

The effects of the fibre environment on the paths taken by regenerating optic nerve fibres in *Xenopus*

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SUMMARY

The paths taken by fibres regenerating to the tectum from various parts of the *Xenopus* retina were investigated in whole-mount preparations, after localized retinal labelling with HRP. The effects of different environments on the fibres were studied by comparing contralateral with ipsilateral regeneration, in the presence of the other eye or after it had been removed in embryonic life.

Under all conditions fibres from the various parts of the retina regenerated to the corresponding appropriate parts of the tectum, but they took a variety of pathways, some grossly abnormal, to get there. Contralaterally regenerating fibres tended to behave less abnormally than ipsilateral fibres; and regeneration in the absence of the other eye tended to be more abnormal than in its presence. In any one category of regeneration the most nearly normal pathways were those of fibres from temporal retina, followed by ventral, nasal and dorsal fibres.

Fibres regenerating from all parts of the retina, in the presence of the other eye, tended to become gathered into the medial brachium as they approached the tectum. All regenerating fibres approached their tectal terminations by one or more of three main pathways: round one or both brachia, thus encircling the tectum to get to their terminal zone; directly across the tectum; or by passing on to the tectum before changing course. The changes of direction required to enable fibres wrongly positioned in the tract to reach their correct terminal zones were frequently sudden and considerable, and took place on the tectum or at the tectodiencephalic junction. The results are discussed in relation to the differing substrates over which the fibres regenerate.

INTRODUCTION

After surgical interruption of an amphibian optic nerve the fibres can regenerate to give a normal retinotectal projection. It has been suggested that the regenerating fibres are grossly disordered at the site of the lesion and that they are enabled to terminate retinotopically by a mechanism involving selective chemo-affinity between individual retinal axons and their target cells in the tectum (Sperry, 1963). The extent of disorder in the regenerating fibre array has, however, been questioned. Horder (1974) has argued that the majority of fibres reinnervate the tectum in approximately correct relative order and terminate with little rearrangement.

Recent studies show that the order in the pathways followed by the regenerating fibres is generally abnormal (Gaze & Grant, 1978; Udin, 1978; Meyer, 1980; Fujisawa, 1981; Gaze & Fawcett, 1983; Stuermer & Easter, 1984), and the role of

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interactions between optic fibres and tectal targets in the re-establishment of retinotopic order in the projection has been re-emphasized (Schmidt, 1978; Fujisawa, Tani, Watanabe & Ibata, 1982). While target affinity is obviously important in the re-establishment of ordered maps by regenerating fibres, there may be other factors involved in regeneration, such as interactions between the fibres themselves and between the fibres and the substrate of the pathway over which they grow. This substrate is probably a complex structure and may include other normal or degenerating fibres.

In the present experiments we have made a detailed examination of the absolute positions of fibres growing to the optic tecta from various parts of the retina after optic nerve cut, in relation to the normal positions of such fibres; and of the relative retinotopic ordering of groups of such fibres growing from various parts of the retina. In this study, as in all previous studies on regeneration in the lower vertebrate visual system, a proportion of newly growing, uncut retinal fibres, arising from the retinal margin during the survival period after nerve cut, is present as well as regenerating fibres. In this paper the fibres said to be regenerating may include an undetermined number of these newly growing fibres. The possible role of such newly growing fibres is considered in the Discussion.

We have sought to examine factors which may influence the arrangement of fibres in regenerating pathways by studying four variations of the regenerating system (Fig. 1). These are:

1. Fibres regenerating in the contralateral tract back to a previously innervated tectum in cases where the other eye and pathway are normal. In this situation the tract up which the fibres regenerate contains the degenerating fibre debris of the previous innervation as well as intact ipsilateral retinodiencephalic fibres from the other eye.
2. Fibres regenerating to the ipsilateral tectum, along a tract containing a normal population of fibres from the other eye. This tract also contains degenerating ipsilateral retinodiencephalic fibres along with the regenerating fibres.
3. Fibres regenerating ipsilaterally to a 'virgin' tectum, i.e. one which has never previously received a normal optic fibre projection. Such a virgin tectum is formed by the enucleation of one eye in the embryo so that the contralateral tectum develops without its normal retinal input (Feldman, Gaze & Keating, 1971; Straznicki & Gaze, 1982). In this case the tract up which the fibres regenerate has never carried a normal retinotectal fibre projection. It does, however, contain degenerating ipsilateral retinodiencephalic fibres from the remaining eye.
4. Fibres regenerating contralaterally in cases where the other eye had been removed in embryonic life. Here the fibres grow up a tract containing the degenerating fibres of the original contralateral projection, but the normal ipsilateral retinodiencephalic fibres which should pass up this tract are absent.

In this paper we describe the pathways followed, in the optic tract and on the tectum, by selected populations of optic nerve fibres in each of the four situations mentioned above, as revealed by horseradish peroxidase tracing methods, using wholemount preparations.

METHODS

Normal animals or animals in which one eye had been removed at larval stage 28 (Nieuwkoop & Faber, 1967) were reared to metamorphosis. Toadlets of less than one month after metamorphosis were anaesthetized in 1:3000 MS222 (Tricaine Methane Sulphonate, Sandoz). The intracranial optic nerve was exposed by removal of the cartilaginous roof of the mouth and fine tungsten needles were used first to section the left optic nerve, and then to separate the cut

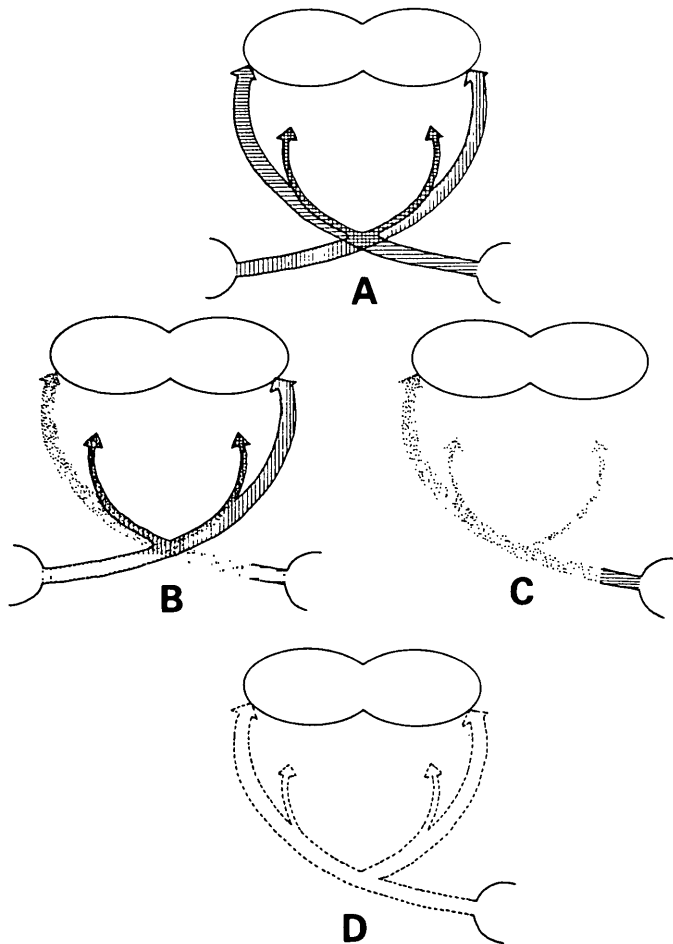


Fig. 1. (A) The normal retinotectal projection. The majority of fibres from each eye cross to the contralateral side at the chiasma and innervate the contralateral tectum and diencephalic nuclei. A subpopulation of fibres from each eye enters the ipsilateral tract, without passing through the chiasma, and innervates ipsilateral diencephalic nuclei. (B) The fibre substrate encountered by regenerating optic fibres when the other eye is present. The optic nerve on the right of this diagram has been cut and the fibres central to the lesion degenerate (stipple). Normal fibres from the other eye are also present as shown. (C) The fibre substrate encountered by regenerating optic fibres when the other eye is absent. Only the degenerating fibres from the lesioned nerve are present. (D) The paths of regenerating retinal fibres (interruption lines) under the conditions of these experiments. Fibres regenerate to both contralateral and ipsilateral tecta as well as to the diencephalic nuclei. These pathways may be compared with the two types of the fibre substrate shown in (B) and (C).

ends. The animals were allowed to recover in oxygenated 50% Niu-Twitty solution, then transferred to 10% Sterns solution for periods ranging from 2 to 14 weeks. Following anaesthesia in 1:3000 MS222, localized retinal lesions were made by crushing the peripheral retina between a fine needle inserted in the vitreous and a pair of forceps held outside the sclera. Recrystallized HRP (Sigma type VI) was then applied to the lesion. 48 h after HRP application the animals were deeply anaesthetized and perfused with 0.25 M-sucrose followed by 2.5% glutaraldehyde in 0.1 M-phosphate buffer (pH 7.2). The brains were dissected out and fixed for a further one hour on ice; the membranes were then removed and the brains processed at 0°C for HRP as wholemounts using the diaminobenzidine tetrahydrochloride method of Adams (1977). The brains were dehydrated in ethanol and cleared in methyl salicylate, and the projections were drawn, using a *camera lucida*. Because of the variations in focus required to permit the fibres to be seen as they pass up round the diencephalon and onto the tectum, it was not possible to show the details of the projections photographically. In some cases the brains were sectioned at 50 µm and the sections were then processed for HRP. To ensure that the positions and extent of the retinal lesions were as intended at the time of HRP filling, the experimental eyes were wax embedded and sectioned at 15 µm. Serial sections were then drawn and the eyes reconstructed three-dimensionally, using a PDP 11/23 computer.

RESULTS

In the normal retinotectal fibre projection in *Xenopus*, the optic tract widens out as it passes from the chiasma to the tectum so that at the tectodiencephalic margin the fibres approach the tectum across its entire rostral extent (Fig. 2A).

A variety of survival times was used in order to ascertain the effect of time on the patterns of fibre distribution in the pathways. At a survival time of two weeks the regenerating fibres had reached the optic tectum. Only a few fibres were seen and these were all extremely fine and appeared to be poorly filled. Patterns of fibre distribution from each retinal quadrant were established by four weeks after nerve section and showed little change when compared with those seen after 6, 8, 12 and 14 weeks survival times.

Regenerating fibres mostly passed up through the diencephalon close to its pial margin (Fig. 3). Fibres regenerating contralaterally grow amongst the degenerating fibre debris of the original projection. In animals with both eyes present, fibres may also have interacted with the ipsilateral retinodiencephalic fibres from the other eye (Fig. 2B). The ipsilateral fibres pass up the diencephalon as far as the rostral border of the tectum, in close association with the rostral margin of the contralateral optic tract. In animals with a virgin tectum, where the other eye had been removed in embryonic life, these ipsilateral fibres are absent. The removal of an eye in early life does not, in our experience, produce a tectum completely devoid of retinal fibres. In control fills of animals with virgin tecta a few, sparse, fibre arborizations have been found on the virgin tectum.

This study is based on 57 experimental animals, distributed as shown in Table 1. In all cases, regenerating fibres were distributed across the optic tract more widely, and were arranged in a less orderly fashion than normal fibres from that part of the retina. Fibres regenerating ipsilaterally were, in general, even more widely spread and disorderly than fibres regenerating contralaterally. Fibres regenerating contralaterally as well as those regenerating ipsilaterally terminated in the region of the tectum appropriate to their retinal origin.

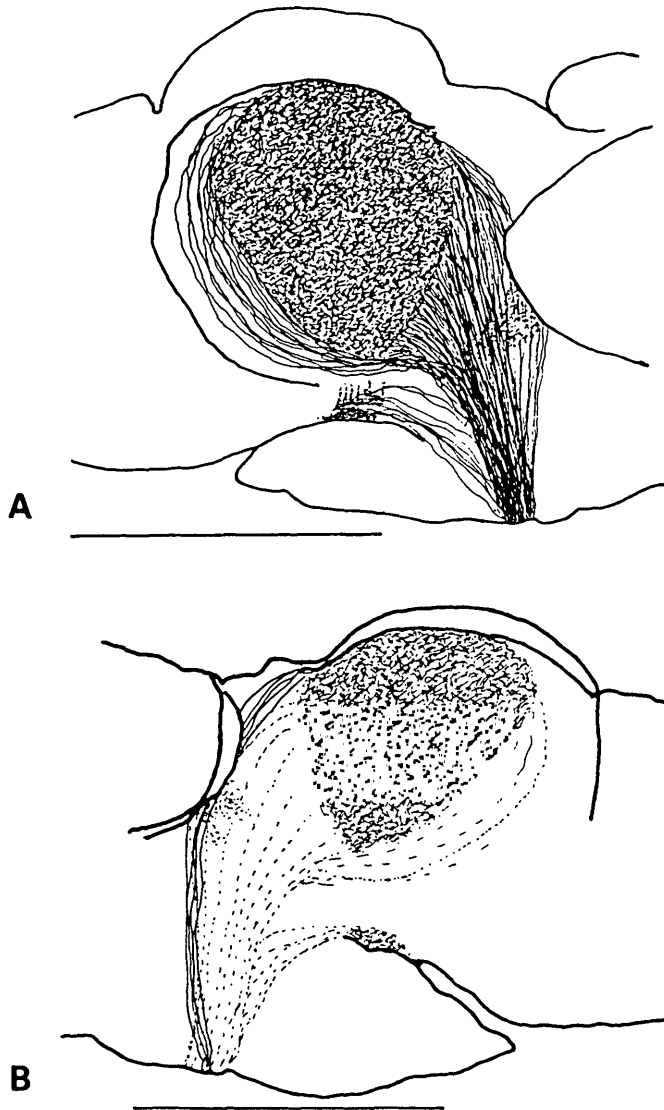


Fig. 2. (A) Normal contralateral projection from an animal in which fibres in the left optic nerve had been filled with HRP. The right optic tract is seen from a dorsolateral viewpoint and rostral is to the right. The neuropil of Bellonci and the basal optic nucleus are shown. Bar = 1 mm. This, and all following illustrations except Fig. 3, are *camera lucida* drawings from wholemount preparations. (B) The ipsilateral diencephalic projection from the same eye as in Fig. 2A. This diagram is based on the assumption that the optic tracts from the two eyes are symmetrical. The ipsilateral retinodiencephalic fibres were first drawn in left lateral view; then the focus was changed to enable the contralateral tract to be seen through the brain and drawn on the same figure. The position of the contralateral optic tract could then be used to indicate the position (interrupted lines) of the unlabelled contralateral tract from the other eye. Rostral is to the left. Bar = 1 mm.

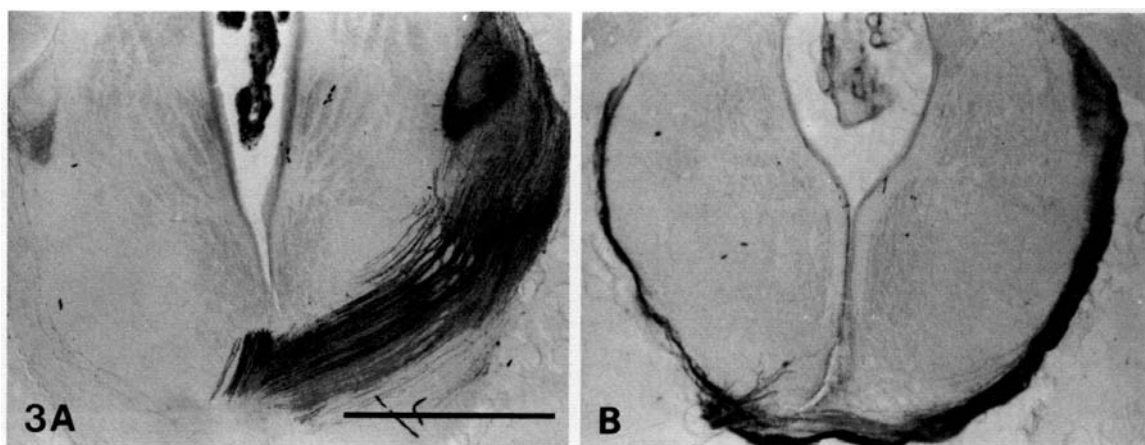


Fig. 3. (A) Transverse section $60\ \mu\text{m}$ caudal to the chiasma, from a normal animal in which the left optic nerve had been filled. The labelled fibres travelled throughout the depth of the contralateral tract. (B) Similar section from an animal in which the left optic nerve was cut 5 weeks before HRP filling. The majority of fibres are confined to the pial margin of the tract, with only a few fibres following abnormal paths through deep tract tissues. Bar for (A) and (B) = $500\ \mu\text{m}$.

Table 1.

Origin of fibres	Nature of experiment	Side	Number of preparations
Temporal retina	Other eye present	Contra	12
Temporal retina	Other eye present	Ipsi	12
Temporal retina	Other eye absent	Contra	5
Temporal retina	Other eye absent	Ipsi	5
Ventral retina	Other eye present	Contra	9
Ventral retina	Other eye present	Ipsi	8
Ventral retina	Other eye absent	Contra	2
Ventral retina	Other eye absent	Ipsi	3
Nasal retina	Other eye present	Contra	6
Nasal retina	Other eye present	Ipsi	6
Nasal retina	Other eye absent	Contra	5
Nasal retina	Other eye absent	Ipsi	5
Dorsal retina	Other eye present	Contra	13
Dorsal retina	Other eye present	Ipsi	13
Dorsal retina	Other eye absent	Contra	4
Dorsal retina	Other eye absent	Ipsi	4

Temporal retinal fibres

Normal temporal fibres travel as a compact group in the central region of the tract and head directly towards the region of rostral tectum, where they terminate (Fig. 4).

Regenerating temporal fibres were less well ordered and more widely dispersed in the tract than normal temporal fibres; however, this was the group of retinal fibres which, during regeneration, gave pathways most closely resembling those of normal, uncut fibres. Temporal fibres regenerating contralaterally were more widely distributed when the other eye was absent (five cases) than when it was present (12 cases). In one animal (with the other eye absent) the regenerating temporal fibres showed an extreme form of a behaviour pattern seen several times among dorsal and ventral retinal fibres – the temporal fibres passed all the way round the tectum to reach finally their rostral terminations (Fig. 5).

Temporal fibres regenerating ipsilaterally in the presence of the other eye were in most cases (seven out of twelve) confined to the central region of the tract, whereas in all five cases when the fibres regenerated to a virgin tectum, the fibre distribution in the tract was wide and disorderly. In some cases when the ipsilaterally regenerating temporal fibres were widely distributed across the tract fibres could be seen forming a medial brachium (Fig. 6; other eye present) but these fibres could not then be followed to their terminations.

Temporal fibres regenerate back to terminate in rostral tectum. As with fibres from ventral and dorsal retina, temporal fibres may also pass on to the tectum for some distance before changing course, suddenly or gradually, to get back to the rostral tectum (Fig. 7; ipsilateral projection to a previously virgin tectum). Even more dramatic changes in direction have been seen when temporal fibres are widely dispersed in the optic tract but terminate in a very localized region at the rostral edge of the (normal or virgin) tectum (Fig. 8). The projection illustrated is that to a virgin tectum and the contralateral projection in this case is shown in Fig. 5.

Ventral retinal fibres

Normal ventral fibres occupy the more rostral part of the tract. At the rostromedial tectum the fibres form the medial brachium in which they travel caudally, before turning laterally and innervating medial tectum (Fig. 9).

In all cases regenerating ventral fibres formed a heavy medial brachium. The tracts formed tended to be wide, disorderly and variable from animal to animal. In the two cases where labelled ventral fibres regenerated contralaterally in the absence of the other eye, the distribution of these fibres in the tract was comparable to the most abnormal ventral fibre distributions found when the other eye was present. In all but two cases ventral fibres regenerating contralaterally could be seen to pass right across the tectum as well as travelling medially and laterally round it (Fig. 10; other eye present). In both the other cases most of the fibres passed medially.

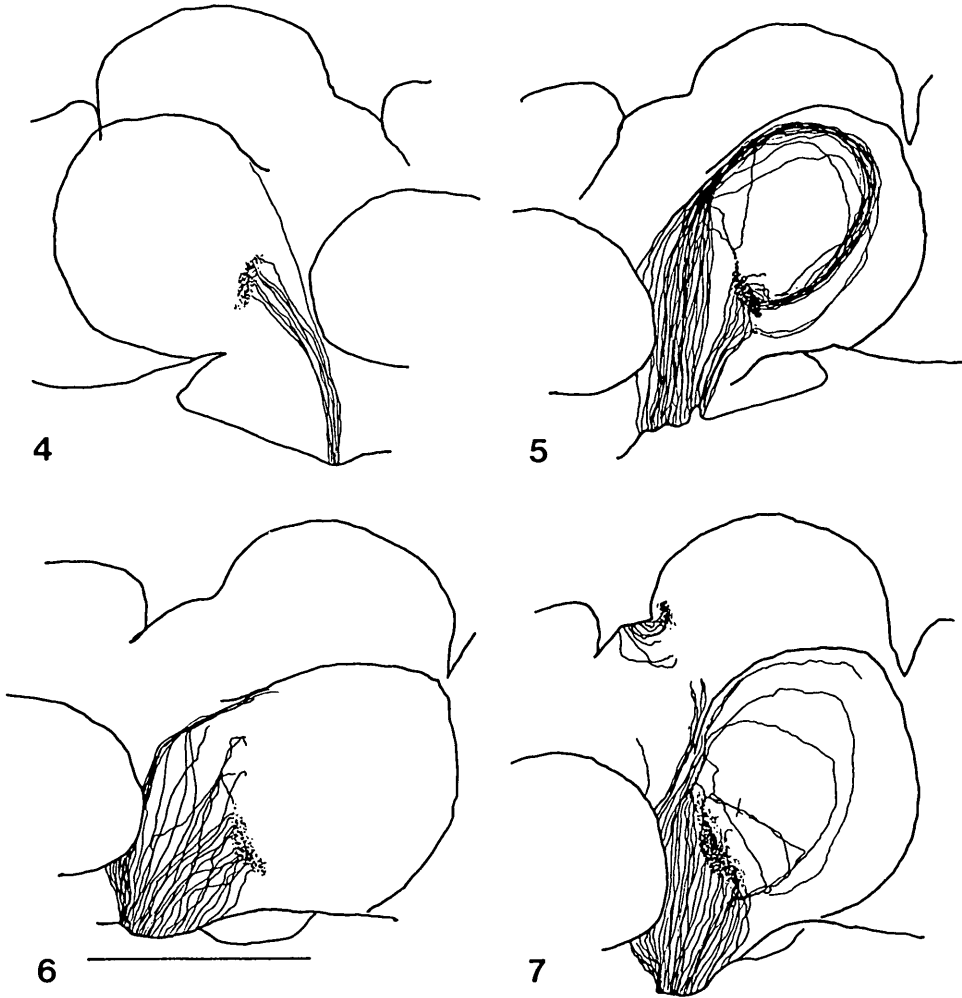


Fig. 4. Control fill of fibres arising in left temporal retina, travelling to rostral tectum in the contralateral optic tract. The fibres form a compact group in the central part of the tract. Dorsolateral view, rostral to the right. Bar = 1 mm. (See Fig. 6.)

Fig. 5. The path of temporal retinal fibres, from the right eye, regenerating contralaterally in an animal with a virgin right tectum. Many fibres were found in the anterior part of the tract from which they were constrained to enter into the medial brachium. Fibres travelled around medial, caudal and lateral tectum, eventually terminating in rostral tectum. Dorsolateral view, rostral to the left. Bar = 1 mm. (See Fig. 6.)

Fig. 6. Temporal retinal fibres from the left eye regenerating to the ipsilateral tectum across the width of the pathway. Fibres meander along the path rather than going directly to their terminations. Those fibres in the anterior part of the tract are gathered into the medial brachium. Dorsolateral view, rostral to the left. Bar = 1 mm.

Fig. 7. Temporal fibres regenerating ipsilaterally to a virgin tectum. The fibres show very wide dispersal. Many fibres grow beyond the rostral tectum and travel across the tectal surface before making turns to return to rostral tectum. Dorsolateral view, rostral to the left. Bar = 1 mm. (See Fig. 6.)

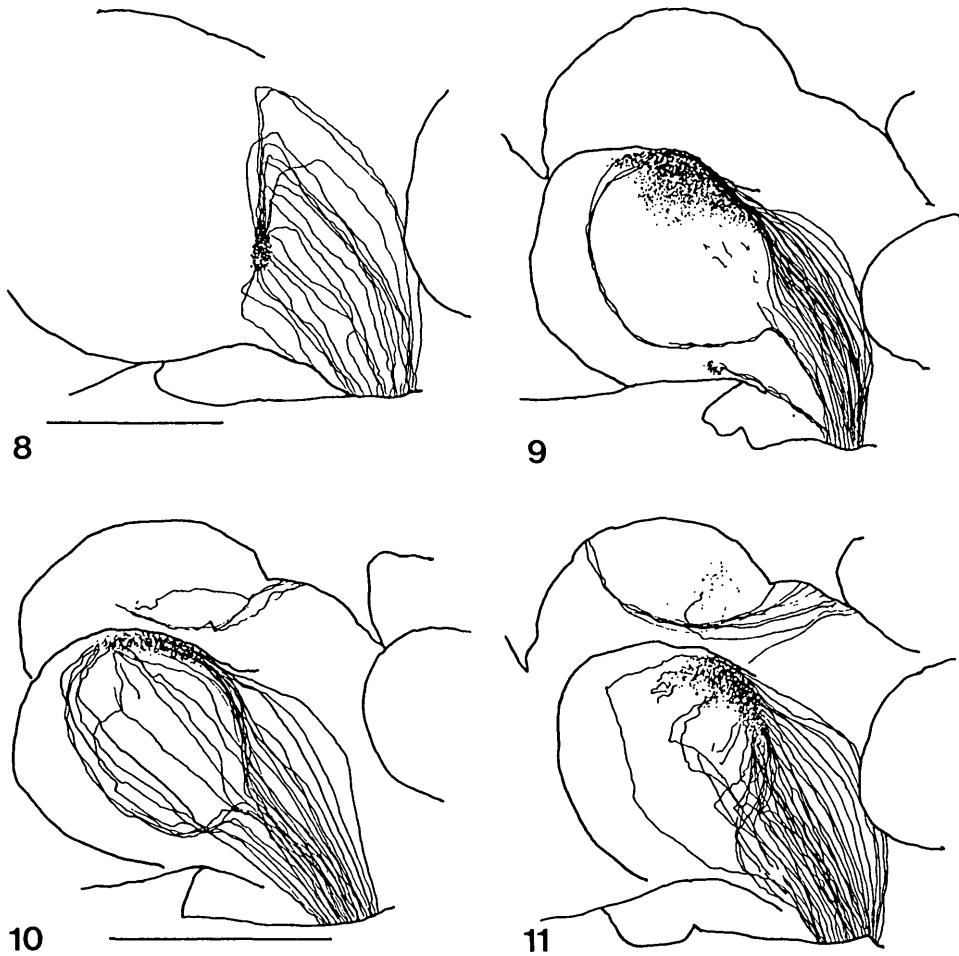


Fig. 8. Temporal fibres regenerating ipsilaterally from the right eye towards a virgin tectum. In this case the fibres approach the tectum widely dispersed in the tract and make abrupt turns at the tectal margin to attain their termination site in rostral tectum. Dorsolateral view, rostral to the right. Bar = 500 μ m.

Fig. 9. Normal control fill of ventral fibres from the left eye; most fibres pass via the anterior half of the tract into the medial brachium and terminate medially. Dorsolateral view, rostral to the right. Bar = 1 mm. (See Fig. 10.)

Fig. 10. Ventral fibres regenerating from the left eye to the contralateral tectum re-form a distinct medial brachium. The fibres are distributed across the width of the tract. Some travel laterally around the tectum and others pass across the tectal surface to terminate medially. Dorsolateral view, rostral to the right. Bar = 1 mm.

Fig. 11. Ventral fibres regenerating ipsilaterally towards a virgin right tectum. There is a very wide distribution in the tract. Although most fibres gather to pass around the rostral tectum to the medial brachium, a few fibres take abnormal paths over the tectal surface, then turn to grow directly into the medial tectum. Dorsolateral view, rostral to the right. Bar = 1 mm. (See Fig. 10.)

Ventral fibres regenerating ipsilaterally to a normally innervated tectum, or to a virgin tectum, may follow all three main paths to reach their destination in medial tectum: they may turn at the tectodiencephalic junction to join the medial brachium, they may pass onto the tectum for varying distances before turning (sometimes sharply) towards medial tectum, or they may pass right round lateral and caudal tectum to get to their destinations (Fig. 11; projection to a previously virgin tectum).

Nasal retinal fibres

Normal nasal fibres are dispersed across the entire width of the tract. At the tectodiencephalic junction the more caudally placed fibres turn abruptly to enter the lateral brachium whilst those in the more rostral regions of the tract turn into the medial brachium. Nasal fibres in both brachia travel around the tectum to caudal terminations (Fig. 12).

All groups of regenerating nasal fibres formed a distinct medial brachium. The tracts were otherwise mostly wide, disorderly and variable, with this trend more noticeable for ipsilateral and contralateral fibres when the other eye was absent. Contralaterally regenerating nasal fibres approached their caudal terminations by passing directly across the tectal surface as well as by travelling round the medial and lateral brachia (Fig. 13; other eye absent).

Nasal fibres regenerating ipsilaterally, along a normal tract, tended to be constrained into following the medial and, to a much lesser extent, the lateral brachia (Fig. 14). When regenerating towards a virgin tectum the nasal fibres may again form a marked medial brachium, but in contrast to the behaviour of such fibres growing up a normal tract they tend to pass also across the face of the tectum, particularly at the medial edge (Fig. 15).

Dorsal retinal fibres

Normal dorsal fibres are confined to the caudal part of the tract. At the rostrolateral tectum they make a characteristic abrupt caudal turn to enter the lateral brachium in which they travel to lateral tectum (Fig. 16). Even in a normal animal a fibre heading too far medially has been seen to make an acute turn as the tectum is approached.

The paths taken by regenerating dorsal fibres tended to be the most abnormal of any group of retinal fibres. In only one out of 17 cases did the fibre distribution in the tract resemble a normal dorsal fibre pathway.

In most cases, contralateral and ipsilateral, and in the presence or absence of the other eye, regenerating dorsal fibres approached their lateral tectal terminations by all three pathways; laterally, medially and across the tectum (Fig. 17; ipsilateral; other eye present). Not only did fibres pass all the way round the tectum to reach their terminal zones, in several cases fibres made sharp turns on the tectal surface (Fig. 18; ipsilateral projection to a previously virgin tectum). In this case, as also in the projections illustrated in Figs 7 and 11, examination of the

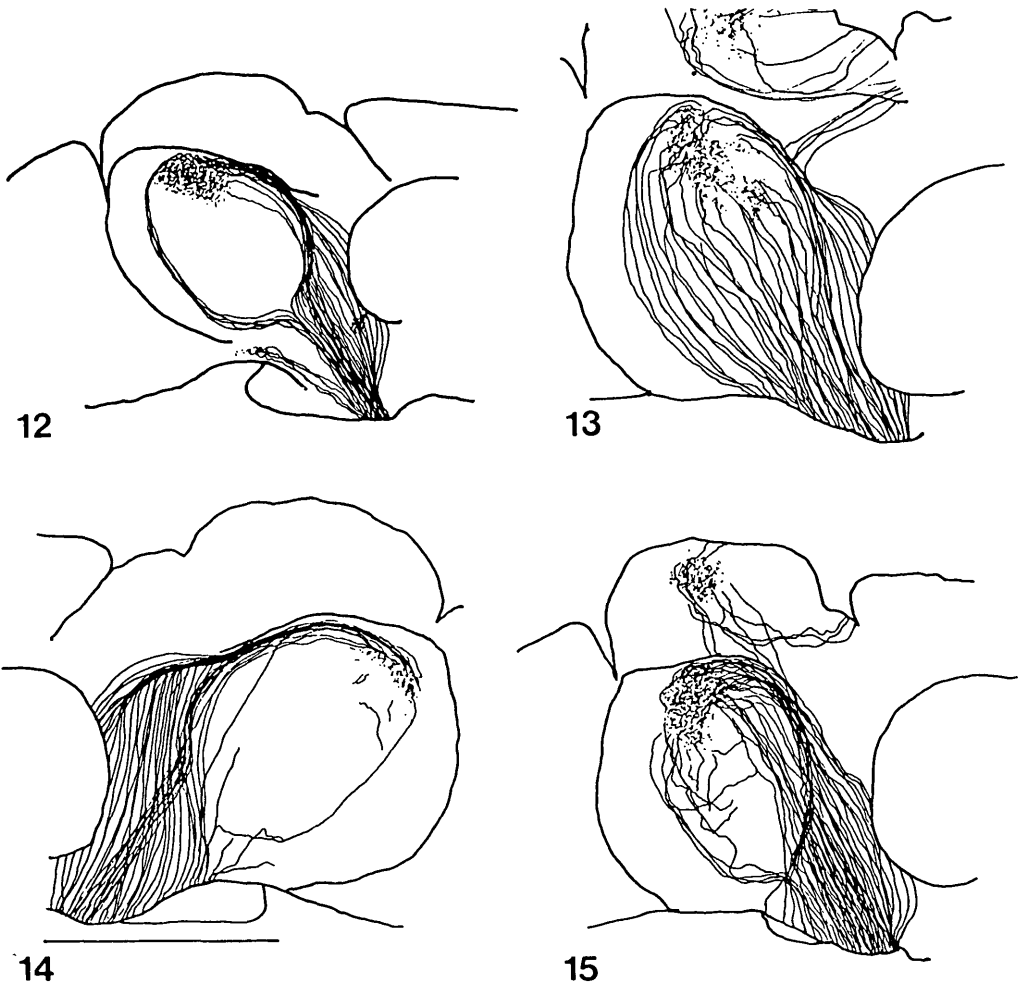


Fig. 12. Control fill of nasal fibres; the fibres are dispersed across the full width of the tract, pass laterally and medially around the tectum and terminate caudally. Dorsolateral view, rostral to the right. Bar = 1 mm. (See Fig. 14.)

Fig. 13. Nasal fibres regenerating contralaterally from the left eye in an animal with a virgin left tectum. Many fibres pass across the tectal surface to their caudal terminations. Those fibres in the anterior part of the tract are constrained to form a medial brachium leading to the caudal tectum. Dorsolateral view, rostral to the right. Bar = 1 mm. (See Fig. 14.)

Fig. 14. Nasal fibres regenerating ipsilaterally, along a normal tract from the other eye, are here distributed widely across the tract and are mostly gathered into a medial brachium. A few fibres also travel laterally and some pass over the tectal surface. Dorsolateral view, rostral to the left. Bar = 1 mm.

Fig. 15. Nasal fibres regenerating ipsilaterally towards a virgin tectum; again most fibres form a medial brachium to reach caudal tectum. However, many more fibres pass over the rostromedial tectum than in an ipsilateral projection along a normal tract. Dorsolateral view, rostral to the right. Bar = 1 mm. (See Fig. 14.)

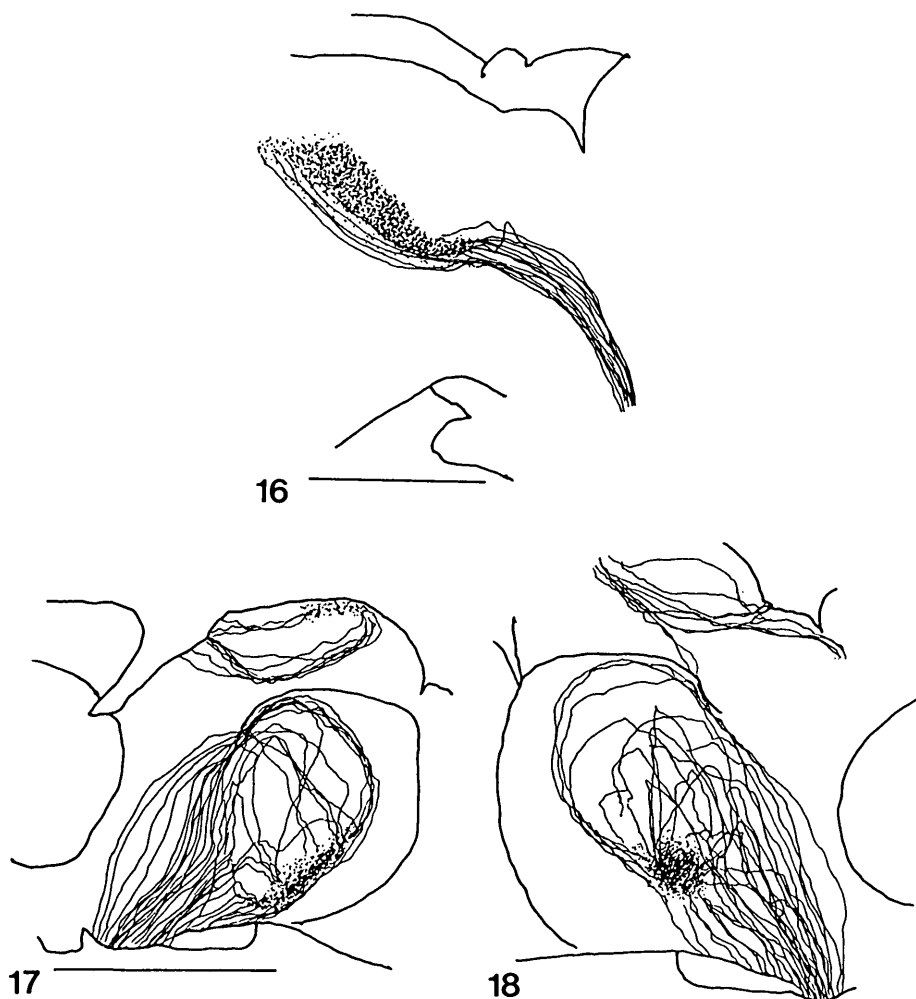


Fig. 16. Control fill of normal dorsal fibres from the left eye; the fibres generally grow in the caudal edge of the tract and turn caudally at the tectodiencephalic junction to form the lateral brachium. A single fibre which has grown too far medially in this projection has made an abrupt turn to gain entry to the lateral brachium. Dorsolateral view, rostral to the right. Bar = 500 μ m.

Fig. 17. Dorsal fibres regenerating ipsilaterally from the left eye up a tract containing intact fibres from the other eye. The distribution of the labelled fibres is highly abnormal. Many fibres pass over the medial tectum, then make turns and reach lateral tectal terminations. Those fibres which enter the medial brachium grow round the tectum before terminating laterally. Dorsolateral view, rostral to the left. Bar = 1 mm.

Fig. 18. Dorsal fibres regenerating ipsilaterally from the right eye to a virgin tectum. In this and other such cases the pathways taken by the labelled fibres are among the most abnormal seen in any category of fibres. Fibres medially placed in the tract grow right round the tectum while fibres which pass on to the tectum make turns, often sharply, to attain their lateral terminations. Dorsolateral view, rostral to the right. Bar = 1 mm.

whole-mount preparations at $\times 200$ magnification showed that the sudden alterations in direction taken by some fibres did not involve a change of depth from the tectal surface.

DISCUSSION

The validity of using a regenerating system to analyse mechanisms involved in the original formation of selective fibre connections (as proposed by Sperry, 1943; 1944; 1945; 1963) has been questioned (Gaze, 1978; Bohn & Stelzner, 1981*a,b,c*). The visual system in amphibians and fishes has, however, provided a generally accepted model for the study of regenerating optic nerve fibres. It now appears that this also may be questionable. At one month after metamorphosis, when many of these experiments have been performed in *Xenopus*, only 50% of the fibres found in the adult are present in the nerve (Wilson, 1971; Straznicky & Hiscock, 1984). Similarly, in most of the cases where the optic nerves of other juvenile lower vertebrates have been sectioned the fibre population emerging from the cut nerve has been a mixture of newly growing fibres from peripheral retina and regenerating fibres.

Gaze & Grant (1978) showed that regenerating optic nerve fibres in *Xenopus* tadpoles grew up the outer edge of the diencephalon rather than following their original paths, which, for fibres from central retina, ran deep. These authors proposed that regenerating fibres grew superficially merely because they were following a mechanism (unknown) which required *all* fibres to grow up the outer edge of the diencephalon. Support for this idea has come from further work (Bunt, 1982; Reh, Pitts & Constantine-Paton, 1983; Fawcett *et al.* 1984). Our present results show that, under these experimental conditions, regenerating fibres in recently postmetamorphic animals also tend to pass up the outer edge of the diencephalon, although this distribution may not be so precise as in tadpoles. It is certainly possible for regenerating fibres in juveniles to pass through the deeper parts of the diencephalon (Fawcett, *in press*). We think it likely that the degree of disorder in the chiasmatic part of the tract (i.e. the prevalence or otherwise of fibres running wild and deep) is related to the degree of injury caused by the lesion close to the chiasma and the resulting alteration in the way the nerve connects with the brain. In the experiments of Gaze & Grant (1978) the nerve section was extracranial and thus the nerve entry point into the diencephalon was to an extent protected from trauma. Comparable differences in the degree of order in the tract after intracranial or extracranial crush in *Hyla* have been reported by Humphrey (personal communication).

At the developmental stage when our experiments were performed the regions of the retina which were growing most rapidly were ventral retina and, to a lesser extent, temporal retina (Beach & Jacobson, 1979; Tay, Hiscock & Straznicky, 1982; Straznicky & Hiscock, 1984). Given the time course of degeneration and regeneration (for *Xenopus*, Ostberg & Norden, 1976; Reier & Webster, 1974; for

newt, Turner & Singer, 1974; for goldfish, Murray, 1976; Lanners & Grafstein, 1980), it is probable that the majority of the first fibres arising from the cut end of the optic nerve during the first 2–3 days, are new (and uncut) fibres originating in ventral or temporal retina. These non-regenerating fibres may grow superficially up the diencephalon (Fawcett *et al.* 1984) amongst the degenerating fibres of the previous projection. The majority of these degenerating fibres will be intact at this time, since 50% of the unmyelinated fibres in the frog may persist for 10 days after axotomy (Lázár, 1980; Matsumoto & Scalia, 1981). In fact, many thousands of unmyelinated fibres have been reported as persisting for 10 weeks, six weeks longer than the time taken to re-establish a functional, ordered projection (Lázár, 1980; Matsumoto & Scalia, 1981). Thus, in the short-term regenerates we may expect that many fibres will not yet have degenerated by the time regenerating fibres reach the tract; and in longer term regenerates there will be some extra uncut fibres, growing from the retinal margin, to mix with regenerated fibres in the tract.

Both the populations of persistent and of newly growing fibres are likely to exert a considerable influence on subsequently regenerating fibres. This will be particularly important if fibre–fibre interactions involving selective fasciculation (Nakai, 1960) are involved in optic nerve fibre guidance (Fawcett & Gaze, 1982). Furthermore, even if the few ipsilateral fibres which have been found to innervate a virgin tectum exert negligible influence, the intertectal projection must be considered as a potential organizing influence. Once on the tectum, the pattern of terminations of the regenerating fibres may also be influenced by the presence and continuing addition of newly growing, non-regenerating fibres (Willshaw & Gaze, in preparation).

In these experiments we have not been able to control the choice between ipsilateral or contralateral pathways made by regenerating fibres at the optic chiasma. In regeneration, although we know that the majority of fibres do not pass through the normal chiasmatic pathways (Gaze & Grant, 1978; Bohn & Stelzner, 1981*a,b,c*; Fawcett, personal communication), our insufficiently detailed knowledge of normal fibre trajectories through the region of the chiasma means that we cannot assess the relevance of the relative abnormality of these pathways, nor how they may vary according to experimental technique. Because extracellular HRP filling of the retina labels a varying number of cells we can only estimate roughly the relative proportion of the total fibre population which grows ipsilaterally or contralaterally. Furthermore, and of great significance to theories of fibre–fibre interaction based on positional differences in the ganglion cell population (Bonhoeffer & Huf, 1980; Trisler, Schneider & Nirenberg, 1981; Fawcett, 1981; Halfter, Claviez & Schwarz, 1981), we cannot assess the relative proportion of fibres from different regions of the retina which contribute to each of the ipsilateral and contralateral projections. The patterns of fibre behaviour identified in this study have been considered in the light of these variables.

In all cases the distribution of fibres regenerating in the diencephalic tract was wider than that of similar retinal fibres in controls, often extending to the caudal

edge of the forebrain, and sometimes to the caudal end of the diencephalon. The paths of all groups of regenerating optic fibres were abnormal but fibres from different parts of the retina behaved differently. There is a hierarchy of normality such that the most normal pathways are those of temporal fibres (Gaze & Fawcett, 1983); then come ventral fibres, nasal fibres and dorsal fibres. In addition the normality of the fibre pathways was related to the nature of the tract up which the fibres were growing. We observed that, in animals with the other eye present, regenerating fibre pathways in the optic tract may be less abnormal, both ipsilaterally and contralaterally, than they are in animals with the other eye absent.

It has been suggested that optic nerve fibres are able to recognize some form of position-dependent information on the surface of the tectum and to utilize this information during development to enable them to terminate retinotopically (Sperry, 1963; Bonhoeffer & Huf, 1982; Halfter, Newgreen, Sauter & Schwarz, 1983). Information which may serve this purpose has been found in the membranes of constituent cells of the tectum (Kern-Veits, personal communication), although its nature is not known. Regenerating fibres are also thought to be able to recognize fibre debris left on the tectal surface by a previous fibre projection (Schmidt, 1978) and it seems very likely that regenerating fibres can also recognize other, intact, optic fibres on the tectum (Schmidt, 1978; Gaze & Straznicky, 1980). If fibres regenerating within the diencephalic optic tracts were able to utilize similar information we would expect them to show patterns of behaviour relating to the nature of the degenerating fibre debris or to the nature of the intact fibres amongst which they have grown. The present findings offer some support for this idea and suggest that there is additionally a tendency for fibres to follow other fibres in a non-specific way.

What is the nature of the fibre substrate on which the regenerating fibres grow? Just prior to section of the optic nerve in recently metamorphosed *Xenopus*, the fibres in the superficial part of the contralateral optic tract will be predominantly from ventral retina with a bias towards temporal rather than nasal retina and with considerably fewer nasodorsal and dorsal retinal fibres (Fawcett *et al.* 1984). Regenerating fibres in the present experiments grow predominantly up the pial surface of the diencephalon and thus fibres regenerating in the presence of the other eye will grow contralaterally on the debris of this (mainly ventral) fibre population, and alongside the intact ipsilateral retinodiencephalic fibres from the other eye; both these substrates, if followed, would lead regenerating fibres into the medial brachium of the tract. Fibres regenerating ipsilaterally in the presence of the other eye will grow over a population of intact ventral retinal fibres and alongside the degenerating debris of the ipsilateral retinodiencephalic fibres. Again, if followed, both these substrates would lead regenerating fibres into the medial brachium. Fibres regenerating ipsilaterally when the other eye had been removed in embryonic life will find the degenerating debris of the ipsilateral retinodiencephalic fibres to act as a possible substrate guide but no intact ventral (or any other) optic fibres, while fibres regenerating contralaterally in such animals will grow along the debris of the previous projection of ventral fibres.

In all four experimental situations the possible non-specific guidance from degenerating or intact optic fibres will thus tend to lead regenerating fibres into the medial brachium.

The greater degree of pathway abnormalities seen among fibres regenerating from dorsal retina in comparison with fibres from ventral retina could be due to the differing access that these two types of fibres have to fibres of similar origin in the tract, or to the debris of such fibres. The changes in the rate of growth of different parts of the retina as the animal approaches and passes through metamorphosis will result in the superficial part of the medial brachium expanding during growth while the superficial part of the lateral brachium will get smaller. Thus ventral, temporal and nasal fibres will be better placed in relation to the tectum if they follow the fibre substrate whereas regenerating dorsal fibres, now present in the superficial tract in much higher proportions than would be normal for this stage of development, may miss the lateral brachium, which is relatively small in its superficial dimension, and not make the rather abrupt turn that is normally needed for fibres to enter it.

The relative frequency with which regenerating fibres from various parts of the retina sweep round the medial brachium is likely to be due to the fibres following the intact or degenerating substrate of previous fibres (Gaze & Fawcett, 1983), as well as the ipsilateral retinodiencephalic fibres when these are present.

The observation that small groups of labelled retinal fibres eventually get back to their correct tectal target zones, even on a virgin tectum, does not allow us to conclude that the fibres are responding to localized markers associated with tectal cells. Once on the tectum it is quite possible that fibres are able to sort into retinotopic order with reference to each other. In all of our preparations the labelled fibres form only a small proportion of the total fibre population regenerating from that eye, so we cannot exclude fibre-fibre interactions as a mechanism for establishing an ordered map. To determine the role of other fibres in permitting a small group of fibres from one part of the retina to terminate at a particular position on the tectum it will be necessary to regenerate these fibres in the absence of others.

There are three main types of route followed by fibres on the tectum in these experiments. 1) Round one or both brachia, thus encircling the tectum to get to the terminal zone. It is surprising that ventral fibres can pass all the way round the lateral brachium past caudal tectum and back along the medial pathway to reach a medial terminal zone; and conversely for dorsal fibres. Even more remarkable is the finding that temporal fibres can run round three sides of the tectum and return to terminate eventually in the rostral part. 2) Running right across the tectal surface; and 3) Passing on to the tectal surface and then, more or less abruptly, changing course towards the terminal zone. In the developing chick visual system, similar abruptly turning axons have been observed on the tectum after intra-ocular injections of an antibody to the neural cell adhesion molecule (Thanos, Bonhoeffer & Rutishauser, 1984). In the present experiments, in some cases fibres were seen to grow past their termination sites for 500 μm or so before making

abrupt, sometimes looping, turns which bring them back to the termination zone. This ability of fibres to grow past the right region, and then to double back to reach it, suggests that if the fibres are searching for their correct tectal markers by the production and retraction of terminal branches, then either the recognition mechanism must be very weak or the signal instructing a passing growth cone to terminate is incorrectly communicated. These sudden reversals of fibre direction seem not to be due to the fibres diving, or climbing, into a different tectal layer.

These observations do not tell us about the intimate mechanisms responsible for the homing of regenerating fibres to their target zones. Two conclusions, however, emerge very clearly. The first is that regenerating optic fibres in *Xenopus* do not reform a normal distribution in the optic tract. The fibre distribution is always abnormal and often grossly so. The second is that, however the targetting mechanism works, it does so on the tectum and not in the tract. Fig. 8 shows that temporal fibres, widely dispersed across a tract leading to a virgin tectum, persist in their abnormal distribution until they reach the margin of the tectum, whereupon they all converge on a small tectal termination zone. The tectal environment must differ greatly from that of the tract, since the behaviour of fibres changes dramatically as they move from the tract onto the tectum.

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