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Fibre organization and reorganization in the retinotectal projection of *Xenopus*

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Summary

This study concerns the retinotopic organization of the ganglion cell fibres in the visual system of the frog *Xenopus laevis*. HRP was used to trace the pathways taken by fibres from discrete retinal positions as they pass from the retina, along the optic nerve and into the chiasma. The ganglion cell fibres in the retina are arranged in fascicles which correspond with their circumferential positions of origin. Within the fascicles the fibres show little age-related layering and do not have a strict radial organization. As the fascicles of fibres pass into the optic nerve head there is some exchange of position resulting in some loss of the retinal circumferential organization. The poor radial organization of the fibres in the retinal fascicles persists as the fibres pass through the intraocular part of the nerve. At a position just behind the eye there is a major fibre reorganization in which fibres arising from cells of increasingly peripheral retinal locations are found to have passed into increasingly peripheral positions in the nerve. Thus, fibres from peripheral-most retina are located at the nerve perimeter, whilst fibres from central retina are located in the nerve core. It is at this point that the radial, chronotopic, ordering of the ganglion cell axons, found throughout the rest of the optic pathway, is established. This annular organization persists along the length of the nerve until a position just before the nerve enters the brain. Here, fibres from each annulus move to form layers as they pass into the optic chiasma. This change in the radial organization appears to be related to the pathway followed by all newly growing fibres, in the most superficial part of the optic tract, adjacent to the pia.

Just behind the eye, where fibres become radially ordered, the circumferential organization of the projection is largely lost. Fibres from every circumferential retinal position, which are of similar radial position, are distributed within the same annulus of the nerve. At the nerve–chiasma junction where each annulus forms a single layer as it enters the optic tract, there is a further mixing of fibres from all circumferential positions. However, as the fibres pass through the chiasma some active pathway selection occurs, generating the circumferential organization of the fibres in the optic tract. Additional observations of the organization of fibres in the optic nerve of *Rana pipiens* confirm previous reports of a dual representation of fibres within the nerve. The difference in the organization of fibres in the optic nerve of *Xenopus* and *Rana pipiens* is discussed.

Key words: optic nerve, fibre organization, *Xenopus*, retinotectal projection.

Introduction

One approach used to investigate the mechanisms by which optic nerve fibres form orderly connections has been to examine the relative order among nerve fibres in the optic pathways (Bunt & Horder, 1983; Walsh & Guillery, 1984; Easter, 1985). For this approach to be most helpful it is necessary to know what the organization of the normal system is both during and beyond its development. Most studies, however, have concentrated on the adult or juvenile system, assuming that the distribution of nerve fibres present is closely related to the manner in which the fibres initially grew in the developing system. This is not an ideal approach, yet because of the technical difficulty in analysing a growing and often minute system, it is expedient.

The early histological analyses of the organization of the amphibian visual pathways were inconclusive. In the newt it was claimed that quadrant-specific,

fascicular organization was maintained throughout the pathway (Ströer, 1940), whilst in *Ambystoma* Herrick (1941) showed that the intraretinal fascicular order broke down in the optic nerve and that there were definite interchanges of fibre position within the nerve and at the chiasma.

With the use of HRP more recent studies have also produced conflicting evidence. In the frog *Xenopus* it was claimed that strict retinotopic order was present in all parts of the projection (Bunt, Horder & Martin, 1978). However, Fawcett (1981) showed that the fibres pass into the nerve in quadrant-specific order, but there is some dispersal of the fibres as they pass along the optic nerve, although a retinotopic organization was restored at the chiasma. He concluded that the circumferential organization is generally retinotopic, but that differing behaviour by nasal axons resulted in their loss of quadrant-specific organization at the chiasma. The radial organization of the developing *Xenopus* optic nerve has also been examined (Cima & Grant, 1982*a*,*b*). In early stages there is a dorsal-to-ventral segregation of large to small calibre fibres, presumed to reflect an age-, and hence radial position-, related order (see also Herrick, 1941, *Ambystoma*). At later stages of development an annular, radial organization of the fibres was shown to exist in the midregion of the optic nerve (Wilson, 1971; Cima & Grant, 1982*a*,*b*). These different organizations were explained as a consequence of metamorphic remodelling and of retinal growth dynamics.

In the frog *Rana pipiens*, a very different organization has been shown to exist (Scalia & Arango, 1982; Reh, Pitts & Constantine-Paton, 1983). Both studies agree that there is an annular, central-to-peripheral, radial organization in the nerve, and report that there exists a symmetrical, duplicate, quadrantic retinotopic representation of the fibres about the dorsoventral midline of the nerve. Reh & colleagues postulated that this organization arises from the growth of the system and the morphology of the optic fissure.

The retinotopic ordering of fibres in the amphibian optic tracts has been well established in the juvenile adult (Ströer, 1940; Herrick, 1941; Scalia & Fite, 1974; Gaze & Grant, 1978; Steedman, 1981; Fujisawa, Watanabe, Tani & Ibata, 1981*a*,*b*; Fawcett & Gaze, 1982; Reh *et al.* 1983; Fawcett, Taylor, Gaze, Grant & Hirst, 1984).

This study has been made with a view to answering the following questions. (i) What is the organization of the *Xenopus* optic nerve. (ii) How does the organization of the optic nerve relate both to the retinal order of fibres and to the tract organization. (iii) How does this organization relate to that found in the optic nerve of *Rana pipiens*? I here present a comprehensive study of the organization of the *Xenopus* optic nerve.

Materials and methods

HRP labelling

The animals used in this study were *Xenopus laevis* and *Rana pipiens*. *Xenopus* were either toadlets of less than one month after metamorphosis (n = 175), or stage 57 (Nieuwkoop & Faber, 1967) tadpoles (n = 12); the *Rana pipiens* were either adult (n = 12) or stage XII tadpoles (n = 8) (Taylor & Kollros, 1946). For HRP labelling, the animals were first anaesthetized in 1:3000 MS222 (Ethyl-m-aminobenzoate, Sandoz) and then prepared for the appropriate filling regime.

To look at the radial ordering five different filling regimes were used (Fig. 1). For labelling fibres in discrete positions of peripheral retinal origin, the majority of lesions were made directly to the retinal periphery (position 1, Fig. 1). A fine tungsten needle was inserted into the vitreous at an angle perpendicular to the retinal periphery, and the retina lesioned by being crushed against a pair of forceps held outside the sclera. Then either a 10% HRP solution (Boehringer, grade I) was injected into the vitreous or a piece of recrystallized HRP was placed at the position of the lesion. A complete annulus of peripheral ganglion cells could be filled by lesion of the superficial layers of the chiasma followed by HRP application (position 2, Fig. 1). To fill the oldest cells in the central retina, the animal was prepared for recording of the visuotectal projection and the

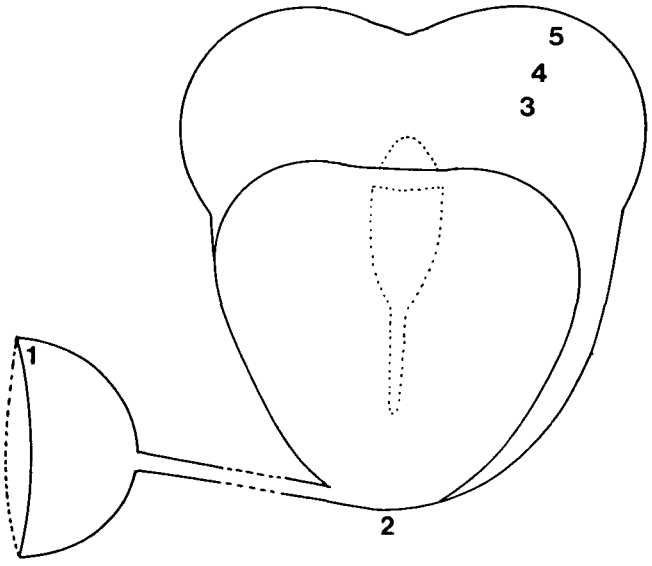


Fig. 1. Diagram showing the positions at which HRP was applied to lesions of the retinal ganglion cell axons. **1**, peripheral retina – labelling discrete, quadrant specific groups of peripheral ganglion cell axons; **2**, superficial chiasma – labelling axons from a complete peripheral annulus of cells in the retina; **3**, central tectum – labelling central axons; **4**, midcaudal tectum – labelling nasal axons of intermediate age; **5**, caudal tectum – labelling nasal peripheral axons only.

position of termination of central retinal fibres determined electrophysiologically (as described below). This part of the tectum was then lesioned (Fig. 1, position 3), and a piece of recrystallized HRP applied to the lesion. For intermediate-aged fibres, a position midway between the central retinal terminations and the caudalmost part of the optic neuropil was determined by visuotectal mapping, and similarly labelled with HRP (position 4, in Fig. 1). In such preparations a group of nasally positioned ganglion cells generated during midlarval life was labelled. Lesions were also made to caudal areas of tectum and HRP crystals applied to the lesion to label peripheral nasal retina (position 5, Fig. 1).

48 h after HRP application the animals were deeply anaesthetized in 1:1500 MS222 and perfused with 0·25 M-sucrose followed by 2·5 % glutaraldehyde in 0·1 M-phosphate buffer at pH 7·2. The brain, optic nerves and eyes were then dissected out and fixed for a further 1 h at 4 °C.

For frozen-sectioned material, the preparations were placed in 20 % sucrose, allowed to sink then transferred to gelatine/albumin mixture overnight. Infiltrated preparations were embedded in gelatine/albumin that had been induced to set by the addition of 1 part 25 % glutaraldehyde to 10 parts gelatine/albumin. 40 μm or 60 μm frozen sections were collected in phosphate buffer, mounted on subbed slides and reacted according to the cobalt DAB method of Adams (1977).

For cryostat sections, the preparations were transferred from 20 % sucrose to Tissue-tek II embedding medium (Raymond Lamb) and rapidly frozen in an acetone/carbon dioxide mixture. 10 μm or 20 μm sections were cut on a Reichardt-Jung cryostat and stained as for the frozen sections.

Reacted sections were either counterstained with neutral red or Harris's haematoxylin and eosin, dehydrated, cleared and mounted.

Retinas for whole mounting were dissected unfixed from eyes that had been perfused with sucrose and dark adapted at 4 °C for 1 h in amphibian Ringer. After brief fixation in 2·5 % glutaraldehyde the lens and vitreous were removed. Four radial cuts were then made in the retina and the whole preparation mounted on a subbed slide using glutaraldehyde-set gelatine/albumin. Following further fixation for 5 min, HRP processing was carried out as above but the incubation times were extended as follows; 1 h cobalt, 90 min DAB and 30 min DAB–H_2O_2.

Whole-mounted brains were processed according to the technique of Adams (1977) as described previously (Taylor & Gaze, 1985).

Electrophysiological recording

Toadlets were anaesthetized in 1:3000 MS222 and the tecta were exposed by craniotomy. Visuotectal responses were recorded using standard techniques (Taylor, Willshaw & Gaze, 1985) and mapped on a perimeter chart. The recording position of responses to central and midnasal retinal stimulation were marked on a photograph of the tectal surface. HRP was then applied to lesions placed in the appropriate position as recorded on the tectal photographs.

Electron microscopy

For electron microscopy, normal animals or those with HRP-filled fibres were anaesthetized and perfused as above using either 2·5 % glutaraldehyde in 0·1 M-cacodylate buffer pH 7·3, or 50 % Karnovsky's fixative. For HRP material the retinae were dissected out intact and reacted as for the whole-mounted retinae. Following washing in cacodylate buffer the retinae were postfixed in 1 % OsO_4, for 1 h, then embedded in Araldite. Thin sections were cut on an LKB III ultramicrotome, stained with uranyl acetate and lead citrate, then examined using a Philips 300 electron microscope.

Computer reconstructions

For retinal reconstructions, camera-lucida drawings were made, then digitized and reconstructed in three dimensions on a PDP 11/23 computer using a program supplied by J. Green (NIMR, London).

Results

(i) *The organization of ganglion cell fibres in the retina*

In *Xenopus* the growth of the retina occurs over a long period, extending throughout embryonic and tadpole life and into juvenile adulthood. New cells are continually added to the retina and axons, arising from the retinal ganglion cells are sequentially added to the visual projection. The position at which new cells are generated is always at the retinal periphery; thus all new axons to be added to the system grow from the peripheral retina. This growth produces a correspondence of the radial position of a ganglion cell in the retina, and hence its axon, with the generation time of the cell. However, the annular growth of the retina in *Xenopus* is not even (Straznicky & Gaze, 1971; Jacobson, 1976). Increased rates of mitosis occur in different regions of the retina during different stages of its development (Beach & Jacobson, 1979*a*; Straznicky & Hiscock, 1984; Tay, Hiscock & Straznicky, 1982), and in consequence cells of a similar generation time interval will be distributed as an uneven annulus in the retina.

Newly growing axons from the most recently formed, peripherally positioned, ganglion cells join fascicles of fibres that have grown from the immediately adjacent central and lateral retina. These fascicles lie beneath the inner limiting membrane and

are ensheathed by processes of glial cells (Fig. 2D). The fascicles are interspersed amongst the cells of the ganglion cell layer and do not form a discrete optic fibre layer. The fibres grow directly within the fascicles to the optic nerve head, located centrally within the retina (Fig. 2A). Observations of HRP-stained fibres in whole-mounted retinae show that the fibres in general do not move from one fascicle to another. Each fascicle will therefore contain fibres of similar circumferential position of origin, but of different generation times, arising from a wedge of retina.

In many species fibres within individual fascicles in the optic fibre layer tend to be arranged in an orderly fashion reflecting their radial position and, in species where the growth of the retina is annular, their age. Thus, the most newly grown fibres from peripheral retina tend to be located at the vitread surface of the fascicles, whilst fibres of progressively more centrally placed (older) cells lie progressively deeper, nearer the ganglion cell layer (Rager, 1980; Easter, Bratton & Scherer, 1984). It was expected that in *Xenopus* a similar organization would exist within the fascicles.

In the light microscope, using semithin sections, clear resolution of the relative position of HRP-labelled peripheral retinal fibres within a single fascicle is difficult (Fig. 2B). However, electron microscopic examination of thin sections cut transversely through fascicles containing HRP-labelled fibres from peripheral retina shows a lack of strong radial organization. An overall weak pattern of deeper older fibres and more superficial younger fibres seems to exist (Fig. 2C). In sections showing growth cone profiles, no clear stratification has been found, with new growing axons interspersed amongst fibres of older generation times (Fig. 2D).

(ii) *The organization of fibres in the intraocular part of the optic nerve*

As the fascicles coalesce at the optic nerve head, the fibres are organized in a readily perceived circumferential order. As the fascicles pass into the optic nerve their entry does not appear to be strictly ordered. Whole fascicles are found to cross over other fascicles as they enter the optic nerve (Fig. 3A,B). At this point in the system there is a degree of loss in the overall organization of the projection. However, the majority of the fascicles pass directly into the optic nerve occupying a position that correlates with the circumferential position of origin of their constituent fibres. Once in the intraocular part of the nerve there appears to be little rearrangement of the fibres. In HRP-labelled preparations fibres arising from specific circumferential positions in the retina tend to travel parallel to one another and in the appropriate sector of the nerve (Fig. 3C,D, see also Fawcett, 1981; Holt, 1984).

Radial positional organization of the fibres within the fascicles is weak as they enter the optic nerve head. There appears to be little internal rearrangement of the fibres as they pass into the optic nerve, so that those fibres that were in the vitread part of the fascicle pass into the central part of the nerve, whilst those in deeper parts of the fascicle pass into the periphery of the nerve. Thus, the weak radial organization that was present in the retinal fascicles is maintained within the intraocular part of the nerve. This means that there is a general trend for more peripherally arising fibres to pass centrally and more centrally arising fibres to pass peripherally. The radial ordering present in the midregion of the frog nerve has been shown to be the opposite of this arrangement. In both *Xenopus* (Cima & Grant, 1982*a*,*b*) and *Rana* (Scalia & Arango, 1982; Reh *et al.* 1983), fibres from peripheral retina are located in the peripheral nerve. To investigate how the change between the organization of the retinal fibre layer and the optic nerve can be explained, the region of the intraorbital optic nerve has been examined.

(iii) *The 'zone of reorganization' (ZOR)*

Just beyond the intraocular part of the nerve a major fibre reorganization occurs. Silver-stained longitudinal sections show that fibres are arranged in parallel fashion before and after this reorganization (Fig. 4A). Fibre reorganization in a similar position in the optic nerve has been described in *Ambystoma* (Herrick, 1941) and in *Rana* (Reh *et al.* 1983). To investigate the relative reordering of the fibres as they pass through this 'zone of reorganization' (ZOR), I have followed the trajectories of small groups of fibres labelled with HRP (Fig. 4B).

As the fibres pass into this ZOR their radial organization is weak. Over a region of some 200 μm fibres are found to reorganize to produce an array which is strongly ordered in relation to their time of entry into the nerve and hence their radial retinal position of origin. Fibres arising in more peripheral retina move to the periphery of the nerve, leaving older, more centrally arising fibres in the nerve core (Figs 4B, 5). The annular growth of the retina produces successive generations of axons which move through the ZOR and deflect to the perimeter of the nerve. This manoeuvre gradually builds up the annular, radial retinotopic, organization of the optic nerve. The radial organization of the fibres within the rest of the optic nerve, chiasma and optic tracts is generated at this point.

What effect does this radial reorganization have upon the circumferential order with which the fibres enter the nerve? In transverse sections through the ZOR, labelled fibres arising from a discrete circumferential position in the peripheral retina, which

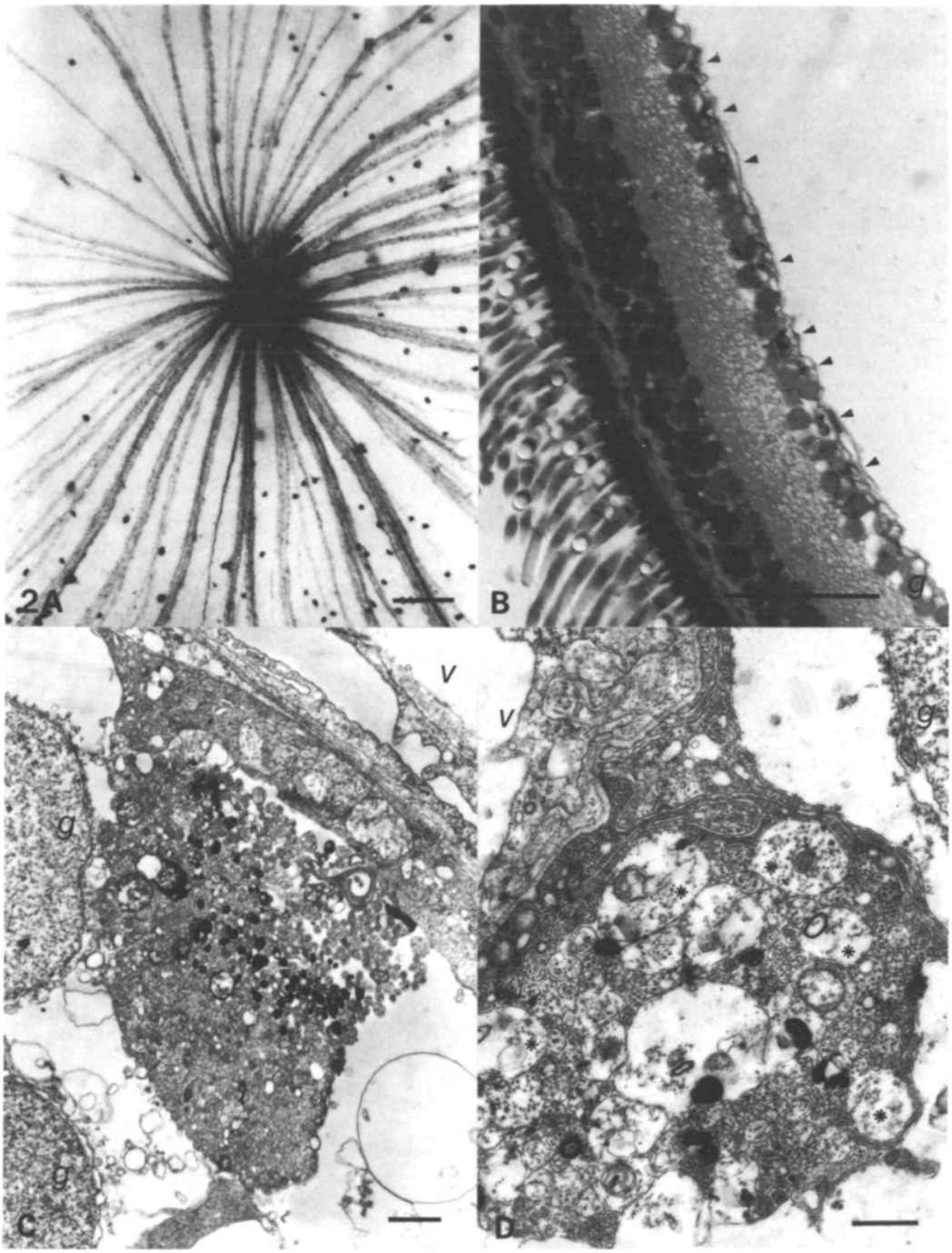


Fig. 2. (A) The fascicular arrangement of fibres in a whole-mounted retina in which peripheral ganglion cells have been labelled with HRP. Fibres arising from cells in a wedge of retina all occupy the same fascicle giving rise to a highly circumferentially ordered array at the optic nerve head. Bar, 50 μm. (B) A 1 μm section through a retina in which peripheral ganglion cells had been labelled with HRP. The fascicles of optic axons (▼) do not form a distinct layer but are interspersed amongst the ganglion cells. Bar, 50 μm. (C) An electron micrograph of a section cut transversely through a fascicle containing HRP-filled peripheral retinal axons. The filled axons are limited to the vitread half of the fascicle. Bar, 1 μm. (D) A micrograph of a fascicle in peripheral retina, cut transversely, showing the intermixing of HRP-filled peripheral retinal axons, growth cones of the most-recently generated ganglion cells and older more centrally positioned ganglion cells' axons. Note that growth cone profiles, some of which are labelled (*), are in contact either with the other fibres or with the glial cell processes ensheathing the fascicle. Bar, 0·5 μm. *g*, ganglion cell; *v*, vitreous.

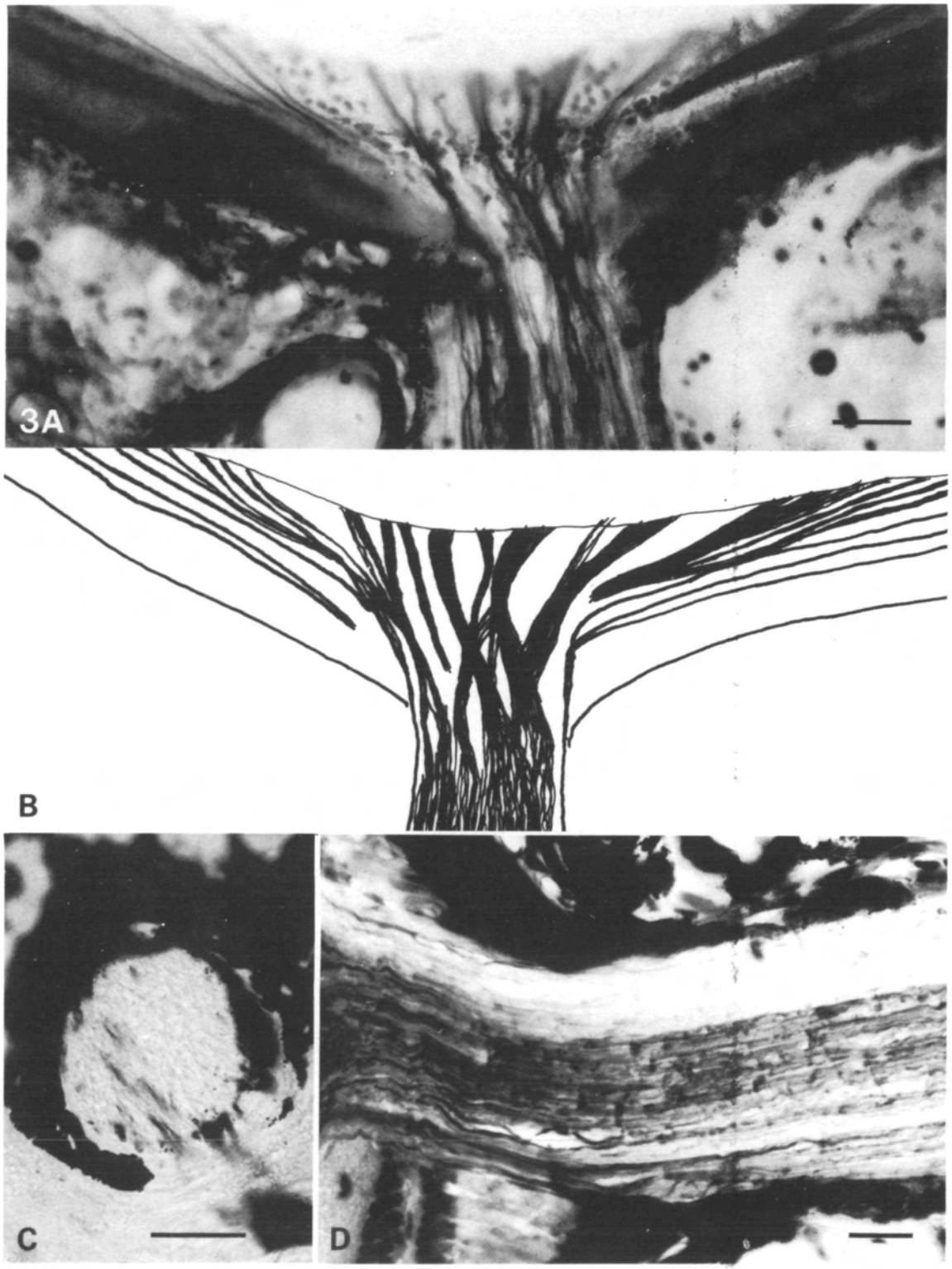


Fig. 3. (A) A photomicrograph and (B) a camera-lucida drawing of a 60 μm frozen section through the optic nerve head of a retina in which peripheral ganglion cells had been labelled with HRP. As the fascicles coalesce at the optic nerve head some exchange of position occurs, thereby degrading the strong circumferential organization of the fibres in the retina. Bar, 50 μm. (C) A 60 μm section transverse through the intraocular part of the nerve. HRP-labelled fibres from peripheral temporal retina occupy a sector of the nerve cross section. Bar, 50 μm. (D) A 60 μm section along the length of the intraocular nerve showing the generally parallel arrangement of the fibres. Bar, 20 μm.

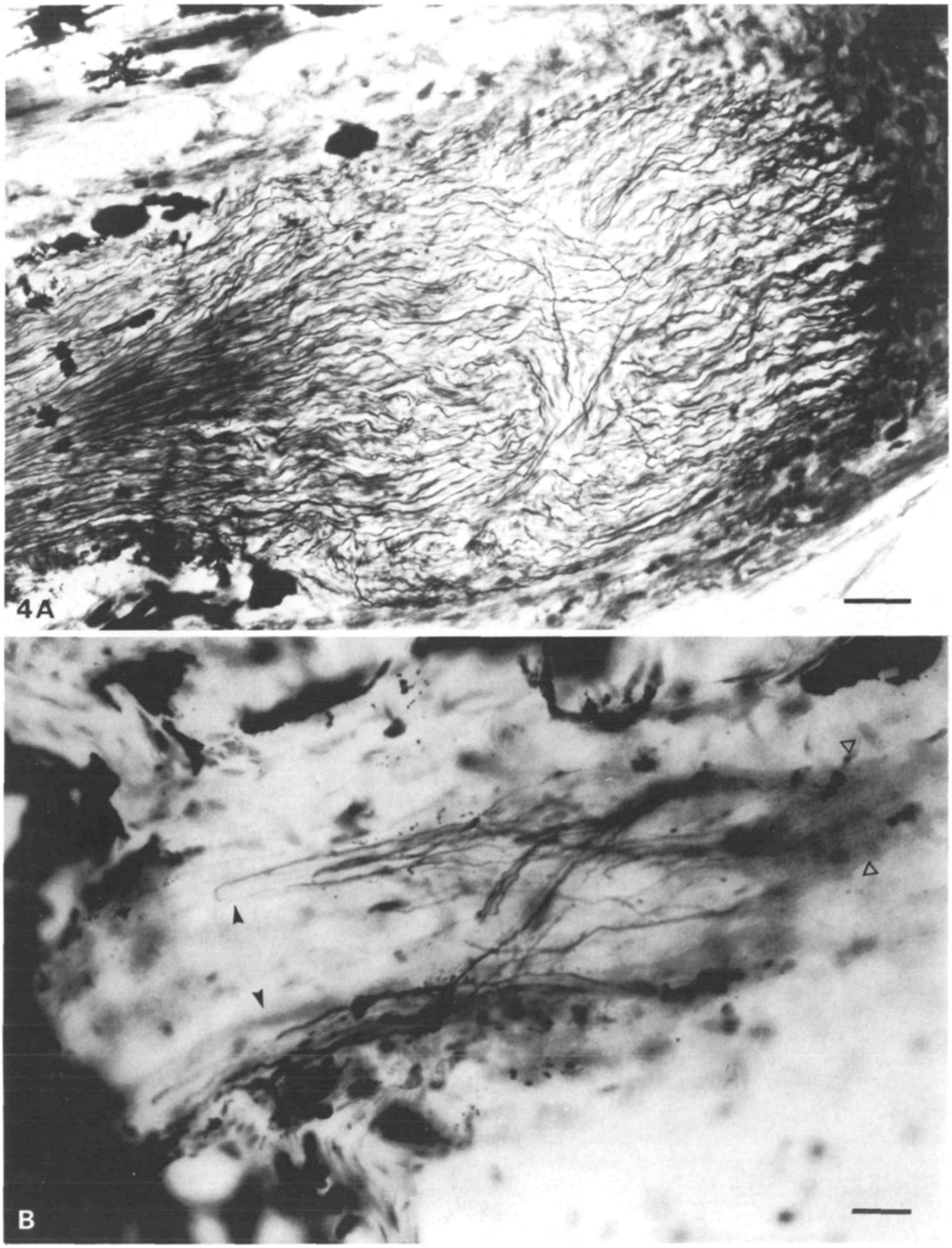


Fig. 4. (A) A Holmes silver-stained section showing the zone of reorganization. Note the parallel arrangement of the fibres in the intraocular nerve (left) and the rest of the nerve (right). Bar, 10 μm. (B) A 60 μm section cut through the ZOR. HRP-filled fibres from central retina, located in peripheral parts of the intraocular nerve (▼) reorganize and come to lie in the core of the nerve (▽). Bar, 25 μm.

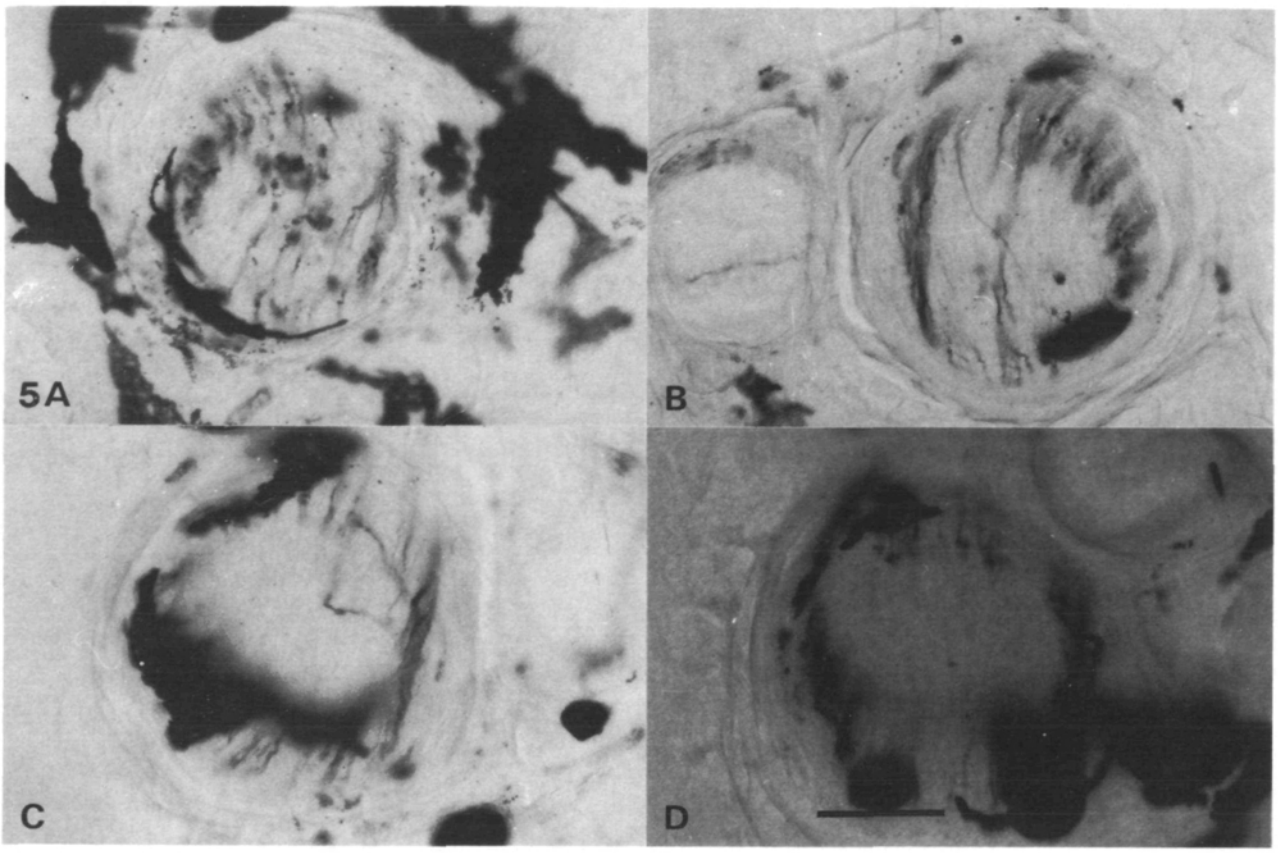


Fig. 5. A sequence of 60 μm transverse sections (A–D) cut through the optic nerve from the ZOR, in which peripheral ganglion cell axons have been labelled. Fibres in the more central regions of the nerve are shown to move to the nerve margin to form a complete annulus. Bar, 50 μm.

passed through the intraocular nerve as a group, were found to subdivide, with fibres travelling obliquely to the nerve periphery. The quadrantic direction of the obliquely running fibres varied in each animal examined, irrespective of which group of fibres was filled. The variation in the direction in which the fibres moved from the nerve core to the periphery resulted in considerable variation in the degree of apparent disorder in the nerve, beyond the ZOR.

(iv) *Fibre ordering within the nerve*

Where HRP was applied to central tectum, reconstructions of sectioned retina revealed that ganglion cells in central retina had been labelled (Fig. 6A,B). Within the midregion of the nerve the fibres arising in central retina were always located in central positions. A subgroup of more peripherally positioned ganglion cells was commonly labelled in these preparations, probably as a result of lesioning fibres of passage in the tectum. Intermediate tectal fills produced intranerve fibre distributions of an intermediate nature, with axons arranged in annuli partway between the centre and periphery of the nerve. In all cases where the HRP-labelled fibres arose in peripheral retina they were located as an annulus in the peripheral nerve (Fig. 6C,D).

Fibres from every circumferential position of the peripheral retina were found distributed around the entire nerve perimeter beyond the ZOR. The pattern of distribution of peripheral retinal fibres was very similar for temporal, nasal and ventral fills (Fig. 7A–F). Fills of dorsal retina often proved difficult, with poor uptake of HRP or only few fibres staining, a result that may be connected to the growth dynamics of the retina. However, successful fills of all retinal quadrants could result in a complete peripheral annulus of fibres being labelled in the nerve (Fig. 7A,B). In many cases more fibres were found at the edge of the nerve corresponding to the position at which the retina was filled (Fig. 7C,D). This uneven distribution of fibres in the nerve's peripheral annulus reflects the sectorial organization of the fibres in the intraocular part of the nerve.

As fibres proceeded along the nerve their circumferential dispersal increased. Where a bias in the fibre distribution was found near the ZOR, this became harder to detect as the fibres spread around the nerve perimeter. In contrast to the reported observations

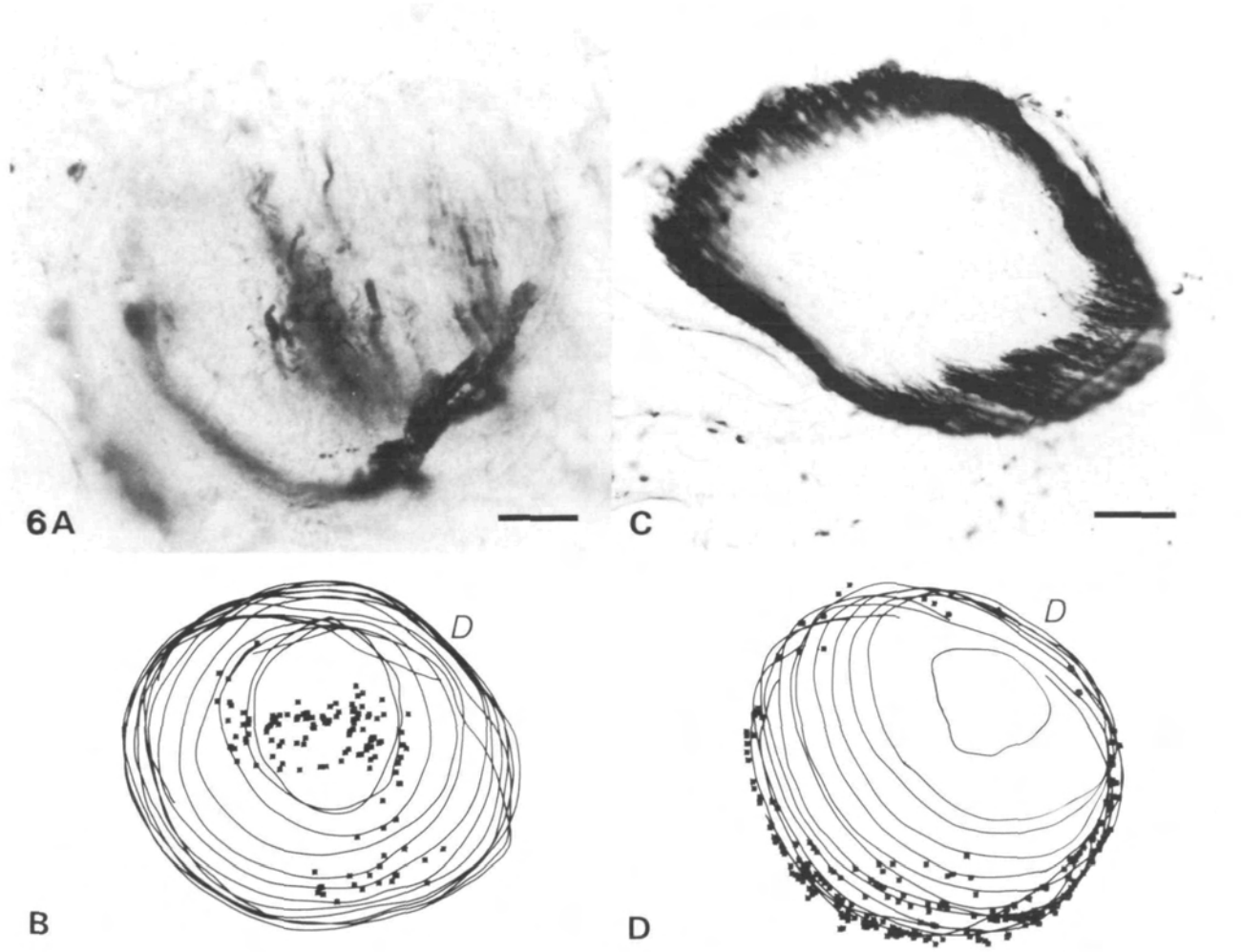


Fig. 6. (A) A transverse section through the midregion of the optic nerve of an animal in which HRP was applied to central tectum. HRP-labelled fibres are located in the core of the nerve. (B) A computer reconstruction of the retina showing the central distribution of filled ganglion cells. (C) A section showing the peripheral annulus of fibres which results from labelling of peripheral ganglion cells. (D) Computer reconstruction of the retina showing the peripheral distribution of the filled cells. D, dorsal. Bar, 20 μm.

on *Rana* (Scalia & Arango, 1982; Reh *et al.* 1983), in this study of *Xenopus* no dual representations of fibres from any retinal positions were observed.

Observations on *Rana pipiens* confirmed previous reports of a dual representation of nasal and temporal axons in the optic nerve (Fig. 8). Axons arising in the nasal and temporal poles of the retina in *Rana* entered the optic nerve head across its full width. This resulted in an intermixing of fibres arising in symmetrically aligned groups of ganglion cells from both nasal and temporal retina (Fig. 8B). At the position of the ZOR, which is within the intraocular part of the nerve, the fibres arising in more peripheral positions again deflected to the nerve periphery. However, the elliptical morphology of the nerve at this point resulted in the majority of fibres deflecting to the nasal and temporal edges of the nerve (Fig. 8C). As the cross-sectional shape of the nerve changed from an ellipse to a circle, the two-dimensional segregation of the fibres produced the dual representation of the fibres either side of the dorsoventral axis of the nerve (Fig. 8E). This dual organization persisted right along the nerve to the point at which the nerve entered the optic chiasma. However, as in *Xenopus*, there was a significant dispersal of the fibre groupings as they passed along the nerve.

(v) *Retinotopic organization of fibres at the nerve–chiasma junction*

The peripheral positioning of the most-recently grown fibres was maintained along the entire length of the nerve up to a position approximately 200 μm from the chiasma. Beyond this point, filled fibres, which had been travelling in all positions in the nerve periphery, were no longer found in the dorsal part of the nerve (Fig. 9).

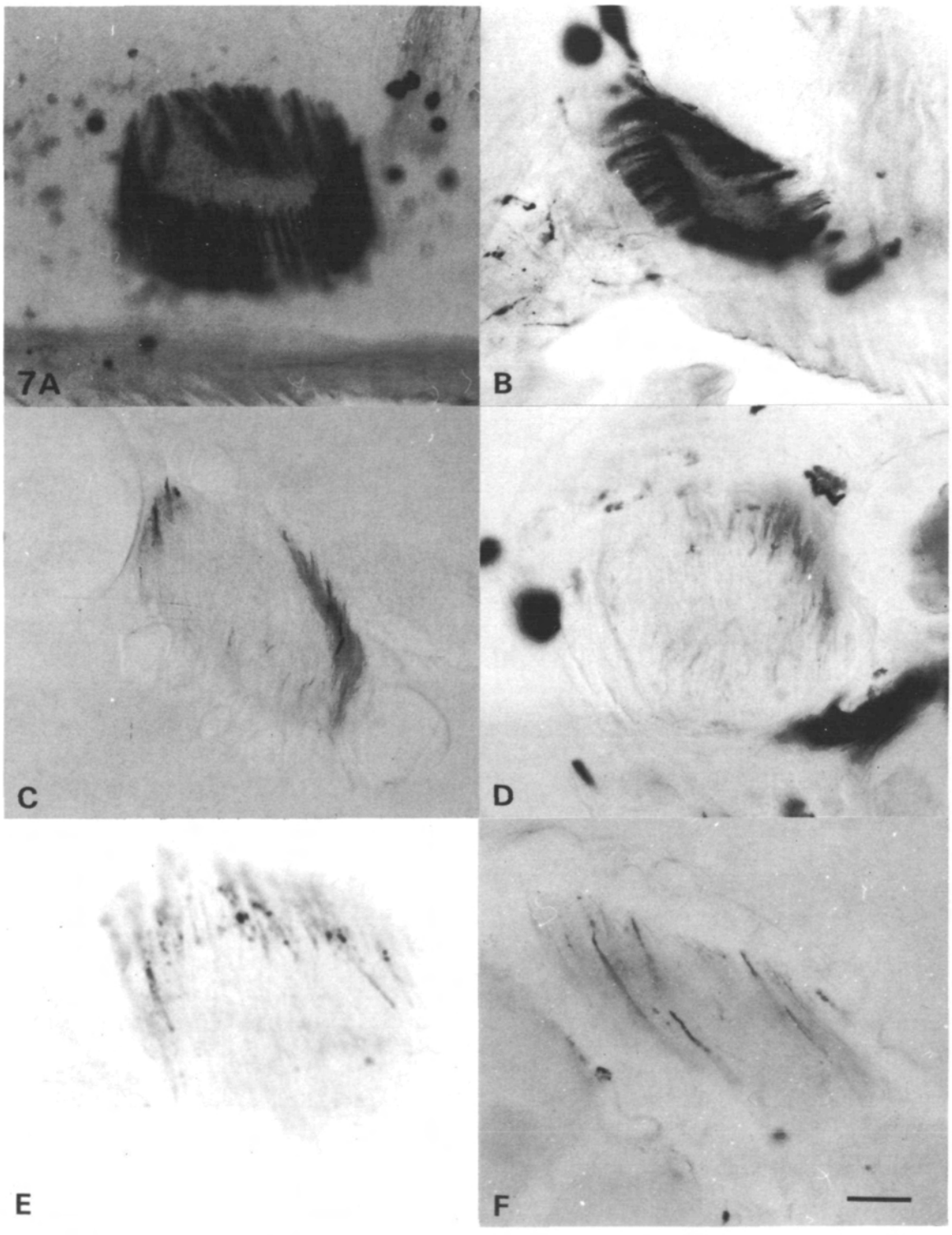


Fig. 7. 60 μm transverse sections through the optic nerves of animals in which quadrant specific HRP fills of ganglion cells had been made. (A) A complete peripheral annulus of filled fibres resulting from a nasal retinal fill in which many fibres have taken up the HRP. (B) A similar annular distribution to that shown in A resulting from a temporal retinal fill. The annular distribution of HRP-labelled fibres in the nerves of nasal (C), temporal (D), ventral (E), and dorsal-filled (F) preparations. In each of these preparations many fewer fibres have been labelled; however in each case the filled fibres are distributed in a similar annular fashion. Note the bias in the distribution of fibres in D resulting from an increase in the number of fibres deflecting to one side of the nerve at the ZOR. Bar, 25 μm.

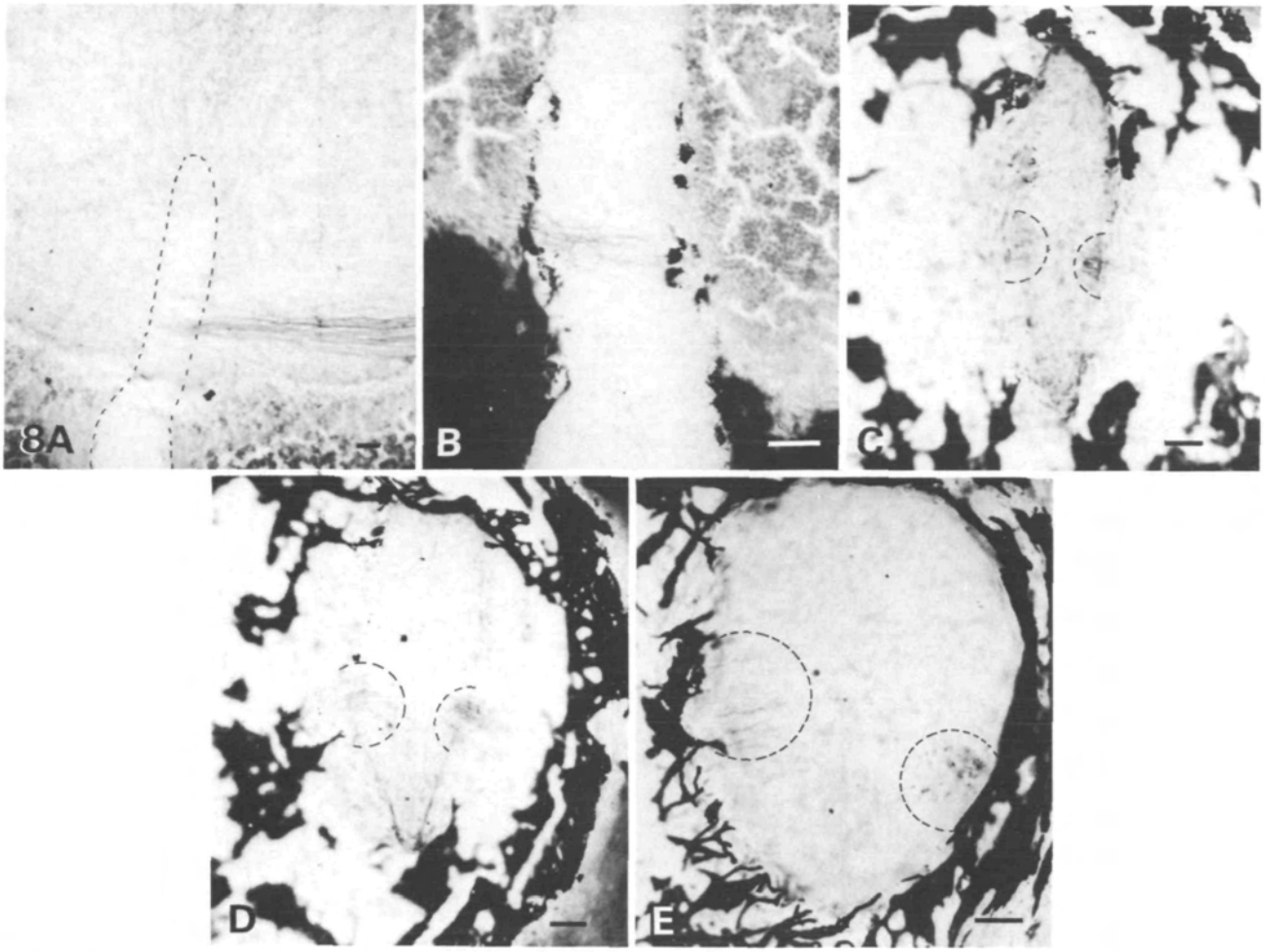


Fig. 8. A peripheral temporal fill of an adult *Rana pipiens*. (A) The axons course in fascicles across the retina to the optic nerve head (dashed outline). (B) 60 μm into the nerve. The fibres enter the optic nerve head across its entire width occupying a band within the intraocular part of the nerve. (C) 120 μm further into the nerve. As the fibres pass through the ZOR they move to the nearest margin of the nerve, thereby dividing into two diametrically opposed groups (dashed lines – also in D and E). (D) As the elliptical intraocular nerve transforms into the circular optic nerve (+120 μm from C), the two groups of fibres become increasingly separated giving the dual representation of fibres seen in the main part of the nerve (E). Bar, 50 μm.

As the nerve entered the brain, filled fibres originating in peripheral retina were found to accumulate exclusively in the ventral part of the nerve (Fig. 9A–D). The findings of Cima & Grant (1982*a*,*b*), that the position of the newly grown fibres was peripheral in the midnerve, but occupied a ventral crescent in the most distal part of the nerve, have been confirmed. The clearest manifestation of this redistribution was found in whole-mounted preparations of the brain and optic nerves, in which the superficial part of the chiasma had been lesioned and filled. Fibres located in the nerve as a ring moved around the outside of the nerve to form a lamina at the point where the nerve entered the brain (Fig. 9B,D). As these peripheral fibres entered the brain they exhibited no dramatic alteration in their position but appeared to rotate slightly, in an anticlockwise direction, to form a wedge in the most superficial layers of the chiasma. Throughout the diencephalic optic tracts, newly grown fibres were always found next to the pial margin (Fawcett *et al.* 1984).

Fibres from the central part of the retina were located in the core of the nerve from beyond the position of the ZOR until just proximal to the chiasma. At this point fibres in the centre of the nerve came to occupy a position at the dorsal edge. This displacement was coincident with the ventral displacement of peripheral retinal fibres. With this rearrangement the oldest fibres, in the dorsal part of the nerve, passed into the deeper parts of the optic tract.

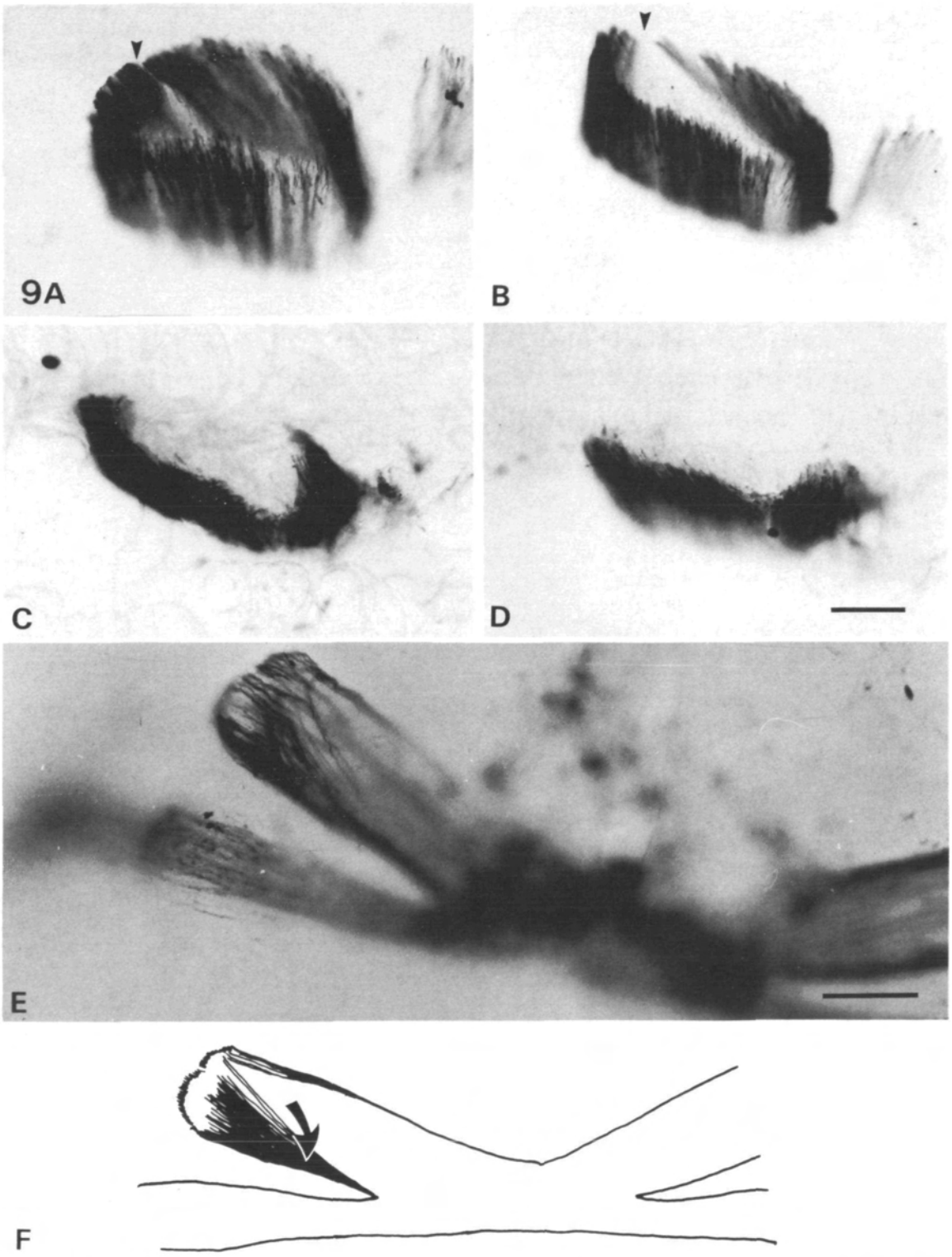


Fig. 9. (A–D) Serial 60 μm sections through the prechiasmatic nerve, showing the transformation of a peripheral annular distribution of temporal retinal fibres in the nerve into a laminar distribution in the optic tract. The annulus breaks dorsally (▼) and fibres move to occupy the ventral region of the nerve, leading directly into the superficial layers of the optic tract. Bar, 50 μm. (E) A whole-mounted preparation and (F) a camera-lucida drawing of the same preparation, showing an annulus of fibres from peripheral retina breaking as the fibres move around the nerve to form a lamina. Bar, 100 μm.

The question of what happens to the non-retinotopic distribution of fibres from circumferential retinal positions is more complex. It was found that fibres from all peripheral circumferential retinal positions accumulated in the ventral crescent of the nerve, resulting in a complete intermixing of these fibres. However, it is well established in a variety of amphibian species that relative circumferential retinotopicity in the diencephalic optic tracts exists (*Xenopus*, Steedman, 1981; Fawcett & Gaze, 1982; *Cynops*, Fujisawa *et al.* 1981*a*; *Rana*, Scalia & Fite, 1974; Fujisawa *et al.* 1981*b*; Scalia & Arango, 1982; Reh *et al.* 1983). As the fibres passed through the chiasma a major circumferential reorganization occurred with the fibres from each retinal quadrant selecting, or being directed into, specific regions of the tract (Fawcett *et al.* 1984). This pathway selection is most apparent for dorsal and ventral fibres which select the posterior and anterior regions of the tract respectively (Fawcett *et al.* 1984). By making small lesions in either dorsal or ventral retina, few fibres were labelled and these could be traced through the region of the chiasma where the pathway selection occurs (Fig. 10).

Discussion

The main intention in this study has been to examine the ordering of retinal ganglion cell fibres in the *Xenopus* retina and optic nerve to see how these relate to the ordering of fibres in the optic tracts (Steedman, 1981; Fawcett & Gaze, 1982; Fawcett *et al.* 1984). It has been established that there are two major reorganizations of the fibre population within the optic nerve. The first is at a position just behind the eye, where fibres become arranged in a radial retinotopic fashion and the second occurs as the nerve passes into the brain, at which point the annular organization of the nerve is converted into the laminar organization seen in the optic tracts. A further organizational change occurs within the chiasmatic optic tract, where fibres select trajectories dependent upon their quadrantic position of retinal origin.

The annular growth of the retina results in fibres of the same age, from all circumferential positions in the retina, entering the projection sequentially throughout development. In contrast to the results from previous studies on other species (chick, Rager, 1980; Krayanek & Goldberg, 1981; Halfter & Deiss, 1984; fish, Cook, 1982; Easter *et al.* 1984; monkey, Minckler, 1980; Ogden, 1983*a*,*b*), the lack of any interchange of growing fibres from one fascicle to another in the *Xenopus* retina results in a highly organized array of fibres at the optic nerve head. The pattern of fascicles that results from this mode of growth resembles a 'cartwheel', in which the fibres converging on the optic nerve head are arranged in an orderly fashion according to the circumferential position of their cells of origin. Examination of thin sections suggests that the lack of an optic fibre layer may be related to this organization. In all other species so far examined adjacent fibre fascicles are bounded by glial end feet, but the proximity of neighbouring fascicles could allow growing axons to pass through the ensheathing glial processes into the next fascicle. The ability of axons to exchange places in the glial-bound fascicles of the mammalian optic nerve has been demonstrated clearly (Silver, 1984; Williams & Rakic, 1985). Indeed, the deformation of glial sheaths has been suggested to enable growth cones to contact the basal lamina of the inner limiting membrane (Easter *et al.* 1984). In *Xenopus* the fibre fascicles are not adjacent to each other in a separate retinal layer but are interspersed in the ganglion cell layer (Fig. 2B). The discrete nature of the fascicles might preclude interchange of fibres anywhere except for positions of coalescence as the fascicles join at the optic nerve head.

The study of the organization of the fibres in the retinae of goldfish and chicks has demonstrated that successive generations of fibres are added to the fascicles at the vitread surface (Rager, 1980; Easter *et al.* 1984). It has been suggested that in the goldfish the newly growing fibres are in fact growing along the basal lamina that surrounds the vitreous rather than upon the fibres in the fascicles (Easter *et al.* 1984). Similar radial ordering has been reported in the retina of the macaque monkey (Ogden, 1983*a*; but, interestingly, good ordering seems not to occur in the owl monkey optic fibre layer, Ogden, 1983*b*). In *Xenopus*, electron microscopic examination of the retinal fibre fascicles which contain HRP-labelled fibres from peripheral retina, does not show good ordering. Where growth cone profiles could be identified, it appeared that the newly growing fibres were growing upon the existing fibres. The lack of a clear radial organization in the fascicles may therefore be a consequence of the relatively free movement of the growth cones amongst other fibres within the fascicle, as they grow to the optic nerve head.

At the optic nerve head, although there is some positional interchange of fascicles, fibres within the intraocular part of the nerve continue to grow in the same general organization as they had in the retina. Thus, fibres from each retinal quadrant tend to be confined to the appropriate sector of the nerve cross section and the oldest fibres, which arise in central retina, generally travel at the nerve margin, whilst successive generations of newly growing fibres enter the core of the nerve.

In the fish, the continuation of the basal lamina of the inner limiting membrane around the ophthalmic

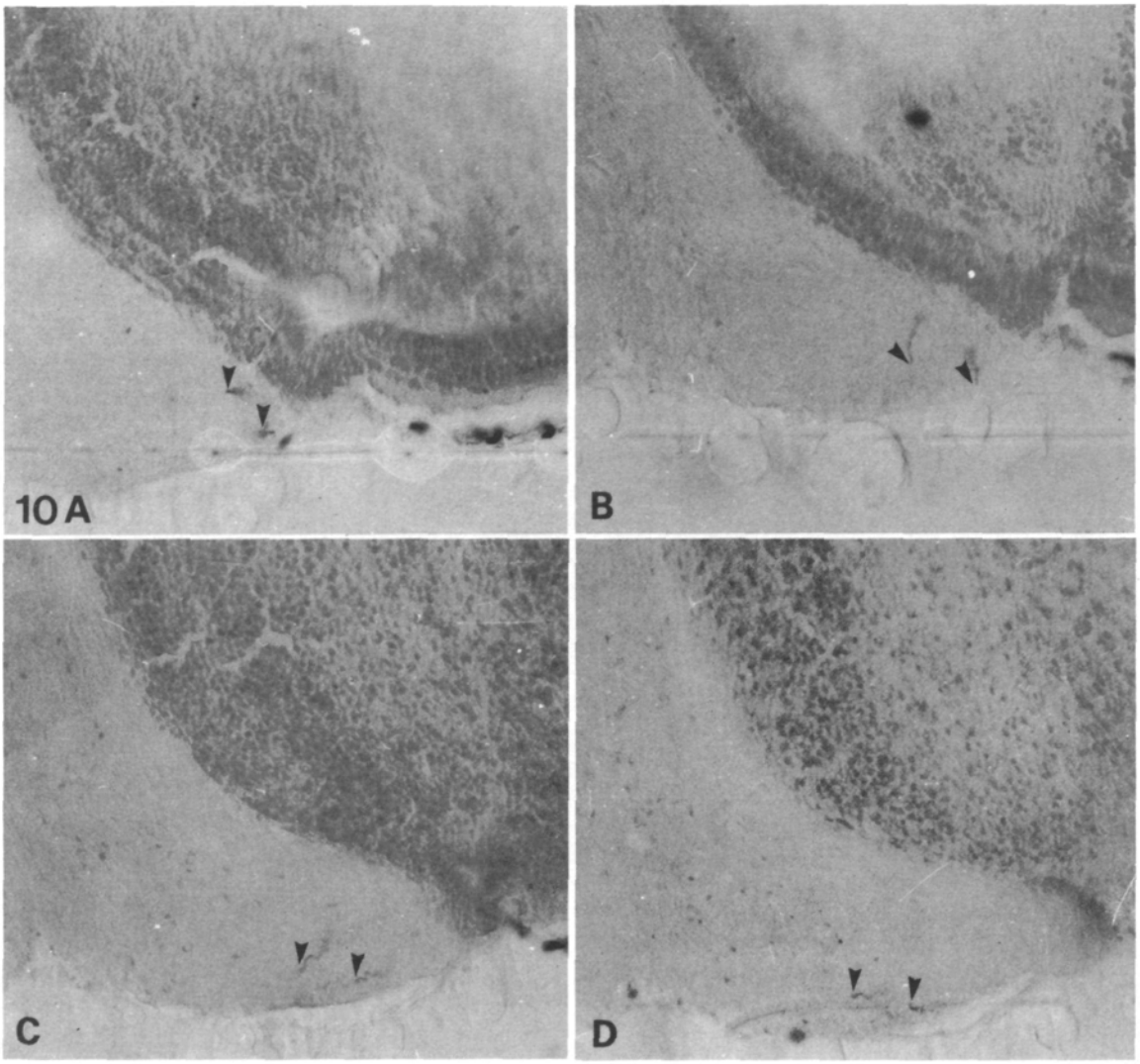
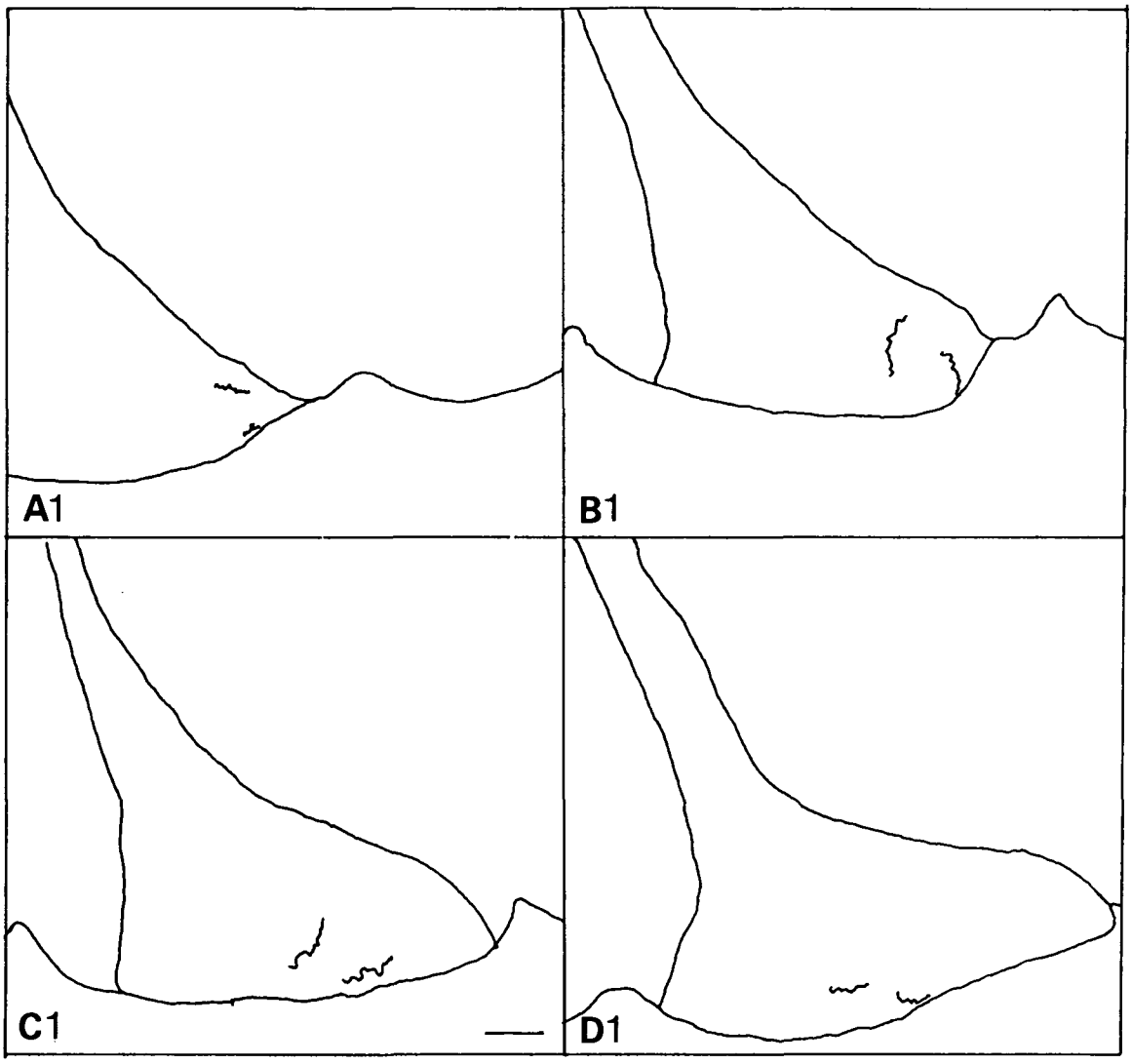


Fig. 10. Photographs (A–D) and camera-lucida drawings (A1–D1) of serial 60 μm sections through the lower optic tract of an animal in which only two fibres (or two small fascicles of fibres) were labelled with HRP (▼). The fibres which are positioned in the rostral part of the tract (A), abruptly move across the width of the tract (C,D), to finally occupy an appropriate caudal position for dorsal fibres in D. Bar, 50 μm.

artery and then the optic nerve, may form a preferred substrate that leads the new generations of fibres from the centre of the nerve head to the ventral periphery of the nerve (Bunt, 1982; Easter *et al.* 1984). This mechanism would enable the good radial retinotopicity of the optic fibre layer to be preserved on entry to the optic nerve. In *Xenopus* a similar mechanism does not exist; the ophthalmic artery penetrates the retina at an adjacent position, but is separated from the nerve (Beach & Jacobson, 1979*b*).

In *Xenopus*, a region of fibre reorganization has been found to occur behind the eye, similar to that found in other amphibians (Herrick, 1941; Reh *et al.* 1983). In contrast to *Rana*, in *Xenopus* the reorganization appears equal in all directions. The central retinal fibres come to occupy the nerve core, with successively younger fibres arranged as annuli around them. It has not been possible to determine what substrata may guide fibres from the centre of the nerve to the periphery. However, given that successive generations of fibres perform similar peripheral movements it is possible that fibre guidance by fibre following could cause the observed reorganization.

Cima & Grant (1982) suggest that the glial elements in the larval midoptic nerve cause centrally growing fibres to choose a 'path of least resistance' by migrating to the nerve periphery. Once a fibre had deflected and attained the nerve periphery, it could form an orientated substratum capable of guiding subsequently growing fibres. It is unclear whether this hypothetical mechanism for optic nerve fibre reordering occurs. Observations of axonal growth cones in the monkey and cat optic nerve suggest that no such physical constraints imposed by glia are likely to exist



(Williams & Rakic, 1985; Williams, Bastiani, Lia & Chalupa, 1986). However, the guidance of growing axons by an existing array of fibres has been shown to be a potent mechanism (Taylor & Gaze, 1985).

The circumferential order of fibres in the intraocular nerve, in which fibres of filled ganglion cells situated in any retinal quadrant remain grouped together in the appropriate sector of the nerve, can be interpreted as a consequence of retinal growth and the fascicular organization of the fibres. During their passage through the ZOR, this originally coherent grouping breaks down as the fibres pass to the nerve periphery. If one assumes that newly growing fibres can move to any position in the nerve periphery, but that they will generally take the shortest route, a bias in the scatter of the fibres would logically arise from a deflection of an initially organized array. For example, if a group of fibres enter the ZOR from the centre of the intraocular optic nerve, they would be expected to deflect to the entire periphery of the nerve exhibiting no bias in any direction. However, fibres arising from a specific retinal quadrant will enter the ZOR at an off-central position and therefore more fibres would be expected to deflect to the nearest edge of the nerve. This will create a bias in the distribution of the axons in the optic nerve beyond the ZOR. Variation in the pattern of filled fibres in any particular preparation was also influenced by the uneven growth of cells at the retinal periphery. At 1 month after metamorphosis, proportionately fewer of the fibres entering the nerve will be from dorsal retina whilst many fibres will arise in ventral retina. Thus, peripheral filling of ventral retina will label fibres that form a high percentage of the most recently grown fibres, and will exhibit a relatively higher degree of scatter in the nerve, forming a more distinct ring. Conversely, dorsal fibres will form a far smaller component of the most peripheral annulus of the nerve and will show a proportionately deeper distribution and a less-pronounced annular distribution.

In *Rana pipiens*, previous reports of a dual representation of nasal and temporal axons in the optic

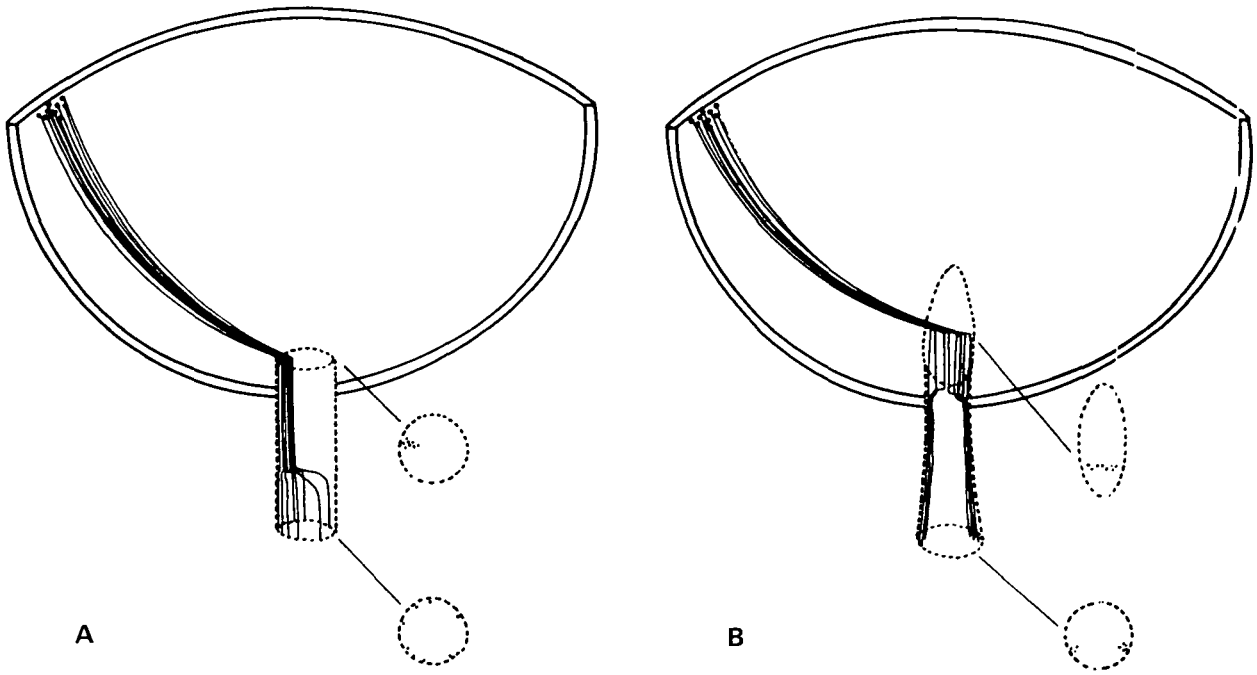


Fig. 11. Diagram to show how the different organizations of axons in the nerves of (A) *Xenopus* and (B) *Rana pipiens* arise. In *Xenopus* fibres from a discrete position in peripheral retina pass across the retinal surface in fascicles and enter the appropriate sector of the optic nerve head. Having passed in parallel arrangement through the intraocular part of the nerve the fibres pass through the ZOR where they all move to the nerve perimeter. Since the nerve is circular in section the resulting distribution of fibres is annular. In *Rana* fibres again course to the optic nerve head in fascicles, but enter the optic nerve head across its full width, giving rise to a band-like distribution. As the fibres pass through the ZOR they again deflect to the peripheral nerve. But because of the elliptical shape of the intraocular nerve fibres move only to the nearest edge of the nerve, splitting the band into two groups. As the fibres pass further along the nerve they continue to separate eventually forming diametrically opposed groupings within the nerve.

nerve have been confirmed. It appears that the different morphology of the optic nerve head is responsible for the differences between *Rana* and *Xenopus* (Fig. 11). The intraocular optic nerve of *Rana* is not organized in sectors as has been found in *Xenopus*, but as a series of bands of fibres running horizontally across the width of the nerve. The position at which the radial organization of the projection is generated occurs within the intraocular nerve. Here fibres are found to deflect to the nerve periphery, but since the cross section of the nerve at this point is elliptical, fibre deflection occurs to either side of the nerve, rather than to its entire periphery. This establishes a dual representation of the nasal and temporal fibres either side of the dorsal-to-ventral axis of the nerve, which becomes more apparent as the nerve cross-sectional shape changes to a circle. However, as in *Xenopus* the fibres continue to disperse as they pass along the nerve resulting in a gradual breakdown in the coherence of the dual groupings at either side of the nerve.

In this study a second major reorganization of fibres in the projection has been shown to occur just as the nerve enters the brain. This appears to be similar for both *Rana* and for *Xenopus*. This reorganization changes the annular intranerve fibre arrangement into the laminar, radially retinotopic organization of the fibres in the postchiasmatic optic tracts. It has been conclusively demonstrated that all newly growing fibres from the ciliary margins of the retina travel at the pial surface of the optic tracts (Gaze & Grant, 1978; Scalia & Arango, 1982; Reh *et al.* 1983; Fawcett *et al.* 1984). When newly growing fibres positioned in the dorsal part of the nerve reach the chiasma region, they only pass into the superficial layers of the contralateral tract at the ventral edge of the nerve–chiasma junction. The result of this manoeuvre is to displace the oldest fibres, previously lying in the central part of the nerve, to a dorsal position in the immediately prechiasmatic part of the nerve. The underlying mechanism of this transformation would thus appear to be a consequence of the growth dynamics of the system and the preference of the newly growing fibres for the pial edge of the tract. It is of great interest that a recent study, showing a similar dorsoventral segregation of older and younger fibres in the ferret optic nerve, has linked the segregation with the position of a tongue of neuroepithelial cells, which extends from the chiasma to the optic

foramen, the position where this segregation occurs (Walsh, 1986).

In all species so far examined there is a change in the organization of the retinal projection as the nerve enters the brain (cat: Guillery, 1982; Torrealba, Guillery, Eysel, Polley & Mason, 1982; ferret: Walsh & Guillery, 1985; fish: Scholes, 1979; Rusoff, 1984; Maggs & Scholes, 1986). In amphibia, studies of the fibre organization in the superficial aspects of the diencephalic tract have shown quadrantic circumferential order across the rostrocaudal dimension (Scalia & Fite, 1974; Steedman, 1981; Fujisawa *et al.* 1981*a*,*b*; Fawcett & Gaze, 1982; Scalia & Arango, 1982; Reh *et al.* 1983; Fawcett *et al.* 1984). This order is established in the region of the chiasma, between the lowest part of the diencephalic tract and the nerve entry point. The region of the chiasma appears to be the 'black box' responsible for the generation of ordered fibre ingrowth to the tectum. Again, we do not know whether these fibres are following previous generations of fibres that have made this manoeuvre, or whether they are individually responding to a cue in the chiasmatic tract.

In an intriguing new study of the Cichlid fish visual system, it has been suggested that the change in glial cell surface properties at the point of entry of the nerve to the brain alters the growth dynamics of the ingrowing axons enabling them to make active choices of pathway based upon such selective fasciculation (Maggs & Scholes, 1986). Such a mechanism would still require that the very first axons to enter this part of the system select their trajectory actively.

In summary, ganglion cell fibres, that leave the eye in a circumferentially position-dependent order, were disordered in the early part of the nerve as they passed through the ZOR. The radial organization of the projection as it left the eye was variable, but at the ZOR the fibres became arranged as annuli. As the projection entered the brain the pattern of radial organization was altered by the morphology of the nerve–tract transformation, but the radial retinotopicity of the projection was maintained throughout the pathway to the tectum. At the midline an active selection of trajectory by the fibres established the circumferential order in which the fibres grew in the diencephalic tracts.

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References

Adams, J. C. (1977). Technical considerations on the use of HRP as a neuronal marker. *Neurosci.* **2**, 141–145.

Beach, D. H. & Jacobson, M. (1979*a*). Patterns of cell proliferation in the retina of the clawed frog during development. *J. comp. Neurol.* **198**, 603–611.

Beach, D. H. & Jacobson, M. (1979*b*). Patterns of cell proliferation in the developing retina of the frog in relation to blood supply and position of the choroidal fissure. *J. comp. Neurol.* **198**, 625–632.

Bunt, S. M. (1982). Retinotopic and temporal organisation of the optic nerve and tracts in the adult goldfish. *J. comp. Neurol.* **206**, 209–226.

Bunt, S. M. & Horder, T. J. (1983). Evidence for an orderly arrangement of optic axons within the optic nerves of the major nonmammalian vertebrate classes. *J. comp. Neurol.* **213**, 94–114.

Bunt, S. M., Horder, T. J. & Martin, K. A. C. (1978). The nature of the nerve fibre guidance mechanism responsible for the formation of an orderly central visual projection. In *Developmental Neurobiology of Vision* (ed. R. D. Freeman). NATO Advanced Study Institute, series A: *Life Sci.* **27**, 331–344.

Cima, C. & Grant, P. (1982*a*). Development of the optic nerve in *Xenopus laevis*: I. Early development and organisation. *J. Embryol. exp. Morph.* **72**, 225–249.

Cima, C. & Grant, P. (1982*b*). Development of the optic nerve in *Xenopus laevis*: II. Gliogenesis, Myelination and metamorphic remodeling. *J. Embryol. exp. Morph.* **72**, 251–267.

Cook, J. E. (1982). Errant optic axons in the normal goldfish retina reach retinotopic tectal sites. *Brain Res.* **250**, 154–158.

Easter, S. S. Jr (1985). The continuous formation of the retinotectal map in goldfish, with special reference to the role of the axonal pathway. In *Molecular Bases of Neural Development.* (ed. G. M. Edelman, W. S. Gall & W. M. Cowan). New York: Wiley.

Easter, S. S. Jr, Bratton, B. & Scherer, S. S. (1984). Growth-related order in the retinal fibre layer in goldfish. *J. Neurosci.* **4**, 2173–2190.

Fawcett, J. W. (1981). How axons grow down the *Xenopus* optic nerve. *J. Embryol. exp. Morph.* **65**, 219–233.

Fawcett, J. W. & Gaze, R. M. (1982). The retinotectal fibre pathways from normal and compound eyes in *Xenopus*. *J. Embryol. exp. Morph.* **72**, 19–37.

Fawcett, J. W., Taylor, J. S. H., Gaze, R. M., Grant, P. & Hirst, E. (1984). Fibre order in the normal *Xenopus* optic tract, near the chiasma. *J. Embryol. exp. Morph.* **83**, 1–14.

Fujisawa, H., Watanabe, K., Tani, N. & Ibata, Y. (1981*a*). Retinotopic analysis of fibre pathways in amphibians. I. The adult newt *Cynops pyrrhogaster*. *Brain Res.* **206**, 9–20.

Fujisawa, H., Watanabe, K., Tani, N. & Ibata, Y. (1981*b*). Retinotopic analysis of fibre pathways in amphibians. II. The frog *Rana nigromaculata*. *Brain Res.* **206**, 21–26.

GAZE, R. M. & GRANT, P. (1978). The diencephalic course of regenerating retinotectal fibres in *Xenopus* tadpoles. *J. Embryol. exp. Morph.* **44**, 201–216.

GUILLERY, R. W. (1982). The optic chiasm of the vertebrate brain. *Cont. Sensory Physiol.* **7**, 39–73.

HALFTER, W. & DEISS, S. (1984). Axon growth in embryonic chick and quail retina whole mounts *in vitro*. *Devl Biol.* **102**, 344–355.

HERRICK, C. J. (1941). Development of the optic nerves of *Amblystoma*. *J. comp. Neurol.* **74**, 473–534.

HOLT, C. E. (1984). Does timing of axon outgrowth influence retinotectal topography in *Xenopus*? *J. Neurosci.* **4**, 1130–1152.

JACOBSON, M. (1976). Histogenesis of retina in the clawed frog with implications for the pattern of development of retinotectal connection. *Brain Res.* **103**, 541–545.

KRAYANEK, S. & GOLDBERG, S. (1981). Oriented extracellular channels and axonal guidance in the embryonic chick retina. *Devl Biol.* **84**, 41–50.

MAGGS, A. & SCHOLES, J. (1986). Glial domains and nerve fibre patterns in the fish retinotectal pathway. *J. Neurosci.* **6**, 424–438.

MINCKLER, D. S. (1980). The organisation of the nerve fibre bundles in the primate optic nerve head. *Archs Ophthalmol.* **98**, 1630–1636.

NIEUWKOOP, P. D. & FABER, J. (1967). *Normal Table of* Xenopus laevis *(Daudin)*. Amsterdam: North-Holland.

OGDEN, T. E. (1983*a*). Nerve fibre layer of the Macaque retina: retinotopic organisation. *Invest. Ophthalmol. Vis. Sci.* **24**, 85–98.

OGDEN, T. E. (1983*b*). Nerve fibre layer of the Owl Monkey retina: retinotopic organisation. *Invest. Ophthalmol. Vis. Sci.* **24**, 265–269.

RAGER, G. (1980). Development of the retinotectal projection in the chicken. *Adv. Anat. Embryol. Cell Biol.* **63**, 1–92.

REH, T. A., PITTS, E. & CONSTANTINE-PATON, M. (1983). The organisation of fibres in normal and tectum-less *Rana pipiens*. *J. comp. Neurol.* **218**, 282–296.

RUSOFF, A. C. (1984). Paths of axons in the visual system of perciform fish and implications of these paths for rules governing axonal growth. *J. Neurosci.* **4**, 1414–1428.

SCALIA, F. & ARANGO, V. (1982). The anti-retinotopic organisation of the frog's optic nerve. *Brain Res.* **266**, 121–126.

SCALIA, F. & FITE, K. (1974). A retinotopic analysis of the central connections of the optic nerve of the frog. *J. comp. Neurol.* **158**, 455–478.

SCHOLES, J. H. (1979). Nerve fibre topography in the retinal projection to the tectum. *Nature, Lond.* **278**, 620–624.

SILVER, J. (1984). Studies on the factors that govern directionality of axonal growth in the embryonic optic nerve and at the chiasma of mice. *J. comp. Neurol.* **223**, 238–251.

STEEDMAN, J. G. (1981). Pattern formation in the visual pathways of *Xenopus laevis*. Ph.D. Thesis, Faculty of Science, University of London.

STRAZNICKY, K. & GAZE, R. M. (1971). The growth of the retina in *Xenopus laevis*: an autoradiographic study. *J. Embryol. exp. Morph.* **26**, 67–79.

STRAZNICKY, C. & HISCOCK, J. (1984). Post-metamorphic retinal growth in *Xenopus*. *Anat. Embryol.* **169**, 103–109.

STRÖER, W. F. H. (1940). Das optische system beim Wassermolch (*Triturus taeniatus*). *Acta. neerl. morphol.* **3**, 178–195.

TAY, D., HISCOCK, J. & STRAZNICKY, C. (1982). Temporonasal asymmetry in the accretion of retinal ganglion cells in late larval and postmetamorphic *Xenopus*. *Anat. Embryol.* **164**, 75–84.

TAYLOR, A. C. & KOLLROSS, J. J. (1946). Stages in the development of *Rana pipiens* larvae. *Anat. Rec.* **94**, 7–23.

TAYLOR, J. S. H. & GAZE, R. M. (1985). The effect of the fibre environment on the paths taken by regenerating nerve fibres in *Xenopus*. *J. Embryol. exp. Morph.* **89**, 383–401.

TAYLOR, J. S. H., WILLSHAW, D. J. & GAZE, R. M. (1985). The distribution of fibres in the optic tract after contralateral translocation of an eye in *Xenopus*. *J. Embryol. exp. Morph.* **85**, 225–238.

TORREALBA, F., GUILLERY, R. W., EYSEL, U., POLLEY, E. H. & MASON, C. A. (1982). Studies of the retinal representation within the cat's optic tract. *J. comp. Neurol.* **211**, 377–396.

WALSH, C. (1986). Age-related fibre order in the ferret's optic nerve and optic chiasma. *J. Neurosci.* **6**, 1635–1642.

WALSH, C. & GUILLERY, R. W. (1984). Fibre order in the pathways from the eye to the brain. *Trends in Neurosci.* **8**, 208–212.

WALSH, C. & GUILLERY, R. W. (1985). Age related fibre order in the optic tract of the ferret. *J. Neurosci.* **5**, 3061–3070.

WILLIAMS, R. W., BASTIANI, M. J., LIA, B. J. & CHALUPA, L. M. (1986). Growth cones, dying axons, and developmental fluctuations in the fibre population of the cat's optic nerve. *J. comp. Neurol.* **246**, 32–69.

WILLIAMS, R. W. & RAKIC, P. (1985). Dispersion of growing axons within the optic nerve of the embryonic monkey. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3906–3910.

WILSON, M. A. (1971). Optic nerve fibre counts and retinal ganglion cell counts during development of *Xenopus laevis* (Daudin). *J. exp. Physiol.* **56**, 83–91.

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