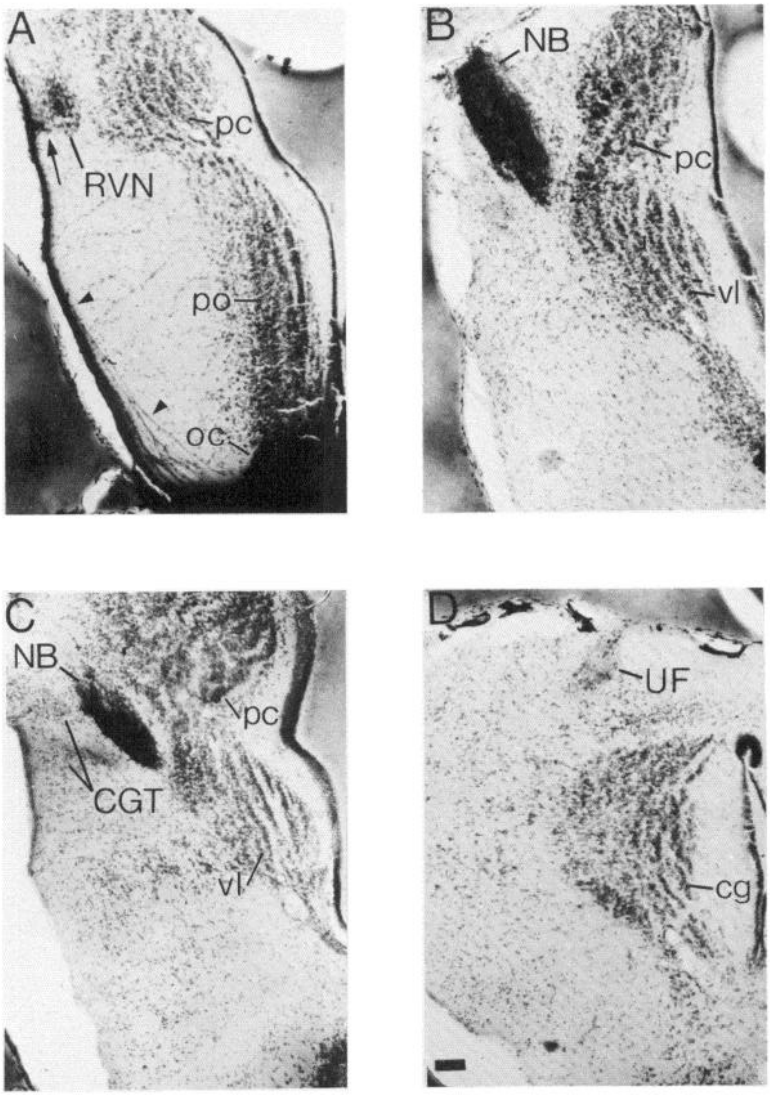

Figure 1. Optic nerve projections to terminal fields in the ipsilateral thalamus of an adult *X. laevis*, as revealed by anterograde transport of HRP applied to a cut optic nerve. *Scale bar* = 400 μm. *A*, Transverse section, taken at the level of the optic chiasm (*oc*), which is stained heavily. Fibers of the optic tract (*arrowheads*) run along the lateral wall of the thalamus toward the rostral visual nucleus (*RVN*) and other targets located more caudally. The projection to the RVN is indicated with an *arrow*. *pc*, nucleus posterocentralis; *po*, preoptic cell masses. *B*, Transverse section taken at a level 160 μm caudal to that of *A*. The ovoid nucleus of Bellonci (*NB*) is densely filled with reaction product. This is the largest ipsilateral terminal field. *vl*, nucleus ventrolateralis. *C*, Transverse section 100 μm caudal to that in *B*. The appendage of the NB lies at the lateral edge of nucleus posterocentralis (*pc*) and nucleus ventrolateralis (*vl*). Lateral to the NB is the corpus geniculatum thalamicum (*CGT*), which is separated from the NB field by a label-sparse region. *D*, Transverse section 360 μm caudal to that in *C*, at the rostral end of the uncinate field (*UF*). The UF lies caudally in the thalamus, just rostral to the optic tectum. *cg*, central gray.

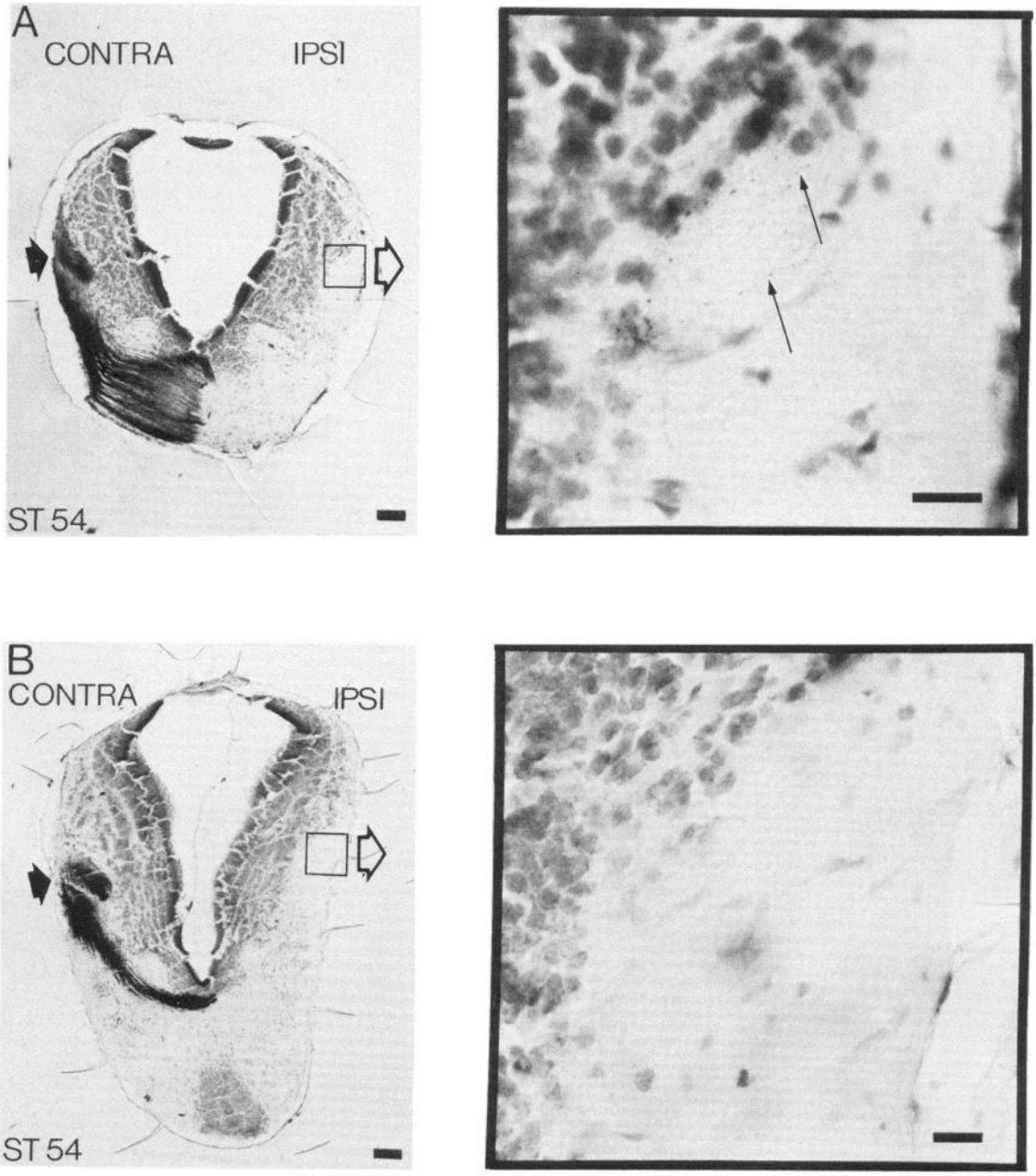

Figure 2. NB terminal fields in tadpoles at stage 54. Stage 54 is the first stage at which reaction product was detectable in any terminal field in the ipsilateral thalamus, although it was not seen in every case. *A*, Transverse section through the rostral thalamus of a tadpole at stage 54. One optic nerve of this animal was filled with HRP. On the contralateral (*CONTRA*) side, reaction product is dense in the NB. On the ipsilateral (*IPSI*) side (*boxed region* and *enlargement* on the *right*), a very small amount of reaction product can be seen. *Scale bar* = 100 μm to *left*, 20 μm to *right*. *B*, Transverse section through the rostral thalamus of a different stage 54 tadpole, prepared like the animal illustrated in *A*. The angle of this section is slightly different from that in *A*; thus, less of the optic tract is visible. As in the above case, reaction product is dense in the contralateral NB. No reaction product was found in the corresponding region in the ipsilateral side (*boxed region* and *enlargement* on the *right*). *Scale bar* = 100 μm to *left*, 20 μm to *right*.

[^{3}H]fucose has been published by Levine (1980). There are four major ipsilateral terminal fields. These are, in rostrocaudal order, the rostral visual nucleus (RVN), the nucleus of Bellonci (NB), the corpus geniculatum thalamicum (CGT), and the uncinate field (UF). Levine (1980) also described two minor components of the ipsilateral projection, a diffuse projection to the thalamopretectal field and a sparse projection to the basal optic nucleus, which were not investigated in our studies. The appearance of the major ipsilateral terminal fields in adults, as revealed by anterograde transport of HRP, is illustrated in Figure 1. It differs from the descriptions provided by Levine (1980) only in regard to the distribution of label in the NB. The NB, the largest and most densely labeled of the ipsilateral terminal zones, has a body which lies along the lateral wall of the thalamus and a caudomedially directed appendage. Levine (1980) described label-sparse areas within central regions of this appendage. Our own observations on full-grown animals show more evenly deposited reaction product throughout this region. It seems unlikely that this difference reflects the differing techniques used since, as described below, label-sparse subregions were apparent in the ipsilateral NBs of younger postmetamorphic animals. A more probable explanation of the difference in the distribution of reaction product is considered under "Discussion."

Development of the terminal fields. We now summarize morphological changes in terminal fields of the ipsilateral thalamus across a period ranging from tadpole stage 49 (approximately 2 weeks after fertilization; staged according to Nieuwkoop and Faber, 1967)

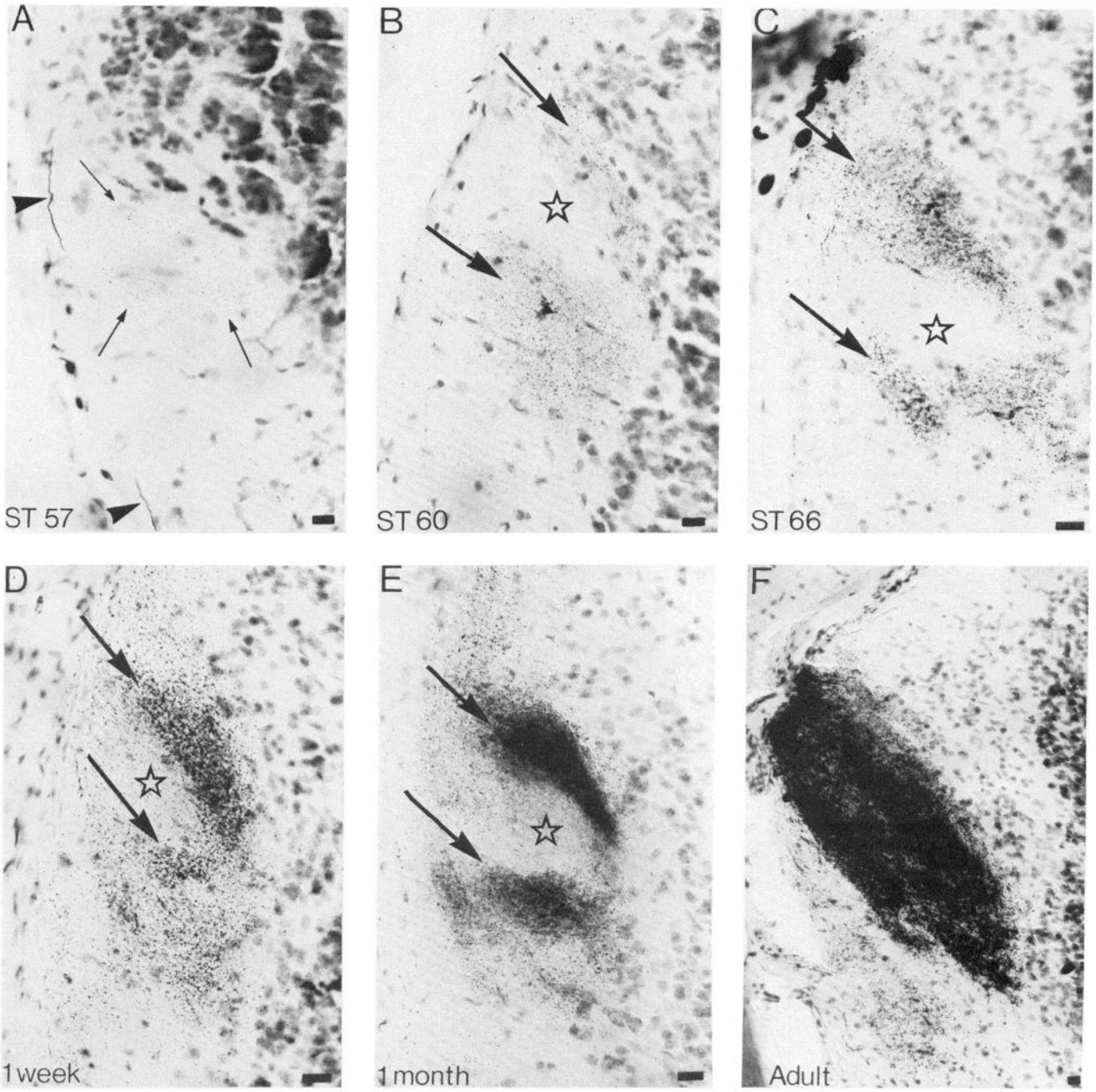

Figure 3. Ipsilateral NB terminal fields of developing *X. laevis*. *A* to *F* are all transverse 40-μm frozen sections through the rostral thalamus of tadpoles and frogs at the stages indicated. *Scale bar* = 20 μm. *A*, At stage 57, sparse deposits of reaction product (*arrows*) were found reliably in the NB, distributed homogeneously in an ovoid region of the neuropil at the lateral edge of nucleus posterocentralis. *B*, At stage 60, deposits of reaction product are more dense, forming a slanted V-shape, with the vertex at the lateral border of the gray matter. The two arms of the V (*arrows*) are separated by a label-sparse zone (*star*). *C*, At stage 66, the density of reaction product has increased in both the dorsal and ventral portions of the terminal field (*arrows*), and the dorsal portion appears to be innervated slightly more heavily. The central area of the terminal field (*star*) remains much less intensely labeled. *D*, At 1 week postmetamorphosis, the distribution of reaction product is similar to the metamorphic case, with intensely labeled regions (*arrows*) separated by a label-sparse zone (*star*). The dorsal portion of the terminal field is labeled more densely than is the ventral border. *E*, At 1 month postmetamorphosis, the density of labeling has further increased in both the dorsal and ventral portions of the terminal field (*arrows*), and the dorsal portion is curved slightly. The label-sparse zone (*star*) is still apparent. *F*, In the adult, the ipsilateral NB field is densely and homogeneously labeled throughout. The label-sparse area which appeared at stage 60 and was still present at 6 months postmetamorphosis (not illustrated) is no longer apparent.

through metamorphosis (approximately 8 weeks after fertilization) and continuing up to 6 months after the completion of metamorphosis. The description is based on cases of most successful anterograde transport of HRP, as judged by the presence of dense reaction product in the optic terminal layer of the contralateral tectal lobe, which is known to be innervated at all of the stages studied.

The development of the projections to the ipsilateral thalamus clearly is delayed relative to that of projections to contralateral thalamus. Clear labeling in the contralateral NB, CGT, and UF was observed at stage 49, the earliest stage examined. The presence of label in the contralateral RVN at stage 49 was difficult to evaluate because of the proximity of this nucleus to the wall of the dienceph-

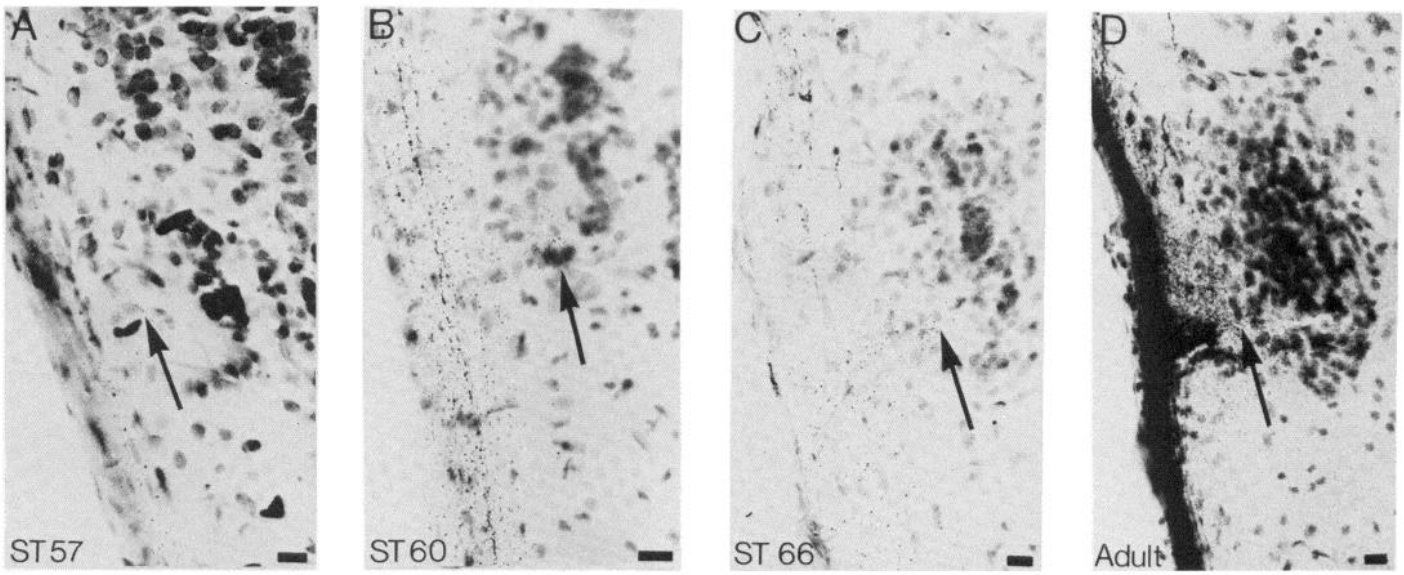

Figure 4. Optic nerve projection to the ipsilateral RVN of developing *X. laevis*, as revealed by anterograde transport of HRP. *Scale bars* = 20 μm. *A*, Ipsilateral RVN of a stage 57 tadpole. At this stage, small deposits of reaction product (*arrow*) can be seen in the central portion of the RVN. *B*, Ipsilateral RVN at stage 60. At this stage (~5 days after stage 57), more fibers are apparent, running along the lateral wall of the brain. More reaction product can be seen in the RVN (*arrow*). Deposits of reaction product were also seen in more caudal sections of the RVN, extending into the cell-free region behind the cellular portion of the nucleus. *C*, Ipsilateral RVN at stage 66 (end of metamorphosis; ~12 days after stage 60). The nucleus is larger and the ipsilateral terminal field contains more reaction product overall than it did at stage 60, but the reaction product is distributed in the same pattern as at stage 60. Reaction product can first be detected in central sections of the ipsilateral RVN, and it extends into the cell-free zone caudal to the nucleus. *D*, Ipsilateral RVN of an adult *X. laevis*. The projection (*arrow*) is substantially more dense than at earlier stages but is distributed similarly.

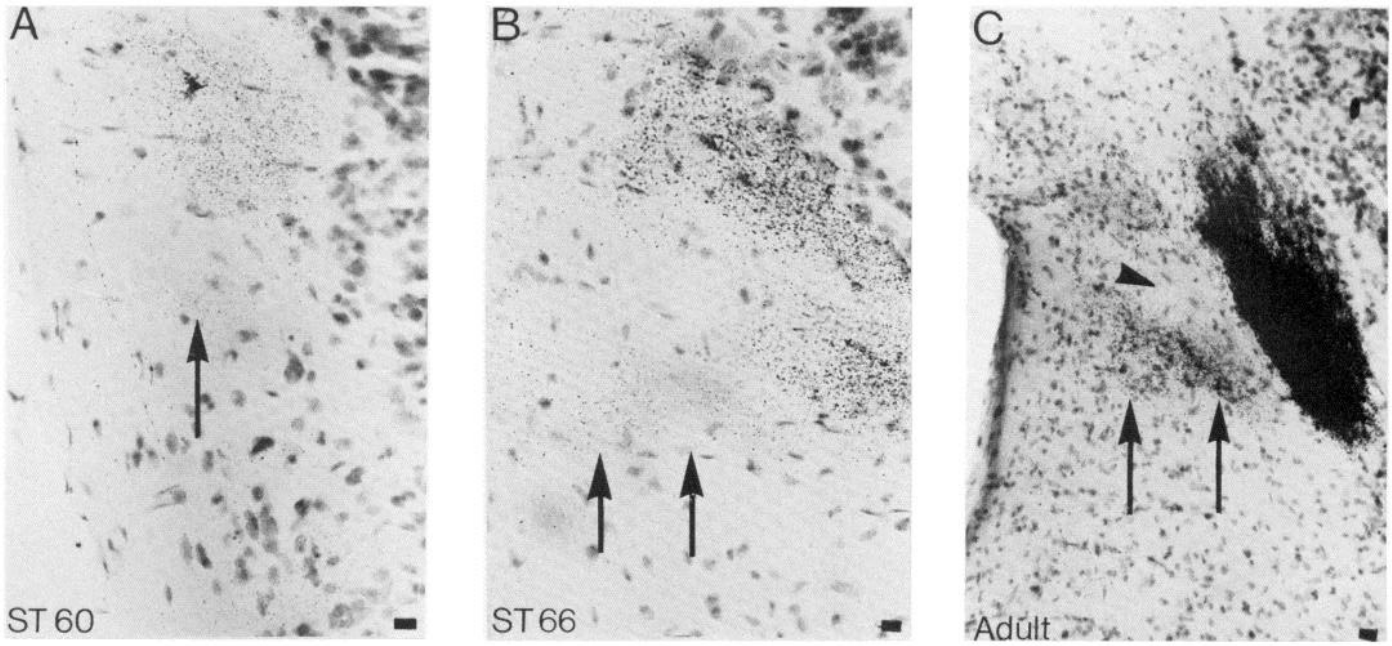

Figure 5. Ipsilateral CGT terminal fields in developing *X. laevis*. *Scale bars* = 20 μm. *A*, CGT in a stage 60 tadpole. The CGT is first detectable (*arrow*) as a small amount of reaction product lying medial to the optic tract and ventral to the NB, separated from it by a label-sparse area. *B*, At the conclusion of metamorphosis (stage 66), the CGT contains somewhat more reaction product, which extends to the lateral wall of the thalamus. *C*, In the adult, the CGT contains substantially more reaction product than at premetamorphic or early postmetamorphic stages, and label-sparse regions have appeared (*arrowhead*).

alon in young tadpoles; however, the nucleus is clearly innervated by stage 54. Projections to ipsilateral terminal fields were not seen before stage 54, although a few HRP-stained fibers were occasionally seen in the ipsilateral optic tract of younger tadpoles. Within the ipsilateral thalamus, as first reported by Khalil and Szekely (1976), projections to the different terminal zones do not develop synchronously. Our observations indicate that the NB is the first terminal zone to be innervated, followed by the RVN and CGT, and later by the UF.

Reaction product in the ipsilateral NB was observed occasionally in tadpoles at stage 54 (Fig. 2) and was present reliably at stages 55 to 56. At early stages, the sparse, evenly distributed reaction product filled most of the terminal zone but did not, as in the adult, extend to the lateral edge of the thalamus. By stage 57, the entire terminal zone was filled (Fig. 3*A*). During metamorphic climax (stages 60 and 63), the density of reaction product increased and its distribution changed somewhat (Fig. 3*B*). The body of the nucleus continued to be homogeneously labeled, but label-sparse regions

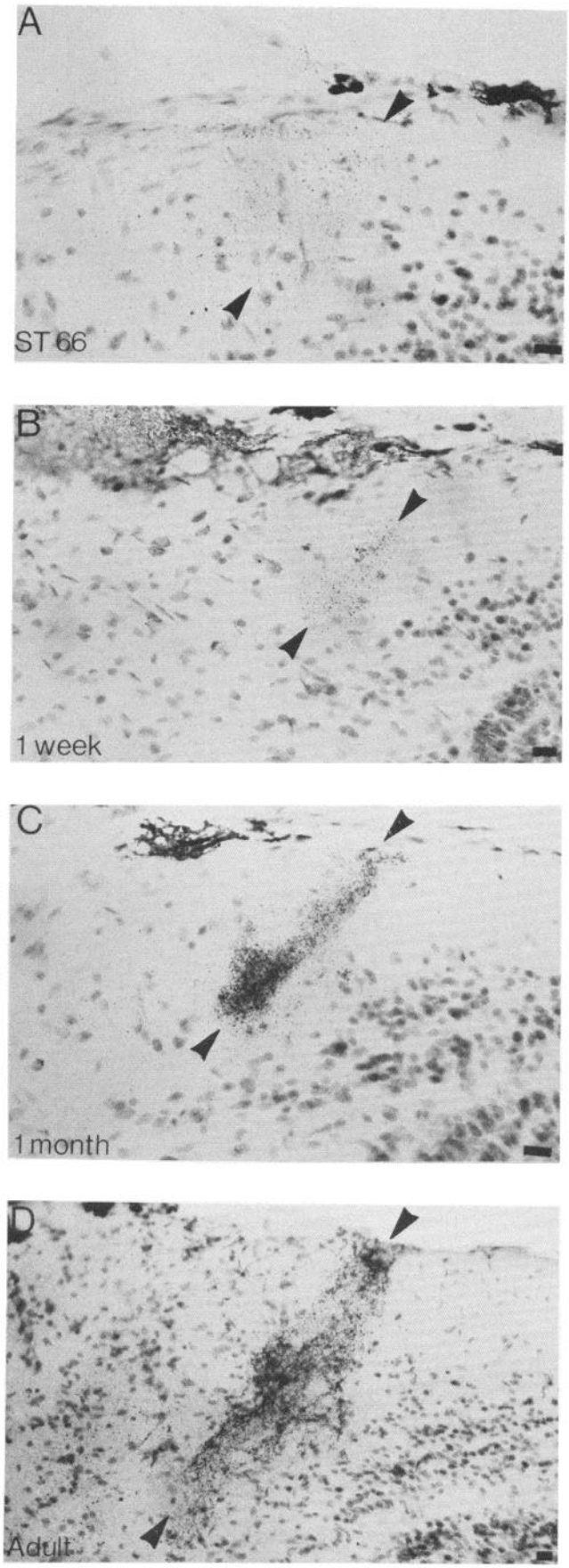

Figure 6. Rostral UF terminal fields in developing *X. laevis*. *Scale bars* = 20 μm. *A*, At stage 66 the UF occupies approximately the same area as it did at stage 64, when it is first detected, but the density of reaction productis greater. The reaction product occupies a wider area at this stage than it does subsequently, being sparsely distributed within an ovoid region (between *arrowheads*). *B*, At 1 week postmetamorphosis, reaction product in the UF (between *arrowheads*) is distributed in a narrow strip, as it is in the adult. The density of reaction product is still low. *C*, One month after metamorphosis, the density of reaction product (between *arrowheads*) has increased, but the distribution has not changed. *D*, In the adult, the density of reaction product is more intense, and the terminal field is larger, but it is otherwise similar in form to that seen at earlier postmetamorphic stages.

appeared in the core of the appendage. Increases in intensity of labeling continued to be apparent during subsequent metamorphic stages (Fig. 3*C*) as well as postmetamorphically (Fig. 3, *D* and *E*). The inhomogeneous distribution of label was still apparent in animals 6 months after metamorphosis, suggesting that there may be continuing development of the terminal zone after this time.

Labeling in the ipsilateral RVN was first observed at stages 55 to 56 and consisted of a sprinkling of reaction product distributed as it is in the adult frog. The intensity of reaction product increased rapidly between stages 56 and 60 (Fig. 4, *A* and *B*). Increases in the intensity of labeling were also apparent postmetamorphically (Fig. 4, *C* and *D*).

Characterization of labeling in the CGT at early stages was difficult since this terminal field lies very close to fibers running up the side of the thalamus toward the NB. Terminal labeling may be present as early as stages 55 to 56, as some sparse reaction product could sometimes be observed ventral and lateral to the NB at these stages. At stage 60 we observed clear labeling in the CGT, in the form of reaction product separated by a label-sparse strip from that in the NB terminal field (Fig. 5*A*). Complete labeling of the terminal zone, so as to give its typical wedge-shaped appearance, is not evident until the end of metamorphosis (Fig. 5*B*). Postmetamorphically, the intensity of labeling increased slightly and the terminal zone developed the label-sparse areas characteristic of the adult (Fig. 5*C*).

Labeling in the ipsilateral UF was first reliably observed at stage 64. The shape and extent of the terminal field at this time were similar to those of adult frogs, but the density of labeling was much less. Labeling continued to be sparse through the completion of metamorphosis (Fig. 6*A*) and clearly increased in intensity postmetamorphically (Fig. 6, *B* to *D*).

Production of ipsilaterally projecting ganglion cells. The studies described above indicate that the projections to terminal fields in the ipsilateral thalamus begin to form at about stage 54 and continue to develop during subsequent metamorphic stages as well as postmetamorphically. Our birth dating studies indicate that this pattern of development is paralleled by the proliferative events in the retina: the vast majority of cells which give rise to ipsilaterally projecting axons are born at or after stage 54, and the production of these cells continues postmetamorphically.

Because new ganglion cells are produced primarily at the ciliary margin of the eye (Straznicky and Gaze, 1971) and there is little or no migration of ganglion cells (Horder and Spitzer, 1973; Jacobson, 1976), cells born at a particular time lie in a ring. This ring initially lies at the retinal periphery but is displaced centrally as new rings of neurons are generated at the expanding periphery. The ring of neurons generated at a particular developmental stage can be marked by injection of [^{3}H]thymidine at that stage. Neurons born before the label was injected will lie central to the ring of labeled neurons, and cells born later will lie peripheral to it. We determined the ages of ipsilaterally and contralaterally projecting retinal ganglion cells by injecting tadpoles with [^{3}H]thymidine at a range of stages from 49 to 66, allowing them to metamorphose, and then injecting HRP into the rostral thalamus of the postmetamorphic frogs. By defining the locations of HRP-stained ganglion cells relative to the ring of [^{3}H]thymidine-labeled cells in the ipsilateral retina, we could determine whether ipsilaterally projecting ganglion cells were born before or after the stage at which the [^{3}H]thymidine was injected.

Analyses of animals which received injections of [^{3}H]thymidine at

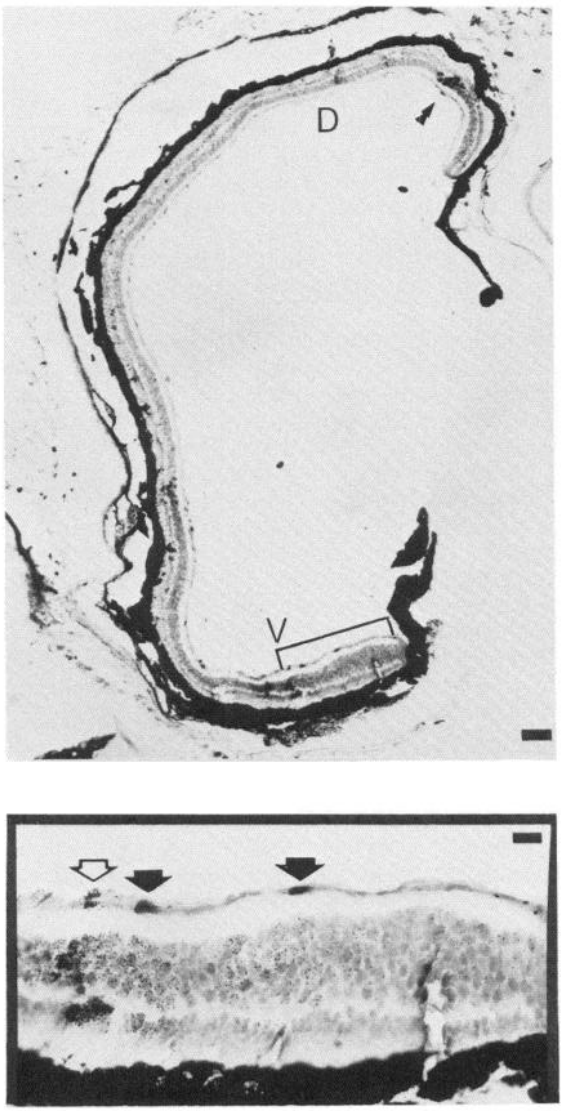

Figure 7. Postmetamorphic production of ipsilaterally projecting retinal ganglion cells. The *upper photomicrograph* is a parasagittal section of the eye of a postmetamorphic frog which received an intra-abdominal injection of 5 μl of [^{3}H]thymidine at stage 66, and an injection of HRP into the thalamus ipsilateral to the eye 12 weeks after metamorphosis. The locations of cells heavily labeled with [^{3}H]thymidine are indicated by the *double arrowhead* in the dorsal (*D*) periphery and by the *boxed region* in the ventral (*V*) periphery. In the enlargement of the *boxed region* (*bottom*), the grain border marking the edge of the retina at stage 66 is indicated by the *open arrow*. Central to the arrow (*left* in the figure), retinal ganglion cells are unlabeled, indicating that they were born before the [^{3}H]thymidine was present. Peripheral to the arrow (*right* in the figure), silver grains (indicative of incorporation of [^{3}H] thymidine into dividing cells) are apparent in neurons of the retinal ganglion cell layer. The *solid arrows* mark the positions of ganglion cells which stained with HRP. Since these cells lie peripheral to the [^{3}H]thymidine grain border, they were produced *after* the conclusion of metamorphosis. Thus, ipsilaterally projecting retinal ganglion cells continue to be produced after metamorphosis is complete. *Scale bars* = 50 μm, *upper*; 10 μm, *lower*.

stage 66 (the end of metamorphosis) provided evidence for postmetamorphic addition of ipsilaterally projecting retinal ganglion cells. Figure 7 illustrates a result in one such case. *Solid arrows* in Figure 7 mark neurons containing HRP reaction product, lying peripheral to the [^{3}H]thymidine-labeled cells which represent the edge of the retinas at stage 66. These cells were born after stage 66, and they made projections to the ipsilateral thalamus. The addition of new fibers from cells such as these may contribute to the alterations in the morphologies of terminal fields observed after the conclusion of metamorphosis.

The distributions of HRP-stained ganglion cells in animals injected with [^{3}H]thymidine at stages near that at which the ipsilateral projection first develops show that the vast majority of ipsilaterally projecting ganglion cells are born at and after these stages. Figure 8 shows the reconstructed [^{3}H]thymidine and HRP labeling patterns in both retinas of an animal which received an injection of [^{3}H]thymidine at stage 54 and an injection of HRP into the thalamus on one side of the brain after metamorphosis. The retinas were reconstructed from serial sections by drawing each section using a Zeiss drawing tube and measuring relevant distances with a map reader. The *heavy outer lines* in Figure 8 correspond to the periphery of the retina 8 weeks after the conclusion of metamorphosis. The *inner rings* correspond to the inner limit of the [^{3}H]thymidine labeling and hence indicate the periphery of the retina at stage 54. *Small dots* mark the locations of ganglion cells stained heavily with HRP. Large numbers of HRP-stained ganglion cells were found both central and peripheral to the [^{3}H]thymidine ring in the retina contralateral to the HRP injection, indicating that contralateral projecting cells are produced both before and after stage 54. In contrast, the vast majority of the HRP-stained cells in the ipsilateral retina were found peripheral to the [^{3}H]thymidine ring, indicating that they were produced at and after stage 54. For clarity in the reconstruction, only the locations of heavily HRP-labeled cells were recorded. Sparsely labeled cells were also present. Their distribution corresponded closely to that of the heavily labeled cells; as with the heavily labeled cells, there was an abrupt drop in the frequency of labeled cells in going from the peripheral to the central side of the [^{3}H]thymidine ring in the ipsilateral retina.

These results indicate that there are few ipsilaterally projecting cells born before stage 54 but that such cells begin to be produced in significant numbers at or shortly after this time. Results from the other animals in this series provide further evidence for this conclusion. A reconstruction of the ipsilateral retina in a second animal, which received an injection of [^{3}H]thymidine at stage 58 and an injection of HRP 10 weeks after metamorphosis, is shown in Figure 9. In this case, despite the fact that fewer HRP-labeled cells were found, there is an increased percentage of HRP-stained cells within the [^{3}H]thymidine ring, indicating that significant production of ipsilaterally projecting cells has begun by stage 58.

Results from the entire series of animals are summarized in Figure 10, in which the percentages of HRP-stained cells central to the [^{3}H] thymidine-labeled population have been plotted. In ipsilateral eyes of animals injected with [^{3}H]thymidine at or before stage 55, less than 10% of HRP-stained cells lay central to the labeled ring. In animals which received thymidine injections at stages 56, 57, or 58, increased numbers of HRP-filled neurons were found central to the border marked by silver grains in ipsilateral eyes. This indicates that substantial numbers of ipsilaterally projecting neurons are born after stage 55, and before stage 58. The percentage of HRP-stained ganglion cells in central ipsilateral retina varied even among tadpoles at the same stage because of slight variations in size of the injection site in the ipsilateral thalamus and the fact that some animals were assayed within the first few postmetamorphic weeks, before the complete complement of ipsilaterally projecting retinal ganglion cells had been generated. It is nevertheless clear from these results that the ipsilateral and contralateral projections to the thalamus originate from groups of neurons born over different time periods. The substantially greater percentages of labeled ganglion cells located central to the ring in the tadpoles injected with [^{3}H]thymidine after stage 55 make it clear that the vast majority of ipsilaterally projecting ganglion cells are born at or after the time when the projection first appears. At the same time, we consistently saw a small percentage of labeled ganglion cells at central locations, implying that they were born prior to stage 54, when the ipsilateral projection is first detectable. It is possible that these cells do project ipsilaterally in the tadpole and that such a projection is not apparent in our anterograde

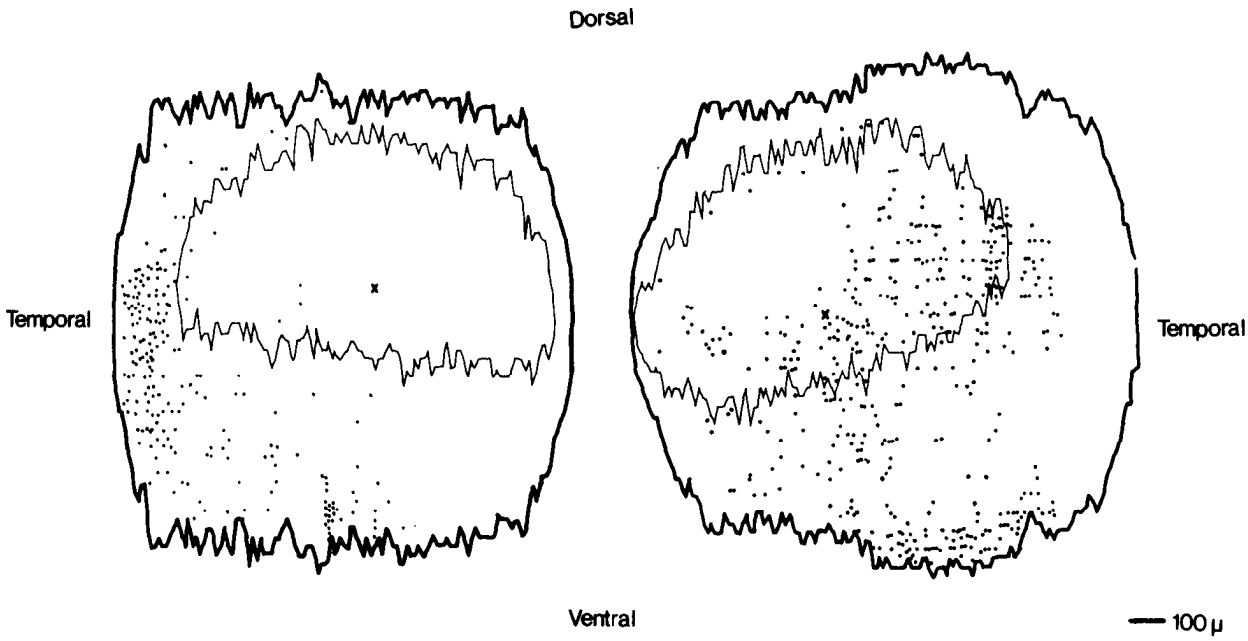

Figure 8. Reconstructions of both retinas of an animal which received a [^{3}H]thymidine injection at stage 54 and an HRP injection into the rostral thalamus on one side of the brain after the completion of metamorphosis. The retina ipsilateral to the HRP injection is to the *left*. Orientation of retinas is as indicated. The *outer heavy line* corresponds to the retinal periphery at the time of sacrifice. The *lighter inner line* corresponds to the location of heavy [^{3}H]thymidine labeling and hence to the retinal periphery at stage 54. *Dots* indicate the locations of ganglion cells stained heavily with HRP. Although stained cells are frequent both centrally and peripherally in the retina contralateral to the HRP injection, they are almost all located peripherally in the ipsilateral retina. *X* indicates the location of the nerve head in each retina.

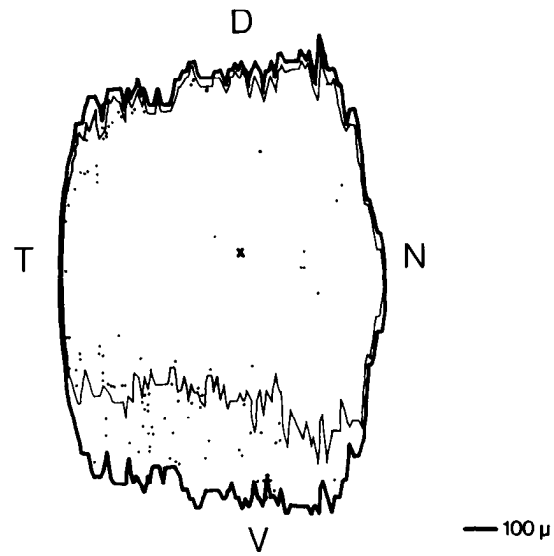

Figure 9. Reconstruction of the ipsilateral retina in an animal which received a [^{3}H]thymidine injection at stage 58 and an HRP injection into the rostral thalamus on one side of the brain after the completion of metamorphosis. Conventions are as in Figure 8. Notice that, although there are fewer labeled cells overall than in the ipsilateral retina illustrated in Figure 8, a greater percentage of the cells lie central to the [^{3}H]thymidine ring.

material because of the small number of cells involved. Alternatively, these cells may develop an ipsilateral projection at the time that older cells are born and produce the majority complement of the projection, or they may represent a small amount of intercalary addition of later born neurons into central retina. Occasional [^{3}H] thymidine-labeled cells were sometimes seen in the ganglion cell layer of the central retina following [^{3}H]thymidine injections at late stages. We are unable to say for certain whether these were ganglion cells.

Discussion

Development of ipsilateral terminal fields. The first study indicating that the appearance of the ipsilateral retinothalamic projection in *Xenopus* is delayed relative to that of the contralateral projection used degeneration techniques and reported the initial appearance of the ipsilateral projection to be at stages 60 to 62 (Khalil and Szekely, 1976). Using anterograde transport of [^{3}H]proline, Kennard (1981) reported stage 56 to be the first at which an ipsilateral projection was observed reliably, without specifying which terminal zones were examined. Our studies using anterograde transport of HRP confirm the delayed development of the projection and indicate a slightly earlier stage of initial appearance (stage 54/55), with the NB being the first terminal field to be innervated. The differences among the studies probably reflect the different techniques used. The earlier stage reported here is of interest since stage 55 corresponds to the time at which overall metamorphic change becomes dependent on thyroxine (Streb, 1967; Dodd and Dodd, 1976).

It is of interest that, not only is the development of the ipsilateral projection to the thalamus delayed relative to the development of the contralateral projection but innervation of the individual terminal zones within the ipsilateral thalamus is also nonsynchronous. The nonsynchrony is apparent in two ways. First, as originally described by Khalil and Szekely (1976), initial innervation of the UF is delayed relative to that of terminal zones in more rostral thalamus. In addition, our observations suggest that the three terminal zones in the rostral thalamus are innervated at slightly different times. Significant deposits of reaction product are detected first in the NB and RVN, later in the CGT, and then in the UF several stages later. Whether this nonsynchrony is a simple consequence of the duration of axon ingrowth or instead indicates that groups of cells born at different times are involved in the projections to different terminal zones is a question worth further investigation.

Our observations on the maturation of the terminal zones also clearly indicate, as suggested by Khalil and Szekely (1976), that the completion of metamorphosis does not mark the completion of development in the visual pathways. Postmetamorphic changes

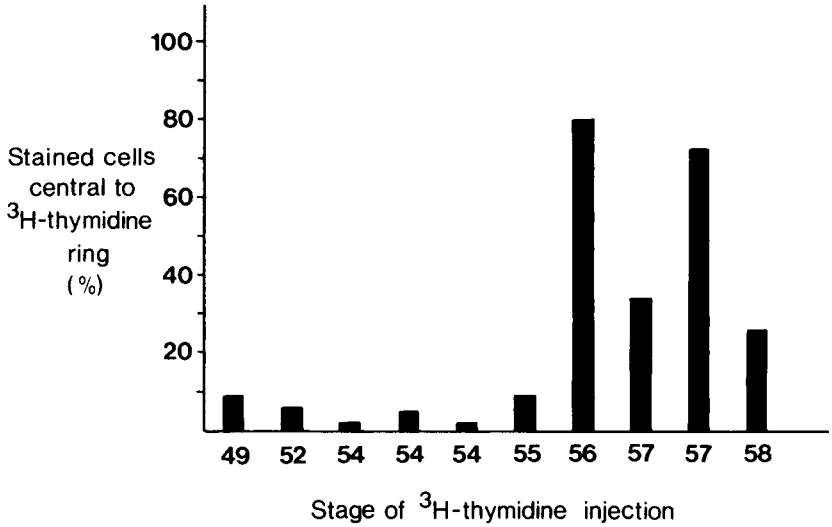

Figure 10. Summary of results of experiments like those illustrated in Figures 8 and 9. Individual animals received [^{3}H] thymidine injections at stages shown on the *abscissa*. Following completion of metamorphosis, the animals received HRP injections into the rostral thalamus on one side of the brain. *Bars* show for each animal the percentage of the total number of HRP-stained cells located central to the [^{3}H] thymidine ring in the ipsilateral retina. Percentages vary somewhat because of variations in the injection and in the effectiveness of retrograde transport. It is nonetheless clear that the percentages are low in animals which received [^{3}H] thymidine injections at or prior to stage 54 and markedly higher for animals which received [^{3}H]thymidine injections at later stages.

include increases in the intensity of labeling in all of the terminal fields, and alterations in the distribution of reaction product in the NB and CGT. The extent to which the postmetamorphic increase in labeling in terminal zones is due to branching of existing axons as opposed to addition of new fibers is not known. Our finding that some ganglion cells born after the completion of metamorphosis contribute fibers to the ipsilateral projection indicates, however, that some of the postmetamorphic changes in the terminal zones are probably due to addition of new fibers. Postmetamorphic changes in the morphology of terminal zones are apparently less prolonged in *Rana pipiens* (Currie and Cowan, 1974) than in *X. laevis*. This difference may be related to the fact that migration of eyes, and associated changes in the boundaries of the binocular visual field, continues for at least 6 months after metamorphosis in *X. laevis* but is virtually complete by the end of metamorphosis in *R. pipiens* (Grobstein and Comer, 1977).

Delayed development in ipsilateral relative to contralateral retinofugal projections is particularly dramatic in *X. laevis*, where a period of several weeks elapses between the development of the first crossed projections during embryonic stages (Grant and Ma, 1983; Holt and Harris, 1983) and the development of the first uncrossed projections more than 3 weeks later. However, in a number of other vertebrates, including the monkey, ferret, cat, rabbit, rat, and hamster (Rakic, 1976; So et al., 1978; Cucchiaro and Guillery, 1982; Bunt et al., 1983; Shatz, 1983; K. L. Chow, personal communication), ipsilateral projections similarly develop later than do the contralateral projections to the same targets, although in these examples the ingrowth of the ipsilateral fibers is delayed by at most a few days. In regard to the development of the final distribution of crossed and uncrossed axons within terminal fields, *X. laevis* shows some similarities as well as a striking difference when compared to other animals. In a variety of mammals (hamster, So et al., 1978; rat, Land and Lund, 1979; opossum, Cavalcante and Rocha-Miranda, 1978; ferret, Cucchiaro and Guillery, 1982; monkey, Rakic, 1976; rat, Bunt et al., 1983; and cat, Shatz, 1983), when the ipsilateral projection is first detected, its terminal field overlaps substantially with that of the contralateral projection to the same region. Later, the ipsilateral and contralateral projections segregate into the essentially nonoverlapped distributions characteristic of the adult. In *X. laevis*, the earliest innervation of the ipsilateral NB seems to be homogeneous. During metamorphic and early postmetamorphic stages, the reaction product is distributed unevenly, indicating that some reshaping of this terminal field occurs during development. However, in the frog, this reshaping does not result in segregation of fibers from the contralateral and ipsilateral eyes, since the projection to the *contralateral* NB remains homogeneously distributed throughout the terminal field (S. G. Hoskins and P. Grobstein, unpublished observations). Subsequent postmetamorphic changes in the NB after 6 months involve not segregation but rather an *increase* in the amount of overlap of the ipsilateral and contralateral projections. At the completion of metamorphosis the core of the NB is primarily innervated by fibers from the contralateral eye. Since in the adult the ipsilateral NB appears to be as densely and evenly filled with reaction product as is the contralateral NB, the core of this terminal field must acquire substantial ipsilateral innervation sometime after the first 6 postmetamorphic months. The functional significance of this "fill-in" is not known, although it may be related to the continuing postmetamorphic shift in eye position characteristic of *X. laevis*.

Production of ipsilaterally projecting ganglion cells. Two main conclusions can be drawn from our studies on the birth dates of ipsilaterally projecting ganglion cells. First, neurons continue to be generated at the retinal periphery after the completion of metamorphosis (see also Jacobson, 1976), and some of these late-born neurons project to the ipsilateral thalamus. Second, nearly all retinal ganglion cells whose axons or axon branches project to the ipsilateral thalamus are born late in development. Very few neurons located central to thymidine rings made at stages 54 to 55 are found labeled with HRP transported retrogradely from ipsilateral terminal zones.

Previous analyses of retinal growth in *X. laevis* have documented a shift from symmetrical to asymmetrical addition of ganglion cells which favors the ventral periphery and occurs at about stage 55 (Hollyfield, 1971; Jacobson, 1976; Beach and Jacobson, 1979a; Gaze et al., 1979). The distributions of ipsilaterally projecting cells described in the previous paper (Hoskins and Grobstein, 1985a) suggested that such cells might be born subsequent to this shift. The present findings provide direct evidence for this conclusion. Although stage 54/55 is the time at which significant numbers of ipsilaterally projecting cells begin to be produced, it is noteworthy that not *all* of the neurons generated after this time project ipsilaterally. As discussed previously (Hoskins and Grobstein 1985a), the density of ipsilaterally projecting cells is low, and the peripheries of retinas *contralateral* to injection sites in the thalamus also contain large numbers of HRP-stained ganglion cells. Thus, the frog retina undergoes two phases of development. The vast majority of cells born during embryogenesis and up to stage 54 project contralaterally in the adult. After stage 54/55, a proportion of the neurons born sends axons ipsilaterally at the optic chiasm, whereas other neurons born during this period project contralaterally, as did their predecessors.

Thus, with regard to the behavior of axons at the optic chiasm, the optic nerve seems to undergo a transition from "all axons project contralaterally" to "some axons project ipsilaterally, some contralaterally," at about stage 55. It is possible that some earlier-born neurons make transient ipsilateral projections (cf. O'Leary et al., 1983, on the

chick), but it seems unlikely that these are present in significant numbers, given the failure to detect ipsilateral projections prior to stage 54, using a variety of techniques (Khalil and Szekely, 1976; Hoskins and Grobstein, 1981; Kennard, 1981). Since stage 55 marks the beginning of the period of time during which the retina undergoes an alteration in its pattern of proliferation (Beach and Jacobson, 1979a), and during which the vast majority of ipsilaterally projecting retinal ganglion cells are born, this suggests that the factors which bring about the development of an ipsilateral projection to the thalamus are of a kind which act on proliferating or recently produced neurons and not on cells produced earlier. Since stage 55 also represents the beginning of thyroxine-induced metamorphic change (Streb, 1967; Dodd and Dodd, 1976), the hypothesis of a critical event which begins at stage 55 and influences a proliferating population is of particular interest because there is evidence that the hormone thyroxine is involved in bringing about the shift from symmetrical to asymmetrical patterns of ganglion cell addition (Beach and Jacobson, 1979b). The role of thyroxine in the development of the ipsilateral retinothalamic projection is discussed in the following paper (Hoskins and Grobstein, 1985b).

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