PATH SELECTION IN THE REGENERATION OF THE TELEOST OPTIC NERVE

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SUMMARY

The work described in this thesis is an attempt to examine the processes which, following section of the goldfish optic nerve, lead regenerating optic fibres to their original, precisely retinotopic, terminal sites in the optic tectum; and thereby to throw light on the nature of the "chemospecific" labels which must be considered characteristic of individual optic fibres and tectal sites if such highly selective regeneration is to be explained.

In the frog it has been suggested that having initially regenerated into the tectum in random array fibre terminals are ordered first along the transverse and later in the longitudinal tectal axis. In the present experiments comparable events were not seen among fish regenerated for varying lengths of time; the earliest detectable regenerated electrical responses (at 22 days after nerve crush) arose from small and normally located visual fields even though they were poor in quality. The subsequent gradual improvement in the responses (by about 50 days they were "normal") supports the general conclusion that in the fish the earliest signals are due to immature but precisely arranged terminal arborizations. Measurement of multiunit receptive field diameters in fish regenerated for less than 70 days indicated an abnormal degree of scrambling of fibre terminals (equivalent to an average random error of 60 μ in terminal position) but after nine months diameters had returned to normal, presumably due to long-term adjustment.

A similar but very much more extensive early scrambling of terminals may underlie Pattern 1 in the frog; if so it is likely that as in the fish fibres destined for one tectal site undergo progressive and gradual long-term refinement rather than two-stage reorganisation. Regenerating fibres in the fish may sometimes make systematic errors of termination; Pattern 2 in the frog may also represent a stable end-point of regeneration.

In normal fish there is complete segregation of optic fibres into either medial or lateral divisions of the optic tract according to whether they project to the dorsal or ventrolateral tectum but during regeneration some fibres that would normally pass exclusively through the medial division have been found to enter the lateral division. These fibres reach their normal terminal sites in spite of having to negotiate novel and longer routes to them. The majority of fibres follow their normal and separate routes through the tract and across the tectum; although this recovery by fibres of their original paths suggests deliberate path selection it is not, in view of the above result, a prerequisite for orderly termination.

Path specificity of regenerated fibres could be the result of selective degeneration of fibres occupying inappropriate paths following initial random growth. The present data is against this possibility since it shows that fibres which happen to terminate at the same site after following widely different routes can both survive.

Electronmicroscopically the growth cone region of the regenerating optic fibre has been identified on the basis of characteristic "wispy" membrane profiles in the cytoplasm; there was no evidence of filopodial extensions of the cone which might play an exploratory role in growth.

Regenerating fibres were seen to advance steadily and in groups and the chance of later fibre realignment was thought to be greatly curtailed by the connective tissue which rapidly forms around them. Degeneration of new fibre processes was only rarely seen.

Nine months after regeneration the central nerve stump contained about 50% of its normal compliment of myelinated axons (which in structure and diameter range appeared to be fully mature) and for each of these there were two unmyelinated fibres (which are almost entirely absent in typical regions of normal nerves), although different nerves varied. The myelinated fibres might be those fibre branches which have successfully formed functional connections in the tectum.

In the normal frog optic fibres approach the tectum in near-random retinotopic array; an electronmicroscopic study of the course of a small degenerating bundle of optic fibres showed considerable scrambling of fibres in the frogs' nerve but perfect retinotopic order in the fish. A small bundle of fibres regenerating through an otherwise normal nerve maintained its retinotopic arrangement throughout the nerve in the fish.

In confirmation of data in the literature it has been found that fibres may form terminals at new sites within the longitudinal tectal axis but, under comparable experimental conditions, do not do so across the tectum. In the course of regeneration following removal of the half-retina normally projecting to the rostral tectum, the remaining fibres form retinotopic connections spaced out along the whole tectum or, if regenerated into a rostral half-tectum, will shift <u>en bloc</u> and form retinotopic terminals within the noncorresponding area of tectum remaining.

A striking correlation has emerged in the present study between the direction of growth of fibres entering the tectum (which is predominantly

rostrocaudal in normal and identical in regenerated animals) and the above described behaviour of fibre terminals. The regeneration process is thought to involve two separate parts;

1). Active and continuous selection by the advancing fibre of the route most appropriate for its designated target. Optic fibres were found to emerge from the optic tract onto the surface of the tectum with their transverse retinotopic order already established and then to pursue independent and straight courses caudally. This pattern did not vary in any of the experimental conditions described above; it may in some way account for the fact that regenerating terminals are not free to shift transversely on the tectum.

2). The fibres destined for a particular narrow rostrocaudal strip of tectal sites occupy a single compact bundle; as a bundle passes caudally fibres have only to peel off in retinotopic sequence and to terminate growth in order to restore the final complete projection. Without positing any change in the normal, unique affinity of individual fibres for their original terminal sites in the tectum, the formation of terminations at more rostral sites may be the result of a tendency for fibres to cease growth prematurely at intervening sites along their normal growth paths when these sites are vacated as a result of retinal ablation.

CONTENTS

INTRODUCTION

> What mysterious forces precede the appearance of the processes (of neurones), promote their growth and ramification, stimulate the corresponding migration of the cells and fibres in predetermined directions, as if in obedience to a skilfully arranged architectural plan and finally establish those protoplasmic kisses, the intercellular articulations, which seem to constitute the final ecstasy of an epic love story.
>
> (Cajal, 1937, p.373).

The work to be described in this thesis is founded on a compelling but untested assumption: that the functional role of neurones is to a large extent dictated by the pattern of their particular interconnections. For the study of the mechanisms underlying the formation of patterned neuronal connections, the regeneration of the retinotectal projection in lower vertebrates is not merely one of the most highly differentiated phenomena available; it is also most amenable to experimental investigation.

It was largely as a result of his work on this system that Sperry proposed the general principle of "chemospecifity" of nerve cells. He argued that individual cells in the nervous system must be characteristically "labelled" and that any connections made between them are the result of complimentarity of specificity. Only in these terms did it seem possible to account for the precision with which otherwise indistinguishable optic nerve fibres come to form terminals on the tectum in exact conformity with their retinal origins.

Sperry's theories have now received fairly general acceptance although the existence of chemospecifities is still strictly an inference. While almost nothing is known of the nature of the specificities there are two views about their arrangement within the retina and tectum. According to one, labels are attached discretely to individual cells of the retina and tectum in a point to point ("patchwork quilt") distribution, while the other view suggests that cells are only specified according to their relative positions within the two arrays of cells and the specifying factors have "gradient" distributions.

These questions are closely related to the problem of cell differentiation in general. According to Wolpert's (1969) appraisal all any cell requires in order to differentiate at any given time in a way consistent with its position within the three-dimensional array of cells that makes up the embryo, is information about its relative position. Just as Sperry introduced the notion of "chemospecificity", Wolpert hypothesises "positional information"; Wolpert's arguments apply equally well to the orderliness of growth of optic nerve fibres.

It is between explanations such as these that we should hope eventually to be able to decide.

Part 1:

The evidence for the orderly regeneration of the optic projection

Following early evidence that visual function might return after section of the optic nerve in some species (Koppanyi, 1923; Matthey, 1925; Stone, 1930) Sperry examined the phenomenon in urodeles (1943), in anurans (1944) and in various teleosts (1948). The basic anatomy of the teleost visual pathways is shown in Fig. 1A. After the nerve has been cut the optic axons degenerate centrally and are replaced by new axonal processes

from the peripheral nerve stump. Visual responsiveness, including the normal ability to locate small lures, was recovered. Sperry (1944) described the scotomata resulting from lesions of the optic tectum which showed that after regeneration the normal retinotopic organisation of the fibre terminals is restored.

If electrodes of the appropriate characteristics (Gesteland, Howland, Lettvin & Pitts, 1959) are placed on the surface of the tectum it is possible to record unitary or multiunit discharge trains; these responses can be evoked by stimuli within the receptive fields of single retinal ganglion cells and are identical to those of fibres of the optic nerve (Maturana, Lettvin, McCulloch & Pitts, 1960).

With this technique it is a relatively simple matter to plot out for a series of positions on the tectum, the corresponding retinal ganglion cells (defined by the positions of their receptive fields) whose fibre terminals are distributed in an orderly retinotopic manner among the recording positions. Gaze (1959), Maturana, Lettvin, McCulloch & Pitts (1959) and Gaze & Jacobson (1963) mapped out the retinotectal projection point by point over the tectal surface in anurans and showed that it was systematically reinstated after regeneration. A similar map was obtained in normal goldfish (Jacobson & Gaze, 1964) and after regeneration (Jacobson & Gaze, 1965; Gaze & Sharma, 1970).

There is no reason to doubt that the localised electrical responses recorded superficially on the tectum originate from the terminals of optic axons and the many arguments in favour of this interpretation can be found in Maturana et al. (1960), Gaze & Jacobson (1963) and Gaze & Sharma (1970). Superficial tectal unit responses have a latency to electrical stimulation of the optic nerve of between one and three milliseconds (Sutterlin &

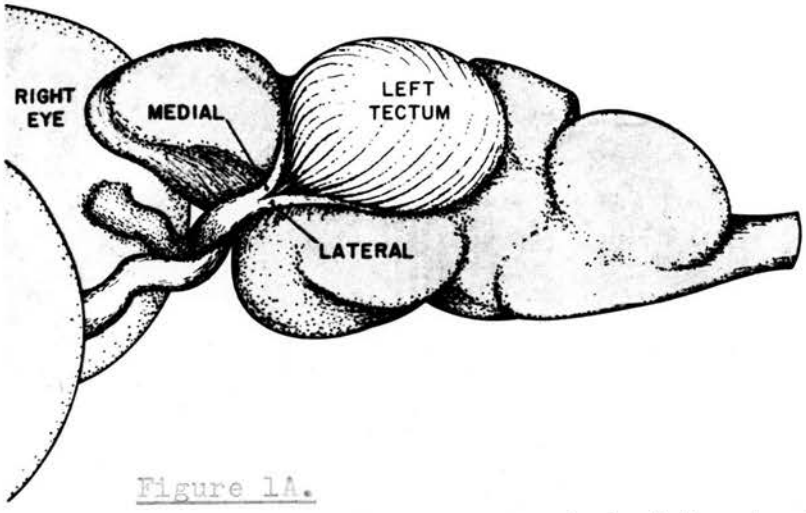
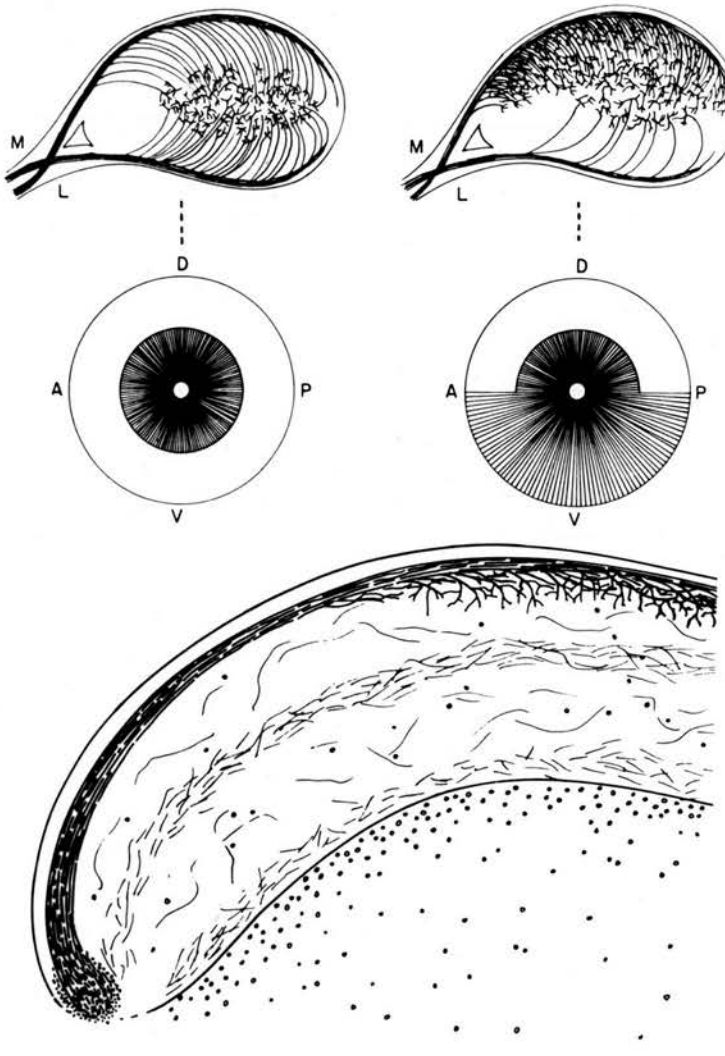


Figure 1A.

Schematic drawing of goldfish optic system showing division of main optic tract into medial and lateral bundles and their relations with midbrain tectum.



Type of regeneration patterns obtained with removal of peripheral retina. Below: Enlarged detail of the result as viewed in a transverse section of tectum.

Figure 1B. (from Attardi & Sperry, 1963)

Prosser, 1970) which corresponds with the range of conduction delay to be expected for goldfish optic fibres on the basis of their conduction velocities (between 5 and 10 m/sec, Konishi, 1960). These units followed high frequency driving whereas all other units encountered had a latency of more than 6 msecs (Sutterlin et al., 1970) and saturated at low frequencies (Konishi, 1960) and are therefore considered to be postsynaptic.

Anatomical evidence was provided by Attardi & Sperry (1963) (see Fig.1B, previous page) who combined ablation of half or circumferential portions of the retina with section of the goldfish optic nerve and found, using Bodian Protargol preparations, that regenerated fibres invariably recover their previous routes as well as terminations in the brain even when this means skipping areas deprived of fibres as a result of the retinal lesions. Jacobson et al. (1965) confirmed electrophysiologically that fibres terminate only at their original sites, during regeneration of only selected parts of the optic nerve or following ablation of parts of the tectum.

In his study of anurans Sperry (1944) showed that if the eye was rotated by 180° when the optic nerve was cut visual localisation behaviour was accordingly and permanently reversed, in exactly the same way as it is after rotating the eye while leaving the retinotectal connections intact. Hence it could be concluded that regenerating fibres do indeed recover their normal arrangement in the brain and this remarkable finding also showed that functional outcome or learning play no part in the process. As a result of the rotation of the eye and its attached peripheral nerve stump (Sperry, 1951) regenerating fibres are initially confronted with inappropriate central channels; that fibres recover their normal terminals in these conditions indicates that an

active search process is involved.

Summarizing the end result of normal regeneration in the fish, the nasal pole of the field (temporal retinal pole) projects to the rostral pole of the tectum and the temporal (nasal retinal pole) to the caudal tectal pole; these points define the "long" axis of the tectum. Only the dorsal half of the tectum, corresponding roughly to the area normally supplied by the medial division of the optic tract (the medial division "territory") is directly accessible for mapping (see Fig.1A). The superior pole of the field (ventral retinal pole) projects to the medial edge of the dorsal tectum while its lateral edge represents the centre of the field. This defines the "transverse" axis of the tectum; points arranged in this axis ("rows" of points) are conventionally joined by lines in retinotectal projection diagrams.

It will greatly facilitate the reader's task if, at this point, I summarize the principal problems to be dealt with in this Introduction and the approach which will then be taken in the experiments to be described thereafter.

Of the possible mechanisms by which regenerating optic fibres might achieve their precise arrangement of terminals in the tectum, one is an initial random ingrowth of fibres followed by the selective survival of those which happen in this way to reach the correct terminal sites. The early behaviour of fibres will be examined in the present experiments by mapping animals at various intervals after nerve section starting at the time of the earliest regenerated electrical responses, and by estimating the precision with which terminal sites are chosen from the size of the

receptive fields of the recorded multiunit responses. An examination of the routes taken by regenerated fibres to their terminals permits inferences as to the mode of initial ingrowth of the fibres.

The major alternative mechanism that has been suggested (see Part 3 of this Introduction) is that fibres reach their targets by actively selecting the appropriate routes to them. While there is considerable evidence that fibres do pursue very precise and direct paths, it has yet to be finally established that this too is not the result of selective degeneration of fibres initially taking inappropriate paths. However, at several points the present results add support to the hypothesis of primary path selection. For example, an attempt has been made to identify the actively growing tips of regenerating fibres and there was little in these observations suggestive of exploratory processes of the growth cone region or of secondary degeneration. As is argued in Part 4 of the Introduction, it is unlikely that it is the function of the growth cone to test out the environment ahead of the advancing axon.

The problem raised in the final section of the Introduction concerns certain major discrepancies in the choice by fibres of their terminal sites in the tectum; under certain conditions the optic projection regenerates in a way which suggests that fibres are specified not with reference to one tectal site but according to their relative positions within the existing retina; this has led to the "gradient" model of retinotectal specificity. The evidence presented in this thesis will confirm that this only applies to the long axis of the tectum; terminals invariably retain their normal positions along the transverse tectal axis. In the Conclusion section of this thesis an attempt is made to fit this data into a unified account of the mode of growth of optic fibres into the tectum.

Part 2:

Possible mechanisms of orderly regeneration

Long before it became necessary to think of chemospecificity evidence was accumulating on the physico-chemical forces which can influence the growth of nerve fibres (Weiss, 1955). It is well established (Harrison, 1910) that fibres require a mechanical substrate. Weiss (1934) showed that under ideal conditions the entire behaviour of fibres could be accounted for by the mechanical constraints existing.

Weiss & Taylor (1944) showed that a rat peripheral nerve forced to regenerate into the base of a Y-shaped section of artery in vivo failed to select the arm containing, among other tissues tested, its own cut distal stump. This experiment appears to rule out the theory, originally proposed by Forssman (1898) to account for the growth of cut peripheral nerve fibres into experimentally deflected distal nerve stumps, that fibres can be "neurotropically" attracted from a distance. Harrison (1910) and Cajal (1893; 1928) held similar views to Forssman, the latter referring to "chemotactic" factors to account for the directed growth of nerve fibres in development and in regeneration.

Arguing that under in vivo conditions and given identical mechanical conditions all fibres have equal chances of reaching a given goal in the brain or peripherally in the sensory or motor systems, Weiss undertook a long series of experiments (reviewed by Hughes, 1968; Gaze, 1970) to show how in that case muscles could acquire their appropriate patterns of contraction in functional coordination with other muscles. In experiments on amphibians he found that muscular coordination was maintained when the fibres which would normally have innervated the muscles were deliberately prevented from doing so - the "homologous response".

A mutual explanation, in terms of the principle of "modulation" (Weiss, 1936; Sperry, 1951) was that the motoneurones whose fibres do come to innervate a given muscle, acquire their incoming dendritic connections in a manner determined by a "modulating" signal from that muscle. The weakness of these experiments was that there was no direct attempt to check, after the homologous response had appeared, whether muscles had not actually become reinnervated by some of their original nerve fibres. In those experiments (Sperry & Arora, 1965; Mark, 1965; Marotte & Mark, 1970) where there is clear evidence of changed neuromuscular connections there is no evidence of modulation. The most recent evidence indicates that the normal timing of a muscle's contraction depends on its having its usual nerve supply; this is true for fish and for mammals provided the effects of learning have been excluded (Sperry, 1941; 1945).

By 1941 and in the face of the difficulty for his theory of accounting for the fact that during normal development nerve fibres do acquire highly individual and invariable patterns of connections, Weiss (1941) began to refer to "selective contact guidance" which emphasizes the responsiveness of individual fibres to specific aspects of the local environment.

The whole position was radically altered by Sperry's demonstration of systematic regeneration of the optic nerve and his formulation of the hypothesis of neural specificity (Sperry, 1943; 1951; 1963; 1965). Originally Sperry was concerned only with the specificity of the fibre terminal for its target site; regardless of the way in which the fibre found the target, both had to possess unique specificities.

Perhaps the nearest approach which has been made to actually isolating them was taken by Székely (1957) and Jacobson (1968). By reversing the dorsoventral and nasotemporal orientations of amphibian eyecups independently and at different stages of development they were able to pinpoint two separate events in which the prospective ganglion cells acquired from their surroundings the characteristics which later determine the rostrocaudal and mediolateral sequences of their axonal terminations in the tectum. So it seems at first sight probable that the "positional information" which is actively transferred to the undifferentiated ganglion cells and which is subsequently disseminated among new cells as they are added to the growing retina, may be no more nor less than the chemospecificity envisaged by Sperry. There is some evidence that the tectum may acquire its matching specificities prior to and independently of the arrival of optic fibres (DeLong & Coulombre, 1965, 1968) and that central nervous tissue is specified similarly at different times in different axes (Roach, 1945).

However, an important extension of Sperry's concepts seems to be called for when one considers how fibres grow to their specific terminal sites. Is fibre growth also selective? It is very probable that the elucidation of this question will itself add to our understanding of the nature and the distribution of the specifying factors. It is for instance possible that the differing functional roles of the growing fibre and the static tectal site in the whole process will entail different forms of neurospecific differentiation in the two.

According to one view fibres grow at random across the tectum in the first instance. If they happen to reach their appropriate terminations in this way they remain fixed while other fibres withdraw from

inappropriate sites and continue to search by trial and error. It has been established that fibres can in certain situations (though not invariably - see Miledi (1963) for an exception) compete for their terminations and inactivate inappropriate fibres (Guth & Bernstein, 1961; Sperry et al., 1965; Marotte et al., 1970). The best evidence that random growth occurs during the regeneration of the optic nerve comes from Gaze & Jacobson (1963) who found in frogs that at the earliest stages at which electrical responses could be obtained after nerve section, isolated areas of peripheral retina projected throughout the tectal surface (see Appendix: Fig.A1).

There are at least three distinct possible mechanisms whereby fibres could achieve orderly termination by trial and error mechanisms. First, the total population of fibres could grow into the tectum entirely at random and thereafter only those ganglion cells whose axons have successfully terminated, survive. Regeneration would then result in a reduction in the number of ganglion cells. That such a mechanism may be of importance in the embryogenesis of nerve connections is suggested by the high turnover of neurones at critical stages in development (Glücksmann, 1951; Prestige, 1965; Hughes, 1968).

A second possibility is that the earliest fibres to enter the tectum, "path-finder fibres" (Harrison, 1910), connect at random and signal in some way as yet unknown the appropriate paths to be followed by the waiting majority of fibres. This is Weiss's (1941) theory of "selective fasciculation". As Cajal (1928, p.382) points out, the "pioneer" fibres which have frequently been observed in developing and regenerating nerve paths, may be no more than precocious fibres. To attempt to draw a functional distinction between early and later fibres

in this way would appear to be unwarranted and artificial. Since every fibre in the optic nerve has its own unique destination it is difficult to see how a limited number of exploratory fibres could convey sufficient information back to provide individual distinguishing signals for the remainder. It should also be mentioned that in ontogenesis the earliest outgrowing fibres seem to have already selected their appropriate paths (Harrison, 1910; Taylor, 1943).

The third possibility is that each fibre puts out a number of exploratory processes and that competitive mechanisms select between these to achieve the most appropriate final pattern of connections. The evidence for such a mechanism will be considered in detail below (p.22 et seq.).

An entirely different approach is, however, suggested by one finding by Attardi & Sperry (1963). They found that fibres tended to select appropriately during regeneration between the two branches of the optic tract which lead separately to the dorsal and lateral tectal surfaces (see Figs.1A, 1B). This raises the possibility that chemospecific labels are not confined to terminal sites in the tectum but that they are available to guide fibre at each potential choice point along the path to the tectum. Indeed the labels which appear to serve as signals to terminate for some fibres at one position on the tectum may also serve to orientate other fibres as they grow further into the tectum. Such a process of active selective fibre growth on the basis of local cues will be referred to as "path selectivity". Before going on to consider the detailed evidence for these theories it must be emphasized that in order to prove that active path selection is the operative mechanism it is not sufficient to show that fibres follow

specific routes when they are examined when regeneration is complete. Such evidence does not exclude the possibility that fibres had initially grown at random and that fibres that had made wrong choices had later degenerated. The end result would be indistinguishable; the attainment of orderly segregation of regenerated fibres will be referred to as "path specificity" regardless of the way in which it was brought about. It is also necessary to distinguish between true "path selectivity" and selective attraction of specific fibres at a distance by their different targets lying at the ends of the alternative paths. For a full account of the processes involved it is necessary to establish the "fineness of grain" of the cues provided for the growing fibre by its environment and the distances over which these cues can influence a fibre.

Part 3

The evidence for and against active pathway selection

Experiments performed on embryonic and lower vertebrate animals have yielded results in which true and active path selection seems to be operating. Harrison (1924), Yntema (1934) and Taylor (1944) removed either dorsal root ganglia or spinal motoneurone anlagen in larval amphibia at the time of outgrowth of peripheral nerves and found later that sensory fibres tend to fill only sensory branches of the peripheral nerves while motor fibres were restricted to motor trunks.

Castro (1963) removed segmental spinal nerve roots in the developing chick and obtained evidence that the remainder grew out into the peripheral nerve plexus in the normal manner without filling the empty channels.

Hamberger (1962) used a similar approach to show specificity in the innervation of the three peripheral trunks of the trigeminal nerve in the developing chick.

Hooker (1930) reported that fibres descending on the spinal cord tend to choose their original fascicles when regenerating into spinal cord segments rotated about the rostrocaudal axis in amphibians.

None of these studies produced evidence as to whether the path specificity was primary or whether it was the result of secondary degeneration following on initial random growth. In the following studies, however, control experiments were performed in order to show that <u>if</u> fibres enter the wrong channel they <u>can</u> successfully innervate the wrong terminations; therefore any initial random connections might have been expected to survive intact. Feng, Wu & Yang (1965) showed that although fibres destined for the fast and slow components of the chick latissimus dorsi muscle <u>could</u> connect with either, they regenerate specifically into the correct branch of their common nerve. Mark (1965) using the nerve to the pectoral fin, and Sperry et al. (1965) the nerves to the extraocular muscles in fish, showed that orderly and normal function was recovered after crush of the mixed nerve trunks while disordered function was the result after individually crossing muscle nerves.

Although this evidence reinforces the idea that the fibres actively select their correct paths in the course of regeneration of the mixed trunk, it is clear (Guth et al., 1961; Sperry et al., 1965; Marotte et al., 1970) that if foreign and matching fibres happened initially to reach a muscle together they could actively compete for functional connections; it has been shown that foreign fibres can be inactivated

after forming functional connections by the later entry of the native fibres. It is therefore a further possibility that where fibres are found to follow specific paths after regeneration, this could arise after initial random ingrowth (and despite the ability of all fibres to establish permanent, functional connections along each path) as a result of competition between fibres at the terminals leading to retrograde degeneration of foreign fibres.

On the other hand it seems most improbable that this mechanism could explain the choice, simple though it might appear, of the correct division of the optic tract by regenerating optic fibres (Attardi et al., 1963). In the first place Attardi & Sperry showed that fibres continue to choose their own division in the absence of fibres destined for the other: this excludes the possibility that direct competition between the sets of fibres destined for the two halves of the tectum is involved either within the tectum or earlier in the pathway. The only remaining, plausible, alternative is that fibres terminating at identical positions on the tectum could compete on the basis of the routes that they had taken to them.

An entirely different line of evidence in favour of path selectivity comes from the consideration of the effects of displacing fibres far from the channels which they would normally follow.

In the above experiments it was always necessary to assume that individual fibres had no preferential access to their targets at the outset which could explain their successful recovery of their terminals. It is usually assumed that as a result of the disarrangement of fibres which occurs at the site of the nerve lesion all fibres have equal initial chances of reaching any given target. By rotating the peripher-

optic nerve stump Sperry (1944) made quite certain that the recovery of orderly terminals was not the result of passive ingrowth of the fibres arranged retinotopically in the stump, because the fibres were initially confronted with parts of the central stump leading to the wrong tectal areas. In other experiments involving eye transplantation (Stone, 1930) there is evidence that the central stump had largely disappeared by the time regenerating fibres were reaching it. In experiments involving actual displacement of nerves the likelihood that regenerating fibres reach their targets by chance rather than by active guidance can be systematically varied according to the amount of displacement.

An example of such a displacement experiment is Sperry & Miner's (1949), where the central processes of the trigeminal ganglion in Triturus regenerated successfully to their central terminations when forced to enter the brain by way of the central stump of the cut facial nerve. In a similar experiment Gaze (1960) and Hibbard (1967) forced one optic nerve in Xenopus tadpoles to regenerate centrally through the oculomotor nerve trunk. Having entered the brain stem and decussated, the majority of optic fibres turned rostrally and followed a more or less direct route to the lateral border of the tectum and the chiasma, a distance of about 500 microns. The fibres then reached their normal retinotopic terminations (Gaze, 1960). Hibbard (1959) reports the case of an optic nerve from an eye transplanted into the cyclopian position in a tadpole, finding its way to the tectum of the host animal.

DeLong & Coulombre (1968) transplanted pieces of retina from known positions in the retina onto the surface of the tectum in chicks at an age prior to the arrival of the optic fibres and removed the contralateral eye. Later histology showed that fibres from the transplants

had grown straight towards the tectal positions which they would normally have supplied and that there was no evidence for any initial random outgrowth of fibres. The ability of fibres to approach their tectal positions from unaccustomed directions was also demonstrated in the regeneration of fibres to their original terminal sites in rotated sections of tectum in fish (Sharma, 1967), by forcing fibres to approach the tectum through the wrong division of the optic tract (Arora, 1963) and by placing tantalum barriers across the normal paths of fibres entering the tectum (Sperry & Hibbard, 1968).

It appears that peripheral nerve fibres can do the same thing; Sperry et al. (1965) noticed a "marked tendency" for displaced oculomotor nerves in Astronotus ocellatus to avoid the most accessible extraocular muscles in favour of growing by a longer and novel course back to their own muscles. The same occurs in mammals after wide deflection of a central stump of a peripheral nerve; fibres regenerate directly towards their peripheral, undeflected peripheral stumps through the intervening connective tissue (Cajal, 1928; Sunderland, 1969).

Unlikely though it may be it remains a possibility that in all these examples path specificity was achieved by way of initial random ingrowth. Where fibres are regenerating along their accustomed routes the need for initial exploration by random processes may be limited by gross mechanical factors and perhaps by the existence of subsidiary, intermediate targets lying along the path to the fibres' final targets. But in the case of displaced nerves, neither of these factors could apply; the fact that displaced fibres can display such "goal directed" behaviour, under experimental conditions which enormously increase the number of alternative and incorrect routes that they might have followed, is strong evidence for primary path selectivity.

Returning to the visual system, there is some preliminary evidence against the suggestion that fibres terminating at identical positions on the tectum could compete on the basis of the routes that they had taken to them. Arora (reported by Sperry, 1965) showed histologically that lateral division fibres transplanted into the contralateral medial division are able to reach their corresponding positions on the contralateral tectum. This result indicates that fibres following different routes (in this case medial and lateral divisions of different sides) can coexist at the same terminal site. This finding is of interest for other reasons too. It is unusual (as has been demonstrated in the case of neuromuscular connections (Miledi, 1963)) for terminal sites to become innervated, as they appear to have done here, by more than the usual compliment of fibres. A similar effect occurs in the case of frogs showing the anomalous form of regeneration designated Pattern 4 (Gaze & Jacobson, 1963). Here one tectum is supplied by direct projections from both eyes due to the indiscriminate growth of fibres from the regenerating optic nerve into the ipsilateral as well as the contralateral optic tract.

In 1962 Arora & Sperry reported the results of cross-uniting the medial and lateral divisions of the optic tract. The "bulk of the fibres" avoided entering the foreign division towards which they had been transplanted. In contrast to their behaviour when transplanted deep into the opposite division (Arora, 1963) they now adjusted their growth so as to re-enter their own division. While it must be borne in mind that this experiment is of considerable technical difficulty it is also the first direct evidence that fibres can respond to specific properties of the tissue associated with their paths in the

divisions of the tract.

There are of course many situations in which fibres fail to show any specific reaction towards the tissues they are growing through. Fibres commonly fail, for example, to distinguish between paired structures in bilaterally symmetrical situations. One example, frog Pattern 4 regeneration, has just been mentioned. The same failure of regenerating optic fibres to discriminate between the two tecta was seen by Sperry (1951, in frogs), Gaze, Keating & Straznicky (1970, in Xenopus) and by Ferreira-Berrutti (1951, in chicks). In the visual system (Szentagothai & Székely, 1956) and in the outgrowth of Mauthner cell axons (Hibbard, 1965) the evidence is that bilateral decussation is determined more by a tendency to cross the midline rather than by left/right specificity.

Hamberger (1928) reported a perplexing case where hind-limb nerves of one side crossed the midline and supplied the other limb when its supply had been removed by spinal cord lesions in the tadpole; here there was spontaneous decussation as well as disregard for any side specificity. Taylor (1944) links this behaviour with the existence in the normal animal of small bilateral components in the lumber plexus.

It is a striking feature of regeneration in mammalian peripheral nerves that fibres appear unable to choose between alternative paths despite the fact that they had selected between them during the course of normal development. Regenerating mammalian peripheral nerve fibres show no preference for the branches of forked nerve trunks which they had previously occupied (Kilvington, 1941; Weiss & Hoag, 1946; Rajkovits, 1953, repeating the experiment of Taylor (1944), where

embryonic fibres did segregate selectively ; Bernstein & Guth, 1961). Whenever the question has been explored fibres have been found to grow at random into all branches despite the fact that in other situations adult mammalian nerve fibres can be shown to select the cells on which they terminate (Langley, 1900; Guth et al., 1961; in the regeneration of preganglionic fibres into the superior cervical sympathetic ganglion in cats).

As Sperry (1945) puts it: "As far as is known, any regenerating fibre of a severed nerve may enter and grow distally within neurilemmal tubes previously occupied by any other fibre". Regenerating mammalian peripheral nerve fibres can apparently invade the tissue of any nerve trunk or any muscle without showing any signs of recognition or incompatibility (see for instance, Weiss & Edds, 1945; Sperry, 1941, 1945, 1951). Mammalian peripheral fibres are even insensitive to their direction of growth, centripetal or centrifugal, within a nerve trunk; they show "polar indifference" (examples are reported in Cajal, 1928; Aitken, 1949). And yet, it seems likely that the fibres themselves retain all the while the ability to select their most appropriate paths, since they can find their peripheral nerve stump after wide deflexions of the proximal from its normal position (Cajal, 1928, p.255; Sunderland, 1969).

So, in lower vertebrates and in embryos, the evidence suggests that nerve fibres select their paths; in adult mammals this potentiality is no longer displayed and the most likely explanation is that mechanical constraints dominate in mammalian regeneration. This follows from the evidence summarized above and is, of course, the conclusion reached long before by Weiss, with regard to fibres studied under tissue culture conditions.

The available data concerning fibre growth in the spinal cord (including Mauthner cell axons) is of some interest because this situation is intermediate between these two extremes. Here it is possible to show that if growing fibres are given the freedom to express it, they will display pathway specificity (i.e. specificity for the appropriate spinal tract) and directional sensitivity (specificity for ascending or descending polarity), but in so far as fibres frequently fail to achieve such specificity, the spinal cord shows parallels with peripheral nerve trunks. Among the features in common between the spinal cord and peripheral nerves the highly oriented nature of the mechanical substrate, the prominence of connective tissue elements or the long distances to be covered to the terminal, might be of significance in limiting the selective behaviour of regenerating fibres.

After rostrocaudal reversal of spinal cord segments in amphibians, descending fibres will readily demonstrate polar indifference and continue downward in conformity to their original direction of growth (Hooker, 1917; Detwiler, 1951; Hibbard, 1965; Swisher & Hibbard, 1967). But fibres will on occasion select their original fascicle within the spinal cord after segments have been rotated about the rostrocaudal axis (Hooker, 1930) and similarly Mauthner axons recover their normal positions in the ventromedial spinal cord after following aberrant routes in the medulla. Swisher et al. (1967) joined two Xenopus tadpoles tail to tail and found that the descending Mauthner axon from one cut spinal cord grew out into the second animal and ascended its spinal cord in the same fascicle as its own descending axon. Here the axons expressed their path specificity with regard to positions across the spinal cord while at the same time growing

diametrically against their normal polarity along the spinal cord. But if fibres are free of the usual mechanical constraints existing along the cord they can demonstrate specificity for polarity; Wieman (1922) rotated cord segments so that they lay at 90° to their original axis; fibres emerging from the cord stumps were found to select appropriately between the two surfaces of the graft, according to their normal direction of growth through it.

The remarkable studies by Hibbard (1965) of Mauthner axon orientation are in many respects comparable. If the rostrocaudal polarity of the cell and its immediate tissue environment was rotated before the emergence of the axon, the earliest response of the appearing axon was to follow its usual route through its immediately surrounding tissue (as do axons in completely isolated midbrain grafts, Piatt, 1944) which leads it now in a rostral direction. Unlike the Mauthner axons in the experiment of Swisher & Hibbard (1967), however, the axons in this case do not disregard polarity when they reach the rostrally-lying border of the grafts; instead they reverse their direction of growth, retrace their passages down through the rotated tissue and thereby recover their normal descending pathways in the spinal cord.

Rather than continue their growth beyond the rostrally-lying border of the graft in conformity to the mechanical orientation of the tissue (as they appear to do in Swisher & Hibbard's experiment) the Mauthner axons are able to select between ascending and descending growth polarities. Their freedom to choose may be the result of the fact that the fibres tend to meet the rostrally-lying border of the graft tangentially rather than perpendicularly because they are simultaneously undergoing bilateral decussation; as in Wieman's experiment the fibres have equivalent access to two alternative channels which, mechanically, are equally favourable. A factor which is

likely to influence the fibres' selection of growth polarity is the gradual anatomical incorporation of the graft into the surrounding nervous tissue (Detwiler, 1951) and the consequent reversion of the chemospecific polarity of the graft to that of the surrounding tissue. If grafting is done early enough re-specification of a graft may be complete (Straznicky & Székely, 1967) and the Mauthner axon may grow caudally from the outset (Detwiler, 1940).

Part 4

The behaviour and structure of growing fibres

> "They (the growth cones) may take the following forms; oval, pencil-point, brush or reversed cone, but the most frequent shape, judging from our preparations, is that of a barley-corn grain having a sharp and, at times, very pale point. Moreover, the shape and dimensions of the terminal mass varies according to the obstacles which it encounters in its progressive movements and whether it remains undivided or whether it is about to give rise to a branch. When the cones do not find obstacles in their route they elongate and become fine, pale and barely undulating."
>
> (Cajal, 1960, p.99)

Since Cajal first described them (1890) it has frequently been stated that the advancing tip of an axon, the growth cone, is akin to an amoeba; that its ceaseless emission of processes in all directions is indicative of goal searching (see, for instance, Sperry (1965); Weiss (1955) from which Fig.2A comes).

There are, however, a number of difficulties in the way of such an interpretation of the function of the growth cone. The most significant of these is that it is by no means certain that <u>in vivo</u> cones resemble those seen in tissue culture; it is under the latter conditions that most observations have been made. Different methods of staining bring out different features of these structures (Cajal, 1960, p.47) and under identical conditions different neuronal types (Godina, 1963; Pomerat,

Hendelman, Raiborn & Massey, 1967) or the same type from different species (Godina, 1963) display different characteristics. Sometimes fibres show no growth cone at all (Cajal, 1960) and even in the simplest situations a uniform population of fibres includes a wide variety of forms (see the drawings of Cajal, 1928).

Moreover, the form of the growth cone is thought to be dependent on the mechanical conditions existing and the speed of advance of the fibre at the time (Cajal, 1960, p.99 (from which the above quotation comes); Cajal, 1928, p.365). Hughes (1953) has suggested that the growth cone processes may be involved with pinocytosis rather than fibre guidance; the gradual transition from growth cone to terminal arborization seen in fibres in the developing trapezoid body (Morest, 1968) even raises the possibility that the growth cone may be no more than an incipient terminal arborization.

Apart from the stem axon itself two types of axonal process are seen during development and regneration; branches, which have life times of the order of hours or days, advance with their own growth cones independently of the stem axon and may subsequently degenerate and, secondly, "filopodia" (Pomerat et al., 1967) or "microspikes" (Taylor & Robbins, 1963) which are reversible projections from the growth cone or elsewhere, which have a life time of the order of minutes (Godina, 1963; Pomerat et al., 1967). Typical microspikes are apparently rigid processes about 15 μ in length and a uniform 0.1 μ in diameter (Porter, Claude & Fullam, 1945; De Robertis & Sotelo, 1952; Taylor et al., 1963). In other situations the processes are tapered, branched and of very variable dimensions. Occasionally growth cones display wide, flattened, membraneous protrusions sometimes called "foliopodia"

(Godina, 1963; Morest, 1968; Boyde, James, Tresman & Willis, 1968). Both types of growth cone process seem, according to tissue culture observations, to be associated with moving cell margins; Godina (1963) suggests that their role is anchorage.

It is not clear what form of growth cone if any exists in vivo (see Table 1, next page). Although Harrison (1910), Speidel (1933) and Tennyson (1969) report that the forms of growth cones are similar in the intact animal and in tissue culture, it is doubtful whether processes as highly differentiated as microspikes can be demonstrated in vivo. The drawings of Harrison (1910) and Morest (1968) show processes which might, if their precise dimensions had been specified, qualify but these are absent in Cajal's (1928; 1960) and in Speidel's (1933). Most of these studies depend on silver staining methods; it is not known to what extent microspikes are resolvable in silver stained material because the method has not been applied where microspikes are characteristic.

There is no electron microscopic evidence for comparable growth cone processes in vivo, even in cells which are known to display prominent microspikes in vitro; compare the studies of dorsal root ganglion cells of Tennyson (1969, EM on rabbit embryo) and Pomerat et al. (1967, in vitro), or of spinal cord axons by Bodian (1966, EM study of foetal monkey cord) and Pomerat et al., (1967) and Godina (1963). But the fact that these processes can readily be observed under the electron microscope in tissue culture material (Taylor et al., 1967) makes it unlikely that the failure to see them in vivo is due to any methodological difficulty.

A wide variety of cell species produce microspikes in tissue

TABLE 1.

A TABULATION OF PREVIOUS OBSERVATIONS ON THE STRUCTURE OF GROWTH CONES

PERIPHERAL NERVES

MATERIAL	GROWTH CONE DIAMETER & FEATURES -MICRONS	LENGTH OF CONE PROCESSES -MICRONS	AXON μ DIAMETER	CONTENTS OF GROWTH CONE	OTHER OBSERVATIONS	METHOD	REFERENCE
FROG-EMBRYO SPINAL N.	2-3 AMOEBOID	<10	1.5		SOME FINE PROCESSES HAVE SIMILAR DIMENSIONS TO MICROSPIKES	LIGHT SILVER	HARRISON, 1910
CAT-SCIATIC N REGEN.	<10 USUALLY BULBOUS	ABSENT, SINGLE OR MULTIPLE			VARIETY OF FORMS OF GROWTH CONE	LIGHT REDUCED SILVER NITRATE	CAJAL, 1928
REGEN. AMPHIBIAN CUTANEOUS N.	<8, "SMOOTHLY, ROUNDED, STELLATE, LANCEOLATE OR FILIFORM."	<10, UPTO ABOUT 8 IN NUMBER, IRREGULAR	3			LIGHT IN VIVO	SPEIDEL, 1933
GUINEA PIG-SCIATIC N REGEN.	ABOUT 2 ROUNDED	NONE APPARENT			FIRST TO EMPHASIZE VESICULAR/TUBULAR FORMATIONS AS PRIMARY CHARACTERISTIC ORGANELLE OF GROWTH CONE	E.M. & THICK SECTIONS	ESTABLE ET AL., 1957
CHICK-LUMBAR MOTOR N	3-4X AXONAL DIAMETER IRREGULAR	A FEW MAJOR PROCESSES			SEE QUOTATION FROM CAJAL, 1960 (PREVIOUS PAGE)	LIGHT GOLGI	CAJAL, 1960
KITTEN SCIATIC N REGEN	<10 BULBOUS	OCCASIONAL SINGLE INDEPENDENT MAJOR PROCESS				LIGHT OSMIUM	KNOCHE ET AL., 1962
RAT-SCIATIC N REGEN.	<10 ROUNDED	NO PROCESSES	5	VESICLES VESICULAR/TUBULAR FORMATIONS		E.M.	WECHSLER ET AL., 1962
MOUSE-SCIATIC N REGEN	ABOUT 4 ROUNDED WITH INVAGINATIONS	NO PROCESSES		MICROVESICLES MITOCHONDRIA MULTI-VESCLR BODIES	POSSIBLY VESICULAR/TUBULAR FORMATIONS	E.M.	WETTSTEIN ET AL., 1963
KITTEN-SCIATIC N REGEN	ROUNDED INDENTED PLUMP PROJECTIONS			LAMELLAR BODIES VESICLES MITOCHONDRIA	VESICULAR/TUBULAR FORMATIONS PRESENT	E.M.	BLUMCKE ET AL., 1965A 1965B
NEWT-REGEN. LIMB N	1.5 SMOOTH	NOT APPARENT	0.5		CHARACTERISTIC GROWTH CONE VESICULAR/TUBULAR FORMATIONS	E.M.	LENTZ 1967
RAT-SCIATIC N REGEN	<10 BULBOUS	NO GROWTH CONE PROCESSES	<10	MITOCHONDRIA TUBULES VESICLES	SIMILAR TO WECHSLER ET AL., VESICULAR ZONE DISTINGUISHED AT TIP OF GROWING AXON VESICULAR/TUBULAR FORMATIONS PRESENT	LIGHT E.M.	ZELENA ET AL., 1968

CONTINUATION OF TABULATION OF OBSERVATIONS ON THE STRUCTURE OF GROWTH CONES

TISSUE CULTURE

FROG-EMBRYO CORD OR D.R.G.	ABOUT 5 AMOEBOID	10	2		GROWTH CONES RESEMBLE IN VIVO STRUCTURES PROCESSES SIMILAR TO MICROSPIKES	LIGHT IN VITRO	HARRISON, 1910
CHICK-SYMPATHETIC MEDULLA	<10	10 TYPICAL "MICRO-SPIKES"			GROWTH CONE SHAPE VERY IRREGULAR FEWER PROCESSES	WHOLE MOUNT E.M. & LIGHT	PORTER ET AL., 1945 DE ROBERTIS ET AL., 1952
CHICK-D.R.G.	<10	10 MANY ACTIVE PROCESSES	5		OBSERVED ACTIVE PINOCYTOSIS AT THE GROWTH CONE	LIGHT IN VITRO	HUGHES, 1953
HUMAN-CHICK-EMBRYO D.R.G.	< 5	<20 UNIFORM 0.3μ DIAMETER	3		1-30 0.3μ PROCESSES PER GROWTH CONE	LIGHT IN VITRO	NAKAI ET AL., 1959
CHICK-D.R.G.	<12 x 5	10 FOLIOPODIA MANY MICRO-SPIKES	1			LIGHT IN VITRO	POMERAT ET AL., 1967
CHICK-SPINAL CORD		NO MICRO-SPIKES APPARENT			OBSERVED ONE GROWTH CONE (FOLIOPODIAL) ABOUT 15μ IN DIAMETER HAVING NO PROCESSES	SCANNING E.M. & LIGHT	BOYDE ET AL., 1968

CENTRAL NERVOUS SYSTEM

MAMMAL-OPTIC N. ABORTIVE REGEN.	<15 BULBOUS RING OBLONG	NONE SINGLE SEVERAL			OCCASIONAL SINGLE INDEPENDENT MAJOR PROCESS	LIGHT REDUCED SILVER NITRATE	TELLO, 1907 CAJAL, 1928
CHICK-D. ROOTS SPINAL INTER NEURONS	ABOUT 5 BIFURCATED IRREGULAR ELONGATED	UP TO 5	1			LIGHT REDUCED SILVER NITRATE	CAJAL, 1960
RAT-D.COLUMN ABORTIVE REGEN	<7 SPHERICAL	SINGLE INDEPENDENT MAJOR PROCESS	2	MITOCHONDRIA DENSE BODIES FILAMENTS MULTI-VESCLR BODIES	VESICULAR/TUBULAR FORMATIONS PRESENT	E.M. & THICK SECTIONS	LAMPERT ET AL., 1964
MONKEY-FOETAL SPINAL CORD	0.5 SMOOTH ROUNDED	NONE		"CLUSTER OF LARGE EMPTY VESICLES" ONLY		E.M.	BODIAN, 1966
RAT-FOETAL CEREBELLUM	UP TO 1.0				SIMILAR TO BODIAN VESICLES SOMETIMES SIMILAR TO VESICULAR/TUBULAR FORMATIONS	E.M.	DEL CERRO ET AL., 1968
MAMMAL-FOETAL TRAPEZOID BODY	<7 "CONE-SHAPED ENLARGEMENT"	UP TO 6 "FILOPODIA, FOLIOPODIA"	2		CONTINUOUS DEVELOPMENTAL SERIES FROM BULBS TO COMPLEX SYNAPTIC CALYCES SOME 0.1μ DIAMETER PROCESSES LIKE MICROSPIKES	LIGHT GOLGI	MOREST, 1968
FISH-SPINAL CORD REGEN.	<5 "BULBLIKE"	NONE APPARENT	<3			E.M. & THICK SECTIONS	BERNSTEIN ET AL., 1969
RABBIT-DEVEL. DORSAL ROOTS	<13 x 5 BULBOUS ELONGATED SMOOTH PROFILE	<12 A FEW MAJOR PROCESSES	1.5	VESICLES FILAMENTS	TUBULES PRESENT IN CONE BUT NOT PROCESSES SIMILAR TO CAJAL, 1960	E.M. & THICK SECTIONS	TENNYSON, 1969
MOUSE-CEREBRAL CORTEX ABORTIVE REGEN	< 3 ROUNDED	NO PROCESSES	1		6μ DEGENERATING END BULBS WITH SINGLE INDEPFNDENT MAJOR PROCESSES WITH OWN GROWTH CONE	LIGHT REDUCED SILVER	WENZEL ET AL., 1969

culture; human conjunctiva cells, HeP cells and cells of various chick tissues including liver and kidney (Taylor et al., 1967), glial cells (Lumsden, 1958), fibroblasts (Porter et al., 1945). Taken together this evidence suggests that these processes are not a characteristic of selective cell growth but rather represent a cell membrane transformation in response to the conditions of tissue culture perhaps related to the transformations reported by Willmer (1961) of amoebae into ciliated forms.

Grainger, James & Tresman (1968) have found that what appear to be single neuronal processes of chick spinal cord in tissue culture when viewed by light microscopy, consist electron microscopically of numerous individual fine processes. This raises the possibility that the branches and growth cone processes described in earlier studies (e.g. Speidel, 1933; Godina, 1967; Pomerat et al., 1967) may be no more than points of separation of the processes comprising such a bundle. This can only be excluded where it has been confirmed that single cells are involved (Cajal (1960), and Morest (1968) did this because they used Golgi methods of preparation; Boyde et al. (1968) and Harrison (1910) traced the out-growing processes back to their cells of origin).

Summarizing the data in Table 1, growth cones <u>in vivo</u> are frequently bulbous in form and less than 10 μ in diameter. Occasionally (and rather more often in developing nerve) fibres display irregularly shaped tapering protrusions up to 10 μ in length, but these have not been observed in the developing CNS as yet (Bodian, 1966; Magnaini & Forstrønen, 1967; Del Cerro & Snider, 1968) and at least in some cases these are due to mechanical accommodation of the fibre to its surroundings (Cajal, 1960). At their most elaborate, <u>in vivo</u> growth cone processes

rarely compare with those seen in tissue culture, where they are always a prominent feature.

In the CNS regeneration is often abortive and this is associated with the formation of enlarged, persistent terminal expansions of axons (Cajal, 1928, p.365). Spontaneously dystrophic axon terminals reaching up to 50 μ in diameter have been described in the nucleus gracilis of normal cats (Hashimoto & Palay, 1965). Another frequently observed form of growth terminal consists of a cone with a single, major process extending forward from it which itself is tipped with a small bulbous growth cone (Tello, 1907; Cajal, 1928; Knoche & Blümcke, 1962; Lampert & Cressman, 1964; Wenzel & Barlehner, 1969).

If growth cone processes play any direct part in pathway selection the extent to which they could explore the tissue in advance of the fibre would, on the basis of their structure, be limited to within a radius of 15 μ of the fibre tip. There is no evidence which actually points to this role; there has, for example, been no report of any correlation between the behaviour of a growth cone and the direction subsequently followed by the fibre. The fact (Hughes, 1968) that the lifetime of growth cone processes is short ("a few minutes" according to Taylor et al.; Harrison, 1910 confirms this) compared to the time needed for the fibre to advance over the distance actually spanned by the process, suggests that the fibre does not directly grow into its processes (a view supported by Godina, 1963).

Numerous reports testify to the proliferation of branches at the site of crushing a peripheral nerve (Ranson, 1912; Cajal, 1928; Shawe, 1955) and each proximal fibre may send as many as 50 branches into the distal stump. The initial impulse to form branches may, like the

collateral branching of normal fibres (Edds, 1953; Causey & Hoffman, 1955), be a generalised response to local injury. On the other hand Speidel (1933) and Cajal (1928, pp.371 & 169) show that regenerating fibres form branches on meeting mechanical obstructions; it may therefore be a reflection of the mechanical conditions existing at the lesion site during regeneration. There is some evidence that branches may later degenerate selectively. Shawe (1955) reported that the number of fibres innervating the distal stump fell progressively having reached a maximum after four weeks regeneration. Weddell (1942) observed and Cajal (1960) inferred degeneration of branches or fibres which had apparently failed to make effective connections. Hughes (1965) suggests that the developing amphibian limb achieves its orderly innervation by the selective degeneration of inappropriate connections made initially by the random invasion of the limb bud by an excess of fibres or branches. Certainly, developmental errors (Cajal, 1960, p.212) and degeneration (Glücksman, 1951) are prominent features of ontogenesis.

In assessing the possible role of axonal branches in selective growth it seems certain that their relatively slow rate of advance and their restricted numbers would not permit them to sample more than a minute fraction of all the potential paths available to the axon at any one time and place. It is important too to note that in embryogenesis the number of axonal branches is much fewer (Harrison, 1910; Weiss, 1936; Cajal, 1960, p.278, shows developing axons of cerebellar Purkinje cells with no more than two or three branches).

Before returning to the data on the regeneration of the optic nerve there remains one impressive feature of nerve growth which must be mentioned; this is its "goal directedness". The tendency for fibres to

follow straight lines was early remarked on (His, 1888; Harrison, 1910; Cajal, 1928, p.363). (In a few cases regenerating fibres are reported to follow gently sinusoidal courses; Cajal, 1928, in the retina; Sperry et al., 1968, in the tectum). In their smooth and gradual separation as they grow through the optic tract the fibres destined for the two divisions of the goldfish tract seem to anticipate their future routes. On the other hand these features of nerve growth could in part be due to an inertial component in the advance of the axon as recognised by Cajal (1928) and otherwise described as vis a tergo (Weiss, 1955). Another possible factor is the "towing" effect (Weiss, 1955) though this can play little part in adult regeneration. Yet the fact that fibres can, on occasion, follow quite tortuous routes through the brain (one example is the path of the motor fibres of the facial nerve in the pons) suggests precise control of fibre growth at these points.

Although it is hardly decisive as to the mechanisms involved, the "smoothness" and "design" inherent in the growth of axons under all the conditions described above, must be taken into account. It appears to indicate further refinement of the active path selectivity which (as was argued in Part 3 of the Introduction) is likely to be a major feature of nerve growth, wherever the nerve fibre happens to lie in the nervous system. If this is true the evidence as a whole shows that the fibre is provided with precise cues at all points along its growth path (hence the smoothness) and that such cues are widely available throughout the tissue (hence the ability of fibres to traverse unaccustomed pathways). On the other hand such smoothness would not be expected if fibres selected their routes by way of a discontinuous series of choices between randomly oriented exploratory branches.

In the case of selective regeneration in the visual system we are effectively left with the following alternative mechanisms; active path selection or selection between random axonal branches. (The possibility that selection is between randomly regenerating ganglion cells, as such, will be discussed in the Conclusions section). If selection between axonal branches plays a part this must be subject to the restrictions mentioned on the previous page but one; sampling of a large number of alternative paths and/or terminal sites would require time; the final paths might not be direct.

A few papers (Gaze et al., 1963; Gaze & Keating, 1970) report direct evidence for an initial random phase of ingrowth of optic fibres into the tectum but all other reports, all for species other than Rana, argue against this (Jacobson & Gaze, 1965; Gaze & Sharma, 1970; Cronly-Dillon, 1968; Attardi & Sperry, 1963; Westerman, 1965; Delong & Coulombre, 1968); the last three papers specifically but unsuccessfully sought histological evidence for initial random growth by fibres.

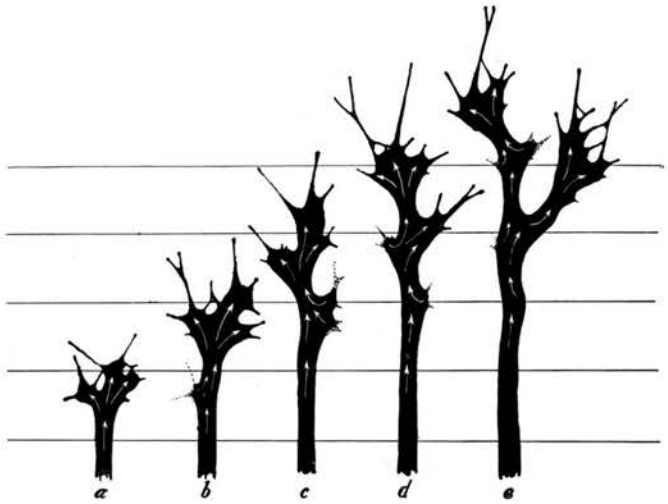
Assuming a dead time required for the cut fibres to cross the optic nerve lesion (both Attardi & Sperry (1963) and Murray & Grafstein (1969,[2] optic tract) estimate about 6 days) and representative rates of fibre regeneration (of the order of 1 mm per 24 hours in poikilotherms according to Lubinska, 1964) fibres should first reach the tectum of a 5-7 cm fish at about 11 days after optic nerve lesioning. Thus Attardi & Sperry (1963) report the first fibres at between 10 and 12 days, on the basis of histological examination. However Murray & Grafstein (1969)[2] comparing the times to first behavioural visual responses after nerve or tract lesions conclude that regeneration is slower, 0.2 mm per day, in which case the first fibres should arrive at about 30 days.

Despite these differences in the estimates of the rates of regeneration, the same authors also report that the first visual responses follow about 5 days on the time when fibres were first thought to have reached the tectum (responses at 14-16 days in Attardi et al., 36 days in Murray et al.). Westerman (1965) reports that behavioural responses were first seen after 29 days, and that electrical responses could be obtained thereafter. In his study there was a close parallel between the appearance of electrical responses and of visible regenerated fibres.

In the frog the available data suggests that electrical signals are obtainable at about the same time as the first functional connections are formed. Gaze & Jacobson (1963) observed the first electrical responses at Day 23 and the first complete map on Day 33. Sperry (1944) observed the first behavioural responses in various anurans between Day 21 and Day 33 with a mean of 25 days.

It can, tentatively, be concluded that the first functional connections are formed within the time (or some time before) needed by fibres advancing at a constant rate to extend to the furthest points across the tectum from their points of entry, and with a promptness which leaves little time for trial and error mechanisms to operate. Electrical responses are probably not obtained prior to the establishment of stable connections, if the assumption is made that stable connections must exist for behavioural responses to occur.

Figure 2B.



Five consecutive phases in the advance of an axon tip (semidiagrammatic). Arrows indicate directions of flow, thrust and drain of neuroplasm. In *e*, dichotomous branching of fiber has been initiated. Dotted portions represent the location of earlier protrusions that have been sucked back by the draining force of the axial stream.

Figure 2A (from Weiss, 1955)

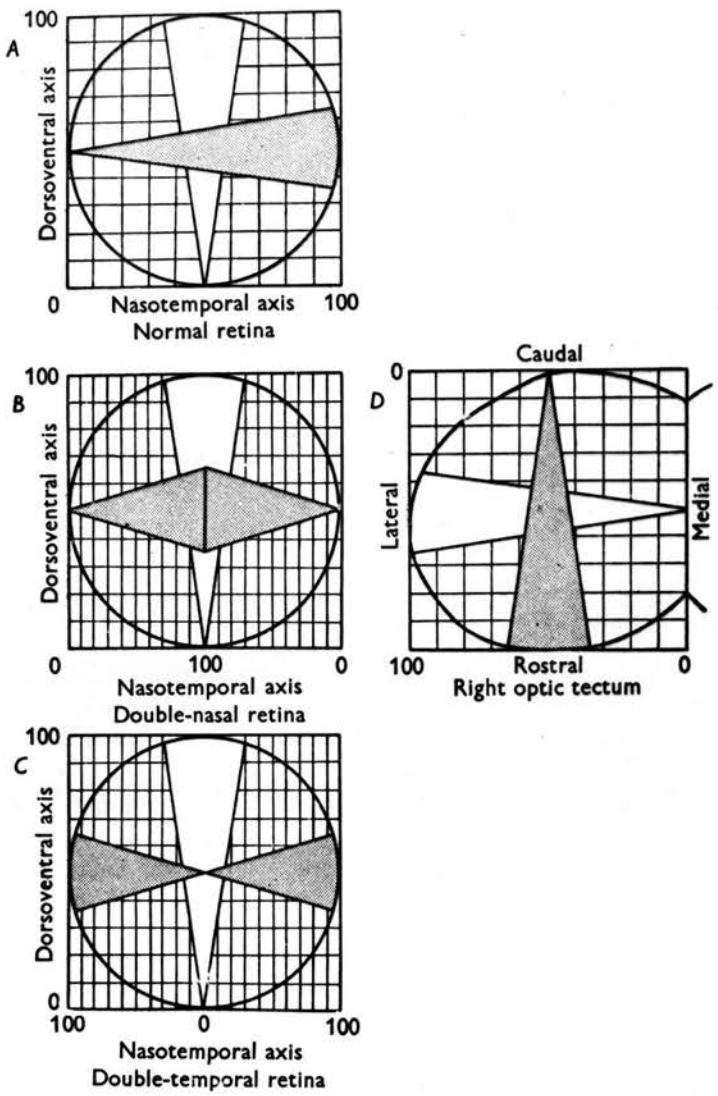


Figure 2B. (from Gaze et al., 1963)

Part 5

Departures from simple point to point specificity in the retinotectal projection

Gaze, Jacobson & Székely (1963) mapped the projection to the contralateral tectum in Xenopus in which one eye had been formed surgically from two temporal or two nasal half-retinae at a time shortly after the nasotemporal axis had been specified (Jacobson, 1968). These are known as compound eyes. As would be expected of two parts of the retina with the same early developmental histories, corresponding points in the two halves of the compound retinae projected to the same tectal positions. However fibre terminals were not confined to the corresponding nasal or temporal areas of the tectum; it was apparent that the double projection had expanded along the longitudinal axis of the tectum to fill the entire available tectal surface. This effect does not involve any detectable abnormality in the tectum because Gaze, Keating & Straznicky (1970) have shown that the same projection results if the nerve from such a compound eye is regenerated into the normal ipsilateral tectum after metamorphosis and the tectum previously supplied from the compound eye showed a normal projection from the normal eye. It was also shown (Straznicky, unpublished) that this expansion is not obligatory; if the caudal tectum was removed at stage 53/54 the full projection from the compound eye was restricted to the remaining rostral half tectum.

In further experiments Gaze, Keating & Straznicky (1971) found that the projections from double-ventral compound eyes did not show the same expansion; they appeared to fill only the dorsal half of the tectum although it is not clear whether the area normally supplied by dorsal retina (lateral and ventral tectum) was completely free of fibre terminals because it is not accessible for recording. In the fish experiments of Jacobson et al. (1965) there was no evidence for either compression or expansion of the projection but Gaze & Sharma (1970) have found that when the caudal half-tectum is removed in fish and the contralateral optic nerve is regenerated into the remainder, the whole projection tends to compress into it.

The results for developing Xenopus and adult goldfish agree well because Straznicky (unpublished) has found that when the rostral or caudal halves of normal 53/54 stage Xenopus tecta are removed and the optic nerves uncrossed at the chiasma, the same compression occurs, but if, as in other fish experiments (Gaze & Sharma, 1970), the nerve supplying the half-tectum is left intact it does not; positions in the field deprived of their tectum remain unrepresented except that in the fish there was a long-term tendency for disconnected fibres to shift into the remaining tectum. A normal projection could be recovered from a half-represented Xenopus eye if, after metamorphosis, the chiasma was uncrossed and the nerve regenerated into the ipsilateral normal tectum.

These various results can be interpreted on the crossed "gradient" model of Gaze, Jacobson & Székely (1963) (See Fig.2B). According to this, a growing optic fibre does not seek to terminate at one fixed region in the tectum; the form of the specification of the retina and the tectum is such that individual fibres locate their terminal positions

on the basis of the relative distance of their ganglion cell between the existing nasal and temporal extremes of the retina, so as to occupy a corresponding relative distance from the existing caudal and rostral extremes of the tectum. When either half of the tectum or half of the retina is removed the cells adjacent to the lesion thereafter occupy the extreme position in that direction and take over the role of the ablated part in the formation of the projection.

Clearly in the data reviewed above this model describes the behaviour of the projection whenever the nasotemporal retinal axis (or correspondingly the longitudinal tectal axis) is concerned, but no modifications of the same kind have been reported with regard to the transverse tectal axis. This has led to the suggestion that there are two geometrically distinct components to this gradient system, perhaps corresponding to the two components discovered in the specification of the eyecup (Jacobson, 1968) and the components observed in the reorganisation of an orderly map in the course of regeneration in frogs (see Appendix, Figs.A1 and A2; Gaze & Jacobson, 1963). However it seems that specification can only take the gradient form in one axis; it is only because the projection behaves differently in the other axis that the two geometric components became apparent.

These various anomalous results conflict with the findings of DeLong & Coulombre (1965) (after retinal lesions made before ingrowth of the optic nerve in chicks, the eventual histological distributions of the surviving fibre terminals in the tectum did not depart from normal), Attardi et al. (1963) and Westerman (1965). The latter author mapped the projections during the early stages of regeneration of infero-temporal or inferonasal retinal quadrants in 21 goldfish. He found no

deviations from the normal point-to-point connections despite the fact that his experiment left open the possibility of readjustment within the longitudinal tectal axis of the kind described above. Part of the explanation might lie in Westerman's use of different recording electrodes (Gaze, personal communication). The same was true in the results of Attardi et al. (1963) where fibres from a nasal half-retina destined for the caudal half of the tectum appeared to skip the rostral half. But the published pictures show that the histological distinctions between innervated and non-innervated tectal regions is less clear in this situation than in parallel experiments involving the transverse tectal axis. It is a possibility that their method cannot reveal the new fibre patterns under these conditions; anomalous fibres might be too few to detect, or the distinction between parallel and plexiform layers may not hold. Sperry (in Sperry & Hibbard, 1968) mentions that while fibres are rigidly confined to individual channels within the parallel layer, they are free to move in all directions in the plexiform layer; if the effects described above were the results of adjustments within the plexiform layer they might not be detectable histologically.

As regards other evidence for the double gradient model there are several reasons for supposing that any parallels in the two-stage specification of the developing eyecup are fortuitous. First, similar two-stage processes are involved in the embryonic determination of many organ systems (Harrison, 1921; 1936; Roach, 1945). The relationship between events at this stage of development and the organisation of adult systems is remote; the complexity of the specificity of cells may have to be greatly increased during later development to accommodate such processes as increase in cell numbers and migration (Hollyfield, 1968;

Gaze & Watson, 1968). Furthermore any geometric correspondence in the orientations of the axes of eyecup specification and the inferred axes of the gradients in the adult retinotectal system (in Xenopus and fish) may be coincidental; in the frog, on the assumption that the eyecup is also specified nasotemporally and dorsoventrally, the corresponding axes in the retinal projection on the tectum are rotated by 35° in relation to the anteroposterior tectal axis as compared to the other species. What is likely to be more significant, this rotation is even greater in relation to the points of entry of the optic tract and hence to the direction of ingrowth of fibres into the tectum.

Gaze & Jacobson (1963) have suggested that in the course of regenerating into the tectum, frog optic fibres recover their retinotopic order in the transverse tectal axis before they become arranged in an orderly manner in the long tectal axis; and that both stages are preceded by an initial stage of random ingrowth. If this were true it would make it easy to see how, as has been shown in the experiments described above, fibres have <u>potential</u> access to all terminal sites along the tectum. This feature is unlikely to be relevant, however, because (in the fish and Xenopus data) flexibility with regard to terminal sites is confined to the nasotemporal projection axis even though (in the frog) there is initial random order of fibres in the dorsoventral axis also. Indirect evidence suggests that far from having initially random access to positions along the tectum, regenerating fish optic fibres lay down their terminals sequentially as they enter the tectum; Gaze & Sharma (1970) showed that in a series of fish in which the whole optic nerve was regenerated into a rostral half tectum, about half showed incomplete projection of the temporal field onto the

remaining tectum; this suggests that fibres normally destined for the rostral tectum (those representing the nasal field) have an initial advantage over others, despite the fact that (without section of the contralateral optic nerve) fibres destined for the caudal tectum tend over the long-term to displace intact terminations in the rostral tectum with the end result of a complete representation of the field.

There are other difficulties with Gaze & Jacobson's interpretation which will be dealt with in Appendix 1. Even if some reason for the differential temporal response of the fibres to the two hypothetical gradients were available, the interpretation leads to a somewhat unlikely prediction. After initial random invasion of the tectum growing fibre terminals are thought to arrange themselves first along the transverse tectal axis; to do this they must follow predominantly transverse growth paths. In order to reach their final terminal sites fibres must later grow to new positions within the longitudinal tectal axis. The paths finally followed by fibres would then be indirect reflecting the two stages of growth.

The adjustments which occur within regenerated projections in the longitudinal tectal axis in fish and Xenopus seem to involve different mechanisms from the homologous developmental phenomena of "regulation" ("size invariance") and "modulation", in which an actual change in the individual characteristic specificities of cells occurs. This was shown by Gaze, Keating & Straznicky (1970) when they uncrossed the optic chiasma in Xenopus with one compound eye; the regenerated projection from that eye to the normal tectum was typical of compound projections; the projection from the normal eye to the tectum previously supplied by the compound eye was similarly unaffected. No irreversible changes in the specificities of individual retinal or tectal cells could therefore explain the spread of the compound projection along the length of the tectum.

An alternative form of explanation, of which the gradient hypothesis is an example, is that the adjustments occurring in the longitudinal axis result from the way in which cells are labelled. The gradient model suggests that cells are specified according to their position in relation to the surrounding population of cells. It follows that the behaviour of individual fibres will be modified by removal of parts of the retina or tectum, presumably as a result of changes in signals received by the fibres from the cells surrounding them.

Against such models is the clear evidence that retinal and tectal tissue does possess fixed and absolute specificity independent of its surroundings; DeLong & Coulombre (1968) showed the retention of retinotopic specificity by small portions of retina transplanted onto an uninnervated tectum; their fibres grew directly to the tectal regions appropriate to the origin of the grafts in the retina. Sharma (1967) similarly showed that regions of tectum possess intrinsic and fixed specificity independent of surrounding tectum by rotating areas of tectum surgically; they became re-innervated with their original patterns of terminations.

A final explanation is in terms of the way in which optic fibres grow into the tectum. On this view the behaviour of fibres within the long tectal axis is not a reflection of the specificities of cells but of the normal mechanism of "read-out" whereby fibres order themselves into the tectum. This possibility will be discussed further in the Conclusions section.

METHODS

Animals

Frogs (Rana temporaria or Rana esculenta) and goldfish (Carassia aurata) of between 3 and 7 cm in length were obtained from a local pet-shop. Fish were kept in batches of up to 20 in plastic tanks containing 20-25 litres of tap water which was changed every 2-3 days. No artificial aeration was used and the temperature was room temperature, about 20°C. The fish were fed twice a week on either minced ox heart or dried Daphnia, with the occasional addition of live white worm.

While the fish tolerated the operations well, there was a continual loss of fish due to endemic diseases. The most common parasitic infestations were due to fungus (Saprolegnia) and flukes (Gyrodactylidae) and both could be treated by bathing the fish in 1-3% NaCl for 30-minute periods. Direct application of strong Malachite Green was found useful in the treatment of superficial fungus while 0.0005% methylene blue was also used against flukes. Dropsy (in which Aeromonas punctata is implicated) could be successfully treated by the addition of chloramphenicol (13 mg/l.) to the tanks. No effective treatment for fin-rot or Ichthyophonus infection was found and these diseases usually proved fatal. Tubifex was never used as food since this was thought to introduce many pathogenic organisms. Postoperatively fish were kept in water to which 0.1% cooking salt had been added to prevent fungus and fluke infection. Subsequent experience has shown that water should be

changed only every 20-30 days and that continuous filtration further reduces fish mortality.

Operative procedures

Operations on the optic tract or tectum were performed in the following way on anaesthetized fish. The epidermis overlying the skull having been scraped clear, a rectangular flap of bone was cut out holding the scalpel blade at an acute angle to the surface of the flap. The tract with its divisions could easily be visualized after the forebrain had been pushed slightly forward with a piece of tissue and the whole dorsal surface of the tectum was immediately accessible.

For chronic operations on the tectum or divisions of the optic tract it was possible to avoid the bleeding which, under normal circumstances, made it very difficult to manipulate parts of the brain. The fish was perfused through the mouth with 7% Urethane and the superficial capillary circulation observed, until it had either slowed or stopped. Bloodless operations were then possible and the fish would recover after perfusion with water in the normal way for about 40 minutes.

Bundles of optic fibres could be cut with tungsten needles under direct vision of the tectal surface or sections of the tectum could be excised using a suction tube. In one series of experiments the lateral division of the optic tract was exposed by medial displacement of the tectum with tissue, freed from the lateral border of the tectum with needles and implanted into the medial division. Similar techniques were used in acute experiments in which one or other of the divisions was cut or lesions were made across the tectal surface in order to study the paths of small groups of optic fibre bundles.

After chronic operation the bone flap was replaced and held firmly in position by the angle of the cut edges. In early experiments the bone flaps were held in place with dental cement or Ethicon isobutyl 2-cyanoacrylate (IBC-1) but these tended to fall off at a later stage.

Fish were anaesthetized in Tricaine (Sandoz MS222) 1 in 3000 until immobile. The fish were then covered in wet tissue and placed in a fish-holder with their mouths over a glass tube through which water was continually perfused from a storage tank.

Operations were performed under a Wild binocular microscope at about 20X magnification. Fine tungsten needles, scalpel, ophthalmic scissors and watchmakers forceps were needed. Special care was taken to protect the cornea from drying due to the microscope light.

For crush or cutting of the optic nerve the eye was drawn forwards and downwards so that the lesion could be made under direct vision. At a point approximately half way between the eye and the orbital fissure the nerve was crushed with forceps or cut with scissors (leaving a small strand of connective tissue to hold the cut ends together; the cut was reinforced with a crush to ensure that all fibres were severed). Unless otherwise stated the <u>left</u> optic nerve or the <u>right</u> tract or tectum were lesioned so that when the animals were later mapped, the left eye was centered in the perimeter and responses were obtained from the right tectum.

For lesions of the retina the eyeball was punctured with a needle and a cut was made with scissors along the scleral-corneal junction. Tissue was used to hold back the cornea and lens in order to give a clear view of the retina. Taking care to avoid the head of the optic nerve the retina was divided with two needles, and, when necessary, part

was removed together with the pigment epithelium with forceps and fine strands of tissue. The cut surfaces of sclera were then simply placed in contact or, in some cases, held together with Ethicon Tissue Adhesive IBC-1.

Recording the retinotectal projection

For the purposes of acute electrophysiological recording the fish were anaesthetized with Tricaine, decerebrated and injected intramuscularly with 0.2 mg tubocurarine. The tectum was exposed and covered with liquid paraffin and the pattern of blood vessels, optic fibre bundles and position of tectal lesions drawn directly onto graph paper using a drawing apparatus attached to the microscope, at a magnification of 31X. The fish was firmly held in the fish-holder and continually perfused with water. Its left eye was centered within an Aimark perimeter. Centering was usually done by eye but for accurate mapping of fish before and after acute lesions it was checked using a reflected beam of light thrown onto the fish's eye from the centre of the perimeter. Centering was judged by the position of the reflected beam within the pupil of the eye.

Electrodes were Woods metal/Iridium filled glass micropipettes tipped with 1-5μ balls of platinum (Gesteland et al., 1959). Tungsten electrodes insulated with Insulex and with tip diameters of about 3μ were also occasionally used. The electrode was placed at a point on the tectum corresponding to a chosen position within the grid on the drawing, and the electrical activity evoked by moving black discs against an illuminated background at 33 cm was fed to a Tektronix oscilloscope and loudspeaker through cathode follower and Type 122 preamplifier. The

electrode was advanced to the optimal depth, and the position in the visual field giving the maximal response was found and noted, together with a brief description of the character of the response; normal, unitary, multiunitary, reduced. Field sizes were estimated at 33 cm from the eye with 6 cm black discs and the average of horizontal and vertical diameters taken.

Normally the retinotopic projection was systematically and completely mapped at the start of the experiment; then, under direct vision, any necessary lesion of the tract or tectum was made. Cuts made in the tectum usually had little effect on the survival of tectal responses other than as a result of cutting incoming optic fibres. The animal was then re-centred and re-mapped; in cases where field positions were recorded a second time they were usually within about 10° of the previously determined position. After recording, lesions originally placed in the optic nerve or elsewhere were inspected.

Histology

Fish were fixed in Susa's fixative, or by the perfusion method used for electron microscopy, in which case the results tended to be improved. Staining was by Holmes silver method modified as follows:

Day 1

Remove wax in xylene (20 mins)
Pass through alcohols to water
Remove mercuric precipitate by putting slides into iodine (3 mins)
Sodium thiosulphate (3 mins)
Wash (5 mins)
Rinse each slide in distilled water
Put into 20% silver nitrate
Seal well and incubate 3 or 3½ hours
Remove silver nitrate from staining trough
Three changes of distilled water
Into impregnating solution, seal well and leave over night.

Impregnating solution

700 ml distilled water
110 ml Buffer A
90 ml Buffer B
10 ml 10% Pyridine
2 ml 1% silver nitrate
Bring to 1 litre mark.

Buffer A: 6.2 gm boric acid in 500 ml water
Buffer B: 9.5 gm Borax in 500 ml water.

Day 2

Put into reducing solution (3 mins).

Reducer

40 gm sodium sulphate
4 gm Hydroquinol in 400 ml water

Wash in tapwater (3 mins)
Wash in distilled water (3 mins)
Put into gold chloride (3 mins) (1 gm gold chloride in 400 ml water)
Wash in distilled water
Put into 2% oxalic acid (3-5 mins)
Look under microscope for appearance of fibres
Wash in tapwater (3 mins)
Fix in Hypo (3 mins)
Tapwater (3 mins)
Dehydrate into xylene and mount in DPX.

Electron microscopy

Two methods gave satisfactory fixation of the optic nerve and tectum of fish and frogs, the second allowing considerably more flexibility.

Dalton's immersion fixation (Dalton, 1953)

Dalton's buffer (4% solution)
20 gm $K_2Cr_2O_7$
500 ml distilled water.

Make neutral by adding KOH
Add equal volume of 3.4% NaCl.

Make up:

5 ml 10% sucrose
5 ml Dalton's buffer
0.1 gm osmium tetroxide.

The fish having been anaesthetized with MS222 the required tissue was rapidly excised and immersed in the fixative for 2-3 hours. The tissue was then dehydrated and embedded in Araldite in the normal way. This method of fixation seemed especially satisfactory for the preservation of cell membranes.

Formaldehyde/gluteraldehyde perfusion. Vaughn & Peters (1967)

The fish was anaesthetized with MS222 and immediately curarized, and, with the fish continuously perfused with water, the heart exposed by cutting through the scapulocoracoids. A suture thread was passed round the aorta, the heart divided at the junction of the ventricle and the conus arteriosus and a fine polythene tube inserted into the aorta and secured. The fish was immediately perfused with approximately 0.5 ml 0.7% sodium nitrite containing heparin (15 Int. Units/ml) followed by 20-25 cc formaldehyde/gluteraldehyde fixative, the fluids and blood being washed out of the cut ventricle by the water leaving the gills.

The perfusion fluid contained 4% formaldehyde and 0.5% glutaraldehyde made up as follows:

0.9 gm NaOH
8.0 gm paraformaldehyde
200 ml water heated to 60°C for depolymerization
Add 3.75 gm $NaH_2PO_4H_2O$
4 ml 25% gluteraldehyde (supplied by Taab Laboratory, Reading)
Adjust pH to 7.4 by adding NaOH
Store in fridge.

The required tissues were immediately postfixed in 1% phosphate buffered osmium or Dalton's fixative for one to two hours, and embedded in Araldite. Sections were cut on a Porter-Bloom microtome and thin sections mounted on wide aperture grids with Formvar films. Thick

sections were prepared and stained in the following manner.

Solution A: 1% Toluidine Blue in 1% Borax solution
Solution B: 1% Pyronin B in distilled water
Make up stain with 4 parts A to 1 part B.

Thin sections were stained with lead hydroxide with or without uranyl acetate and viewed in a GEC EM6B.

Presentation of the results

In the results which follow ELECTRONMICROGRAPH PLATES ARE NUMBERED SEQUENTIALLY AND ARE INCLUDED AS CLOSE AS POSSIBLE TO THE TEXT TO WHICH THEY REFER; RETINOTECTAL PROJECTION MAPS ARE INCORPORATED AS NEAR AS POSSIBLE TO THE POINT IN THE TEXT AT WHICH THEY ARE FIRST REFERRED TO; EACH MAP IS GIVEN A THREE-PART CODE NUMBER. A PAGE INDEX TO PROJECTION MAPS IS GIVEN IN APPENDIX 2. The first part of the code number specifies the nature of the operation first performed on the fish;

Series Tr>mTe-	Removal of the lateral half of the tectum and implantation of the lateral division of the tract into the medial division.
Series IRet-	Removal of the superior half-retina together with section of the optic nerve.
Series NSRet-	Removal of the infero-temporal half-retina together with section of the optic nerve.
Series NSRet>rTe-	Removal of the infero-temporal half-retina and the caudal half-tectum together with section of the optic nerve.

The second part of the code describes the conditions under which regeneration subsequently occurred including its duration.

R	followed by a number indicates the number of days between section of the optic nerve and recording.
RetR	refers to regeneration after section of a proportion of optic fibres within the retina.
TrR	refers to regeneration following section of the optic tract.
3rdR	refers to regeneration three times completed.
DelayR	refers to regeneration delayed by repeated crush of the optic nerve.

In addition N designates an intact normal fish.

The third part of the code number specifies the examination that was made of the regenerated projection after initial mapping.

Series Tr Acute section of the medial division of the tract and mapping of the surviving projection.

Series Te Acute transection of the rostral tectum sparing a selected and restricted group of entering optic fibres and mapping of the surviving projection.

Throughout these experiments, points on the tectum explored in the course of the initial mapping of the animal are numbered and marked with small dots. Where field positions were re-plotted the new positions in the field are distinguished with a circle. In the diagram of the field the four poles are indicated, S (superior), I (inferior), N (nasal) and T (temporal) and any retina which had been ablated is shown in terms of the areas of the field it previously represented. In the diagram of the tectum the arrow lies in the midline and points rostrally.

In many cases the data includes the results of re-recording tectal positions after a selective lesion. Where this has been done, each recorded position is marked on the tectum either as a large dot (representing an intact response), a cross, if necessary, superimposed on an initial small dot, (representing the absence of any response), or as a large dot with white cross (designating a reduced response). Empty circles represent positions at which responses could never be obtained. Intact areas are indicated by grey shading and, where available, the observed orientations of the visible tectal striations are indicated. The position of the tectal lesions is shown by a double line.

In the case of animals in which the medial division of the tract was acutely sectioned, surviving tectal responses were surveyed, and the

extent of the corresponding field positions (based on the previously determined fields of those tectal positions) was marked with stippling. In the diagram summarizing this series of experiments, surviving fields are combined for all available animals; filled circles represent normal responses; empty, subnormal.

Where parts of the tectum or retina were removed these are shown as accurately as possible on the basis of gross observation after recording. The extent of tectum ablated was assessed by directly comparing the dimensions of the ablated tectum with the contralateral intact tectum.

RESULTS

Part 1

The electron microscopy of the normal optic pathway of the teleost

Montage 1 (Plate 6) shows the general features of the goldfish optic pathway from the start of the optic nerve to the division of the tract, a length of approximately 4 mm in a 6 cm fish. The diameter of the nerve is about 0.6 mm. As the optic fibres leave the eye they acquire myelin and become grouped into more than 50 bundles of varying sizes each of which is bounded by the sheet processes of one or two astrocytes and, external to these, a basement membrane and collagen with associated fibrocytes (Plates 1, 2A, 2B). A bundle may be internally partitioned by transversely oriented extensions of the astrocyte sheath. The outermost basement membrane surrounding the nerve is continuous with the membranes of the bundles; the connective tissue spaces between the bundles are therefore connected with the space outside the nerve. Occasionally points can be seen at which fibres appear to be passing between bundles, but in general, neighbouring bundles are kept separate by the ample intervening connective tissue, at least until the level of the chiasma. It is not possible to follow individual bundles very far through the nerve (see Montage 1) though one interesting case was observed in which the nerve remained divided into two distinguishable halves for some distance from the eye; a feature which is prominent in other fish species (Stroer, 1940).

Soon after crossing the midline at the chiasma the optic fibres lose their astrocytic partitions and become embedded within the lateral wall of the diencephalon in a single bundle indistinguishable from other central tracts. Desmosomes are no longer seen and astrocytic processes are rare.

As can be seen in Plate 4 optic fibres pass through the tectum in solid bundles occupying a particular stratum corresponding to piano della fibre afferenti of Leghissa (1955), Layer B of Jacobson & Gaze (1964) and Layer r of Attardi & Sperry (1963). With the exception of the edges of the tectum where they occupy a larger volume, bundles lie below about 40 μ from the surface and no deeper than 65 μ. The optic layer can very easily be distinguished because of its characteristic concentrations of myelinated fibres. Attardi & Sperry's "plexiform" layer below it contains very few myelinated fibres while Jacobson & Gaze's Layer D could be recognised as a deeper layer of scattered myelinated fibre fascicles. The optic fibre bundles are thought to account for the longitudinal striations of the tectal surface which can be seen under the dissecting microscope radiating out from the medial division of the optic tract at the rostromedial tectal border.

Because of the intimate relationship between the optic fibres and the tectal neuropil it is difficult to draw conclusions about the later courses and structure of optic fibres. Occasionally small groups of unmyelinated fibres are present towards the periphery of the predominantly myelinated optic bundles but these may be of tectal origin. The proportion of unmyelinated fibres among the myelinated fibres in an optic bundle in the tectum did not appear to be any greater than in the

PLATE 1.

Plate 1.

Cross section of normal goldfish optic nerve fixed by perfusion and postfixed in Dalton's fixative; stained with uranyl acetate and lead citrate; the field includes myelinated fibres, a few unmyelinated fibres (unM) and astrocytic processes (AP) containing fibrils and terminating in desmosomes (D). The edge of the fibre bundle is shown lower left; the bundle is surrounded by basement membrane (B.M.) and collagen.

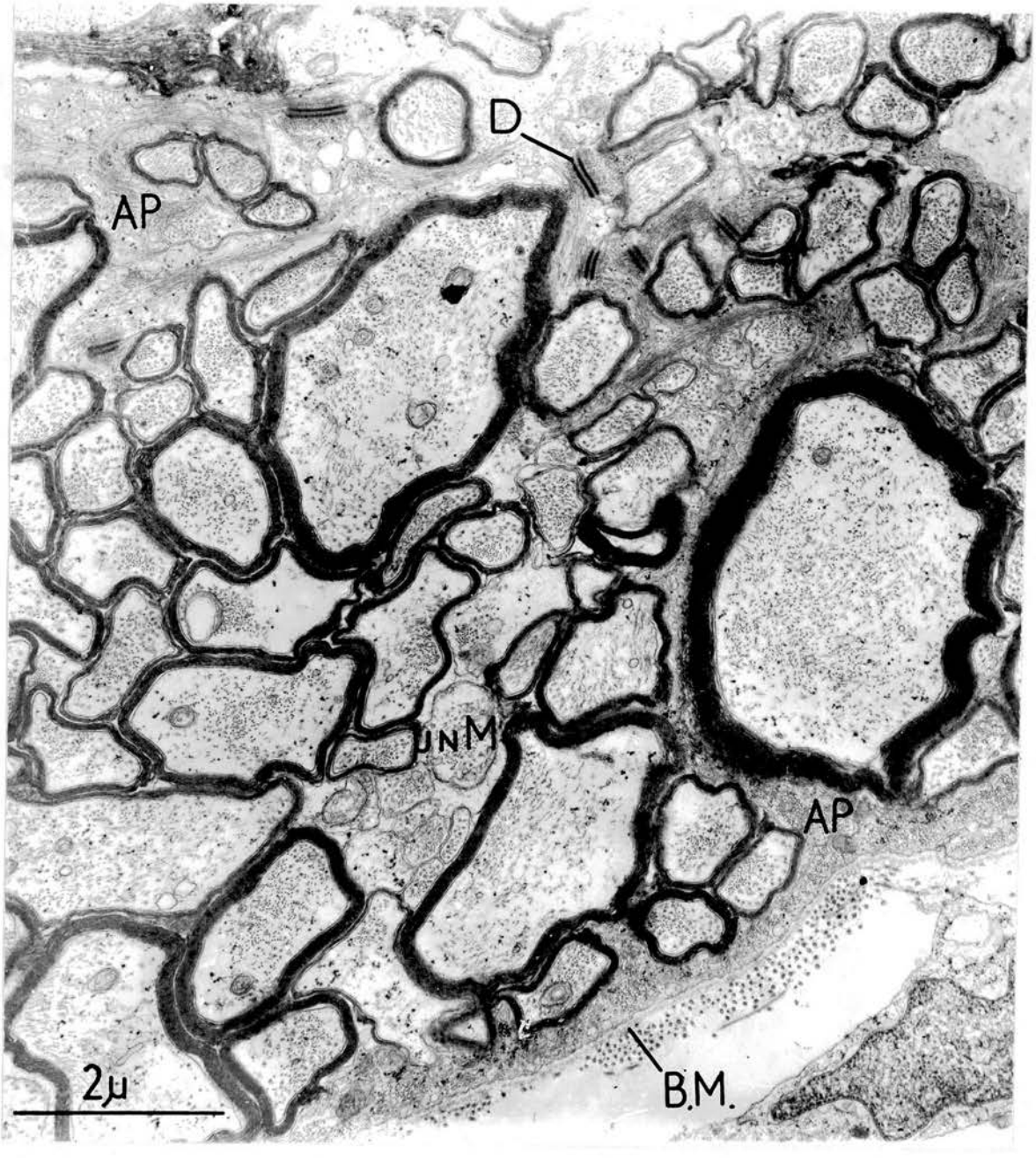

D
AP
μNM
AP
2μ
B.M.

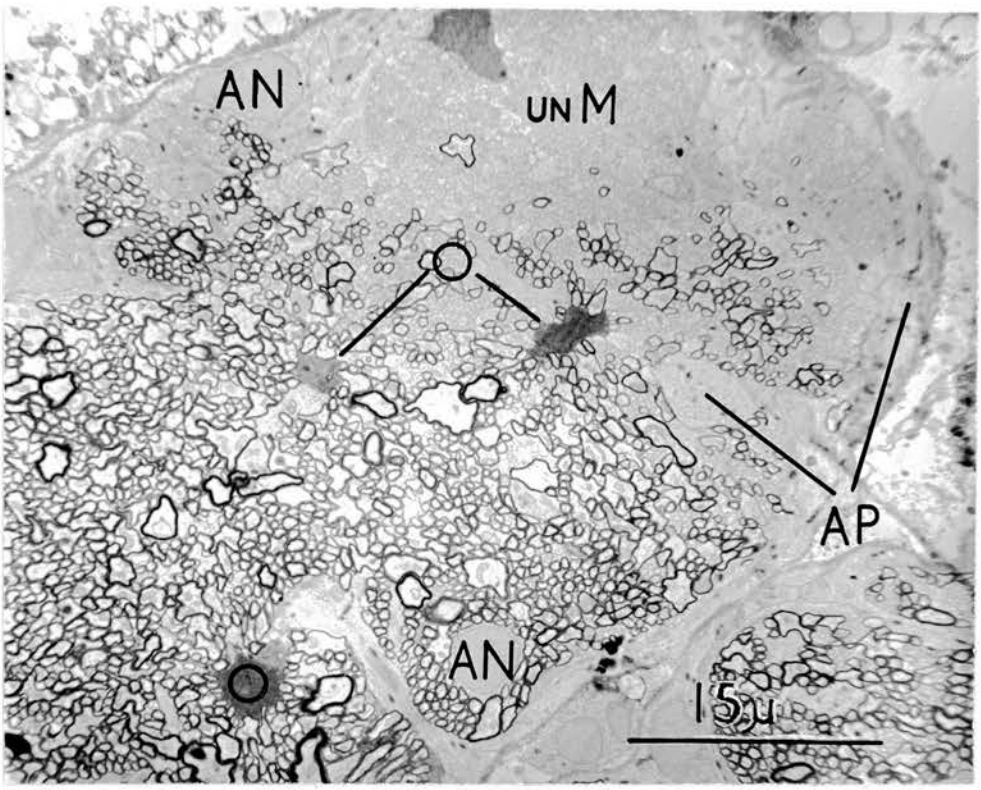
Plate 2A.

Cross section of normal optic nerve showing one bundle part of which is made up exclusively of unmyelinated fibres (unM); astrocytic processes (AP) surround the bundle; two types of cell nuclei are visible, of oligodendrocytes (O) and astrocytes (AN).

Plate 2B.

The same tissue as Plate 2A; a totally myelinated fibre bundle includes one myelinated fibre in process of spontaneous degeneration (D); open connective tissue spaces surround the bundle.

PLATE 2.


AN
UNM
AP
AN
15μ

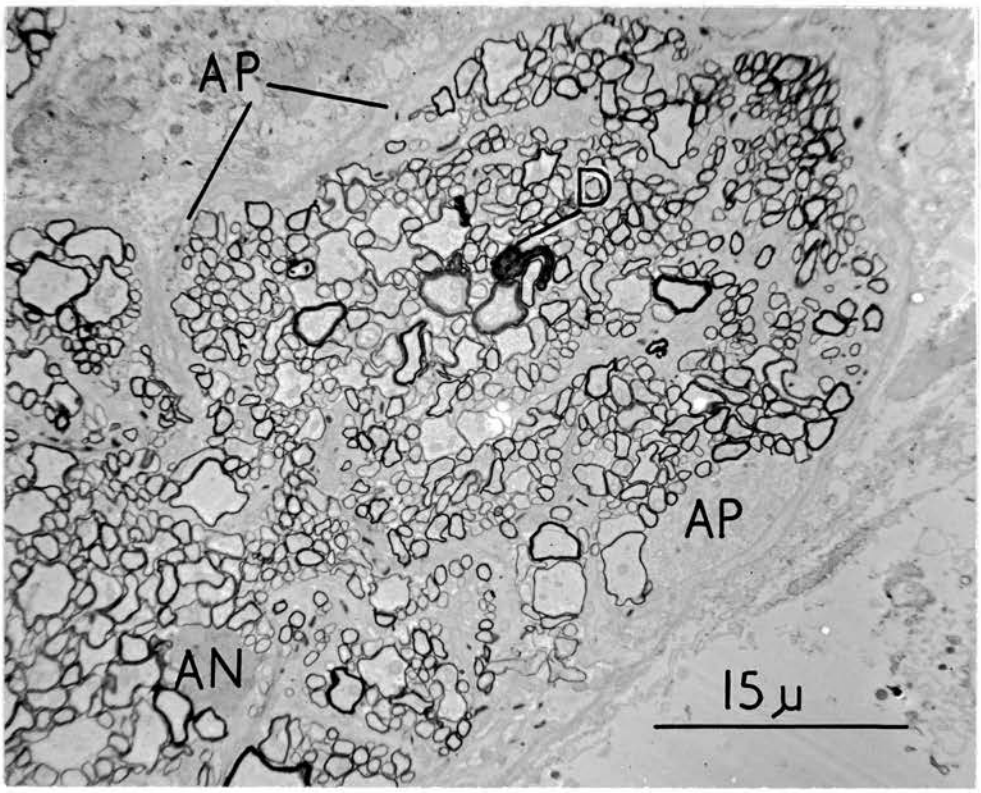

AP
D
AP
AN
15μ

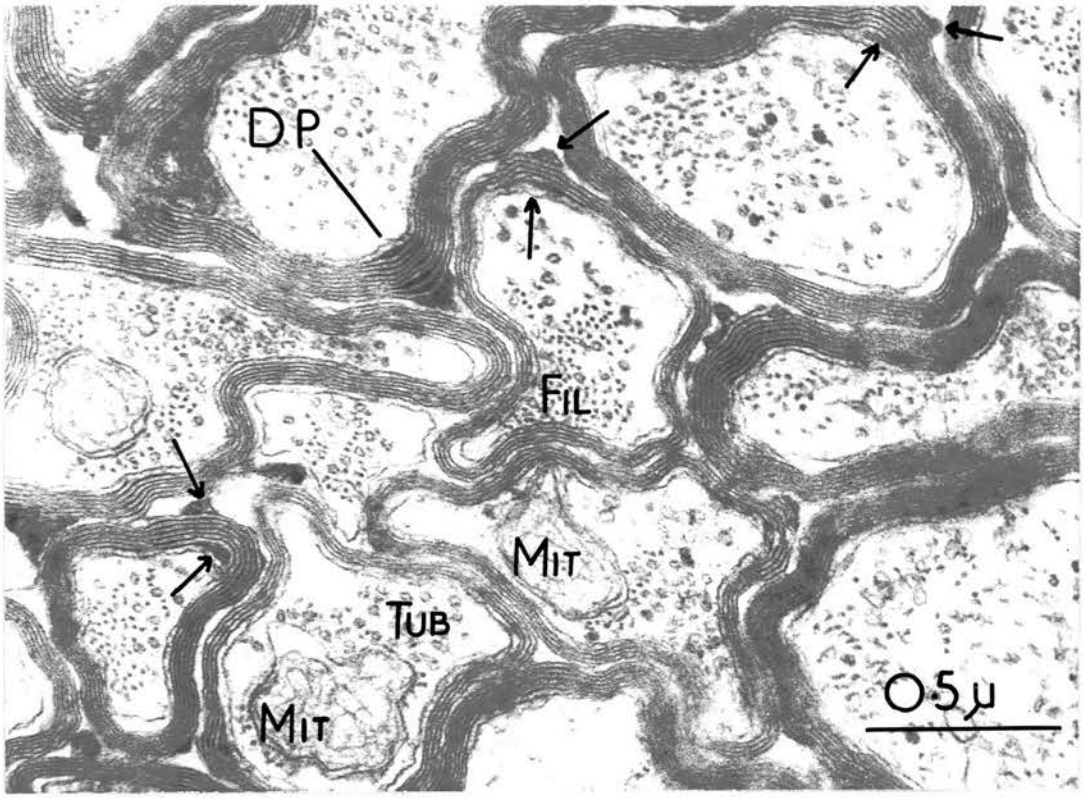
PLATE 3.

Plate 3A.

High power view of cross section of normal optic nerve fibres; arrows show internal and external mesaxons of myelin sheaths; axons contain mitochondria (Mit), neurotubules (Tub) and clumped neurofilaments (Fil); one myelin sheath incorporates intefperiodic condensations (DP).

Plate 3B.

Light micrograph of the region of the optic chiasma of a fish in which the right optic nerve (R.O.N.) had been disconnected for 203 days; the left optic nerve (L.O.N.) contains numerous fibres, which are absent from the right nerve. Holmes silver staining.


DP
FIL
MIT
TUB
MIT
0.5μ

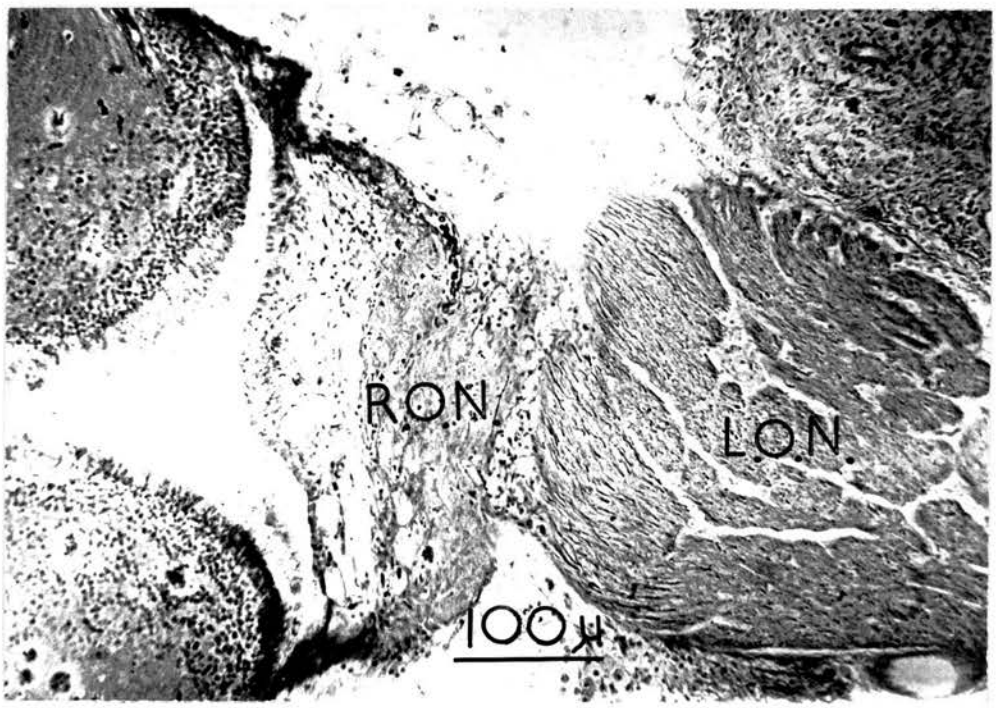

RON
LON
100μ

PLATE 4.

Plates 4A & 4B.

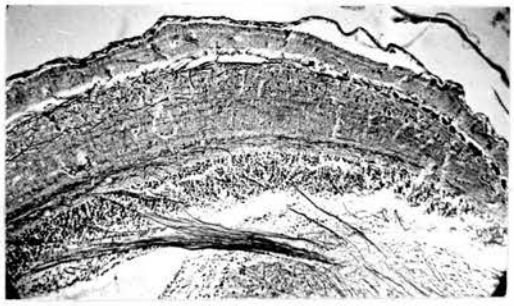
Comparable regions of the lateral edge of the tectum of a normal fish stained by Holmes silver method and osmium respectively; showing concentrated optic fibre bundles in the optic stratum near their origins in the lateral division of the tract.

Plate 4c.

203 day denervated tectum; contralateral nerve was prevented from regenerating by repeated crushing for 135 days; fixed 68 days later, recording having shown no evidence for successful regeneration. Same animal as Plate 3B.

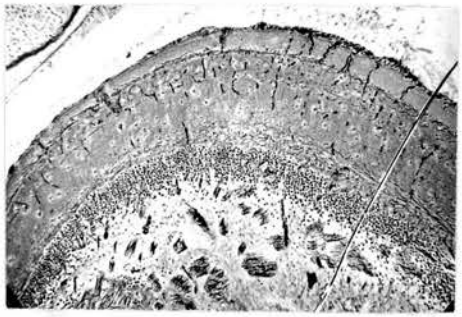
Plate 4D.

Comparable area in normal tectum of the same animal.

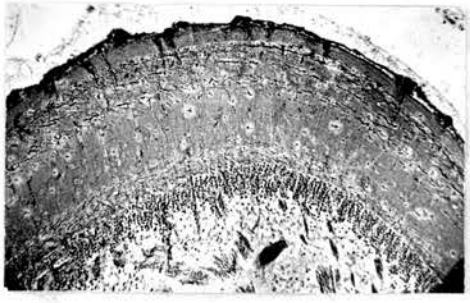


4A

4B



4C



4D

200μ

optic nerve or to increase as the bundle passed further across the tectum. There was no evidence for loss of myelin by fibres within this stratum but few vertically oriented myelin fibres were seen below it. Since responses presumed to originate from fibre terminals are obtained between Layers B and D it seems likely that fibres lose their myelin immediately after leaving the bundle as suggested by Attardi et al. (1963). Surprisingly few myelinated fibres remain by the time bundles have reached as far as the centre of the tectum.

Detailed examination of whole mounts of the optic nerve of four fish showed, in marked contrast to the frog (Maturana, 1960), that over 99% of the cross-sectional area contained only myelinated fibres. When they were present unmyelinated fibres were usually confined to one or two individual bundles (see Montage 1 and Plate 2A), which were usually peripherally placed in the nerve and followed retinotopic courses though they did not seem to occupy any particular segment. In one, younger fish, unmyelinated fibres were more common, especially peripherally, and more scattered.

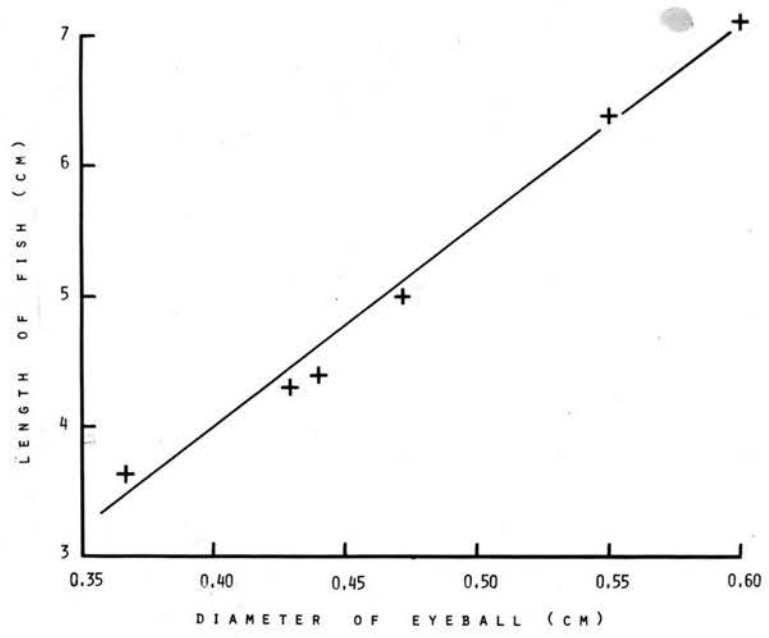
In fish the unmyelinated fibres showed a similar size range to those in the frog's optic nerve (0.15 - 0.6 μ) ranging from 0.14 to about 0.5 μ. Very occasionally, exceptionally large unmyelinated profiles were seen among unmyelinated fibres, one case being 1.3 μ across. As in the frog the unmyelinated fibres are closely packed together with spaces between their membranes of 90 to 250 Å and no intervening processes. Occasionally astrocytic processes of similar dimensions divide the fibres up into smaller fascicles, though such grouping is much more common in the frog. In the fish neurotubules are, if anything, more common than neurofilaments in unmyelinated fibres

whereas in the frog only filaments are common.

Myelinated fibres have diameters (excluding myelin sheaths) from about 0.2 to 6 μ and a maximum of 10 μ, with the majority having a diameter in the range 0.4 - 0.9 μ (see Fig.3B). As can be seen in Plate 3A the outermost layers of the myelin of neighbouring fibres run separately and in parallel with an intervening space ranging from the interperiodic distance of the sheaths up to about 0.2μ at the corners between three axons. The number of myelin layers depends approximately on the axon diameter with the largest fibres having some 25 turns with a periodicity of 100 Å. Interperiodic lines were quite clear here, though they were not in the frog (Maturana, 1960).

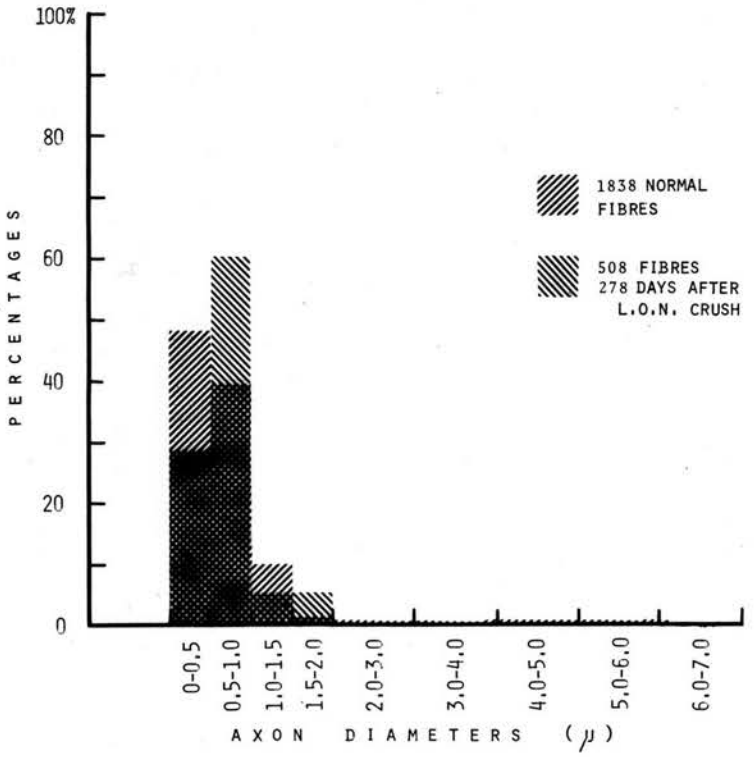
Neurotubules and filaments are common in the axoplasm of myelinated fibres in varying though usually equal proportions, the former sometimes displaying a regular geometric arrangement, and the latter frequently clumped together (Plate 3A). Occasionally smooth endoplasmic reticulum and vesicles were seen. Mitochondria were frequently present; they were very elongated reaching, in one case seen in the tectum, 7-8 μ in length with a diameter of 0.3 μ. Nodes of Ranvier were only rarely seen, and Schmidt-Lantermann clefts never, though the condensations seen in Plate 3A may, judging by Hall & Williams' (1970) paper, be related to these. Occasionally in each bundle a degenerating myelin form was visible (Plate 2B).

Two types of glial cell are present throughout the optic pathway (Plates 2A and 2B) conforming to the characteristics of fish glia described by Kruger & Maxwell (1967). In any cross section taken midway along the optic nerve there might be two astrocytes related to the sheaths of each typical bundle, with two or three others lying within



RELATION BETWEEN BODY LENGTH AND EYEBALL DIAMETER IN 6 FISH OF DIFFERENT AGES
BODY LENGTH: DISTANCE BETWEEN SNOUT AND BASE OF TAIL FIN.

Figure 3A.



HISTOGRAM COMPARING THE MYELINATED OPTIC FIBRE DIAMETERS OF A NORMAL AND NINE MONTH REGENERATED NERVE
DIAMETERS DO NOT INCLUDE THICKNESS OF MYELIN SHEATHS.

Figure 3B.

the bundle, and between one and three oligodendrocytes among the contained fibres. The latter were recognised by their extremely dark, ribosome-rich cytoplasm and dark irregularly-shaped nucleus containing randomly distributed nucleotide condensations. There is a tendency for these cells to lie at the outer edges of bundles and for myelinated fibres to lie in rows radiating out from the cell between its short processes. These cells were not noticeably more common near unmyelinated areas. Although it was very difficult to trace oligodendrocytic processes for any distance, their involvement in the process of myelination is indicated by the dark cytoplasmic fingers which were often seen at one point round the internal and external circumferences of myelin sheaths. As noted by Peters (1964) these points often lie close to one another (three cases can be seen in Plate 3A).

Astrocytes are characterised by their large, rounded nuclei, which have contents of uniform density apart from darkening beneath the nuclear membrane. The cytoplasm is clear and in some cases may appear to be completely empty. Elsewhere it contains glycogen granules and densely-packed strands of fibrils which frequently contribute to the formation of desmosomes at the junctions between astrocytic cell processes (Plate 1). At this point fibrils travel perpendicularly towards the junctional membrane and appear to terminate near or within submembrane condensations. The condensations are approximately 0.5 μ in diameter and 100 Å in thickness and their outer border appears to be formed by the outer leaflet of the astrocytic unit membrane. The inner leaflet is embedded in the condensation. The unit membranes of the cells composing the desmosome are separated by some 400 Å instead of the usual 150 Å, and within the cleft transverse and parallel condensed

formations can be seen. Nerve fibres were never found to be involved in the structural makeup of a desmosome.

No ultrastructural differences were noted in fibres or in their relations at different points along their paths from retina to tectum. In the tectum glial processes are few and each bundle remains distinct from the surrounding tectal neuropil though there is no evidence for any specialised intervening structures. Occasionally astrocytes were encountered lying within the bundle. The characteristic connective cells of the tectum, the ependymal cells (Kruger & Maxwell, 1966), were apparently not involved.

Part 2

Efferent fibres

The uncertainties in identifying efferent optic nerve fibres anatomically have been fully discussed by Brindley (1960). The persistence of apparently normal fibres central to the point of nerve crush (as reported by Maturana (1958) in the toad, and Kruger & Maxwell (1969) in the alligator but not apparent in the present study of the frog; normal fibres are entirely absent within the degenerating bundle in Plate 5A) cannot be taken as evidence because Stefanelli (1968, p.215) reports that fish Mauthner cell axons survive separation from their cell bodies. In any case all normal optic fibres disappear rapidly in the fish (Plate 6); they were never seen in nerves regenerated for more than a month and, as can be seen in Plate 3B, nerves in which regeneration was prevented were almost structureless after several months.

Although Arey (1916) reported physiological evidence for efferent fibres in the fish, Ogden (1966) mentions that Arey himself was later unable to confirm this. The impression was gained here that peripheral to the nerve lesion there was a progressive fall in the number of degenerating fibres from a point near the lesion where most fibres showed retrograde degeneration to points nearer the retina where only a few degenerating fibres could be seen. However, the retrograde response was probably exaggerated in some fibres; section of the optic tract resulted in a proportion of degenerating fibres in the nerve while section of fibres in the tectum had no such result.

Part 3

Retinotopic organisation in the optic nerve and tract in fish and frog

In mammals there is much evidence suggesting retinotopic arrangement of fibres in the optic pathways (Hoyt, 1962). Stroer (1940) was able to follow single fascicles throughout the length of the nerve in Triturus while in Salmo each nerve is completely divided throughout its length into dorsal and ventral parts which interdigitate at the chiasma. However, Herrick (1941) argues that in Amblystoma the fibres are randomly distributed across the nerve. As for the frog, Maturana (1960), Maturana et al. (1960) briefly argue that fibres are randomly arranged on the basis of the orientation of fibres in the nerve and the distribution of degenerating fibres following a small localised retinal lesion.

In one frog (Rana esculenta) and one goldfish, small localised lesions were placed in the dorsal retinae of the right eye and the animals were fixed after intervals of 15 and 4 days respectively. In a second fish a few fascicles of the nerve were cut immediately behind the left eyeball and the animal was fixed on the 25th day after allowing time for regeneration of the lesioned fibres. In each case the entire nerve and tract was embedded with an attached flap of dorsal retina for orientation purposes and sections were taken at intervals of approximately $\frac{1}{2}$ mm.

In Plate 5 the results for the frog are shown; 5A (inset) shows the whole nerve immediately behind the eye with the area of degenerating fibres marked; within this area all fibres are in advanced states of degeneration but elsewhere the nerve was entirely normal and free of

degenerating fibres. The region of degenerating unmyelinated fibres is discrete and clear and the absence of normal fibres confirms that a retinotopic organization of fibres exists at this point.

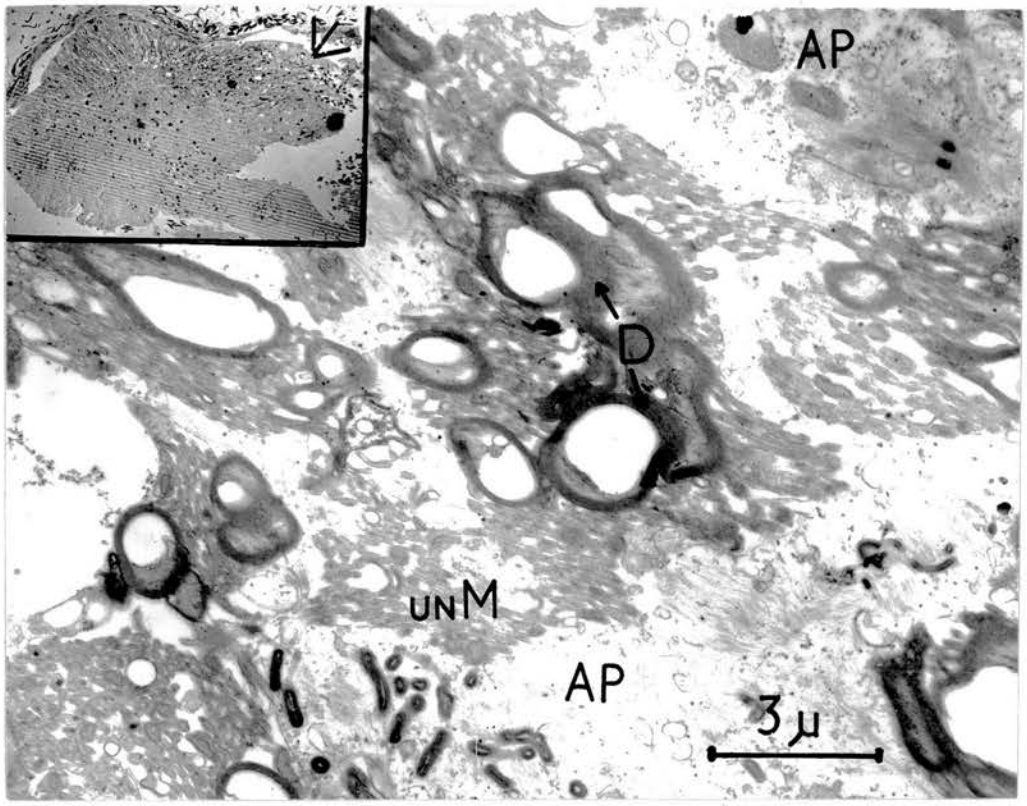
However the fibres which occupy a discrete area at the dorsal pole of the nerve begin to spread out within 0.5 mm from the eye, and the situation reached at 1.25 mm (approximately half way between retina and chiasma) is shown in thick section in Plate 5B (inset) and at high power. In the thick section the dark granulations due to degenerating myelin and reactive glial cells are spread throughout the dorsal third of the nerve; Plate 5B shows groups of degenerating myelinated fibres between fascicles of normal fibres. Outside the dorsal third only normal fibres could be seen. It is concluded that there is a partial breakdown in the retinotopic organization of the fibres as they pass further along the optic nerve, due to the interdigitation of small fibre fascicles as first described by Maturana (1960).

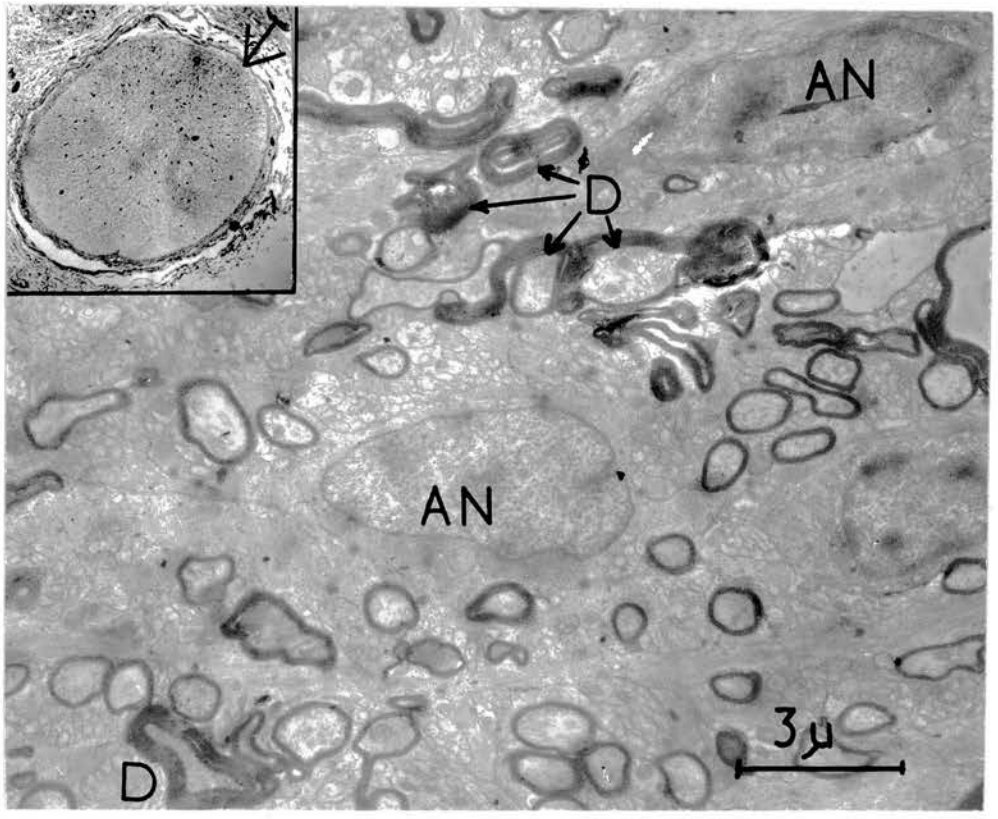
However fibres do not become quite randomly scattered throughout the nerve; from this point on, fibres group themselves together again so that by the time they reach the tectum retinotopic order has been at least partially re-established.

The fish with the dorsal retinal lesion confirmed the data already available (Jacobson & Gaze, 1965) that on entering the optic nerve fibres preserve their retinotopic arrangement. In the second fish the later course of fibres was investigated as shown in Montage 1. In each section of the nerve orientation was established by reference to a dorsal flap of retina or to a tendon which runs dorsally along the optic nerve from the eyeball to the cranium.

The dotted areas were found (Plate 6B) to contain only degenerating

PLATE 5.


AP
D
UNM
AP
3μ


AN
D
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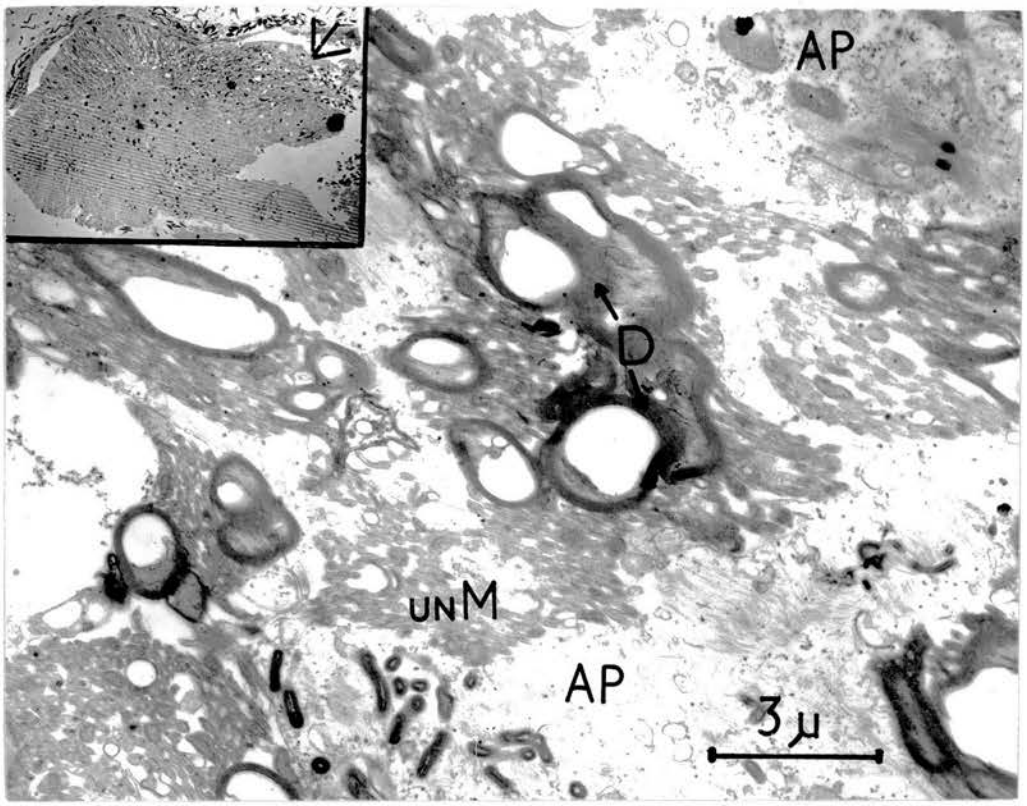
D

Plate 5A.

Inset shows complete cross section of optic nerve of frog taken immediately behind the eye, fixed 15 days after placing a small lesion in the superior retina; bundles of fibres can be seen entering the optic nerve upper left; arrow marks superior pole of nerve and area from which the high power picture was taken. This area contains only the degenerating remains of myelinated (D) and unmyelinated (unM) fibres affected by the lesion and astrocytic processes (AP).

Plate 5B.

Inset (X60 magnification) shows thick section of the same nerve taken 1.25mm central to the previous section; arrow marks the corresponding point on the nerve. The electronmicrograph taken here shows mixed normal fibres and groups of degenerating myelinated fibres (D) and astrocytic nuclei (AN) which presumably give rise to the granulation visible in the superior third of the nerve.


AP
D
UNM
AP
3μ

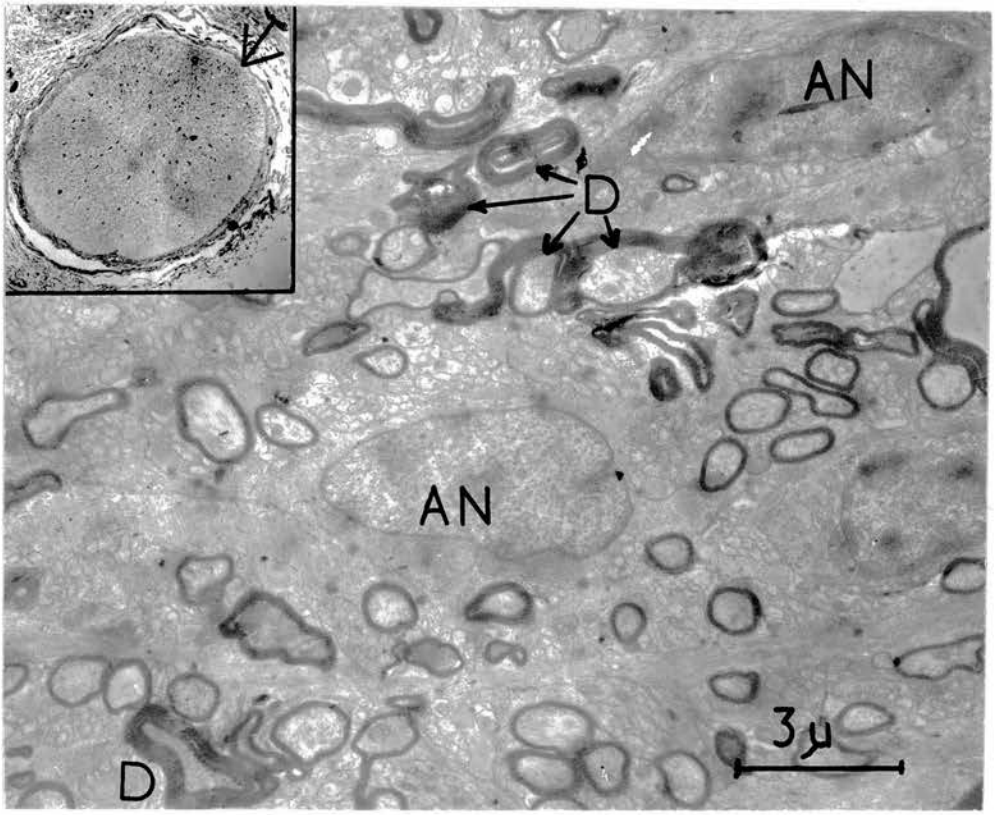
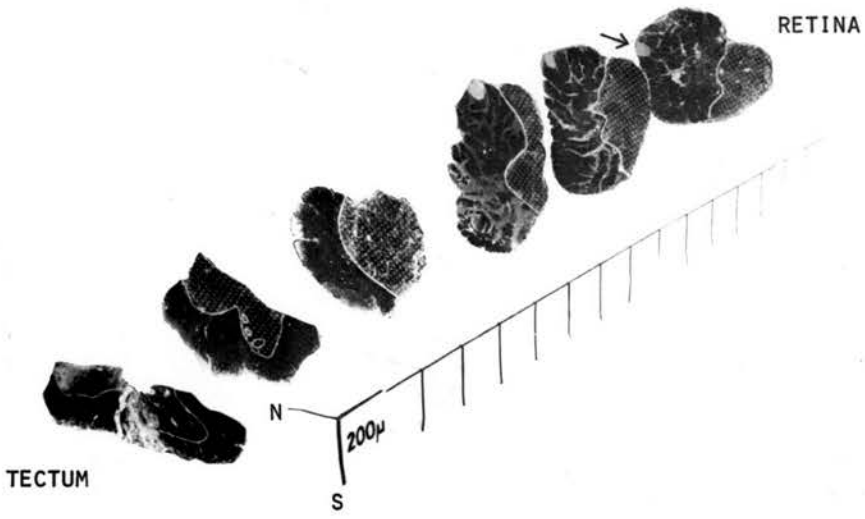

AN
D
AN
3μ
D

PLATE 6.

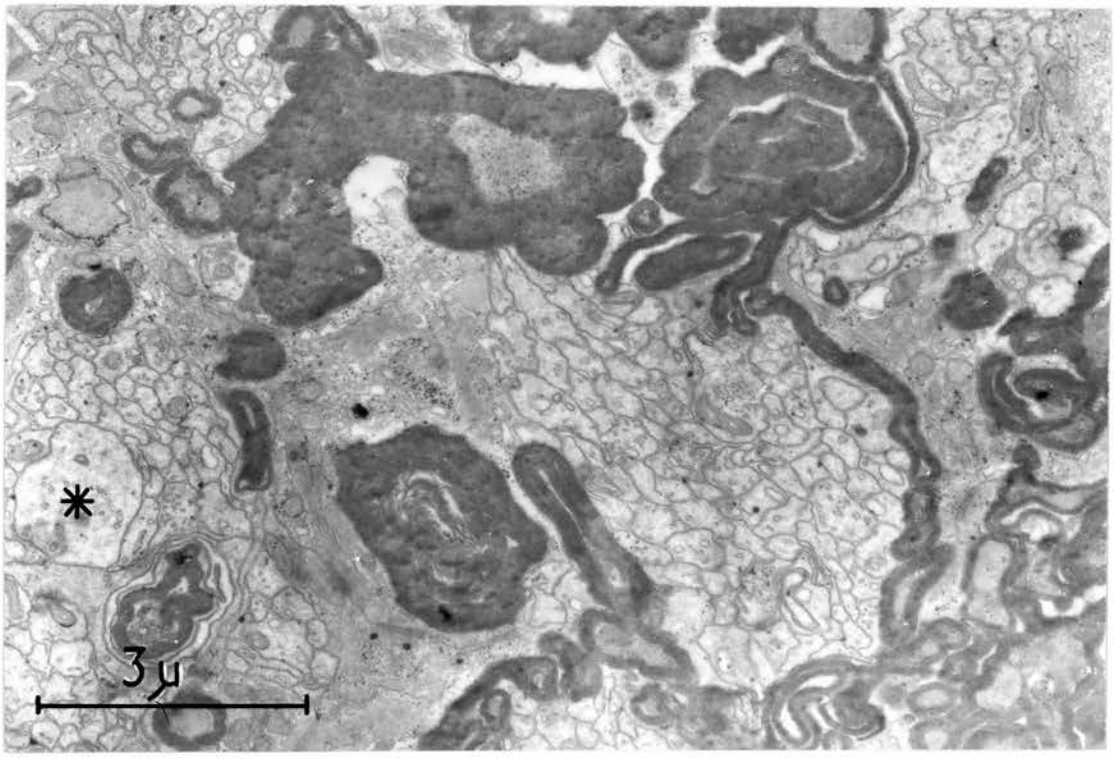
MONTAGE 1.

Plate 6B.

Typical section taken from the stippled area half way along the nerve shown in Montage 1. This area contains only the degenerating remains of the fibres cut 25 days earlier near the eye and groups of regenerating fibres which are deplacing them; asterisk marks an exceptionally large regenerated fibre.



SEQUENTIAL 600μ SECTIONS OF THE GOLDFISH OPTIC PATHWAY FROM THE DIVISION OF THE OPTIC TRACT (LOWER LEFT) TO THE HEAD OF THE LEFT OPTIC NERVE NEAR WHERE THE TEMPORAL REGION HAD BEEN CUT 25 DAYS EARLIER. THE STIPPLED AREAS SHOW THE REGIONS OCCUPIED BY DEGENERATING AND REGENERATING OPTIC FIBRES. THE ARROW REFERS TO A BUNDLE OF NORMAL UNMYELINATED FIBRES. THE SUPERIOR POLE OF THE NERVE IS DOWNWARDS.



myelin forms and regenerating fibres; outside these areas there was a sharp change in the appearance of the nerve to normal. Beyond the level of the chiasma (between third and fourth sections from the retina in Montage 1) and as the nerve looses its fasciculated structure the regenerating fibres move gradually and smoothly round from their original temporal position to a ventral one. Although there are minor shifts in the spatial relationships of fascicles which result in changes in the shape of the lesion area retinotopic organisation is evidently rigidly maintained even within individual bundles because the border between degenerating/regenerating and normal areas cuts straight through them.

The final section in Montage 1 shows the complete separation of the tract into two divisions with dorsal fibres occupying the deepest positions within each; retinotopic organisation is therefore preserved among fibres entering the tectum, and those fibres which leave the tectal bundles first lie ventrally within them.

Part 4

The number of fibres in the optic layer of the normal tectum

A transverse strip of normal tectum was taken from a point approximately half way along the length of the lobe. A thick cross section of the whole tectum was prepared (Plate 4) and the consecutive thin section photographed at 22,500X. Fibre numbers per unit area of the optic layer were determined using representative plates covering about 2.75% of the total area. The areas occupied by myelinated fibre bundles in the optic layer can easily be distinguished in a thick section; these were cut out of a photograph of the thick section and weighed in order to determine the percentage of the total represented by the counted bundles. On the assumption that the density of myelinated fibres was uniform within the bundles across the tectum the total number of fibres passing through this sagittal level of the tectum was estimated to be 40.000.

On the further assumption that the terminations of the fibres are evenly distributed throughout the longitudinal axis of the tectum it follows that the same number of fibres terminate in the rostral half of the tectum as pass on to the caudal. The total number of fibres traversing the tectum is therefore approximately 80,000.

Part 5

Numbers of fibres in the normal fish optic nerve

Among other species, the optic nerve in man contains about one million fibres (all myelinated) (Arey & Bickel, 1935); the cat 85,926 (Donovan, 1967); or 119,000 (Breusch & Arey, 1942) (all myelinated); the rat, 117,000 (Forrester & Peters, 1967) (all myelinated); the newt, 28,000 unmyelinated and 6,000 myelinated and Xenopus, 45,000 unmyelinated and 7,235 myelinated fibres (Wilson, unpublished); Rana pipiens, 500,000 (Maturana, 1959) (3% myelinated); Nikrui (1969) estimated 11,755 myelinated and 234,000 unmyelinated fibres in Rana esculenta.

For the goldfish Breusch & Arey (1942) estimated a total of 53,000 and 52,000 from 10-15% samples of two nerves. Gaze (unpublished light microscope study) counted 68,000 myelinated fibres in one nerve. Fig. 3A shows that among other things the number of fibres in the fish optic nerve may depend on the age of the fish; the size of the eye (and presumably the retina also) increases as the fish continues growth. In the newt (Gaze & Watson, 1968) there is continual addition of retina at the ciliary margin throughout life.

A section of the right optic nerve from a 3.65 cm fish was mounted on a Formvar film and a montage of electron micrographs produced at a magnification of approximately 4,500X. Fibres numbers were counted bundle by bundle, using an electronic counter, and the areas of each bundle estimated by weighing cutouts from a 500X complete photograph of the same section. Two bundles containing regions of unmyelinated fibres were found which occupied less than 1% of the total cross-sectional area of the nerve. Some 50% of the unmyelinated fibres were counted and the remainder estimated on the basis of the concentration of fibres per unit

area. 93.8% of the myelinated fibres were counted. Repeated counts showed an error of well under 5%. Bundle counts were arranged in ascending order of bundle cross-sectional area and the densities of fibres per unit area were determined (the unit was not given any exact magnitude in terms of the actual dimensions of the nerve) in each bundle. The following table summarizes the results. All estimates of fibre numbers were based on one of several alternative determinations of the mean of the fibre densities of a number of bundles.

1). Myelinated fibres; estimate based on small samples	2). Myelinated fibres; idealised estimate (excluding bundles less than 0.56% of total area and two bundles near unmyelinated bundles which have exceptionally high densities)	3). Counted myelinated fibres (93.8% of total)	4). Unmyelinated fibres (50% counted)
Considering 7 small bundles occupying the size range, 0.56-1.0% of the total area	These and the uncounted areas amount to 17% of total area 69,000 counted fibres remain	81.292	Bundle(1) approx. 5,370 Bundle(2)4,890 Among myelinated fibres; 400
Mean of bundle densities; 9,750 fibres per unit area (S.D. 1,270)	Mean of bundle densities now; 9,460 fibres per unit area (S.D. 1,229)	(Mean bundle density; 10,000 fibres per unit area) (S.D. 2,040)	(Density approx. 20 fibres per μ^2)
Total estimated from mean of bundle densities: 84,000 (S.D. 10,900)	Total estimated from mean of bundle densities: 81,500 (S.D. 10,600)	Total including estimate for uncounted areas: 84,500	10,660

TOTAL : 95,160

On the basis of the numbers of fibres actually counted (Columns 3 and 4) the nerve contained 95,160 fibres (including 10,660 unmyelinated fibres). It was found that bundles having areas less than 0.56% of the total cross-sectional area of the nerve and two bundles lying adjacent to the unmyelinated bundles had exceptionally high fibre densities. If these are excluded (Column 2) the standard deviation of bundle fibre densities is reduced; estimates of the number of fibres in the remaining 83% of the nerve based on the new mean value of fibre densities (68,000) are now within 1.5% of the value obtained from the actual count (69,000).

It was also found that bundles as small as 0.56 to 1.0% of the total cross-sectional area had densities which were equally consistent (Column 1) and that estimates of the total number of fibres in the nerve based on the mean of their densities were remarkably accurate; in this case the estimated total of myelinated fibres was 84,000. It appears that one would be justified in basing estimates of the numbers of myelinated fibres in the fish optic nerve on counts of small bundles, provided that the smallest bundles and areas near the unmyelinated fibre bundles had been avoided. The only source of error remaining in reaching this estimate is the measurement of the total cross-sectional area of the nerve; this can be measured with an error of less than 0.86% (Forrester & Peters, 1967).

Part 6

Ultrastructural features of regenerating optic fibres

The immediate result of cutting or crushing the fish optic nerve is the acute destruction and structural disruption of a 0.3 mm segment of the nerve, a retrograde degeneration of fibres which may extend for several hundred microns towards the retina and a centripetal degeneration of all axons and myelin sheaths severed by the lesion.

The course of degeneration in the central nerve stump is in general similar to that recently described for reptiles by Kruger et al. (1969) although in the fish the process seems to be more rapid. Thus Plate 3B shows that all fibres have disappeared from the nerve by 7 months while 5% of the myelinated fibres remained after 20 months in the alligator. In both species, unmyelinated fibres disappear after about one week the most prominent sign of degeneration being disruption of the axolemma. In the frog, where the frequency of unmyelinated fibres is considerably greater (Plate 5B) advanced stages of degeneration are present later than two weeks. In the fish the first sign of degeneration among myelinated fibres is increased granularity of the axonal cytoplasm at about 24 hours. By two days the cytoplasm becomes darkened and the axolemma and myelin sheath have begun to assume abnormal forms, similar to those described in the alligator except that "honeycomb formations" were not seen. (On one occasion similar formations were seen associated

with the outer myelin lamellae of normal fibres lying near recently cut fibres). However it should be said that many of the features of degeneration could be reproduced in normal nerves which were poorly fixed. In degenerating nerves it is especially difficult to assess the fixation. A remarkable feature of the course of degeneration in this study and in that of Kruger & Maxwell is the variability of degenerative forms in any one section. While this may in part be a reflection of the variation between fibres in the normal nerve, it must also be due in part to the complex forms of longitudinal breakdown which occur in the myelin sheath; this is clearly seen in Cajal's (1928) pictures; it would make transverse sections difficult to interpret.

By about three weeks about half the myelin has been removed, and of the remainder 50% appears to be intracellular; 20% retains some axonal remains, which are dark and structureless. The phagocytic cells have large rounded nuclei containing scattered chromatin and moderate density cytoplasm, and commonly contain myelin at many different stages of digestion. Even at two weeks very fibres remain that could be considered normal. In the optic fibre bundles in the tectum degeneration may be somewhat faster but some normal and degenerating myelinated fibres persist for long periods. The visible striations on the tectal surface (which are considered to correspond to the optic fibre bundles) are observable for several months after removing the contralateral eye; this is presumably due to long-lasting myelin fragments and/or non-optic myelinated fibres. This being the case it is perhaps surprising that the results of Attardi et al. (1963) were as clear as they were.

Regenerating fibres can apparently arise from the peripheral stump fibres in two way (Cajal, 1928). Plate 7 shows enlarged myelin sheaths

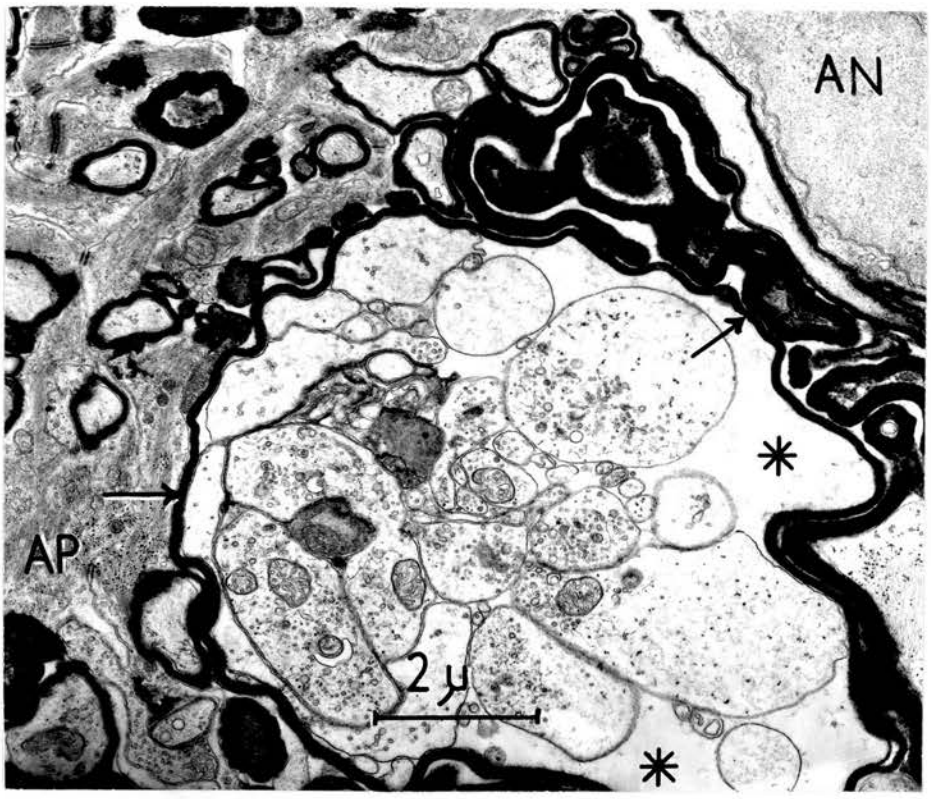
PLATE 7.

Plate 7A.

Section taken near site of nerve crush performed 7 days before fixation. Arrows point to an enlarged myelin sheath containing numerous cell processes some of which contain "wispy" membrane profiles and spaces (asterisks).

Plate 7B

Another part of the same section. A myelin sheath contains what are presumably axonal sprouts (P) of various dimensions.


AN
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2 μ

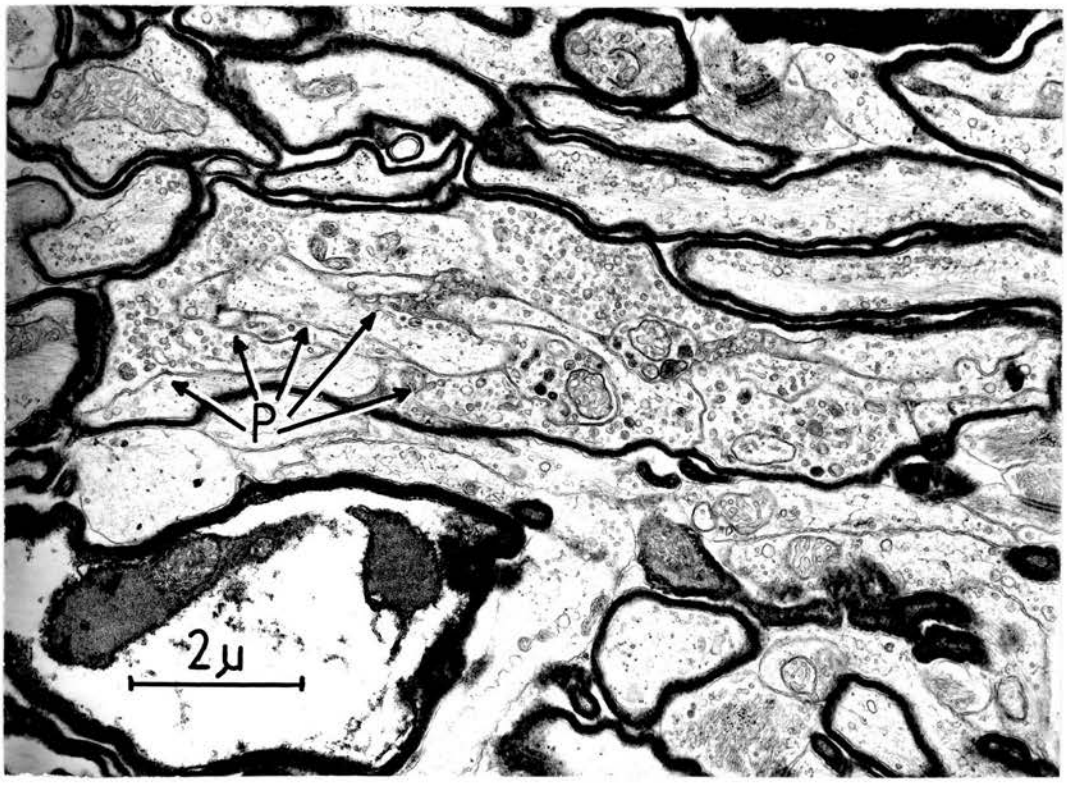

P
2μ

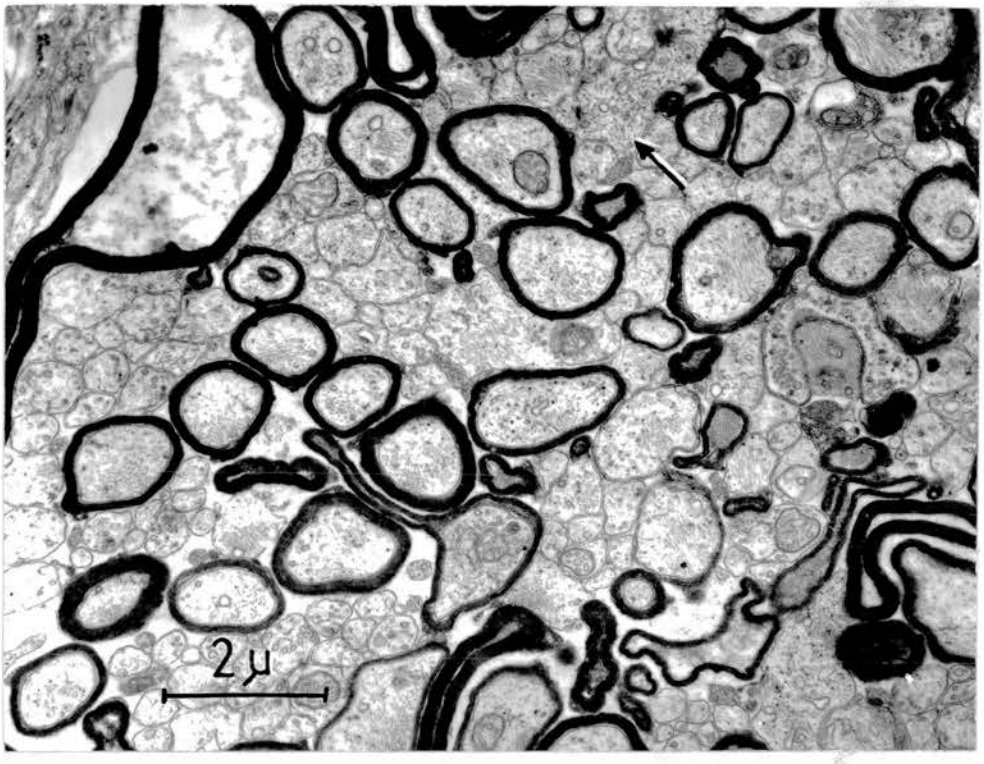
PLATE 8.

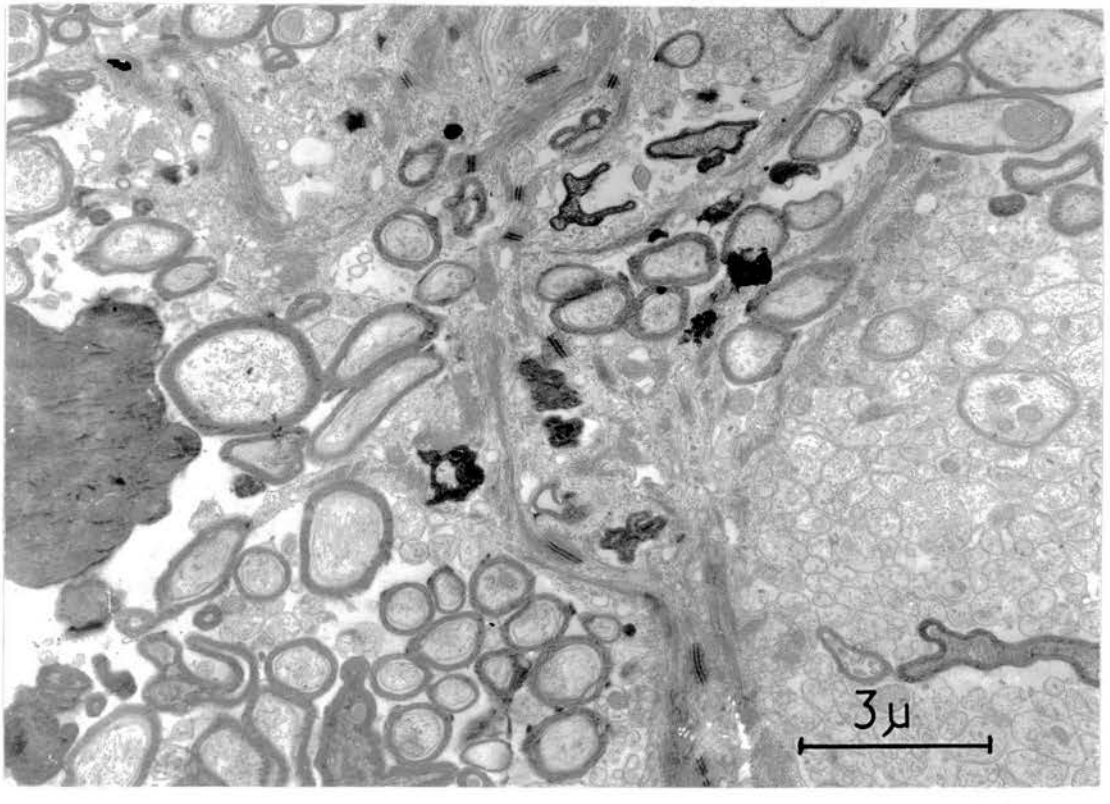
Plate 8A.

Peripheral nerve stump fixed 13 days after nerve crush. Some myelinated fibres show retrograde degeneration; others are normal; between these are numerous axonal sprouts newly formed nearer the eye. Arrow shows apparent growth cone region containing characteristic membrane formations.

Plate 8B.

Optic nerve fixed 155 days after incomplete intraretinal section of optic fibres; areas of degenerating, normal, and regenerating fibres are relatively distinct.


2μ


3μ

containing some twenty axonal sprouts observed in the peripheral stump of a nerve crushed 7 days earlier. Zelena, Lubinska & Gutmann (1968) show similar formations in peripheral nerve fibres and the appearance of the bundle of sprouts is reminiscent of the processes seen in tissue culture by Grainger et al. (1968). Thus the damaged fibre may extend a number of fine processes along the remaining myelin sheath towards the lesion. Alternatively sprouts may originate at a node of Ranvier. An apparent instance of this is Plate 14B seen in tectum 11 days after a lesion of the tectal striations.

It is of some interest that collateral sprouts were not produced by nearby normal fibres in response to axonal degeneration; during the degeneration of a small optic nerve bundle (Montage 1) no axonal changes were seen in normal regions of the nerve. Causey & Hoffman (1955) report collateral sprouting from intact fibres during partial degeneration of a mammalian peripheral nerve.

Regenerating fibres were identified primarily on the basis of the fact that elongated processes containing filaments, mitochondria and sometimes tubules, of regular and constant diameter and orientated longitudinally, which are common in a regenerating nerve, are absent under conditions which are comparable but for the fact that the formation of regenerating axonal processes has been prevented; e.g. after enucleation. Though regenerating fibres are similar to unmyelinated fibres (which range between 0.14 and 0.5 μ in diameter) there can be no doubt that the processes in the central stump during regeneration are due to regeneration because unmyelinated fibres are very few throughout most of a normal nerve. Newly regenerated fibre processes could also be distinguished structurally; they are less

regular in shape than unmyelinated fibres, and contain vesicles but not neurotubules (mitochondria and neurofilaments are common even in the earliest regenerated fibres). Their diameter is similar (0.2 - 0.6 μ) though it may be increased at the growth cone region up to some 2 μ.

Although expanded regions which appear to be terminating centrally-oriented regenerated fibres are seen (Plates 9 & 11A), a new criterion for the identification of the "growth cone" region became apparent. It was frequently found that the earliest fibre processes (in both the nerve and the tectum) contain a variety of vesicles and smooth tubular profiles of which one form is particularly characteristic; these are short, flattened and sometimes branched tubules, very approximately 100 Å across and 0.4 μ long in typical cases. They seem to belong to the same population of membrane forms as the vesicles in that there are many intermediate forms and their general appearance could perhaps best be described as "wispy". In other cases (Plate 14A) there are similar formations which are less flattened and appear to be more closely related to endoplasmic reticulum.

That these formations are characteristic of the growth region is suggested by the fact that they are common in the earliest regenerating processes (Plates 7, 9, 10, 14) though rare in cross sections of recently regenerated fibres (Plates 11, 13, 15). In some sections it was possible to observe expanded, central regions of a bundle of regenerating processes containing these formations while more peripheral fibres already have the characteristics of established regenerated fibres and contain filaments and a few vesicles only (Plates 9, 11A).

The nature of these vesicular/tubular formations was discussed by Estable, Acosta-Ferreira & Sotelo (1957) who were the first to draw

PLATE 9.

Plate 9.

Growing region of regenerating fibre bundle near site of nerve crush performed 13 days earlier; the group of axonal processes (P) is enclosed in a basement membrane (Bm); processes of surrounding cells (arrows) make specialized contacts; expanded regions of the processes (asterisks) contain "wispy" formations.

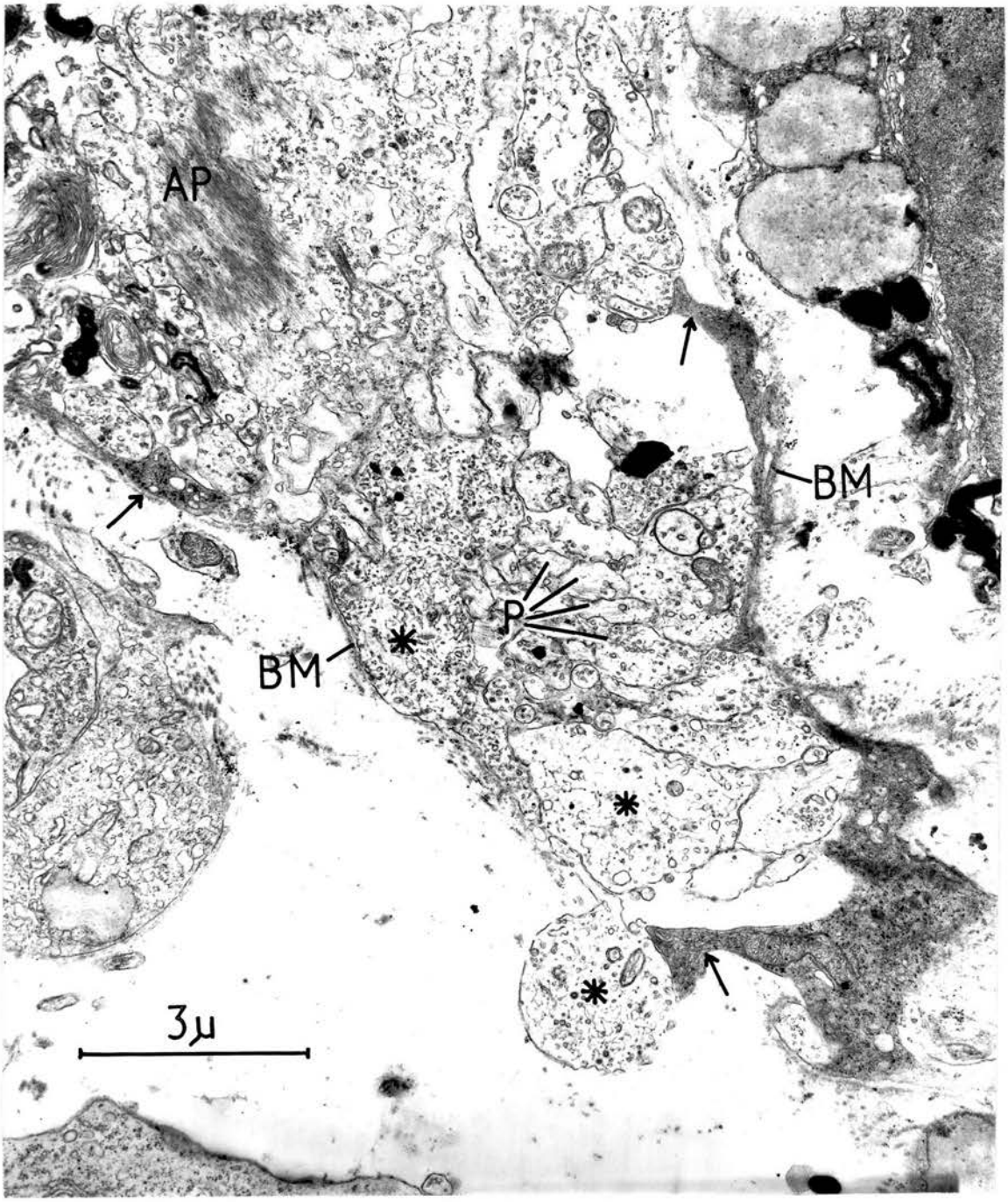
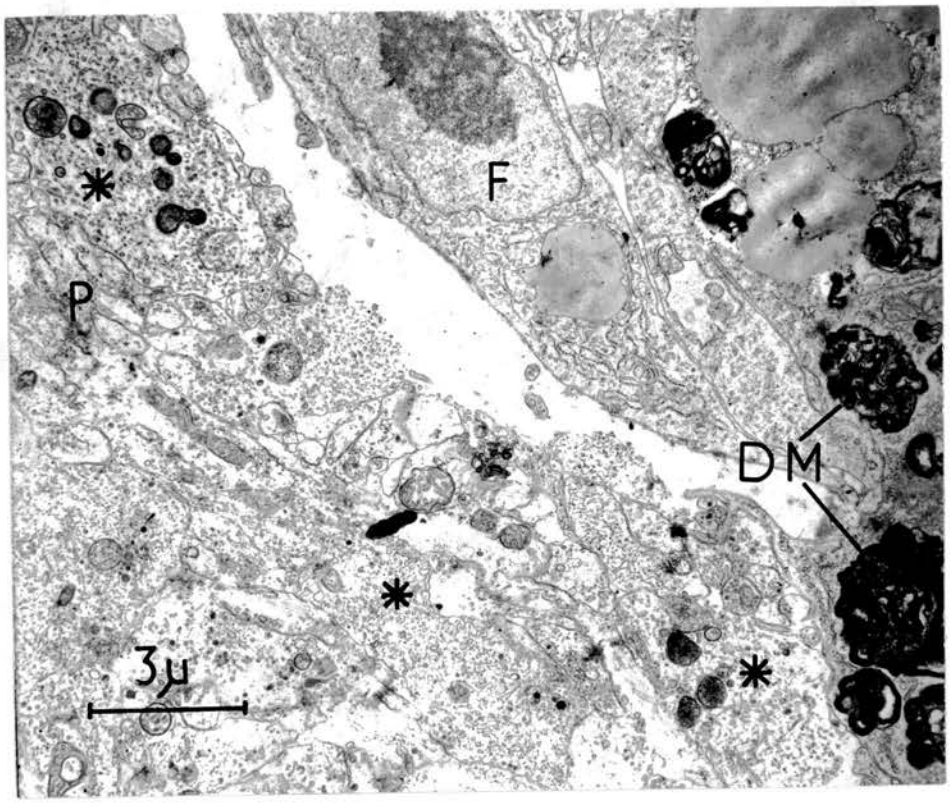

AP
BM
P
BM
3μ

PLATE 10.

Plates 10A and 10B.

Further views of growth cone region of the nerve shown in Plate 9; fibroblast (F); intracellular myelin remnants (IM).


F
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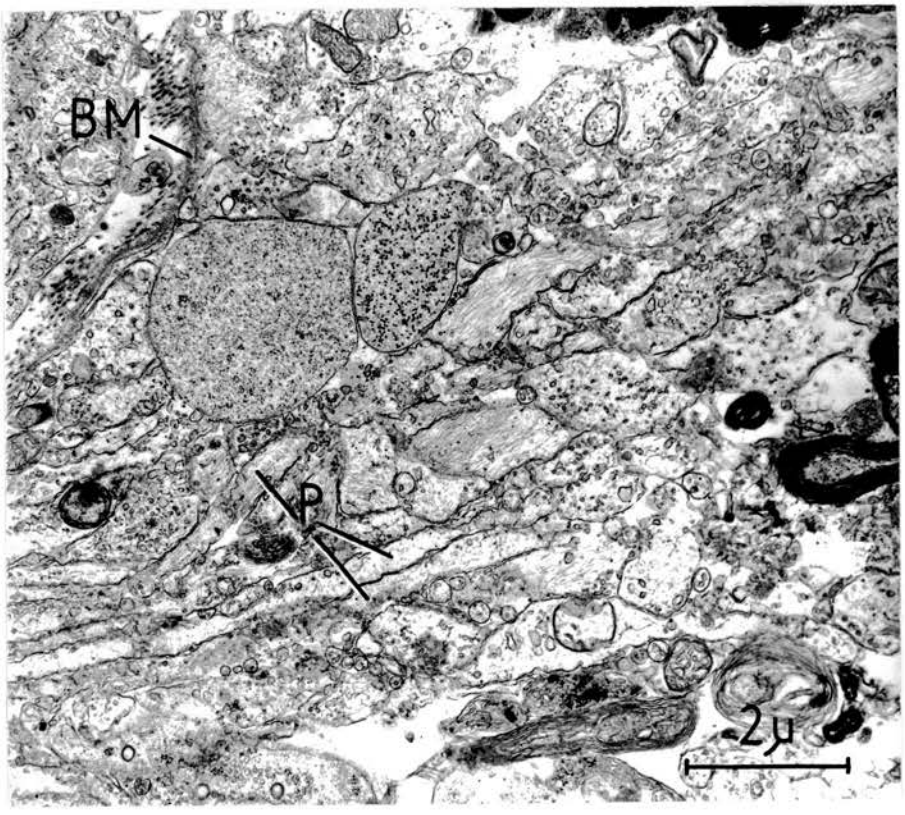
DM
3μ


BM
P
2μ

attention to them in this case in regenerating mammalian peripheral nerve. Identical formations are apparent in the work of Lentz (1967), in amphibian peripheral nerves under regeneration, Lampert & Cressman (1964), Del Cerro et al. (1968), and Bernstein & Bernstein (1969), in regenerating and developing central nervous system, and in several studies of regenerating mammalian peripheral nerve (see Introduction, Table 1). Mugnaini et al. (1967) observed similar structures in developing chick mossy fibre terminals and reported their gradual replacement by synaptic vesicles; Estable et al. (1957) ascribe to them the function of vesicle formation. Whether these formations originate from endoplasmic reticulum, neurotubules or neurofilaments cannot be determined but they do not appear to be related to the axolemma; they are most common within the cytoplasm. They may be a consequence of the damming up of axoplasmic flow since similar formations have been seen prior to regeneration in divided fibres (Rodriguez-Echandia, Zamora & Piezzi, 1970; Zelena et al., 1968) and in dystrophic dorsal column terminals (Hashimoto et al., 1965). This is suggested here by the fact that the formations can be seen in the cytoplasm of otherwise normal myelinated fibres peripheral to the lesion (Plate 8A).

A related phenomenon may be the vastly expanded axons seen proximal to the site of a partial transected nerve (Plate 12). These appear to be dystrophic responses; the axons are full of neurofilaments.

Plate 8A, shows normal, retrograde degenerating and vesiculated myelinated fibres and, between them, recently regenerated fibres originating peripheral to the lesion, some of which contain vesicular/tubular formations. In the present studies the first fibres to cross the lesion area were seen between 6 and 15 days, in the nerve as well

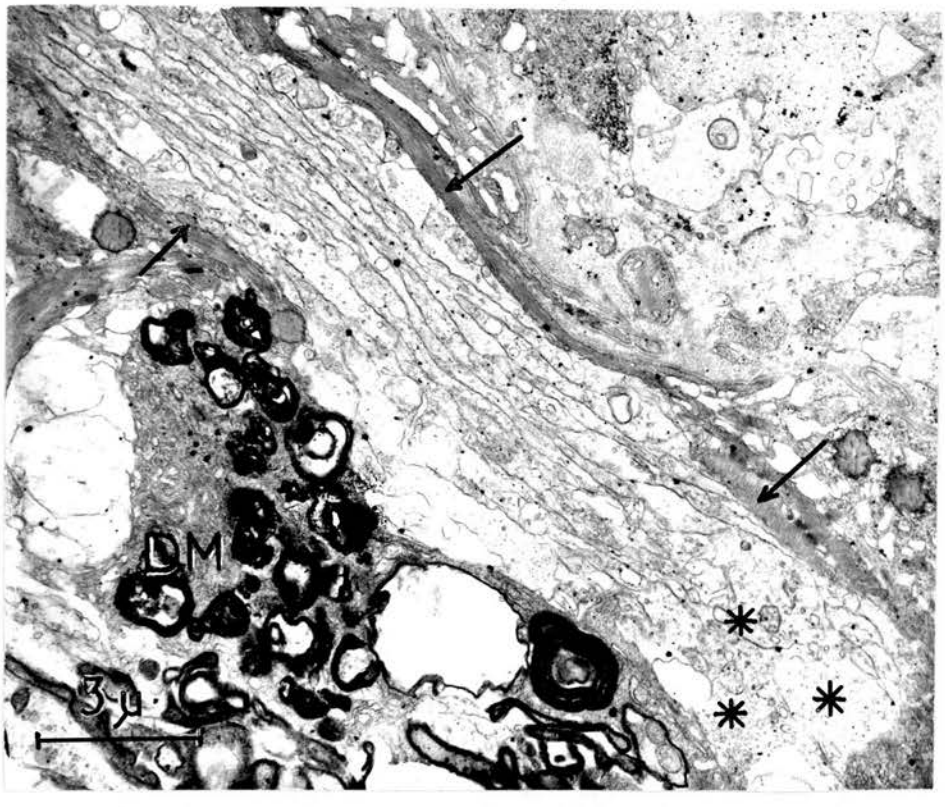
PLATE 11.

Plate 11A.

Longitudinal section of newly regenerated optic fibre bundle; from nerve crushed 6 days previously; arrows show compact astrocytic sheath; asterisks show expanded regions of axons.

Plate 11B.

Transverse section of comparable bundle: from nerve crushed 29 days previously.


DM
3μ

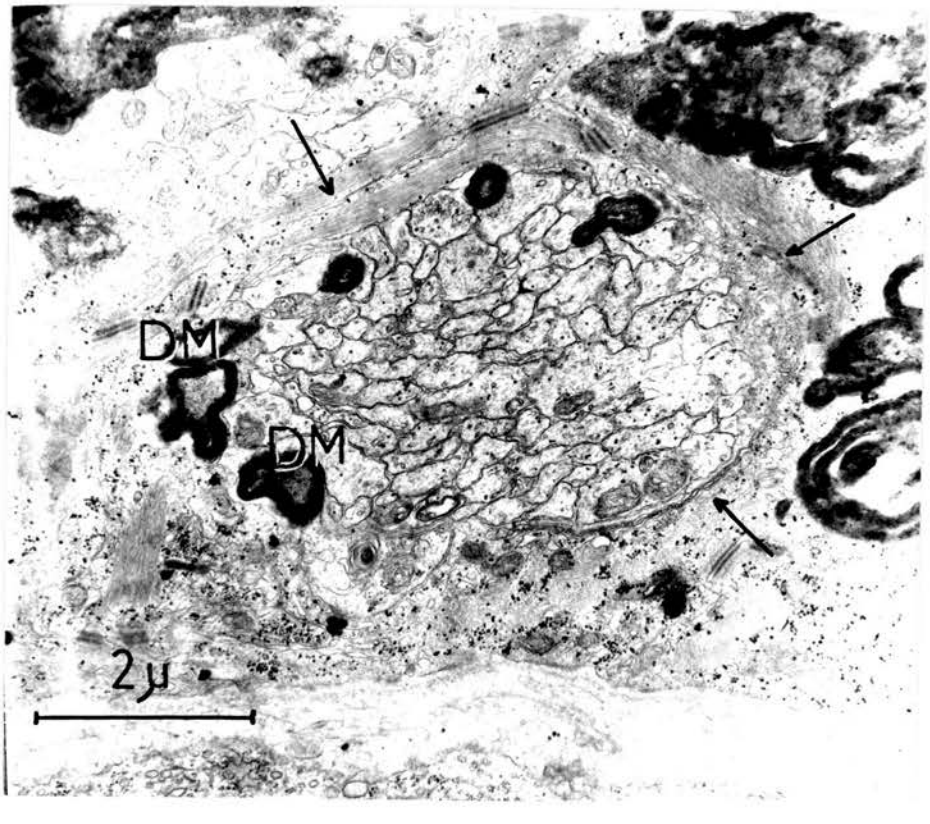
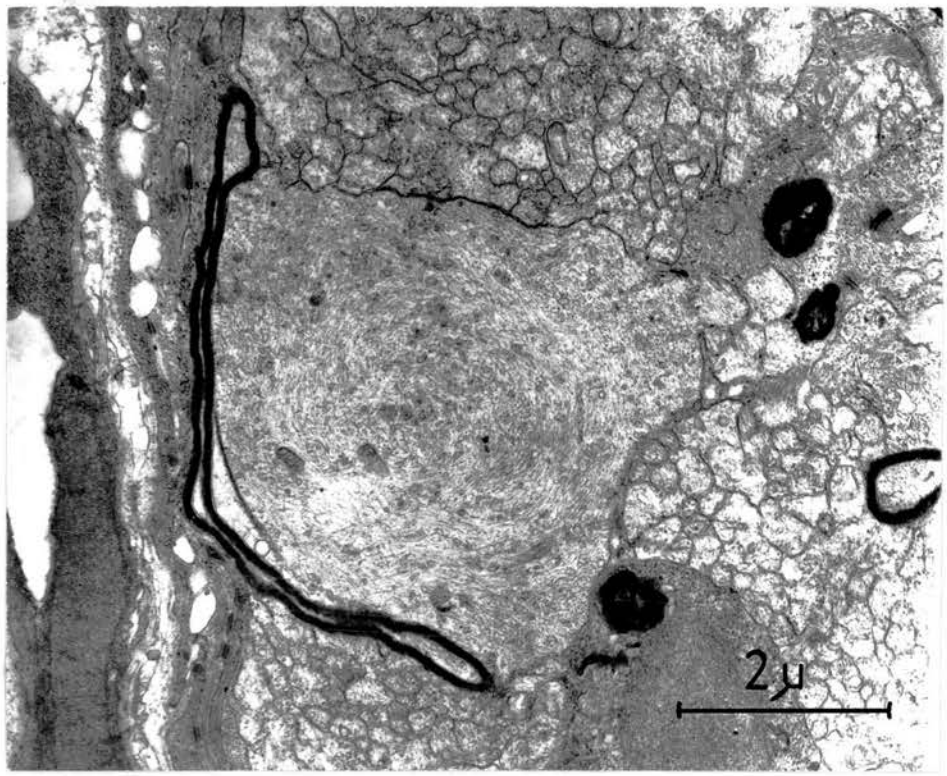
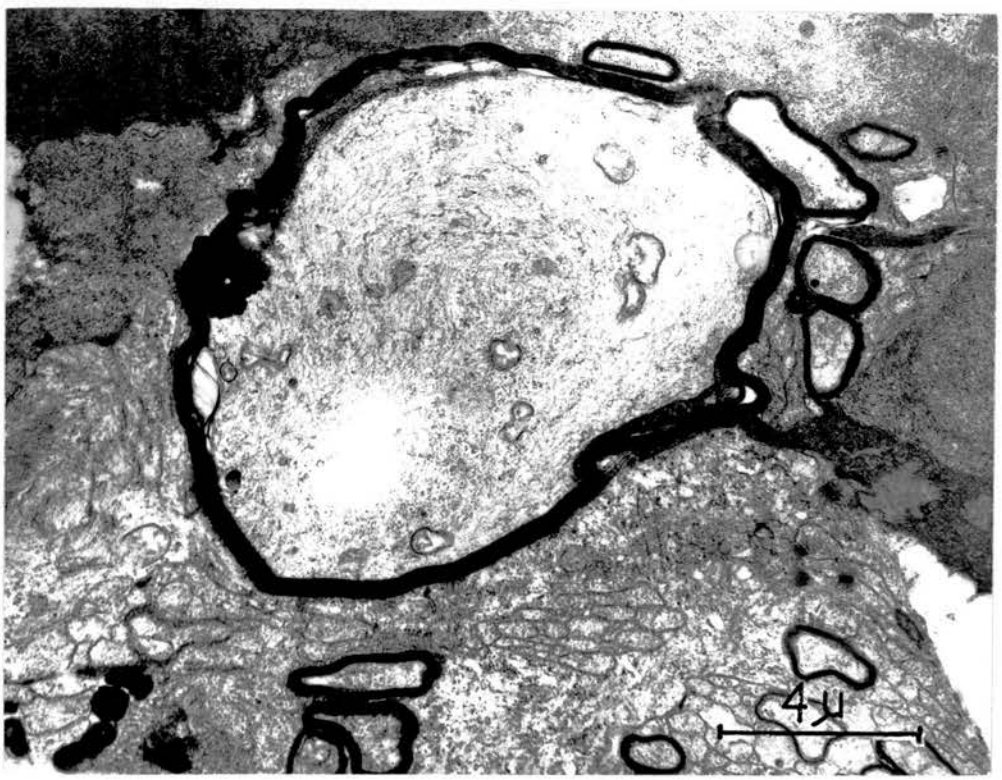

DM
DM
2μ

PLATE 12.

Plates 12A & 12B.

Two greatly enlarged "dystrophic" axons, one still inside its myelin sheath, containing densely packed, regularly arranged neurofilaments; both from region near site of nerve transection performed together with removal of half the retina, 85 days before fixation.


2μ


4μ

as in the tectum. At least a part of this time delay is due to the time required for the formation of sprouts by damaged fibres. Regeneration may also be delayed by the mechanical conditions existing after damaging the nerve. The tissue met by the new fibres as they enter the lesion area can be seen in Plates 9, 11, 13A.

In the central stump regenerating fibres normally meet tissue which retains its normal, partitioned framework made up of astrocytic processes, although, as can be seen in Montage 1, this may be partially obliterated by the collapse of myelinated fibres and the invasion of reactive glial processes. At the lesion site, however, there appears to be only a randomly arranged assemblage of reactive cells including fibrocytes. The earliest stages of penetration of this region are shown in Plates 9, 10, 13A. As seems to be the case in the developing nervous system (Tennyson, 1969; Hughes, Egar & Turner, 1969), fibres initially penetrate a very open structure including many apparently empty spaces, and have no special relationships with surrounding structures (Plate 9). At a very early stage, a group of fibres acquires a surrounding basement membrane sometimes with associated collagen (Plate 9, 13A). The profiles of the earliest fibre processes are relatively smooth and there was no evidence either for specialised indentations or for growth cone processes in anyway similar to microspikes or filopodia (see Introduction). A number of fine rounded profiles were sometimes visible between fibres, which are more likely vesicular than cylindrical, with diameters of about 0.2 μ (Plates 9, 10, 13A) but as is apparent in Plate 13A these are not confined to the neighbourhood of fibres.

As discussed by Cajal (1928), some features of the growth cone may be peculiar to the particular conditions existing at the lesion site but

the present description of the growth cone region applies equally to lesion site, central stump (Plate 13B) and tectum (Plate 14A). This is despite the fact that the surroundings of the fibre are quite different in these places; as fibres penetrate the central stump the basement membrane is replaced by a tight astrocytic sheath compounded in much the same way as the sheaths of bundles in the normal optic nerve. This sheath in general isolates the fibre bundle from reactive and degenerating regions of the old nerve.

In the tectum (Plates 14, 15) there was no evidence for any such form of sheath at any stage of the regeneration process and the growing point of the axon appeared to come into immediate contact with any structures lying in its path. In the optic nerve astrocytic sheaths were never seen prior to the entry of fibres; the fact that the basement membrane and astrocytic sheaths are adjusted to the exact shape and size of the individual bundle also argues that these structures are deposited around the fibres <u>after</u> their outgrowth. There was no evidence here for the existence of <u>preferential paths</u> for regenerating fibres. Similar sheaths are absent in the peripheral stump (Plate 8a).

In some cases nerves were cut a second time shortly after regeneration in order to study the degeneration of regenerating processes. In general this process was similar to that seen in unmyelinated fibres with noticeable cytoplasmic granularity evident by two days. By six days little more than disrupted membrane outlines remained. Similar forms were not seen during the early stages of normal regeneration and there was no evidence to support the hypothesis that selective regeneration is achieved by the selective degeneration of incorrectly growing fibres after an initial massive random invasion by

fibres. Degeneration was, however, seen in one regenerated nerve (Plate 17).

The most striking positive finding of this study was that fibres grow out from the peripheral stump in groups even at the earliest stages. The constituent fibres are tightly packed and there are no other components of the bundle, which very soon becomes insulated from the surrounding tissues by astrocytic processes. The bundle appears to follow a relatively straight course (see Plates 11A, 13B, 15); a useful comparison is the frog's optic nerve in which the irregularity of the growth paths of groups of fibres can easily be seen in TS or LS (Maturana, 1960). Although the growth cone regions of individual fibres are less than 2 μ in diameter the total diameter of the tip of a regenerating bundle (Plate 9) commonly reaches the dimensions and form of growth cones reported elsewhere. In short, the regenerating optic fibre bundle may well reproduce many of the features previously ascribed to single fibres; the process of advance of fibres in the optic nerve is similar to that described by Grainger et al. (1968) in tissue culture.

A likely consequence of these structural features is that fibres cannot readjust their growth paths once they have entered a bundle and if an axon forms branches during its later advance all of these must follow the same course. It follows, though there is no direct evidence to support the suggestion, that fibres must establish their retinotopic order before entering such bundles; the most likely site would be before entering the central stump of the nerve. Although silver preparation show considerable apparent disorganization of fibres in the lesion area, interpretation is complicated, among other things, by the

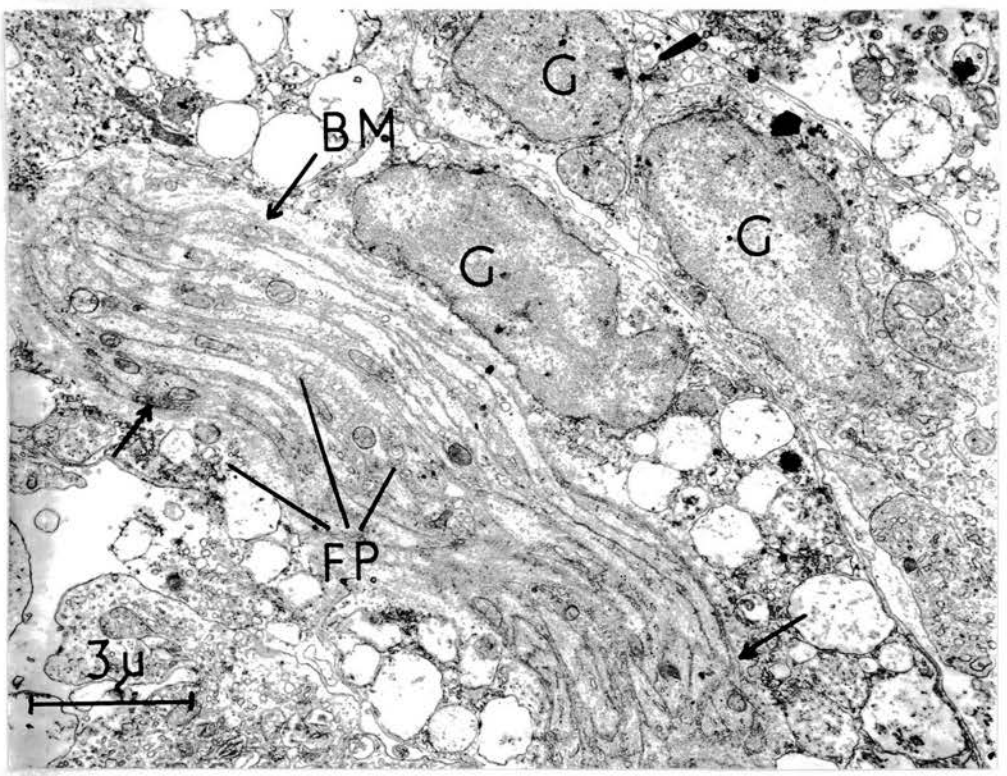
PLATE 13.

Plate 13A.

Early regenerating fibre bundle crossing region of nerve crush performed 12 days before, surrounded by basement membrane (BM) only, which may have been deposited by nearby, unidentified glia cells (G); both inside and outside the bundle some very fine (0.2μ) membrane profiles (F.P.) can be seen which are apparently extracellular.

Plate 13B.

Central portion of nerve partially transected 29 days before fixation; rows of astrocytes form partitions between regenerated fibre bundles; the myelinated fibres may be fibres which remained intact after lesioning.


G
BM
G
G
FP
3μ

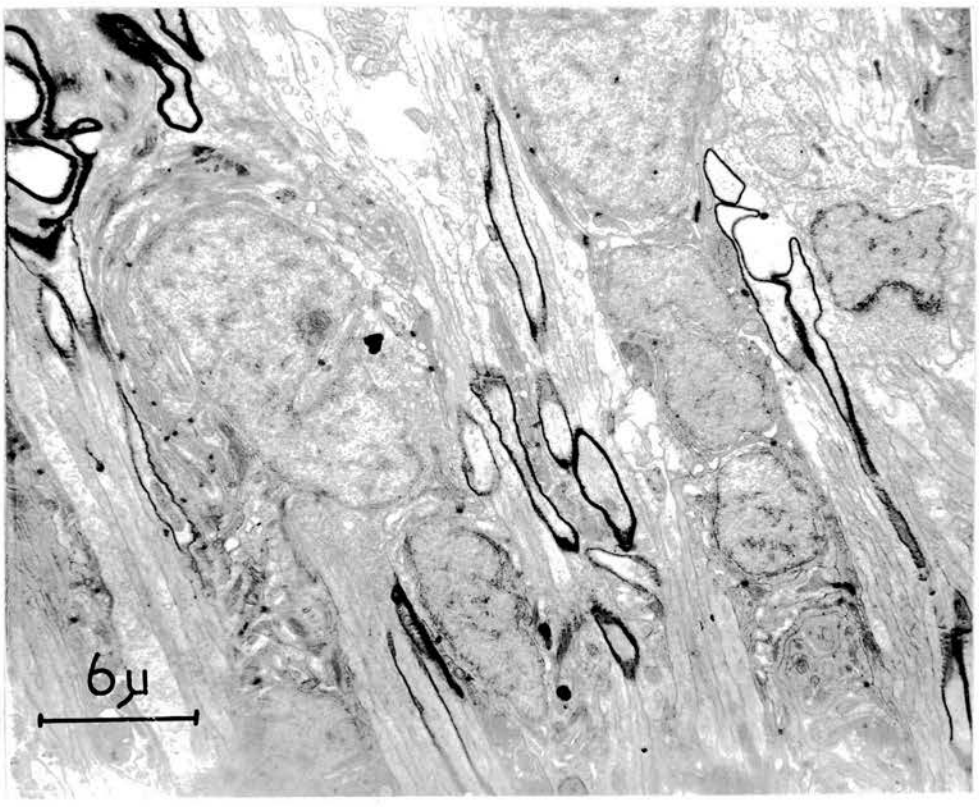

6μ

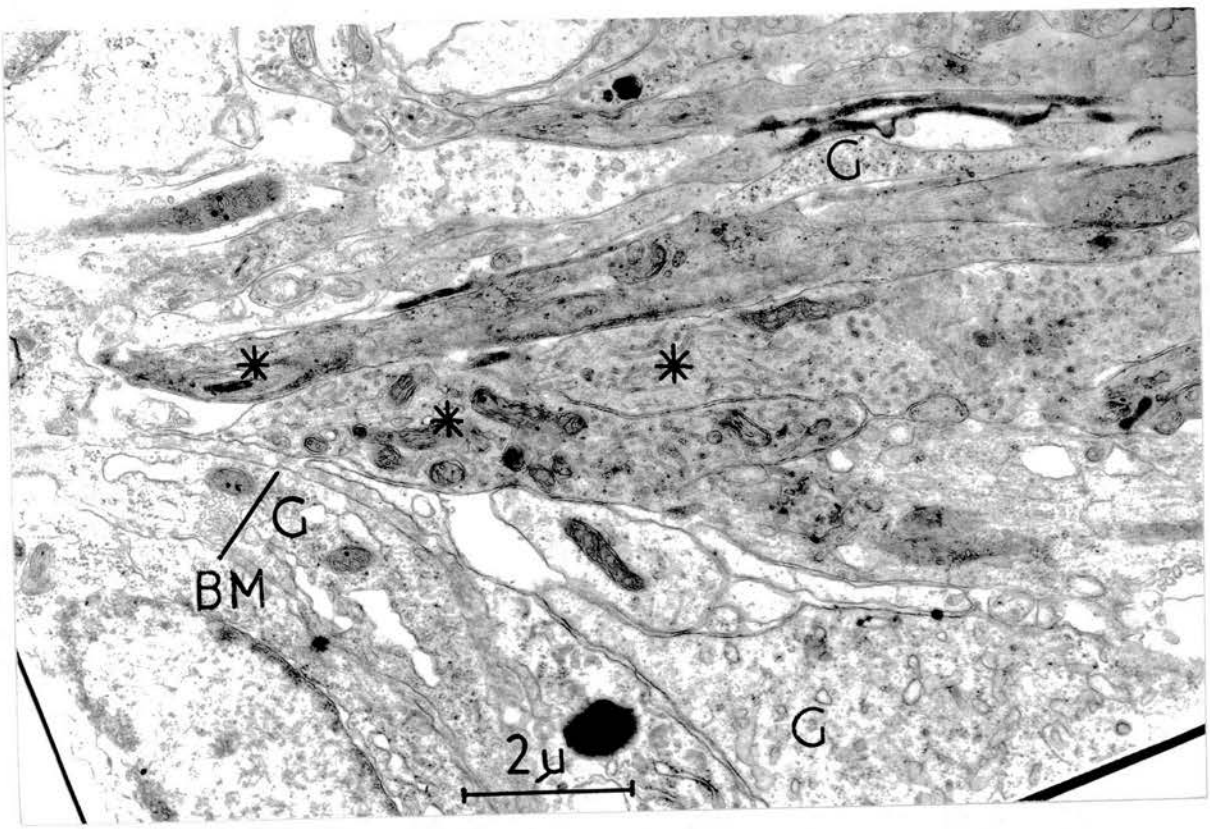
PLATE 14.

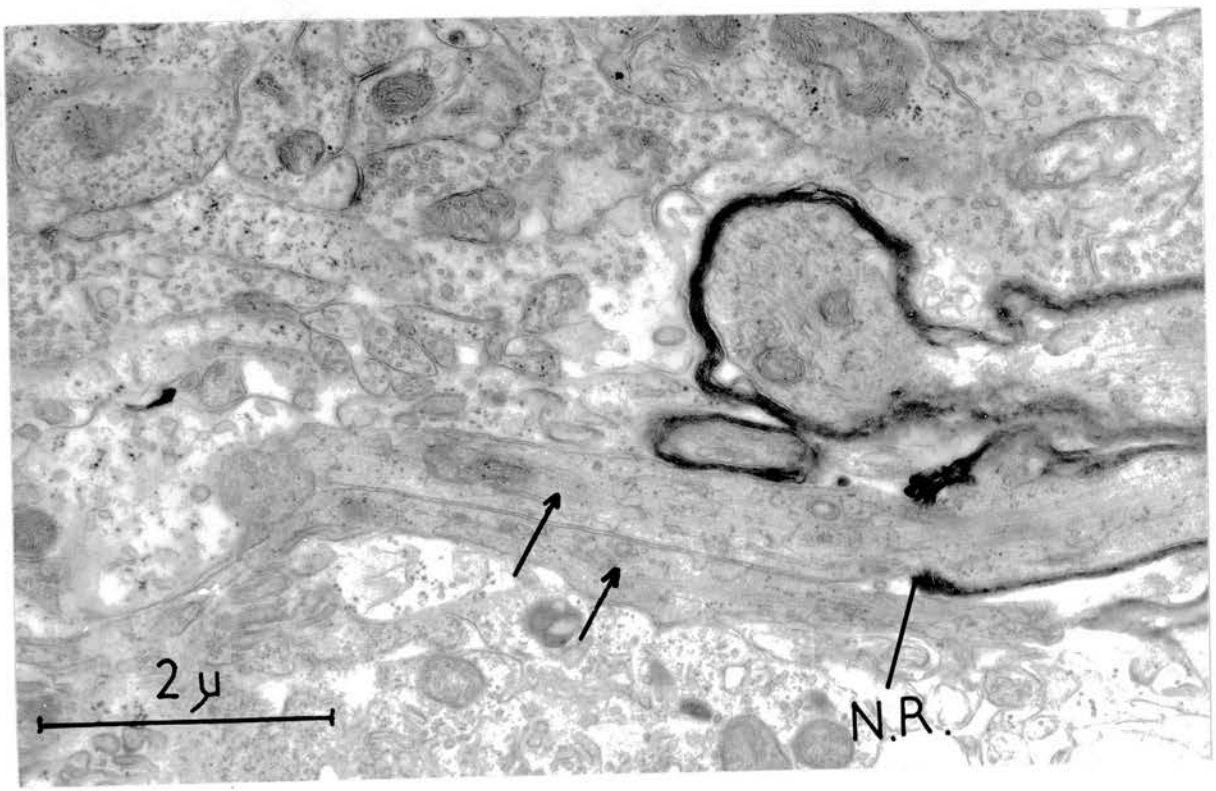
Plate 14A.

From a region in the goldfish optic tectum near the site of section of a tectal optic fibre bundle; fixed 11 days after operation; asterisks show advancing axonal sprouts containing characteristic organelles; these are partially surrounded by glial cell processes (G) and possibly by basement membrane material (BM).

Plate 14B.

Axonal sprout (arrow) emerging from Node of Ranvier (N.R.); same tissue.

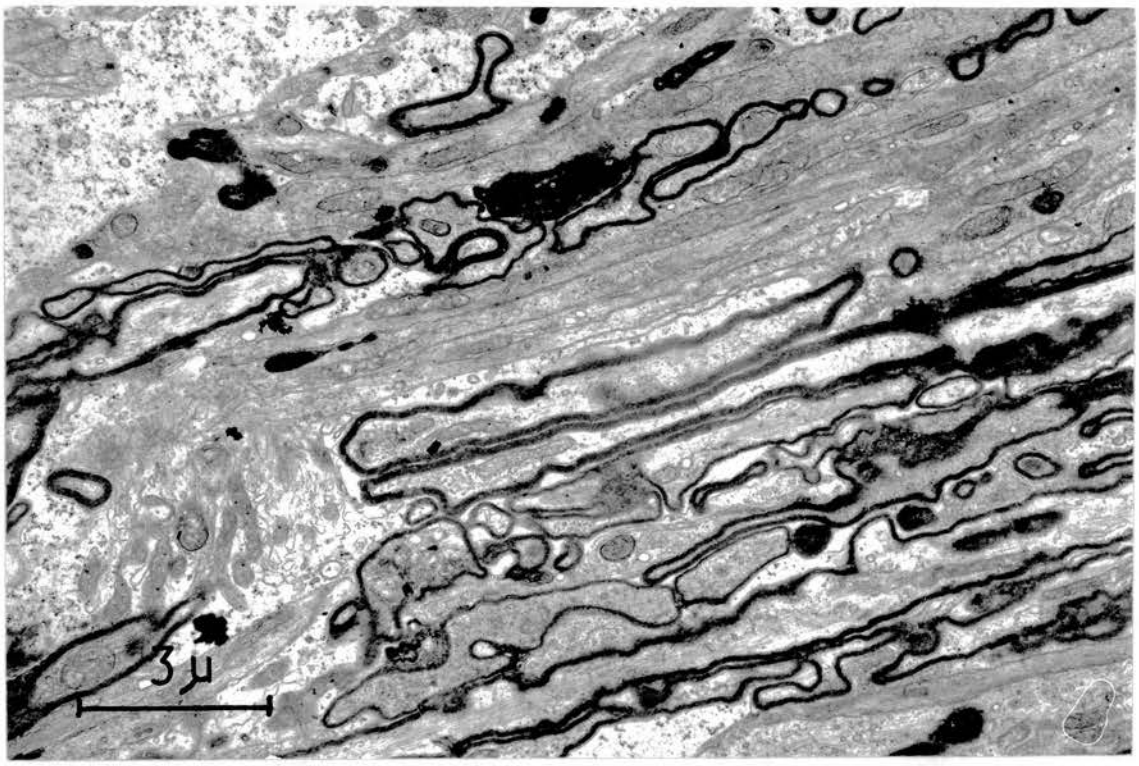

G
G
BM
G
2μ

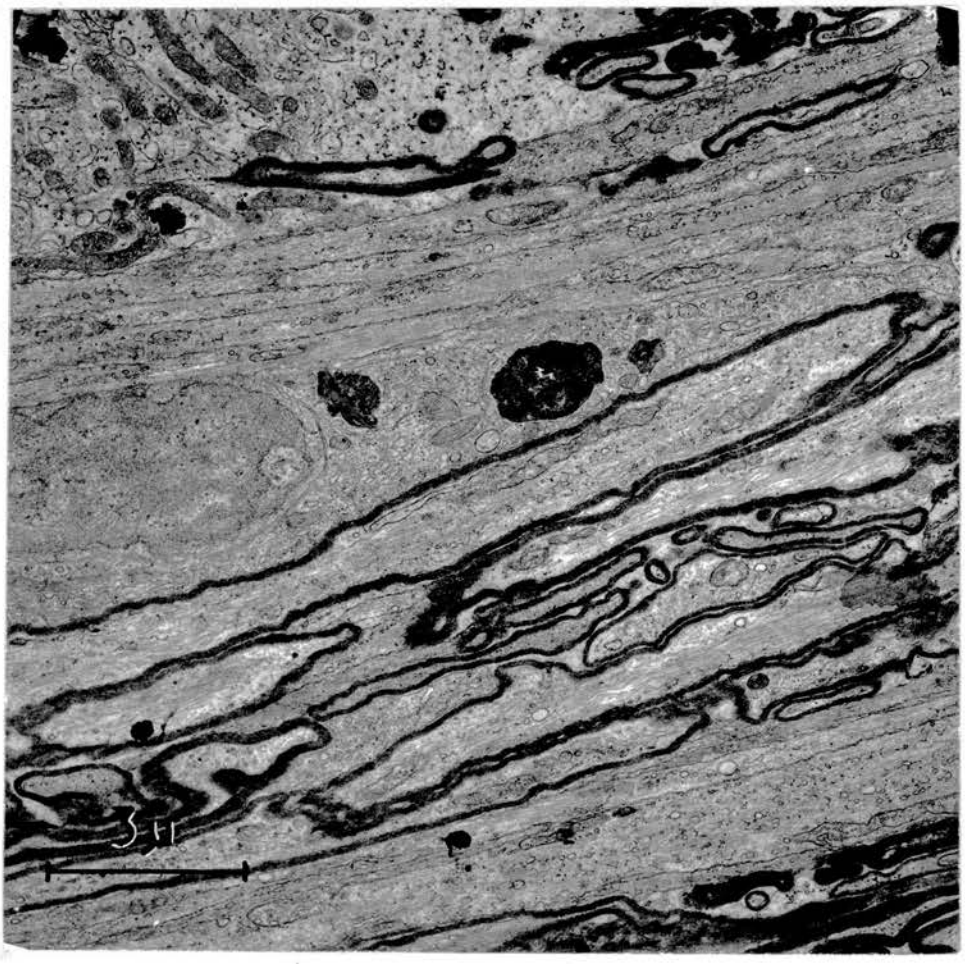

2μ
N.R.

PL TS 15.

Plates 15A & 15B.

Two views of a tectal fibre bundle cut longitudinally near the site of bundle transection performed 13 days before fixation; regenerated fibres can be seen running parallel to the remains of degenerating myelinated fibres and intact fibres.


3μ


3μ

fact that some of the stained "fibres" might be collagen. It is likely that fibres are able to preserve their order as they cross the lesion area. Consistent with this hypothesis is the observation (Montage 1) that regenerating fibres follow exactly and only the degenerating regions of the central stump after partial transection of the nerve. These arguments do not apply to the tectum or the optic tract.

The tendency for regenerating fibres to bunch together can be seen in Plate 6 (regeneration of part of optic nerve), and 8B (regeneration of small proportion of fibres). Under these conditions regenerating fibres seem equally to avoid degenerating and normal intact fibres; there seems to be no question of new fibres following exactly in their old paths.

If the conclusion that path selection in the optic nerve is pinpointed and confined to the lesion site is justified this adds significance to the observation (Plate 9) of certain specialised contacts between growth cone regions and processes of local cells. In this Plate three contacts are seen from a cell, characterized by its dark cytoplasm and lying within the basement membrane. These were the only form of specialized contact seen between regenerated fibres and their surroundings; at first sight they appear to be strong candidates for a possible role in guidance of the fibre.

Part 7

Maturation of regenerated nerves and the significance of residual unmyelinated fibres

From two weeks after nerve section, the central stump of the optic nerve becomes progressively filled with tightly packed bundles of regenerated fibres each rapped around with substantial astrocytic sheaths. Again using the normal frog nerve as a standard for comparison the courses of these bundles appear to be quite straight. During the earliest stages of maturation there is an increase in the number as well as the sizes of the bundles; by four weeks the central stump is densely and uniformly packed with fibres and astrocytic processes. Only at a later stage do individual fibres begin to increase in diameter and assume the normal characteristics of fish optic nerve fibres.

The Plates presented in this Part are from four animals and concern the central stump of the optic nerve only;

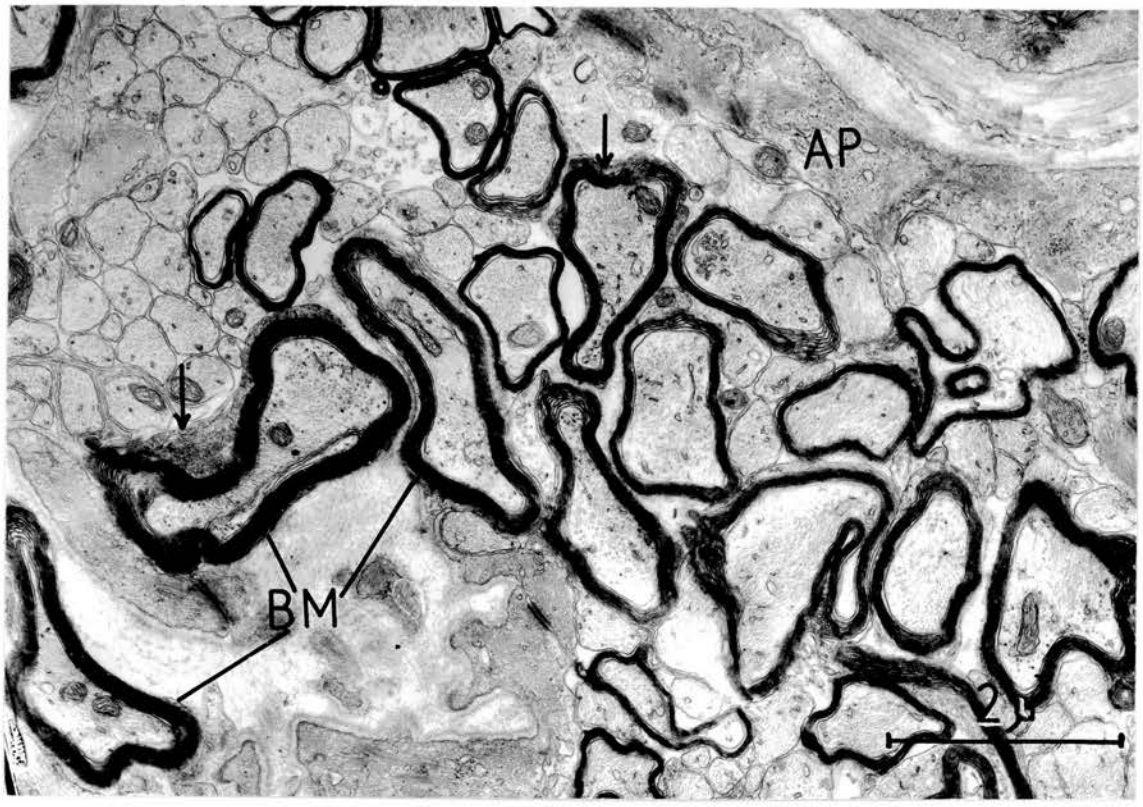
Plate 16A & 16B: Left optic nerve crushed 278 days before.
Plate 17A & 17B: Right optic nerve cut 126 days before.
Plate 18A & 18B: Right optic nerve cut 330 days before and crushed 7 days later.
Plate 19 : Fish in which the left optic nerve had been cut 119 days before.

In Plate 19 (119 days) it can be seen that between a third and a half of the fibres have become remyelinated with sheaths of up to 10 lamellae each. There is surprisingly little difference in the sizes of myelinated and unmyelinated fibres, both of which vary greatly in

PLATE 16.

Plates 16A & 16B.

These plates are taken from the central stump of a goldfish optic nerve which had undergone regeneration for 278 days following nerve crush; arrows refer to densely staining external mesaxons; asterisks show spaces surrounding individual remyelinated fibres which are bounded by basement membranes (BM) and astrocytic processes (AP).


AP
BM
2μ

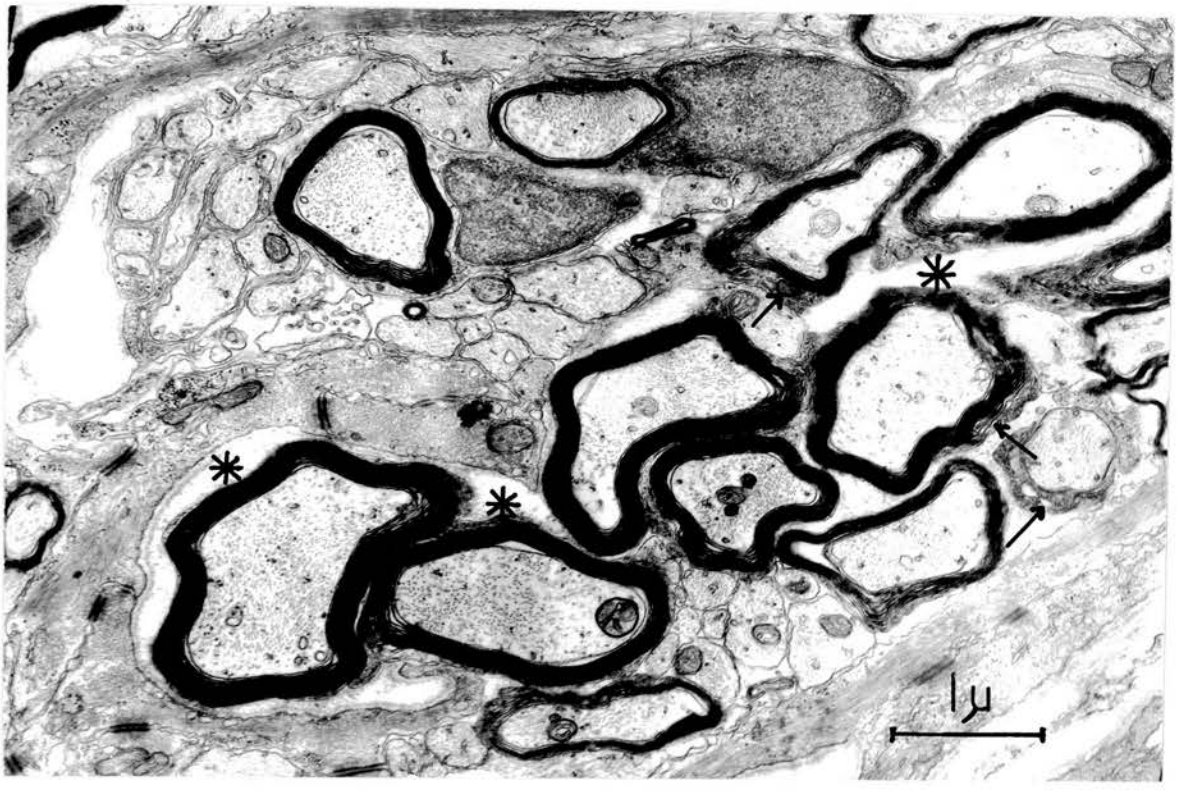
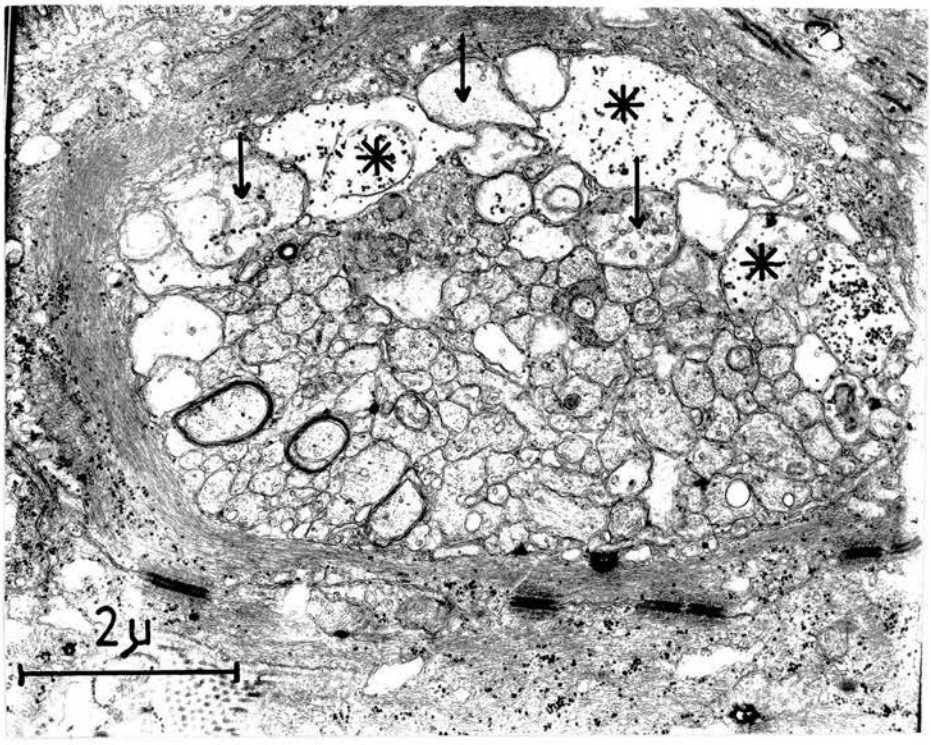
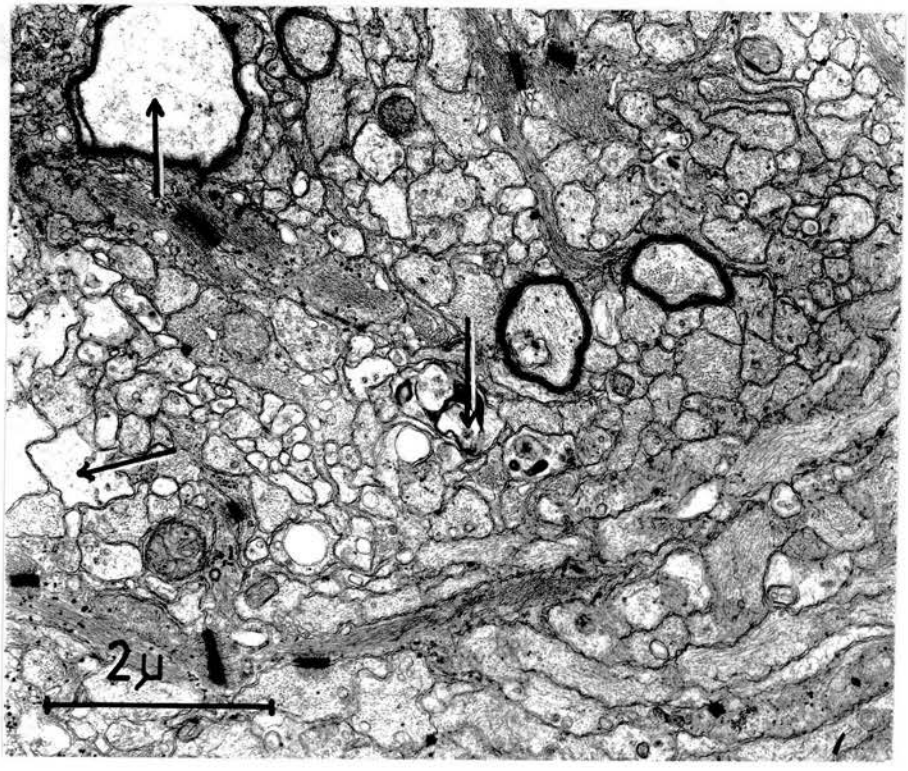

1μ

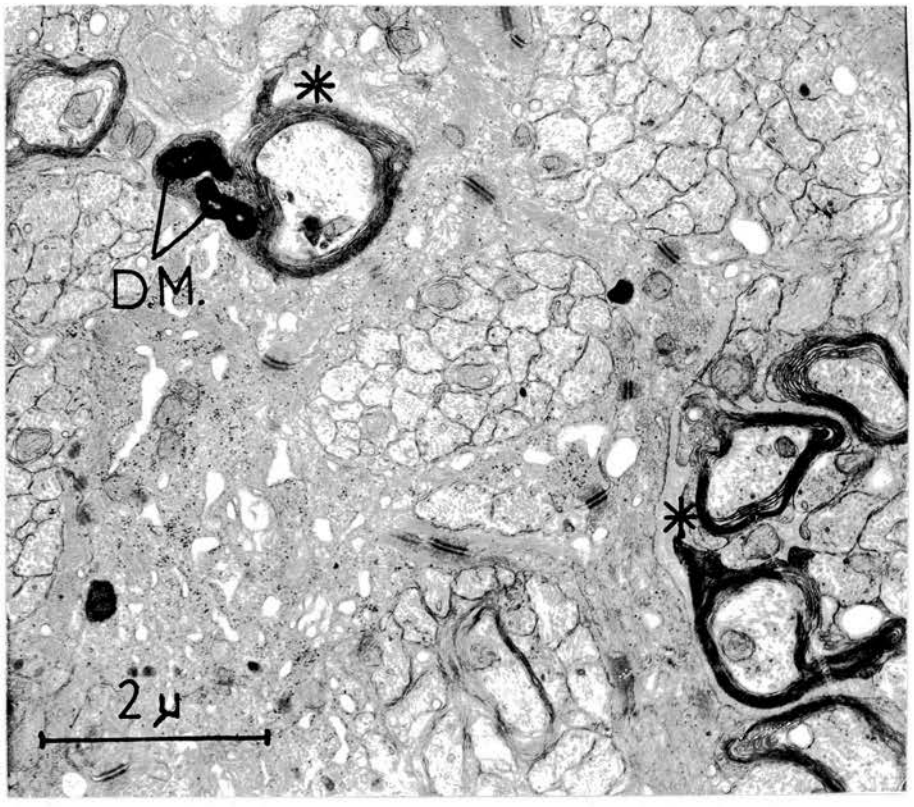
PLATE 17.

Plates 17A & 17B.

This nerve regenerated for 126 days shows poor myelination and signs of degeneration among the regenerated axons (arrows); astrocytic processes are common and may occur inside the fibre bundles (asterisks).


2μ


2μ


D.M.
2 μ

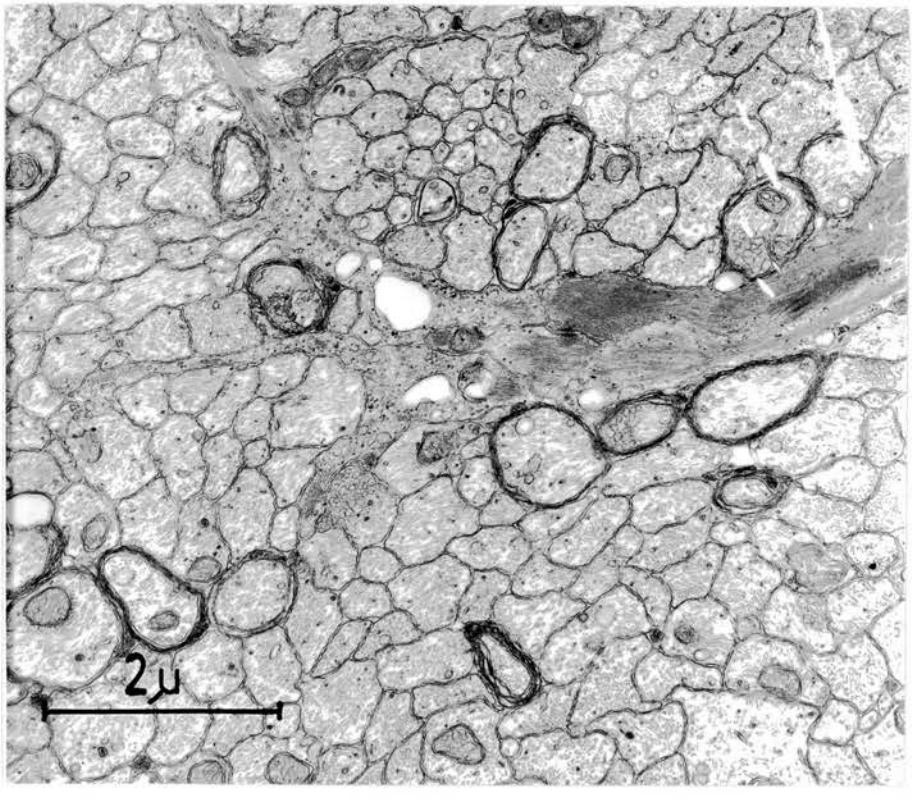
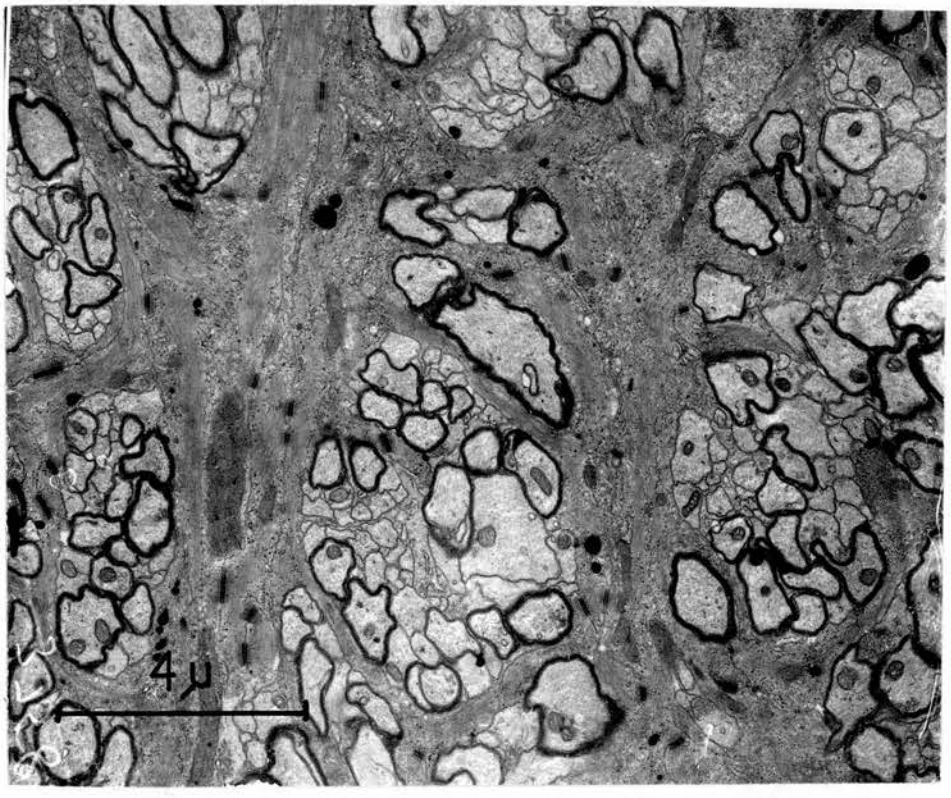

2μ

PLATE 19.


4μ

diameter up to over 1 μ. The two types are randomly mixed. Astrocytic processes are very common but oligodendrocytic processes and cell bodies are apparently no more common than in the normal nerve despite the active myelination in progress. There were no signs of degeneration among the regenerated fibres and axoplasmic contents were predominantly neurofilametns. Remnants of earlier myelin have been almost completely removed.

In Plate 17 (126 days) the situation is very different, despite the fact that the animal had been treated almost identically to the previous one. Although the general disposition of bundles and astrocytes is comparable, myelination has hardly begun in this nerve. Secondly there is evidence of degeneration among the regenerated fibres, including empty fibre profiles and deposition of glycogen. Other apparently empty profiles containing glycogen and lying within the bundles, may be invading astrocytic processes. One gets the impression that the larger fibres are those showing the degenerative signs.

Plate 16 (278 days) presents a picture similar to Plate 19 but more matured. An entire section of the nerve was taken near the chiasma (and at a fair distance from the site of the lesion) and approximately 2.2% was counted from prints at 9000X while the total cross-sectional area was estimated from a single photograph at 511X. For the count, 9 fields were selected at random over the nerve and the fibre density was determined in the same way as in the count of normal nerve.

	Counts	Densities	S.D. of densities	Estimated totals for whole nerve
Myelinated fibres	890	13.4	6.4	40,500 (26.7%)
Unmyelinated fibres	2549	38.0	13.4	116,000 (73.3%)

A second sample representing 0.87% of the total cross section yielded similar results:

Estimated total myelinated fibres	34,300 (20%)
Estimated total unmyelinated fibres	137,300 (80%)

This sample is, according to the arguments presented in connection with the count of the normal nerve, adequate to get a realistic estimate of the total. However the greater variance of the present figures suggests that the distribution of fibres is less even; in part this may be the result of the greatly increased glial component.

As can be seen in Fig.3B there is no tendency for smaller fibre diameters to predominate among the myelinated fibres in this nerve as compared with a normal nerve; in fact the majority here fall in the range 0.5 to 1.0 μ whereas in the normal the majority are about 0.4 to 0.8 μ. The very largest diameters are absent in the regenerated nerve.

Thus although this nerve shows many signs of having reached full maturity (many fibres for instance have up to 20 myelin lamellae and both myelinated and unmyelinated fibres have apparently normal neurofilaments and tubular contents) it retains one major atypical characteristic; a majority of fibres are unmyelinated. In part this accounts for the fact that there are more fibres in this nerve than in the normal nerve.

A second feature of this nerve of some interest is the very common deposition of basement membrane material (often with associated collagen) along astrocytic processes. Associated changes are the wider tissue spaces between fibres and more frequent partitioning of fibre groups. The result is that individual fibres frequently appear to be partially or totally suspended within a tissue space surrounded by their own basement membranes. External mesaxons are more frequent than in normal

myelinated nerve fibres though these are not continuous around the whole myelin sheath. Oligodendroglia are common in this nerve and signs of active myelination are frequent including relatively wide separation between myelin lamellae. Degenerating myelin forms were apparent.

Plate 18 shows the structure of a nerve regenerated for 330 days. In many respects this nerve is comparable to that shown in Plate 17 (126 days); the proportion of fibres undergoing myelination is well under 10% and unmyelinated fibres are closely packed in large bundles. However there are no signs of degeneration and at some points there are fibres which have reached the same stage of maturity as the myelinated fibres in the previous case (Plate 16), including the appearance of basement membrane containing tissue spaces around fibres. The unmyelinated fibres showed signs of relative maturity themselves, containing tubules as well as filaments.

When compared with Plate 16 (278 days) however, this nerve shows a remarkable predominance of unmyelinated fibres. This may be related to the fact that the nerve was lesioned twice at slightly different positions; Aitken et al. (1947) found that this procedure (which might prevent the retrograde spread from the terminal of a motor fibre of a stimulus to mature) delayed maturation in the nerve to the medial head of gastrocnemius in rabbits. A second nerve similarly treated and examined after 343 days appeared similar to Plate 19 (119 days). It is clear from these results that the maturation of the regenerated central stump shows considerable individual variability, if not actual dependence on the exact characteristics of the lesion.

As was suggested in the Introduction fibre branches may be a response to the mechanical conditions at the site of the nerve lesion;

thus the more severe the lesion the higher the expected proportion of unmyelinated fibres. This may explain the high proportion of unmyelinated fibres in Plate 18 (330 days); in animals in which the nerve had been repeatedly crushed, the nerve became enlarged into a "neuroma" which was found in the electron microscope to be due to a massive proliferation of unmyelinated processes. It is also likely that the number of processes decreases as the distance from the lesion increases (Cajal, 1928; Shawe, 1955) since the proportion of unmyelinated fibres among the myelinated fibres in a tectum nine months after regeneration began is considerably smaller than in Plate 16.

The fact that a fibre is unmyelinated does not preclude its having established connections in the tectum. At the time the first electrical responses are detectable in the tectum (between 25 and 30 days) and at the time of the first behavioural responses there are few signs of myelination in the nerve. Plate 11B (29 days) for example, shows myelinated fibres within the regenerating bundle which are probably degenerating. Plate 13B (29 days) shows some myelinated fibres which may be newly regenerated fibres.

Therefore a proportion at least of the residual unmyelinated fibres in long-term regenerated nerves (such as in Plate 16) may have reached the tectum and successfully established terminations. It is well known that regenerated fibres fail to mature to their normal dimensions and to myelinate fully when prevented from contacting their normal type of end organ, i.e. denervated muscles (Weiss & Taylor, 1944; Aitken et al., 1947; Aitken, 1949) and that such fibres may later show signs of retrograde degeneration (Cajal, 1928, p.369; Aitken et al., 1947). Degeneration was not a dominant feature in most of the present material;

surprisingly matured, myelinated fibres showed signs of degeneration as often as unmyelinated fibres in Plate 16. For this reason the electron microscopic evidence is, if anything, against the suggestion that multiple branches in regenerating nerve fibres are concerned with path selecting by way of withdrawal of aberrant branches. Plate 17 in which there were signs of extensive degeneration, may represent a nerve in an exceptional state; in this nerve, compared to Plate 19, myelination was greatly retarded.

Gutmann & Sanders (1943) showed that after simple crush of the rabbit's nerve the normal number of myelinated fibres was recovered by 150-200 days, while the normal fibre diameter distribution had been restored by 250-300 days. Aitken et al. (1947) showed in the rabbit's medial gastrocnemius nerve that although the number of myelinated fibres may be increased above normal by multiple lesioning of the nerve or disconnection from a muscle, during straightforward regeneration after crush the fibre number and diameter distributions are near normal by 100 days. In the case of the nerve counted here, the number of myelinated fibres after 278 days was approximately half the normal number, although there was a 50% increase in the total number of fibres. The fibre diameter distribution was approximately normal. There is therefore some reason to think that the number of fibres that have myelinated after about 250 days may represent the fibres which have successfully re-established connections in the tectum.

Maturation of regenerated fibres seems to proceed independently among fibres destined to become myelinated and those remaining unmyelinated; thus in Plate 19 unmyelinated fibres seem to have a similar diameter range to those undergoing myelination, in both cases

ranging to larger diameters than newly regenerated processes. In Plate 16 both types of fibre have acquired neurotubules. However, the formation of basement membranes at later stages of maturation (Plate 16) is characteristic of myelinated fibres and cannot be seen in neighbouring unmyelinated areas in Plate 18. This response may well be a reflexion of the tendency of astrocytes to form basement membranes around the bundles of normal nerve; but it seems in the case of regenerated nerve to have been greatly increased perhaps due to the greater profusion of reactive astrocytic processes.

Part 8

Numbers of myelinated fibres in the optic fibre layer of the tectum in long-term regenerated fish

A preliminary attempt was made to confirm the above estimate of the number of fibres successfully reinnervating the tectum by counting the number of myelinated fibres in the optic layer of a cross section of tectum in a second animal also regenerated for 278 days following nerve crush.

The procedure was modified from that used to estimate the equivalent number of fibres in the normal tectum in order to take into account the increased vertical spread of optic fibres after regeneration (Attardi & Sperry, 1963). Counts were made of the myelinated fibres present in 9000X electron micrographs of two vertical strips of tectum. All fibres lying between 35 and 100 μ below the surface of the tectum were included. To reach an estimate of the total number of fibres in the entire cross section of the tectum, these counts were divided by the fraction of the total optic bundle cross-sectional area represented by bundles occupying exactly equivalent areas in the normal tectum.

It was estimated that approximately 70,000 myelinated fibres traversed the whole tectum in the optic fibre layer compared to 80,000 in a normal tectum. A very small sample (about 1% of the total fibre population) was used to arrive at this estimate which can only be regarded as very approximate.

Part 9

Features of the restoration of the retinotectal projection in the course of regeneration of the optic nerve

The animals listed in Appendix 2 include 42 animals in which the restored retinotectal projection was mapped between 20 and 250 days after transection or crush of the left optic nerve.

The earliest electrical responses to visual stimuli were detected after 22 days. However the responses obtained at this stage and during the subsequent two weeks were very slight and difficult to characterise. Frequently the responses seemed to consist of irregular 'bursts' and followed the passage of the stimulus with an abnormal delay. In some respects these earliest responses were similar in quality to those observed in Pattern 1 (Gaze & Jacobson, 1963) in frogs. The first clear sign of the usual form of "unit" response appeared after 31 days. In each case in which responses were obtained, the position and the size of their receptive fields were as far as could be assessed, near normal. In one animal regenerated for 31 days, separate experimenters positioned the electrode and located the receptive field in order to avoid any possible bias; receptive fields were approximately in the correct positions.

The earliest case in which systematic retinotectal mapping could reasonably be attempted is shown in Map R34; even here the responses were still very faint compared to normal. Again a double-blind technique was used (for some of the points) and it is clear that retinotopic order exists among the tectal positions giving responses.

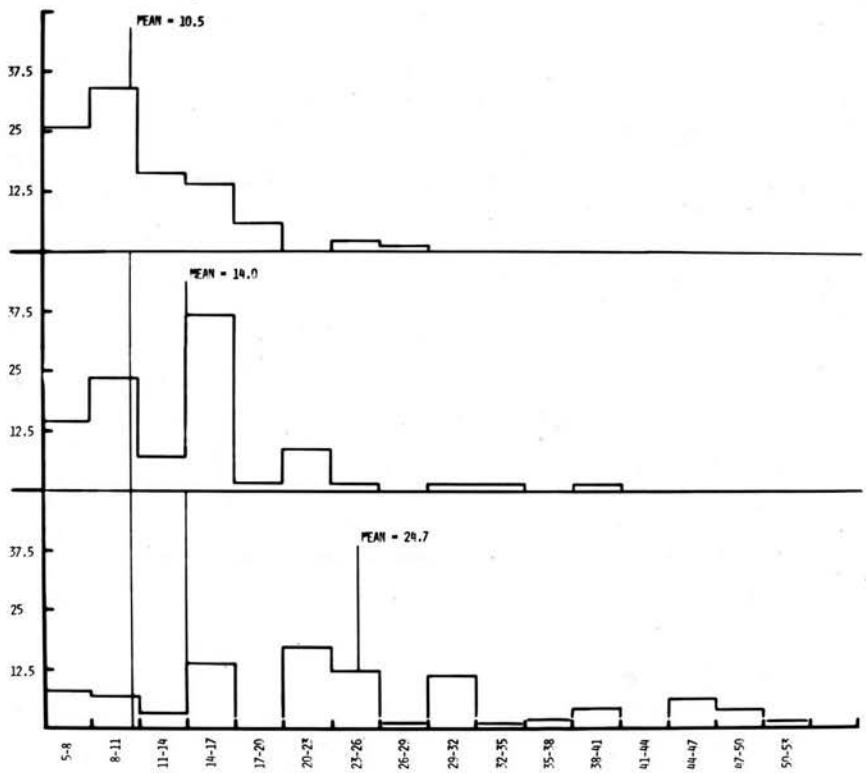
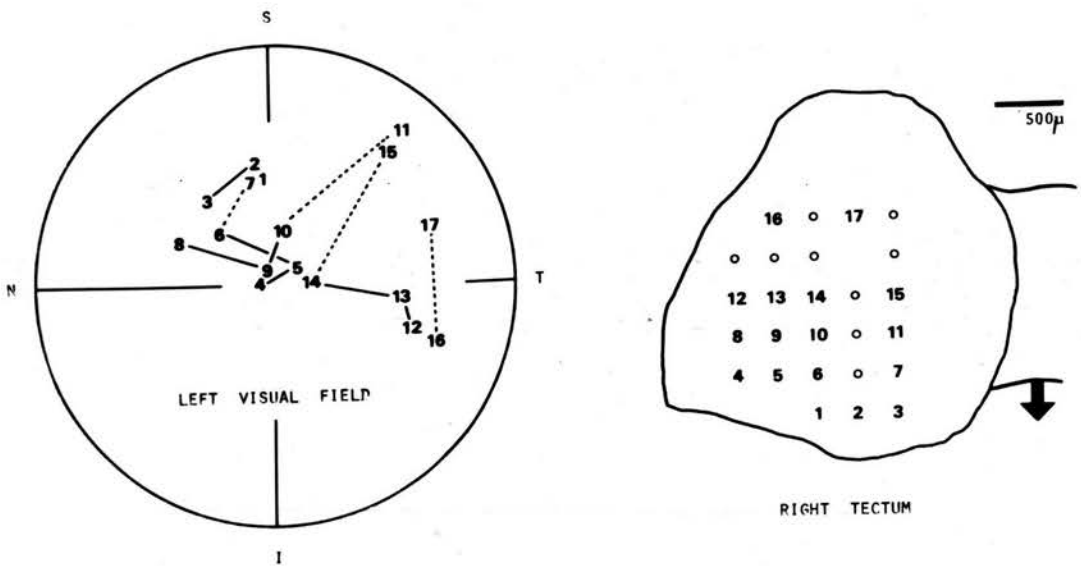


FIGURE 4.



R34

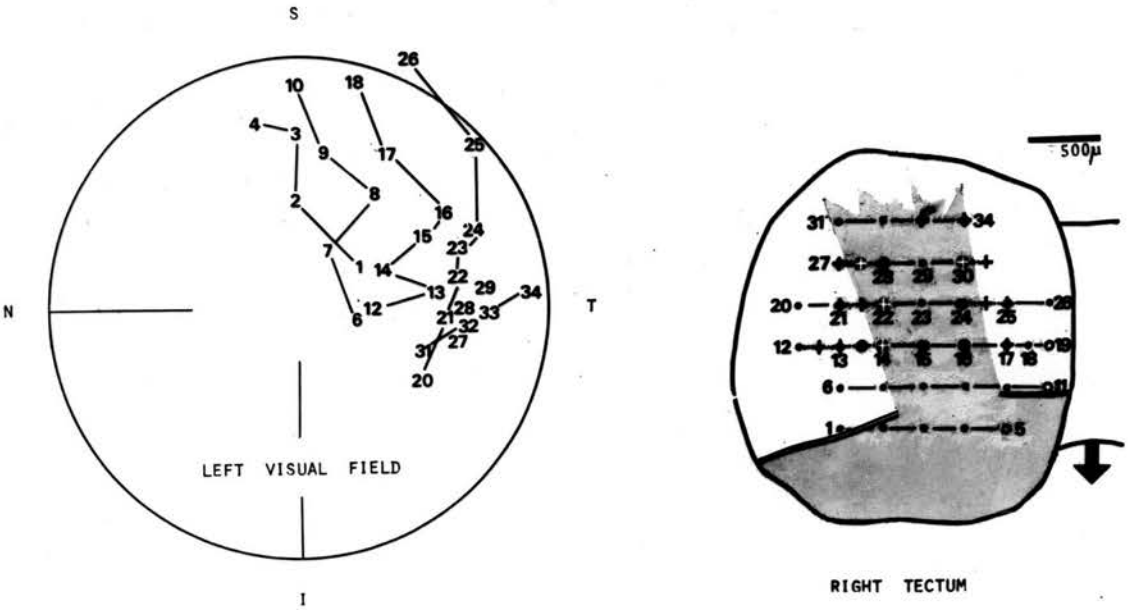
REGENERATED VISUAL PROJECTION 34 DAYS AFTER L.O.N.CRUSH

The gradual maturation of responses continues for 20 days more; in this time some animals still give very poor responses indeed but by 50 days it is usually difficult to describe any qualitative differences between them and normal responses.

It did not prove possible to show that any one region of the tectum became innervated earlier than any other, although in several animals the data is consistent with the possibility that the earliest responses are concentrated rostrally; the early responses are so poor that it is often difficult to say with any certainty whether there is a response present or not.

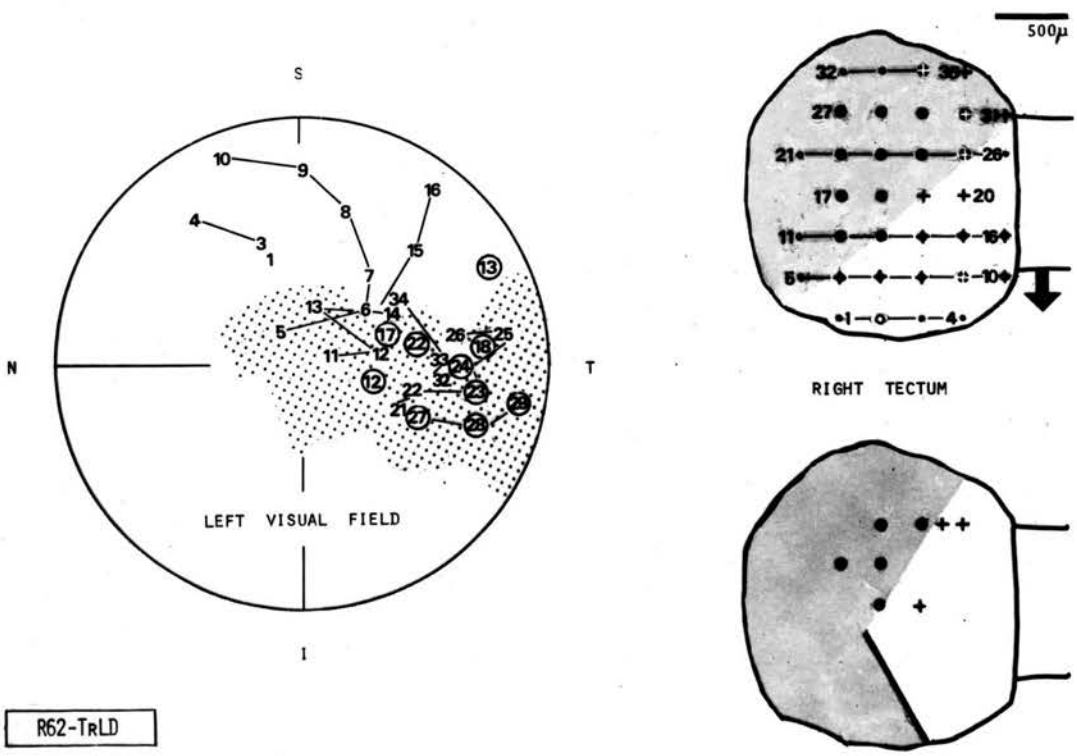
A most sensitive method for detecting the relative consistency with which fibres terminate at a given point in the tectum in accordance with their retinal origins is to measure the multiunit receptive field diameter of the response at that point; if this is enlarged compared to normal this means that fibres from ganglion cells distributed more widely than usual in the retina are reaching this point. Fig.4 shows the general pattern of results obtained (details of individual animals are given in Appendix 2). The upper graph shows the distribution of multiunit receptive field diameters from three normal animals and the lower shows the same measurements averaged over eight animals in which regeneration had been in progress for less than 70 days. In normals the mean field diameter is 10.5° (S.D. 9.8, 85 points measured) whereas in "short term" regenerated animals it is 24.7° (S.D. 13.9, 86 measurements).

There is therefore a detectable but small degree of error in the early restoration of connections, which is evidently corrected over the course of the following eight months. The mean for animals undergoing



R54A-TE

REGENERATED VISUAL PROJECTION 54 DAYS AFTER L.O.N. CRUSH AND THE PATTERN OF TECTAL RESPONSES SURVIVING ACUTE SELECTIVE TRANSVERSE LESION



R62-TRLD

REGENERATED PROJECTION 62 DAYS AFTER L.O.N. CRUSH AND SURVIVAL OF RESPONSES FROM THE LATERAL OPTIC TRACT DIVISION FOLLOWING LESION OF MEDIAL TRACT FIBRES

UPPER TECTUM SHOWS EFFECTS OF SECTION OF THE MEDIAL DIVISION OF THE TRACT. LOWER SHOWS SURVIVING RESPONSE AFTER SELECTIVE LESION OF LATERAL DIVISION FIBRES. CIRCLED FIELD POSITIONS WERE RELOCATED AFTER MEDIAL DIVISION SECTION.

regeneration for between 278 and 292 days was 14.0° (S.D. 6.7, 53 measurements in three animals).

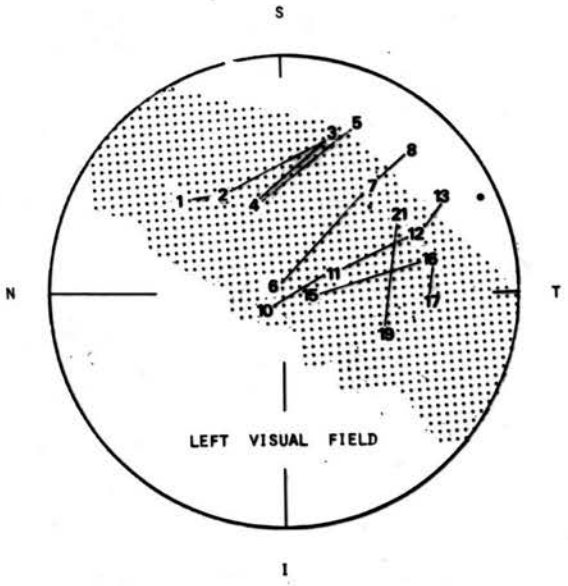
Apart from this no further progressive changes were seen among the retinotectal projections mapped after about 50 days. It was noted that the visible optic fibre striations on the tectal surface underwent a progressive dissolution over a period extending up to about 100 days after the initial nerve transection but they became clearer later and by ten months were hardly distinguishable from normal.

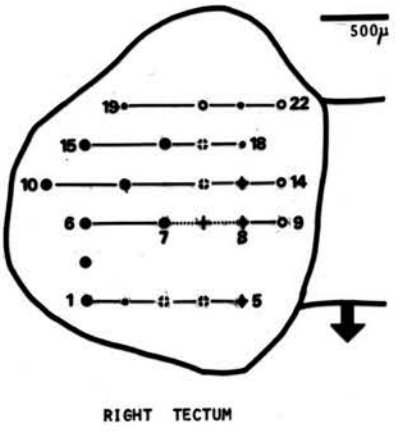
Of the final 29 fish (regenerated for between 54 and 293 days) in the series, 16 yielded projection maps which were indistinguishable from normal maps in any way (examples are R278-Te, R286-Te). The remainder showed anomalies whose significance is difficult to assess. In five animals (e.g. R54A-Te, R62-TrLD, p.83) fewer field positions than normal fell within the nasal half of the field and, in contrast to the situation in the normal animal, a row of points lying half way along the rostro-caudal tectal axis was supplied by field positions far into the temporal field. It is unlikely that this feature is due to poor centering of the animals because the reverse condition was not seen - i.e. an apparent shift of the projection caudally along the tectum did not occur.

There is therefore suggestive evidence that fibres may in some animals terminate at more rostral tectal positions than is normally the case, but it must be emphasized that the method employed for mapping these projections does not permit absolute identification of tectal loci; the positions of an electrode on the tectum is only defined with respect to the visible borders of the optic tectum and both the lateral and rostral edges are buried below the visible surface.

A second possible class of anomalies are maps with irregular arrangements of field points. In assessing the significance of these it is important to bear in mind that the centre of a receptive field cannot be pinpointed more precisely than to within an area with a radius of about 5° and when errors in positioning the electrode on the tectum and errors due to the enlarged fields in short-term regenerated fish are included, the margin for error in the final mapping of a field position may rise by a factor of 2 or 3. Consequently the irregularities to be seen in Maps R54A-Te and R286-Te (p.86) involving cross-overs of lines of field positions, are probably insignificant. The irregularity in the rostral row in Map R191-Tr, the superposition of the 3rd and 4th rows in Maps R54B-TrLD (p.87), and 3rd, 4th and 5th rows in R170 (p.87) and the abnormalities in R55B are probably all significant errors; they were checked by repeated mapping in most cases and in all these animals the responses were good and localised. The errors in Map R63-Te were probably due to the difficulties of mapping this animal which had exceptionally large multiunit fields. Similar types of irregularity were noted in 4 of the 9 animals mapped by Gaze & Sharma and included in the present series for consideration.

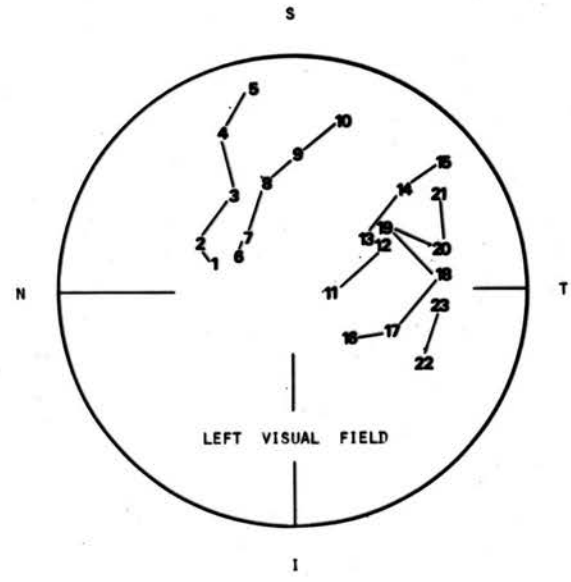
The present evidence puts the arrival of regenerating fibres at the tectum at earlier than 22 days, which is in agreement with the findings of Attardi & Sperry (1963). In view of the quality of the earliest responses and their only gradual improvement over the following two or three weeks, it is not surprising that no sequence was detected in the order of recovery of responses in different tectal areas, even if fibres took 10 days or so to cross the longest axis of the tectum from their points of entry.

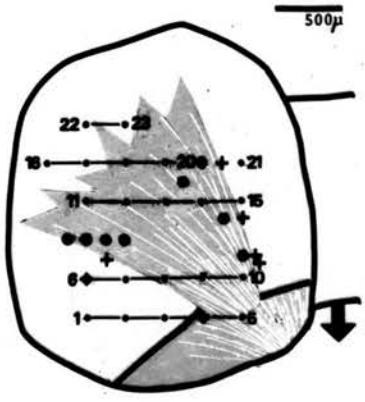




R191-TR

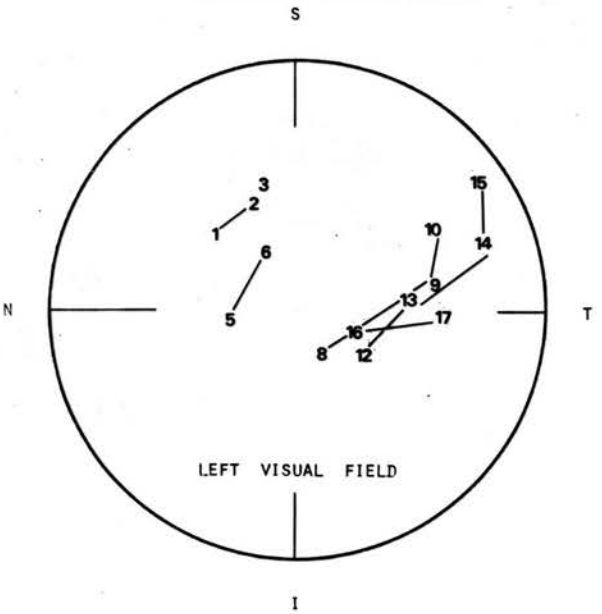
REGENERATED PROJECTION 191 DAYS AFTER L.O.N. CUT SHOWING THE FIELD SUPPLIED BY FIBRES OF THE LATERAL TRACT DIVISION AFTER ACUTE SECTION OF THE MEDIAL DIVISION

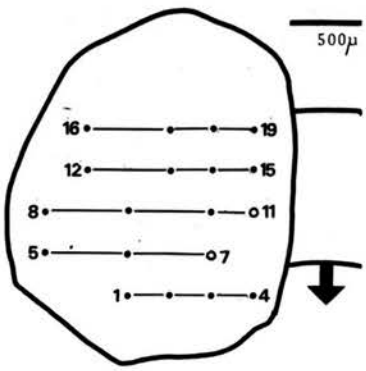




R286-TE

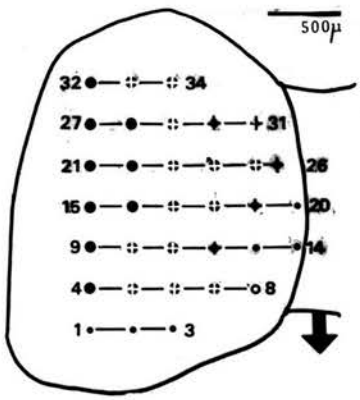
REGENERATED PROJECTION 286 DAYS AFTER L.O.N. CRUSH AND THE PATTERN OF TECTAL RESPONSES SURVIVING ACUTE SELECTIVE TRANSVERSE SECTION

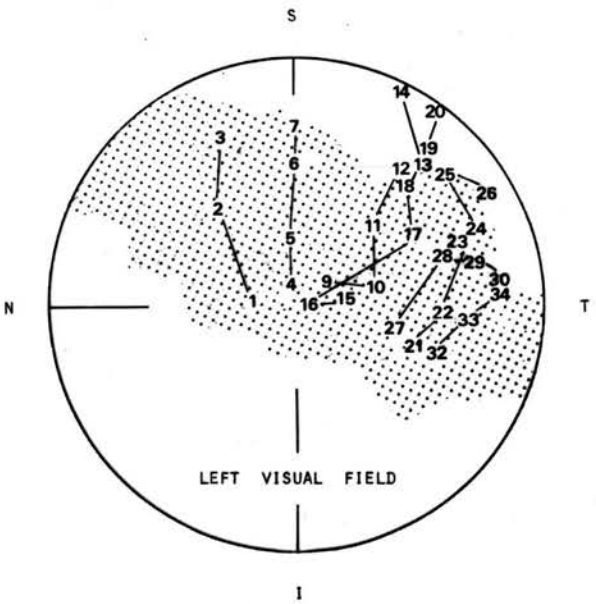


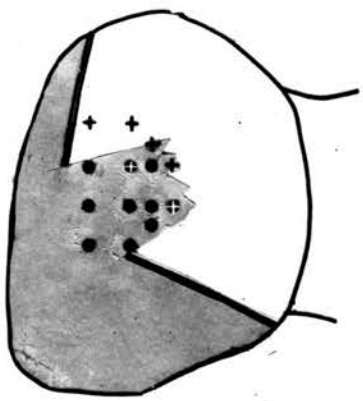


R170

REGENERATED VISUAL PROJECTION 170 DAYS AFTER L.O.N. CUT







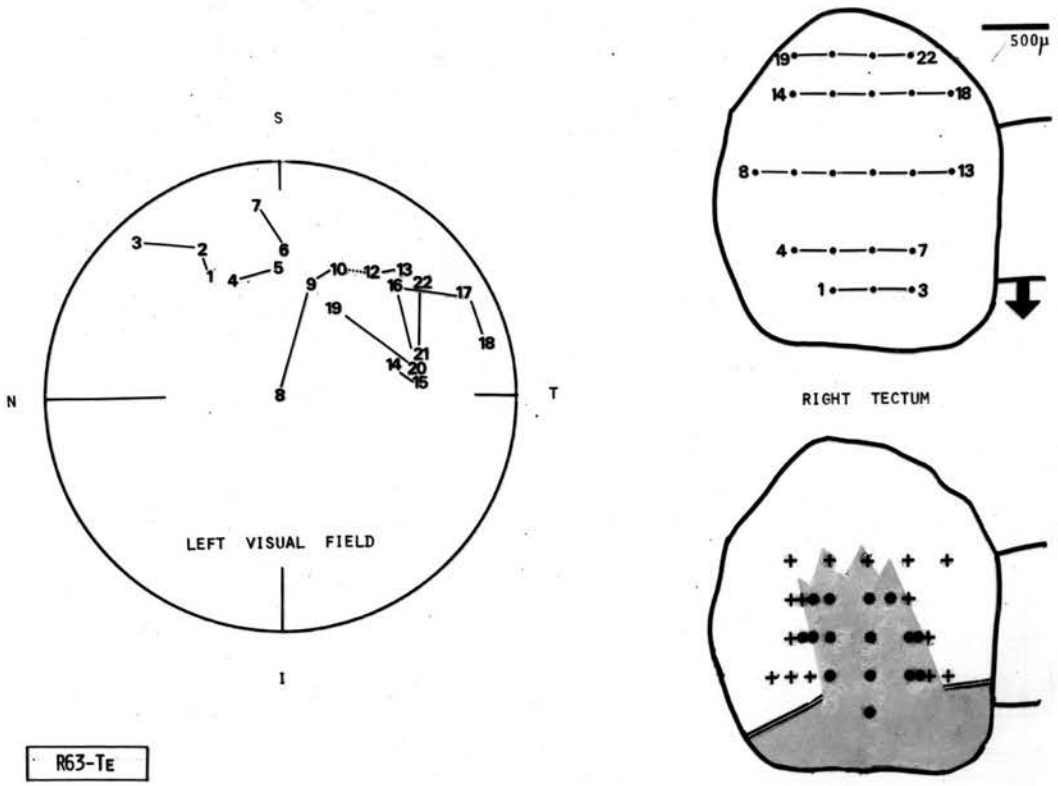
R54B-TRLD

REGENERATED PROJECTION 54 DAYS AFTER L.O.N. CRUSH INCLUDING EXAMINATION OF THE FIELD SUPPLIED BY LATERAL DIVISION FIBRES.
UPPER TECTUM SHOWS SURVIVING RESPONSES AFTER ACUTE SECTION OF MEDIAL DIVISION. LOWER SHOWS RESPONSES OBTAINED AFTER SELECTIVE LESION OF LATERAL DIVISION FIBRES.

The very gradual appearance of normal responses together with the fact that even the earliest, immature responses were retinotopically organised, suggest that from the start the responses are being picked up from the same structures as normal responses.

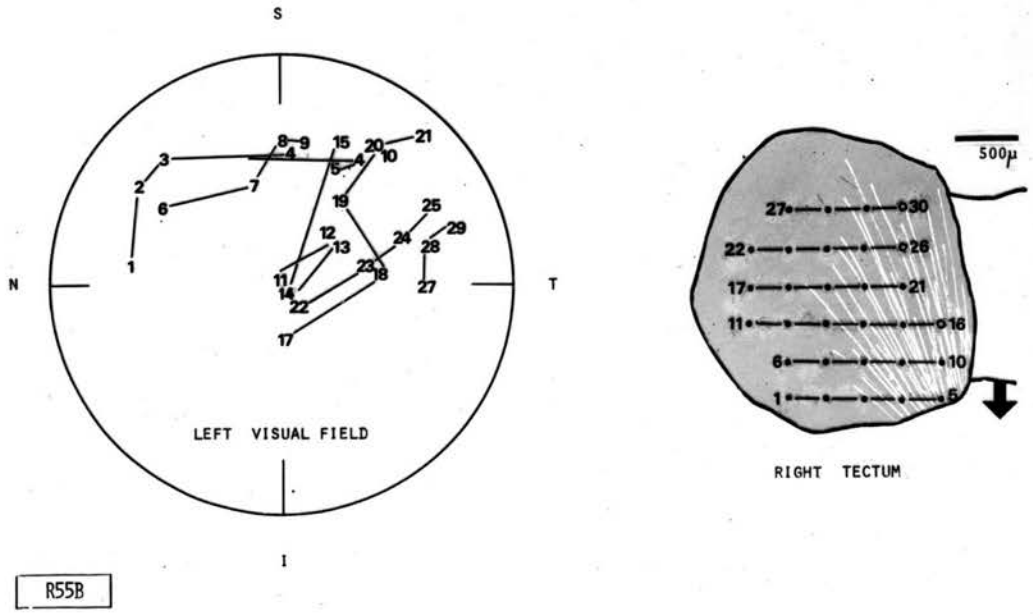
It is extremely unlikely that the absence of any instances of Pattern 1 in the present experiments is due to inadequate sampling of fish regenerated for the appropriate length of time. The time scales for recovery of electrical responses in the frog and the fish are similar; in both, the first responses are detected by about 23 days, and the first "normal" maps are obtained by 33 days. In the results of Gaze & Jacobson, only half the cases of Pattern 1 which they reported were seen before day 50; the same number (7 animals) were seen later. Of the 30 animals examined by them earlier than 50 days, 16 were negative, 7 were Pattern 1 and 5 were Pattern 3 ("normal"). Therefore, as a rough comparison, of the 10 animals successfully recorded earlier than 50 days in the present fish experiments, about five of them might have been expected to show Pattern 1.

Although it remains a possibility that fibres are randomly distributed prior to the time at which electrical responses are obtainable - on the basis of Attardi & Sperry's (1963) data about 10 days might intervene between the earliest arrival of optic fibres in the tectum and the first responses - the time needed for early enlarged multiunit response fields to reach normal sizes argues against it. The data obtained here suggests that some degree of trial and error may occur in the choice of terminals by the earliest fibres; this may be comparable tp Pattern 1 in the frog but it is of an altogether smaller magnitude.



R63-TE

REGEMERATED PROJECTION 63 DAYS AFTER L.O.N. CUT
LOWER TECTUM SHOWS SURVIVING TECTAL RESPONSES FOLLOWING ACUTE SELECTIVE LESION OF ENTERING MEDIAL DIVISION FIBRES.



R55B

REGENERATED VISUAL PROJECTION 55 DAYS AFTER L.O.N. CUT

The present results also indicate that regenerating fibres may occasionally terminate erroneously. The measurements of multiunit receptive fields show that small-scale errors are corrected by long-term re-arrangements taking several months. Other irregularities in the retinotopic pattern may occur and in some cases resemble Pattern 2. These are not associated with abnormal fields and probably represent real "errors" in the way fibres select their terminals.

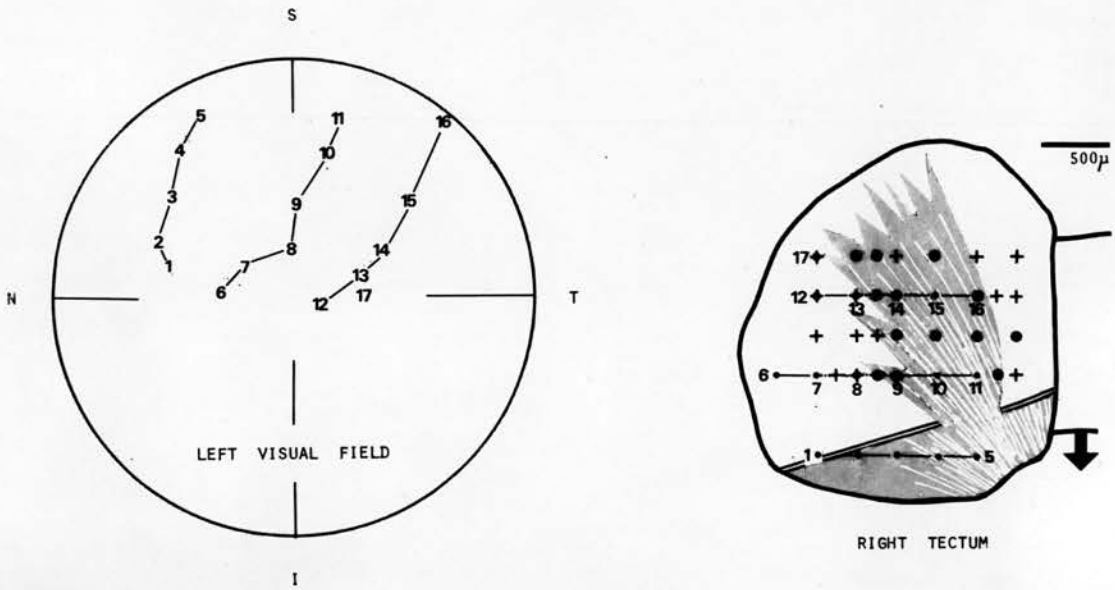
Part 10

The normal anatomical segregation of fibres at the division of the optic tract

As can be seen in Fig. 1A and in Plate 6 (Montage 1) the goldfish optic tract undergoes gradual division into quite distinct medial and lateral brachia which pass round the rostral border of the tectum and radiate out onto the surface of the lobe from, respectively, the rostromedial and rostrolateral poles. These structures can easily be observed under the dissecting microscope.

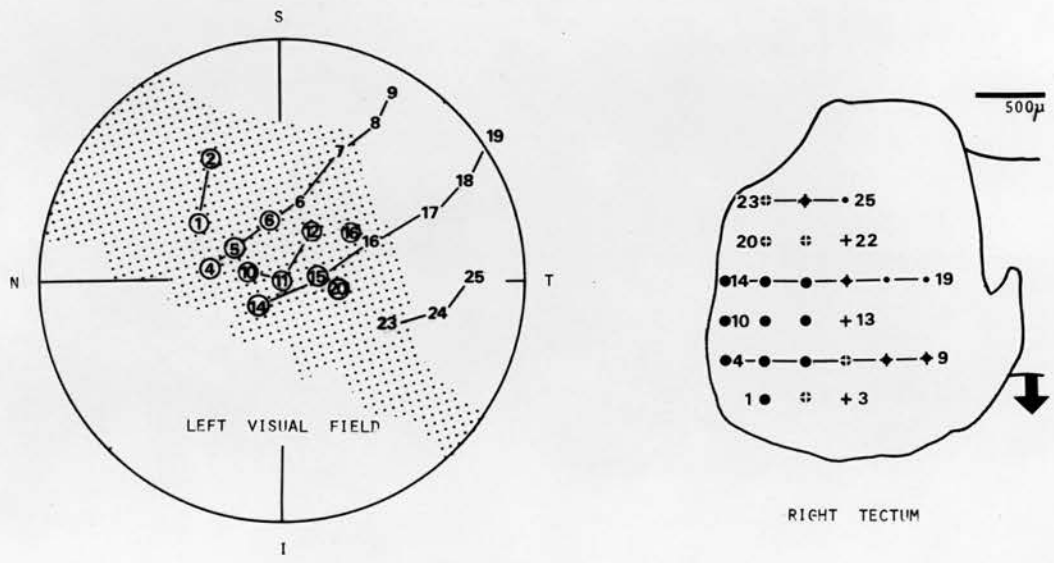
With a view to identifying the fibres occupying the two brachia, the medial division was cut during recording, with a generous stroke of a sharp tungsten needle and the surviving retinotectal map explored. Ten normal fish were used in this series and a typical result is shown in Map N1-Tr. In this case the full retinotectal map was plotted first; the selected tectal positions are shown in A and the corresponding field positions are shown on the left. After the medial division had been cut the animal was re-centered and re-mapped with the results which are shown in C. At the lateral edge of the tectum normal responses survived (marked by large filled circles) but as the electrode was moved medially there was a relatively sudden disappearance of responses; over most of the medial surface no responses whatsoever could be obtained; a few intermediate points were met (marked by crossed dots) where responses were reduced in intensity. Commonly the transition from normal to absent responses occurred within 300 μ.

The stippled area in the field map shows the area corresponding to the surviving responses on the tectum. Expressed in this way in terms



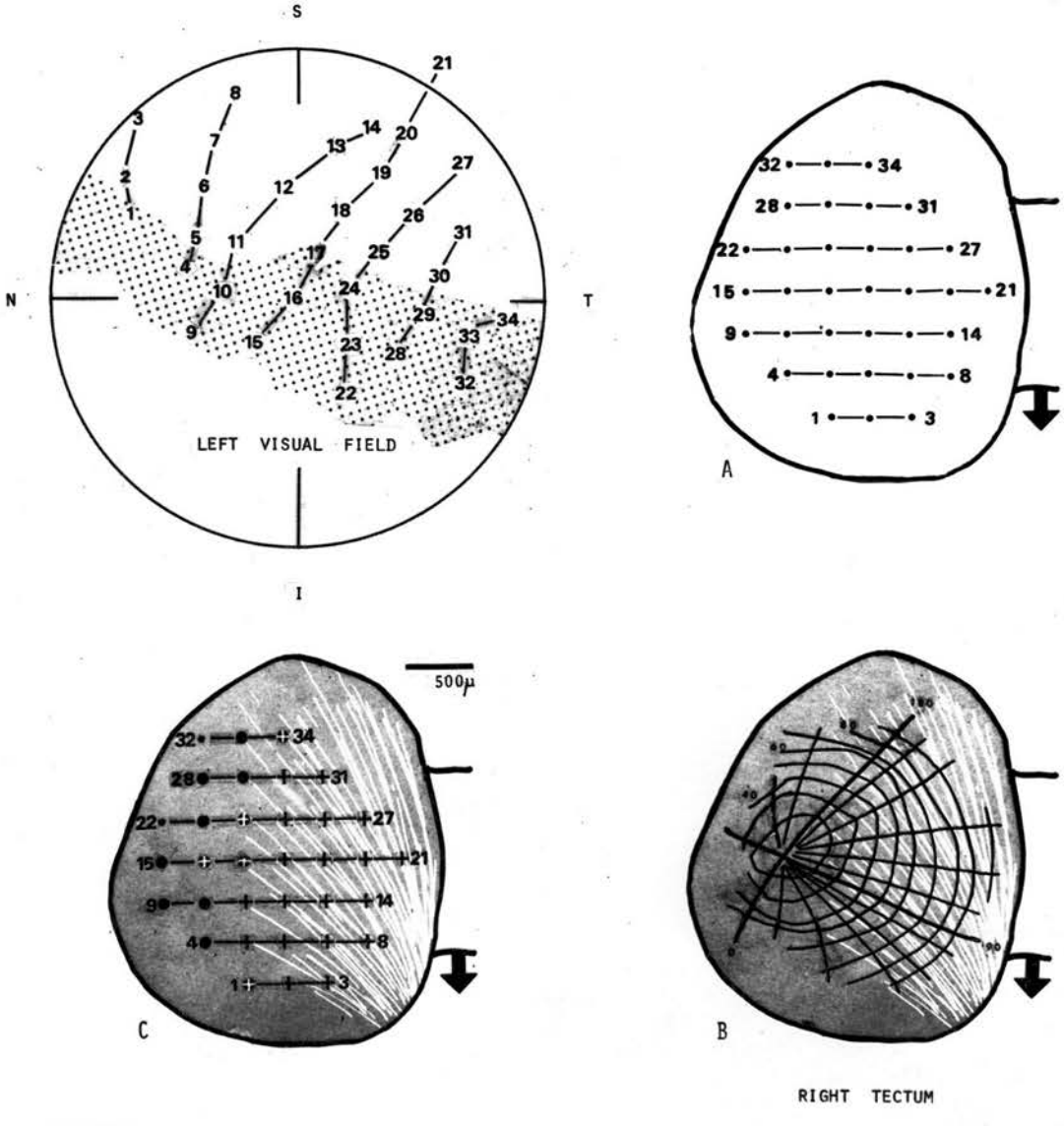
R278-Te

REGENERATED PROJECTION 278 DAYS AFTER L.O.N. CRUSH SHOWING PATTERN OF RESPONSES SURVIVING ACUTE SELECTIVE TRANSVERSE LESION OF ENTERING FIBRES



N7-Tr

NORMAL ANIMAL IN WHICH THERE IS AN EXCEPTIONAL SPREAD OF RESPONSES (SHOWN BY LARGE DOTS ON TECTUM AND STIPPLED AREA AND CIRCLED POINTS IN THE FIELD) AFTER ACUTE SECTION OF THE MEDIAL DIVISION OF THE OPTIC TRACT



N1-TR

NORMAL GOLDFISH VISUAL PROJECTION SHOWING RELATION OF TECTAL STRIATIONS TO THE RECORDING POSITIONS AND THE VISUAL FIELD LINES. A TYPICAL PATTERN OF POINTS SURVIVE ACUTE SECTION OF THE MEDIAL OPTIC TRACT DIVISION.

A SHOWS THE TECTAL RECORDING POSITIONS FROM WHICH RESPONSES AT THE CORRESPONDING NUMBERED FIELD POSITIONS WERE OBTAINED.

B SHOWS THE INFERRED MERIDIANS AND PARALLELS OF THE VISUAL FIELD ON THE TECTUM.

C SHOWS THE EFFECTS OF ACUTE SECTION OF THE MEDIAL DIVISION OF THE OPTIC TRACT. CROSSES MARK POINTS WHERE NO RESPONSES COULD BE OBTAINED; LARGE DOTS MARK SURVIVING RESPONSE POINTS WITH A WHITE CROSS DESIGNATING A RESPONSE OF REDUCED INTENSITY. THE CORRESPONDING SURVIVING FIELD IS STIPPLED.

of field positions, results are comparable for different animals; the irregularities and arbitrariness of comparing positions on the tecta of different animals are avoided. The accompanying diagram (Fig.5A) combines the surviving fields positions from the maps of six animals of this series. Field positions were determined either before or after section of the medial division; centering of the left eye on the perimeter was carefully checked and the results were consistent. The thick line marks the normal extent of the goldfish visual field and the empty area between it and the marked points represents the area of retina projecting exclusively through the medial brachium. In most cases surviving points were explored with the usual spacing of electrode positions on the tectum, occasionally at half the distance between positions.

Of the ten animals in the series nine were entirely consistent but one showed an exceptionally wide medial spread of responses surviving the lesion; this animal is shown in Map N7-Tr. The most likely explanation for this anomolous result is that the lesion was incomplete because the exceptional responses lie only at the front of the tectum; according to evidence elsewhere in this thesis these positions are supplied by fibres which occupy the deepest positions in the medial division of the tract. This animal was one of the first of the series; in a later case an attempt was made to reproduce the anomaly by making a lesion as restricted as possible, but the results were no different from those of the majority of animals.

In two other normal fish the lateral division of the tract was cut during recording and it was found that a completely normal map could be

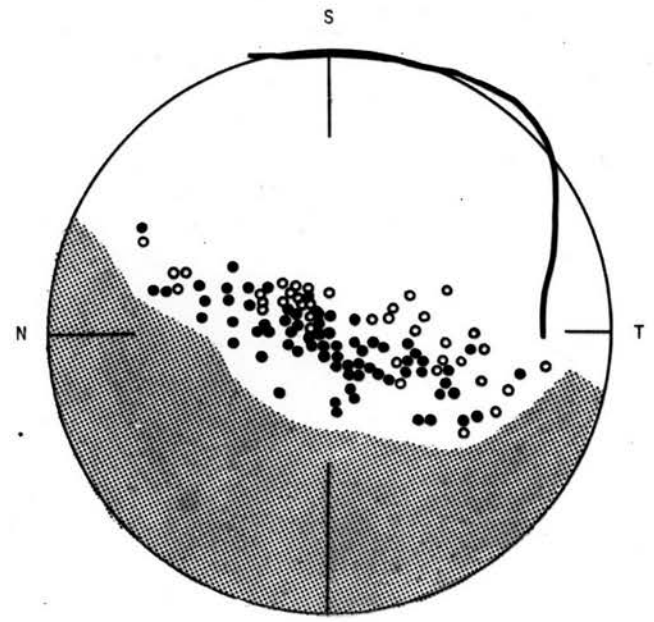


Fig. 5A.

FIELD POSITIONS OF RESPONSES SURVIVING ACUTE SECTION OF THE MEDIAL DIVISION OF THE OPTIC TRACT: 6 NORMAL ANIMALS

SHADED AREA NOT ACCESSIBLE FOR RECORDING. THICK LINE MARKS NORMAL EXTENT OF RECORDABLE FIELD. FILLED CIRCLES SHOW NORMAL RESPONSES; EMPTY REDUCED.

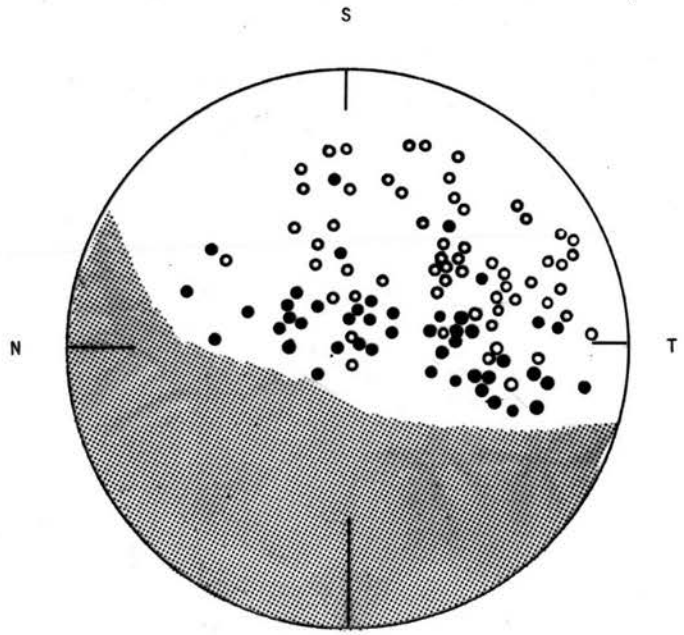


Fig. 5B.

8 ANIMALS AFTER OPTIC NERVE REGENERATION: FIELD POSITIONS OF RESPONSES SURVIVING ACUTE SECTION OF THE MEDIAL DIVISION OF THE OPTIC TRACT

obtained covering the entire dorsal tectal surface. On the reasonable assumption that the hidden surface of the tectum serving the ventral visual field had been denervated as the dorsal surface is denervated after section of the medial division, it follows that points along the lateral tectal edge are supplied through both divisions.

Fibres along the nasotemporal meridian of the retina are therefore not exclusively assigned to either brachium while the majority of fibres from the dorsal and ventral halves segregate completely between the brachia serving their respective halves of the tectum. These results are entirely consistent with the description of the goldfish optic pathway given by Attardi & Sperry (1963) and confirm experiments briefly described by Konishi (1960). They also conform with the present anatomical description of fibre paths in the nerve and tract (Montage 1) which shows that retinotopic organisation exists up to and beyond the division of the tract.

Part 11

Path specificity of regenerated fibres in the optic tract divisions

On the basis of their histological observations Attardi & Sperry (1963) conclude that, in general, fibres correctly recover their original paths in the tract during the course of regeneration although, because it was necessary to avoid the immediate vicinity of the head of the optic nerve in making the retinal ablations, it was never possible to achieve the ideal clear-cut result in which one tract division was entirely devoid of regenerated fibres: this is the result of complete removal of either superior or inferior retinal halves that they would predict. An earlier study of regeneration in Hyla cineria (Sperry, 1955) had concluded that there was only poor path selection in the tract, although Jacobson (see Jacobson, 1966) has found that in cases of Pattern 1 in Rana temporaria responses could be abolished over the corresponding half of the tectum by acute transection of one division.

Methods identical to those used for normal fish were employed to examine the distribution of fibres in the optic tract <u>after</u> regeneration in a series of 11 fish. Representative results are shown in Maps R191-Tr (p.86) and R54B-TrLD (p.87). The combined results are shown alongside the combined results for the series of normal animals (Fig.5). It can be seen that although responses originating from the most superior part of the field had been abolished, the surviving field was noticeably enlarged; it now includes responses from field positions outside the field which survives section of the medial division in normal animals.

These additional responses were almost always poor in quality. Regenerated animals were characteristically different from normal animals in that the cutoff between intact and silent response areas on the tectum was very gradual.

Of the 11 fish investigated 9 showed responses originating from positions outside the surviving field area of normal animals. The combined results illustrated here represent the whole sample equally; in 8 animals between 4 and 11 points were obtained during the normal course of re-mapping the projection which fell outside the normal area of surviving field positions. Details of the number of such points which are contributed by individual animals in Fig.5B are given in Appendix 2.

One animal contributed only one abnormal point to the combined results (R66); this essentially negative result may be due to the fact that in this case the lesion of the medial division had been extended across most of the tectum which entails more direct damage to the tectum. Two additional animals in the series could not be included because field positions were not recorded; the results in one case were representative and in the other were difficult to interpret. Map R62-TrLD (p.83) is an atypical case; although it includes four points lying outside the normal survival area these were not markedly abnormal and they were all in the temporal field. The significance of these results is difficult to assess and all the more so because the field map is unusual. As is indicated in the diagram it may be that the lesion was larger than intended; the possible extent of the first lesion is suggested by the second tectal diagram in Map R62-TrLD which shows that even when the

lesion was deliberately extended across the tectum a similar distribution of surviving points in the caudal tectum remains.

Summing up the results of this series of experiments; in 8 out of 11 animals there was clear evidence that fibres that, in the normal animal, reach the tectum exclusively by way of the medial division of the optic tract, follow a different route during regeneration. It is important to emphasise that fibres destined for the most medial tectal regions appear to follow entirely normal routes through the medial division and that, judging by the poor quality of most aberrant responses, only a minority of fibres destined for any given tectal site take a new route. The same observations have been made in a number of other animals to be described below in which the conditions of regeneration had been experimentally varied.

Before attempting an interpretation of these findings it is necessary to consider first, the significance of the aberrant responses and the fact that they gradually diminish in a medial direction, and secondly, the question of whether identical lesions in normal and regenerated animals are truly comparable.

The method of electrophysiological recording used here does not allow any quantitative assessment of the number of fibres terminating within the area occupied by the electrode tip; at best it is justifiable to describe the responses obtained as normal, reduced or totally absent. When, at a given position, no responses can be detected throughout the usual range of tectal depths, fibres can be assumed to be absent or in very much lower concentration than normal. The present results therefore confirm Attardi & Sperry (1963) in showing that after regeneration fibres supplying the more extreme medial portions of the tectum pass

only through the medial division.

In the zone intermediate between this and the lateral edge of the dorsal tectum the responses obtained after section of the medial division were of diminished intensity suggesting a relative sparseness of terminals; sometimes only single units could be found at one recording site and responses would sometimes be scored "absent" as frequently as they were scored "present".

The simplest interpretation is that the gradual fall off of responses medially reflects the likelihood that fibres destined for these areas chose correctly in the tract. The probability of entering medial or lateral division is equal for fibres destined for the lateral edge of the tectum but for fibres destined for more medial tectal regions the probability of entering the medial division progressively increases.

This is not the only possible interpretation however. It could be that fibres initially select routes at random and that fibres required to travel the greatest distances to reach their destinations are subsequently at some disadvantage.

The further possibility must be considered that a minority of fibres fail to follow the usual restricted routes of fibres in the two divisions but grow into the tectum by channels intermediate between these; such aberrant fibres might escape the lesion to the medial division and account for the anomalous distribution of surviving responses in regenerated animals. When observed with the dissecting microscope and in histological sections the segregation of the two divisions is as clear after regeneration as before. As will be shown in the following paragraphs it is clear that, as in normal animals, the majority of fibres destined for the medial tectum enter in a retinotopic

array from the rostromedial tectal pole (it is this fibre population that has been examined in the present experiment) but this does not exclude the possibility that other fibres simultaneously follow different routes to the same destinations.

In four animals the lesion to the medial division was extended across the front of the tectum and in all cases there was clear evidence (Maps R54B-TrLD, R62-TrLD) that responses within the anomalous surviving response area persisted behind and medial to the end of the extended lesion; it is therefore certain that some of the aberrant fibres do indeed follow a long route into the medial tectum by way of the lateral tectum. In one animal the lateral division was cut after having mapped the area of responses surviving section of the medial division. In this case all surviving responses were abolished; this is the only direct evidence that the aberrant fibres do indeed follow the path of the incorrect division rather than follow paths intermediate between the two divisions.

Part 12

Some factors affecting the segregation of regenerating fibres at the division of the tract

The following series of experiments were undertaken in an attempt to obtain further variations in the behaviour of regenerating fibres in the optic tract. As was pointed out in the foregoing section the method of plotting the distributions of fibres surviving acute section of the divisions which is being used here is hardly quantifiable. But these distributions can be classified as normal, or like those of straightforward regenerated animals already described, and the more extreme case might be obtained in which fibres were randomly assigned to either tract division and section of one would have a uniform effect over the whole projection.

Experiment 1

In six fish the left optic nerve was crushed at intervals of seven days for periods of up to 89 days. In this way fibres were effectively prevented from regenerating until prolonged degenerative changes had occurred in the optic pathways. After subsequent regeneration periods of some six months the animals were mapped. The time over which regeneration could be delayed by repeated crushing was limited by the increasingly severe bleeding of the scar tissue which formed around the optic nerve, and the formation of a neuroma around the peripheral nerve stump. In three animals of the series fibres evidently failed to penetrate the scar region; no responses were obtained at recording and

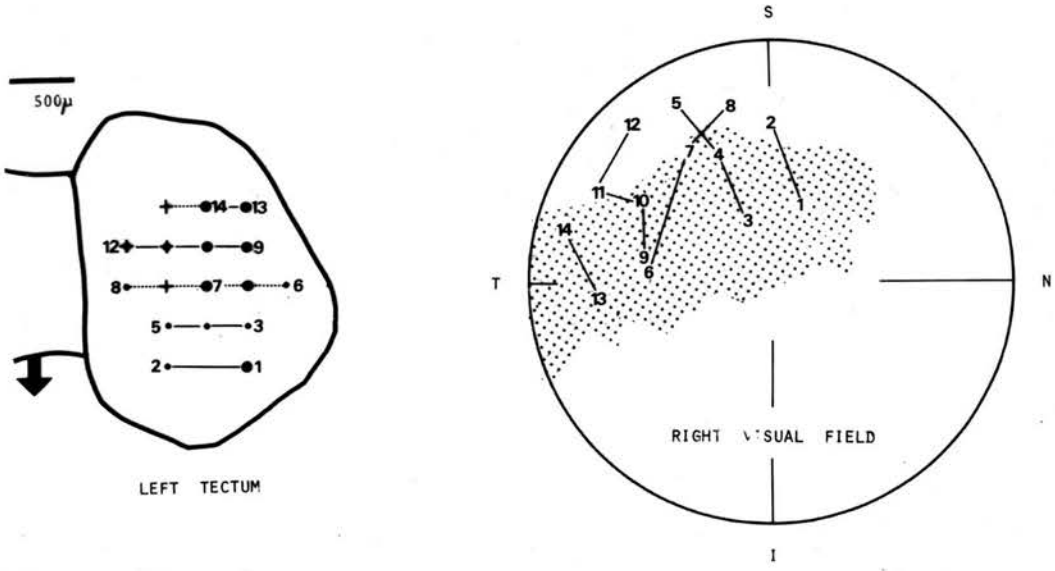
no fibres were visible in the tract (Plate 3B)

Map Delay-R160-Tr shows an animal which was successfully mapped six months after a delay period of 76 days (11 crushes). This and two other animals showed quite normal projections.

In two animals the medial division was cut acutely and the projection re-mapped; as in animals regenerated in the normal way, responses could be obtained from an abnormally extensive area of the medial half of the tectum. Electron microscopy of an animal after 85 days in which regeneration had been prevented showed that at this time small numbers of both normal and degenerating myelin sheaths remained in the optic layer. Plate 4 shows that even after 203 days the optic layer remains distinct, while in the same animal the tract was apparently structureless (Plate 3B). Electron microscopic evidence concerning the optic nerve suggests that myelin and cytoplasmic fragments are removed more rapidly and are virtually absent at the time regeneration began in the animals of this experimental series.

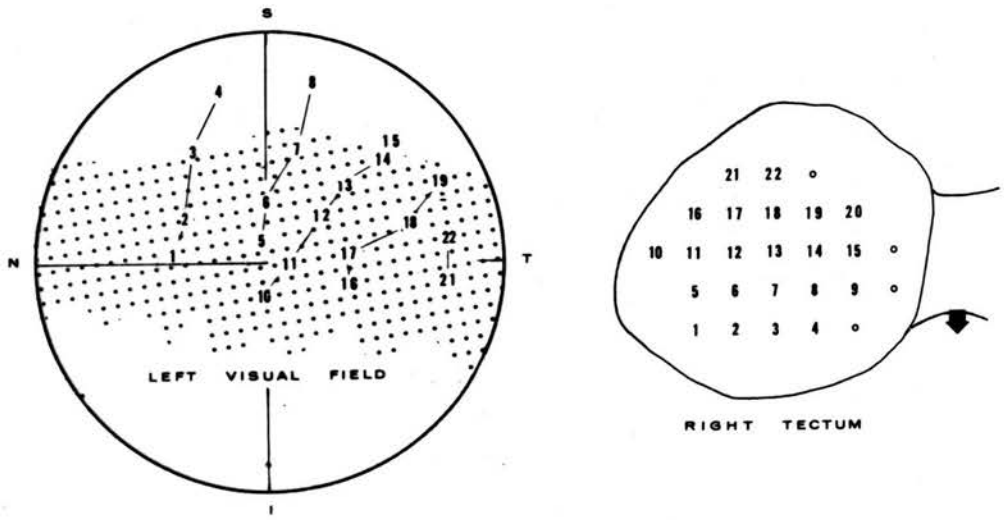
Experiment 2

In four animals the right optic nerve was crushed a second time 75 days after an initial crush when time had been allowed for regeneration. 192 days later one of these was mapped; the map and the spread of fibres after section of the medial tract division were not detectably different from normal. The remaining animals were lesioned once more two days later and mapped after 89 days. One animal failed to regenerate; the remaining two showed regenerated maps which were comparable with those of animals regenerated once. In Map 3rdR89-Tr the spread of fibres surviving section of the medial division is also comparable. In animals in which regeneration had occurred three times and final



3RDR89-TR

PROJECTION FROM THE RIGHT EYE TO THE LEFT TECTUM AFTER THE R.O.N. HAD BEEN PERMITTED TO REGENERATE THREE TIMES, FOR 75, 194 AND 89 DAY PERIODS CONSECUTIVELY. THE RESPONSE AREA SURVIVING ACUTE SECTION OF THE MEDIAL DIVISION IS SHOWN STIPPLED



DELAY-R160-TR

REGENERATED VISUAL PROJECTION AND FIELD SUPPLIED BY LATERAL DIVISION FIBRES MAPPED 160 DAYS AFTER REGENERATION HAD BEEN PREVENTED FOR 76 DAYS BY REPEATED OPTIC NERVE CRUSH

regeneration had been effectively delayed by some 269 days (during which time the original myelin and fibre remains underwent progressive degeneration) there was no evidence for any progressive degradation of either pathway or terminal selectivity.

Experiment 3

In two animals in which the dorsal half of the retina had been removed at the same time as the left optic nerve was being cut (Maps IRet-R76 and IRet-R85) the spread of regenerated responses surviving section of the medial division was again similar to that seen after regeneration of the whole optic nerve; it was therefore concluded that under conditions in which fibres destined for one division of the tract were absent (this was also a feature of the experimental design used by Attardi & Sperry, 1963) the remaining fibres behave much as they usually do. There was no evidence that fibres depend for their selection of one path on the presence and/or competitive influence of other fibres. The proportion of fibres taking the unaccustomed route to the medial tectum by way of the lateral tectum is unaffected by the absence of the fibres that would normally accompany them and their ability to recover their terminals correctly in the medial tectum is apparently also unaffected by the fact that they have to pass through unsupplied areas of tectum, a conclusion already reached by Attardi & Sperry.

In animal IRet-R76 the lateral division of the tract was cut after mapping of the distribution of responses surviving section of the medial division; this abolished all responses and confirmed that all fibres pass into the tectum in one or other of the two divisions.

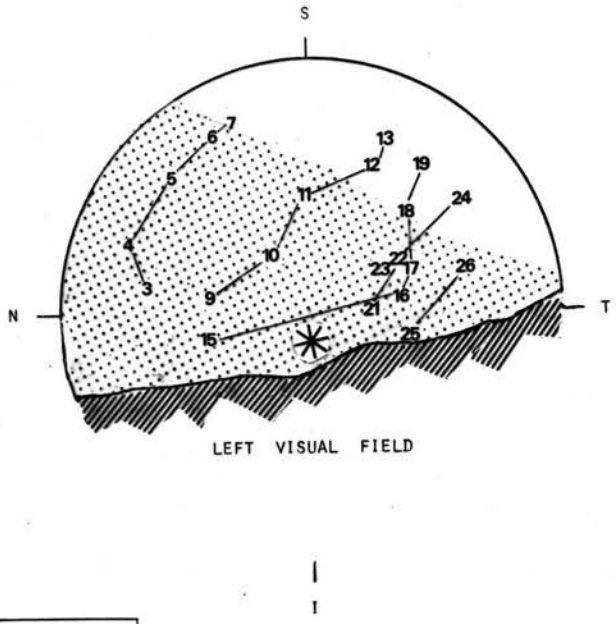
Experiment 4

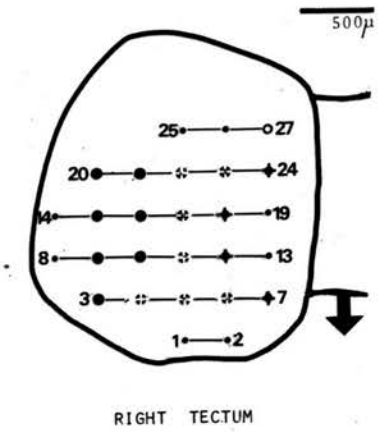
In six animals a random selection of optic fibre fascicles were cut as they passed across the retina towards the fundus. These fibres, representing very approximately a third of the total, were then allowed to regenerate in the company of the remaining intact fibres through the optic nerve which was not further lesioned in any way. Later electron microscopy confirmed that extensive regeneration occurred through the nerve (see Plate 8B).

One animal died at recording; four gave entirely normal projection maps and distributions of fibres after acute section of the medial division which were like those of normal animals; Animal RetR155-Tr was mapped after section of the medial division and showed some evidence of an expanded area of surviving responses comparable to regenerated animals. The significance of these findings is difficult to assess because the likelihood of detecting aberrant regenerated fibres is reduced due to the smaller number of fibres involved in regeneration.

The electron microscopic evidence (p.58) suggests that regenerating fibres growing in the presence of intact fibres maintain their retinotopic order rigidly. In the present experiment there is no question of regenerating fibres being displaced from their normal retinotopic arrangement as they might otherwise be by the mechanical effects of cutting the optic nerve.

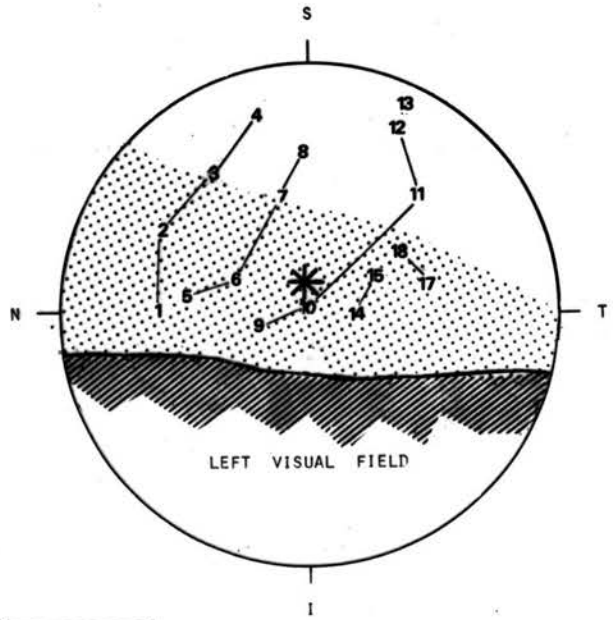
In general conclusion to this section, it would appear that under a variety of conditions which would be expected to affect the composite structure of the tissue they are growing through, fibres behave much as

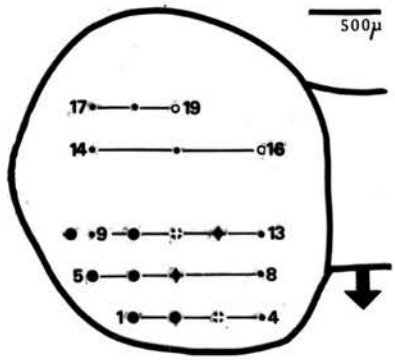




IRET-R76

PROJECTION OBTAINED 76 DAYS AFTER L.O.N.CUT AND REMOVAL OF THE SUPERIOR HALF OF THE LEFT RETINA



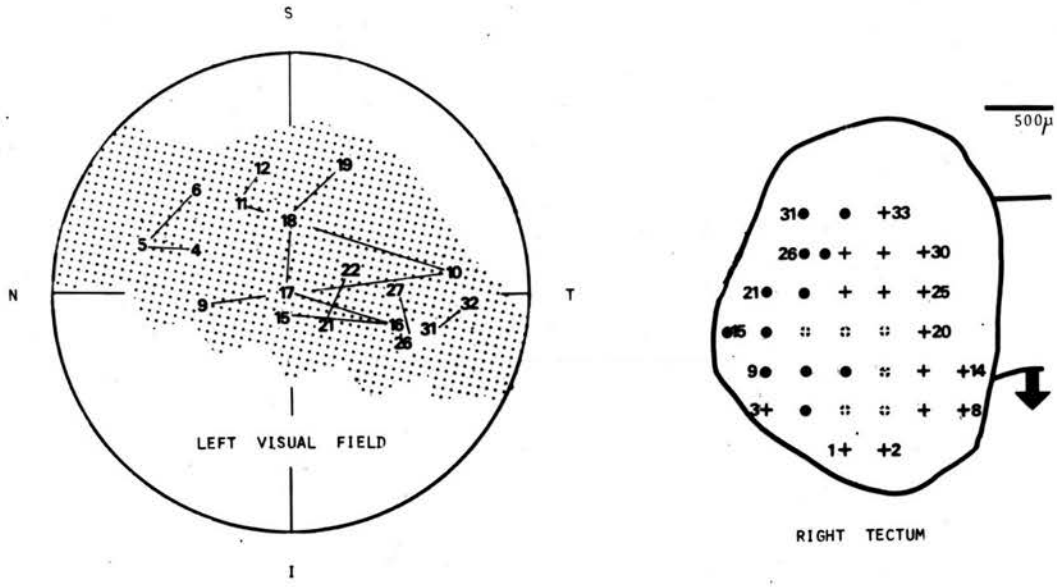


IRET-R85

PROJECTION OBTAINED 85 DAYS AFTER L.O.N CUT AND REMOVAL OF THE SUPERIOR HALF OF THE LEFT RETINA
RESPONSES COULD BE OBTAINED FROM THE VENTRAL UNDERSIDE OF THE TECTUM FROM THE CENTRAL VISUAL FIELD.

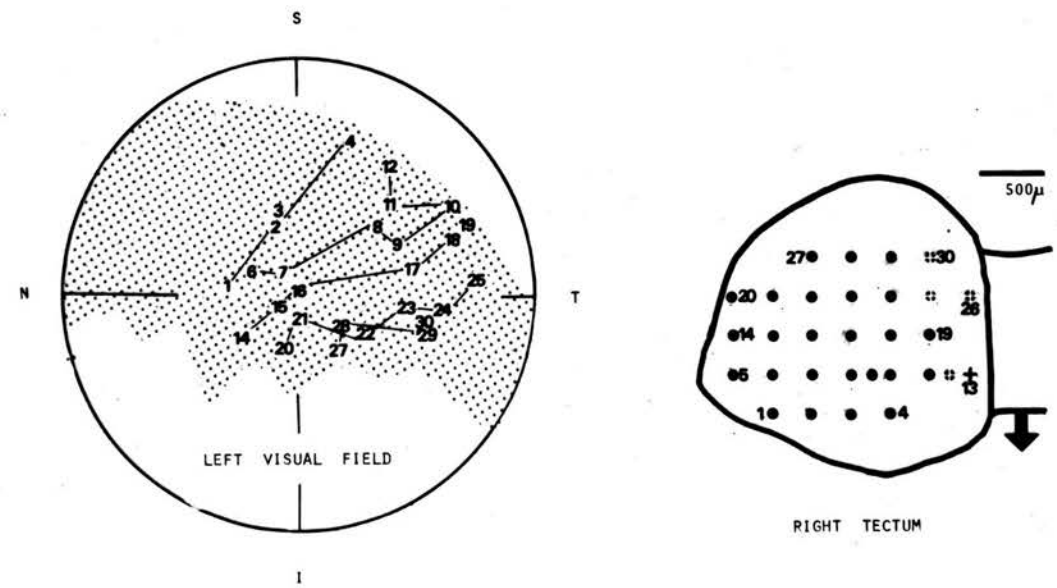
they do during straightforward regeneration. In no cases were either the retinotectal map or the selective behaviour of fibres in the tract significantly degraded or modified; under a variety of conditions of regeneration, fibres displayed an almost invariable affinity for routes in the tract and in no cases were conditions met in which fibres showed as high a degree of path specificity as exists in the normal animal or complete absence of path preference. The operation which probably results in the greatest disturbance to fibres' choice between the divisions of the tract is transection of the optic tract in place of the optic nerve. Map TrR329-Tr shows that responses survive throughout the tectum of such an animal after acute section of the medial division of the tract; retinotopic order among the surviving responses was good.

In the course of these studies fibres were lesioned in a variety of ways; they were cut in the retina, in the nerve, in the tract and in the medial division, and, in other cases, crushed once in the nerve or repeatedly crushed and mechanically disrupted throughout the length of the nerve. Despite the variety of conditions that were met by fibres here retinotopic order was restored to the same degree.



RETR155-TR

PROJECTION SURVIVING ACUTE SECTION OF THE MEDIAL DIVISION IN AN ANIMAL WHERE A PROPORTION OF OPTIC FIBRES HAD BEEN CUT IN THE LEFT RETINA 155 DAYS BEFORE



TRR329-TR

REGENERATED PROJECTION OF ANIMAL IN WHICH LEFT OPTIC TRACT HAD BEEN CUT 329 DAYS PREVIOUSLY SHOWN AFTER ACUTE SECTION OF THE MEDIAL DIVISION OF THE OPTIC TRACT

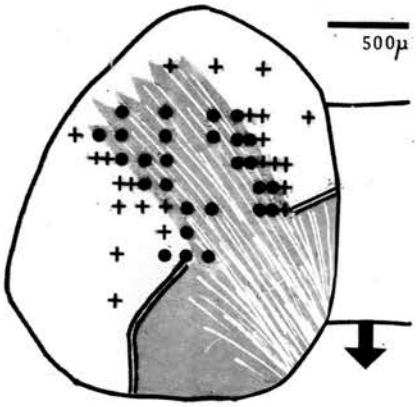
Part 13

The paths of optic fibres across the tectum in normal fish

With a view to investigating the retinotopic arrangement of fibres entering the tectum from the medial tract division, transverse cuts were made near the rostromedial pole leaving a small bridge of tissue connecting rostral and caudal parts of the tectum. The retinal origins of the optic fibres passing through the intact portion could then be examined by mapping the tectum caudal to the lesion and the distribution of the responses gives further information about the paths followed by the fibres across the tectum.

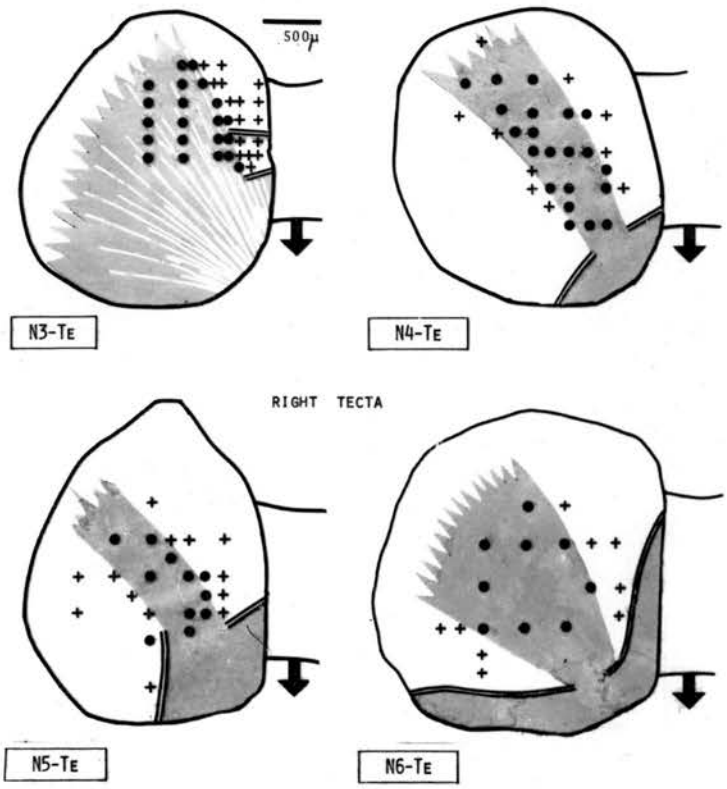
Map N2-Te shows a typical result of such an experiment in a normal fish. The position of the lesion, which usually extended through the entire thickness of the tectum including the lateral division, is shown by the double line. Circles mark positions of responses and crosses mark points at which no response at all could be detected; the grey shading marks out the inferred area of surviving responses. The receptive fields of the surviving responses were sometimes checked in order to confirm that the usual retinotopic arrangement of terminals applied under these conditions.

In some experiments the alignment of the visible optic fibre bundles on the tectum was drawn onto the tectal map and as the electrode was being positioned it was noted whether it lay over a bundle that had been cut or one that had been left intact by the lesion. In this case



N2-TE RIGHT TECTUM

PATTERN OF RESPONSE POINTS IN A NORMAL OPTIC TECTUM AFTER A TRANSVERSE LESION SPARING A SELECTED GROUP OF ENTERING OPTIC FIBRES
LESION MARKED BY DOUBLE LINE. CIRCLES SHOW SURVIVING RESPONSES; CROSSES NO RESPONSES.



PATTERNS OF RESPONSE POINTS IN FOUR NORMAL TECTA AFTER SELECTIVE TRANSVERSE LESIONS

and consistently in eight other fish investigated the area of surviving responses extended caudally from the lesion following the same contours as the bundles. Lateral movement of the electrode by some 50μ was sufficient to take it across the sharp boundary between areas of normal responses and no responses. The optic bundles diverge gradually as they pass from the front of the tectum to the back; at half way they lie about 35 μ apart.

These results show that fibres form their terminals at sites lying along their line of growth across the tectum. Responses were never obtained more than about 75 μ outside a line separating cut and uncut bundles. The more usual 50 μ spread of responses beyond this line may reflect the size of the terminal arborizations of optic fibres; consistent with this interpretation is the fact that the spread of responses from an individual bundle is constant throughout its length. If a second lesion is placed (as in N3-Te) caudal to the first, cutting only those bundles that had already been cut, the line of demarcation of the surviving responses is unchanged. This confirms the conclusion that the fibres supplying the response area travel within the same bundle throughout their course across the tectum. Fibres only rarely move between neighbouring bundles; this can occasionally be seen in the most rostral tectum; evidently fibres never jump more than two bundles to either side of their original bundles.

Transverse lesions were placed on the tectum which were carefully restricted in depth so that only the visible optic bundles were transected. Caudally all responses were then absent; this confirms that incoming optic fibres are confined to the visible tectal striations.

It is concluded that in the normal goldfish optic fibres enter the tectum from the optic tract in a highly orderly manner, each being already assigned to a particular and separate bundle which follows a precise and regular path across the tectum supplying a narrow rostrocaudal band of terminal sites.

Part 14

The paths of optic fibres across the tectum in regenerated fish

The same procedure was repeated in a series of 11 fish which had previously undergone regeneration of the left optic nerve for varying lengths of time. Broadly speaking the results were the same as those obtained in normal fish. As can be seen in the four regenerated animals presented here (R278-Te, R63-Te, R54A-Te, R286-Te) the area of surviving responses extended back from the transverse tectal lesion with the line of demarcation between it and the area in which no response could be obtained following the usual orientation of the optic bundles.

The course of degeneration and disappearance of the visible optic bundles after cutting the optic nerve is slow. By forty days after unilateral enucleation, degenerating bundles are indistinguishable from normal, and at three months they can be seen extending throughout their normal ranges in the tectum though less distinctly. After seven months they are absent from most of the tectum although faint traces of them can be detected near the entrance of the medial division. After regeneration there is a slow recovery of the usual pattern of bundles; by nine months the regenerated side is barely distinguishable from the normal side in clarity and in orderly arrangement. An incidental observation was that after ablation of the caudal half of the tectum together with section of the optic nerve, visible bundles disappeared much faster than has just been described.

These two lines of evidence (mapping after tectal lesions and the recovery of visible optic fibre bundles) show that as a result of the regeneration process optic fibres recover their normal routes into the tectum, as well as their normal terminations. Again, lesions restricted to the vertical level containing the bundles abolished responses beyond, but this method is not of sufficient precision to reveal any small increase in the depth distribution of incoming fibres.

In one respect the situation differed in regenerated animals; the sharpness of cutoff from areas of normal response to areas in which responses had been completely abolished was less and responses could, on occasion, be detected 300 μ or more beyond the line dividing cut and uncut bundles - these responses were usually noticeably poorer than responses within the expected response area. Whether poor responses are detected depends among other things on qualities of the electrode, amplification and background noise. These factors vary between different animals and this is almost certainly one explanation for the fact that deviant responses were only detected occasionally in some of the animals studied.

According to the results of Part II some regenerated fibres may reach positions on the dorsal tectum by way of the lateral division of the tract and would therefore not be included in lesions confined to medial division fibres. In all the present experiments the lateral division was cut to avoid this complicating factor. In a number of experiments the tectal lesion was completed transversely in order to confirm that all the responses previously mapped were actually due to fibres passing through the gap in the lesion. A further variable that

might have a bearing on the results is how near the origin of the bundles the lesion had been placed; lesions placed rostrally might give more variable results because fibres might be less specifically arranged within bundles near their start. None of these considerations account for the present deviations from the normal situation.

In cases where the lesion was placed at the medial edge of the tectum responses were observed up to 300 μ medial to the line of the most medial of the uncut group of bundles (an example is R278-Te) while with laterally placed lesions deviating responses were observed up to some 600 μ beyond the line dividing cut and uncut bundles and there was evidence that they deviated further at increasing distances caudal to the lesion. It should be emphasized that in all cases, including regular and deviant responses alike, responses were correctly arranged within the retinotectal projection map.

The most likely explanation for these findings is that just as a minority of regenerating fibres take the "wrong" path at the division of the optic tract so some fibres initially select an inappropriate tectal bundle as they leave the tract; in order to reach their correct terminal sites these fibres would then have to move further out of the line of their original bundle than occurs in normal animals. Nevertheless signs of such aberrant fibres were entirely absent in some animals and where they were recorded they were only weak responses; few fibres are involved compared to the number which are correctly assigned to their tectal bundles.

Like the lateral division it is probable that after regeneration the medial division contains fibres usually assigned to the other.

This may account for the large deviations seen with laterally placed lesions. Indeed in two cases where the lesion was lateral and rostrally placed, some deviant responses were seen for some distance along the caudal edge of the lesion and in the area beyond; these represented the greatest deviations observed and may be related to the passage of fibres, wrongly assigned in the tract, towards the lateral tectum.

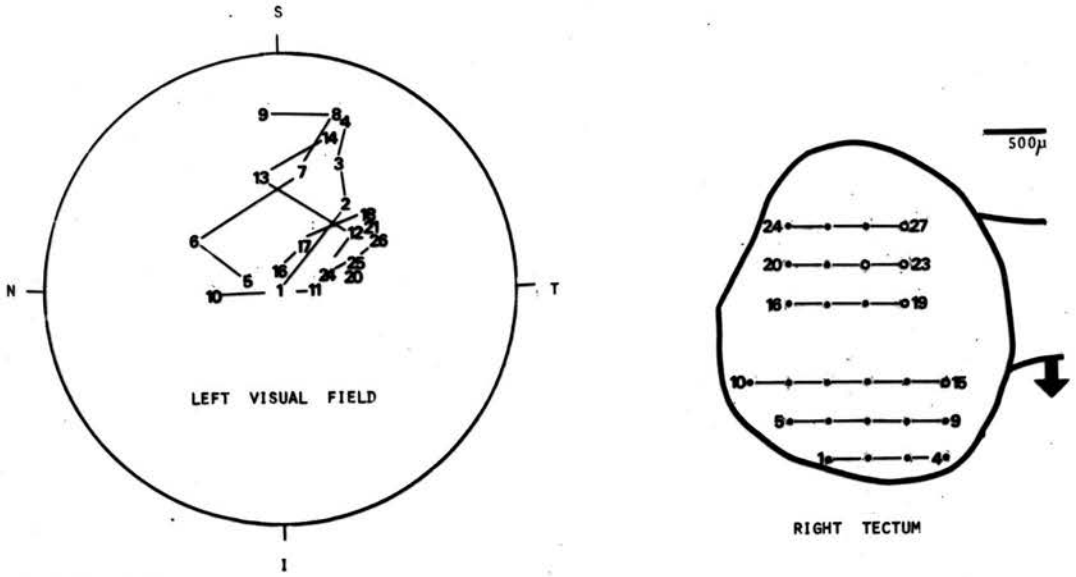
Part 15

Specificity of fibre terminations in the transverse tectal axis

As was shown in the Introduction there is evidence that in the fish, as in Xenopus, fibres always display their normal predisposition to form their termination at a fixed point along the transverse tectal axis, even under experimental conditions comparable to those which induce fibres to terminate at new sites longitudinally on the tectum. Jacobson & Gaze (1965) found no evidence for abnormal connections when one half of the optic nerve was delayed in its regeneration relative to the other, or when either medial or lateral halves of the tectum were removed prior to normal regeneration. These results might be accounted for, however, by saying, in the first experiment, that the "delayed" fibres were in fact present at the tectum at the same time as the other fibres but in insufficient numbers to be detected. In the other case the tectal lesion may have prevented fibres from the corresponding medial and lateral tract divisions from having access to the surviving tectal areas.

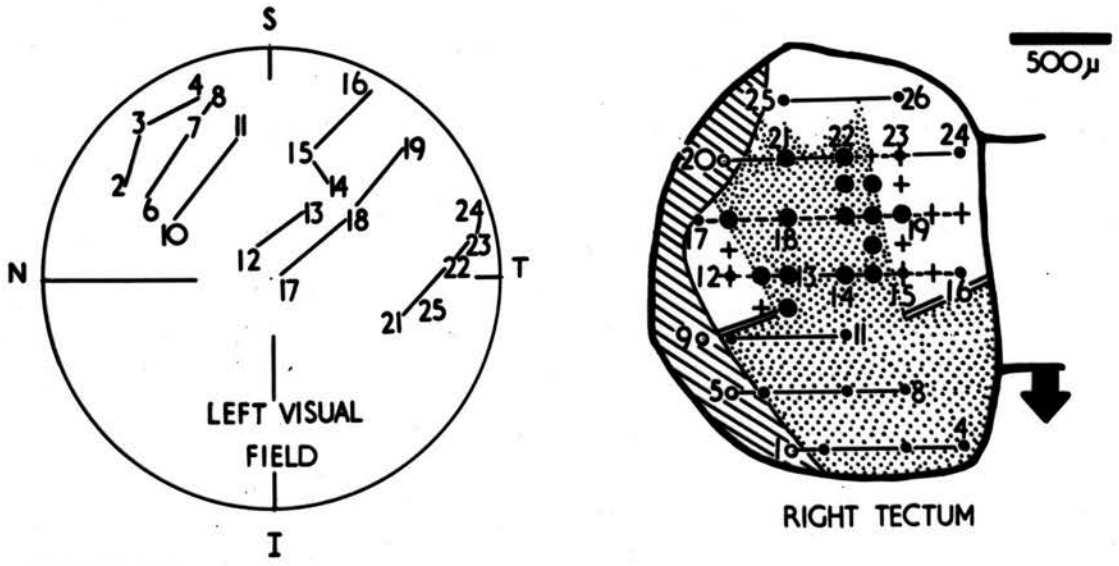
Experiment 1

In an attempt to avoid this problem the lateral half of the right tectum was widely ablated in three surviving animals and the lateral division of the right optic tract was freed and implanted directly into the medial division which itself was transected in the process. At recording 79 days later Animal Tr-mTe-79-Te showed a normal regenerated projection from the surviving tectum (although responses became weaker



NSRET-R82

PROJECTION MAPPED 82 DAYS AFTER L.O.N. CUT TOGETHER WITH REMOVAL OF THE TEMPORO-INFERIOR HALF RETINA



TR>MTE-79-TE

PROJECTION OBTAINED 79 DAYS AFTER REMOVAL OF LATERAL HALF OF RIGHT TECTUM AND IMPLANTATION OF LATERAL DIVISION INTO CUT MEDIAL DIVISION OF RIGHT OPTIC TRACT. THE EFFECT OF ACUTE SELECTIVE TRANSVERSE SECTION OF THE TECTUM IS SHOWN

laterally and more difficult to map because the tectum had tended to shift laterally as a result of the lesion) with no evidence whatsoever that terminations had shifted in the medial direction as a result of the transplantation of the lateral division. In fact the fibres of the lateral division appear to have been unable to terminate on the surviving tectum, despite the fact that they had access to it which was equivalent to the other fibres. Further evidence that the behaviour of fibres was unaffected by removal of part of the tectum is the fact that fibres take normal routes across the medial tectum from the medial division of the tract.

In the second animal the map was similarly unaffected (there were gaps in the field corresponding to the area of tectum ablated) although there was slight nasotemporal compression of the final rows in the map. The third animal was consistent but the map was incompletely explored.

Histology does not reveal the destinations of the lateral division fibres; the disorganisation of the region of the transplantation was considerable. It is unlikely that the operation was entirely successful in its aim of transplanting the lateral into the medial division and there was a suggestion in the histology that, as in the experiments of Arora & Sperry (1962), the transplanted fibres had attempted to regenerate away from the medial division in a lateral direction.

Experiment 2

In three animals the dorsal half of the retina was ablated at the same time as the left optic nerve was cut. Although there was no evidence for compression in the transverse tectal axis in the above experiment, this experiment attempted to induce spreading of a half-retina along the whole extent of the transverse axis of the tectum.

The maps, IRet-R76 and IRet-R85 (p.107), show normal reinstatement of the projection to the dorsal tectum. The measured field positions probably reflect ganglion cell positions in the retina wall because there was minimal mechanical distortion to the eye and shift in the orientation of the retina (as can be judged by the position of the fundus) as a result of the operation.

In an attempt to discover whether the ventral tectum was entirely devoid of terminals the recording electrode was lowered through the whole tectum to its underlying surface. Where responses could be obtained they were weak and invariably originated from the zone of the horizontal meridian of the field. It is not possible to determine the extent of responses on the ventral tectum but the impression was that fibres representing the centre may have extended further ventrally than would normally be the case; this part of the field projection might therefore be somewhat expanded but there was no graded shift of the whole projection comparable to that seen in double nasal or double temporal compound eye projections.

Part 16

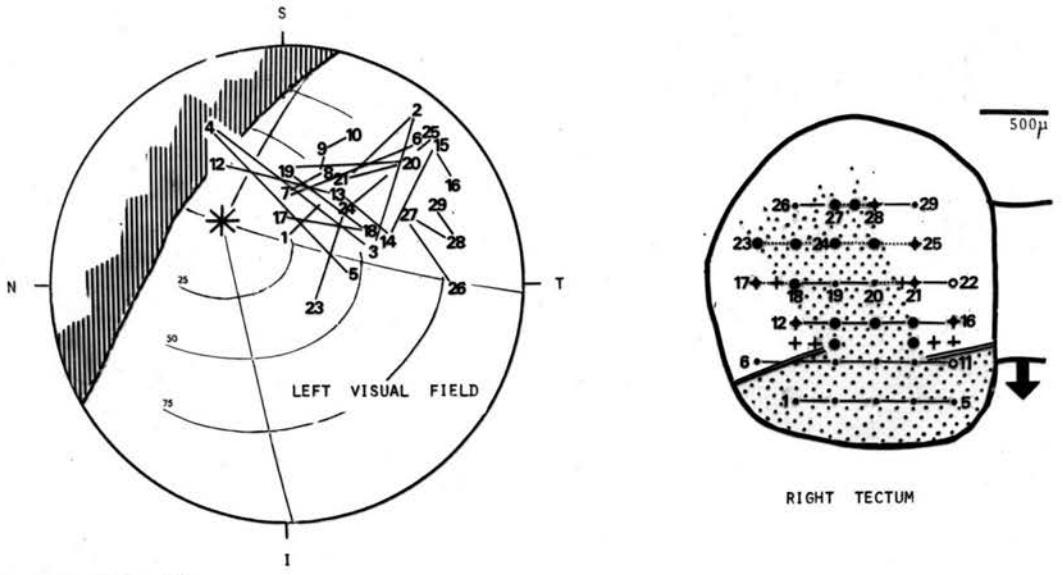
Specificity of terminations within the longitudinal tectal axis

Experiment 1

This experiment duplicates Experiment 2 above except that a different axis of the retinotectal projection is involved. In eight animals the inferotemporal half of the left retina was removed and the left optic nerve cut. Five animals were successfully recorded and gave consistent results; three in which complete regenerated maps were obtained are shown here with an indication of the area of retina that had been removed shown in terms of the field it would normally represent (NSRet-R82, NSRet-R69, NSRet-R63-Te).

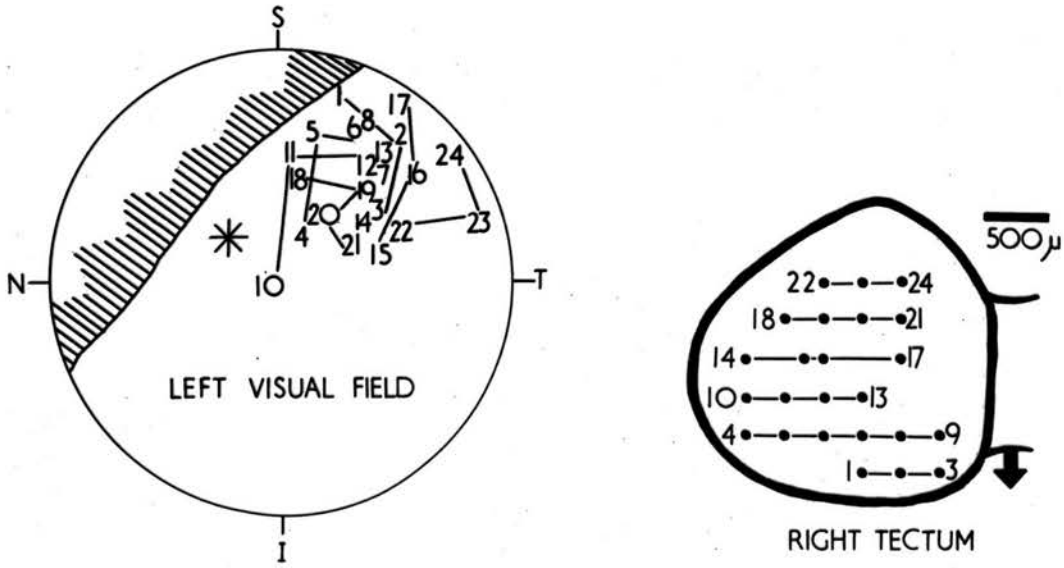
In all cases responses were obtained equally from the area of tectum to which the surviving part of the retina would normally project and from the remaining, rostral tectum. The responses were frequently from large receptive fields, but the location of the fields was reproducible and maps were systematically organised. Retinotopic order was intact within the transverse tectal axis but poor in the longitudinal axis.

There was no evidence here for preferential regeneration of the surviving optic fibres to their normal terminal area in the tectum. The arrangement of terminals was no more orderly in the caudal half of the tectum than in the rostral. The absence of retinotopic order in the nasotemporal axis may in part be the result of the small area of the surviving field; the mapping method used in the present experiments may be insufficiently discriminating to detect differences of receptive fields within this. This was not the case, however, in the following experiment.



NSRET-R63-TE

PROJECTION REGENERATED 63 DAYS AFTER SECTION OF THE L.O.N. AND REMOVAL OF THE TEMPORO-INFERIOR HALF OF THE LEFT RETINA.
THE EFFECT OF ACUTE SELECTIVE TRANSVERSE SECTION OF THE TECTUM IS ALSO SHOWN.
NUMBERED FIELD POSITIONS ARE ARRANGED AS RECORDED WITH SUPERIOR POLE OF FIELD UPWARDS.
SUPERIMPOSED ARE THE APPROXIMATE, OBSERVED POSITIONS OF THE OPTIC NERVE AND THE BORDER OF THE LESION, AND THE INFERRED RESULTANT POSITIONS OF FIELD LINES; ALL ROTATED SO AS TO CORRESPOND WITH AXES OF RECORDED PROJECTION.



NSRET-R69

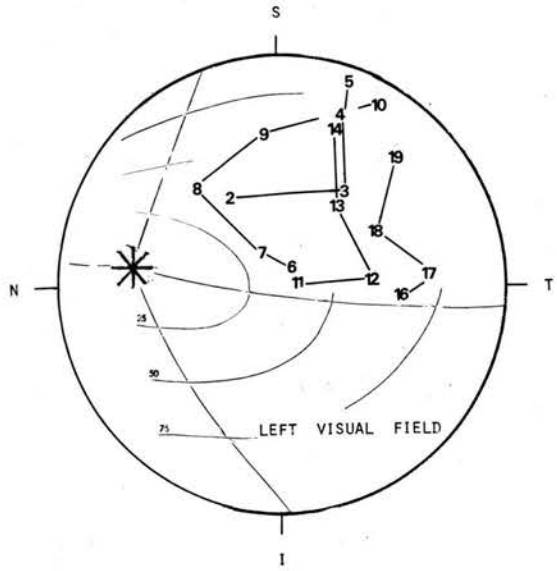
PROJECTION MAPPED 69 DAYS AFTER L.O.N.CUT TOGETHER WITH REMOVAL OF THE INFERO-TEMPORAL HALF RETINA

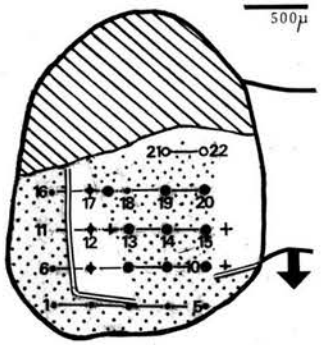
Experiment 2

In this experiment an attempt was made to confirm and to isolate one factor which contributes to the results seen in Experiment 1; the behaviour of fibres destined for the caudal tectum within the non-corresponding rostral half tectum; in order to do this the same retinal operation was combined with removal of the caudal half-tectum in 11 animals, 5 of which yielded too few points at recording to be useful. All were consistent.

Again there were no points in the rostral tectum at which responses were absent and the whole of the surviving retina appeared to be represented in the surviving tectum. In all cases retinotopic order was good transversely and in some cases (NRet-rTe-R107-Te, NRet-rTe-R87-Te) it was clearly established along the tectum also. Although the retinal lesions were so placed that there might have been some overlap between the surviving central retina and areas of the surviving tectum which it would normally supply, there is clear evidence for a systematic shift of the projection forwards. The most caudal rows on the surviving tectum which would normally be supplied by fibres originating near the vertical meridian of the retina are now supplied from the extreme nasal retina which itself would normally project to the ablated tectum. The most rostral sites on the tectum which would normally be supplied exclusively from areas of the retina which have been ablated are now supplied from near the vertical meridian of the retina. Map NSRet-rTe-R57 is more similar to the maps seen in Experiment 1 because there was no clear ordering of the rows along the tectum.

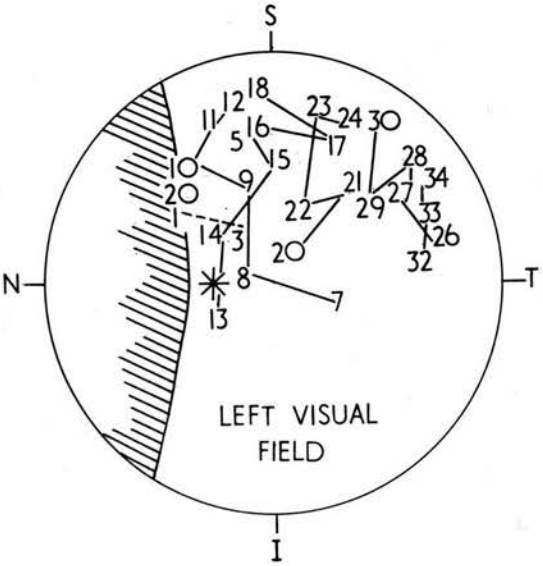
There is no direct parallel between these results and those of

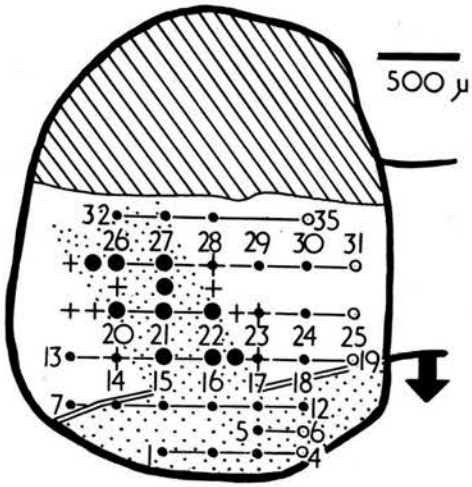




NRET > RTE-R107-TE

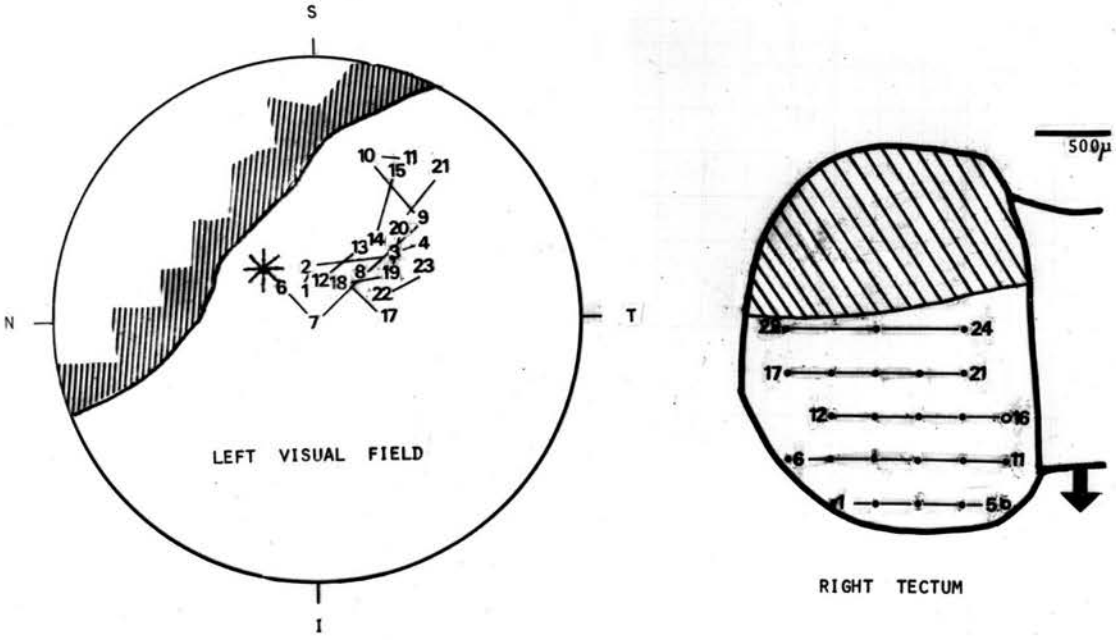
PROJECTION OBTAINED AFTER 107 DAYS REGENERATION (L.O.N. CUT) OF THE NASAL HALF RETINA INTO THE NONCORRESPONDING, ROSTRAL HALF TECTUM, INCLUDING THE EFFECTS OF ACUTE SELECTIVE TRANSVERSE SECTION OF THE TECTUM





NRET > RTE-R87-TE

PROJECTION OBTAINED AFTER 87 DAYS REGENERATION (L.O.N. CUT) OF THE NASAL HALF RETINA INTO THE NONCORRESPONDING, ROSTRAL HALF TECTUM, INCLUDING THE EFFECTS OF ACUTE SELECTIVE TRANSVERSE LESION OF THE TECTUM



NSRET > RTE-R57

PROJECTION OBTAINED AFTER 57 DAYS REGENERATION (L.O.N. CUT) OF THE NASO-SUPERIOR HALF RETINA INTO THE NONCORRESPONDING, ROSTRAL HALF TECTUM

Experiment 1; in the latter the surviving half-retina did not project in the same orderly manner to the rostral tectum as it does after removal of the caudal half-tectum. This raises the possibility that the mechanism determining the ordering into the rostral tectum may be the same as that observed by Gaze & Sharma (1970) in an experiment also involving removal of the caudal half-tectum; in that experiment the fibres destined for the caudal tectum superimposed their terminals retinotopically over part of the intact projection to the rostral tectum.

Again in this experimental series fibres appear to follow precisely the same paths into the tectum as they do in a normal and intact animal.

CONCLUSIONS

The completeness of recovery of retinotectal connections in regeneration

The electron microscopic studies described in this thesis have shown that goldfish possess some unique advantages over other species (p.60) for the purpose of investigations of orderly regeneration of the optic pathway; among other lower vertebrates in which regeneration occurs teleosts may be unique in having an optic nerve in which the unmyelinated fibres are confined to one small compartment while the greatest part of the nerve is composed entirely of myelinated fibres. It may not be a coincidence that, as in mammals where the optic nerve is totally myelinated, the goldfish optic pathway is arranged in a precise retinotopic manner throughout its length; it is this feature which accounts for the clear cut results of mapping the projection after selective lesions to the divisions of the tract or the tectal bundles (Parts 10 & 13). The majority of regenerating fibres follow their original paths (Parts 11 & 14).

Because the goldfish optic nerve is almost totally myelinated it is possible to estimate the number of fibres which successfully re-establish connection with the tectum by counting the number of remyelinated regenerated fibres in the nerve, having made the assumptions that myelination is a consequence of reconnection with a suitable terminal site (Aitken et al., 1947; Aitken, 1949) and that every fibre reaching the tectum will tend to become myelinated. The counts (Parts 7

& 8) suggest that at least 50% of fibres reach the tectum and possibly as many as 100%. The time allowed in the present experiment should be sufficient to permit all those fibres that had been successful in this respect to myelinate fully. The fibre diameter histogram confirms that smaller fibres are not in process of myelination. However it is known that the number of regenerating fibres depends on the form of the lesion (Aitken et al., 1947) as does the rate of myelination (Glimstedt & Wohlfart, 1960).

The nature of the residual unmyelinated fibres which are a prominent feature of long-term regenerated nerves is unknown. The work of Young and his colleagues on regeneration in peripheral nerves does not take unmyelinated fibres into consideration. If myelination is a consequence of connection with the tectum, fibres must be unmyelinated when they first re-establish terminals there and this seems to be the case (Part 7). It is certainly a possibility that some reconnected fibres remain unmyelinated, but it is unlikely that these are related directly to the group of unmyelinated fibres which exist in the normal nerve because they are uniformly distributed throughout the regenerated nerve.

Compared to the maps obtained from normal animals, regenerated maps show various degrees of irregularity and in some cases evidence for <u>en bloc</u> displacement of the projection forward on the tectum. The fact that irregular projection maps have been observed in long-term regenerated fish in which the recorded electrical responses were normal in quality and had normal sized receptive fields, suggests that these maps represent stable end-points of the regeneration process and that this process may be liable to certain minor errors. Similar errors might account for some features of Pattern 2 maps in frogs (see Appendix 1).

It became apparent in the work described in this thesis that regenerating fibres make "errors" in their choice of paths at the division of the optic tract and possibly later as they are assigned to tectal bundles. It was clear, however, that a wrong choice of path does not affect a fibre's ability to reach its original terminal site successfully; so errors in path selection as such are unlikely to account for irregularities of the projection maps or for the initial enlargement of the receptive fields of multiunit responses.

An unexpected finding was that long-term modifications of optic fibre terminals can occur; this is clearly shown by the reduction of multiunit receptive field sizes which occurs after 70 days. The same phenomenon had been noted in newts by Cronly-Dillon (1968) and it may well be related in mechanism to the process of rearrangement of terminals which may occur in the intact rostral half tectum (Gaze & Sharma, 1970) after removal of the caudal half (without cutting the optic nerve); similar time scales are involved.

The relevance of these findings to the visual function of the animal is difficult to assess (Gaze, 1970, p.150). Murray & Grafstein (1969)[2] report that there is no detectable deficiency in the visual acuity of goldfish after regeneration. Weiler (1966) demonstrated a 78% recovery of normal visual acuity in Astronotus, but it is not clear what the quantitative significance of this estimate is; the figure has little meaning unless 100 and 0% acuity have been defined. Acuity is likely to depend on two main factors; the number of successfully regenerating fibres and the amount of their terminal overlap in the tectum.

Various estimates of the sizes of optic fibre terminal arborizations have been given; in 2-day-old chick, 75 μ (Sperry & Hibbard, 1967); frog, 10-50 μ (increasing with depth) (Lazar & Székely, 1967); chick, 200-300 μ (Cajal, quoted by Székely, 1966). The evidence in the present experiments (Part 13) suggest an upper limit to the transverse extent of terminal arborizations of between 50 and 100 μ in goldfish. Jacobson & Gaze (1964) report that single optic nerve fibres have receptive field diameters of between 10 and 20° and on average across the retinotectal projection, 10° of the field occupies about 160 μ on the tectal surface. The area of the fish tectum is very approximately 10^9 microns^2. Assuming an even distribution of terminals of the 95,000 fibres reaching the tectum from the optic nerve in a normal goldfish and terminal arborizations with diameters of 75 μ, an electrode should come into contact during its descent at any one tectal position with approximately 80 fibre terminals representing about 10° of the field.

A reduction in the numbers of projecting fibres after regeneration (the figures given above suggest that about 40 of the above 80 might remain) would not directly affect the size of the multiunit receptive field; if anything it should reduce it but any such reduction would not be detectable because single fibres themselves have field diameters of the same order of magnitude as the normal multiunit field. The enlarged multiunit fields seen early in regeneration could be due to enlarged terminals (in regenerated animals (Part 14) this could not be directly measured) but if the above line of argument is reversed terminals would have to increase to about 400 μ in diameter to account for the enlarged multiunit fields observed in short-term regenerated

animals. If this does happen it does not make it any more easy to see the terminals in histological preparations!

Alternatively an explanation for enlarged multiunit fields might be a low-level scatter of terminals which would normally lie at the same site. This may also be the explanation for Pattern 1 in the frog (see Appendix 1). In order to correct such an excessive overlap, fibre terminals must, in effect, reposition themselves by an average of 60 μ on the tectum.

Mechanism of recovery of orderly retinotectal connections

The present experiments allow certain conclusions to be drawn regarding the major alternative mechanisms of selective fibre growth outlined in the Introduction, Part 2. The present evidence is against the hypothesis that fibres enter the tectum in random array at the outset and that orderly connections are achieved by the selective withdrawal of inappropriate fibre processes but it is short of conclusive.

The orderliness of the earliest recorded retinotectal maps and the gradual maturation of electrical responses and multiunit receptive field sizes are all consistent with the idea that fibre terminals are laid down at their appropriate sites from the outset and that, as far as the electrophysiological method of observation is concerned, the earliest stages of this process may be undetectable. In contrast to the situation for the frog, there was no direct evidence in observations on goldfish for any intervening processes like Pattern 1; as was argued in Part 4 of the Introduction, what evidence there is suggests that

functional, and therefore presumably stable, terminals are established very soon after fibres first reach the tectum and probably before the first electrical signals can be recorded.

One might have hoped in these experiments to get direct evidence that fibres are laid down sequentially from their point of entry into the tectum, in accordance with the relative accessibility of individual tectal positions to their matching fibres; the data is not incompatible with the prediction that localised responses are detectable earliest near the tract divisions, and the frog data has been interpreted along the same lines (Appendix 1). As pointed out on page 24, the evidence of Gaze & Sharma (1970) can be taken as confirming this.

The anatomical data throws further light on the regeneration process. The description of the form of the growth cone (pp.64-71) ties in with the conclusions reached in the Introduction (Part 4) that the growth cone is simply of insufficient size to explore territory much in advance of the growing axon, although it may well do this over distances of a few microns. Even this is unlikely, because there was no evidence whatsoever for exploratory processes of the optic axon growth cone; the picture that emerges is very different. From the earliest stages axonal processes are grouped together into distinct channels, encapsulated by connective tissue processes. Whatever processes, selective or non-selective, determine the paths of the leading members of these fibre groups, others quickly follow and there is little likelihood that precocious fibres can act as "path finders" (p.10) either by sampling the tissue far in advance or by selective survival based on the successful outcome of advanced growth; growth is simply not advanced enough.

Axonal branching was observed in the course of these investigations, as an immediate response to cutting optic fibres (Plate 7) and in long-term regenerated nerves where counts (Part 7) showed that on average half the original fibres of the peripheral optic nerve stump maintained two fibre processes central to the lesion. The persistent unmyelinated fibres observed in all regenerated nerves may be branches of the fibres which successfully reach the tectum and become myelinated. The evidence is consistent with the hypothesis that fibre branching is a response to the mechanical conditions met by the growing fibre rather than an exploratory mechanism (pp.26-27);

1) The excess of fibres counted in the 278-day regenerated nerve (Part 7) arose near the level of the lesion and if anything the proportion of unmyelinated fibres fell further along the optic pathway.

2) If the nerve is repeatedly crushed there is a massive and progressive local increase in the number of fibre processes leading to the formation of a neuroma.

3) It was exceptional to see degenerating axonal processes in regenerated nerves at any stage, over and above the few degenerating fibres seen in any normal nerve.

4) There was direct evidence from serial sections of a regenerating bundle that regenerating fibres are rigidly consigned to their appropriate retinotopic position throughout the nerve (Montage 1).

5) There was indirect evidence (pp.70-71) that fibres order themselves retinotopically immediately on entering the central nerve stump because later rearrangement is likely to be prevented by the connective tissue compartments. The fact that fibres recover their connections equally well regardless of the level in the pathway at which

the lesion has been placed confirms that no great distance of travel is needed by the fibres to sort themselves.

6) All the histological evidence (reviewed in the Introduction, p.29) has failed to show initial random growth by fibres into the tectum.

7) The straightness of the paths of regenerated optic bundles in the tectum (Part 14) is itself evidence (Introduction Part 4) against early random growth.

There is one other version (p.10) of the theory that selective connections are achieved by trial and error; it might be that the entire retinal ganglion cell degenerates selectively if, as a result of random growth, its processes make inappropriate tectal connections. If growth into the two divisions of the tract were entirely random such a mechanism would predict a 50% reduction in the number of ganglion cells each time the nerve regenerates. Even more extensive selection would be required to achieve the segregation of fibres between the optic fibre bundles on the tectal surface. But there was no apparent reduction in the precision or quality of electrical responses in animals regenerated several times (Part 12). Further evidence against this mechanism is the fact that acuity is barely affected by regeneration (Weiler, 1966; Murray & Grafstein, 1969[2]). Murray & Grafstein (1969)[1] observed the responses of ganglion cells during axonal regeneration and did not report degeneration.

Evidence in this thesis supports the suggestion (see Introduction Part 3) that regenerating fibres follow highly specific paths to their targets and that these paths must be actively selected by the advancing fibre. It is clear (Parts 11 & 14) that after regeneration the majority of fibres occupy their original branches of the optic tract. Before

concluding that these paths are the paths followed by the earliest fibre processes to cross the tectum it is necessary (p.12) to show that, had any fibre followed other abnormal routes and also succeeded in reaching its correct terminal site, it would have survived intact in the presence of fibres following the normal route to the same site. There is already evidence that optic fibres in the fish can successfully terminate after growing through the inappropriate tract division in the absence of fibres following the correct route (Arora, 1963) and after growing through the contralateral inappropriate division in the presence of fibres following the normal routes to the same terminals (Afora quoted by Sperry, 1965). In the present experiments there was clear evidence (Part 11) that a small proportion of fibres destined for the medial tectum take lateral paths from the tract, grow by indirect routes across the tectum and successfully terminate in their normal positions together (as far as can be determined by the methods used) with fibres following the normal routes. This shows that if fibres do initially select their paths at random, fibres following anomalous routes can survive.

There remains the possibility that there is an overall survival advantage for fibres taking the correct route; this is one explanation for the fact that fibres taking the longer route are in the minority according to the present results. This new evidence is clearly not decisive but it does reduce the small remaining margin of doubt regarding active path selection as a primary mechanism in the orderly regeneration of nerve fibres. The evidence concerning selection at the division of the tract probably applies directly to selection by fibres of their routes across the tectum except that, in this case, it would be even more difficult to imagine any possible basis on which fibres following

incorrect routes along the tectum would be at any selective disadvantage; the difference in the distances travelled by fibres would, for instance, be minimal. It therefore seems certain that the precise arrangement of regenerated fibres within tectal bundles is the result of active path selection, a mechanism of nerve growth for which there is now considerable evidence.

The fact that fibres take several quite different routes to the same terminal site, for example through both medial and lateral tract divisions, does not conflict with the evidence for path selection. On the contrary, it is unlikely that fibres taking an anomalous route through the lateral division could successfully reach their correct sites in the medial tectum other than by path selection; over the increased distances that the fibres now have to travel, the probability of finding a terminal site by trial and error would be diminished.

The differences between frog and fish (Part 3) as regards retinotopic arrangement of fibres in the optic pathways is of some interest. The progressive refinement of retinotopic order as the optic nerve approaches the tectum in the frog may indicate a path selection similar to that which arranges fibres beyond a nerve lesion in fish, but it is evidently poor at the start of the frog's nerve where retinotopic order is initially lost.

Against the possibility (p.12) that path specificity is the result of fibres being individually attracted at a distance by factors specific to their terminal sites (perhaps carried to them by chemical diffusion) is the evidence (Part 16) that fibres destined for the caudal tectum undergo specific regeneration in the absence of the caudal tectum. Although Arora & Sperry (1962) offer direct support for specificity of the tissues of the pathways it is likely (Part 15) that the damage entailed by their operations would make it

difficult to cross the division stumps effectively; it is more likely that fibres would undergo retrograde degeneration and approach the two central stumps of the division much in the way they usually do during nerve regeneration. The fact that some fibres take the wrong route anyway in regeneration further complicates any interpretation of Arora & Sperry's results.

The substrate of neurospecificity in the retinotectal system

Some of the present experiments were undertaken in an attempt to define the characteristics of the hypothetical neurospecific labels associated with individual tectal sites and the fibres that seek them. The results throw some light on the location of these signals and on their susceptibility to modification as a result of various experimental procedures.

Attempts to change the conditions met by the regenerating fibres in the tectum, including the prior degeneration of the remains of the original fibres for an effective 269 days, produced no detectable change in the ability of fibres to select their terminal sites. It is therefore almost certain that, like fibres first reaching the tectum during embryogenesis, regenerating fibres must be guided entirely by structural components of the target tissues. It is also possible to say that these components and the ability of optic fibres to recognise them do not change as a result of repetition of the recognition process (Results Part 12); there was no progressive degradation in the precision of the regeneration process as a result of the repeated penetration of the tectum by regenerating fibres or of the successive connective tissue and glial cell reactions induced by repeated degeneration of new optic fibres.

Experiments in which parts of the retina were removed prior to regeneration (Parts 15 & 16) show that although a fibre's selection of terminal site may be modified by the absence of certain other fibres, its choice of pathway is invariable. No conditions were met during the course of these experiments which led to any modification in the assignment of regenerating fibres to divisions of the tract or to tectal bundles. This is one argument in favour of a "patchwork quilt" model of neurospecificity. As far as path selecting is concerned individual fibres appear to be guided in a characteristic way by unique cues which have a fixed location in the target tissues (see Introduction Part 5).

There is some evidence that in their selection of terminal site fibres also retain a unique and fixed affinity for their original site despite the fact that they terminate at other sites (Part 16); the alternative explanation for the experimentally induced redistribution of fibre terminals (instances of this were reviewed in Part 5 of the Introduction) is that fibres are directed to tectal sites by signals which are modified by removal of parts of the retina or tectum. However fibres regenerating from half-retinae reveal that they retain the information needed to recognise their original terminal sites in the following respects (Horder, 1971); as has been demonstrated in this thesis they invariably terminate at a constant position with respect to the transverse axis of the tectum. Secondly, in experiments complimentary to those described in Part 16 involving removal of the nasal half of the retina instead of the temporal half, it has been found that terminals of fibres from the intact temporal half-retina are confined retintopically to the rostral half of the tectum. Therefore fibres are only free to choose new terminal sites within the

longitudinal tectal axis if the site is rostral to their normal terminal site. As far as the goldfish is concerned redistribution of terminals is evidently far more restricted than the processes embodied in models such as the gradient hypothesis; a different kind of explanation is called for.

Like the results of rotating small portions of tectum (Sharma, 1967) and regenerating fibres from small isolated portions of retina (DeLong & Coulombre, 1968) this evidence suggests that neurospecific cues exist as discrete and independent attributes of individual ganglion cells and tectal sites. Although it remains possible that the cues are arranged in a graded form across the tectum, the idea of gradients may well be inappropriate; the present evidence calls into question the interpretation of the observations which originally gave rise to the idea (see Part 5 of the Introduction).

As has already been noted fibres recover their correct terminals wherever they have been cut along the optic pathways, and no matter how extensive the lesion is. Although it is not known how much the retinotopic order of fibres is disarranged as they regenerate across the region of a nerve lesion (either by direct mechanical displacement or as a consequence of the inevitable disruption of the spatial arrangement of the guiding connective tissue framework of the nerve) this observation demonstrates a feature of the regeneration of optic fibres shown more clearly by fibres assigned to the inappropriate division of the tract; fibres can recover their designated terminal sites regardless of any displacement from their normal channels of approach. If it is true that in selecting their paths to their terminals fibres utilise specific cues associated with the tissue they are traversing, it follows that any

given fibre can identify cues throughout the visual pathways and respond to any one of them in a way which takes it nearer its own eventual destination.

One can speculate that the most economical distribution of neurospecific information would be that in which a single site in the nervous system is differentiated by a single label. If in addition it is possible for a fibre which would never in normal circumstances come near that site to identify it, this suggests that all labels are organized according to a unified code which can take in all sites in the nervous system. The precise function of the labels appears to be to make the sites identifiable. But since fibres utilise this information to locate themselves in the tissue it could be described as "positional information". Wolpert's concept pinpoints what is perhaps the simplest form the neurospecific code could take; every site might actually be specified solely on the basis of its anatomical position according to a unified set of parameters.

It would be very difficult to estimate the amount of neurospecific information needed to achieve the orderly regeneration of the fish optic nerve because of problems in assessing the completeness of the regeneration process; in particular the proportion of fibres that regenerate successfully and the amount of random overlap between fibre terminals. However there is evidence (see next section) that the orderliness of fibres and their terminals in the transverse tectal axis can be wholly accounted for by the orderly arrangement of fibres into separate tectal bundles. If this is true it is possible to obtain a rough estimate of the amount of information needed to order optic fibre terminals in one axis of the tectum from the number of alternative paths available to a

fibre entering the tectum. A fibre regenerating through the medial tract division has to select between about 30 options; on this basis about five bits of information are needed to order the whole projection transversely on the tectum.

The "readout" of neurospecificity in the process of regeneration of the optic nerve

As mentioned in the previous section the regeneration of optic fibres to anomalous, new tectal sites occurs under such limited circumstances that an explanation in terms of the way in which fibres regenerate seems probable. There is a striking correlation between the paths taken by fibres into the tectum (Parts 13 and 14) and the direction in which fibre terminals are free to rearrange themselves (Part 16). It follows from the fact that fibre paths are rostrocaudal and the fact that fibres only move to sites rostral to their normal terminal sites that new sites are chosen from among those that the fibres would traverse in the course of their normal growth across the tectum.

This offers an immediate explanation for the access of fibres to new sites; no element of random exploratory growth needs to be supposed. What fibres appear to be doing is to form their terminals prematurely during growth across the tectum, an occurrence which is presumably only possible because the fibres normally occupying the intervening sites are absent.

The results of this thesis lead to the following account of the way in which fibres become ordered onto the tectum. Before they reach the tectum growing optic fibres sort themselves into separate bundles which are retinotopically arranged. By the time they emerge from the optic

tract fibres are committed to the particular rostrocaudal track followed by its bundle across the tectum and retinotopic orderliness is already established as far as the transverse tectal axis is concerned in the transverse arrangement of the individual bundles. In order to fully restore the final retinotectal projection fibres composing individual bundles have only to peel off in retinotopic sequence as the bundle passes further in a caudal direction. Whereas the assignment of fibres to bundles and the growth of the bundle across the tectum presumably involve a process of active path selection, the ordering of terminals in the longitudinal tectal axis involves cellular mechanisms of a very different kind; a decision by the growth cone to cease growth. The geometry of the situation permits the isolation of the two components of a fibre's behaviour; the way fibre terminals are ordered in the long axis reflects the rules which control the fibres' decision to terminate while order in the transverse axis may reflect the path selecting behaviour of the fibres and this as we have noted is invariable.

We arrive finally at the following tentative explanation for the anomalous termination of optic fibres; it is the result of peculiarities of the mechanism which determines when a regenerating fibre ceases growth. One possibility is that the termination response is triggered by tectal cues which are separate from, and less well defined than, the unified system of site-specific cues which are thought to mediate path selection. But if one makes the simpler assumption that there is only one system of tectal cues it follows that while fibres can respond to these in a fixed and highly discriminating way in path selection they may respond at any available site by terminating indiscriminately. How in that case do fibres come to terminate

in retinotopic order along the longitudinal tectal axis during the course of normal regeneration and during the redistribution of the projection which follows regeneration of fibres from a nasal half-retina (Part 16)?

When fibres approach the medial tectum from the lateral division of the tract (Part 12, Experiment 3) they do not appear to terminate prematurely if the lateral tectum is unoccupied as a result of removing the dorsal half-retina. This suggests that the reported anomalies in the patterns of termination of fibres are peculiar to the conditions under which fibres enter the tectum in their normal paths and it can be inferred that these conditions are incidental to the way in which fibres normally select their correct terminal sites. Perhaps it is a feature of the bundle structures in which fibres normally enter the tectum that leads to anomalous termination.

Although fibres from nasal half-retinae do not appear to have an exclusive affinity for any one site along their paths they tend under all conditions examined (Part 16) to retain their retinotopic sequence along the tectum. One way in which this could be achieved is suggested by the results of Gaze & Sharma (1970); after removal of the caudal half-tectum in a normal fish fibres tend to redistribute themselves along the tectum so that eventually the entire retinal projection is compressed in retinotopic sequence into the rostral half-tectum. This suggests that a population of fibre terminals has the potential to order itself by a process of internal rearrangement quite independent of tectal cues; this may well be the mechanism which allows the projection from a nasal half-retina to accommodate itself to the available longitudinal extent of the tectum.

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APPENDIX 1

A reinterpretation of the course of events in regeneration of the frog retinotectal projection

When Gaze & Jacobson (1963) and Gaze & Keating (1970) mapped frogs electrophysiologically from the time of the first appearance of regenerated visual responses, the earliest projection maps were apparently totally disorganised; these maps were designated Pattern 1(See Fig.A1) and characterized by the fact that responses were only found to originate from restricted areas of the field, the nasal and/or temporal poles, and were randomly distributed throughout the corresponding medial and/or lateral half tectum. The earliest such case was recorded after 23 days regeneration and the latest after 103 days.

If the supposed direct contralateral projections seen in Pattern 4 animals are considered patterns very similar to Pattern 1 occurred later than this, on Days 153 and 165 (Gaze & Jacobson's animals F153 and F165). All cases recorded between 23 and 33 days were Pattern 1; the earliest "normal" map, Pattern 3, was recorded after 33 days and the evidence suggests that Pattern 1 represents the earliest stages of the regeneration process to be detected.

It has been argued that the prerequisite for obtaining the electrical responses from the normal tectum is a structural feature of the terminal arborisations of optic axons. A priori, therefore, it seems likely that Pattern 1 responses have a similar underlying structure and from evidence in the fish it seems probable that the earliest electrical signals obtained originate from maturing forms of terminals.

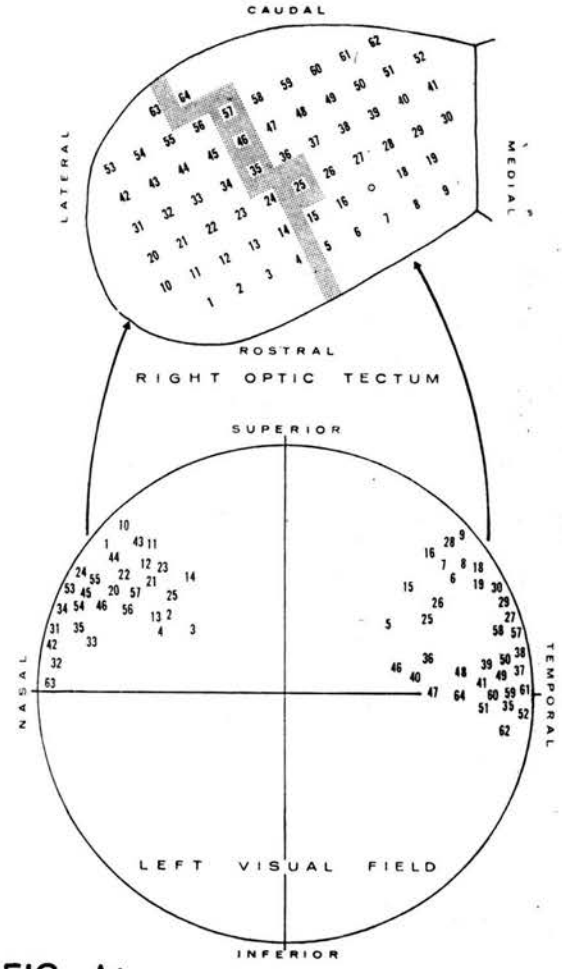
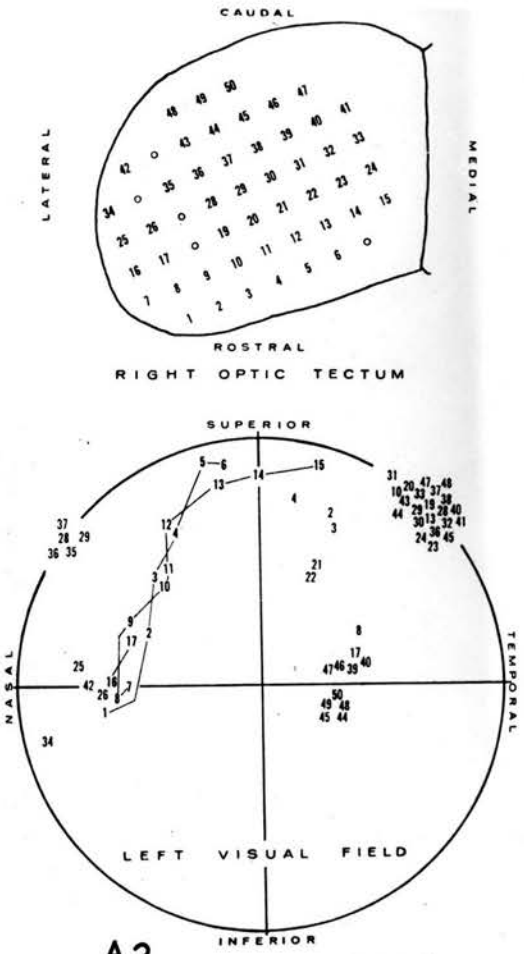
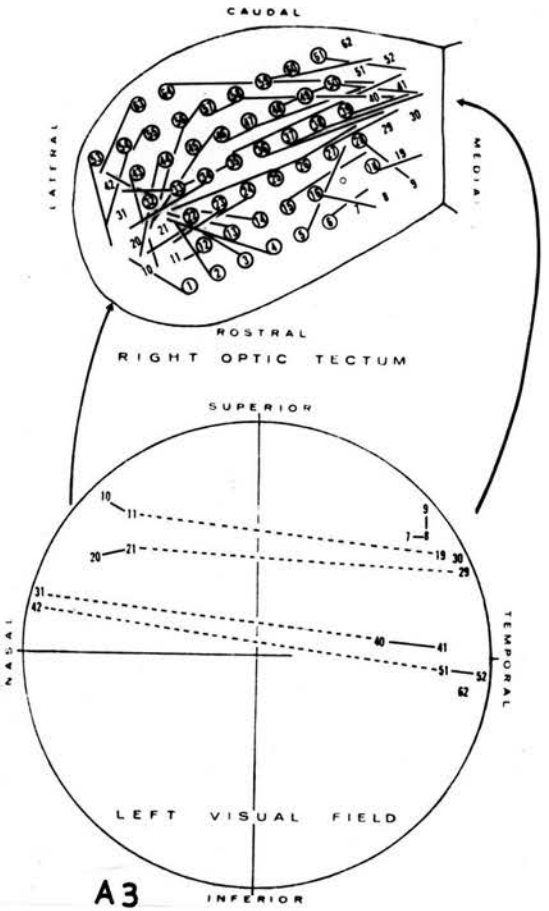


FIG A1 Pattern 1.



A2 Pattern 2.



A3

The alternative also remains that a species difference exists; it is conceivable that in the frog, optic fibres in the course of ingrowth possess structural features which permit electrical responses to be recorded from them, as well as from established terminals, and it is further possible that these ingrowing fibre processes are initially randomly distributed in the tectum.

In the frog the divisions of the optic tract approach the tectum from approximately the medial and rostrolateral poles as shown by the arrows in Fig.A3, and it is evident that in Pattern 1 it is precisely those fibres which have the shortest distances to travel from the divisions to their corresponding tectal areas that are represented in the map. It is therefore a possibility that relative accessibility of the fibres of the optic nerve to their own tectal areas is the explanation for the restricted origins of the responses in Pattern 1.

Gaze & Jacobson (1963) suggest that in Pattern 1, fibres from the nasal and/or temporal poles are randomly distributed in their half tecta. One difficulty with this account of the earliest stage of ingrowth of optic fibres is that, in view of the above consideration, these fibres would have to avoid terminating in their correct tectal positions in order to reach other areas further towards the centre of the tectum.

If the tectal areas which are normally supplied from the field positions of Pattern 1 are considered in isolation (unringed positions in Fig.A3 (upper)) and the field positions supplying them are replotted from the data in Fig.A1 of Gaze & Jacobson (1963) retinotopic order can be seen to be very well established in these areas (Fig.A3 lower).

This indicates that Pattern 1 is not simply due to a random

distribution of regenerating fibres. It suggests that in the tectal areas which fibres would be expected to reach first, retinotopic order exists among terminals earliest while in other areas terminals are poorly organized. There is other evidence that after ablation of the caudal tectum (Gaze & Sharma, 1970) fibres destined for tectum nearest the tract are laid down there preferentially during regeneration though they may later be displaced by other fibres. It would be predicted that stable terminals exist in Pattern 1 animals because behavioural responses to visual stimuli are observed from the time of the first cases of Pattern 1 in anurans (Sperry, 1944: 23-33 days).

Why responses from the nasal and temporal poles of the field should be obtainable outside the normal tectal areas is not clear. In view of the present findings in the fish it would not be surprising if terminals were to some extent retinotopically scattered early in regeneration. The enlarged multiunit receptive fields of Pattern 1 animals (up to 70°, Gaze & Jacobson, 1963) suggest that considerable scatter does exist sufficient to account for the extension of responses from the peripheral field as far as the central tectum. The lines on the tectum in Fig. A3 join recording sites with the points to which their receptive fields would normally project and indicate the extent of displaced responses. If Pattern 1 is comparable to early fish regeneration later maturation would be expected to occur by gradual and progressive retinotopic refinement among terminals.

In Pattern 2 there is retinotopic order within the nasotemporal axis of the regenerated projection in the absence of order in the rostrocaudal tectal axis. It was seen in 4 cases by Gaze & Jacobson (1963) as against 45 cases showing "normal" direct contralateral projections. In the 1970 paper there were 2 as against 15 cases. Although their early occurrence suggests that Pattern 1 is a transitional stage in regeneration this is not true of Pattern 2; it occurs at a constant rate among Patterns 3 & 4 animals; the last animal of the entire time series (315 days regeneration) was Pattern 2.

Fig.4.26 (Gaze & Jacobson, 1963) (see Fig.A2 here) is representative of Pattern 2 maps. However order is not entirely absent within the long tectal axis in this map as inspection will show. The interpretation of such maps is complicated by the fact that large areas of the field do not figure in them at all. It cannot be said that the three rostral rows on the tectum in Fig.A3 are supplied from random positions in the superior-inferior field axis because only a narrow transverse strip of the field is represented. They are in fact all supplied by the same restricted set of fibres which are abnormally spread out in the long tectal axis and the projection of the remaining fibres that would normally supply them is unknown.

Pattern 2 responses were, in marked contrast to those in Pattern 1 animals, "normal" in quality (Keating, personal communication), and not enlarged. If, according to the original interpretation of Gaze & Jacobson, fibres are mobile within the long tectal axis as they were in both axes in Pattern 1, it follows that the Pattern 2 response should retain some of the characteristics of Pattern 1 responses and in particular the enlarged fields which are the result of randomized terminations. Followed through to its logical conclusion the prediction would be that Pattern 2 receptive fields should be oblong; narrow across the field in the ordered axis and elongated in the axis in which fibres are random, but this was not observed.

Pattern 2 may in part represent a stable end-point in the regeneration process comparable to the more irregular of the maps obtained in the series of regenerated fish reported in this thesis. Map R170 in this thesis is quite similar to one of the published Pattern 2 maps (Fig.2 of Gaze & Keating, 1970).

However Pattern 2 maps are also likely to include portions of the anomalous ipsilateral projection of Pattern 4 which reaches the denervated tectum by way of the ipsilateral tectum; like Pattern 2 this projection is usually confined to the rostral tectum. The significance of Pattern 2 cannot be assessed unless this possible origin for the responses has been excluded.

Appendix 2

List of Animals

Time series of animals mapped after transection or crush of the left optic nerve

Days regeneration	Nerve cut	Nerve crush	Multiunit response field diameter			Number of points outside normal field surviving medial division cut	Visible tectal bundles	"Normal" map	Comments	Code no.	Page no.
			Mean	S.D.	N						
20		+	-	-	-	-			Responses doubtful	-	-
22		+	-	-	-	-	+++		Possible unitary responses	-	-
25		+	-	-	-	-			Responses doubtful	-	-
27		+	-	-	-	-	++		Nothing	-	-
31		+	-	-	-	-			Definite unitary responses restricted to correct positions	-	-
32		+	-	-	-	-	++		Definite unitary responses	-	-
33		+	-	-	-	-			Localized responses in rostral tectum	-	-
34		+	-	-	-	-	++		Poor responses; approx. normal map	R34	81
35		+	-	-	-	-			Poor responses	-	-
39		+	-	-	-	-	+++		Very poor responses	-	-
41		+	-	-	-	-	+		" " "	-	-
43		+	19.3 (10.9)	4.9 (3.0)	7 (22)	-	+		Clear unitary responses. Normal field diameters in brackets	-	-

47		+	25.0	4.1	6	-			Poor responses, correctly localized	-	-
50	+		-	-	-	-		+	Projection shifted forward on tectum	-	-
54A		+	-	-	-	-		+	" " "	R54A -Te	83
54B		+	-	-	-	11			Some crossed rows in temporal fields	R54B -TrLD	87
55A	+		19.1	9.1	7	-	+	+	A little irregular	-	-
55B	+		14.4	4.2	8	-	++		Irregular map	R55B	89
57	+		-	-	-	-	+	+		-	-
62		+	30.0	10.9	15	4	++		Irregular projection shifted forward on tectum	R62-TrLD	83
63	+		37.8	14.0	20	-	+		Some wide receptive fields	R63-Te	89
66		+	20.0	11.8	12	1	+	+		-	-
67	+		-	-	-	-			Some irregularities; projection shifted forward on tectum	G & S	-
69	+		15.2	8.6	11	-		+		-	-
92	+		-	-	-	-			Some irregular and enlarged receptive fields	G & S	-
93	+		-	-	-	-		+		G & S	-
95		+	-	-	-	11			Enlarged fields in caudal tectum	-	-
96	+		-	-	-	-		+		G & S	-

113	+		-	-	-	-		+	Published in Gaze & Sharma (1970)	G & S	-
120	+		-	-	-	-		+	Projection shifted forward on tectum	G & S	-
123	+		-	-	-	-			Slight temporal irregularities	G & S	-
124A	+		-	-	-	-		+		G & S	-
124B	+		-	-	-	-			Only organized in transverse tectal axis	G & S	-
146		+	-	-	-	4		+		-	-
150	+		-	-	-	6		+		-	-
170	+		-	-	-	-			Three temporal rows compressed	R170	87
176	+		-	-	-	4		+		-	-
191	+		-	-	-	6			Some irregularities	R191-Tr	86
220		+	-	-	-	6		+		-	-
278		+	12.6	3.7	16	-	++	+		R278-Te	92
286		+	17.1	7.7	23	-	++	+		R286-Te	86
292		+	10.0	4.6	14	-	++	+			
293		+	-	-	-	-		+		-	-

(G & S: Gaze & Sharma, 1970)

Other animals