The diencephalic course of regenerating retinotectal fibres in *Xenopus* tadpoles

By R. M. GAZE and P. GRANT

*From the National Institute for Medical Research, Mill Hill, London*

**SUMMARY**

The normal retinotectal path in the diencephalon of *Xenopus* tadpoles is widely distributed in the form of a wedge of fibres extending from the central grey to the outer margin of the diencephalon. Regenerating optic nerve fibres were shown, by silver-staining and proline autoradiography, to follow an abnormal path up the extreme lateral edge of the diencephalon. Study of tadpoles at various stages of development, and of optic nerves allowed to regenerate for various periods, indicates that all new incoming optic fibres pass up the lateral edge of the diencephalon. The inner/outer order of the fibres in the normal diencephalon thus reflects the radial distribution of the retinal cells of origin.

**INTRODUCTION**

Retinal fibres going to the optic tectum in *Xenopus* tadpoles cross in the optic chiasma and pass dorso-caudally through the diencephalon. In studies of the regeneration of tadpole optic nerves we have observed that the time of arrival of the fibres at the chiasma has an effect on their distribution in the diencephalon. In a normal tadpole, fibres from the central retina, near the optic nerve head (the oldest retinal fibres and thus the first to reach the chiasma), traverse the diencephalon most medially, while fibres from peripheral retina (the youngest retinal fibres, and thus most recently arrived) travel most laterally. We describe here observations which indicate that the inner/outer distribution of retinotectal fibres in the diencephalon of the late-stage tadpole is dependent upon the stage of development of the animal at which the fibres enter the diencephalon.

**METHODS**

*Xenopus* tadpoles were bred in the laboratory by standard methods involving the subcutaneous injection of chorionic gonadotropin. Animals were fed on Heinz baby soup (beef and liver), in dilute suspension, strained through muslin. Tadpoles were staged according to the criteria of Nieuwkoop & Faber (1967).

1 *Author's address:* The National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA, U.K.

2 *Author's address:* University of Oregon, Eugene, Oregon 97403, U.S.A.
Under anaesthesia produced by immersion in 1:3000 tricaine methanesulphonate, one optic nerve, usually the left, was cut close to the entry into the cranium, by insertion of a knife made from a piece of razor blade. The operation was performed under direct vision through a dissecting microscope and a small gap in the nerve could be seen after the cut was made.

Operated animals were kept in small aquaria (approximately 25 cm by 10 cm by 10 cm) with up to ten animals per aquarium, at either 20 or 27 °C, and with a normal day length.

In most experiments, regeneration of the optic nerve was demonstrated by electrophysiological mapping after times ranging from 10 days upwards. The techniques used for mapping the visuotectal projection have been described previously (Gaze, Keating & Chung, 1974).

After electrophysiological mapping (and in some cases, unmapped) the animals were fixed in Susa fixative and 15 μm paraffin sections were cut in various orientations and the sections stained with Holmes' silver method.

Eleven normal tadpoles, of various stages, and nine operated animals were used for autoradiographic analysis of the retinotectal projection following intraocular injection of [3H]proline ([3H]P; Radiochemicals Centre, Amersham; specific activity 40 Ci/mmol); standardly 1 μCi was given in 0.25 μl. In some operated animals the proline was injected on the day prior to the recording experiment. This did not appear to interfere with the mapping. In other animals the proline was injected after the map had been made and the tadpoles were then kept in Niu-Twitty solution (Niu & Twitty, 1953) for 6–24 h before being used for autoradiography. This arrangement was not satisfactory since the prolonged exposure of the brain to the saline solution appeared to wash out the tectal label. Autoradiographs were made from 10 μm sections, coated with Ilford K2 emulsion and exposed for 18 days.

A series of normal tadpoles was examined histologically and autoradiographically.

This paper is based on the study of 106 silver-stained preparations of tadpoles at stages 50, 52, 54, 55, 56, 57, 58 and 62; and a group of 15 silver-stained embryos and early tadpoles; also on autoradiography of 11 normal tadpoles of stages 54, 55, 56 and 57, and on autoradiography of nine experimental animals of stages 54 and 56.

RESULTS

At 27 °C we obtained fully recovered visuotectal maps in 10 days in a number of tadpoles. By this we mean that the apparent extent and order of the map was normal; we have no means of knowing what proportion of the fibres had regenerated at this time. At 20 °C few tadpoles showed visual responses after 10 days but the great majority had reformed complete maps by 20 days. The nature and progression of the maps obtained will be described elsewhere. Here we concern ourselves with the anatomical results.
The normal retinotectal path in the diencephalon

The normal path followed by the optic fibres in a tadpole of mid-fifties stages is shown schematically in Figs. 1a and 7, and in a photomicrograph in Fig. 2. Darkly staining fibres pass from the region of the chiasma laterally, dorsally and caudally. The distribution of these fibres mediolaterally extends from near the central grey matter to the lateral margin of the diencephalon and the optic fibres run in bundles interspersed with fibres of other kinds which, in coronal sections, are mainly cut transversely. These fibre bundles running latero-caudo-dorsally from the chiasma are optic in origin in that they degenerate over a period of days following removal of the eye (Fig. 3) and they are labelled after the injection of [3H]P into the contralateral eye (Fig. 4).

Optic fibres in the diencephalon include those ending in various regions of diencephalic neuropil as well as those going to the tectum. In this paper we take no account of fibres ending in the diencephalon. We are only concerned with retinotectal fibres; and the distributions we describe are those of fibres going to the tectum. Fibre fascicles from the lateral to the medial boundaries of the
The path of regenerating retinotectal fibres in the diencephalon

As soon as the regenerating visuotectal projection can be demonstrated electrophysiologically (i.e. by 10–20 days after nerve section) regenerated optic fibres can be seen, in silver-stained preparations, in the diencephalon. In all cases the pathway followed by the regenerating fibres is abnormal. Instead of forming an extended array from near the central grey to the lateral margin of the brain, regenerating fibres all pass up the far lateral edge of the diencephalon, forming a compact band (Figs. 1b, 2, 6 and 7). The fibres forming this compact band presumably include some destined to end in the diencephalon. In our preparations, however, the main part of the band can be followed up to, and into, the tectum.

Regenerating optic nerve fibres do not, in these animals, follow the paths of degenerating optic fibres through the substance of the diencephalon. Degener-
Fig. 3. Regenerating optic fibres (optic nerve cut 21 days previously) and degenerating optic fibres (eye removed 11 days previously) in the diencephalon. Tadpole EDTR 3–2, stage 55. (a) Low-power photograph of chiasmatic region. (b) Higher-power view of degenerating tract. 15 μm section. Holmes' silver method.
Fig. 4. Dark field photograph of tritium-labelled optic tract in a normal tadpole of stage 57. The contralateral eye had received an injection of 0.25 μCi [3H]proline 24 h before the animal was killed.

Degenerating bundles of fibres can be found for several weeks after nerve section; and it is often possible to see, in the same section, the band of degenerating fibres with the band of regenerating fibres diverging from it (Fig. 8). The only degenerating optic fibres which may lie in the path of the regenerating fibres are those which run up most laterally at the edge of the diencephalon.

This abnormal distribution of regenerating optic fibres indicates that these fibres are not being guided by place-related chemospecific cues on other diencephalic structures, or by traces remaining from the previous fibre projection. It looked as if the regenerating fibres were growing across the chiasma and then merely following the lateral edge of the diencephalon. We therefore decided to look at very young animals, at the time when the first retinal fibres arrive at the chiasma.

The path of optic fibres in the very young animal

Light microscopy of silver-stained sections shows that the first optic fibres to reach the chiasma do so at about state 33/35 (Fig. 9). These fibres, which are difficult to identify at these early stages, cross at the chiasma and pass up the
Regenerating retinotectal fibres in Xenopus tadpoles

Fig. 5. The diencephalo-tectal junction in a young tadpole. The section was cut in a plane lying between coronal and horizontal, thus showing optic fibres over a considerable length of the diencephalic tract. Fibres (fibre bundles) from the entire latero-medial extent of the tract can be seen entering the tectum. Dorso-caudal is to the top of the photograph, lateral to the right. The arrow indicates the diencephalo-tectal junction. Holmes’ silver stain.
extreme lateral edge of the diencephalon, which at this early stage consists only of a sack of neuroblastic cells. At the next few developmental stages, incoming optic fibres appear to run up the lateral edge of the diencephalon. However, both eye and diencephalon are growing rapidly and by stage 40 the optic path in the diencephalon shows some latero-medial extension.

It seemed likely, therefore, that all newly arriving optic fibres, whether during normal development or during regeneration after nerve section, course up the extreme lateral edge of the diencephalon. If this is so, then if we cut an optic nerve in mid-larval life and leave the animal for as long as possible before making a histological examination, we should find that there is now a band of regenerated fibres which runs up the lateral diencephalon, separated from the lateral edge by another band of tissue which contains all the new retinal fibres that have developed since the cut was made.

The reasons for this are straightforward. The retina grows, throughout larval life, by addition of cells at the ciliary margin (Straznicky & Gaze, 1971). The diencephalon also grows during larval life and the continuing arrival at the diencephalon of new fibres from the ciliary margin results in the extended
Regenerating retinotectal fibres in Xenopus tadpoles

Fig. 7. Diagram of retinotectal fibre pathways through the diencephalon, reconstructed from camera lucida drawings of horizontal sections of 15 μm thickness, stained with Holmes' silver method. Tadpole LONC 4, stage 54. Sections run from most dorsal, showing the tectal outline, at the bottom of the figure, to more ventral at the top of the figure. The right of the diagram shows the diencephalic distribution of fibres on the normal side and their entrance into the lateral and medial branches of the optic tract. On the left of the figure is shown the compact band of regenerated optic fibres at the lateral margin of the diencephalon (black). The contralateral optic nerve had been cut 12 days previously and the animal kept at 27 °C.

mediolateral array of optic fibres which can be seen in the mid-larval diencephalon (Figs. 1 a, 2 and 7). If, now, the optic nerve is cut, all optic fibres which had already grown past the site of the lesion will be severed, while some fibres from the most peripheral retina will not yet have reached the site of section and will thus be uninjured. Thus the mass of fibres growing towards the brain from the cut end of the nerve will mainly comprise regenerating fibres from the greater part of the retina, but will also include a proportion of uninjured fibres
from the retinal margin. These uninjured fibres can be expected to have a slight advantage (Reier & Webster, 1974) and may thus be able to reach the chiasma first. However, in comparison with the time-span over which the retinal fibres reach the chiasma during normal development this time difference must be small, and after nerve section the diencephalon thus receives within a short period of time fibres not merely from newly grown peripheral retina, but also from the rest of the retina; and all these behave as newly arriving fibres and pass up the lateral edge of the diencephalon (Figs. 1b, 2, 6 and 7). This may take place within 10 days, under optimal conditions. The retina and the diencephalon, however, continue to grow. After the compact band of regenerated fibres has been established at the lateral edge of the diencephalon, new retinal fibres arrive from peripheral retina which has grown since the nerve lesion. These new retinal fibres add to the lateral margin of the diencephalon, which is itself growing.

Observations consistent with this postulated sequence of events have been made in a series of 54 tadpoles in which the time between section of the nerve (at stages 50, 54 and 56) and histological examination ranged from 10 to
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Fig. 9. Coronal section showing early optic fibres crossing in chiasma and passing up laterally to diencephalon in a stage 35 Xenopus. Holmes’ silver stain, 15 μm section.

125 days. The consistent finding was that, with intervals of 30 days or more between nerve section and histological analysis (or even less in young tadpoles), a compact band of regenerated fibres was seen lying laterally in the diencephalon, separated from the pial surface by a band of tissue containing yet other optic fibres (Fig. 10).

Thus retinal fibres in the diencephalon show retinotopic order in relation to the radial positions of the cells of origin. Whether diencephalic retinotectal fibres are ordered in relation to the circumferential retinal distribution of their cells of origin is not yet known for Xenopus, but seems likely in view of the findings of Scalia & Fite (1974) in Rana. Certainly, the fascicles of retinal fibres show circumferential retinotopic order as they enter the optic nerve head (Fig. 11).

DISCUSSION

The main problem underlying this work is the nature of the influences which lead to the formation, by optic fibres, of a retinotopic map on the tectum. The most widely held view at the present time is that ordered nerve connexions of this sort are established by the action of selective affinities between pre-synaptic
Fig. 10. Regenerated optic fibres 63 days after optic nerve section. Tadpole EDTR 1-9, stage 57. The compact band of regenerated fibres that resulted from the initial regeneration of the nerve is now separated from the lateral edge of the diencephalon by further fibres which represent the new retinal margin which has grown since the optic nerve was cut. Holmes' silver stain, 15 μm coronal section.
Regenerating retinotectal fibres in Xenopus tadpoles

Fig. 11. Fascicles of optic nerve fibres entering the optic nerve head in a tadpole at stage 57. Reconstructed from parasagittal sections (15 μm) stained with Holmes' silver method. D, Dorsal; A, anterior.

optic fibres and their post-synaptic tectal partners (Sperry, 1951; Gaze & Hope, 1976). There has recently appeared, however, evidence suggesting that tectal specificity markers (responsible for the tectal end of the differential affinity system) may be imposed on the tectum by the incoming optic fibres (Schmidt, 1977) and that therefore the fibres may be able initially to establish a map in the absence of such tectal markers (Gaze, 1977). If this turns out to be the case, then some means of forming the retinotopic map on the tectum must be sought, other than differential retinotectal affinities. The most likely candidates for this role would then be either a fibre-sorting mechanism whereby the retinal fibres were able to establish or preserve a retinotopic order in the pathway, or a time-position mechanism whereby the distribution of fibres in the pathway was closely related to the position and time of development of each ganglion cell in the retina (Gaze & Hope, 1976); or some combination of both mechanisms. The observations described in the present paper relate to this general problem.

It has been shown (Attardi & Sperry, 1963) that, in adult goldfish, optic fibres select the appropriate branch of the optic tract (medial for dorsal tectum; lateral for ventro-lateral tectum); and there is evidence that a comparable choice is made by the developing Xenopus visual system (Straznicky, Horder and Gaze, unpublished; see also Gaze, 1977). Attardi & Sperry (1963) argued that the selection of the appropriate tract by optic fibres indicated the action of selective affinities in the optic pathway as well as in the tectum; and this could be the case in Xenopus as well. The present results, however, indicate a major
Fig. 12. Suggested mode of representation of the retina in the optic tract of the *Xenopus* tadpole. Left: Plan of left retina. Right: Coronal section through right optic tract in diencephalon. The retina is assumed to be represented as if cut open along a line joining ND to the optic nerve head; in the tract the various parts of the retina are shown according to the positions indicated by Scalia & Fite (1974) for *Rana*. N, D, T, V, nasal, dorsal, temporal, ventral; U, upper; L, lateral.

role for timing in the ventriculo-pial distribution of retinal fibres in the diencephalon of *Xenopus* tadpoles.

It seems that, no matter when the optic fibres reach the chiasma during larval life, they always go up the lateral margin of the diencephalon. In the case of optic nerve regeneration, since all the fibres grow back at approximately the same time, they all go up the lateral diencephalic margin in the form of a compact band. This distribution of regenerated fibres is abnormal in the ventriculo-pial sense, but we have as yet no indication that the regenerated fibres are abnormally positioned in either the rostro-caudal or the dorso-ventral axes of the diencephalon. Since the diencephalon in these animals contains structural elements (the ependymo-glial cells) which individually span the entire thickness of the tissue, it is possible that the regenerating fibres are still growing back in close association with the same cells that they would normally associate with, but now in contact with more peripheral parts of these cells. Certainly, the regenerating optic fibres are not following cues provided by the degenerating remains of the original fibres. Whatever may be the case in the tectum, where it has been suggested (Murray, 1976; Schmidt, 1977) that the regenerating fibres in goldfish may follow traces left by their degenerating predecessors, in the tadpole diencephalon regenerating optic fibres seem to pay no attention to the degenerating remains of the main part of the previous innervation.

It is clear that the ventriculo-pial arrangement of the fibres (and thus their central-peripheral arrangement in retinal terms) is grossly displaced in regeneration. Whether the relative position, or ordering, of the individual fibres in the abnormally situated band of regenerated fibres is normal, is not yet known.

The present results show that, in normal *Xenopus* tadpoles, the ventriculo-pial ordering of retinotectal fibres in the diencephalon is decided by the time of arrival
of the fibres at the chiasma. The first fibres to arrive, those from the earliest part of the retina to form, near the optic nerve head, lie closest to the ventricle; and later fibres, arriving from younger parts of the retina, further from the optic nerve head, lie further from the ventricle. The most recently arrived fibres at any stage of development, those from nearest the ciliary margin of the retina, lie closest to the pial margin of the diencephalon.

The optic fibres in *Xenopus* tadpoles enter the optic nerve head in fascicles which are ordered retinotopically in the circumferential dimension of the retina (Fig. 11). The optic nerve may be thought of as a cylindrical group of fibres which come, naturally, from all parts of the retina. It is not known whether the fibres maintain a retinotopic arrangement in the nerve; in the diencephalon, however, the present work suggests that the total array of optic tract fibres is distributed in the form of a wedge-shaped segment of a circle, such as would be obtained if the retina were opened along a line running from a point on its periphery to the optic nerve head, and then partially closed up about the optic nerve head as a hinge (Fig. 12).

The position at which the retina is opened in Fig. 12 is on the naso-dorsal margin, as would be suggested by the work of Scalia & Fite (1974) on *Rana*. It is not yet known whether the situation is the same in *Xenopus* and experiments to determine this matter are in progress. It may be seen from the present results, however, that fibres in the dorsal (anterior) margin of the diencephalic tract enter the tectum via the medial optic tract (Figs. 1a and 7) whereas fibres from the ventral (posterior) margin of the diencephalic tract enter the tectum via the lateral optic tract (Figs. 1a and 7). This distribution agrees with the findings of Scalia & Fite (1974). Moreover, we can say that, of the fibres placed more medially (centrally) in the diencephalon, some enter each branch of the optic tract (Figs. 1a and 7).

While a distribution such as that shown in Fig. 12 is likely to be found in *Xenopus*, it is to be expected that the detailed distribution of the retinal circumference along the outer edge of the wedge will not be uniform in spacing, because of the preponderance of ventral retinal growth at certain stages of larval development.

Experiments to establish the extent of retinotopic ordering in the normal optic tract and in the tract after various operations on the visual system, are in progress.

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REFERENCES


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