Factors involved in the development of ipsilateral retinothalamic projections in *Xenopus laevis*

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**SUMMARY**

The extent, and the development, of the ipsilateral retinothalamic projection in the frog *Xenopus laevis* have been studied using terminal degeneration and autoradiographic techniques. This ipsilateral projection derives only from those retinal areas receiving visual information from the binocular portion of the visual field. In *Xenopus*, the ipsilateral retinothalamic projection arises from a larger area of the retina than was found to be the case in earlier studies on *Rana*. This correlates with the fact that *Xenopus* has a larger binocular visual field than does *Rana*.

The ipsilateral retinothalamic projection is just detectable at about stage 56 of larval life, considerably later than its contralateral counterpart. Experimental manipulation of the developing eye vesicle at early larval stages followed by histological studies of the ipsilateral retinothalamic projections showed, however, that the retinal areas which give rise to this projection are determined by stage 32 of larval life. Further studies, in which monocular enucleation was performed at different larval stages with subsequent examination of the retinothalamic projections from the remaining eye, indicated that the selective pattern of decussation and non-decussation of retinothalamic fibres at the optic chiasma does not require interactions, at the chiasma, between optic fibres from the two eyes.

**INTRODUCTION**

The amphibian visual system has played a major role in the analysis of developmental factors governing the formation of selective neuronal connections. Attention in this respect has been concentrated on the totally decussated projection from the retina to the contralateral optic tectum (Sperry, 1951; 1963; Gaze, 1970; Jacobson, 1978). Neuroanatomical studies in the last decade have, however, revealed that in anuran amphibians, in addition to the crossed retino-fugal projections to the contralateral optic tectum and to various contralateral diencephalic areas, there also exist uncrossed components which project to ipsilateral diencephalic centres (Knapp, Scalia & Riss, 1965; Scalia, Knapp, Halpern & Riss, 1968; Lázár & Székely, 1969; Lázár, 1971; Scalia & Fite, 1974; Levine, 1978, 1980).

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In mammals, the degree of uncrossed retinofugal projections correlates with the degree of binocular overlap of the visual fields of the two eyes (Walls, 1942). It was of interest to observe whether a similar correlation obtains in anuran amphibians. In *Rana*, which has its eyes arranged such that there is a moderate overlap of the visual fields of the two eyes, the ipsilateral retinothalamic projection arises only from the peripheral temporal retina (Scalia & Fite, 1974). *Xenopus* has a much larger binocular visual field particularly superiorly (Grobstein & Comer, 1977) and thus if the above correlation is true, the ipsilateral retinothalamic projection in this species should derive from larger areas of the retina than in *Rana*. It has also been observed by Currie & Cowan (1974) that the ipsilateral retinothalamic projections in *Rana* appears later in development than the corresponding contralateral projections, and that the appearance of this ipsilateral retinothalamic projection coincides with the appearance of a significant frontal binocular visual field. The ipsilateral projections in *Xenopus* were studied to see if a similar correlation operated in this anuran. In addition, experiments were carried out to elucidate the developmental stage at which retinal areas which will give rise to the ipsilateral retinothalamic projection are determined, to see if this latter projection is determined at a later developmental stage than that at which the contralateral retinal projections are determined.

Finally, the existence of bilateral retinal projections in amphibians provides an opportunity to investigate in these species, the possibility that the patterns of decussation and non-decussation of optic fibres from the two eyes at the optic chiasma develop by interaction between fibres from the two eyes, as has been suggested by Lund and his colleagues (Lund, Cunningham & Lund, 1973; Lund, 1975; Lund & Miller, 1975) on the basis of their studies on neonatal rats.

**MATERIALS AND METHODS**

To determine the retinal area which gives rise to ipsilaterally projecting retinothalamic fibres, localized retinal lesions were made with a heated tungsten needle in one of eight different retinal locations situated around the periphery of the retina in adult *Xenopus laevis*, anaesthetized with ether. The animals were allowed to recover from the anaesthetic and were sacrificed 5 days later. The distribution of degenerated optic fibres and optic terminals in the ipsilateral diencephalon were demonstrated by the technique of Fink & Heimer (1967).

The maturation of the retinothalamic projections was studied by autoradiographic analysis of the distribution of silver grains following intraocular administration of [$^3$H]proline to one eye of 40 *Xenopus* of differing larval and early post-metamorphic stages, staged according to the criteria of Nieuwkoop & Faber (1967). The amount of labelled amino acid injected varied according to the age of the animal from 1 $\mu$Ci in 0.25 $\mu$l to 4 $\mu$Ci in 1 $\mu$l and was administered by microinjection in animals anaesthetized with tricaine methane sulphonate (MS 222, Sandoz). The animals were permitted to recover from the anaesthetic
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and were then sacrificed from 6 to 24 h later again depending upon developmental stage. The brains were dissected free, fixed in Carnoy’s solution and embedded in paraffin. Serial sections (10 μm) of the brain were cut in the transverse plane and were processed for autoradiography by the method of Rogers (1972).

Operations on one eye vesicle or eye of animals of various embryonic or larval stages were carried out to assess the developmental stage at which are determined those retinal areas which will give rise to the ipsilateral retinothalamic projections. Thus rotations of an eye by 180° were performed in Xenopus embryos of stage 32 or 37 (Nieuwkoop & Faber, 1967). In other embryos of stage 32 one ‘compound eye’ was constructed by methods similar to those of Gaze, Jacobson & Székely (1963). In the animals considered in this paper a cut was made in the eye vesicle along a meridian which permitted the temporoventral portion of the developing retina to be left in place while the nasodorsal portion was removed and replaced by the temporoventral retinal portion from the eye of another animal. The graft retina was placed in one of two orientations (Fig. 1) in different animals. The operated eye thus derived entirely from retina which was of temporoventral origin. The retinofugal projections from these rotated or temporoventral ‘compound’ eyes were studied when the animals were aged 3–6 months post-metamorphosis. Localized retinal lesions were produced in these animals as described for normal adult controls above and the projections from such lesioned eyes studied both by degeneration and autoradiographic techniques.

The possible role of interactions at the optic chiasma between optic fibres from the two eyes in determining the pattern of decussation and non-decussation of optic fibres was investigated by studying the retinal projections from an eye in animals in which such interactions were impossible. Enucleation of one eye was carried out in embryonic Xenopus of stage 30 or 32, or larval Xenopus of stage 50. Three to six months after metamorphosis the retinal projections from the remaining eye were studied either by analysis of degeneration products following localized retinal lesions or by autoradiographic techniques.

RESULTS

(a) Normal diencephalic retinofugal projections in adult Xenopus laevis

Diencephalic retinofugal projections in normal adult Xenopus laevis were examined in 10 animals by autoradiographic methods and in 11 animals by observing the distribution of degeneration products following monocular enucleation. Both techniques yielded similar results (Fig. 2). Seven diencephalic sites of optic fibre termination were identified, all of which received an input from both eyes. Projection sites in the dorsal thalamus were, from rostral to caudal, the nucleus entopeduncularis posterior (rostral visual nucleus, Levine, 1980), the neuropi of Bellonci, the corpus geniculatum thalamicum, the posterior
Fig. 1. Diagram to show the method of construction of right compound eyes used in this study. In stage-32 *Xenopus* embryos a cut (dotted line) was made across the developing eye along a meridian from temporodorsal to nasoventral. The nasodorsal portion of the developing eye was removed and replaced by the temporoventral portion from a donor animal. If the donor portion came from a left eye the result was a TT/VV eye, if from a right eye a TV/TV eye. In the lower part of the diagram are represented the two types of compound eye, the portion marked by open circles indicating host tissue and that by closed circles indicating the donor tissue.

thalamic nucleus, the pretectal nucleus, and the uncinate nucleus. In addition ventrally at the diencephalic junction the basal optic nucleus receives bilateral retinal input (for details see Kennard, 1978). In contrast to the binocular nature of the retinal fibre input to all diencephalic projection sites, the optic tectum received input only from the contralateral eye.

In the remainder of this paper analysis of the ipsilateral retinothalamic projections has been confined largely to the dense ipsilateral projections of the rostral thalamic areas, the neuropil of Bellonci and the corpus geniculatum thalamicum.
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Fig. 2. Retinofugal projections from the left eye of a normal adult *Xenopus laevis*. The left eye received an injection of \(^{3}H\)proline 24 h before the animal was sacrificed. Camera-lucida drawings were made of sections taken at 100 \(\mu\)m intervals and the location and approximate density of silver grains within the sections were plotted. The lower series of drawings represent the ipsilateral (left) side of the brain while the upper series represents the contralateral (right) side of the brain, drawn mirror-reversed to facilitate comparison. The sections pass rostrocaudally from left to right. NE, nucleus entopeduncularis posterior; NB, neuropil of Bellonci; C, corpus geniculatum thalamicum; PN, posterior thalamic nucleus and pre-tectal nucleus; UN, uncinate nucleus; T, optic tectum; BON, basal optic nucleus.

(b) Retinal areas giving rise to ipsilateral retinothalamic fibres

Since localized retinal lesions of central retinal areas inevitably interrupt optic fibres travelling from more peripheral retinal areas to the optic nerve head, it is not easy to obtain direct evidence about the projection of these central areas from such lesions. Retinal lesions were thus restricted to peripheral retinal areas. Localized retinal lesions were made in 18 adult *Xenopus* in one of eight different retinal sites, at the nasal, nasodorsal, dorsal, temporodorsal, temporal, temporoventral, ventral or nasoventral retinal periphery (Fig. 3).

In ten animals the distributions of degeneration products were mapped using the Fink–Heimer technique. In the other eight animals, 24 h prior to sacrifice \(^{3}H\)proline was injected into the lesioned eye. In these animals serial sections of the brain were processed alternately for autoradiography and for silver staining.

The extent of the retinal lesion was checked both on the retina itself and by analysing the distribution of degeneration products in the contralateral optic tectum (Fig. 3). Degeneration was found in the contralateral neuropil of Bellonci and corpus geniculatum thalamicum following retinal lesions at all eight locations. Terminal degeneration in the ipsilateral thalamic areas was seen only
Fig. 3. (a) Diagram illustrating the location of various lesions at the retinal periphery of the left eye, V coincides with the ventral iridial notch. (b) Outline drawing of the right optic tectum, the arrow points rostrally, medial is to the left and lateral to the right. On the tectum are illustrated the locations of regions of terminal degeneration following localized retinal lesions to the contralateral eye. Key: D, dorsal; TD, temporodorsal; T, temporal; TV, temporoventral; V, ventral; NV, nasoventral; N, nasal; ND, nasodorsal.

after lesions of temporodorsal, temporal, temporoventral, ventral and nasoventral retinal regions (Fig. 4). By contrast, no ipsilateral retinodiencephalic degeneration was seen after nasal, nasodorsal or dorsal retinal lesions (Fig. 5).

Reconstruction of the neuropil of Bellonci was made and the distribution of degeneration products following the localized retinal lesion was made. As may be seen from Fig. 5 a single lesion yielded degeneration products quite widely distributed throughout this area. Comparison of the areas occupied following lesions at different retinal sites did not reveal evidence of a topographically ordered projection. It was noted that ipsilateral retinothalamic projections were, in the main, restricted to caudal regions of the neuropil.

(c) The maturation of the retinothalamic projections

The development of the retinothalamic projection was studied autoradiographically in 40 larval and early post-metamorphic *Xenopus* following intraocular administration of \[^{3}H\]proline. In larvae of stage 50, a mid-larval stage at which limb buds are just appearing, the retina projected to the contralateral tectum and to all the contralateral thalamic visual areas. In such animals there was no evidence of an ipsilateral retinothalamic projection (Fig. 6). As animals of progressively greater age were examined ipsilateral retinothalamic projections

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**Fig. 4.** Photomicrographs showing discrete areas of terminal degeneration in (A) the contralateral and (B) the ipsilateral neuropil of Bellonci following a localized temporal retinal lesion in *Xenopus*. Fink–Heimer stain, transverse sections. Scale = 20 μm.
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Fig. 5. The distribution of terminal degeneration in the contralateral (C) and ipsilateral (I) neuropils of Bellonci and in the contralateral optic tectum (T) following a localized retinal lesion in a 6-month post-metamorphic Xenopus. 7 days before sacrifice a lesion was made in the temporal periphery in (A) and in the nasal periphery in (B). 24 hours before sacrifice tritiated proline was injected into the lesioned eye. The brain was sectioned and alternate sections processed autoradiographically and stained by the Fink–Heimer method. Study of the autoradiographic series permitted the extent of the neuropil of Bellonci to be delimited. The distribution of degeneration products from the lesion was plotted with camera lucida reconstruction, and is illustrated in sequential sections (at intervals of 40 μm) with rostral sections displayed at the top and caudal sections at the bottom. The outline of the contralateral tectum (T) at the bottom of the figure, with the arrow pointing rostrally, shows the tectal distribution of degeneration products. M, Medial; L, lateral.

Fig. 6. (A) Dark-field illumination of an autoradiograph of a transverse section through the rostral thalamus of a stage-50 Xenopus tadpole. 6 h before sacrifice 1 μCi of [3H]proline had been injected into one eye. Dense projections to the contralateral thalamic region (left side of photomicrograph) are already evident. The two dense areas represent the neuropil of Bellonci superomedially and the corpus geniculatum thalamicum ventrolaterally. The thin band of label passing mediolaterally in the ventral region is the optic tract. No evidence of an ipsilateral retinofugal projection was observed at this stage. Scale = 100 μm. (B) Retinofugal projections from the eye of a stage-52 Xenopus tadpole: 6 h before sacrifice 1 μCi of [3H]proline had been injected into the eye. Camera-lucida drawings of transverse sections at 100 μm intervals are shown, together with the distribution of silver grains. The drawings labelled 1–7 progress from rostral to caudal. It may be seen that the contralateral retinofugal projections were present at this time but no ipsilateral retinofugal projections were observed. Key, as in Figure 2.
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Fig. 8. The distribution of terminal degeneration in the contralateral (C) and ipsilateral (I) neuropil of Bellonci following a localized retinal lesion in the left eye of a six-month post-metamorphic *Xenopus*. Conventions and methodology as in Fig. 5. (A) At stage 37 the left eye had been rotated by 180° and had remained in this position. Seven days before sacrifice a lesion was made at the nasal pole of the retina which derived from retina that was embryologically temporal. It may be seen that ipsilaterally a retinothalamic projection from this area was present. (B) At stage 32 the left eye had been rotated by 180° and had remained in this position. Seven days before sacrifice a localized lesion was made in the temporoventral retinal periphery which derived from embryological nasodorsal retina. Terminal degeneration was found in the neuropil of Bellonci only contralaterally.

Fig. 7. Dark-field illumination of autoradiographs of transverse sections through the rostral thalamic region of the brain of *Xenopus* at different developmental ages: 6 h before sacrifice [3H]proline was injected into the left eye. (A) stage-57/58 tadpole; (B) stage-64 tadpole; (C) 6 weeks post-metamorphic animal. It may be seen that the ipsilateral retinofugal projection is present at all these stages but increases considerably in density with age. Scales: (A), B, 150 μm; (C), 300 μm.
were first seen in animals of stage 55 but were not seen constantly until stage 57. As development proceeded further the ratio of the densities of the ipsilateral and contralateral retinothalamic projections gradually increased until about 6 weeks after metamorphosis when the ratio appeared similar to that observed in the adult (Fig. 7).

**(d) The retinal origin of the ipsilateral retinothalamic projection following embryonic eye manipulation**

Eye rotations were performed in *Xenopus laevis* embryos of either stage 32 or 37. Nine of these animals matured satisfactorily until 3–6 months after metamorphosis at which time the left eye of eight of the animals was in a position of 180° rotation and in one animal the left eye was rotated by 90°. This degree of eye rotation was assessed by the position of the iridial notch which in normal animals is situated ventrally. In four of these animals autoradiography following intraocular injection of [3H]proline to the rotated eye showed that the eye had failed to connect with the brain. Retinal projections were thus studied in the five remaining animals. The rotated eye received a localized retinal lesion 5 days before the animal was sacrificed. In two of the animals, in both of which the eye was rotated by 180°, the lesion was placed in the nasal retina which, of course, because of the rotation derives from embryonically temporal retina. In both cases Fink–Heimer study of the projection revealed that the lesioned retina projected to the ipsilateral thalamus (Fig. 8 A). Thus although peripheral nasal retina in normal animals does not project to the ipsilateral thalamus, it does so in animals in which the eye was rotated at stage 32 or 37 of embryonic or larval life. The distribution of degeneration in the contralateral tectum was found to be at the rostral pole, normally innervated from the temporal retinal pole (Fig. 8 A). The converse experiment was carried out on the other three animals with rotated eyes. In these cases the localized retinal lesion was placed in a retinal area which in the normal animal does project to the ipsilateral thalamus but which in the rotated eye derives from retinal areas which do not normally project to the ipsilateral thalamus. In two of these three animals the eye was rotated by 180°. The localized lesion was placed in temporoventral retina which because of the rotation derives from retina which was embryonically nasodorsal retina. In the third animal, the eye was rotated by 90° in a clockwise direction so the localized retinal lesion was placed in temporodorsal retina which derived from embryonically nasodorsal retina. In none of these three animals was there any sign of terminal degeneration in the ipsilateral thalamus (Fig. 8 B), whereas in normal animals temporoventral or temporodorsal retinal lesions invariably yielded degeneration products in the ipsilateral thalamus. The conclusion that emerges from these animals with an early eye rotation is that the portion of the retina that is going to project to the ipsilateral thalamus is determined by the embryonic position of the retina before stage 32 rather than
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the retinal position after this stage, even though the ipsilateral retinothalamic projection does not appear until stage 56.

The results obtained following localized retinal lesions made in 'compound' eyes composed of two tempororoventral halves led to a similar conclusion. Thus localized retinal lesions in the current nasodorsal retinal periphery of such eyes were made in five animals. The terminal degeneration resulting from such lesions were studied with Fink–Heimer techniques and the overall retinothalamic projections from the 'compound' eye were studied autoradiographically. In four of the animals the retinal lesion resulted in terminal degeneration in both ipsilateral and contralateral anterior thalamic neuropil (Fig. 9). A similarly positioned lesion in a normal animal would give rise only to degenerating terminals in the contralateral thalamic neuropil. In the fifth animal, no degeneration was seen in the ipsilateral thalamus. In this case, however, examination of the distribution of terminal degeneration in the contralateral optic tectum revealed that it was localized to caudal tectal areas which would have received input from the nasodorsal area in a normal eye. This, together with the failure of the lesion in this animal to produce ipsilateral retinothalamic degeneration could well mean that the surgical attempts to create a 'compound' eye at stage 32 had failed and that embryonic regulation to produce a normal eye had occurred. This phenomenon has previously been described in a small proportion of 'compound' eye operations in which subsequent electrophysiological mapping of the retinotectal projection yielded a normal projection (Gaze, Jacobson & Székely, 1965).

(e) Ipsilateral retinothalamic projections from monocular Xenopus

Monocular enucleation was performed in 21 Xenopus at stage 30 (n = 11), stage 32 (n = 7) or stage 50 (n = 3). The retinofugal projections that developed from the remaining eye were studied subsequently either by autoradiography or by analysis of terminal degeneration following localized retinal lesions. In three of the animals enucleated at stage 32 the remaining eye received an intraocular injection of [3H]proline at stage 52 to determine whether the ipsilateral retinothalamic projection developed earlier than normal under these circumstances. No evidence for the premature appearance of such an ipsilateral retinothalamic projection was found in any of these animals.

In the remaining 18 animals the distribution of the retinothalamic projections were studied by autoradiography when the animals were between 3–6 months post-metamorphosis. In all of these animals the ipsilateral retinothalamic projections appeared normal and did not spread into those thalamic regions normally innervated only by the contralateral eye (Fig. 10). In eight of these 18 animals the remaining eye received a localized retinal lesion 4–6 days before receiving an intraocular injection of [3H]proline. The localized lesions were made in the temporodorsal (TD) retinal periphery in four cases and in the nasodorsal (ND) periphery in four cases. In these animals alternate serial sections were prepared
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Fig. 10. Retinofugal projections to the rostral thalamus from the remaining eye of a 6-month-old post-metamorphic *Xenopus laevis* from which the right eye had been removed at stage 32 of larval life. The left eye received an injection of [³H]proline 24 h before sacrifice. Camera-lucida drawings of alternate sections of a series of 20 μm transverse sections were made. The upper series represents the contralateral (right) side of the brain, drawn in mirror-image to facilitate comparison with the lower series representing the ipsilateral (left) side of the brain. Key as in Fig. 2.

for silver staining and autoradiography so that the topography of the projections from the lesioned area could be studied at the same time as the overall projection from the remaining retina. In those animals with ND retinal lesions localized areas of terminal degeneration were seen in the usual positions in the contralateral thalamic neuropil but none was found in the ipsilateral anterior thalamic neuropil. This retinal region, which does not project to the ipsilateral thalamus in normal animals, did not do so in monocular animals. All animals with a TD retinal lesion showed bilateral terminal degeneration in the anterior thalamic neuropil.

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Fig. 9. Photomicrographs showing terminal degeneration in (A) the contralateral and (B) the ipsilateral neuropil of Bellonci following a nasodorsal retinal lesion in a TV/TV compound eye which had been constructed at stage 32. The retinal lesion was made when the animal was three months post-metamorphosis and the animal was sacrificed 7 days later. Fink–Heimer stain. Scale = 20 μm.
Several points emerge from these results. The retinothalamic projection in *Xenopus* appears later in larval life than the contralateral retinothalamic projection. The extent of the ipsilateral retinothalamic projection correlates with the degree of binocular overlap of the two eyes but the retinal areas which give rise to this projection are selected by a developmental programme the spatial properties of which are determined by stage 32 of larval life. The selective patterns of decussation and non-decussation of retinothalamic fibres at the optic chiasma do not depend upon an interaction at the optic chiasma between fibres from the two eyes.

The distribution of degeneration products in ipsilateral retinothalamic areas after localized retinal lesions in normal adult *Xenopus* indicates that, in *Xenopus*, the ipsilateral retinothalamic projections arise from larger areas of the retina than they do in *Rana* (Scalia & Fite 1974). This supports the findings of Khalil & Székely (1976) and Levine (1980) of an enlarged ipsilateral retinothalamic projection in *Xenopus* compared with that in *Rana*. For technical reasons it was necessary to limit retinal lesions to the retinal periphery and from these results no definitive statements can be made about central retinal areas participating in these projections. It is likely, however, that central retinal areas, situated between those peripheral areas which are known to project ipsilaterally, also do so. Electrophysiological studies on the ipsilateral retinothalamic projection (Kennard & Keating, submitted for publication) indicate that this is so in *Rana*. If this is the case in *Xenopus* then ganglion cells from more than half the retinal surface project to the ipsilateral thalamus, only those in the nasodorsal third of the retina failing to do so. The binocular overlap of the two monocular visual fields in *Xenopus* is considerably larger than in *Rana* (Keating, 1974; Grobstein & Comer, 1977). Thus the correlation that has been noted in mammals between the extent of the binocular visual field and the degree of ipsilateral visuotectal projections may be extended to anuran amphibians.

The delay in the appearance of the ipsilateral retinothalamic projection when compared with the contralateral projection in *Xenopus laevis* is similar to that which has been described in another amphibian *Rana pipiens* (Currie & Cowan, 1974) and has also been described in *Xenopus* by Khalil & Székely (1976). These latter authors, on the basis of degeneration techniques claimed that the ipsilateral retinothalamic projection did not appear until stage 60–62 in *Xenopus*, but the autoradiographic techniques used in this study indicate a somewhat earlier appearance. The first appearance of the ipsilateral retinothalamic projection in *Xenopus* occurs just prior to the beginning of metamorphic climax during which time the laterally placed eyes migrate in an anterosuperior direction, resulting in an overlap of the monocular visual fields. One possible explanation for this delay is that the ganglion cells from which the projection derives may not appear until late larval life.
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Retinal histogenesis in *Xenopus* occurs by the addition of rings of neurones at the retinal periphery (Straznicky & Gaze, 1971; Jacobson, 1976, 1977; Gaze, Keating, Ostberg & Chung, 1979) this accretion continuing at least into early juvenile life. As discussed above it seems most likely that the retinal areas projecting to the ipsilateral thalamus include central retinal areas in which reside the oldest retinal ganglion cells that have been present since stage 30. It therefore seems most unlikely that the explanation for the delayed appearance of the ipsilateral retinothalamic projections is the delayed appearance of the retinal ganglion cells giving rise to this projection.

The development of these ipsilateral retinothalamic fibres clearly requires an activating trigger which could either be an intrinsically preprogrammed maturational phase or could be a response to some extrinsic factor. Since the projection appears just prior to the onset of metamorphic climax at a time when the circulating level of thyroid hormone rises (Etkin, 1968) this hormone is a possible candidate. This is however, made unlikely by the finding of a similar delayed appearance of the ipsilateral retinothalamic projection in a urodele *Ambystoma mexicanum* which is neotenic and does not undergo metamorphosis due to a lack of thyroid hormone (Kennard, 1978) and also by the failure to induce prematurely an ipsilateral retinothalamic projection in *Xenopus* with intraocular pellets containing tri-iodothyronine (Kennard, 1978).

An alternative explanation for the delayed ipsilateral retinothalamic projection is that the developmental programme selecting retinal ganglion cells which will project their axons ipsilaterally operates at a later developmental stage than that controlling the contralateral retinotectal and retinothalamic projections. This explanation is not supported by the results of experiments in which localized retinal lesions were made in rotated or compound eyes before stage 32 of larval life. The distribution of terminal degeneration from these localized lesions was such as to indicate that spatial coordinates of the developmental programme determining those retinal areas which will project ipsilaterally had already been established by stage 32. The polarisation of the retina which controls the pattern of connections in the contralateral retinotectal system is known to have occurred by stage 32 (Jacobson, 1968) although it may well in fact occur considerably earlier (Gaze, Feldman, Cooke & Chung, 1979). A similar conclusion as to the early specification of the ipsilateral retinothalamic projections in *Xenopus* was reached by Tay & Straznicky (1977) who studied the ipsilateral retinothalamic projections from double-nasal and double-temporal compound eyes. The interpretations of Tay and Straznicky need to be modified slightly since they assumed that ipsilateral retinothalamic projections derived only from temporal retina and they were surprised to obtain autoradiographic evidence of ipsilateral retinothalamic projections from double-nasal eyes. In fact as described in the results section the nasoventral retinal sector does project ipsilaterally in the normal animal and this adequately accounts for the
ipsilateral retinothalamic projections described by Tay & Straznicky (1977) in double-nasal eyes.

The finding of a normal overall distribution of the ipsilateral retinothalamic projection from the remaining eye following a unilateral enucleation at an early larval stage suggests that interactions between the growth cones of developing retinothalamic axons from the two eyes at the optic chiasma are unnecessary for their normal guidance to the ipsilateral or contralateral optic tract. Similarly, rotation of the eye vesicle would be expected to result in a misalignment of optic nerve fibres arriving at the optic chiasma which, if interactions were important, would have disrupted the normal pattern of decussation. This was not found and these results are therefore in contrast to the results suggesting chiasmal interaction of optic nerve fibres obtained by Lund et al. (1973) in rats following unilateral enucleations.

One is led to the conclusion that the developmental programming of each retinal ganglion cell contains precise information on whether or not its axon will decussate at the optic chiasma or pass into the ipsilateral optic tract as well as information determining the visual centre in which it will eventually terminate.

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