The central pathways of optic fibres in Xenopus tadpoles

By J. G. STEEDMAN, R. V. STIRLING and R. M. GAZE

SUMMARY

A cobalt chloride impregnation technique was applied to the optic nerve in Xenopus tadpoles and the central optic pathways were examined in cleared, whole-mounted preparations, and in thick sections. The overall plan of the optic input was visualized in relation to the outlines of the parts of the brain and details of the structure of the tectal optic neuropil, the neuropil of Bellonci and the basal optic neuropil were seen. The fibres in the main retinotectal tract maintained an orderly disposition with respect to each other, in contrast to the fibres of the basal optic tract, in which no order was apparent. Optic fibres were seen passing caudally from the region of the basal optic neuropil.

INTRODUCTION

Over the past 20 years the development and regeneration of nerve connexions in the amphibian visual system has been extensively studied by electrophysiological means (Gaze & Jacobson, 1963; Gaze, 1970; Gaze, Keating & Chung, 1974). Such studies, primarily concerned with the input to the mid-brain optic tectum, demonstrate the existence of a highly ordered visuotopic map on this structure and imply ordered connexions between the retinal ganglion cells and the tectum.

Electrophysiological methods cannot, however, directly reveal the mode of growth by which retinal ganglion cell axons find their appropriate central target cells. To obtain a comprehensive picture of the phenomena of development and regeneration in the optic pathway, and enable us to assess properly the nature of the factors which control the establishment of ordered maps, we need to know not only the sites of origin and of termination of a fibre, but also the particular path it takes to get from one to the other.

Several histological methods have been used to trace fibre pathways in the amphibian visual system, such as degeneration techniques (Knapp, Scalia & Riss, 1965; Scalia, Knapp, Halpern & Riss, 1968; Scalia & Fite, 1974), autoradiographic tracing (Scalia, 1973; Currie & Cowan, 1974) and anterograde movement of horseradish peroxidase (Scalia & Colman, 1974). The drawback to these methods is that they have involved sectioning of the preparation. Consequently any three-dimensional impression of the shape of the optic tract as a whole, or of the paths of individual fibres, must be built up by laborious reconstructions and fibre-following from section to section.

1 Authors' address: National Institute for Medical Research, London, NW7 1AA, U.K.
To enable us to see the *Xenopus* optic tract in high contrast in cleared, whole-mounted preparations, we have used a modification (Stirling, 1978) of the cobalt impregnation technique in larval and juvenile *Xenopus*. These preparations allow us to see the optic tracts stained throughout their length from the chiasma to their terminal zones in thalamus and tectum. The brain can be viewed stereoscopically in any desired orientation, either as a whole brain or dissected into parts, and can finally be sectioned (for example, at 100 μm) if higher power viewing and analysis are required. It is frequently possible to follow individual fibres, in the whole-mounted preparations, from near the chiasma into the tectum. By this technique we are able to see, for the first time, precise details of the optic pathway, including the mode of entry of optic fibres into the tectum and their distribution therein.

Since the growth of the optic axons from the retina to the tectum is ordered both in space and time and takes place throughout the whole of larval life, details of the paths followed by the various fibres may give valuable information on the kinds of forces acting on the growing axon tips during development. In this paper we present a description of the central parts of the visual system in mid-larval *Xenopus*. This is preparatory to further studies on the details of the retinotopic arrangement of fibres in the optic tract, and of its development in larval animals with and without early operative interference with the visual system. These will be presented in further papers.

**METHODS**

This study is based on the examination of 42 tadpoles between stages 51 and 66 (Nieuwkoop & Faber, 1956). Larvae of *Xenopus laevis* obtained from induced spawnings were raised on nettle-powder at 22 °C. A preliminary account of this method for filling optic axons has been published (Stirling, 1978). The optic nerve of the animal is dissected free from surrounding tissue and cut immediately behind the eye. A boat of Vaseline soft petroleum jelly is then constructed around the nerve such that the cut end of the dissected nerve is contained in a water-tight hollow. A drop of distilled water is placed in the boat and the nerve is cut below the water. This procedure causes the ends of fibres to open up and aids good filling. After 1 min the distilled water is removed and replaced by aqueous cobaltous chloride solution (130 mM-CoCl₂). The drop of cobalt is then roofed over and sealed in with more Vaseline. The preparation is left moist at 4 °C for 13 h. Excess cobalt and the Vaseline are then removed. Cobaltous ions are precipitated as the sulphide by soaking the specimen in cold 0·35% saline, saturated with hydrogen sulphide, for 10 min. After rinsing in saline, the tissue is soaked for 6 h at room temperature in Stieve’s fixative (140 ml saturated aqueous picric acid, 10 ml 5% trichloracetic acid and 10 ml formalin) during which time the brain is dissected out and all membranes removed. After fixation the brain is rinsed overnight in 70% ethanol (three
Central pathways of optic fibres in *Xenopus* tadpoles

Changes). The staining is then intensified by a silver substitution method based on that developed by Bacon & Altman (1977). At 60 °C throughout, the specimen is pre-soaked for an hour in a solution consisting of 100 ml 25% gum arabic (cleaned sorts), 3.5 g citric acid, 0.34 g hydroquinone, 10 g sucrose and 100 ml distilled water. The specimen is then transferred to fresh solution containing 0.1% silver nitrate for intensification. The intensifier solution is changed approximately every 20 min to avoid indiscriminate silver precipitation. The degree of intensification has to be assessed by inspection and is usually complete in 30–45 min. The process is stopped by washing in hot distilled water. Specimens are then dehydrated and cleared in methyl salicylate. They can be viewed mounted in methyl salicylate or Canada balsam between spaced coverslips. Selected specimens can be subsequently embedded in celloidin and sectioned at 70–100 μm.

**Observations**

The optic pathway of a stage-57 *Xenopus* tadpole revealed by cobalt filling of the left optic nerve, is shown in dorso-lateral view in Fig. 1. Fig. 2. shows a ventro-lateral view from stage 55 tadpole with the right nerve filled. The optic nerve crosses the mid-line at the chiasma and the main tract passes caudally and dorsally up the side of the diencephalon towards the optic tectum. Just beyond the chiasma some fibres leave the tract to innervate the basal optic nucleus. As the optic tract turns towards the optic tectum the neuropil of Bellonci is seen. The tectum itself is covered with a dense meshwork of fibres. Just rostral to the tectum, and medially placed, is the pre-tectal (posterior thalamic) neuropil. Fine fibres (not shown in this figure; see later) leave the tract shortly after the chiasma to pass up through the ipsilateral diencephalon. A synoptic diagram of the main elements of the organization of the optic pathway is shown in Fig 3.

There follows a description of each of these areas as seen in the whole-mounted preparations, from different angles of view, and from serial sections cut at 100 μm.

**Optic tract**

The fibres are densely stained in the region of the optic chiasma but appear as ordered groups of evenly spaced fascicles as they fan out and pass laterally from the chiasma (Fig. 4.). Previous work (Gaze & Grant, 1978) suggests that the distribution of these fascicles reflects the sequential arrival of retinal fibres during development.

The optic tract, as it opens up after leaving the chiasma, is wedge-shaped in cross-section with the base of the wedge lying most laterally, on the wall of the diencephalon, and the apex of the wedge lying closest to the central axis of the brain (Fig. 2). The whole of this wedge-shaped tract passes caudally and dorsally, following the curve of the diencephalic wall. Fig. 5 shows a lateral view of the
bisected brain of a stage-56 tadpole. From the chiasma (bottom left) fibres open out to form the main retinotectal tract, leading to the tectum at upper right. As the optic tract approaches the tectum, it dips inwards from the surface at the diencephalo-tectal junction. On the tectum the fibres give rise to the tectal optic neuropil, some details of which can be seen caudally on the tectum in the photograph.

**Basal optic neuropil**

Fibres to the basal optic neuropil leave from the ventral posterior side of the chiasma (Fig. 5). Many of these fibres are large and branch repeatedly (Fig. 6). In contrast to the orderliness seen in the main optic tract these fibres in the basal optic tract interweave with one another without apparent order. In a parasagittal section the basal optic neuropil shows clearly a layer and column structure (Fig. 7). Fine cobalt-filled fibres can be seen passing caudally from the neuropil (Fig. 8a–c). These can be followed into the ventral medulla where they become progressively finer and more difficult to see and eventually disappear.

**Neuropil of Bellonci**

The Bellonci neuropil appears as a hollow cone-shaped collection of fine fibres and silver precipitate which sits medial to the optic tract and passes dorsally through the diencephalon (Fig. 9; see also Figs. 1, 2 and 5). Some of the optic fibres supplying the neuropil appear to project solely to that region of the brain while others are clearly side-branches of fibres which continue in the main tract towards the tectum and pre-tectal regions. The conical shape of the neuropil is well seen in stereo view (Fig. 11). In sections counter-stained with cresyl violet, which reveals the distribution of cell groups, it is clear that this cone of neuropil is situated in a cell-free zone. The position of the neuropil in relation to the tract is shown in a parasagittal section in Fig. 10.

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**Fig. 1.** Top: stereo pair showing dorso-lateral view of the brain of a stage-57 tadpole with a cobalt-filled optic pathway. Bottom: Diagram identifying the structures in the photographs. F, forebrain; D, diencephalon; T, tectum; N, optic nerve; CH, chiasma; OT, optic tract; B, neuropil of Bellonci; BON, basal optic neuropil; R, rostral; C, caudal. The filled optic nerve may be seen through the brain as it enters the chiasma.

**Note.** This and the other stereo pairs shown may be viewed with the aid of a stereoscopic viewer. Or alternatively it is possible to see the stereoscopic effect with the naked eye, in the following manner. Hold the figure in front of the eyes at the closest distance for clear vision and then attempt to look through the picture into the distance. This will result in three out-of-focus images being seen, the middle one of which is the left and right pictures superimposed. Concentrate on the middle image and, while keeping its two components in register, slowly move the figure away to arm’s length. Now the image must be brought gradually into focus with the eyes, whereupon the full three-dimensional effect will be obtained. This last focussing of the image is an ability that may be difficult to achieve at first, but improves rapidly with a little practice. It is advisable to use conditions of good light, preferably daylight.
Fig. 1. For legend see opposite page.
Fig. 2. Top: stereo pair showing a ventro-lateral view of the brain of a stage-55 tadpole with a cobalt-filled optic pathway. Bottom: explanatory diagram. Lettering as in Fig. 1.
Central pathways of optic fibres in Xenopus tadpoles

Fig. 3. Synoptic diagram taken from a camera-lucida drawing of the brain of a stage-57 tadpole in which the left optic nerve had been filled with cobalt. The specimen is viewed from the right, slightly rostral and dorsal. Key: I, olfactory nerve; II, optic nerve; CH, chiasma; IF, ipsilateral optic fibres; OT, optic tract; B, neuropil of Bellonci; P, pre-tectal neuropil; T, tectum; BON, basal optic neuropil; C, cerebellum.

Fig. 4. Transverse section through the diencephalon of a stage-57 tadpole, showing the fan of optic fibres spreading out from the chiasma. The section, approximately 1 mm thick was cut by hand. Dorsal is uppermost. The outline of the optic nerve has been drawn in to indicate its position. Bar = 1 mm.
Fig. 5. A half-brain from a stage-56 tadpole, viewed from the lateral aspect. The brain had been cut in half along the mid-sagittal plane. The main optic tract curves dorsally and caudally from the region of the chiasma at the bottom left. At the caudal end of the tract the tectum is seen with its dense meshwork of optic fibres. The most ventral optic fibre approaching the tectum makes a sudden turn dorsally to get there (arrow). Half way along the tract the neuropil of Bellonci can be seen protruding above its dorsal edge. Ventrally a bundle of fibres (the basal optic tract) may be seen running caudally to reach the basal optic neuropil. B, neuropil of Bellonci; OT, optic tract; BOT, basal optic tract; BON, basal optic neuropil; T, tectum; R, rostral; C, caudal; D, dorsal; V, ventral. Bar = 500 μm.

Fig. 6. Higher magnification, with different focus, of the basal optic tract and neuropil shown in Fig. 5. Extensive branching and interweaving of the fibres is seen. Bar = 200 μm.

Fig. 7. Basal optic neuropil from a stage-61 tadpole. Dorsal is uppermost, rostral to the right. Parasagittal section cut at 100 μm. The layer-and-column structure of this neuropil shows clearly. Bar = 100 μm.
Fig. 8. Fibres passing caudally from the region of the basal optic neuropil. (a) Low power photograph of lateral view of the brain of a stage-57 tadpole. Within the inset box is the basal optic neuropil, shown also in (b). M, medulla; T, tectum; OT, optic tract; B, Bellonci neuropil; F, forebrain. Bar = 500 μm. (b) High power view of the basal optic neuropil. Bar = 200 μm. (c) Montage showing cobalt-filled fibres passing caudally along the ventral margin of the midbrain and medulla.

**Pre-tectal neuropil**

The pre-tectal neuropil is shown in dorsal view in a whole-mount of the same brain (Fig. 12), and parasagittal sections of the same preparation show that a fine skein of optic fibres can be seen entering the dorsal end of this neuropil after making an abrupt turn in the main optic path (Fig. 13). Fibres within this neuropil are usually lightly stained and appear to branch repeatedly.

**Tectum**

Some of the relationships between the optic fibres and the tectum are shown at high magnification in Fig. 14. In general, fibres closest to the surface of the rostral end of the tectum pass the furthest caudally before turning into the tectal mesh. The mode of distribution of optic fibres as they enter the tectum is well seen in the stereo view (Fig. 16) taken from a section of the same brain as Figs. 5 and 6. Here sparse superficial fibres have been cut as they run towards the caudal tectum and the tectal optic innervation clearly comprises three layers. Nearest the surface are found large fibres travelling caudally. Beneath this is a complex meshwork of fibres with the interstices of the mesh apparently free of
Fig. 9. The Bellonci neuropil protruding dorsally from the optic tract of a stage-60 tadpole. Whole-mounted brain, viewed from the dorsolateral aspect. V, ventral; R, rostral. Bar = 100 μm.

Fig. 10. Low power view of parasagittal (100 μm) section through the optic pathway in a stage-61 tadpole. The chiasma is at the bottom right, tectum is at the top. The neuropil of Bellonci is at the right and the basal optic tract and neuropil is at the bottom. Bar = 500 μm.

Fig. 11. Stereo pair showing the hollow conical structure of the Bellonci neuropil as seen with cobalt. Same brain as Fig. 10. Bar = 100 μm.
Central pathways of optic fibres in Xenopus tadpoles

Fig. 12. Dorsal view of whole-mounted stage-61 tadpole brain. The chiasma (CH) is seen through the brain. Fibres pass up through the optic tract (OT) to the tectum (T). Out of focus ventrally is the basal optic neuropil (BON) and just rostro-medial to the tectum is the pretectal neuropil (P), with fibres entering from the optic tract. This neuropil is also shown in Fig. 13. C, caudal; R, rostral. Bar = 500 \( \mu \text{m} \).

Fig. 13. Parasagittal (100 \( \mu \text{m} \)) section through the tectodiencephalic junction in a stage-61 tadpole. The same region of this brain is shown in dorsal view in Fig. 12. D, dorsal; R, rostral. The fibres of the optic tract may be seen approaching the tectal neuropil (top) from the right. The pretectal neuropil is the vertical structure at the right of the photograph (arrow). Bar = 200 \( \mu \text{m} \).

fibres. This meshwork has a finely granular appearance, since each fibre appears to have a halo of precipitate around it. In the lowest layer fibres form another mesh, less densely stained, where individual optic axons can be easily traced. These axons follow an erratic course and give off small side branches at irregular intervals. It is commonly found that the most medial and most lateral of all optic fibres approaching the tectum do so at a wide angle and then finally swing in towards it (Fig. 15). Sometimes this turn is very sharp as shown in Fig. 5.

Ipsilateral diencephalic fibres

In well-stained preparations fine optic axons can be seen leaving the base of the chiasma to innervate the ipsilateral diencephalon. They travel laterally (Fig. 17) before running dorsally up the lateral margin of the diencephalon. Some such ipsilateral fibres can be seen to give off sets of horizontal branches to the neuropil of Bellonci (Fig. 18) before going on to the pre-tectal region.

DISCUSSION

Cobalt impregnation, as used in these experiments, fills optic nerve fibres and reveals their pathways and areas of terminal arborization. The present observations show clearly the advantages of cobalt impregnation over previously used methods. Particularly valuable is the fact that, in a small brain such as that of the tadpole, it is possible to study the optic pathway in cleared, whole-mount
Fig. 14. Parasagittal (100 μm) section showing optic fibres entering the tectal neuropil in a stage-61 brain. Bar = 200 μm.

Fig. 15. The lateral edge of the tectum in a whole-mount of the brain from a stage-60 tadpole. R, rostral; C, caudal; D, dorsal. Bar = 200 μm.

Fig. 16. Stereo pair showing fibres of the optic tract approaching the tectum in a tadpole stage 56. The photographs are of a 100 μm section cut in an orientation between horizontal and parasagittal. The outermost part of the tectum was included in the next adjacent section and is not shown. The figure shows three layers of optic fibres at the rostral part of the tectum. Most superficially are large fibres passing caudally (upwards in photograph); next is a dense black meshwork of fibres, and deeper still is a lightly stained meshwork of fine fibres. Bar = 200 μm.
Central pathways of optic fibres in Xenopus tadpoles

Fig. 17. Ipsilateral fibres passing up the wall of the diencephalon in a whole-mount from a stage-57 tadpole. The large black object at the left is the cobalt-filled optic nerve. The chiasma is just off the picture at the bottom. Bar = 300 μm.

Fig. 18. Ipsilateral fibres branching in the region of the Bellonci neuropil in a stage-57 tadpole. In this tadpole, for purposes unrelated to this paper, a partial retinal lesion, leaving temporal-ventral retina intact, had been made three days before the animal was killed. Bar = 100 μm.

preparations, thus permitting individual fibres to be followed for considerable distances and their relationships to other fibres and to general brain structures to be seen.

Much detail can also be seen in the whole-mount preparations since they permit the use of objectives up to × 40. The amount of information that can be obtained from such a preparation is indicated by the fact that Fig. 7, 10, 11, 12, 13 and 14 are all taken from the same animal. The fibre tracts leading to thalamic and mid-brain optic centres have been clearly shown, as have optic fibre components of terminal regions in the neuropil of Bellonci (Figs. 1, 2, 5, 9, 10, 11, and 18), the posterior thalamic neuropil (Figs. 12, 13), the neuropil of the basal optic nucleus (Figs. 1, 2, 5, 7, 8 and 10) and the optic neuropil of the tectum (Figs. 1, 2, 5, 10, 14, 15 and 16).

The present experiments provide no evidence that cobalt is transported trans-synaptically; in fact the results suggest otherwise, since no labelling of cells in the tectum or the diencephalic nuclei associated with the optic pathway was seen. In this connexion it is relevant to comment on a surprising result of this work; that is, the demonstration of impregnated fibres which pass caudally from the region of the basal optic neuropil (Fig. 8). In view of the novelty of this observation, one might suspect that these fibres had been trans-synaptically labelled, since optic fibres certainly go to the basal optic nucleus and most of the caudally running fibres appear to issue from the related optic neuropil. However, some of these caudally running fibres can be followed from the basal optic tract, bypassing the basal optic neuropil (to which they may give branches) and then passing further caudally.
This observation thus indicates a previously unreported direct optic input to the hind-brain and perhaps further caudally. We have seen these fibres in animals as young as stage 51 and in the oldest specimens examined, newly metamorphosed toads. Lázár (1973) showed, in Rana, that the basal (accessory) optic tract was probably the pathway exclusively responsible for the optokinetic movements of an animal in response to rotation of a striped drum. It is tempting to hypothesize that this prolongation of the tract is also involved in such responses. Lesioning and electrophysiological experiments are being performed in an attempt to decide this question.

It is possible that these fibres have not been reported in previous autoradiographic and degeneration studies because the fibres are sparse and fine. We have observed (unpublished results) that when the optic pathways are studied by autoradiography, following the labelling of one eye with $[3\text{H}]$proline, the fibres of the basal tract themselves can frequently not be distinguished, even though the basal optic neuropil is well labelled. The sparse and fine fibres passing caudally from the basal optic neuropil would thus be expected to be even more difficult to find with this method. The ability to see individual fibres in continuity leads to a significant increase in the sensitivity with which fibres and their branches can be identified and followed.

The anatomy of the adult anuran diencephalon and optic tracts has already been studied extensively. The most detailed and comprehensive descriptions recently are those of Knapp et al. (1965), Scalia et al. (1968), and Scalia & Fite (1974), who used degeneration-staining combined with silver-staining of adjacent sections. Most of the structures described in those papers as being associated with the optic pathway in adult Rana pipiens we can here identify in Xenopus laevis.

The only area of optic neuropil described in the adult frog by previous authors and not shown here is the corpus geniculatum thalami. Our preparations show, on close inspection, a small number of fibres in the main tract that branch before reaching the area of the Bellonci neuropil. This is in the right area of the thalamus to be homologous with the corpus geniculatum thalami of adult Rana. With regard to the pre-tectal neuropil reported here, we have not sought to distinguish between the separate areas identified functionally and anatomically by previous authors (Scalia & Fite, 1974). The incomplete coverage of the larval tectum by optic afferents revealed in our preparations seems in accord, stage for stage, with what is detected by electrophysiological mapping of visually evoked responses in Xenopus (Gaze et al. 1974).

Cobalt impregnation of optic axons, as used here, reveals fibres and neuropil but not cellular structures. The central regions of optic neuropil already described and discussed are all associated, in the anuran brain, with certain nuclei or cellular groupings, with which they form dendritic or in some cases axosomatic contacts. For details of these nuclei the reader should see the papers cited and Scalia & Gregory (1970). Our failure to find cobalt-impregnated cells in the
Central pathways of optic fibres in Xenopus tadpoles

tectum (or anywhere else) after cobalt treatment of the central end of the optic nerve, suggests that there are no efferent fibres passing to the retina in the optic nerve. This agrees with the findings of Scalia & Teitelbaum (1978) who used horseradish peroxidase in the frog and toad. In our preparations, the cone-shaped sheath of the Bellonci neuropil was the only part of this structure to be visible. The central core of the neuropil appeared empty (Fig. 11). This result is comparable to the findings of Knapp et al. (1965) who used a Nauta–Laidlaw method. However, the later work of Scalia et al. (1968) using Cajal and Fink-Heimer methods showed a fine degeneration in the central region of the Bellonci neuropil. These latter authors argued, reasonably, that the difference between the two results could be due to a tendency for the Nauta-Laidlaw method to show selectively large fibres, while the Cajal method also showed up the degeneration of fine fibres.

On this basis it would seem likely that the cobalt impregnation in the present series is restricted to the larger fibres in the optic pathway. This could account for the fact that at the developmental stages investigated the projections ipsilaterally to all thalamic centres and contralaterally to corpus geniculatum thalami show faintly or sometimes not at all. This would also account for the main advantage of the present method, which is the (relatively) small number of fibres seen. This is what permits the cobalt method to be useful: the optic nerve of a stage-57 tadpole contains some 23000 fibres (Gaze & Peters, 1961), and if all of them were stained none would be individually distinguishable. In this paper we use the terms ‘larger’ and ‘finer’ fibres without any attempt at measurement of the actual fibre diameters. This is because, since the intensification used is a silver-deposition process, such measurements at light-microscopic level could be misleading.

While it is likely that the cobalt impregnation, as used here, reveals particularly the larger fibres in the optic pathway, the method is capable of showing finer, unmyelinated fibres. Preliminary observations on tadpoles with newly regenerated optic fibres show that the retinotectal fibres (or a proportion of them) can and do become stained. The intensity of the reaction, as shown by the darkness and contrast of the impregnated fibres, is much less in such cases than in normal animals of the same stage. Similarly, tadpoles as early as stage 49 also show optic fibre impregnation, again of a lesser intensity than in older animals. The first myelinated fibres appear in the developing optic nerve at about stage 49 (Gaze & Peters, 1961) and all fibres in a newly regenerated optic nerve are probably unmyelinated (Gaze & Grant, 1978). We can say, therefore, that some at least of the smaller and unmyelinated fibres can be revealed by this method.

It is possible that all the fibres in the nerve take up the cobalt, and whether or not the impregnation may be seen depends upon the extent of the intensification that is permitted. Alternatively, the finest fibres either may not fill or may fill but lose the cobalt thereafter. With the methods used in the present work, in a
normal tadpole of mid-larval stage, some optic fibres show up as black wires against a totally structureless background. The last is the optimal situation for following individual fibres, but it is obtained at the price of a very selective visualization of the optic fibres. On the basis of their appearance, especially when arborizing terminally, and of electronmicroscopic observations of the (normal, unimpregnated) tadpole diencephalon, we believe that the cobalt-stained fibres that we see are single axons rather than fascicles: and that the branching points seen represent individual axonal branchings rather than diverging fibres.

The fibres which are well stained by the use of cobalt, show a high degree of orderliness throughout the retinotectal tract. It seems likely that the finer fibres which are not visible with the use of the present technique, will also show a comparable order. Retinotopic order already existing at the level of the optic tract (Scalia & Fite, 1974, Rana) may thus considerably simplify the developmental task of forming a properly organized retinotectal map.

Details of the trajectories of the individual fibres, and details of the retinotopy of the projection, are presently being investigated and will be presented in a further paper. The method of cobalt impregnation is also being used to analyse the fibre pathways in situations in which various operations on the embryonic eye have previously been performed, such as rotation, transplantation and the formation of various types of 'compound eye'.

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REFERENCES

Central pathways of optic fibres in Xenopus tadpoles


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