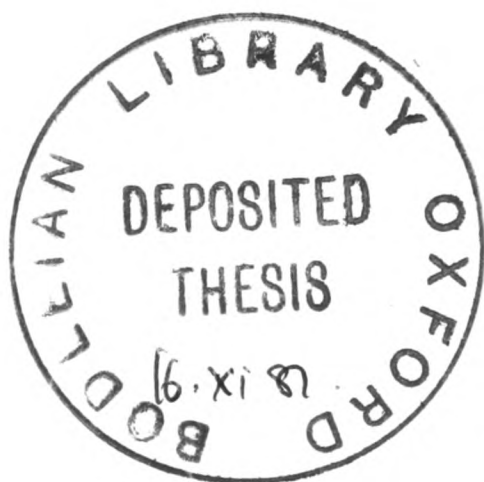


STUDIES ON THE FORMATION
OF
SPECIFIC NERVOUS CONNECTIONS

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ABSTRACT

Studies On The Formation Of Specific Nervous Connections

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This thesis is concerned with the way in which precise patterned connections are formed between groups of neurones and their targets. Two theories are discussed: the chemospecificity hypothesis, according to which these connections are formed by the matching of detailed labels carried by the neurones and their targets; and the morphogenetic model in which the orderly and passive growth of nerve fibres determines the pattern of synapses formed.

Two pathways previously thought to be disorderly, peripheral nerves and the mouse optic nerve, were studied and their fibres were found to be actually arranged in an ordered fashion. The goldfish retinotectal pathway was also studied, and the apparently complex reorganization of the fibre array needed to generate the observed projection was found to be explicable by the passive guidance of optic axons over the optic cup and stalk.

Regeneration of optic fibres within the goldfish retinotectal system was investigated. It was found that distortions of the normal fibre ordering could be corrected within the pathway and also at the tectum. Some means of active fibre guidance was therefore indicated, but this was found incapable of correcting certain instances of gross disordering.

In some experiments fibres were forced to project to the ipsilateral tectum in the absence, presence, or during the regeneration of, the normal contralateral projection. In the tecta of two-eyed fish interactions between the two fibre populations were detected, leading in some fish to the incomplete segregation of the two groups into patches.

In conclusion the development of specific neural connections was considered to be more than the consequence of the passive maintenance of fibre order within neural pathways. Active self-ordering of fibres on the basis of their positions of origin could explain additional phenomena present in regeneration, and would be an economical way of correcting mistakes made in development.

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PREFACE

The work described in this thesis is concerned with elucidating the mechanisms by which precise connections are formed between groups of neurons and their targets, whether they be other neural populations or peripheral structures such as muscles. For a number of years one theory has dominated this field: the chemospecificity hypothesis of Sperry (1963). This proposed that such precise connections were formed by the matching of individual labels borne by both the neurons and their targets, a theory which required that a large amount of information of a very detailed kind be built into the system.

Over the last ten years a considerable amount of experimental work has been conducted in this field, mainly on the retinotectal system of lower vertebrates. This has produced results which have called into question the existence of the rigid point to point matching of cells envisaged by Sperry, and the chemospecificity model has been modified to allow a considerable amount of flexibility in the formation of neural connections. However some workers have become disenchanted with the entire theory, considering it to incorporate factors either completely unnecessary for the explanation of experimental results, or required only in the explanation of regeneration of connections and not in their initial formation. This view culminated in the "Morphogenetic" model of Horder and Martin (1978) which virtually rejected the labelling of chemospecificity, emphasizing instead the role of passive forces such as fasciculation and contact guidance of nerve fibres.

The fundamental assumption made in the Morphogenetic model was that nerve fibres maintain a highly ordered arrangement within neuronal pathways. This order would then lead to the formation of precise and orderly connections. If fibres become scrambled as they pass from one part of the brain to another or from the spinal cord to the limb then some active sorting-out process would

be essential. Thus if any nervous pathway were found to contain fibres which were "muddled-up", whose organization did not reflect, directly or indirectly, the relative arrangements of their cell bodies then the Morphogenetic model could not apply to that pathway and doubt would be thrown on its validity in other situations.

This thesis is thus organized as follows: in Chapter 1 the relevant literature is reviewed. First the various theories concerning the formation of specific nervous connections are considered. Then the evidence for an orderly arrangement of fibres in peripheral nerves and in the primary visual pathway is described. Finally experimental investigations of the development and regeneration of the primary visual pathway are reviewed, with particular emphasis on the minimum amount of fibre guidance and labelling necessary to explain the results.

Because the literature review revealed some doubt about the orderly arrangement of fibres in vertebrate peripheral limb nerves and in the mammalian optic nerve these two systems were investigated experimentally in Chapters 3 and 4 respectively. In Chapter 5 the nature of the retinal representation in the goldfish optic nerve and tract was studied as the complex reorganization of the fibre array apparently required to generate the observed retinotectal projection seemed to pose a problem for the Morphogenetic model. The results obtained were combined with existing data to produce a model in which the necessary rearrangement could be easily achieved.

In Chapter 6 the morphology of goldfish optic terminals in the tectum was studied in the normal animal and after optic nerve regeneration. This was of interest because subsequent chapters employed electrophysiological recording from those terminals yet little information existed in the literature concerning their shape and size. Furthermore the simplest experimental paradigm for testing models such as chemospecificity has been regeneration of the optic

nerve. Several explanations were possible for certain aspects of regeneration and a study of the size and shape of regenerated terminals provided a possible means of distinguishing between them.

Chapters 7 to 9 involved experimental manipulations of the goldfish retino-tectal pathway. In view of the highly ordered fibre arrangement reported in the literature in various pathways and that seen here in the peripheral nerve and in the mouse optic nerve, these experiments were designed to study the effect of that fibre ordering on the projection produced. In Chapter 9 the possible contribution of active interactions between and within fibre groups to the formation of connections was also investigated.

Finally the significance of these results and their implications for theories of neuronal development and regeneration were discussed in Chapter 10.

Chapter 1

LITERATURE REVIEW

Introduction

The ultimate aim of developmental neurobiology is to provide a complete understanding of the way in which the fertilized ovum of a vertebrate can give rise to an adult possessing a nervous system composed of many millions of cells and capable of receiving, processing and responding appropriately to a vast wealth of information about the internal and external environment. Since Ramon y Cajal and his contemporaries (see Ramon y Cajal, 1955, for references) succeeded in demonstrating neurons to be separate cells showing a variety of morphologies and behaviours the problem has been not only one of explaining the control of differentiation of particular groups of neurons but also, and perhaps of far greater import, of explaining how connections are formed between these neurons and with other organs such that the whole nervous system can function correctly.

Initially this latter problem was avoided. It was believed that within the constraints of the grossly predictable nervous pathways seen by Ramon y Cajal the growth process in the embryo gave rise to an unstructured, even random, network of nervous connections which was then moulded by experience into the precise system seen in the adult. The idea that "function precedes form in the development of the nervous system" found many adherents. Much evidence was produced to support this view; for example the recovery of apparently normal function in an axolotl limb innervated by inappropriate nerves (Weiss, 1937) and the ability of even a sensory nerve to form connections with a muscle (Weiss, 1934a). Both of these examples suggested the existence of little, if any, specificity in the growth of nerves. Instead a mechanical, or "contact guidance" theory of nerve growth was proposed which seemed, when combined with other factors such as correct timing and varying

rates of axonal outgrowth, to be adequate to explain the formation of the basic, gross pathways of the nervous system. In the words of Weiss:

"a neuroblast, though being the source of the axon, cannot also provide its itinerary. A growing nerve fibre has no inherent tendency to move in any particular direction, nor to change its direction at certain points, nor to branch and terminate after having reached a certain length. For all these actions it is dependant on directive influences of the environment, and its own endowment in this respect consists merely of the faculty of letting itself be guided".

(Weiss, 1939)

Precision of communication in the nervous system would not, therefore, be based on the formation of highly selective synapses, but instead upon some kind of specificity in the signals themselves. First proposed by Hering in 1913 and extended by Weiss (1928, 1939), this resonance principle suggested that certain cells, while receiving a diffuse innervation from many other cells, would respond selectively to signals of a particular quality.

Evidence for the existence of precise neuronal connections

A major challenge to this hypothesis, suggesting that synaptic rather than signal specificity was the major factor in the development of the functioning vertebrate nervous system came in the early 1940s with the work of Sperry. His investigations of the lower vertebrate visual system were to revolutionize thinking about neuronal development. Matthey (1927) had previously reported the successful restoration of vision in newts after optic nerve section and even eye transplantation, work which was confirmed and extended by Stone and his colleagues using larval and adult newts and salamanders (Stone, et al., 1937; Stone and Zaur, 1940). Sperry was able to show that not only was the ability of a newt to strike accurately at a lure restored after optic nerve regeneration (1942, 1943a) but also that if the eye was rotated around the optic axis by 90° or 180° before regeneration then visually-mediated behaviour, when recovered, was misdirected by an amount corresponding roughly to the degree of eye rotation. Moreover, even

after extensive experience the animals were never able to readjust their behaviour (1943b).

Derotation without further nerve section of the eyes of newts which had previously shown misdirected visual strike responses for several months gave the immediate restoration of correct strike behaviour. Thus Sperry demonstrated that the inappropriate behaviour had not led to reorganization of central nervous system connections. Two explanations remained for the effects of eye rotation; possibly ganglion cells carried labels (either chemical or as some property of the signals which they transmitted) which remained unchanged after random regeneration of the optic nerve. A cell which had previously seen nasal visual field was therefore still "resonant" with the tectal cells appropriate for a nasally-directed strike even though the cell now saw temporal field. Alternatively ganglion cells were regenerating to their original positions in the tectum, which again were inappropriate to the visual field now surveyed by those ganglion cells.

In extending his work to anurans Sperry (1944, 1945) produced evidence which he took to support the latter hypothesis - of regeneration of highly ordered, though now inappropriate, connections between retina and tectum. He found that lesions in the tectum produced similar scotomata whether made before or after regeneration of the optic nerve. Thus ganglion cells seemed to be regenerating their axons back to the same place on the tectum. This conclusion did of course, ignore the fact that behavioural testing for visual field defects relied upon the integrity of the tectal efferent connections which could have been disrupted by the lesions. Though somewhat shaky at the time, Sperry's conclusions have since received substantial support from more direct experimentation.

The retinotectal projection of lower vertebrates is a system which has been utilized extensively in the study of the formation of nervous connections

because it allows direct experimentation on the developing amphibian larvae and indirect investigation of development using regeneration as a model in adult teleosts. In this system this direct evidence came with the introduction of electrophysiological recording techniques. These allowed the detection of action potentials in the terminals of ganglion cell axons in the optic tectum. (Evidence that the action potentials do originate in presynaptic terminals rather than in the postsynaptic tectal cells is discussed in appendix I). In 1958 Gaze used such techniques to record from the optic tectum of the frog the electrical activity produced by visual stimulation. By inserting a fine electrode at a sequence of tectal positions and locating the part of the visual field at which stimulation would produce electrical activity detectable via the electrode he was able to construct a visuotectal map. This showed the visual field, and hence the retina, to be represented in a highly ordered topographic fashion on the tectal surface. It must be noted that this technique was designed to detect the summed activity of a number of adjacent ganglion cells and not to pick up the responses of individual cells so that small random errors in the ordering of ganglion cells as they terminate on the tectum should not affect the position of the centre of the multiunit receptive field recorded at any particular tectal position but would merely increase the size of that field.

The technique was then applied to the regenerated visuotectal projection in Xenopus laevis (Gaze, 1959) and Rana pipiens (Maturana, et al., 1959). Little difference was seen between the normal and the regenerated projections except that a slight enlargement of receptive field sizes was noted in the case of Rana pipiens suggesting reduced accuracy in the termination of regenerated ganglion cells. Moreover Gaze found that after eye rotation plus optic nerve regeneration the visuotopic map was rotated by a similar amount. The retinotopic map must have been unchanged. Sperry's original conclusion that nervous system function relied upon the formation of specific connections between neurons was thus vindicated.

In the mammalian visual system similar electrophysiological techniques have shown a topographic representation of the visual field upon the various visual nuclei of the central nervous system (see Lund, 1978 for references). Similar highly ordered maps of the body and its environment within the central nervous system have been recognized for the other sensory modalities (Barr, 1972).

With regard to the effector part of the nervous system, the motor output to the skeletal musculature, the means by which precise functional control was achieved was similarly a subject of much controversy. As discussed above, it was the early experiments of Weiss (1937), in which he found that transplanted axolotl limbs could function apparently normally despite an incorrect innervation, which led to the idea of disorderly nervous connections functioning by resonance, finding favour. Again Sperry provided evidence against this hypothesis. He found that whenever he caused muscles to become incorrectly innervated, usually by crossing the nerves of two antagonistic muscle groups, the muscle activity was reversed (Sperry, 1941, 1947, 1950; Sperry and Doupre, 1956; Sperry and Arora, 1965). Even after long survival periods in which he attempted to induce reeducation by forcing the animal to use the cross-innervated muscles no learning was observed (Sperry, 1941). However, straightforward regeneration of motor nerves led to recovery of correct function (Sperry and Doupre, 1956; Sperry and Arora, 1965). There was thus no support for either Weiss's original observation that an incorrect innervation could produce correctly coordinated movement or his postulate that this occurred because of reordering of central connections within the motor system. Furthermore a reexamination of the experimental system employed by Weiss showed that the innervation of supernumerary limbs in the axototl was by nerves which were, on the whole, not inappropriate as nearly every muscle in the axototl limb is normally supplied by all three of the limb-supplying spinal segments (Czeh and Szekely, 1971). Nor was the movement well coordinated. Instead the homologous response could simply have been the consequence of the geometry of the limb combined with a slight preference of

the innervating root for particular muscles and hence their stronger activation (Czeh and Szekely, 1971).

Again Sperry's hypothesis that the correct functioning of the nervous system depended on the formation of correct and very precisely defined connections, between neurons and either other neurons and target organs such as skeletal muscle, triumphed. Sperry himself was unable to demonstrate the reforming of such precise connections after experimental disordering, merely showing that functional adaptation did not follow nerve cross (1941, 1947; Sperry and Doupre, 1956). More recently the ability of nerves to return to the correct muscles despite deflection has been demonstrated in lower vertebrates (Grimm, 1971; Cass and Mark, 1975).

Anatomical evidence of a highly ordered representation of the skeletal musculature within the nervous system has come from the work of Romanes in constructing spinal cord maps. Using the chromatolytic response of ventral horn motoneurons to section of their axons he was able to determine which cells supplied the various muscles. He thus built up detailed plans of the spinal cords of rabbit (1941), mouse and rat (1946), and cat (1964) and revealed the motoneurons supplying a particular muscle to be grouped together in a characteristic position in the cord. A similar map of the human spinal cord was produced by correlation of the muscle paralysis of polio myelitis victims with the spinal cord structure seen at necropsy (1964). More recently the response to axotomy and the uptake of the axonal tracer horseradish peroxidase (HRP) have been used to map the spinal cord ventral horns of chick (Hollyday et al., 1977; Landmesser, 1978a) and frog (Rana catesbiana - Cruce, 1974; Xenopus laevis - Lamb, 1976). In every case a highly ordered and repeatable arrangement of motoneurons was found.

Postulated mechanisms for the formation of specific nervous connections

Dominating discussion of how such neuronal connection specificity arises during development and after regeneration has been the chemospecificity hypothesis of Sperry (1963). This argued that growing nerve fibres were actively guided to particular termination sites and induced to form synapses there by the matching of labels carried by both the nerve fibres and their targets. Active choice was envisaged since the highly ordered connections were formed in conditions, for example, regeneration after experimental manipulation, where there was thought to be ample opportunity for the fibres to terminate elsewhere. Initially proposed to explain retinotectal connection formation, this theory has also been applied to the formation of neuromuscular connections, each motoneurone supposedly labelled according to the muscle it innervates.

In its original form (Sperry, 1963) the chemospecificity model made great demands upon the genetic information content of the nervous system for it required that each retinal ganglion cell and each tectal termination site (not necessarily a single cell) be very precisely labelled as to its position in the retinal and tectal cell arrays. It also proposed considerable rigidity in the nature of the connections, a ganglion cell being able to synapse only with the tectal area bearing a matching label. In the neuromuscular system there must obviously be less exclusivity for Sperry had ample evidence that "incorrect" connections could be formed and would persist unchanged for long periods (1941, 1947, 1950; Sperry and Doupre, 1956; Sperry and Arora, 1965).

Indications that the early hard-line chemospecificity model needed modification with regard to the retinotectal system as well as the neuromuscular system, came with the size-disparity experiments of the early 1970s. A study of the normal development of the amphibian retinotectal pathway also threw doubts upon the ability of rigid point to point matching to explain the formation and regeneration of specific nervous connections and both sets of experiments are reviewed in greater detail later in this chapter.

The chemospecificity hypothesis was consequently superseded by theories which took into account factors such as the interaction of neighbouring retinal ganglion cells and their competition for synaptic space on the tectum. (For example of such a multifactorial approach to the problem see Cook and Horder, 1977). Exchange of information was postulated to occur between ganglion cells such that they maintained a highly ordered arrangement of terminations. How such information might be carried, whether by a chemical marker within the axon, as the impulse pattern transmitted by the axon, or in some other way, and the means by which it was utilized to create fibre order, was left undefined. A considerable amount of information was still required from the genome in order to allow labelling of the ganglion cells according to their retinal position. In the tectum the information requirement was less clear. Certainly these later models presumed the retention of markers for tectal polarity in order to control the orientation of the retinal projection with regard to the anatomical axes of the tectum. This was necessary to explain the way in which a small graft of rotated tectum received a projection rotated by the same amount as the graft (Sharma, 1969). A certain amount of tectal regional marking was also required to explain the innervation of pieces of caudal tectum transplanted unrotated to a rostral position by their original ganglion cells (Martin, 1978a, 1978b). The precision of this labelling was not determined as the grafts used were large. Competitive interactions between ganglion cells were then invoked to explain why a retinal projection should expand or compress to fill the available tectal space.

A problem with the use of experimentally-induced regeneration as a model for development was raised by the work of Schmidt (1978). Though regeneration was an accessible process on which to experiment it involved the formation of a retinotectal projection on a tectum which had already received such a projection. That a previous projection might have altered the tectum in some way so that it behaved differently on receiving a subsequent projection - a process known as "modulation" - was first tested by Cook and Horder (1974)

and analysed in detail by Cook (1979). No evidence was found to support it. In these experiments a compressed projection was induced on a rostral half tectum. The optic nerve was then cut again and on second regeneration compression was found to occur in the same progressive manner and with the same time course as initially. In contrast Schmidt reported immediate expansion by a half retina on to a tectum which had previously received an expanded projection. If, however, the second regeneration was delayed for five months, sufficient time to allow degeneration and removal of all fibre debris on the tectum, then the reexpansion was found to occur gradually. Yoon (1976) has reported immediate compression after an optic nerve section, if optic nerve regeneration was delayed for 40 days or more whereas he found gradual compression after a lesser delay.

Both these results can be criticised on the grounds that an early normal projection - from only appropriate retina to matching tectum - might have occurred transiently but not have been detected for early regenerated projections are often difficult to map electrophysiologically (Horder, 1971b). Even so it is unlikely that this provides a complete explanation of the disparity. It is possible that the presence or absence of axonal debris might have altered the time course of regeneration and terminal formation without altering the overall sequential nature of the size disparity adjustment. Thus although the existence and nature of any previous retinotectal projection has not yet been assigned a conclusive role in the determination of the nature of any subsequent projection this controversy serves to highlight the need for care in the use of a regeneration model for development.

A similar evolution of the chemospecificity hypothesis concerning the formation of specific nervous connections has occurred in the field of neuromuscular synapses. Functional connections were found to be possible between virtually any skeletal muscle and any nerve in any group of vertebrates (fish - Sperry and Doupre, 1956; amphibia - Sperry, 1947; birds - Hollyday et al, 1977;

mammals - Sperry, 1941) whether such incorrect innervation was induced before or after the muscle had received an initially correct innervation. Evidence that a strong preference was expressed for the correct nerve when it was competing on equal terms with a foreign nerve was actually quite rare (Grimm, 1971; Cass and Mark, 1975) and again the problem of the effect of any previous innervation arose since some histochemical properties of muscle have been shown to be influenced by the innervation (Salmons and Sreter, 1976). It was therefore possible that a nerve might have labelled a muscle in such a way that the muscle preferentially accepted synapses from the same nerve on reinnervation (Hoh, 1975).

Thus the chemospecificity model has expanded to include explanations for the plasticity of neuronal connections seen in both development and regeneration. It still presumed a large quantity of information, however, in order to label cells, if not with their absolute position, then at least with their position relative to other cells in the array. This labelling was needed in order to match each array of cells with its target, whether that be a second array of neurons or the muscles of the limb. Because experimental data on development are relatively sparse it is possible that this model has sought to explain phenomena which would not occur in development and which have been seen in regeneration only because the system is making reconnections and not new connections. A reluctance to accept a model which makes such heavy demands on the genome and a feeling that it may have sought to explain too much has led to the formulation of an alternative to chemospecificity, the morphogenetic model.

The morphogenetic model was first formally presented as an alternative to chemospecificity only in 1978 (Horder and Martin, 1978), though in some respects it antedates Sperry's hypothesis. The phenomena which it presumes to be of prime importance, contact guidance of nerve fibres by the substrate, and fasciculation, the tendency of fibres to form parallel bundles, were

described by Harrison in 1914 and extensively investigated by Weiss in the 1930s (Weiss, 1934b, 1955). These workers found that nerve fibre growth was preferentially directed parallel to an aligned substrate such as the fibrin within a blood clot and that once pioneer fibres had established a route other fibres would grow rapidly parallel to the first axons. The purpose of the morphogenetic model has been to explore how far such phenomena, seen in neurites both in vivo and in vitro, might contribute to the formation of ordered connections within the nervous system. Thus the minimum amount of extra information required could be defined. It was never the purpose of "morphogenetics" to provide the complete solution to developmental neurobiology but instead to provoke reconsideration of existing models with the elimination of unnecessary complexity. Moreover if passive and non specific phenomena were found to be able to contribute substantially to the formation of ordered connections this would not preclude the existence of some form of chemospecific labelling within the system. It would merely leave its existence unproven.

Evidence for the operation of morphogenetic forces within the primary visual pathway and in the neuromuscular system

Within the developing retina optic fibres normally form fascicles which take a direct radial path to the optic nerve head (Ramon y Cajal, 1960; Goldberg and Coulombre, 1972; Hinds and Hinds, 1974). If the retinal sheet was deformed experimentally the aberrant paths of fibre growth so produced could be predicted from the mechanical nature of that deformation (Goldberg, 1976, 1977), suggesting strongly that morphogenetic forces were involved. Furthermore in the developing mouse eye inter-cellular channels have been identified which have been implicated in fibre guidance (Silver, 1979; Silver and Robb, 1979; Silver and Sidman, 1980). These channels were found to appear before the outgrowth of ganglion cell axons and could extend without interruption into the optic stalk. They could thus provide a favourable exit route from

the eye. Axon growth cones have been identified within these channels and the absence of such channels in a mutant mouse strain (or^J) correlated with a failure of the optic nerve to exit from the eye (Silver and Robb, 1979). However the channels did not, in the normal animal, extend into the diencephalon and so could not provide guidance throughout the path from retina to primary visual nucleus.

A requirement for such a preferential substrate for successful growth may explain why axons deflected away from their normal depth in the retina grew randomly (Goldberg and Frank, 1979). Furthermore while retinal lesions in young mice (less than sixteen days postnatally) led to orderly growth of axons in fascicles around the lesion and confined to the ganglion cell fibre layer, such lesions in older mice produced random growth in all retinal layers with no fasciculation. This might be explained by loss both of substrate guidance channels - to direct pioneer fibres - and of an ability to fasciculate. The growth of fibres in an incorrect direction, either turning peripherally at the vitreal surface or overshooting the optic nerve head, has also been observed in normal animals (Ramon y Cajal, 1960; Goldberg, 1977) suggesting the guidance to be mechanical rather than chemical. This is because chemical guidance would probably be in one direction along a concentration gradient whereas mechanical guidance would probably favour growth along the substrate in opposite directions.

Within the optic nerve the morphology of the growing fibres has provided an indication of prevailing growth conditions. When neurite growth is slow and against considerable resistance, Ramon y Cajal (1928, 1960) has described the growth cone as typically large and irregular. In more favourable conditions growth cones were slender and hard to detect. Optic axons, though capable of forming large irregular growth cones in culture (Agranoff et al, 1976; Landreth and Agranoff, 1976) have rarely been seen to do so in either the developing (Goldberg, 1974) or regenerating (Grafstein, 1971) retinotectal

pathway. This suggests that growth is occurring rapidly through an orientated environment. Thus the mechanical forces central to the "morphogenetic" model do seem to be in operation in the primary visual pathway. To what extent they are able to contribute to the formation of precise and highly ordered neuronal connections within that pathway will be discussed later.

Within the neuromuscular system there is evidence that mechanical forces play at least some part in the formation of ordered connections. In a long series of experiments Piatt has caused amphibian limbs to become innervated after limb regeneration. This regeneration was induced either before or after the limb had received a normal innervation (Piatt, 1939, 1957b). He also caused limbs to become innervated by inappropriate parts of the central nervous system by transplanting either the limb or part of the spinal cord (Piatt, 1941, 1956, 1957a). Though many such limbs showed abnormalities in the gross pattern of nerves - the only aspect studied by Piatt - the vast majority showed an approximately normal pattern of major nerves suggesting that mechanical conditions in the limb had played a large part in controlling and directing the growth of the main nerve trunks. Moreover if such manipulations were carried out early in larval life before the limb had become innervated then on regeneration of the limb the innervation appeared normal whereas later amputation produced an abnormal pattern on regeneration (Piatt, 1957). Thus many of the abnormalities seen may have occurred because the later-regenerating limb lacked a nerve-guidance factor - perhaps an orientated substrate - present earlier in development.

In the light of Piatt's results and Weiss's own work in which transplanted axolotl limbs became innervated by inappropriate nerves Weiss has placed great emphasis on the role of mechanical forces in the control of limb innervation (Weiss, 1937, 1955). Direct evidence for the favourable nature of the substrate which might guide nerve fibres into the limb is lacking.

However the direct path taken by ventral root fibres out of the cord (Ramon y Cajal, 1955) their convergence into segmental spinal roots in the region of the somites (Detwiler, 1936), and the convergence of these roots to form a limb plexus at the narrowed limb bud base do suggest the channelling of fibres away from relatively dense and impenetrable regions towards more accessible favourable areas. Fasciculation of nerve fibres in the limb has also been observed with motor and sensory fibres of different classes tending to form separate groups (Weiss, 1955). This may indicate that each class of neurons has sent out its own pioneer fibres which are followed by other neurites from the same part of the cord.

A further morphogenetic force which may help to generate the apparent complexity of the adult nerve pattern is the early innervation of primitive muscles which then undergo elongation, rotation of their origins and insertions, and even further division (Landmesser and Morris, 1975), pulling or "towing" their nerves with them. Thus the complexity of the adult may conceal a simple pattern at the time when neuromuscular connections were first formed.

Prerequisites if morphogenetic forces are to be viewed as important in the generation of specific nervous connections

Morphogenetic forces, guidance of nerve fibres by contact with their environment and by fasciculation with other, earlier-growing nerve fibres, have been shown to act within the primary visual pathway and in the neuromuscular system. These factors are undoubtedly involved in the direction of groups of axons to their targets. However they could only contribute to the formation of specific connections within these targets if they allowed the maintenance, throughout the axon pathway, of an ordered representation of the cell array of origin. The nearest neighbour relationships of cells in the retina must be reflected in the nearest neighbour relationships of their axons in the optic nerve and tract. Ventral motoneurons which

have cell bodies close together in the spinal cord must have axons in close proximity in peripheral nerve trunks. This does not mean that the optic nerve has to be an exact replica of the retina nor the peripheral nerves of the cord. The map might become distorted in some way; though such a distortion and its subsequent correction, must also be amenable to a mechanical explanation.

This review will therefore consider evidence for the ordering of fibres within both the primary visual pathway and the peripheral nerve trunks. If such order is lacking then "morphogenetics" cannot be considered a major factor in the formation of specific nervous connections. It will then consider what, if any, reorganization of nearest neighbour relationships is required to explain the precise pattern of such orderly connections, the distribution of fibres to several targets and the formation of branches; and how these have been, or might be, explained in terms of phenomena such as contact guidance which could be operating in development. Finally regeneration will be considered as a model for development: can the behaviour of regenerating retino-tectal fibres in lower vertebrates be explained in terms of the order present in the growing pathway? How has the effect of the ordering in arriving fibres been tested to determine what influence it might have on the pattern of connections formed? What light can such experiments throw on embryonic development?

Are optic fibres arranged in an orderly manner in the primary visual pathway?

Lower vertebrates: evidence for order

Though the anatomy of the primary visual pathway in lower vertebrates has been the subject of fairly extensive investigation over the past century, the emphasis has, until fairly recently, been placed on the distribution of optic fibres to various nuclei in the central nervous system and on whether those are innervated by the ipsilateral or contralateral eye, with little attention paid to the arrangement of fibres of various retinal origins within that

pathway (for example see Huber and Crosby, 1926; Armstrong, 1950).

The first consideration of the problem of fibre arrangement was by Stroer in 1940 who concluded that fibres were arranged in the optic nerves of fish, reptiles, amphibia and birds on the basis of their retinal origin and that this ordering could contribute substantially to the very precise projection of the quadrants of the retina on to the optic tectum. In contrast, Herrick (1941a, 1941b, 1941c, 1942) could find little evidence of significant grouping together of fibres of similar retinal origin in the optic nerves of fish (Ameiurus), and amphibia (Ambystoma tigrinum and Necturus.) He described a continuous weaving, shifting and anastomosing of fibre fascicles throughout the optic nerve from eye to chiasm and suggested that individual fibres must be following extremely convoluted routes. This contemporary evidence of a disordered, even random, fibre arrangement in the optic nerve must have contributed substantially to Sperry's conviction of the need for an active sorting process to produce the precise nervous connections which he had demonstrated experimentally. The later suggestion that the snake (Natrix natrix) had a retinotopically ordered optic nerve served only to confuse the issue further (Armstrong, 1951).

However these early studies were based on the interpretation of sectioned histological material usually stained with a reduced silver method to demonstrate nerve fibres. Where fascicles of fibres appeared to run essentially unaltered from eye to chiasm, order was presumed to exist. (Stroer, 1940; Armstrong, 1951). If considerable change was seen in the fascicular pattern over this distance, due to fusion and division of fascicles, then it was assumed that individual fibres must be following such indirect paths that they could not possibly maintain contact with other fibres of a similar retinal origin. These studies did not allow a detailed description of the course of fibres in the optic nerve head and into the nerve so that the retinal origins of fibres within a particular fascicle were never clear. Nor did

they allow selective staining of a small group of fibres and the tracing of these through the pathway to see if they remained together or became widely separated.

Advances in histological techniques, particularly the development of electron microscopy for direct identification of degenerating fibres after small retinal lesions, the use of Nauta and other staining methods for light microscopic visualization of degenerating fibres, and the use of tracer molecules such as horse-radish peroxidase (HRP) which can be selectively introduced into small groups of fibres, have allowed a more direct approach to the problem of fibre ordering in the primary visual pathway. Even so considerable controversy has arisen over the results, particularly those obtained in the frog.

In fish retinotopic ordering of the optic fibres within the nerve and tract is now accepted, having been shown using electron and light microscopic tracing of degeneration after retinal lesions (Horder, 1974a; Anders and Hibbard, 1974; Roth, 1974; Dawney, 1979a; Scholes, 1979). Further confirmation has come from the use of HRP injections into the tectum (Dawney, 1979a) and the optic nerve (Rusoff and Easter, 1980) and from the tracing of the unmyelinated fibres, which originate from peripheral retina only (Dawney, 1979a).

While the amphibians Triturus vulgaris, alpestris and cristatus have all been shown to have highly ordered nerves using light and electron microscopic tracing of degeneration, the same technique has failed to show order in the optic nerves of Rana temporaria and Xenopus laevis (Bunt, 1980) and Rana pipiens (Maturana et al, 1960). Maturana and his colleagues also failed to detect any order when recording electrophysiologically within the optic nerve of Rana pipiens, for successive microelectrode positions responded maximally to stimulation of widely separated parts of the retina. A possible

explanation for the failure of the degeneration technique comes from the observation that in the frog optic nerve glial cells exist which send long processes radially. These may enclose degenerating debris and move it to the periphery of the nerve so that by the time the products of degeneration are detectable microscopically they may have been moved considerable distances in all directions away from an initially discrete patch (Horder and Martin, 1978). The apparent disorder recorded electrophysiologically may reflect a reorganization of the retinal map within the nerve, perhaps involving folding of the nerve so that while a high degree of order is retained some fibres of greatly disparate retinal loci come to lie together (for an example of such a reorganization in the cichlid fish optic nerve see Scholes, 1979). More recently the maintenance of nearest neighbour fibre relationships in the frog optic nerve has been demonstrated using HRP (Bunt, et al., 1979).

In birds there is both indirect and direct evidence of order in the optic nerve. Correlations of varying ganglion cell size and density across the nerve with the differential distribution of fibres of various sizes in the nerve have suggested the existence of retinotopic ordering in the chick (Botto et al, 1975; Rager, 1979) and pigeon (Binggeli and Paule, 1969; Duff and Scott, 1979). A parallel course for fibres between eye and tectum has been shown directly using HRP (Horder et al, 1979).

Thus in every group of lower vertebrates studied there is at least some, and in many cases a large body of evidence for the occurrence of some form of order in the primary visual pathway. It now remains to consider the precise nature of that order.

Lower vertebrates: the nature of the retinal representation within the primary pathway

The only lower vertebrate group which has been examined in detail with a view to determining the arrangement of fibres in the retinotectal pathway is fish. These have an almost completely crossed projection, the whole retina being represented very precisely on the contralateral tectum (Jacobson and Gaze, 1964; Sharma, 1972). Two major problems have to be overcome in this pathway in order to produce the observed projection. Firstly there needs to be an independent axis reversal of fibre arrangement between retina and tectum. Secondly there is a need to incorporate additional fibres into the pathway as both retina and tectum grow throughout adult life (Johns and Easter, 1977; Mayer, 1978).

The need for an axis reversal arises because of the way in which the retina maps on to the tectum, dorsal retina projecting laterally, temporal retina rostrally, ventral medially and nasal caudally (Jacobson and Gaze, 1964). Such a projection cannot be achieved by directly superimposing the ganglion cell arrangement in the retina on to the tectum. Instead a mirror-image reversal, best envisaged as involving inversion of one axis independent of the orthogonal axis, is required. Obviously chemospecific matching of fibres with their tectal targets could achieve such a reorganization but could it be achieved without such labelling, using only mechanical forces?

On the basis of degeneration tracing after retinal lesions in the goldfish, when dorsal fibres were found to occupy an increasingly ventral position in the centre of the nerve while ventral fibres split into two groups which passed dorsally on either side of the nerve, Bunt and Horder (1977) have proposed a model based on the contact guidance of fibres in the optic cup and choroid fissure. Because the optic cup is initially divided with the ventral fissure in continuity with a groove in the optic stalk dorsal fibres formed early would

tend to grow directly through the fissure and be guided ventrally by the groove. Ventronasal and ventrotemporal fibres would then enter the fissure on either side of the more dorsal fibres and would tend, as the groove in the optic stalk flattens out towards the brain, to be displaced dorsally on either side of the nerve. If later-added fibres merely followed neighbouring earlier fibres such a reorganization would be perpetuated throughout retinal fibre outgrowth producing a nerve in which fibres of dorsal retinal origin lie ventrally and those of ventral origin dorsally whilst nasal and temporal fibres remain on their own sides of the nerve. Moreover peripheral fibres would lie around the circumference of the nerve. Thus a mirror image reversal would be achieved in the optic nerve head and the region immediately behind it.

That such a model was not a complete description of what was happening in the fish visual pathway was evident when further data became available on the arrangement of fibres in the nerve. In 1974 Anders and Hibbard provided the first clue with their description of the ribbon optic nerve of the teleost Cichlasoma biocellatum. The ribbon nerve was so named because the optic nerve cross section seemed to have been stretched out to form a narrow band which was then extensively pleated to give an approximately circular outline. In this nerve each retinal quadrant was found to occupy a narrow strip extending the whole length of the ribbon. Scholes (1979) has confirmed the existence of such convoluted nerves in a number of cichlid species and shown that the narrow axis of the ribbon represents the complete circumference of the retina opened up about a ventral point. Moreover since the newest, most peripherally-originating fibres are always clustered at one edge of the ribbon even though the eye continues to grow in the adult he concluded that the long axis of the ribbons cross-section represented the radial distribution of ganglion cells in the retina.

Such a "chronotopic" representation of the retina within the optic nerve seems also to apply to the goldfish for again newest, unmyelinated fibres are always

clustered together on the opposite side of the nerve to the oldest central fibres (Dawmay, 1979b). This is supported by the annular labelling seen in the retina after a small injection of HRP into the optic nerve indicating that ganglion cells formed at a similar time (for the retina grows by concentric addition of cells) (Johns and Easter, 1977; Johns, 1977) have fibres laying together in the nerve (Rusoff and Easter, 1980).

The means by which the concentric organization of cells of various ages in the retina becomes converted into the ribbon may very closely resemble the model of Bunt and Horder (1977) for certainly the point of opening of the retinal circumference is located ventrally (Scholes, 1979). A chronotopic ribbon would represent the halfway stage of the process envisaged by Bunt with ventral fibres remaining on either side of the nerve rather than passing round to rejoin dorsally. Stretching of this compact arrangement and subsequent folding would then produce the ribbon seen in cichlids. The tendency of fibres within the retina to pass over the vitreal surface of earlier-formed fibres would also contribute to their guidance towards the ventral edge of the fissure and into one end of the chronotopic nerve. Incidentally this model allows easy incorporation of new fibres as the retina grows for by following the preceding generation of fibres they would reach the correct position in the nerve.

There still remains the problem of reassembling from the ribbon a direct representation of the retina and this must be achieved before the optic fibres reach the tectum for whereas the ribbon represents the retina opened along a ventral radius, the division of the optic tract into two brachia corresponds to the nasotemporal retinal axis. Thus the ribbon cannot simply be divided into two halves. Instead ventral fibres lying on opposite edges of the ribbon must be brought together so that they can pass into the same brachium.

One description of the way in which optic fibres might become rearranged in the pathway to facilitate formation of the normal retinotectal map is that of Scholes (1979). His model is based upon the study of fibre degeneration after retinal lesions in cichlid fish. He found that whereas near the eye lesion of a small section of retina produced a narrow strip of degeneration extending most of the length of the ribbon, near the tectum this strip broke up into a number of crossways bands extending through most of the width of the ribbon. He interpreted this to mean that as the fibres approach the tectum they reorganized not as a whole but in small groups with each group acting independently. Within each group ventral fibres were brought together so that nasal fibres were left at the margins of the ribbon which still constituted an opened out representation of the retina. This manoeuvre, he believed, would bring fibres into the correct order from which they could grow straight out into the tectum.

Certain problems do arise however, on detailed consideration, both of the rearrangement problem as Scholes viewed it, and of his solution to it. Firstly Scholes recognized no disparity between the basic handedness of the retina and tectum and thus saw no need for an axis reversal. However he seems to have viewed the fibre array within the optic nerve from a point near the tectum but the tectal array from a position near the eye. The retina and tectum thus appeared reconcilable. However this change in viewpoint is itself equivalent to an axis-reversal by the fibres.

Secondly within the Scholes model the whole fibre array undergoes a rotation through 180° between the eye and the point of subunit reorganization. Though having no effect on fibre-fibre relationships it must be noted that at the start of the major reorganization peripheral fibres lie dorsally in the nerve, central fibres ventrally and nasal and temporal fibres on opposite sides of the nerve to those at which they entered it.

Figure 1.1 This diagram seeks to explain how the Scholes (1979) model fails to generate a correct retinotectal projection.

Step 1: conversion of retinal fibres into a ribbon nerve.

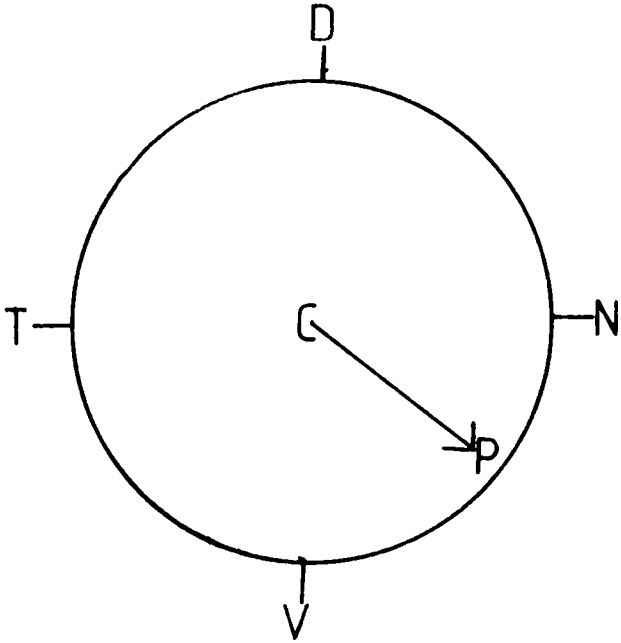
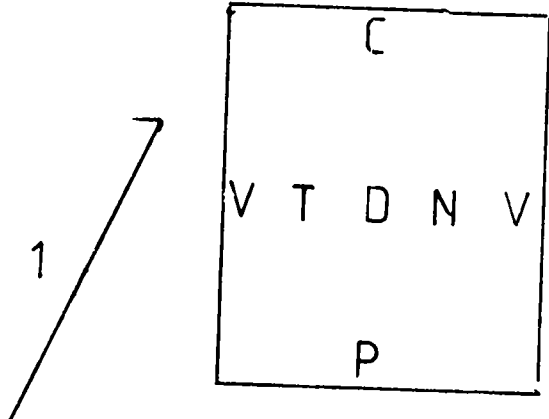
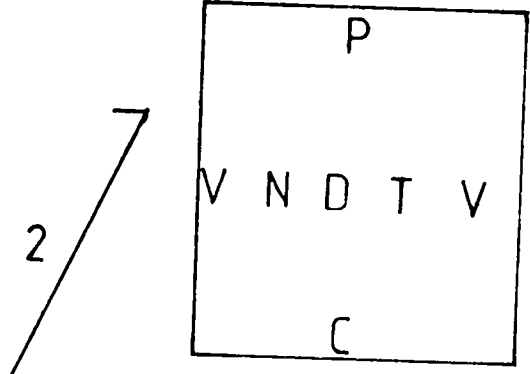
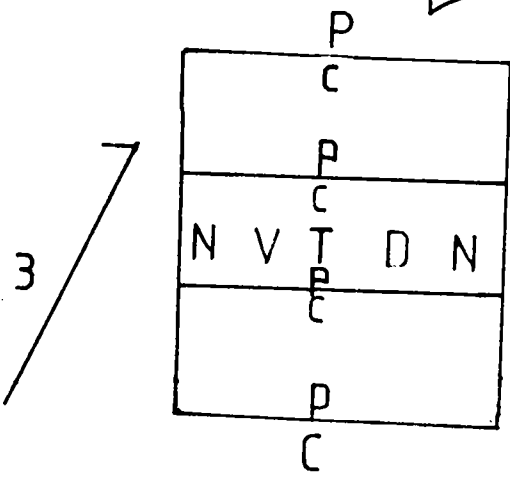
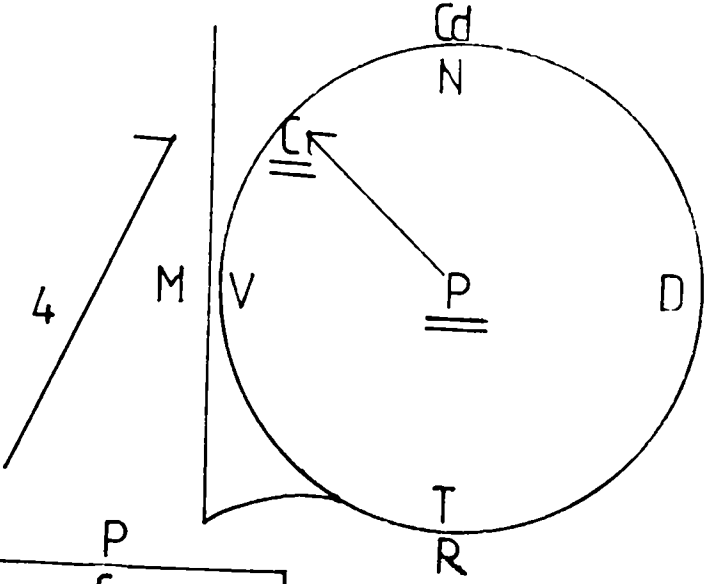
Step 2: 180° rotation of ribbon nerve.

Step 3: subunit reorganization as proposed by Scholes (1979; fig.4). While reorganizing each subunit so that its center-periphery axis is reversed and its circumferential order changed from a ventral division point to a nasal one the overall centre-periphery axis is unchanged. Thus each subunit is incorrectly orientated within the complete ribbon.

Step 4: Direct projection on to the tectum. This gives a map with its centre-periphery axis reversed from that observed.

fig 1.1

L Tectum



R Retina

Reorganization of fibres then occurs in small subunits, each subunit representing an annulus of several hundred retinal ganglion cells formed at about the same time in development. Within each subgroup nearest neighbour relationships are maintained (except at the new nasal point of split) as ventral fibres are brought from one side of the subunit to the other. However this manoeuvre as pictured by Scholes (diag. 1.1) would have the effect also of reversing the centre-periphery axis of the subunit. Yet each subunit remains in the same position within the overall centre-periphery axis of the ribbon. Thus a ribbon running from centre to periphery would consist of a number of subunits each running from periphery to centre. Whether those discontinuities at the subunit boundaries would affect the map would depend on whether each subunit represents an annulus of retina thick enough to be considered to have definite central and peripheral edges. If the annuli are only one ganglion cell thick there is no problem and even if they are thicker, as they would have to be in central retina to give the roughly uniform subunits observed, then the small errors introduced could either persist in the map unnoticed, or be corrected at the tectum.

Finally reorganization of the fibres as small groups will lead to disruption of fibre-fibre relationships at the group boundaries with fibres criss-crossing. This is not consistent with substrate contact guidance and would require a specific signal acting upon fibres according to their origin on the retinal circumferential axis. Some means is also required for dividing the fibres into the several hundred subunits observed and Scholes suggests this mechanism to be time. Each subunit would represent the fibres reaching the reorganization point within a given period of time. To be effective this would require retinal ganglion cells to send out fibres in highly synchronized bursts for any asynchrony would lead to incorporation of fibres into the wrong subunit. Though goldfish retinal growth is by circumferential addition (Johns and Easter, 1977) it is not clear whether sufficient synchrony occurs, particularly after the first few generations of cells are added, to ensure that a fibre is

assigned its correct place during fibre reorganization. In view of these problems with the interpretation of the Scholes model it is unfortunate that more data are not available concerning the observed patterns of degeneration.

A far less extensive investigation of nerve fibre topography in the retino-tectal pathway has been conducted in amphibia, for only recently has even the existence of ordering been confirmed (Bunt et al, 1979). In Rana ssp degeneration studies following retinal lesions suggest that the optic tract represents the retina opened out along a dorsonasal axis (Scalia and Fite, 1974). Dorsal retina is believed to be represented ventrally followed by temporal ventral and finally nasal as one moves dorsally. This organization resembles that of the cichlid fish optic tract (Anders and Hibbard, 1974; Scholes, 1979). Furthermore a chronotopic representation of the centre-periphery retinal axis is suggested by the finding in Xenopus laevis larvae that whereas the retina grows in rings (Straznicky and Gaze, 1971) optic fibres always grow along the extreme lateral edge of the diencephalon. Thus youngest fibres from peripheral retina come to lie dorsolateral to older fibres from central retina (Gaze and Grant, 1978).

Thus the problem of the detailed arrangement of fibres in the primary visual pathway of lower vertebrates has not yet been resolved. It remains an important question for it may throw light upon the mechanisms which operate to ensure the correct formation of neural connections in development. If the normal adult pathway could be explained in terms of simple phenomena such as contact guidance then the significance of more specific guidance by chemical labelling which may have been postulated to explain the results of regeneration, would have to be questioned.

Mammals: evidence for order in the primary visual pathway

Attempts to determine how fibres are arranged in the primary visual pathway of mammals have been concentrated on the optic nerve between eye and optic chiasm. As will be discussed below there are a number of features of the subsequent part of the pathway, notably the partial decussation of fibres from the two eyes in the chiasm, the probable occurrence of fibre branching and the presence of extensive and retinotopically ordered projections to more than one nucleus, which are either absent or much less extensive in lower vertebrates. Thus a satisfactory explanation of the mammalian pathway in purely morphogenetic terms is intrinsically less likely. However the aim of the "morphogenetic" approach is to identify how far such simple phenomena as contact guidance might contribute to the production of ordered visual projections. One of the major contributions which such forces might make is the maintenance of fibre ordering within the optic nerve.

The first region in the visual pathway in which the nearest neighbour relationships of fibres might change from those of their parent ganglion cells is within the eye at entry to the optic fibre layer of the retina, and within the optic nerve head. Studies on this part of the pathway have been beset with methodological problems mainly caused by the small size of the optic axons. Early work employing Marchi staining of degenerating myelin after retinal lesions suggested a deep location for peripheral fibres in the rabbit though with some scatter (Sjaaff and Zeeman, 1924), a finding supported by Wolff and Penman (1950) using a similar technique with careful identification of the lesion site. These latter workers did, however, fail in their attempts to identify fibre positions in the monkey retina. Clinical support for such a scleral location for peripheral fibres came from those human subjects with exudative choroiditis who occasionally showed areas of normal vision between the central scotoma caused by rod and cone damage and the peripheral scotoma due to fibre damage. This suggested that fibres from intermediate ganglion cells were separated from the choroid by those of a more peripheral origin (Wolff and Penman, 1950).

More recently the problem has been reexamined using autoradiography. Ogden (1974) made small injections of ^3H -proline into the rhesus monkey retina in the region of the arcuate bundle and then traced the paths of these arcuate fibres into the optic nerve head. Near the optic nerve head, where the nerve fibre layer contains many fibres of area centralis origin, which should be unlabelled, the label was distributed uniformly throughout the thickness of the fibre layer. Ogden consequently concluded that there was no topographic organization within the fibre layer - a fibre's vertical position bearing no relation to the site of its parent ganglion cell. The method is however open to criticism in that it relies upon there being no spread of label away from the injection site. Moreover it is assumed that arcuate fibres enter the optic nerve head at the same point on its circumference as do area centralis fibres, whereas it may be that the latter displace the arcuate fibres to either side. Sections passing through the arcuate fibres as they enter the optic nerve head would in that case be expected to show labelling throughout the fibre layer. Alternatively Ogden's result may reflect a reordering rather than a disordering of fibres in the nerve head.

Minckler (1979) by injecting primate nerves and retinae with either HRP or ^3H -fucose has attempted to trace the intra-retinal paths of fibres from peripheral retina. He found that, regardless of distance from the disc, ganglion cells appeared to send their axons vertically to the internal limiting membrane and then horizontally to the disc, with little if any crossing of fibres between bundles, or overlap of bundles though it is not clear from his brief publication what the consequences of this arrangement would be for the distribution of fibres of various retinal origins in the depth of the fibre layer as it is impossible for all fibres to pass up to and then simultaneously to run along the vitread surface of the fibre layer. Thus the exact arrangement of fibres within the depth of the fibre layer is unclear.

A similar degree of confusion surrounds the behaviour of optic fibres within the optic nerve head, the only available evidence being a Marchi study suggesting a peripheral location for peripherally-originating fibres in both the rabbit and the monkey (Wolff and Penman, 1950).

In the optic nerve itself experimental studies of the arrangement of optic fibres have been numerous and are supplemented by clinical evidence though mainly of an anecdotal nature. From human postmortem material Leber (1877) and Henschen (1893) supported the idea of a direct representation of the retina within the optic nerve though Ingesheimer (1919) was unable to confirm this. The concentric diminution of the visual field after treatment of the optic nerve in a human with novocaine led Seidel (1919) to propose a peripheral location in the optic nerve for peripheral fibres though Van der Hoeve (1920) took the enlargement of the blind spot in nasal accessory sinus disease to indicate the contrary.

Experimental studies utilizing Marchi staining similarly produced a variety of results. In the rabbit retinal lesions were found to produce either localized degeneration corresponding approximately to the position of the retinal lesion (Dean and Usher, 1896; Usher and Dean, 1896; Pick, 1896; Loddoni, 1930), or diffuse degeneration (Sjaaff and Zeeman, 1924). This latter finding was, however, interpreted by the authors as possibly due to folding and consequent distortion of the retinal map (such as occurs in some fishes - Anders and Hibbard, 1974) so that the general consensus was for the existence of some degree of order though perhaps not a direct representation of the retina. The existence of the visual streak, a specialized horizontal strip of retina with an increased ganglion cell density, might also have contributed to the disparity of results for if it distorted the map it might well cause some lesions to give more scattered degeneration than others.

In primates the problem is further complicated by the presence of the area centralis around, rather than over, which fibres from a more peripheral origin in temporal retina course. Lesions between the area centralis and the optic disc thus interrupt only relatively central fibres, yet those were found to produce, near the eye, a patch of degeneration extending to the edge of the nerve (Dean and Usher, 1896; Usher and Dean, 1896; Brouwer and Zeeman, 1926). Nearer the chiasm degeneration was found only in the central part of the nerve. Thus it seems that not only does the optic nerve contain a distorted representation of the retina, but that the nature of this representation changes between eye and chiasm. Both Polyak (1957) and Ramon y Cajal (1955) have suggested that this reorganization occurs because at the optic nerve head the fibres from the area contralis occupy a small but complete sector, displacing arcuate fibres to either side. Near the chiasm the arcuate fibres come together, by some unknown mechanism, to enclose the centralis fibres.

Thus the early histological and clinical evidence is in favour of some degree of orderliness of fibres in the optic nerve, reflecting their retinal origins. However Marchi staining is in-sufficiently imprecise, with its tendency to give a high background and occasional false positive results, to provide any degree of precision as to the nature of the map. Fairly localized degeneration could indicate a very precise map with the blurring of edges due to a diffuse retinal lesion or the inadequacy of the staining technique, or it could result from a degree of order no better than crude localization to appropriate quadrant and either a central or peripheral position.

An attempt to resolve the question of the degree of ordering in the monkey optic nerve was made using electrophysiology by Hubel and Wiesel (1960). A tungsten microelectrode was used to record from fibres within the optic nerve while stimulating the retina with a small spot of light. In any penetration through the nerve successive units were found to respond to widely separated receptive field positions with no finer degree of ordering than a

crude segregation to the correct quadrant. However the failure of a similar method to detect order in the frog optic nerve (Maturana et al, 1959) while anatomical methods suggested a high degree of order (Bunt et al, 1979) indicates that there are problems in the interpretation of such microelectrode studies. Firstly it is not certain that the units so detected form a representative sample of optic fibres so that any disorder demonstrated might be present in only a small, though highly responsive, fibre subgroup. Secondly penetration of the electrode might itself considerably distort the fibre arrangement. Finally if the arrangement of fibres in the nerve is not a direct map of the retina but is transformed in some way (for example the ribbon nerve of cichlid fishes, Anders and Hibbard, 1974) then fibres from disparate retinal origins may lie close together even though the vast majority of fibres retain the same neighbours as their cell bodies possess in the retina.

The introduction of Nauta staining for degenerating axons themselves rather than for the degenerating myelin, as with Marchi, allowed Hoyt and his colleagues to reexamine the problem (Hoyt, 1962; Hoyt and Luis, 1962; Hoyt and Tudor, 1963). In monkeys they made retinal lesions using a photocoagulator and traced the degeneration so produced through the optic nerve. The results confirmed the earlier histological studies - fibres of peripheral retinal origin were found to have a peripheral location in the optic nerve, while more central fibres lay towards the middle. Macular fibres extended to the periphery of the nerve just behind the eye but moved to a more central position nearer the chiasm. However the fairly large size and poorly defined location of the lesions used means that these studies provide little information as to the accuracy of the retinal representation in the nerve.

A method potentially able to resolve the uncertainties about the nature and accuracy of the retinal map in the primary visual pathway of mammals is the use of HRP to trace small groups of fibres over considerable distances. HRP can be introduced at any point in the visual pathway as it is transported both

retrogradely and anterogradely by neurons and their processes. Thus it can be used to label a small group of axons selectively and follow them back to the retina or forward to their target nucleus. Horton and co-workers have employed this method in the cat (Horton et al, 1979) where the results suggest little if any order while Minckler's work in the monkey (1979) indicates a high degree of order. This controversy and its possible resolution will be discussed in chapter 4 where the results of HRP staining in the mouse visual pathway will be described.

Mammals: the nature of the fibre ordering and its possible contribution
towards the formation of specific nervous connections

If a morphogenetic approach is to be at all useful in considerations of the mammalian primary visual pathway then particular attention must be paid to the nature of the orderly neuronal connections, which ganglion cells project to which visual centres, how they reach those targets and in what arrangement. The first point on the pathway at which the behaviour of fibres might be influenced by their arrangement is the optic chiasm. Whereas in lower vertebrates the visual fibres pass almost exclusively to the contralateral side of the brain, in mammals at least some, and in some species almost half, of the visual fibres project to the ipsilateral side of the brain. What determines whether a fibre crosses or remains uncrossed at the chiasm?

The need for a partial decussation arises if mammals are to make use of their binocular overlap whereby some regions of the visual field can be viewed by both eyes at the same time. It allows information about this part of the visual field, obtained through both eyes, to be brought together in the brain. If the division of fibres into those going ipsilateral and those going contralateral were simply straight down the vertical meridian all fibres nasal to this line crossing, and all fibre temporal to it remaining uncrossed then the explanation could lie in simple, mechanical splitting of a direct retinal map, the position of the split depending on the point at which the vertical

meridian projects on to each retina. However there is considerable evidence that the situation is far from simple. Firstly the accuracy with which fibres divide seems to depend upon the type of fibre. Optic fibres have been classified by a number of workers according to the way in which they respond to visual stimuli. Using the classification of Fukuda and Stone (1974) in the cat, X-cells, projecting primarily to the dorsal lateral geniculate nucleus (dLGN) were found to divide along the vertical meridian within a zone only 1° across. In contrast Y-cells, supplying both dLGN and superior colliculus (SC), and W-cells, projecting mainly to the midbrain, were found to show a much greater degree of overlap, 16.5° and 10° respectively, in which the fibres might project to either side of the brain. Thus fibres seem to vary in the accuracy with which they respond to guidance cues in the chiasm. If such cues were mechanical it is difficult to see why different fibre classes should respond differently unless they differ in either their arrangement in the nerve, their time of arrival at the chiasm or some property such as adhesivity.

A further complication came with the finding that some ganglion cells in rats though not adult cats (Kreutfeldt - personal communication) send branches to both sides of the brain (Cunningham and Seagraves, 1976; Cunningham, 1976; Cunningham and Freeman, 1977; Cowey and Perry, 1979). Moreover these ganglion cells were found to occur not only along the vertical meridian, where the decision to project ipsilaterally or contralaterally might be most difficult, but over a considerable part of temporal retina (Cowey and Perry, 1979). Such bilateral branching is amenable to experimental manipulation, removal of one eye in the neonatal rat, though not the adult, causing the whole of the remaining retina to branch (Cunningham and Spears, 1975; Cunningham, 1976). The nature of the branching stimulus is unclear though the existence of the phenomenon poses problems for both morphogenetic and chemospecificity models. In morphogenetic terms the stimulus to branch might be the existence of a mechanical barrier to fibre growth though how this could act on a large

proportion of the optic fibres to produce two separate sets of branches, one for each side of the brain, is unclear. Similarly it is difficult to envisage a more specific signal for the stimulation of branching and the subsequent direction of the branches which could act independently on the two branches of one axon unless one tract is formed first with branching and growth of the new branches to a separate target occurring as a much later event. Ordering of fibres within the optic nerve might certainly contribute to this phenomenon, however, by determining which fibres are exposed to the branching stimulus.

It had been hoped that light might be thrown on the mechanisms operating to determine fibre pathways at the chiasm by studying a wide range of species and varieties of animals. Some limited correlation has been demonstrated between the pigmentation of the animal and the proportion of fibres which pass ipsilaterally at the chiasm, (Sanderson et al, 1974; Wise and Lund, 1976; LaVail et al., 1978), suggesting a biochemical control system. However these, and other studies using abnormal ipsilateral projections produced by early eye removal (Lund et al., 1973; Lund and Miller, 1975), or treatment with teratogens (Lund et al, 1976) share the problem of interpretation that they may be identifying factors not causally related to the control of the normal ipsilateral projection. They may produce some structural alteration or interfere with fibre contact guidance in a way not normally seen in development.

Information on the arrangement of fibres within the optic tract of mammals is sparse. In the monkey upper peripheral field is believed to be located dorsally and lower peripheral field ventrally with the macular fibres central and lateral (Brouwer and Zeeman, 1926; Hoyt and Luis, 1962). There is evidence for mixing of fibres from the two eyes (Brouwer and Zeeman, 1926; Ramon y Cajal, 1955; Polyak, 1957; Hoyt and Luis, 1962) and Cunningham and Freeman (1977) have attempted to describe this in detail in the rat. Using small retinal

lesions and a silver stain for degenerating axons (Hink-Heimer) they found that temporal peripheral retina sends fibres dorsomedially in the ipsilateral tract but ventrolaterally in the contralateral tract. Thus fibres subserving the same area of visual space, and hence from different retinal origins, are brought together in the optic tract. Such intermeshing could be achieved without any need for reorganization of fibres according to their naso-temporal origins, suggesting that a morphogenetic explanation might be adequate.

However from the optic tract at least two major representations of the retina, those to the dorsal lateral geniculate nucleus (dLGN) and the superior colliculus (SC) are separated out. Both targets receive projections from ganglion cells viewing the entire contralateral visual field. To some extent the basis of the separation may be the differential distribution of the various fibre types, X-cells supplying the dLGN, W-cells the SC (Fukuda and Stone, 1974). However Y-cells supply both targets. Possibly the Y-cells form a heterogeneous group and might therefore vary in their response to guidance signals towards dLGN or SC. However some at least of those cells send branches to both nuclei (Barris et al., 1935; O'Leary, 1940; Ramon y Cajal, 1955; Hayashi et al., 1967; Sefton, 1968). Again both morphogenetic and chemospecific zones seem inadequate to explain the distribution of branches of one fibre to different targets unless some other factor such as timing is involved with the branching occurring after one pathway had become well established.

The topography of the retinocollicular projection has been extensively investigated using both anatomical and electrophysiological methods in a wide range of mammals, and considerable variations have been observed between species. In rodents the major projection is from the entire extent of the contralateral retina with a minor ipsilateral projection to the front of the SC (rat - Hayhow et al, 1962; mouse - Drager and Hubel, 1975; rabbit - Giolli and Guthrie, 1969; guinea-pig - Johnston and Gardner, 1959; Giolli and Creel, 1973; hamster-Finlay et al., 1978), though the ipsilateral projection was often

difficult to detect and seemed to vary with the strain of animal, often being absent in albinos (Giolli and Guthrie, 1969; Giolli and Creel, 1973). In cats the existence of a substantial ipsilateral projection to the SC is well established (Apter, 1945; Altman, 1962; Laties and Sprague, 1966), retinal fibres seeming to divide along a vertical line lying slightly temporal to the area centralis, with fibres originating temporal to this division projecting ipsilaterally and the remainder contralaterally. In primates the nasotemporal division of retinal fibres seems to be even more precise, occurring with great accuracy along the vertical meridian so that the collicular map is of the contralateral half field only (Wilson and Toyne, 1969; Schiller and Koerner 1971; Cynader and Berman, 1972; Lane et al., 1973; Hubel et al., 1975; Pollack and Hickey, 1979).

Two aspects of these retinocollicular projections are of major interest in the consideration of the role of morphogenetic forces in their development, the map orientation and the relationship between ipsilateral and contralateral fibres. In all therian mammals investigated the orientation of the retinal projection on to the contralateral SC is of dorsal retina going laterally and nasal retina posteriorly (rat - Siminoff et al., 1966; mouse - Drager and Hubel, 1975; rabbit - Masland et al., 1971; hamster - Finlay et al., 1978; Thompson, 1979; cat - Sprague et al., 1968; monkey - Wilson and Toyne, 1969, 1970; Schiller and Koerner, 1971; Lane et al., 1973). Ipsilateral fibres, in contrast, show the same orientation of the dorsoventral retinal axis but nasal-most fibres go rostrally and more temporal fibres caudally. The effect of the differing modes of ipsilateral and contralateral mapping is to bring fibres representing homonymous points in the visual fields of the two part retinae to the same collicular location (Schiller and Koerner, 1971; Cynader and Berman, 1972; Berman and Cynader, 1972; Gordon, 1973; Lane et al., 1973; Thompson, 1979). This mode of superposition of the two sets of fibres is that which would be expected if the two sets are in retinotopic order in the chiasm and come together with no major reorganization of their ordering.

The incompleteness of mixing of the two sets of terminals in the tectum which has been detected in a number of species (rat - Lund and Lund, 1976; hamster - Chalupa and Rhoades, 1979; cat - Graybiel, 1975, 1976; tree shrew - Hubel, 1975; macaque - Hubel et al., 1975; rhesus - Pollack and Hickey, 1979; chimpanzee - Tigges et al., 1977) might then simply represent a tendency of fibres from one eye to remain in groups throughout the pathway and hence terminate together.

The orientation of the retinal map on to the SC may also be a consequence of the orderly arrangement of fibres within the pathway. If the upward direction of growth of terminals from the deep stratum opticum into the more superficial stratum griseum superficiale and stratum zonale is taken into account (Kruger, 1970) and reflects the vertical organization of fibres as they grow into the SC (i.e. more superficial fibres at the entry into the SC terminate near that point of entry while deeper fibres terminate later), then the map could be produced directly from a direct retinotopic arrangement of fibres in the tract without need for reorganization.

Thus in mammals there is some evidence for an orderly arrangement of optic fibres within the optic nerve though this still remains a controversial matter. Of the detailed nature of that ordering little is known though there are suggestions of a direct representation of the retina within the optic nerve and tract. How this might be produced is not clear. The highly orderly growth of the retina seen in lower vertebrates (Straznicky and Gaze, 1971; Johns, 1977) with sequential outgrowth of optic fibres over a considerable period of time, seems not to occur in the mammal. Instead ganglion cells are produced rapidly with a general trend towards addition of cells at the circumference (Sidman, 1960; Morest, 1970; Braekevelt and Hollenberg, 1970) though with considerable intercalary addition of ganglion cells or possibly displaced amacrine cells (Perry - personal communication).

Whether timing of fibre outgrowth can be correlated with retinal location is unknown. Considering the short period over which it occurs, sequential fibre outgrowth is unlikely to provide as high a degree of precision as it seems to do in the goldfish (Dawney, 1979b).

Guidance of optic fibres into the optic nerve in a relatively orderly manner may be aided by the appearance of intercellular channels within the optic cup and stalk along which pioneer fibres could progress under guidance by contact with the substrate - the "blueprint hypothesis" (Singer et al, 1979), though this raises the question of the stimulus for channel development. Morphogenetic forces might thus be adequate to produce an orderly representation of the retina within the optic nerve, the bringing together of fibres viewing the same area of visual space and the formation of the retinocollicular map. However it is far from clear that the fibre arrangement within the primary visual pathway is sufficiently well ordered to be important in the formation of visual projections. Furthermore some aspects of the pathway, notably the division of fibres into ipsilateral and contralateral at the chiasm, the occurrence of branching and the production of several retinotopic projections in different nuclei make great demands upon a model invoking only mechanical forces such as contact guidance and fasciculation.

Are nerve fibres arranged in an orderly manner in vertebrate peripheral nerves?

Within the spinal cord the motoneurons which supply a particular muscle are grouped together in the ventral horn in a manner which is fairly consistent within each species (frog - Cruce, 1974; chick - Hollyday et al., 1977; mammals - Romanes, 1941, 1946, 1964). When the axons first leave the spinal cord they travel, without intermixing, across the surrounding mesenchyme (Ramon y Cajal, 1955, 1960). Thus fibres supplying a particular muscle remain close together. Obviously these fibres must be grouped together,

though with the addition of sensory fibres, where they enter the muscle. Do they remain together throughout the intervening distance, in which case their arrangement within the nerve trunk might help to determine which muscle they supply, or do they intermingle with fibres going to other muscles, in which case some more complex mechanism, for example chemospecific matching, must be invoked to explain the development of the limb innervation?

As with the primary visual pathway a survey of the literature on the organization of fibres within nerve trunks reveals a plethora of contradictory information from a variety of sources, clinical, pathological and experimental. In the late nineteenth century an attempt to resolve the problem was made by Herringham (1886), whose ability to trace individual motor roots through the entire limbplexus in human foetal and adult material suggested an orderly arrangement of fibres. This was compatible with the contemporary observation that natural variations in nerve structure such as complete longitudinal division of the sciatic nerve were not accompanied by any functional loss (Paterson, 1887).

However the major impetus to such research came with the first world war and the need to find a satisfactory surgical approach to the repair of peripheral nerve lesions. In 1913 Stoffel had developed the concept of the "cable structure of nerves". He regarded the proximal part of a nerve as merely the systematic aggregation of the peripheral branches so that at proximal levels the fasciculi representing peripheral branches could be precisely identified. Support for such a view came from correlations of the nature and extent of motor and sensory loss with the degree of damage to peripheral nerves (Dejerine et al., 1915; Putti, 1916; Barile, 1917), from experimental studies on humans in which nerve trunks exposed at operations were stimulated with surface electrodes and the motor responses observed (Marie et al., 1915; Kraus, 1920; Kraus and Ingram, 1920), and from studies of the effects of ischaemic block of nerves (Lewis, 1931; Denny-Brown, 1944).

Despite this apparently precise disposition of fibres within nerve trunks the results of regeneration after nerve injury were disappointing. Though the fibres grew fairly rapidly (Seddon et al., 1943) the connections made within the limb were often incorrect, mistakes being particularly evident in regions such as the intrinsic musculature of the hand where normal function requires very precise motor control (Stopford, 1920). To resolve this apparent contradiction a number of groups attempted microdissection of nerve trunks. Langley and Hashimoto (1917) were the first to demonstrate clearly the existence of intraneural plexi though they interpreted them as the means by which motor and sensory fibres to a particular muscle were brought together. Subsequent workers have been so impressed by the extent of such plexi that they have postulated a much more extensive intermixing of fibres supplying different muscles (Compton, 1917; Dustin, 1918; Goldberg, 1923; O'Connell, 1935). A particularly extensive series of such microdissections and reconstructions from sectioned material of human nerve trunks has been conducted by Sunderland and his colleagues (Sunderland, 1945; Sunderland and Ray, 1948; Sunderland et al., 1959; Sunderland, 1975) which apparently showed again considerable intermixing of fibres within the trunks. However these analyses were based on the assumption that whenever two fascicles merge their contents become so intermixed that on subsequent division each daughter fascicle contains a sample of the fibres present in both parents. The possibility that fascicle fusion and division is a secondary structural feature of the nerve due to the laying down of connective tissue around the orderly array of fibres was not considered.

An experimental approach which it was hoped might throw more light on the precise extent of fibre inter-mixing in intraneural plexi was the tracing of degeneration after partial nerve lesions. McKinley (1921), in the dog, and Kilvington (1940), in the cat, followed distally the degeneration occurring after a partial cut of the proximal trunk. In both cases degeneration was initially localized but became more scattered as the distance from the lesion increased. However apart from the general problems of using stains for

degenerating myelin with their high background and false positive staining, both these studies suffer from the fact that the lesion was of fibres which happened to lie together at one point in the trunk rather than constituting a functional group. It is possible that while fibres to a particular muscle remained together fibre groups serving different muscles might show changing relationships due to limb rotation (Bardeen, 1906) and nerve towing by muscles (Weiss, 1955). Thus a lesion which interrupted several groups of fibres might give scattered degeneration while in fact each individual group preserved its integrity. A similar explanation might apply to the widespread motor activity caused by local stimulation of a nerve trunk (McKinley, 1921).

More recently Veyama (1978) has studied the distribution of spinal roots within the nerve trunks of the dog using a degeneration method and has found little evidence of intermixing of fibres. This concurs with the regular distribution of fibres within the limb according to their segmental origins noted by Horder (1978). However in terms of the fibres supplying a particular muscle and their course through the nerve trunks towards the spinal cord there is a remarkable dearth of information.

Even if a highly ordered arrangement of fibres within limb nerves were demonstrated there remains some facets of muscle innervation which pose severe problems for a "morphogenetic" approach. A good example comes from the study of Burke and his colleagues (1977) of the distribution of labelled motoneurons within the spinal cord after injections of HRP into various muscles of the cat. The motoneurone groups supplying the muscles soleus and medial gastrocnemius were found to overlap extensively, not surprisingly as both belong to the triceps surae muscle group and are adjacent in the limb.

However medial gastrocnemius is a fast muscle and soleus a slow muscle - the properties of fastness and slowness being controlled by the innervating nerve (Salmons and Sreter, 1976). This suggests some basic difference between the two groups of motoneurons. How do they come to find the correct muscle? Such fine tuning in the development of limb innervation may well require some information above that available from morphogenetic sources.

Experimental investigations of the formation of specific nervous connections between retina and tectum in lower vertebrates

The retinotectal projection of lower vertebrates has proved an extremely convenient system in which to investigate the ways in which precise connections are formed between groups of cells within the nervous system. Amphibian larvae have a developing retinotectal pathway accessible to study and manipulation while the good regeneration of the optic nerve in fish and amphibia facilitates investigation of the adult system. Moreover both fish and amphibia show surprisingly good recovery after extensive operative disturbance of the system and the results of such surgery are readily studied using electrophysiological, behavioural and anatomical methods. In contrast the retino-collicular pathway of mammals (the colliculus being the mammalian structure homologous to the optic tectum of lower vertebrates) is much less easily studied. Prenatal operations have a dauntingly high mortality while post-natal operations are only effective if performed very soon after birth as regeneration of the mammalian visual system, once formed, is extremely poor. Thus the literature to be reviewed below, concerning the response of the retinotectal pathway to experimental disturbance, concerns mainly work on the goldfish (Carassius auratus) and the frog (usually Rana ssp. and Xenopus laevis). Where data are available from mammalian studies they will be discussed with the results of similar experiments on lower vertebrates.

Rigid chemospecificity - its experimental basis and subsequent disproof

Regeneration of the optic nerve

As discussed above, the classical chemospecificity theory of Sperry, proposing that each optic fibre carried a label of its position of origin in the retina and formed synapses with an area of tectum bearing a corresponding label, arose from the observation that in lower vertebrates optic nerve section and regeneration led to full functional recovery (Matthey, 1927; Stone et al, 1937; Stone and Zaur, 1940; Sperry, 1942, 1943a). When combined with the results of eye rotation experiments this suggested that fibres from a particular retinal locus were reconnecting with the same tectal area to which they initially projected.

Electrophysiological mapping later confirmed Sperry's original conclusions (Gaze, 1958, 1959; Maturana et al., 1959). The need to postulate some form of labelling to explain how fibres were able to return to their original tectal positions arose from Herrick's suggestion (1941a, b, c) that the normal anuran optic nerve is disordered and from Sperry's observation of considerable disorder in the arrangement of fibres in the regenerating optic nerve (1943a, b, 1945, 1948). However there is now considerable evidence for an orderly pattern of fibres in the optic nerves of lower vertebrates. Might not such order be retained in the regenerating optic nerve and contribute to the formation of an orderly retinotectal projection?

In silver-stained sections of the scar region of the regenerating optic nerves Sperry observed fibres running across the nerve obliquely suggesting that considerable intermixing of fibres was occurring (1943a). However it is possible that these aberrant fascicles represented fibres escaping from the nerve to innervate abnormal targets, or no targets, at all (Gaze and Jacobson, 1963; Gaze and Keating, 1970; Reier and Webster, 1974; Beazley,

1977) and which did not, therefore, contribute to the regenerated retinotectal projection. Further distortion might have arisen, as suggested by Horder and Martin (1978), due to swelling of connective tissue at the lesion which would displace fibre bundles from a parallel arrangement without actually causing them to intermix. Thus histological sections might have appeared to demonstrate a degree of disorder not actually present. Moreover a tendency for optic fibres to regenerate in an orderly manner has been found in the rabbit (Ortin and Arcuate, 1913) and toad (Maturana, 1958) after nerve section, and in the newt after a cold lesion (Stensaas and Feringa, 1977) though whether any distortion of fibre arrangement would be expected in this latter case is doubtful as the cooling did not disrupt the basal lamina. Attempts to trace small groups of fibres through an optic nerve lesion in the goldfish have been made recently. Horder (1974) found the majority of fibres to pass undisturbed through the region of the lesion while Roth (1972) showed "some" scrambling of fibres near the lesion with rapid restoration of retinotopic ordering more centrally.

Thus the arrangement of fibres in the regenerating goldfish optic nerve might well contribute substantially to the reformation of the retinotectal map. That such ordering is not sufficient to produce a highly ordered map after regeneration is indicated by a number of results. Firstly, in the frog (Gaze and Jacobson, 1963; Gaze and Keating, 1970), though not the goldfish (Jacobson and Gaze, 1965; Horder, 1971), the electrophysiologically-recorded map showed an initial period of disorder from which an ordered map gradually evolved. Furthermore in fish (Horder, 1971) the receptive fields recorded with a microelectrode showed a gradual reduction of size with time after regeneration. Autoradiographic results are compatible with an improvement in the ordering of the regenerated goldfish projection with time (Meyer, 1980). Thus it seems that both fish and frogs go through a disordered phase early in the regeneration of the retinotectal projection which subsequently undergoes tidying up.

Such "tidying up" may have to involve active re-routing of at least some fibres back to their correct tectal locus rather than just death of fibres which have regenerated to the wrong place, for both Horder (1974b) and Udin (1978a) detected some fibres following highly abnormal routes back to the correct tectal region. That the tectal termination was approximately appropriate was shown using electrophysiological mapping while the persistence of the response after cutting through the normal access route to that tectal position showed their paths to be highly anomalous. How many fibres underwent such rerouting was unclear - Horder inferred there to be few from response strength assessments while Udin suggested there to be a much higher number. Meyer's autoradiographic method may allow quantification of this process though it is not clear that the method is sufficiently sensitive to allow retinal and tectal lesions to be combined in the same animal thus showing that the routes are abnormal but the termination sites correct.

Thus although regeneration studies, which provided the inspiration for the classical chemospecificity model with its rigid point-to-point matching, do indicate the need for something more than maintenance of fibre ordering in the pathway to produce the regenerated projection they have given little information on the nature of that force. If, as has been suggested (Horder and Martin, 1978), lesions of the optic nerve and tract cause little disturbance of fibre relationships then the tidying up of the projection might be achieved by a relatively weak, short-range process unable to correct larger disturbances to the system. Regeneration studies throw no light on the nature of the mechanism involved which could be very crude or as precise as that postulated in the classical chemospecificity model.

Size disparity experiments

The experimental technique which was to provide the first strong evidence against rigid chemospecificity was actually introduced by Attardi and Sperry (1960, 1963) in support of their model. This was the production of a "size disparity" between retina and tectum by ablating part of one or other structure, and then studying the resulting retinotectal terminations. Attardi and Sperry ablated part of the retina of goldfish, crushed the optic nerve and then allowed fibres to grow back into the complete, denervated contralateral tectum. Using histological methods they found that the remaining optic fibres terminated only in the appropriate part of the tectum and would cross unoccupied tectum to do so. For example if peripheral retina was ablated then fibres from the remaining central retina crossed the empty rostral tectal pole to reach the central area before terminating.

Electrophysiological confirmation of this result came from Westerman (1965) who found that ventral retinal quadrants would regenerate only to the correct medial tectal region, and Horder (1971a) obtained a similar result with dorsal, ventral and temporal hemiretinae. Delaying the arrival of some fibres, rather than actually removing them, was found to give the same result (Jacobson and Gaze, 1965). Similarly, removing part of the tectum gave a regenerated projection containing only fibres appropriate to the tectal remnant (Jacobson and Gaze, 1965).

However this apparent support for rigid chemospecific matching of each ganglion cell to a particular tectal termination site was shortlived. Gaze and Sharma (1968, 1970) demonstrated that after ablation of the caudal half of the goldfish tectum and crush of the optic nerve the electrophysiological map at 50 days survival showed a projection from only appropriate, temporal retina. However mapping of other fish after long survival times showed the complete retina to be represented in a highly ordered, though compressed, manner across the tectal remnant. Ganglion cells were therefore forming connections with

tectal sites other than those to which they would normally project. Subsequently compression was also shown to be possible across the mediolateral axis of the tectum after medial or lateral half tectal removal (Yoon, 1971) and to occur in other animal groups (amphibia - Jacobson and Levine, 1975a; Udin, 1977; Freeman, 1977; neonatal hamsters - Finlay et al, 1976; Jhaveri and Schneider, 1974; foetal rats - Miller, 1975; Miller and Lund, 1975). Even more surprisingly, connections were found to be possible between non-corresponding retinal and tectal remnants (Horder, 1971a; Yoon, 1972b).

In order to explain compression of the retinal projection on to a half tectal remnant while retaining the idea of very precise and rigid matching of labels between retinal ganglion cell axons and their tectal targets Meyer and Sperry (1973) and Yoon (1975a, 1976) proposed that such ablations caused the remaining tectal tissue to undergo "regulation" whereby the complete set of tectal chemoaffinity labels became expressed over the remaining half tectum. Evidence against regulation came both from Sperry's laboratory (Meyer, 1975a, b) and, more convincingly, from Cook and Horder (1974, 1977; Cook, 1979), who, after inducing compression on to a rostral half tectum, recut the optic nerve and analysed the earliest subsequent regenerated projections. If regulation had occurred then an initial fully compressed projection would be expected. Instead the first projection was uncompressed and from appropriate temporal retina. Only gradually were more nasal fibres accommodated on the rostral half tectum. No regulation was apparent.

Compression was only observed in the presence of fibres deprived of a termination site. If, after caudal tectal ablation, the disconnected nasal fibres were prevented from reaching the tectum by repeatedly cutting the nasal side of the optic nerve the remaining fibres showed no tendency to shift forwards on the tectal remnant (Cook and Horder, 1977; Cook, 1979). Similarly autoradiography showed the innervation of a half tectum by the appropriate half retina to be uniform so that in the absence of disconnected fibres the

remaining fibres had no stimulus to move their termination sites (Meyer, 1975b; Meyer and Sperry, 1976).

A further modification of the chemospecificity hypothesis was then proposed in which tectal chemoaffinity cues were assumed to be graded (Meyer and Sperry, 1976). Disconnected fibres which would have terminated just beyond the cut edge of the rostral tectal remnant were then able to terminate at the edge of the remnant because cues there nearly matched their own. Once connected they were postulated to "modulate" that part of the tectum, causing its labels to become closer to their own, that is, more caudal. This would leave the edge of the remnant labelled in a way which nearly matched the next row of disconnected fibres which could then edge their way on to the remnant. In this way fibres would be added progressively to the caudal edge of the tectum and would gradually alter the tectal labels until finally all, or nearly all, were accommodated and the part tectum gained a complete range of chemoaffinity cues (Meyer and Scott, 1977; Meyer, 1977).

Again the recompression experiments of Cook and Horder (1974) provided the convincing evidence against a change in tectal labels. Instead a purely competitive interaction between optic fibres for tectal space seemed the likely cause of progressive compression seen initially and after section of an optic nerve which had already formed a compressed projection. It is interesting to note that evidence against any alteration of tectal labels during compression was available in the first full account of compression (Gaze and Sharma, 1970). Instead of an orderly compressed map some fish had coherent retinotopic projections from the disconnected nasal retina which was superimposed on the normal temporal projection to the half tectum. Such a projection from two retinal regions to the same area of tectum, a phenomenon confirmed by many other workers (Sharma, 1972c; Meyer, 1977; Marotte et al., 1977; Martin, 1978b) clearly violated the principle of precise retinal-tectal label matching whether or not the labels had been modified after partial tectal ablation.

Compression has been extensively investigated under a variety of conditions; with or without optic nerve section and after varying degrees of tectal ablation (Cook, 1977, 1979; Martin, 1978); on a tectum which had already received a compressed projection (Cook and Horder, 1974; Yoon, 1976; Cook, 1979); when the tectum had been denervated long previously (Sharma and Romeskie, 1977); under various lighting conditions (Yoon, 1974, 1975c; Arora and Grinnell, 1976; Scott and Meyer, 1976; Meyer and Scott, 1977; Marotte et al, 1977); and in fish of various sizes and ages (Cook and Horder, 1974; Schmidt et al., 1978). However because the order in which fibres arrive at the tectum in such size disparity experiments is changed little, if at all, the extent to which that order might contribute to the nature and order of the map remains unchallenged. The sole problem posed is of accommodating an abnormal number of fibres in a given area of tectum and the only mechanism suggested by the experiments is of some form of competitive inter-action between fibres.

Normal development of the retinotectal projection-changing synaptic relationships

The precise and inflexible matching of a retinal ganglion cell with a particular area of tectum required by the chemospecificity hypothesis as originally formulated (Sperry, 1963) has been contradicted not only by the results of experimental manipulation but also by the events of normal development. The discovery that in Xenopus laevis the retina grows by addition of cells at the ciliary margin (Straznicky and Gaze, 1971) while the tectum undergoes cell division in a curvilinear manner from rostral to caudal (Straznicky and Gaze, 1972) retinotectal connections being formed before retinal and tectal growth have been completed (Gaze, Keating and Chung, 1974), raised the problem as to how synapses from later-formed retina could be accommodated on the tectum.

To explain this the concept of sliding connections, originally proposed to account for the development of projections of compound eyes (Gaze, 1970), was invoked. This postulated that the first connections formed were between central retina and rostral tectum but that these were gradually shifted backwards on to later formed tectum, so leaving room rostrally for fibres from more peripheral temporal retina to terminate. Later-formed nasal fibres posed no problem as they would be expected to terminate caudal to more central fibres and hence would pass automatically to newly-formed tectum.

Electrophysiological studies of the earliest retinotectal projections of larval Xenopus laevis appeared to confirm such a shift (Gaze et al, 1974; Chung et al., 1974) as did anatomical work (Scott, 1974; Scott and Lazar, 1976; Currie and Cowan, 1975; Longley, 1978). In terms of precise point-to-point label-matching such a shift was difficult to explain, requiring a gradual evolution of those labels as development proceeded.

Subsequently the need for sliding connections has been questioned. Retinal growth in Xenopus laevis over the period in which retinotectal synapses are forming was shown to be assymetric so that fewer cells that had previously been thought needed to be accommodated on rostral tectum. (Jacobson, 1976). Furthermore using the autoradiographic double-label method of Scott and Lazar, Jacobson failed to detect any relative movement between retinal axons and tectal cells (1977). However a recent reexamination of the problem has provided clear and careful evidence of a shift (Gaze et al., 1979a). Furthermore the continued growth of retina and tectum in the goldfish throughout adult life, the retina growing in annuli (Johns, 1977; Johns and Easter, 1977), the tectum by cell division at the caudal end only (Meyer, 1978a) requires a similar shifting of connections to explain incorporation of new fibres into a coherent map. Thus the gradual displacement of the projection during normal development is now widely accepted and, like the size disparity experiments, is hard to explain without some modification of the classical hypothesis of chemo-specific matching of retinal and tectal labels.

Evidence for the existence of guidance cues involved in the formation of retinotectal connections

In order to determine how much extra information is available to optic fibres, other than that provided by their ordering in the pathway, a number of experimental stratagems have been employed ranging from the investigation of fibre paths when the position of origin of a group of fibres is changed or changing the relative arrangement of retinal and tectal loci by means of fibre diversion and tectal graft exchange or rotation, to diversion of part of all of the fibre complement into the ipsilateral, and hence mirror-image, tectum. The majority of these studies have involved regeneration of the visual pathway of adult goldfish though some data are available from work on amphibia and mammals and where appropriate these are considered below together with related goldfish results. A detailed discussion of the multitude of conflicting data concerning experimental manipulation of the larval amphibian visual pathway will not, however, be attempted.

Guidance of optic fibres by cues lying outside the optic tectum

Sperry's original hypothesis (1951, 1963) proposed not only the existence of labels within the tectum which governed the termination of retinal fibres at particular locations but also that cues were present along the pathway to guide fibres towards the correct tectal site. The existence of pathway guidance signals seemed to be supported by the apparent growth of regenerating optic fibres directly towards their terminal sites from the point of nerve crush (Attardi and Sperry, 1963). Three aspects of the paths of optic fibres need to be considered in a search for guidance signals prior to the tectum; the general orientation of fibre growth towards the tectum rather than other parts of the brain; the decision whether to cross at the optic chiasm; and the division of the optic tract into two brachia just before entry into

the tectum.

1. Routing of fibres towards the tectum

In 1959 Gaze observed that optic fibres reaching the tectum via an abnormal route could form a correctly orientated map there. In one Xenopus laevis larva which had undergone eye rotation the regenerated optic nerve failed to follow its normal path but instead joined the oculomotor nerve and reached the tectum only after crossing the midline at an abnormally ventral position. To study the guidance signals suggested by Gaze's observation Hibbard (1967) deliberately deflected cut optic nerves of frogs into the severed oculomotor nerve stump. However though some fibres did reach the tectum they did so by crossing at the chiasm and following a normal route thereafter. Fibres pursuing a more deviant course seemed to get lost and never reach the tectum.

Hibbard and Ornberg (1976) have studied the route taken by optic fibres from supernumerary eyes grafted on to normal axolotls and on to genetically eyeless axolotls. In both cases the fibres entered the brain high on the lateral diencephalic wall rather than at the normal ventral position and instead of passing straight to the tectum ran forward until they reached the normal optic tract position in the diencephalon. They then followed the normal optic tract pathway to the tectum. Similarly Sharma (1972d) observed that a supernumerary eye, placed midway between the normal eyecups of a larval Rana pipiens, would send fibres via an abnormal route to form a "normal" projection on the tectum.

However if the transplanted eye was placed some distance away from its normal position the chances of fibres reaching the optic tectum were remote. In an extensive series of papers Constantine-Paton and Capranica (Paton and Capranica, 1974; Constantine-Paton and Capranica 1975, 1976a, 1976b; Constantine-Paton, 1978) studied the paths of optic fibres after transplantation of the eye to the ear region of Rana pipiens embryos. Such fibres failed to

innervate the optic tectum, tending instead to enter the hindbrain and then to travel dorsocaudally without decussating and so pass into the spinal cord. Since the normal direction of growth of optic fibres is dorsocaudal this led to the idea of a three-dimensional system of polarity cues throughout the central nervous system utilized by many groups of fibres travelling in different directions, rather than there being specific cues for each fibre group. However optic fibres occasionally pursued a rostral course, and where fibres passed caudally into the spinal cord they did so at a variety of positions. This suggested that control of fibre paths by such universal cues must be relatively weak.

The idea of universal polarity cues was further contraindicated by the finding that more extreme translocation of eye primordia in Xenopus laevis larvae resulted in optic fibres travelling both rostrally and caudally (Katz and Lasek, 1978, 1979; Giorgi et al., 1979), occasionally even reaching the tectum, though from completely the wrong direction. Instead it seemed likely that the rostrocaudally-orientated sensory and motor tracts were providing a suitable substrate along which the heterotopic optic fibres would grow, by contact guidance, with equal facility in either direction (Katz and Lasek, 1979).

Though the evidence for guidance cues distributed widely in the brain is thus rather tenuous this does not preclude the existence of specific cues for optic fibres within the normal pathway. A better test for such cues would be to study the paths of fibres which have been deviated from their normal course and yet remain within the normal route to the tectum. The only such experimental deviation so far employed has been that of eye rotation and though the fibres are known to reach the correct termination sites in the tectum the point at which they become reorganized is unclear (Sperry, 1943a, 1943b, 1944, 1945b, 1948) though there is some suggestion that this occurs in the tract. The question of the existence of specific guidance signals on

the route from eye to tectum which direct fibres to the correct termination sites is thus unresolved.

2. Pathway selection at the optic chiasm

There is considerable variation between species of animals, and even within species, in the proportion of optic fibres which cross the midline at the optic chiasm and pass to the contralateral side of the brain. Furthermore this proportion can be altered by experimental manipulation. Is the decision of a fibre to cross or not to cross determined by special signals in the chiasmatic region and what form might such signals take?

In lower vertebrates the vast majority of optic fibres, including all those destined for the optic tectum, pass contralaterally at the chiasm (goldfish - Sharma, 1972a; frog - Scalia and Fite, 1974). However there is no absolute ban on synapse formation on the ipsilateral side of the brain for in 1927 Matthey observed restoration of visuomotor responses in newts after regeneration of the optic nerve to the ipsilateral tectum. Similarly Szentagothai and Szekely (1956) found no overwhelming laterality preference of optic fibres in embryonic urodeles after left-right eye exchange and this was confirmed by Beazley using electrophysiology (1975). It might be argued that these results occurred because the manipulations were carried out early in development, before laterality preferences had been established, for Cronly-Dillon and Glaizner (1974) had great difficulty in persuading the optic nerves of adult goldfish to innervate the ipsilateral tectum. However it seems likely that this was a reflection of the technical problems of diverting an optic nerve away from its normal course.

There is thus no clear evidence of specific guidance of fibres either ipsilaterally or contralaterally at the chiasm of lower vertebrates. The difficulty in explaining the complex events at the mammalian chiasm, with different ganglion cell classes decussating with varying accuracy, in terms of chemical guidance cues has already been discussed.

Important in determining events at the chiasm may be the alignment of fibres along a favourable substrate such as the optic stalk for optic fibres entering the brain by unusual routes and encountering substrates aligned in a non-crossing manner showed no tendency to decussate (Katz and Lasek, 1978, 1979). Timing of fibre outgrowth may also play a role by determining how fibres will interact with those from the other eye, for Springer and his coworkers (Springer and Landreth, 1977; Springer et al., 1977; Springer, 1980b) have found the formation of an aberrant ipsilateral projection by a regenerating optic nerve in goldfish to depend on the presence, absence and timing of regeneration of the other nerve. It is not clear however, whether this variation in occurrence and extent of the ipsilateral projection reflects variation in the number of fibres passing ipsilaterally at the chiasm initially or whether it depends on the ability of those fibres to survive long term (Springer, 1980b). In the latter case an interaction between the two fibres at the tectum based on, among other factors, their times of arrival, would be the important phenomenon rather than interaction actually at the chiasm.

Similar experimental variation in the proportion of fibres passing ipsilaterally at the chiasm under various timing conditions have been demonstrated in frogs (Beazley, 1975; Stelzner, 1976; Glastonbury and Straznicky, 1978) and mammals (Lund, 1965; Chow et al., 1973; Goodman et al., 1973; Lund et al., 1973; Cunningham and Speas, 1975; Lund and Lund, 1976; Finlay et al., 1979; Thompson, 1979) though similar problems of interpretation arise. Thus the control of fibre pathway at the chiasm may involve a number of factors such as substrate guidance, timing of fibre arrival and interactions between fibres but there is no clear evidence for specific chemical guidance signals.

3. Pathway selection at the optic tract division

The third major region of the pathway, prior to the tectum, at which cytochemical guidance cues might be expected to act is at the division of the optic tract into the medial and lateral brachia. Certainly regenerating optic fibres in goldfish seem to select the appropriate branch (Roth, 1972, 1974) though this finding alone does not distinguish between an active choice by fibres and a purely passive split of the tract into two parts. In the normal animal it seems that the arrangement of fibres in the tract is such that a simple division down the middle would give the correct distribution of fibres into the brachia (Scalia and Fite, 1974; Gaze and Grant, 1978) and regeneration alone does not affect this splitting to any great extent (Horder, 1974a).

Stronger evidence for an active choice by fibres at this point comes from experiments involving retinal lesions. After removal of half of the retina the remaining fibres were found to enter the tectum by the appropriate brachium rather than spreading out to fill all the available space (Attardi and Sperry, 1963; Horder, 1971a). Moreover after cross-union of the medial and lateral branches Arora and Sperry (1962) noted that fibres appeared to regenerate back to the right position prior to entry into the tectum. Furthermore, all the fibres from a double ventral compound eye created in an early *Xenopus* embryo entered the tectum via the medial brachium which normally carried ventral fibres (Straznicky et al., 1979).

However any guidance cues present are not completely effective, at least in regeneration, for in both goldfish (Horder, 1974b) and frog (Udin, 1976) a number of fibres were found to regenerate into the tectum via the incorrect tract brachium.

Active guidance of optic fibres by cues within the tectum

A considerable body of experimental work has been directed towards the detection of information within the tectum which is used to guide fibres actively towards the correct termination site. This information may be conveniently divided into that determining "polarity" - the orientation of the retinal map with respect to the tectal axes - and that determining the actual place on the tectum at which a particular fibre synapses. Such a distinction is necessarily an artificial one but it is important when considering the interpretation of experimental data if a higher degree of information than actually exists is not to be attributed to the system.

Obviously precise matching of retinal and tectal labels, as envisaged by the classical chemospecificity hypothesis, would produce a projection of the correct polarity. However it is possible to generate a model in which the polarity of the map, the correct relationship of fibres to their neighbours, is generated without any tectal inhomogeneity. Every locus on the tectum could bear the same polarity markers, perhaps as an asymmetry of the dendritic trees of tectal cells, and the map then formed by the internal ordering of the ganglion cell array. Such ordering might be achieved by means of position markers, whether chemical, electrical or some other form, on the ganglion cells but no place labelling of tectal cells would be required. An example of such a system is the "Arrow model" of Hope et al., (1976). Alternatively the relative arrangement of ganglion cell terminals might be defined by the order generated by contact guidance within the pathway, with the orientation of the map determined by direction of ingrowth or by a polarity marker.

1. Evidence for polarity-defining cues within the tectum.

The basic experimental paradigm employed for the detection of polarity markers has been the confrontation of a group of optic fibres with an area of tectum of inappropriate polarity with investigation of the resulting projection. Such a

confrontation has been provoked in a number of ways starting with the simple eye rotation and transplantation experiments of Sperry and Stone. Rotation of the eyes of adult anurans and urodeles with optic nerve regeneration produced visuomotor responses rotated to the same degree as the eye (Sperry, 1942, 1943a, 1943b, 1944) suggesting that the retinotectal map was forming according to the original axes of the eye rather than to its new orientation. Similar experiments on late anuran and urodele larvae produced a concordant result since confirmed with electrophysiology (Stone et al., 1937; Stone, 1960; Gaze, 1959). Results of experiments on early embryos have suggested a similar control of projection polarity though some controversy has arisen concerning the determination of axes within the retina (Hunt and Jacobson, 1974; Gaze et al, 1979b).

Exchange of eyes between the two orbits, and uncrossing the chiasm with direction of fibres into the ipsilateral tectum were also employed by Sperry (1945) in the investigation of retinotectal connection formation in anurans and urodeles. Both situations produced behavioural responses suggesting that the map was orientated according to the original axes of the eye and the polarity of the tectum. Electrophysiological confirmation of the orientation of these projections has been obtained in adult goldfish (Easter and Schmidt, 1977) and larval Xenopus laevis (Straznicky et al., 1971; Beazley, 1975; Feldman et al., 1975; Sharma and Hollyfield, 1980). Closely related is the experiment of Sharma (1973) in which one eye and the ipsilateral tectum were ablated. The remaining eye formed a projection of the "correct" polarity to its ipsilateral tectum. Thus nasal retinal fibres connected to caudal tectum and dorsal fibres medially but in the ipsilateral and not the normal contralateral tectum. Similarly the few fibres seen to project ipsilaterally after unilateral enucleation and optic nerve regeneration in the goldfish did so with a polarity appropriate to the ipsilateral tectum (Springer et al., 1977).

Alternatively the tectum, rather than the eye, may be rotated. When performed on a virgin (never previously innervated) tectum in a metamorphic Xenopus the resulting projection was orientated according to the original tectal axes (Straznicky, 1978). Earlier neural tube rotations confirmed an influence of tectal orientation on the orientation of the map though they suggested that the tectal axes were a consequence of the tectal relationship to the diencephalon during development rather than its relationship to the body axes (Chung and Cooke, 1978).

The above results certainly demonstrate that the polarity of the retinotectal projection is determined by axes set up in the retina at some, as yet unclear, point in development and by a mechanism not yet understood. Since each eye was projecting alone to one tectum in most cases (though see Beazley, 1975) there is no complication introduced into the interpretation of the results by interactions between two projections, as happens in experiments to be described below. However in none of these experiments was the precise arrangement of fibres within the pathway clear. It is thus possible that the reorganization of fibre arrangement necessitated by the manipulations might be achieved outside the tectum.

Two classes of experiments do however suggest that polarity control exists within the tectum for the fibres are known to be normally arranged until they are confronted with inappropriately orientated tectum. These involve either rotated tectal grafts or the ipsilateral diversion of fibres taken from the contralateral optic tract or tectum. Since Gaze et al. (1966) demonstrated that tectal tissue from an adult goldfish could survive excision and reimplantation and still support a retinal projection the technique has been extensively utilized by a number of workers to investigate tectal polarity. Such grafts were rotated through 90° or 180° so altering both tectal axes, or reimplanted upside down, with or without rotation so altering one axis.

In each case electrophysiological mapping showed the graft to bear a retinal projection orientated, in the vast majority of cases, according to the original axes of the graft while the surrounding tectal tissue carried a normal projection. In both goldfish (Sharma and Gaze, 1971; Yoon, 1972a, 1973, 1975b) and Xenopus laevis (Levine and Jacobson, 1974) pieces of tectal tissue as small as 10-15% of the total were able to achieve reorientation of their projection. This control of polarity by a small graft was particularly striking in the case of inverted grafts which, despite a severe derangement of the graft laminar structure, were able to cause reversal of one axis of their projection independent of the other (Yoon, 1975b). Entry of fibres into such tectal grafts seemed to be in the normal rostral to caudal (in terms of the body axes) direction as responses persisted within the graft when medial, lateral and caudal edges were cut through (Yoon, 1975b). Fibres should therefore have been arriving at the graft in their normal topographic arrangement and thus needed to undergo considerable reordering to produce the observed rotated map. How this was achieved is not clear though in mammalian tectal grafts fibres have been seen to follow extremely circuitous routes before terminating, perhaps exploring the graft (Lund and Hauschka, 1976). It is not clear whether the termination sites eventually adopted by these fibres were correctly ordered.

The second experimental stratagem designed to induce polarity changes in a group of fibres known to have an internal ordering appropriate to the production of a normal map was to take fibres from one optic tract or tectum and divert them into the other tectum. Such a fascicle deviation in the goldfish gave an ipsilateral map showing extremely poor ordering though with a polarity crudely appropriate to the previously denervated host tectum in the experiments of Meyer (1978b). Other workers have employed a similar operation though diverting fibres into an innervated host tectum.

In these latter cases, the likelihood of interaction between the two fibre populations makes the identification of tectal polarity cues impossible (Cronly-Dillon and Glazner, 1974; Levine and Jacobson, 1975; Meyer, 1979a, 1979b).

Similar problems have arisen in the interpretation of experiments designed to detect polarity cues in the mammalian superior colliculus. Removal of one eye at around the time of birth caused the remaining eye to send an increased component of fibres into the almost emptied ipsilateral tectum, probably by increased branch formation at the chiasm (Cunningham and Speas, 1975; Cunningham, 1976; Cunningham and Seagraves, 1976). The resulting map seemed to have two components, one resembling the normal ipsilateral map, and a more extensive one from most of the retina, whose polarity has not been agreed upon (Cunningham and Speas, 1976; Lund and Lund, 1976; Thompson, 1979).

The second stratagem employed in mammals has involved removing part or all of one superior colliculus so causing fibres to recross the midline into the remaining tectum. Again the arrangement of fibres was unclear though they were known to cross directly into the ipsilateral tectum rather than going back round the chiasm (Schneider, 1971). Such projections were found to be a mirror image of the normal contralateral projection rather than showing the normal ipsilateral orientation (Miller and Lund, 1975; Schneider et al., 1975; Finlay et al., 1979). Again possible interactions between the fibre populations made interpretation of these results very difficult.

Thus the clearest evidence for the presence of polarity cues within the tectum comes from the tectal graft rotation experiments and from the crude polarity of an ipsilateral projection induced in a denervated tectum (Meyer, 1978b). Such polarity might however be the consequence of precise place matching between retinal ganglion cell axons and tectum rather than of a more general polarity-defining mechanism. That polarity can be controlled independent of place

matching has been shown by the ability of rotated grafts to reorientate a projection derived from inappropriate optic fibres. This was first demonstrated by Yoon (1977) who obtained compression on to a rostral tectal remnant containing a 180° rotated graft. Fibres which compressed on to that graft followed its polarity. It was unclear whether the new fibres innervating the graft after compression were aligning themselves with the polarity of the graft itself or with fibres already in the graft. More convincingly Martin (1978) obtained polarity control by a graft in a rostral half tectum innervated only by inappropriate nasal fibres, having removed temporal retina. However it remains possible that deflection of fibres by the edges of the graft played some role in the production of such rotated maps for at least some fibres were found to enter the graft at its caudal edge.

2. Evidence for place-defining cues within the tectum

In order to detect place-defining labels within the tectum over and above any general polarity-defining mechanism indicated above, it is necessary to show that optic fibres will terminate preferentially in the appropriate place when a choice of termination site is available. The first approach adopted in order to seek such position labels was that of allowing a small group of fibres to innervate an empty tectum. Attardi and Sperry (1963) used silver staining to show that fibres from a part of the retina projected exclusively to the correct tectal region, growing over empty tectum to do so. Subsequently Bunt and co-workers (Bunt et al., 1978) have detected fibre terminations, using electrophysiological mapping within abnormal regions of the tectum though there remained a preference of fibres for the correct area. Similarly on deflecting a small group of fibres into an empty tectum Meyer detected a preference, though not an exclusive one, of fibres for the correct tectal area (1978b).

An alternative approach has been to exchange, without rotation, grafts between different tectal areas. Such translocated grafts have been found to vary in their innervation. In one series, two out of four animals were found to have appropriately translocated map regions whereas the remainder had normal maps

(Hope et al., 1976). In an extensive series Martin (1978a, 1978b) found that grafts could select out the appropriate fibres but only if the graft lay within the normal route of ingrowth of the fibres. Thus ventrolateral fragments, placed dorsally, became innervated by fibres appropriate to the surrounding dorsal tectum, correct fibres never having access to the graft. However caudal grafts placed rostrally could, having the opportunity to "sample" the majority of incoming fibres, select out their correct fibres. Rostral fragments placed caudally rarely received the correct fibres which seemed to have been deflected away by the more rostrally-placed implant.

The concept of tectal place labels is also supported by the work of Jacobson and Levine (1975a, 1975b) in which large, non-corresponding grafts were exchanged between left and right tecta in Rana catesbiana. In half the animals not only was the polarity of the projection appropriately reorientated within the graft but also some retinal regions were found to project to both the graft and to the normal position in the host tectum. A similar result was obtained in compound double-rostral or double-caudal tecta created in goldfish (Sharma, 1975) though whereas the reduplicated projection was found to be stable in frog it was later overridden in these goldfish and replaced by a normal map extending over the entire compound tectum. Further support for crude tectal place labelling came from the finding that optic fibres deprived of a termination site by partial tectal ablation would only pass ipsilaterally in significant numbers on to a half tectum, rather than compressing on to the remaining contralateral remnant, if the ipsilateral remnant was appropriate for the displaced fibres (Sharma, 1980).

However in all these cases the required level of tectal differentiation is low, probably no better than rostral versus caudal and medial versus lateral.

Possibly as important in determining the termination site of optic fibres is the area of tectum to which they have access when following the normal pattern in ingrowth. There is certainly no evidence for the precision of tectal locus definition proposed by classical chemospecificity.

3. Tectal polarity and place-labelling cues may be over-ridden

The polarity and place labels described above certainly do not exert absolute control over the formation of the retinotectal projection. A number of cases have been described in which they have apparently been overridden. The first of these is the projection to rotated grafts. In a number of animals the projection to the graft-bearing tectum was normal with no sign of any effect of graft rotation (Levine and Jacobson, 1974; Jacobson and Levine, 1975a, 1975b). This may have occurred because the graft was lost and the gap healed by remaining tectum or it may represent an interaction between fibres going to the normal tectum and fibres going to the graft such that the polarity of the former overrode that of the latter and forced fibres to the graft to form part of a normal map. Furthermore in some induced ipsilateral projections the map polarity has been found to be that of the normal contralateral projection rather than reorientated to fit the host tectum (Bunt et al, 1978). Thus polarity cues in the host tectum were ignored, possibly due to an overwhelming control by the incoming fibre arrangement. Similar disregard for tectal cues was seen in the reversed maps seen by Martin when a retinal fragment was forced to project to a mainly incorrect tectal fragment (Martin, 1978b).

Tectal position cues are obviously disregarded in cases of compression and expansion but even more dramatically in those cases where two projections from one or two eyes were found to overlies each other on a single tectum (Gaze and Sharma, 1970; Sharma, 1972c; Meyer, 1977, 1979b; Marotte et al, 1977; Martin, 1978b; Sharma and Tung, 1979).

Conclusion

Extensive experimental investigation has thus shown the original rigid chemospecificity hypothesis of Sperry (1963) and its subsequent variants involving regulation or modulation to be untenable. A wealth of examples exists for the formation of terminals by retinal ganglion cells in inappropriate tectal areas. As an alternative, therefore, morphogenetic forces have been suggested to play a large part in the formation of the normal retinotectal projection and the normal neuromuscular connections in both development and regeneration. Great emphasis was placed on the orderly arrangement of nerve fibres within the optic nerve and in peripheral nerve trunks (Horder and Martin, 1978).

Evidence for the existence of such order in the normal animal has been extensively reviewed in the first part of this literature review. It seems likely that neither simple regeneration of the optic nerve nor the group of size disparity experiments would cause much disturbance of such order and so these do not provide a clear test of its importance. Only by investigating the effects of more extensive disruptions of the pathway will the ability of morphogenetics to explain the formation of specific nervous connections be made clear.

A search for guidance cues for optic fibres on the way to the tectum has revealed little except a preference at the optic tract division for a particular route depending on retinal origin and there is evidence that this choice is not made with complete accuracy. Within the tectum itself evidence purporting to demonstrate the existence of polarity- and place-defining cues has been considered. With regard to polarity many experimental stratagems involve production of an ipsilateral projection. The point in the pathway at which polarity is controlled is unclear. Only rotated grafts and ipsilateral diversion of tectal fibre fascicles suggest strongly that

polarity cues reside within the tectum. Similarly the search for place labels has been hampered by a lack of knowledge of fibre arrangement in the pathway; and where, as in the case of translocated grafts, it can be predicted with some certainty, it has been found to be important in influencing the innervation of the graft. Moreover numerous experiments have shown that both polarity and place cues can be overridden.

Thus there is considerable doubt as to the possible contribution which fibre ordering, produced by the action of morphogenetic forces such as contact guidance during development, may make to the final retinotectal map. Nor has there been any extensive investigation of fibre pathways after experimental manipulation in order to determine at which point(s) in the pathway polarity and position are controlled. The nature of polarity and place cues and the role of these and any interactions between and within groups of fibres remain ill understood.

Chapter 2

2. MATERIALS AND METHODS

Experimental animals and their maintenance

Fish

Common goldfish (Carassius auratus) were obtained from an importer (L. Cura and Sons, Hemel Hempstead). In order to minimize the effects of size on the rate and nature of recovery from surgical manipulation, size limits of 50-65 mm (snout to base of tail fin) were adopted for the experimental series. Larger fish were used to develop techniques and smaller fish were maintained until they reached the required size.

On arrival, healthy fish were transferred directly to 30 or 45 litre tanks containing filtered, aerated tap water. The fish density never exceeded 1 fish/2 litres water. Sick fish were placed in a salt bath (5 g sodium chloride/litre tap water) for twelve hours before transfer into fresh tap water as this was observed to increase survival. Obviously unhealthy fish were not used in the experimental series. Fish were then kept at room temperature, 18-22°C according to season. At such a temperature optic nerve regeneration can restore a complete visual projection within 34 days (Horder, 1971). The normal light/dark cycle of the laboratory was not modified. Fish were fed daily with "Tetramin" fish flakes which provide a low residue complete diet.

After surgery fish were kept singly or in pairs in 5 litre polyethylene tanks for at least 2 weeks before transfer to the larger tanks. The small tanks were not filtered or aerated but the water was changed when necessary, usually every fortnight. Food was withheld for 3 days after surgery to reduce infection.

Evaporative losses from all tanks were made good with distilled water to avoid increasing the ionic concentration of the water.

Each operative batch of fish was kept in a separate tank to avoid identification problems. When individual fish needed to be identified, for example to allow comparison of the retinotectal maps obtained at different times, they were kept singly in the small 5 litre polyethylene tanks.

Mice

Adult common brown housemice (Mus musculus - a gift from Dr. H.M. Charlton) of both sexes weighing between 20 and 35 g were used in this study. They were kept on sawdust in white polypropylene cages in groups of 2-6 and given water and food (Lab Chow) ad libitum. Individuals were distinguished by marking their tails with a felt-tipped marker pen. The mice were kept in the normal laboratory light/dark cycle at room temperature, usually 18-22°C, except after surgery when a lamp was placed over the cage to prevent hypothermia.

Frogs

Small frogs (Rana temporaria) with a snout to rump length of 5-10 cm were obtained from a local supplier (Gerrard Ltd.). They were kept in groups of 4-10 in 20 litre polyethylene tanks which were tilted at 10-15° to allow a small pool of tapwater to be maintained at one end. The tanks were kept at room temperature, 18-22°C, in the normal laboratory light/dark cycle and were cleaned weekly. Frogs were fed by hand every 3 days with small pieces of ox heart.

Voles

Fresh cadavers of adult voles (Microtus agrestis) weighing 20-50g and captured in Wytham Woods, Oxfordshire, were obtained from Dr. H.M. Charlton.

General Surgical Techniques

In all cases surgery and dissection were performed using a binocular dissecting microscope with focussing by foot control (Carl Zeiss). Clean instruments, washed in 70% ethanol, were used throughout though strict aseptic precautions were never deemed necessary.

Fish

1. Anaesthesia.

Fish were anaesthetized for all surgical procedures by immersion in a 1:1000 solution of tricaine methane sulphonate (MS222. Sandoz) in tapwater until all gill movements had ceased. Each fish was then wrapped in damp tissue paper and placed on a Perspex holder where it was secured with "Plasticine". Fresh water was passed over the animal's gills via a tube in its mouth at a rate of 100-500 ml/min and drained away from the base of the holder, which was designed to stand on a bench during surgery or to be mounted at the centre of a perimeter during electrophysiological mapping. Anaesthesia would last for 10-20 minutes, sufficient time for any of the operations performed in this study to be completed. After surgery the fish was placed in a small tank of fresh water and observed until respiratory and swimming movements returned which was usually within ten minutes. No fish failed to recover from anaesthesia though some (between 5 and 80% depending on the nature of the operation and the health of the fish before surgery) died during the first post-operative weeks. It was not possible to ascertain the cause of most of these deaths.

2. Exposure of the optic nerve: optic nerve section

The anaesthetized fish was mounted on the holder at an angle of about 20° dorsal-up from the horizontal. The dorsal conjunctiva was torn using sharpened watch-makers forceps and the eye pulled forward. After removing fluid and fat using fine tissue swabs the entire intra-orbital extent of the optic nerve could be visualized.

Nerve section was performed with fine iridectomy scissors at a point about halfway between the eye and the orbital wall avoiding the large blood vessels which join the nerve at its temporal side. The nerve ends were then parted to confirm the extent of the lesion, then reapposed and the eye pushed gently back into place. The conjunctiva healed well within a few days without need for sutures.

3. Exposure of the optic tecta

The tecta were exposed by cutting along the suture lines of the overlying skull using a sharp scalpel. By undercutting the skull a hexagonal plate with bevelled edges was removed which could later be replaced securely without need for adhesives. Fat deposits and fluid were then removed using swabs so exposing the tecta.

4. Tectal surgery

Details of the tectal surgery performed on the various experimental series are given in the relevant chapters.

Mice

1. Anaesthesia

All operations were carried out under chloral hydrate anaesthesia, 0.2ml of a 3.5% solution in distilled water per 10 g body weight being injected intraperitoneally. Premedication with 0.2-0.3ml of a 10 μ g/ml solution of atropine in distilled water was found to alleviate respiratory distress. A single injection of anaesthetic consistently gave 2-3 hours of deep anaesthesia and the mouse remained sedated for several hours more.

During recovery from surgery hypothermia proved the major cause of death. To prevent this the animals were kept under a lamp for at least 12 hours.

2. Exposure of the optic nerve

The anaesthetized animal was placed on its side with its head supported and immobilized by Plasticine. Its whiskers were clipped and a dorsal incision made in the upper eyelid and supraorbital skin. The edges of the incision were held apart using Spencer-Wells forceps and the conjunctiva carefully incised with iridectomy scissors. The extraocular muscles and extensive veins were then cautiously parted along a mid-dorsal line and wedged apart with fine tissue swabs. It was usually possible to expose the dorsal aspect of the optic nerve without causing significant bleeding though rupture of the orbital vessels, if it occurred, gave torrential bleeding which usually necessitated sacrifice of the animal. When surgery was complete the extraocular muscles were eased gently back into place and the skin incision closed with one or two fine silk sutures.

3. Exposure of the optic tecta

The anaesthetized animal was placed prone with its head supported by Plasticine. The skin over the cranium was shaved and cleaned with a 1% solution of Hibitane (ICI) in 70% ethanol. A mid-sagittal incision was then made in the skin using a sharp scalpel, and the two edges retracted. A small rectangle of bone, lying lateral to the sagittal suture and between the lambda and bregma suture points was removed using a dental drill and bone cutters. Care was taken to avoid the major intradural venous sinuses. The meninges were then incised and a small area of cortex removed using suction until the superior colliculus was visible. Haemostasis was achieved by plugging bleeding points with gelatine foam (Sterispon - Allen and Hanbury). After surgery the skin was closed with a number of silk sutures. The wound was then sealed with a plastic spray-on dressing (Nobecutane - Astra Chemicals Ltd.) to prevent infection.

Frogs

1. Anaesthesia

Anaesthesia was achieved by immersion in a 1:100 solution of MS222 in water.

It was maintained for up to 24 hours by keeping the animal in a fridge at 4°C. During all periods of anaesthesia the animal was wrapped in moist tissue paper to prevent dehydration and facilitate respiration through the skin.

Electrophysiological Mapping of Goldfish

Preparation

The fish was anaesthetized with a 1:1000 solution of MS222, wrapped in damp tissue paper and secured to its holder with Plasticine. Throughout the mapping session (3-7 hours) the fish was perfused via the mouth with a 1:8000 solution of MS222 in tap water at a rate of 50-100 ml/min. One litre of anaesthetic solution sufficed for each fish, being recycled using a peristaltic pump (Shuco Ltd.) and aerated.

The tecta were exposed and the tectum to be mapped was photographed in monochrome using a Polaroid camera attached to a dissecting microscope which had been fitted with a green filter to enhance the contrast of the blood vessels. An orthogonal grid of lines was then drawn on the photograph aligned with the midline. The grid intersections corresponded to tectal spacing of 250 μm and were used to define the mapping positions by reference to nearby blood vessels.

The tecta were then dried and covered with liquid paraffin (B.P.) to provide electrical insulation. Where both eyes projected to one tectum the eye not being mapped was covered with a black plasticine occluder held in place with a paste of carbon powder in Vaseline. This proved a more effective way to reduce activity from that eye than did intra-vitreous and intraorbital injections of local anaesthetic.

Centring the fish

The fish on its holder was mounted at the centre of a modified Aimark visual perimeter of diameter 66 cm. The fish was transilluminated and the optic nerve head was aligned with the axis of the perimeter by viewing the unpigmented nerve head through a sighting tube mounted along the perimeters major axis. The optical axis of the eye then lay at the coordinates $13-18^\circ$ radial, $60-75^\circ$ circumferential (Easter et al., 1977) so that maps obtained with this centring showed a much greater proportion of temporal than nasal visual field represented on the accessible dorsal tectum. The rotation of the eye was measured as the angle between a line joining the nasal and temporal marks, seen always to occur on the iris, and the horizontal. Visual field response positions were then corrected for this rotation, the magnitude of which was monitored during and after mapping to ensure the fish had not moved. Centring of the eye was checked after mapping for the same reason.

Electrodes

Electrodes were of tungsten wire insulated with glass by the method of Merrill and Ainsworth (1972) leaving a conical tip of length $100-120\mu\text{m}$ exposed. The electrodes had an impedance of $50-250\text{K}\Omega$ measured at 1000 Hz with the electrode at a recording site within the tectum.

Electronics

The electrode was mounted in an active guard of 26 SWG stainless steel tubing in order to screen it from mains interference without increasing its input capacitance. The electrode was connected directly to a JFET input low noise operational amplifier (National Semiconductors LF 356) configured for a voltage gain of 10 throughout the audiobandwidth. This unit had an input resistance of $10\Omega\text{M}$, a total input capacitance (including electrode) measured as 10pF , and was mounted directly on the micromanipulator. With the electrodes used sensitivity was limited only by their impedance, amplifier noise being negligible. (Equivalent input noise voltage, input shorted, over the 1-10 KHz

bandwidth was $1.1\mu\text{Vrms}$. Thermal noise at 20°C for an electrode was:
 impedance $50\text{K}\Omega = 2.7\mu\text{V}$, $100\text{K}\Omega = 3.8\mu\text{V}$, $250\text{K}\Omega = 6.0\mu\text{V}$.

The signal was then passed via a low noise coaxial lead to a preamplifier and filter unit giving a maximum voltage gain of 1000 and including a 50 Hz notch filter and a high pass filter (slope = 12dB/octave: roll off = 300Hz). It then passed to a conventional audio power amplifier and a wide band speaker.

The fish was connected to a common earth using a short length of insulated silver wire, the free end of which was inserted into the wet tissue around the fish.

Responses and Fields

Mapping was carried out in a darkened room by one operator and one observer, who checked all dubious or unexpected field positions. The fish was mounted in the holder, centred, and the eye rotation measured. The electrode was then positioned using the photo-grid and lowered slowly until the tip just penetrated the tectum - this was indicated by a change in the character of the noise generated by the equipment. Tip depth was then adjusted to give maximum response strength, responses almost certainly representing the action potentials in the terminal arborization of several optic fibres (see Appendix 1).

Stimulus positions giving the best response were then found by exploring the visual field either with a white disc subtending 7° of visual angle at 33 cm and illuminated by a light placed on the far side of the fish as it was moved against the grey background of the perimeter arms, or with a flashing stimulus. This latter consisted of an array of 12 light emitting diodes (TIL 223. Texas Instruments Ltd.) subtending a total of 4° of visual angle at 33 cm and flashing at 5Hz with a square waveform and a peak current of 30 mA/diode. The LED array was employed in a dark room and facilitated localization of receptive fields where the response was very weak as there

was no confusion with shadows generated by the equipment or operator. However the LED array did occasionally generate a high degree of background activity in other parts of the retina than that giving the main response. Many fields were located using both methods and close agreements between the positioning was found.

In either case the centre of the receptive field was located by moving the stimulus back and forth across the field in several directions until the position of loudest response was found. Field size was similarly assessed by noting the distance from the field centre in several directions at which the response became detectable above background noise.

Though the majority of responses seemed to reflect activity in a large number of optic fibres single units were occasionally detected, particularly in sparsely innervated tecta and when using the LED stimulus.

Measurements

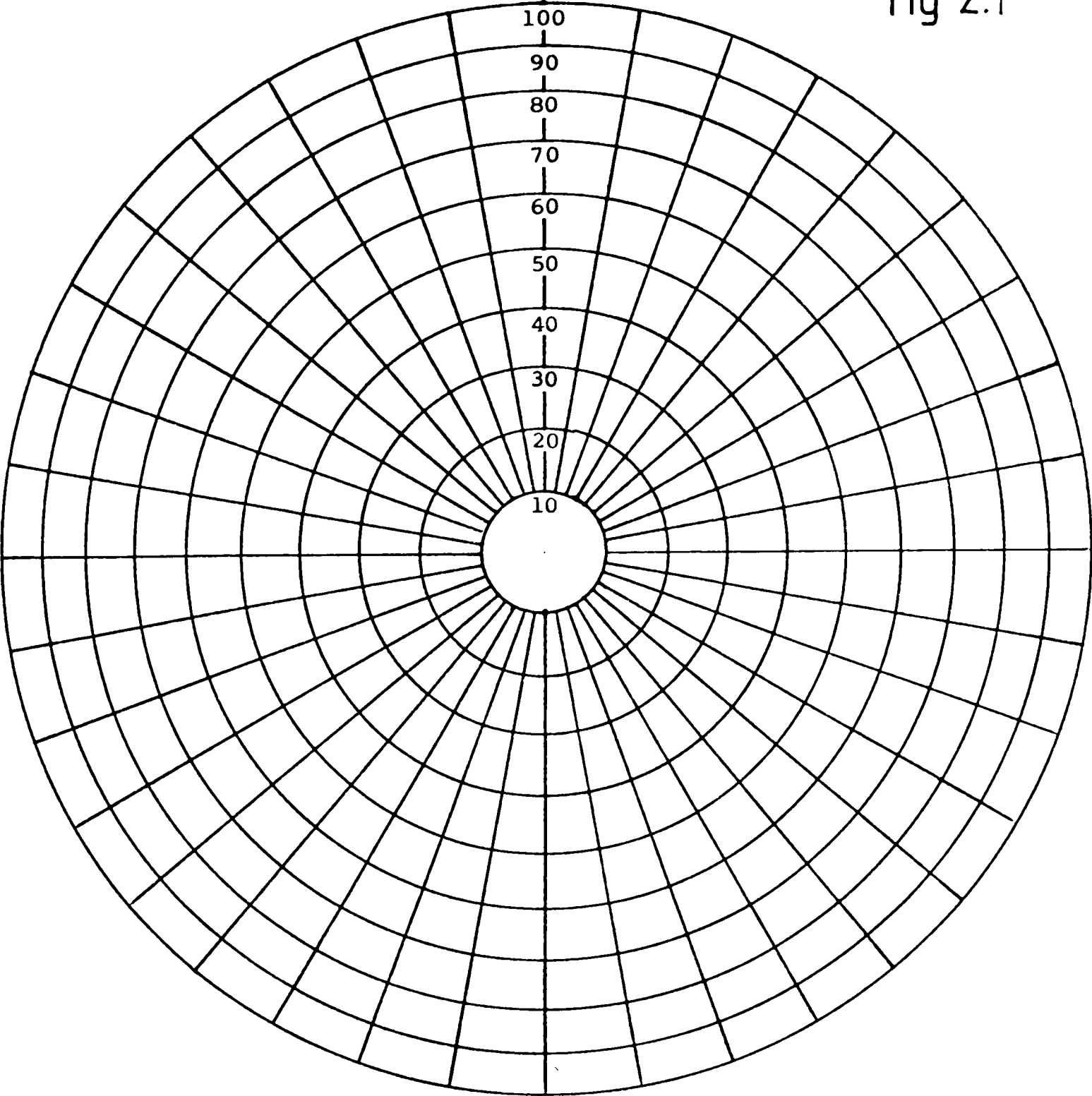
Field centre positions were expressed in terms of the two polar coordinates as determined using the Aimark perimeter. One was radial, the other the angle between the horizontal, nasal to the fish eye, and that perimeter arc passing through the field centre. At each electrode position these two field coordinates were noted as was a subjective assessment of the field size, response strength and single/multi unit nature.

Analysis

After correction for eye rotation the positions of the visual field centre were plotted on a perimetric chart extending out to 100° . On this chart the radial distance of a point (representing a visual field centre) from the centre (representing the optic nerve head axis) was proportional to the sine of half the visual angle between the field position and the optic nerve head axis (Cook, 1979). Thus any chart area was proportional to the solid angle of vision.

Fig. 2.1. The chart used to plot the visuotectal projection detected electrophysiologically. The radial distance of a point from the centre is proportional to the sine of half the visual angle between the field position represented by the point, and the optic nerve head.

fig 2.1



An example of the chart used is shown in fig. 2.1. However the general appearance of a projection map using this chart was little different from that using a chart on which the radial distance was directly proportional to the visual angle between the field position and the optic nerve head axis (for example as used by Jacobson and Gaze, 1964.)

Limitations of eye-in-air mapping

When the goldfish eye is underwater the cornea is optically inactive but in air it becomes a highly refractive surface and renders the eye myopic. Furthermore the cornea is not spherical but is irregular and astigmatic, being flattened towards the edges (Meyer, 1977). This causes several problems in the construction of an accurate retinotectal map if the animal is mapped with the eye in air.

Firstly, the high degree of myopia causes enlargement of apparent receptive field sizes. The exact extent of this enlargement is unclear for though Charman and Tucker (1972) calculated that a distant point source would appear to subtend 13° when mapped in air, Cronly-Dillon (1964) measured receptive field sizes of $5-8^{\circ}$. To some extent the size of the field detected will depend on the noise of the apparatus for a field is only identifiable when the activity induced in the terminals can be heard above that noise. The greater the size of the field the more room there is for error in determining the position of its centre.

More important, the optical activity of the cornea leads to a displacement of the field centre away from the optic axis. Assuming corneal sphericity Charman and Tucker (1972) estimated that as 5° maximum but of course the situation is greatly complicated by the non-spherical corneal shape, and Meyer (1977) found, by mapping the same fish at the same electrode positions in both air and water, displacements of up to 25° . This displacement increased

with increasing distance from the optic axis, though probably not in a simple proportional manner and furthermore was greater in the naso-temporal than the superio-inferior axis.

Distortions imposed by the cornea also limit the extent of the field which can be mapped in air. In water the visual field subtends $99 \pm 2.5^\circ$ from the optic axis (Charman and Tucker, 1972), though in air it subtends only $10-15^\circ$ more than this, rather than the extra 25° of the field position shift. Thus there exists in air a $10-15^\circ$ wide annulus of peripheral retina which cannot be stimulated. In water the whole field can be mapped (Meyer, 1977).

A further error in the mapping procedure arises with the use of planar coordinates for determining electrode positions on a curved tectal surface. Particularly at the downcurving anterior tectal edge electrode spacings are unequal. However in this series of experiments the extreme edges of dorsal tectum were not usually mapped as peripheral field positions were difficult to determine accurately (see Appendix II). The main concern was the determination of the polarity and ordering of the retinotectal projection so that small non-linearities in the map did not usually pose any great problem of interpretation.

Histological Methods

Horseradish peroxidase (HRP)

HRP is an enzyme of molecular weight 40,000 which has been shown to be transported both retrogradely (Kristensson and Olssen, 1971; LaVail and LaVail, 1972) and anterogradely (Colman et al, 1976) by neurons. Extremely low concentrations of HRP can be detected by making use of its enzymic activity to oxidize a chromagen to a coloured reaction product using hydrogen peroxide as an oxygen donor. Moreover a number of workers report that, at least within the central nervous system, intact fibres, and particularly intact myelinated

fibres, do not take up a significant amount of HRP (LaVail et al., 1973; Lynch et al., 1974; Nauta et al., 1974; Turner and Harris, 1974). Thus HRP has proved an extremely valuable tool in the study of neuronal connections as it appears to avoid the problem of distinguishing fibres en passage through an area from those terminating there. However in peripheral nerves there is some evidence of uptake of HRP by intact fibres so that some caution is required in the interpretation of results (Krishnan and Singer, 1973).

1. Mode of administration of HRP

There is evidence that some isozymes of HRP are taken up and transported along the axon more readily than others (Bunt et al., 1976). However the isozymal composition of the commercially available HRP used in this study (type VI, Sigma and grade I - Boehringer) is not specified by the manufacturer. In both cases the HRP was obtained as a lyophilized powder of activity about 250U/mg and was injected as a 30% solution in either 0.1M phosphate buffer at pH 7.4 alone or the same buffer with 0.5% dimethyl sulphoxide.

2. Survival times.

A survival time of 18-48 hours was allowed between administration of HRP and sacrifice of the animal. LaVail and LaVail (1972) have suggested a retrograde transport rate of 72mm/day within the chick optic nerve and in these studies a maximum transport distance of 15 mm was required. Attempts to improve labelling quality and distance by increasing the survival time were not successful.

3. Fixation and preparation.

In accordance with the findings of Avramcas (1973) and Malmgren and Olsson (1978) paraformaldehyde was avoided and all tissue fixation was performed using a 2%

solution of gluteraldehyde in phosphate buffer (0.1M at pH 7.4). The mode of fixation and preparation varied according to the type of tissue.

Goldfish and mouse retinae for wholemounts: the eyes were removed from the anaesthetized animal by cutting through the conjunctiva and extraocular muscles and severing the optic nerve as close to the eye as possible. In each case the ventral edge of the cornea was marked with a small cut before enucleation. The eyes were placed in individual dishes of 0.9% saline solution, the cornea incised and a cut made at the ventral mark from the retinal margin to the optic nerve head to define the orientation of the wholemount subsequently. The retina was then dissected free and several small cuts made around the margin to facilitate flattening between filter paper and a coverslip, vitreal surface against the glass. This retinal sandwich was then placed in 2% gluteraldehyde solution in phosphate buffer and weighted with a 10g weight. After 30 minutes fixation the retina could be gently prised free from the paper and glass as a flat disc, washed in phosphate buffer and processed. In the case of goldfish retinae the dark pigment was removed from the scleral surface of the retinae prior to fixation - this was facilitated by dark adapting the animal for 30 minutes prior to anaesthetization.

Goldfish tecta for wholemounts: the anaesthetized fish was perfused through the heart with 5 ml of 0.9% saline at 4°C to remove as much blood as possible. The tecta were then dissected out and placed in 2% gluteraldehyde in phosphate buffer and left for 30 minutes. The underlying tissues and the pial covering were carefully removed using iridectomy scissors and fine watchmakers' forceps and the tecta left for a further 30 minutes fixation.

Goldfish and mouse brains for sectioning: the anaesthetized animals were perfused through the heart with 0.9% saline and then 2% gluteraldehyde in phosphate buffer, both at 4°C (5ml of each for goldfish, 10-20 ml of each

for mice). The brains were dissected out and fixed in 2% glutaraldehyde in phosphate buffer for a further 2 hours at 4°C. They were sunk overnight at 4°C in a solution of 30% sucrose and 0.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.4. This aids the cutting of frozen sections. Brains were embedded in a melted mixture of 3% gelatine and 30% albumen in distilled water which was cooled to set it. The block was fixed and sunk overnight in the same solution used to sink the brains.

40 μ m thick coronal sections were cut on a sledge microtome, collected from phosphate buffer (0.1M at pH 7.4) on to gelatinized slides, dried slightly and processed. Where, as in the case of some fish, both HRP and ³H-proline had been injected into the same animal alternate 40 μ m frozen sections were taken for HRP processing and for autoradiography.

4. HRP development

The method employed depended on the type of tissue. Goldfish retinae and tecta for wholemounts: this method is extensively modified in this laboratory from the method of Hanker et al., (1977). The tissues are processed as follows:

- | | |
|-------------------------------------|---|
| (1) Wash 4°C | Tris buffer (0.05M at pH 7.6) |
| (2) Pre-soak 4°C 18-24 hr | 1 mg: σ -catechol
0.5 mg ρ -phenylene diamine
/100 ml tris buffer |
| (3) Develop 4°C 2 hr | 100 mg σ -catechol
50 mg ρ -phenylene diamine
4 drops 100 mol hydrogen peroxide
/ 100 ml tris buffer |
| (4) Dehydrate 20°C 1 hr | 70% ethanol |
| 20°C 1 hr | 95% ethanol |
| 20°C 1 hr | 100% ethanol |
| 20°C 1 hr | 100% ethanol |
| (5) Clear 20°C 10-24 hr | methyl salicylate |
| (6) Mount on dimple slides in DePeX | |

Mouse retinae for wholemounts: this method is slightly modified from that of Graham and Karnovsky (1968) with a cobalt chloride presoak as suggested by Adams (1977).

(1)	Wash	20°C		Distilled water
(2)	Presoak	20°C	30 min	Cobalt chloride - 10% in distilled water
(3)	Presoak	20°C	1 hr	50 mg diamino benzidine (DAB) /100 ml tris buffer (0.05M at pH7.6)
(4)	Develop	20°C	1 hr	50 mg DAB and 1 drop 100 vol hydrogen peroxide/100 ml tris buffer
(5)	Dehydrate	20°C	30min	70% ethanol
		20°C	30min	95% ethanol
		20°C	30 min	100% ethanol
		20°C	30 min	100% ethanol
(6)	Clear	20°C	6 hours	methyl salicylate
(7)	Mount			DePeX

Sectioned mouse and goldfish material: originally a method modified from that of Colman, Scalia and Cabrales (1976) was employed with a cobalt chloride presoak as per Adams (1977).

(1)	Wash	4°C		Distilled water
(2)	Presoak	4°C	20 min	Cobalt chloride (10% in distilled water)
(3)	Presoak	4°C	20 min	40 mg σ -dianisidine/100 ml distilled water
(4)	Develop	4°C	20 min	40 mg σ -dianisidine 90 mg sodium nitroprusside 4 drops 100 vol hydrogen peroxide /100 ml distilled water
(5)	Wash	20°C		Distilled water
(6)	Dehydrate	20°C	2 min	70% ethanol
		20°C	2 min	95% ethanol
		20°C	2 min	100% ethanol
		20°C	2 min	100% ethanol
(7)	Clear	20°C	2 min	Xylene
(8)	Mount using DePeX			

Later work utilized the method of Mesulam (1978) which employs a non-carcinogenic chromagen 3,3', 5,5', tetra methyl benzidine (TMB). The only modification was to increase the presoak, development and stabilization times to 30 minutes each. Sections were then dehydrated, cleared for 5 minutes in methylsalicylate and mounted with DePeX. No fading problems were encountered with this method and the staining was of a less granular nature than with the DAB method.

All these developing methods were subject to the problem of a high background level of staining due to the action of endogeneous peroxidase in red blood cells and capillary endothelia. Though inconvenient in the case of tectal wholemounts, as it increased the difficulty of transilluminating the thick preparations, the stained fibres could easily be distinguished on morphological grounds from the blood vessels.

Autoradiography

The take-up of amino acids by nerve terminals, their incorporation into proteins and their transport along the axon by an active process has been extensively employed by neurobiologists in the study of neuronal connections. By introducing radioactively labelled amino acids, leaving the animal for a time, and then studying the distribution of the radioactivity by making use of its ability to expose a photographic emulsion, fibres can be traced over considerable distances. In a series of experiments (reviewed in Grafstein, 1977) the rate of this transport process has been studied and found to have at least two components. One is very rapid, about 50mm/day, and the other much slower, 0.5mm/day in goldfish optic nerve. The fast component seems to be largely in the form of particles and penetrates the axonal terminals well while the majority of the slow component is composed of soluble protein and is largely retained in the axon trunk. This differential distribution of the two transport components has been confirmed by Landreth et al (1975) who also showed that the fibre pathways outlined with this autoradiographic method agree well with those revealed by degeneration studies.

It was therefore decided to employ autoradiography in two ways to look at the goldfish retinotectal system. In both cases the label was introduced into ~~the eye and~~ transported back towards the tectum. However in one case a short survival time of 18-30 hours was allowed and the purpose was to look at the distribution of terminals from that eye within the tectum. In the second case the fibre pathways were of interest and a much longer survival time, more than 20d, was allowed to make use of the slow component of axonal transport.

1. Label.

The label employed was L- $[3, 4(n)^3\text{H}]$ -proline of specific activity 41Ci/mmol , at a radioactive concentration of 1m Ci/ml , obtained from the Radiochemical Centre, Amersham. $5-7\mu\text{l}$ of this was introduced into the anterior chamber of the eye in a small puncture hole. Injection was by means of a micropipette connected to a manual microdrive and was performed over a period of about 2 minutes while the animal was anaesthetized.

2. Survival times.

In animals where the distribution of terminals was of interest the survival time was 18-30 hours. Where pathways were of interest at least 20 days survival post-injection were allowed. In the latter animals ARG investigation of the pathway was always combined with HRP tracing of a selective part of the pathway. In the former case ARG was sometimes used alone and sometimes combined with HRP.

3. Fixation and Preparation.

Where HRP was employed in the same animal fixation and preparation was as described for HRP. Alternate $40\mu\text{m}$ frozen sections were taken and stored on gelatinized slides in a dessicator until ready for coating.

In those animals where only ARG was required the animal was anaesthetized and the tecta removed and fixed for at least 24 hours in 10% formal saline. The brains were then dehydrated, embedded in wax and serial 10 μ m sections were cut and mounted on gelatinized glass slides. Before coating with emulsion these were dewaxed through xylol and a descending ethanol series to water.

4. Autoradiographic processing.

Both groups of slides were processed by the same method.

Coating: slides were coated with ARIO stripping film (Kodak) which was floated out on 0.05% potassium bromide solution at 22°C. Slides were then air dried overnight in total darkness and one of each batch was exposed as a control for negative chemography. This was excluded in both normal and experimental animals.

Exposure: slides were sealed in light-tight boxes and exposed for 12 weeks at -20°C.

Development:

Kodak D19 developer	18°C	20 min
Rinse (fresh distilled water)	18°C	30 sec
Johnsons Fixsol diluted 1+9	18°C	10 min
Wash (running tap water)		30 min

Air dried

Staining:

Cresyl violet (0.05% in water)	4 min
Distilled water	2 min
Distilled water	2 min

Air dried

Stored over silica gel overnight

Mounted with hystomount

Cobalt

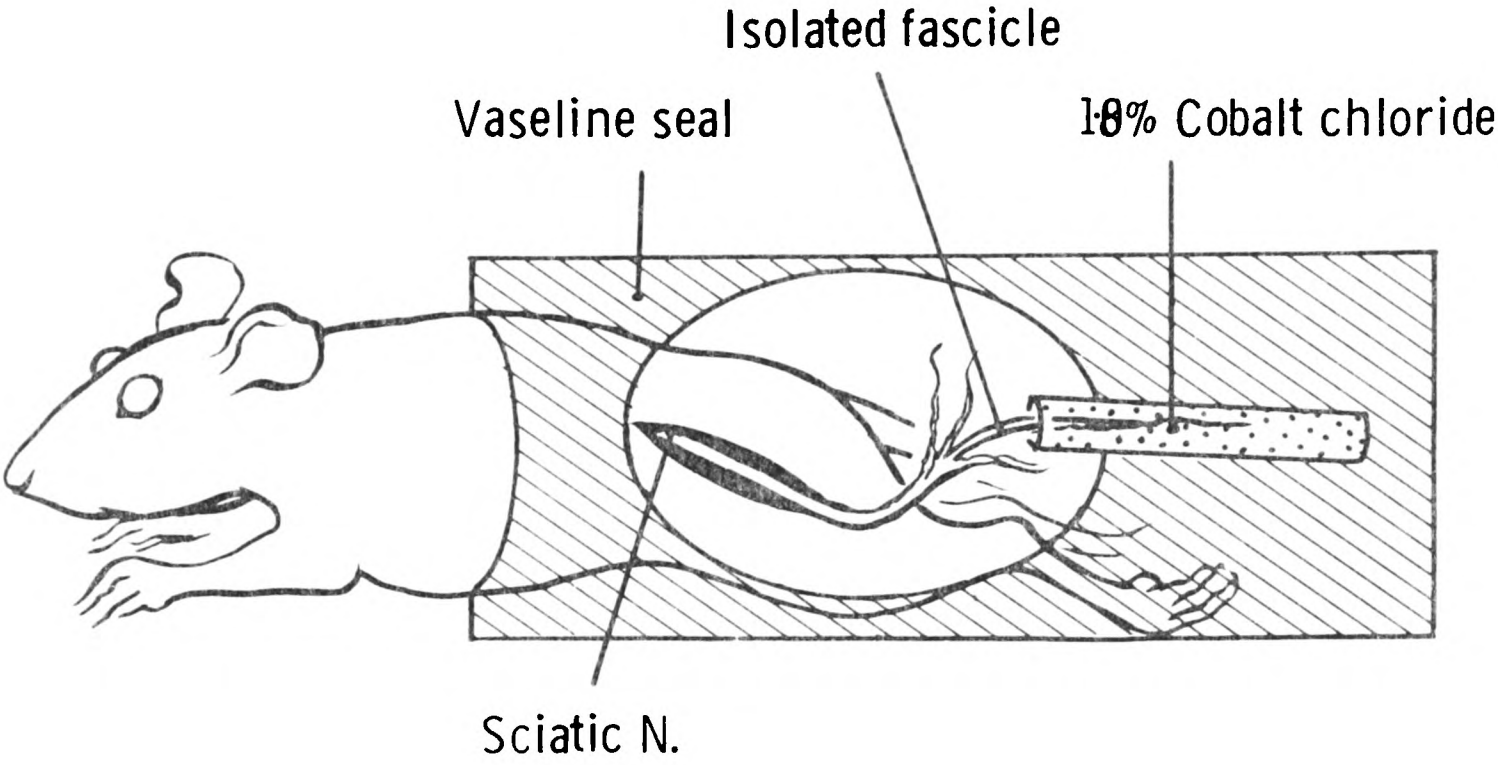
Cobalt salts were first used to study neuronal morphology and connections in insect material (Pitman et al., 1972) though they have subsequently been employed in vertebrates. Cobalt ions, usually as a solution of cobalt chloride in water, are introduced into the cut ends of axons, along which they will travel for up to 25 mm (Szekely and Gallyas, 1975). They can then be detected by precipitating the sulphide of cobalt as a black solid. Use of a silver intensification method has been reported to give improved sensitivity in some cases (Timm, 1958; Bacon and Altman, 1977).

Preparation: in these studies either freshly killed voles, mice and frogs or frog anaesthetized with MS222 and maintained unconscious by cooling to 4°C or mouse tissue fixed for less than 2 hours in 10% formol saline, were used. The nerve to be studied was cut and dissected free for several millimetres. The cut end was then placed in a tiny tube of a 1.8% solution of cobalt chloride in distilled water. Surrounding tissue was covered with Vaseline to protect it from the cobalt solution and to provide support for the tube (see fig. 2.2). The preparation was then left in the fridge for 12-48 hours.

Development: the filled nerve and its major trunks were dissected free and washed well in saline (0.9% sodium chloride in distilled water). The cobalt ions were then precipitated by immersing the nerve in either ammonium sulphide solution (10% W/V hydrogen sulphide) or phosphate buffer (0.1M at pH 7.4) saturated by bubbling with hydrogen sulphide for 30 minutes. A silver intensification method was not employed as it was not found to enhance the quality of the staining in these preparations. Nerves were then dehydrated through a series of alcohols, (10 min in each of 50, 70, 95, 100 and 100%), cleared for at least 2 hours in either methyl salicylate or cedarwood oil, and mounted in DePeX. A tendency for the staining to fade over a period of months was noted and this was not prevented by storage in the dark.

Fig 2.2. This diagram illustrates the method by which cobalt ions were introduced into a small branch of the mouse sciatic nerve.

fig 2.2



Chapter 3

3. Are nerve fibres arranged in an orderly pattern within vertebrate peripheral nerves?

Introduction

As discussed in the literature review, there is considerable evidence that within the vertebrate spinal cord motoneurons supplying a particular muscle are grouped together (for example see Romanes, 1941, 1946, 1964) and that on leaving the cord the axons follow an initially non-crossing course through the adjacent mesenchyme (Ramon y Cajal, 1955, 1960). Do the axons remain together as they travel through the peripheral nerves to their muscle or do they become intermingled with fibres supplying other muscles?

This problem assumes great significance when attempting to determine the role of passive morphogenetic forces in the control of limb innervation (Horder, 1978). However studies on man conducted in the first decades of this century yielded conflicting results, some workers finding an orderly, and others a disorderly, arrangement of nerve fibres. Attempts to resolve the controversy by microdissection of nerve trunks merely confused matters further for they revealed the existence of complex intraneural plexi where fascicles of fibres repeatedly fused and separated. The assumption was made that wherever two fascicles fused their component fibres became completely mixed so that on subsequent division each daughter fascicle contained a sample of the fibres in each parent fascicle (Sunderland, 1945). It was not considered that the fascicles might represent a secondary division of the nerve trunk by connective tissue partitions imposed upon a primary, highly-ordered fibre arrangement. Such a secondary distortion of fibre ordering might also explain the scattered degeneration seen after partial nerve lesions (McKinley, 1921; Kilvington, 1940) for the lesions were not of fibres supplying a particular muscle but merely of fibres which happened to lie together at some point along the nerve trunk.

It was therefore decided to reinvestigate the problem of fibre arrangement in peripheral nerves by using the recently-developed cobalt method (Pitman et al., 1972; Szekely and Gallyas, 1975) to trace fibres from limb muscles back into the major limb nerve trunks in several vertebrate species.

Methods:

These were described in chapter 2. Cobalt was used to trace fibres from the following nerves back into the limb trunks:

Frog	a) foreleg	Radial nerve Ulnar nerve Coracoclavicular nerve
	b) hindleg	Common peroneal nerve Soleus muscle nerve Cruralis posterior nerve Large collateral of the Sciatic nerve Nerve accompanying the lateral circumflex artery of the knee

Vole and Mouse

a) foreleg	Radial nerve Ulnar nerve
b) hindleg	Soleus nerve Common peroneal nerve

Results:

In agreement with Szekely and Gallyas (1975) the distance of travel of cobalt ions detectable within the axon was limited, rarely exceeding 15 mm in these preparations and often substantially less. No difference was observed between the length of staining in freshly killed and anaesthetized material. In both cases the staining was intra-axonal. Fig. 3.1 shows a teased preparation of cobalt filled axons prepared from a freshly killed mouse.

In unfixed preparations showing good intraaxonal staining fibres supplying a particular muscle could be traced 10-12mm into the parent nerve trunk. Whichever nerve branch was followed the fibres were found to pursue a parallel course, remaining together and travelling on that side of the trunk at which

they entered. This is illustrated in Fig. 3.2 which shows a teased mouse sciatic nerve preparation in which cobalt had been introduced into the common peroneal nerve. In no preparation was significant intermingling of fibres observed over the stain length of 10-12mm, a distance forming a substantial part of the pathway between limb musculature and cord in the small vertebrates studied.

Such a parallel organization of fibres in peripheral nerves is also supported by the appearance of extracellularly-stained preparations obtained from lightly fixed material. In these preparations axons can be seen to maintain their nearest neighbour relationships for several millimetres (Fig. 3.3). The mechanism leading to the spread of cobalt between axons in fixed tissue rather than within the axons, as in fresh tissue, is unclear.

In both anaesthetized and freshly killed preparations particularly dense accumulations of cobalt sulphide were seen at the nodes of Ranvier (Fig. 3.1). This may reflect a preferential permeability of sulphide ions at the nodes or alternatively membrane specializations at the node (Landon and Hall, 1976) may provide an advantageous site for the precipitation of cobalt sulphide from solution.

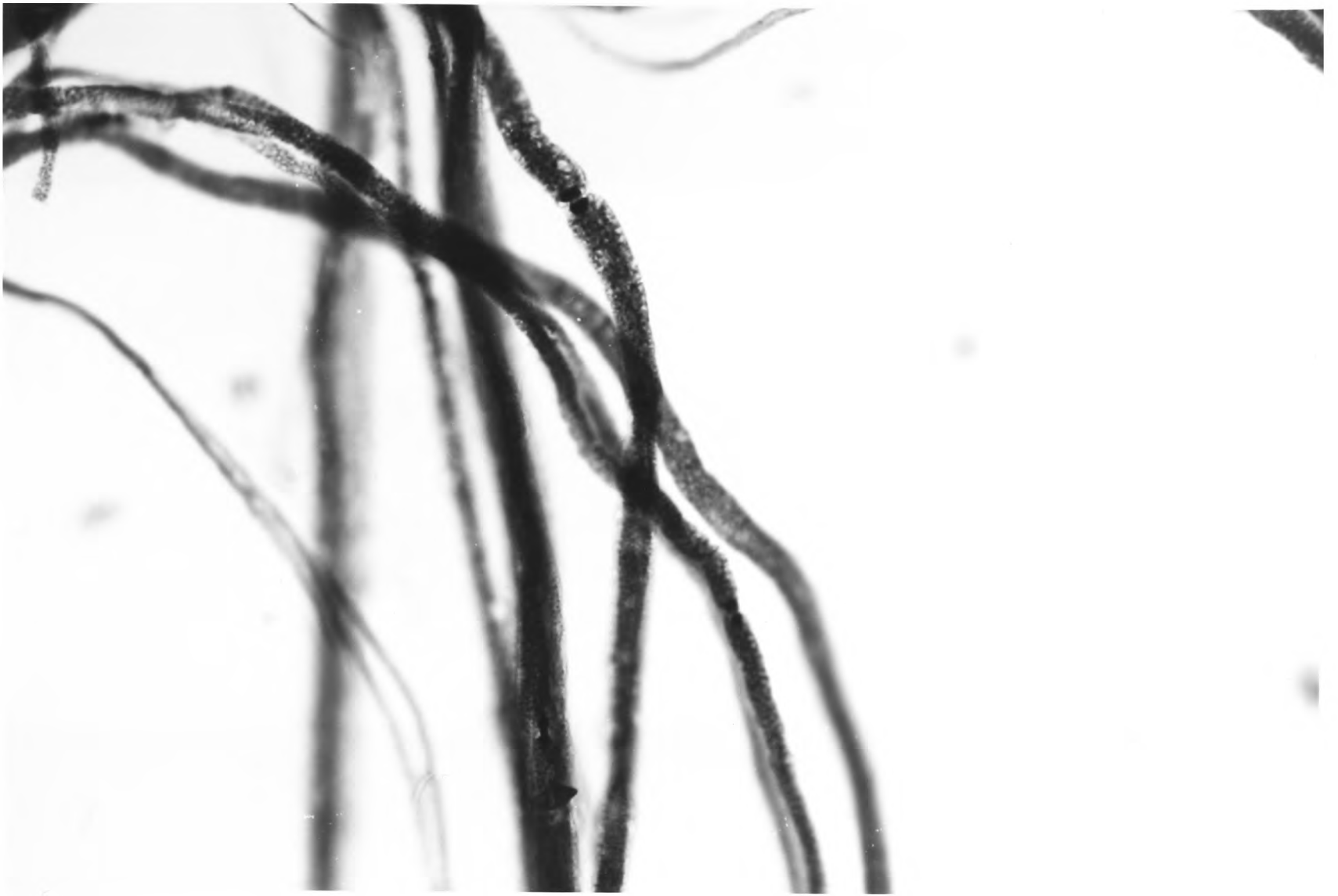
Discussion

Within the distance limitations imposed by the cobalt method these results do provide strong support for an orderly arrangement of fibres within the peripheral nerves of frogs, voles and mice. The "cable structure" as proposed by Stoffel in 1913 seems to be present. The maximum travel of the cobalt ions obtained in these experiments was only 10-15 mm and thus the tracing of fibres the whole way from the distal musculature to the spinal cord was not possible. However, regardless of which species or nerve was studied the fibres were seen to remain together in a discrete bundle whether the labelled group entered the nerve trunk proximally near the limb girdle or distally towards the foot.

Fig. 3.1. Photomicrograph of teased cobalt-filled axons from a fresh mouse peripheral nerve. Note the intracellular nature of the staining and the heavy deposition of cobalt sulphide at the nodes of Ranvier.

Fig. 3.2. Photomicrograph of cobalt-filled axons within the mouse sciatic nerve after introduction of cobalt ions into the common peroneal nerve in a freshly killed preparation.

fig 3.1



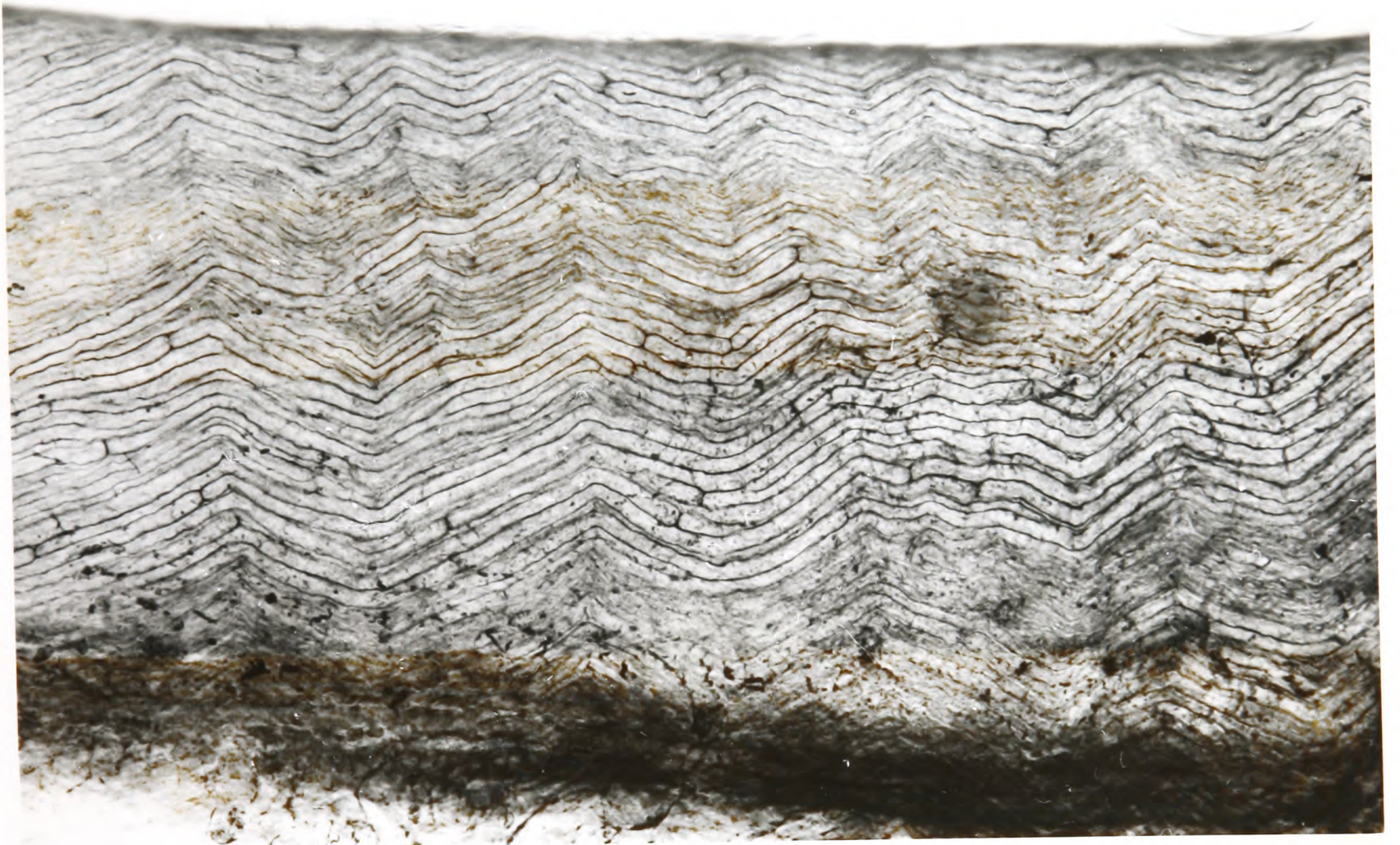
20 μm
150 μm

fig 3.2



Fig. 3.3. Photomicrograph showing cobalt staining of a mouse forelimb nerve which had been lightly fixed with 10% formal saline for 10 minutes before introduction of the cobalt. Cobalt has travelled between the axons, highlighting the parallel arrangement of fibres and also the slight folding of the axons to allow for length changes during movements.

fig 3.3



50 μm

Thus it is extremely unlikely that the region over which fibres were traced in any particular case was untypical of the overall organization of the trunk.

These results agree well with the finding of Veyama (1978) of little intermixing between fibres of different spinal segments within the sciatic nerve of the dog and that of Coggeshall et al., (1978) on the stingray in which afferent and efferent fibres were seen to run together for considerable distances through the musculature without intermixing.

Such an orderly arrangement of fibres within peripheral nerves is of interest to the developmental neurobiologist as it makes tenable those theories of limb innervation in which the distribution of nerve fibres is determined by morphogenetic forces rather than precise chemospecific labels. Horder (1978) has proposed that the initial pattern of nerve fibre distribution is controlled by the ease of access of pioneer fibres into the developing musculature with later fibres being guided to the same targets by contact guidance and fasciculation. Subsequent distortions of the simple basic pattern might then arise due to the rotation of the limb bud during development and to partition of the nerve trunks by connective tissue. Nerve fibres might retain a parallel arrangement despite the repeated division and fusion of fascicle observed by Sunderland in his microdissections (1945, 1948, 1959, 1975).

The morphogenetic model does not, however, seem able to provide a complete explanation for all facets of the development of limb innervation. The difficulty of explaining how overlapping motoneuron pools can provide separate and physiologically distinct groups of nerve fibres to adjacent muscles with a high degree of accuracy, for example the case of gastrocnemius and soleus muscles in the mammal, has been discussed in chapter 1. A similar problem arises when considering the formation of the Ia monosynaptic reflex arc for such a pair of muscles as this requires that afferent fibres from nearby muscles accurately

connect with those motoneurons of the joint pool supplying the same muscle. A possible explanation may be in terms of a differential timing of maturation and axonal outgrowth of the two motoneuron populations though it is unlikely that the temporal separation could be large enough to ensure that no errors occur in the innervation of the limb. If errors do occur then a mechanism must exist for their elimination and this requires that the mistakes be recognized as such.

The search for chemospecificity cues in limb innervation has frequently utilized regeneration of limb nerves in the adult. However any demonstration of a preference of a nerve for the "correct" muscle in regeneration is open to the objection that such a preference occurs because the muscle had been modified in some way by its previous innervation (Salmons and Sreter, 1976) and does not represent a preference present in early development. Only the chick and frog have proved accessible to study during development of the limb innervation. In the chick the events of normal development have been contested, Landmesser and Morriss' (1975) finding the correct (adult) pattern to be established initially whereas Pettigrew et al (1979) found the pattern of spinal root distribution to be modified during development with the loss of some connections. At first sight this latter observation suggests some sort of specificity mechanism operating to remove mistakes though it could equally reflect simple competition between axons with those connections seen in the adult persisting because those fibres have better or earlier access to the muscle in question.

Experimental manipulations of the chick limb bud - spinal cord relationship have mainly failed to demonstrate a preference of a spinal root for the "correct" muscle, innervation normally occurring from the first/nearest nerve to contact the muscle (Stirling and Summerbell, 1980; Lance Jones and Landmesser, 1980a, 1980b). Only in the case of spinal cord rotation was a preferential rerouting of axons to the "correct" muscle observed. In this case the small size of the rotated cord fragment and the inevitable distortion occurring at its boundaries

means that the identification of motoneuron pools within the graft, upon which this demonstration of specificity relies is extremely difficult and the significance of this result is unclear (Lance Jones and Landmesser, 1980a, 1980b).

In the case of the frog there is stronger evidence for some form of specificity acting between motoneurons and their muscles. Lamb has shown modification of the innervation pattern during normal development, suggesting that mistakes are being eliminated. Again this might be based on trivial advantages of one nerve over another due to its proximity to the muscle etc. However he found that when one leg of a larval Xenopus laevis was amputated and the nerve diverted into the remaining leg those neurons which persisted on the amputation side of the cord were those appropriate to the muscles which their axons came to innervate on the contralateral side. Furthermore the normal number of motoneurons survived on both sides of the cord. Thus normal cell death seems to involve loss of inappropriate rather than supernumerary neurons (Lamb, 1974, 1976, 1977, 1979, 1980).

In conclusion, the orderly parallel arrangement of axons seen here in the frog, vole and mouse peripheral nerves, is consistent with a morphogenetic explanation of limb innervation. Though such an orderly arrangement of fibres may contribute to the development of the limb it does seem unlikely that it can provide a complete explanation. An example of the high precision in innervation of muscles by overlapping motoneuron pools which would be difficult to generate morphogenetically has been discussed. Furthermore there is some experimental evidence, good in Xenopus though less clear in the chick, for an active process involved in the generation or selection of specific neuromuscular connections.

Chapter 4

Are nerve fibres arranged in an orderly pattern within the mouse optic nerve?

Introduction

As discussed in chapter 1, a morphogenetic model for the formation of specific nervous connections requires that some form of order be present in the arrangement of fibres within nerve pathways. The model would not be capable of explaining the formation of highly ordered connections from an incoming randomly mixed axon population. Order based upon, though not necessarily a direct representation of, the retinal positions of the ganglion cells has been demonstrated in the primary visual pathway of a number of lower vertebrate species (Bunt, 1980). However in the mammal a considerable amount of controversy has arisen as to the existence of order within the optic nerve.

Human clinical and post mortem studies suggested an orderly fibre arrangement though with some disagreement about the precise relationship of various groups of axons (Seidel, 1919; Van der Hoeve, 1920). Experimental work on animals gave a variety of results in the hands of different workers, varying from a precise retinotopic arrangement (Dean and Usher, 1896; Usher and Dean, 1896; Pick, 1896; Loddoni, 1930) to a more diffuse pattern which might reflect disorder or distortion of an ordered map by folding (Sjaaff and Zeeman, 1924). Polyak's (1957) extensive degeneration study on the primate certainly indicated the optic nerve to contain a highly ordered representation of the retina though with some modification in the arrangement as one progressed along the pathway.

The existence of order in the mammalian optic nerve would thus probably have been widely accepted had not Hubel and Wiesel reexamined the problem with electrophysiological methods (1960). They studied the receptive field positions of successive responses encountered during electrode penetrations of the optic nerve of the spider monkey. The results suggested a localization of fibres within the nerve no better than to the correct quadrant. More recently a combined electrophysiological and anatomical study on the cat

(Horton et al., 1979) produced a similar result - that the degree of order within the optic nerve was extremely low.

In view of those uncertainties about the existence of ordering in the mammalian optic nerve, and of the importance of the problem in the light of the morphogenetic model for nervous connection formation (Horder and Martin, 1978) it was decided to use the neuronal tracer horseradish peroxidase in an attempt to detect order in the primary visual pathway of the mouse.

Methods

The mouse was chosen for this study because the relatively short length of the optic nerve (4-6mm from optic nerve head to anterior edge of the chiasm) greatly increased the chance that it would be possible to introduce sufficient HRP into the optic fibres to allow them to be followed from retina to chiasm and possibly into the optic tract. Furthermore the absence of a fovea in this species (Chievitz, 1891) meant that any order present in the pathway was unlikely to be distorted by the need to fit into the pathway a large number of fibres from a small atypical retinal region.

HRP was chosen as the neuronal tracer because it could be introduced into a small proportion of the fibre pathway and then followed both retrogradely into the parent ganglion cells and anterogradely towards the brain. Moreover its detection is less subject to the false positive results which posed a large problem with the Marchi staining method for degenerating myelin. Three main sites of administration of the HRP were chosen: into the retina; into the optic nerve just behind the eye; and into the tectum.

1. Retinal injection: The conjunctiva was incised and the posterior surface of the eye exposed dorsally. A small incision was made through the sclera into the retina which was lesioned with a sharpened tungsten needle. $0.1\mu\text{l}$

of HRP solution was then absorbed into a small piece of gelatine foam and inserted into the lesion. This route of administration allowed HRP to be introduced into fibres originating in a particular region of the retina. The fibres were filled by HRP transported anterogradely from the cut axons. As a control for fibre labelling due to HRP uptake by undamaged retinal ganglion cells a similar volume of HRP was introduced into the eyes of some animals via a small incision anterior to the retinal margin without a retinal lesion being made.

In some of the experimental animals the posterior part of the eye was sectioned with the optic nerve and brain in an attempt to obtain information on the paths of fibres within the optic nerve head. In others the optic nerve and brain were sectioned while the retina was processed as a wholemount. However in both cases the adhesion of HRP to the vitreal surface of the retina gave a diffuse but strong staining which prevented determination of the extent of the retinal ganglion cells labelled due to the lesion.

2. Nerve injection: Injection of HRP into the optic nerve immediately behind the eye, though a difficult proposition surgically and not allowing fine control of the particular subgroup of fibres labelled, did allow determination of the retinal origins of the labelled fibres. These were studied in retinal wholemounts while the paths of fibres were traced through serial frozen sections. It is likely that HRP was taken up only by those fibres damaged by the injection and not by those left intact (LaVail, et al., 1973) as packing HRP-soaked Sterispon around an exposed but undamaged mouse optic nerve gave no staining in either the nerve or retina.

3. Tectal injection: The superior colliculus was exposed as described in the general methods, and a small volume (0.05-0.1 μ l) of HRP solution injected into the posterior edge using a 1 μ l Hamilton syringe with a sharpened, bevelled tip. This injection site was chosen in order to avoid optic fibres passing

posteriorly over the colliculus and so minimize the number of fibres labelled and confine them to a small region. After injection into such an area of optic fibre terminals, HRP should be taken up by damaged, and possibly by intact, axon processes and transported retrogradely to the cell bodies (Kristensson and Olsson, 1971).

Criteria for the Determination of the Existence of Order

With these techniques the number of optic fibres stained (often a considerable proportion of the total) meant that it was not possible to trace the paths of individual fibres from retinal ganglion cells body to the chiasm and beyond. Therefore it was decided to look at the proportion of the cross sectional area of the optic nerve stained with HRP at various points between the eye and chiasm and to compare this, where possible, with the proportion of the retinal area occupied by stained ganglion cells. This comparison is based on the assumption that there exists no major inhomogeneity in the distribution of ganglion cells in the retina (Chievitz, 1891) and of various sized axons in the optic nerve. However the tendency of both retinal and nerve injection to label sectors of the retina means that in the absence of a marked fovea each injection should sample a representative group of ganglion cells and their axons.

If substantial disruption of the ordering of retinal ganglion cell axons is occurring as the fibres progress towards the brain then a gradual increase in the area of nerve occupied by stained fibres as one moves away from the injection site is to be expected. Furthermore if the nerve contains only a poor representation of the retinal arrangement of the ganglion cells then a substantially greater proportion of nerve than of retina should be stained in the case of retinal or tectal injection and vice versa in the case of nerve injection.

Measurement of the area of HRP labelling in retina and nerve

Retinal wholemounts and nerve and brain sections were traced using a projector microscope and the region of HRP labelling marked. The total areas of the retinae and the nerve cross sections, and the area of labelling were then determined by tracing on to squared paper or by use of a computerized image analyser (Quantimet 720). Percentage areas occupied by staining were then calculated.

Accuracy:

Several factors limit the accuracy of this means of detecting order in the optic nerve.

1. At the borders of the area of HRP staining there is a problem in determining whether a particular region is or is not stained. Particularly when computing areas by hand a certain error is likely to arise through chance. An indication of the magnitude of any purely statistical error can be obtained as follows:

Each retina was subdivided into at least 10,000 subunits and each nerve cross section in at least 100 subunits. For each subunit a decision was recorded as either stained or not stained. Thus each retina or section provides a purely binomial statistic. In each case n (the number of subunits) is large so that the mean percentage area stained in each nerve section should be given by $np \times \frac{100}{n} \%$, where p is the probability that any particular subunit is stained. Where the retina was available, p was taken as the proportion of the retina occupied by stained cells. When the retina was not available p was taken as the mean proportion of the nerve cross section stained for all the approximately transverse sections available for that animal.

Standard deviation of the mean = $np(1-p)$

Thus the 95% confidence limits lie at $\pm 2 \sqrt{\frac{p(1-p)}{n}} \times 100\%$

The maximum purely statistical error will arise in cases where the probability of any particular subunit being stained is 0.5.

2. Not all sections were cut exactly transverse to the long axis of the optic nerve. This occurred because the optic nerves showed a tendency to adopt a sinuous course during fixation and were too small to be pinned out. In such oblique sections the stained fibres might occupy a deceptively large or small proportion of the nerve and for this reason any obviously oblique sections were disregarded when calculating the mean area of staining for any one animal.

Results

In the case of retinal injection of HRP good staining was obtained between the optic nerve head and chiasm but as discussed above the area of retinal labelling could not be determined. As introduction of HRP into the eye without retinal lesion gave no staining in the nerve it was assumed that HRP was not being taken up in significant quantities by intact ganglion cells. The region of cells labelled was therefore taken to be those lying peripheral to the lesion.

Nerve injections gave good labelling of both ganglion cells and fibres as far as the chiasm though the proportion of the fibre population filled was often large and fibres from a particular retinal region could not be selectively labelled with any degree of precision. HRP injected around an intact nerve gave no labelling so that the enzyme seemed to be taken up only by damaged axons.

Though potentially the best site for HRP administration as it left both pathway and retina intact, tectal injection failed to give good results. Fibres were well stained in the optic tract but this faded out as the chiasm approached and was too faint in the optic nerve to be of use. Retinal ganglion cells were, however, well stained. This may reflect limited uptake by the fibre terminals so that the amount of HRP present at any point along the axon at one time was inadequate for detection by the staining methods

employed even though, over a period of time, enough HRP was transported to label the cell body well.

The results described below are, therefore taken from fourteen animals, seven labelled by retinal injection and seven by nerve injection of HRP, in which high intensity intraaxonal staining was obtained. These represent less than 10% of the total number of operated animals, the remainder showing little, if any, HRP transport within the visual pathway. Tables 4.1 and 4.2 give details of the mean area of staining for each animal and the standard deviation of that area (obviously oblique sections being excluded from both calculations.) The smallness and consistency of the standard deviations is remarkable for the mean areas of nerve stained cover a wide range (11-80%). Only two of these fourteen animals show a wide variation in the percentage staining with progression along the nerve. In the first case this was probably due to the highly irregular shape of the area of staining which made tracing the data into the Quantimet extremely difficult. In the second case stain intensity declined markedly as one moved away from the eye.

Fig. 4.1 shows camera lucida tracings of the HRP labelled fibres in the optic nerve in three animals after retinal injection of label. Each reconstruction is accompanied by a schematic diagram of the position of retinal labelling which should have arisen from the injection and by a graph of percentage area of the nerve cross section stained against distance from the eye. Fig. 4.2. a-c show similar diagrams for three animals after nerve injection of HRP though in these cases the retinal diagram shows the approximate region of labelling seen in the retinal wholemount of that animal. These diagrams demonstrate that the area of staining remained almost constant through any one series of sections though with some change in shape and position within the section.

Table 4.1: Mice labelled by retinal injection of HRP

Animal	Site of Labelling	Method of area computation	Mean area of nerve stained %	SD %	No. of sections used	Length of nerve examined $\times 1000 \mu m$
M1-R	N-V	Quantimet	14.4	3.8	18	4.6
M2-R	N	Quantimet	60.0	4.7	11	4.0
M3-R	T	Quantimet	11.7	2.1	7	2.8
*M4-R	V	Quantimet	34.2	-	10	3.5
+M5-R	V	Quantimet	21.9	-	5	2.0
M6-R	V	Quantimet	15.5	3.1	7	2.5
M7-R	T	Quantimet	14.9	2.6	4	1.2

*Patch of label very irregular - Quantimet measurements inaccurate

+ Stain fades greatly towards chiasm - measurements not reliable

Table 4.2: Mice labelled by nerve injection of HRP

Animal	Area of retina stained %	Method of area computation	Mean area of nerve stained %	SD %	No. of sections used	Length of nerve examined $\times 1000\mu m$
M1-N	59.3	Hand	61.9	3.2	7	1.4
M2-N	72.7	Hand	75.5	3.3	13	2.6
M3-N	19.7	Hand	18.9	2.0	12	2.4
M4-N	84.8	Hand	79.8	3.7	10	2.0
M5-N	72.5	Hand	75.1	7.4	13	2.6
M6-N	49.1	Hand	48.1	4.6	11	2.2
M7-N	61.6	Hand	59.7	3.9	11	2.2

Fig. 4.1a-c Camera lucida tracings of the area of HRP labelling seen within the nerve cross sections of three mice labelled by introduction of HRP into a small retinal lesion.

The approximate retinal region whose fibres should be labelled by the lesion is shown in the top left corner of each diagram.

Fig. 4.2a-c Camera lucida tracings of the area of staining within the nerve cross sections of three mice labelled by introduction of HRP into small optic nerve lesions.

The approximate retinal area of staining, as seen in the retinal wholemount is shown in the top left corner of each diagram.

The graph in each case shows the percentage area of the nerve stained against distance from the eye.

fig 4.1a

M2R

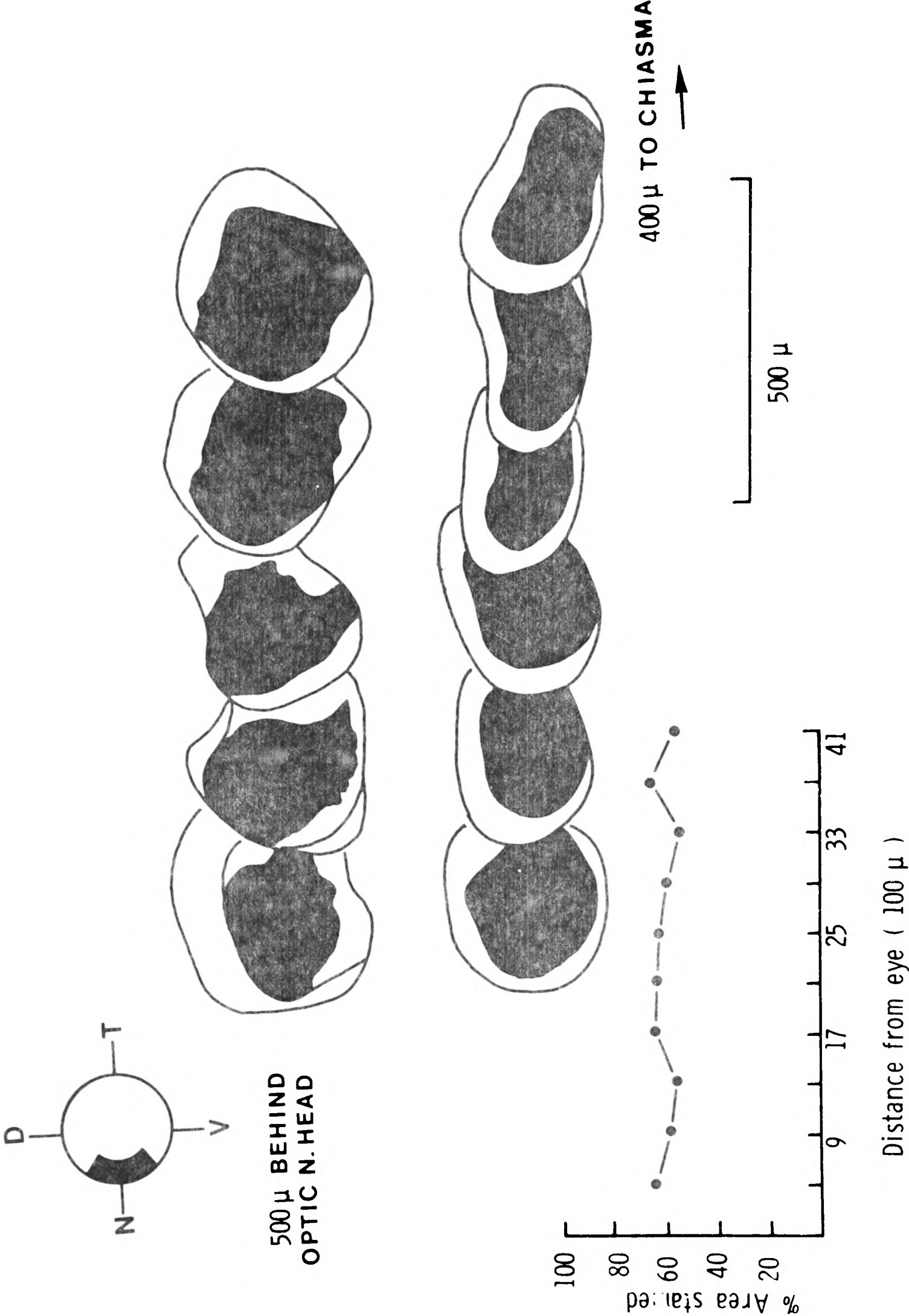


fig 4.1b M3R

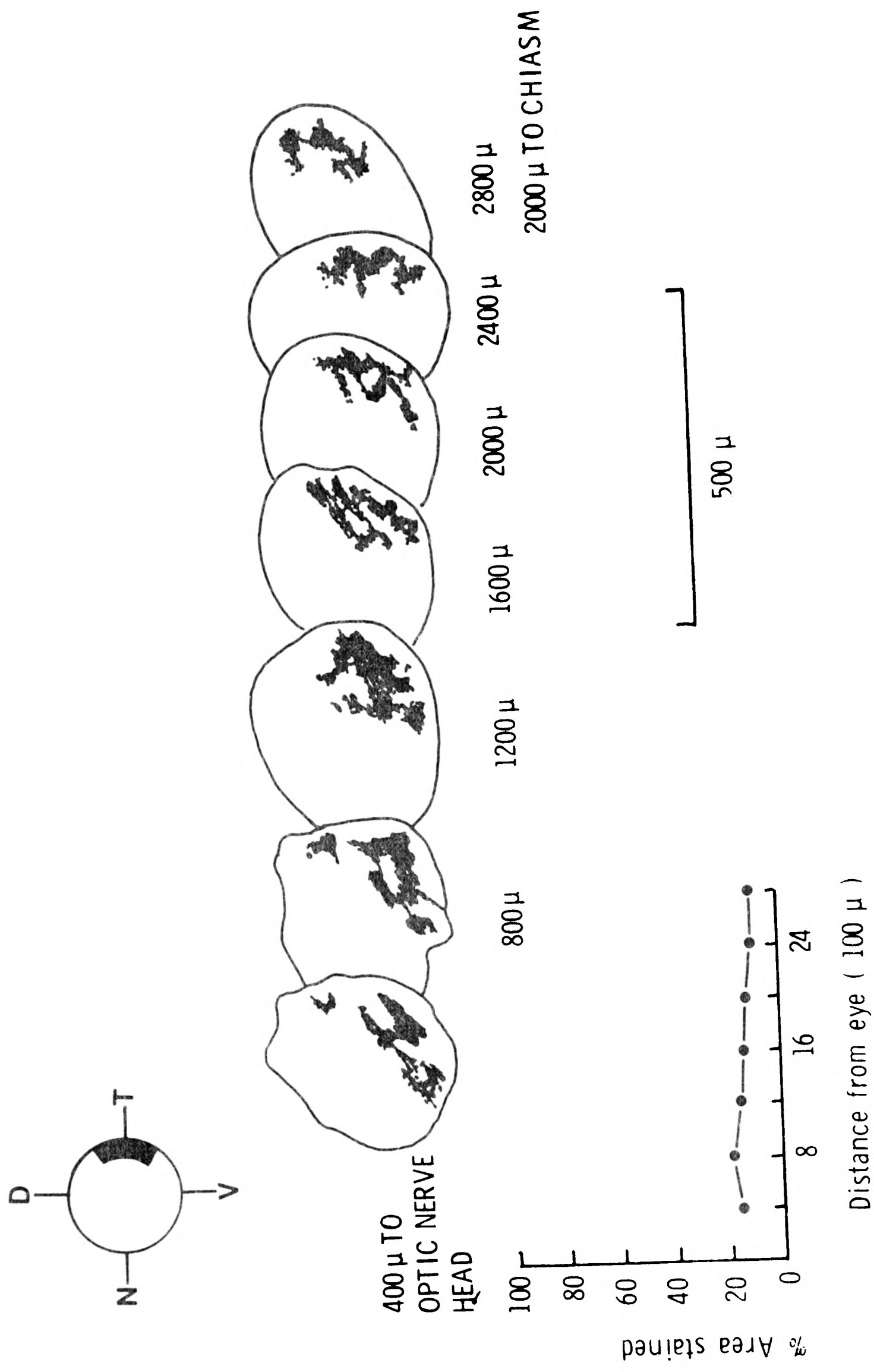


fig 4.1c M6R

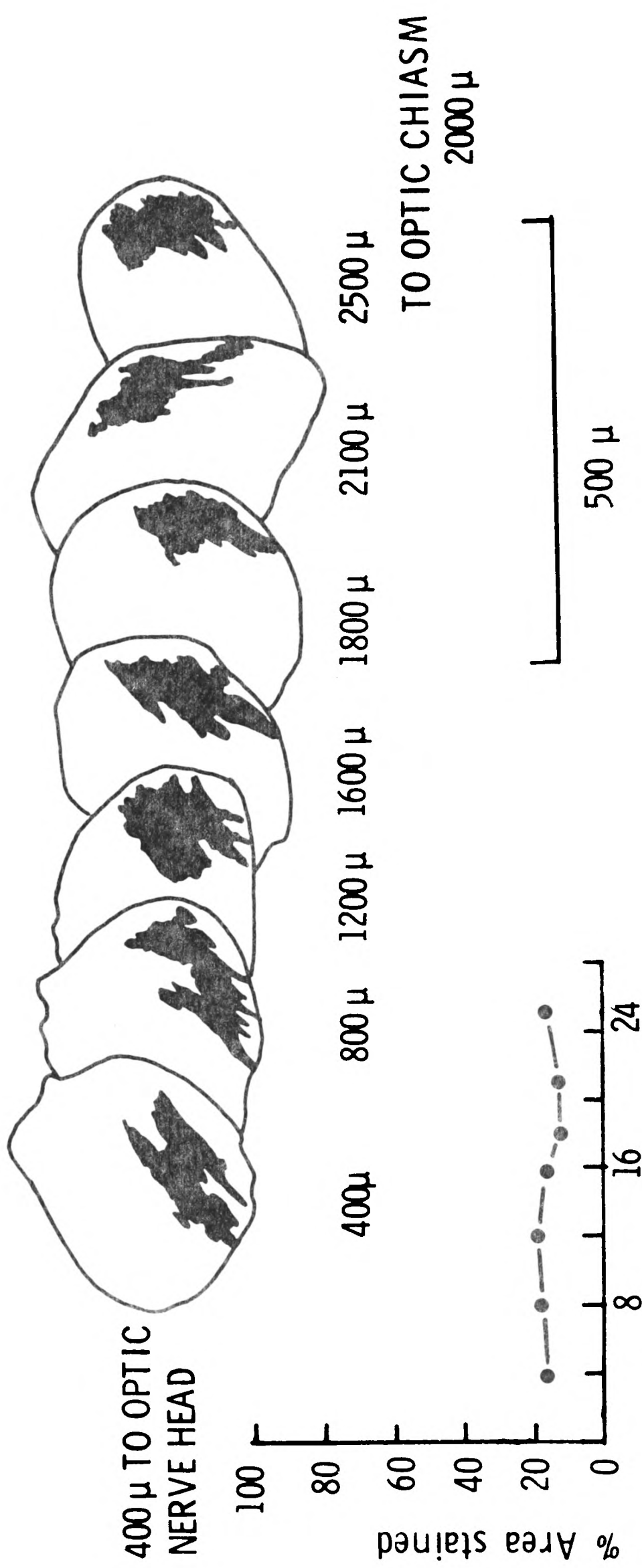
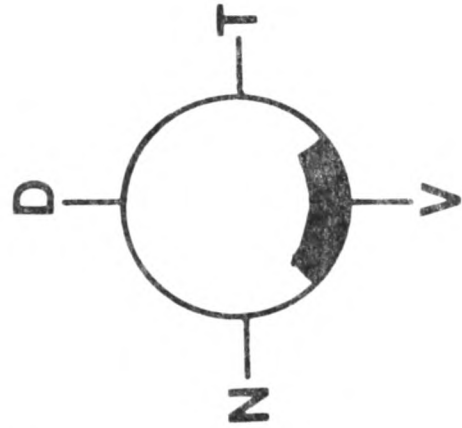


fig 4.2a M2N

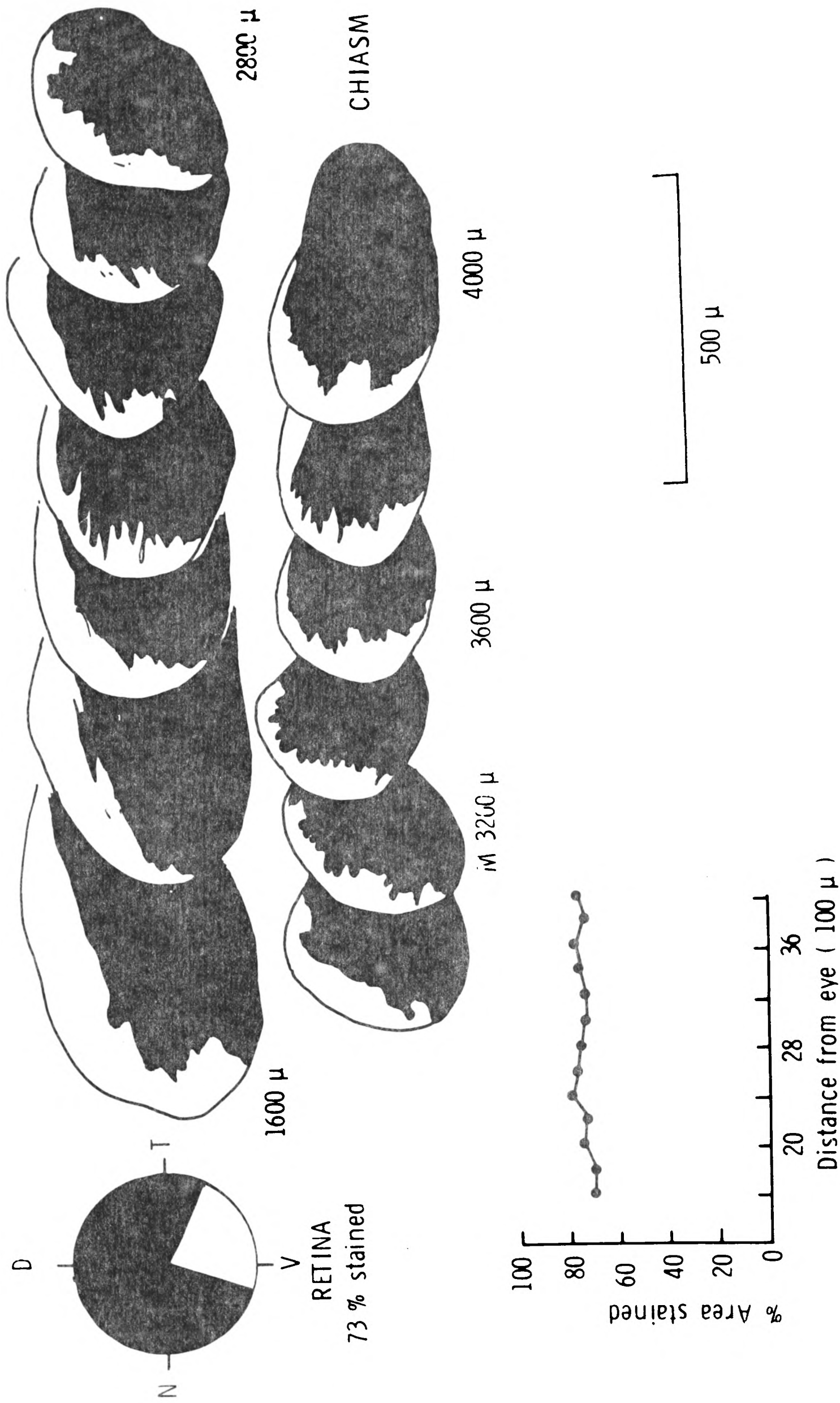
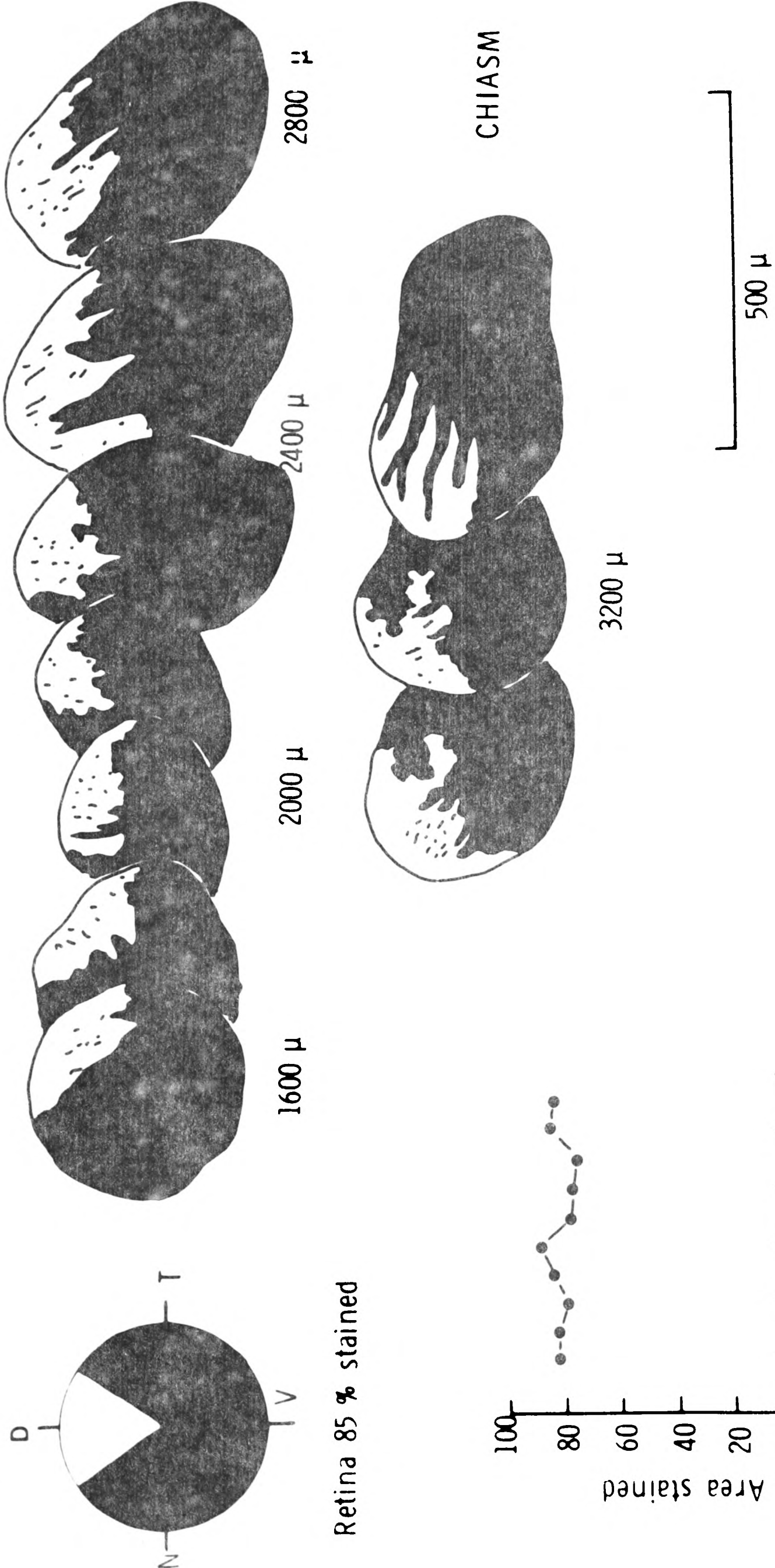


fig 4.2b

M4N



Retina 85 % stained

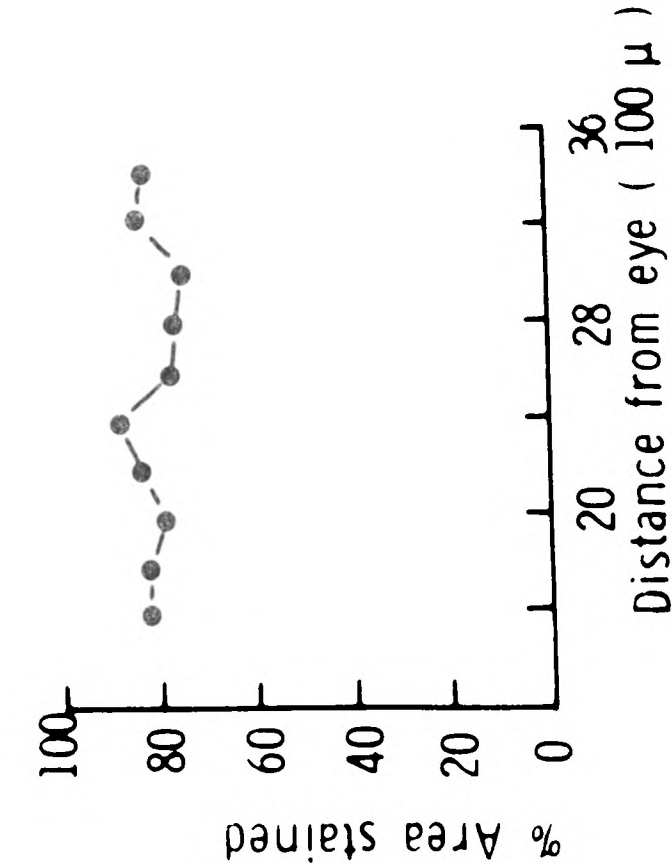


fig 4.2c **M7N**

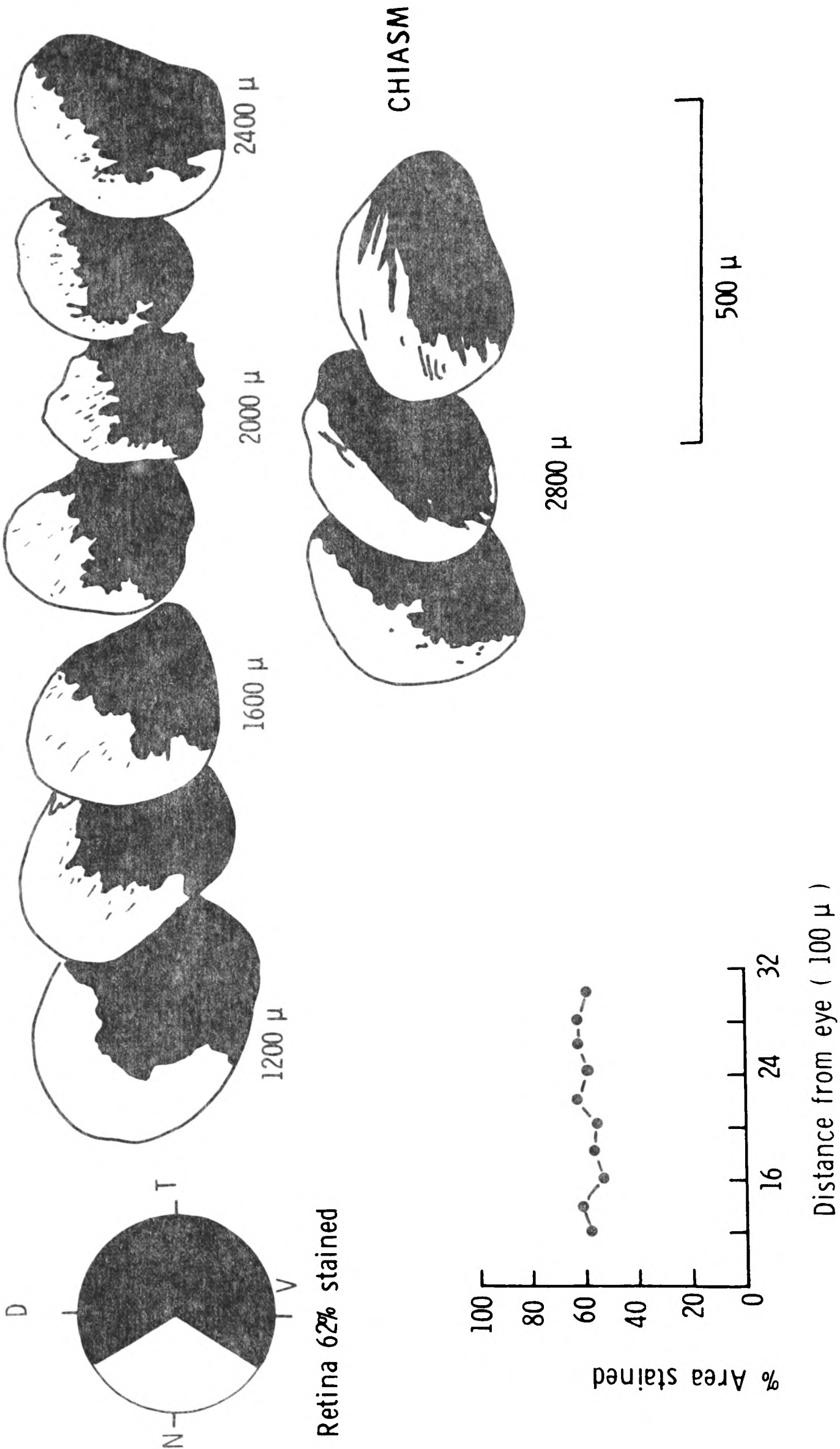


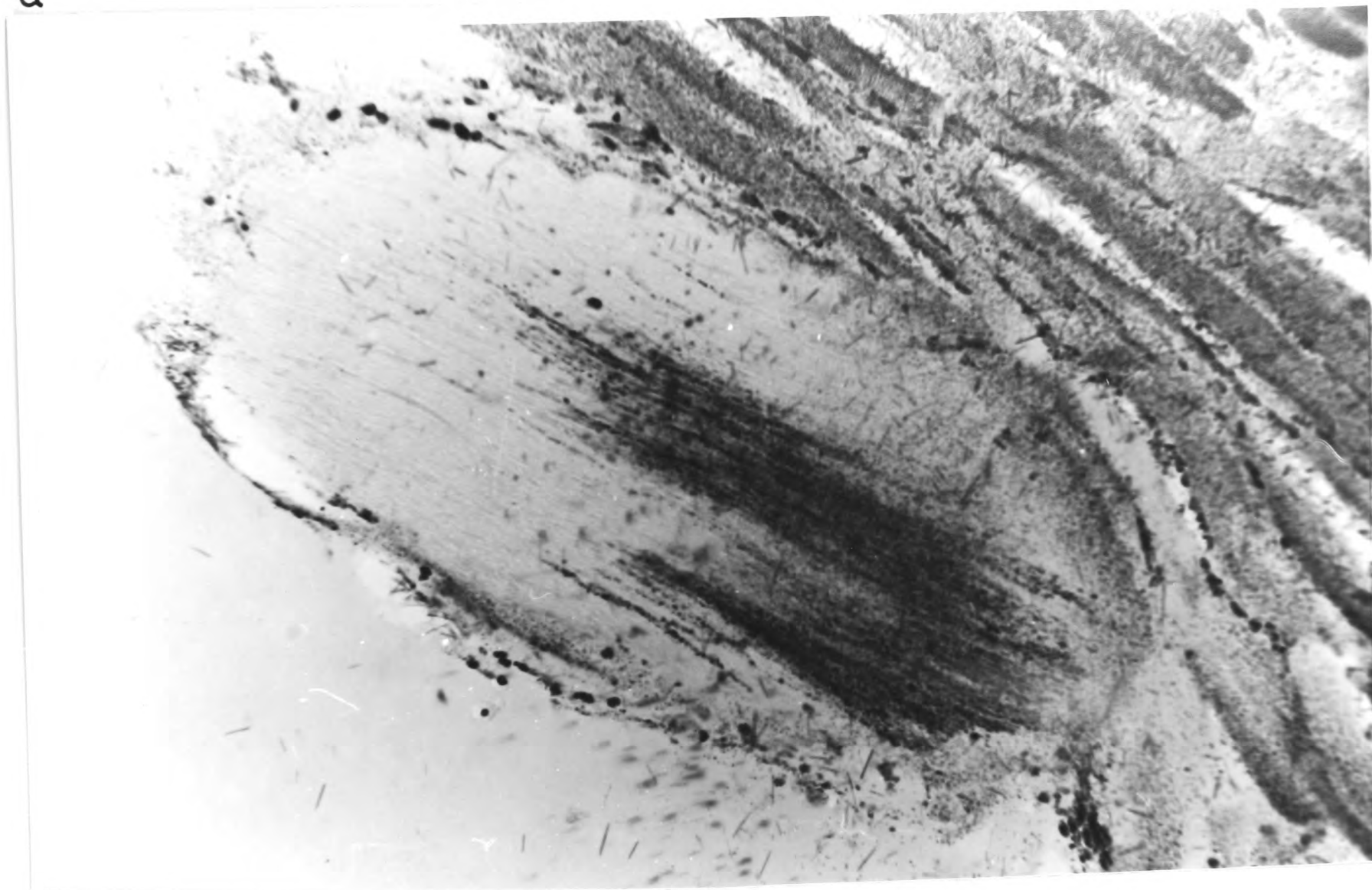
Fig. 4.3 a and b. Photomicrographs of optic nerve sections from mouse M3N showing the pattern of labelling obtained after a nerve injection of HRP.

(a) 1200 μm behind the eye.

(b) 2800 μm behind the eye.

fig 4.3

a



500 μm

b

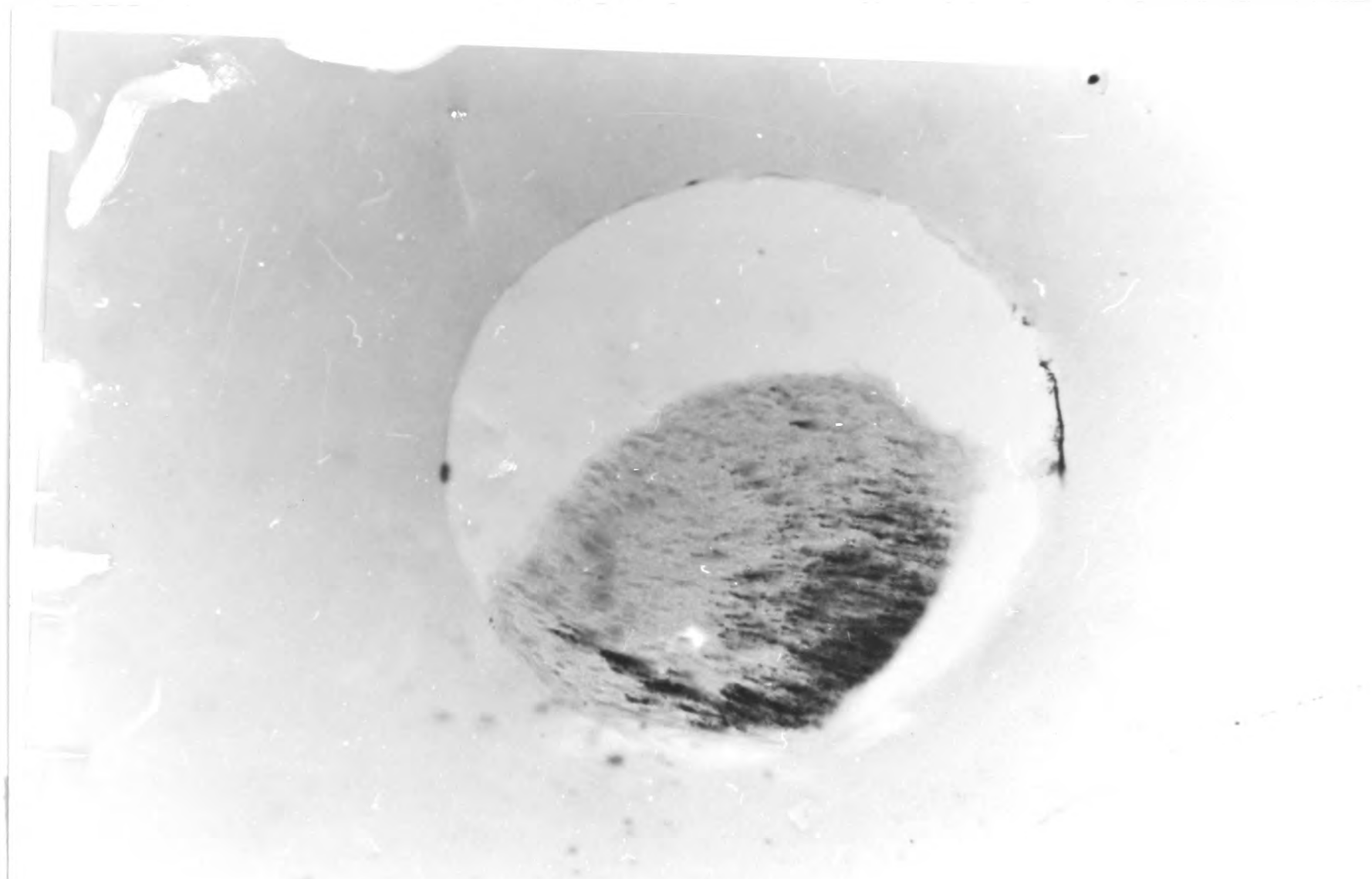


Fig. 4.3 a and b. Photomicrographs of optic nerve sections from mouse M3N showing the pattern of labelling obtained after a nerve injection of HRP.

(a) 1200 μm behind the eye.

(b) 2800 μm behind the eye.

fig 4.3

a



500 μm

b

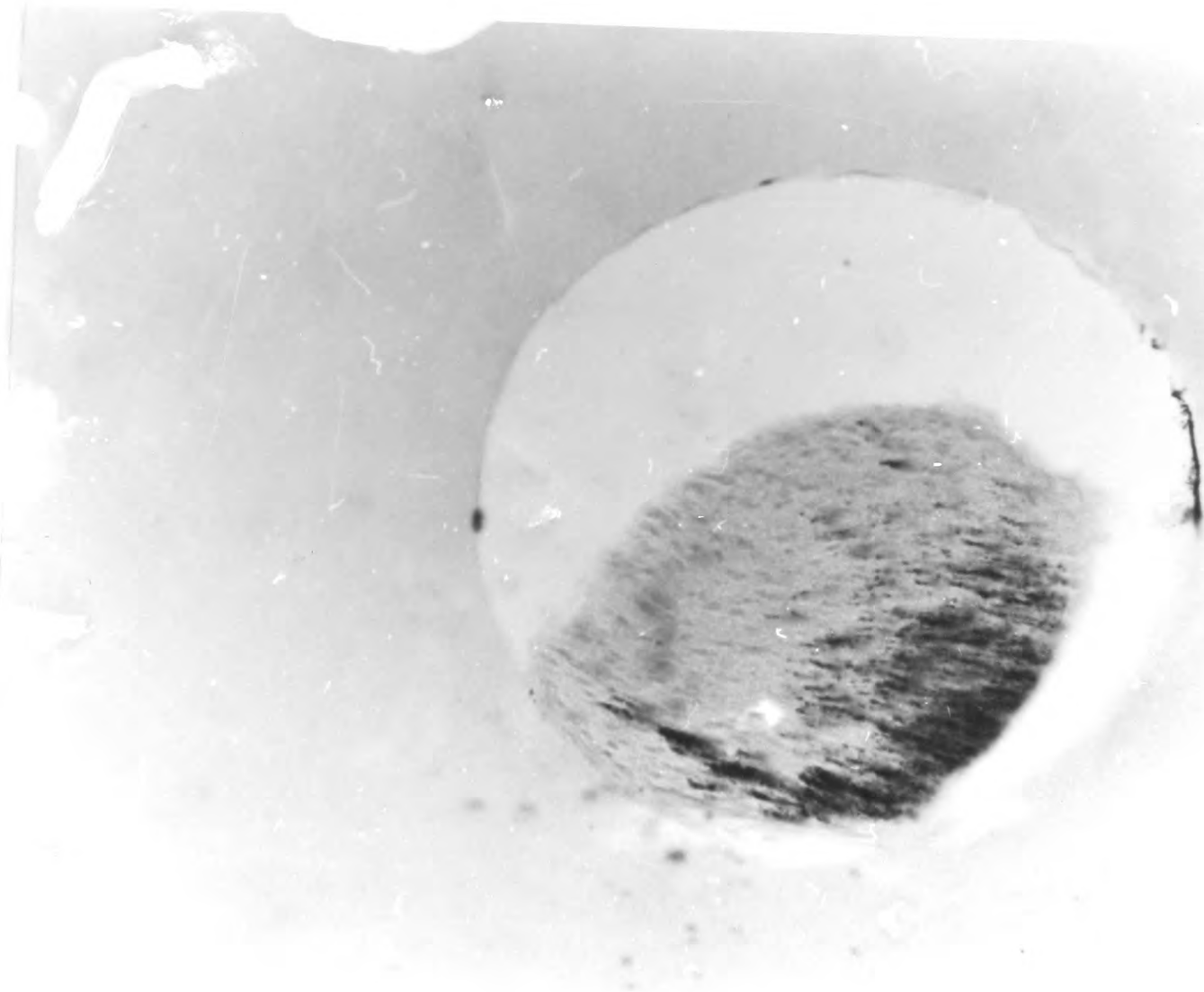


Fig. 4.4. Diagram summarizing the distribution of HRP labelled fibres and cell bodies in a typical nerve section taken between 1000 and 2000 μ m behind the eye, and the retina for the 14 animals studied.

fig 4.4

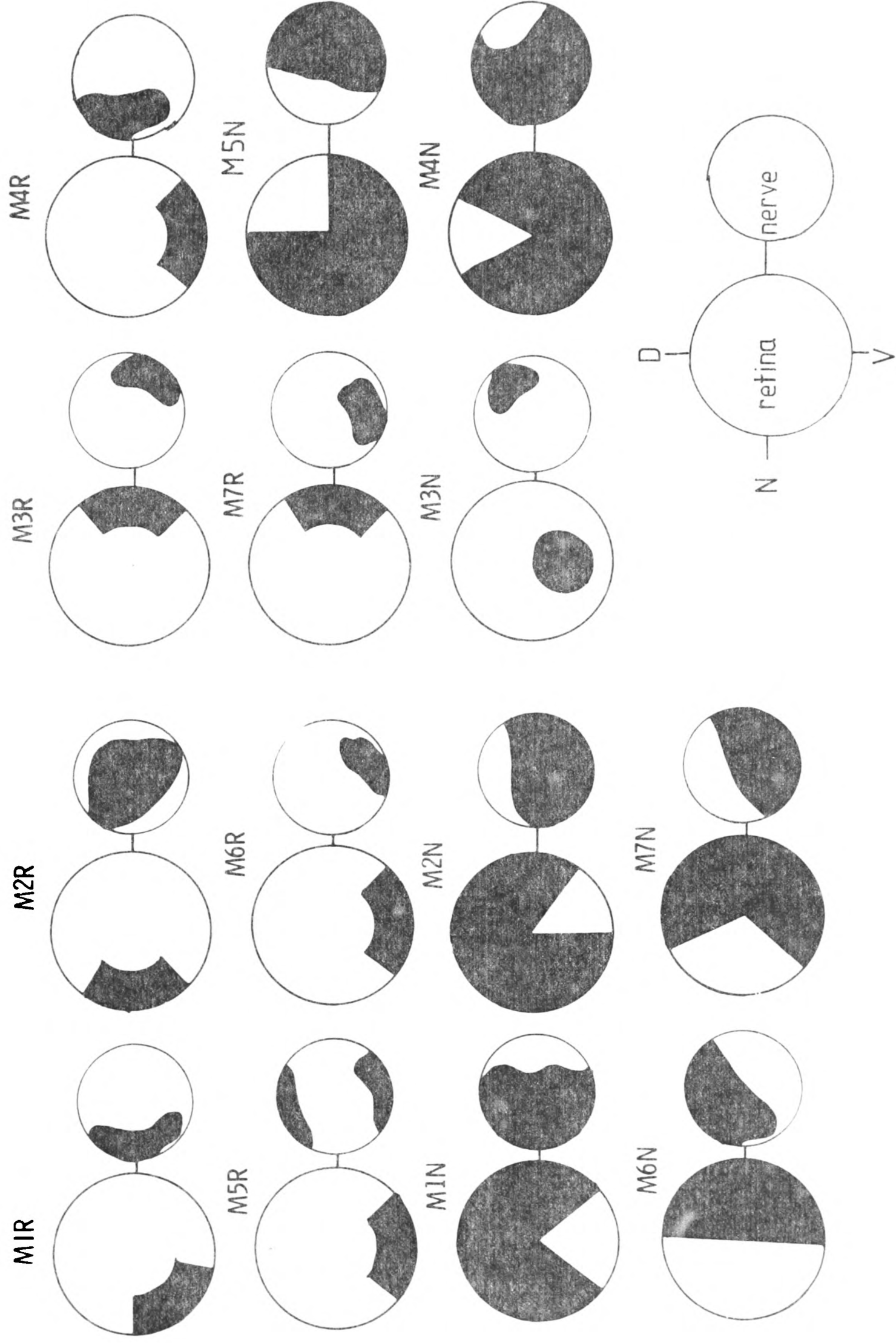


Fig. 4.5. Photomicrographs of coronal sections through the optic chiasm of a mouse after introduction of HRP into the right optic nerve just behind the eye.

(a) at the anterior edge of the chiasm;

(b) 400 μ m more caudally.

Note the uncrossed fibres adopting a dorsal position while the crossing fibres pass laterally and ventrally in wide fascicles. The right of the animal is shown at the left of these photomicrographs.

a



500 μm

b



Typical optic nerve sections showing the staining obtained after injection of HRP into the nerve of animal M3N are shown in Fig. 4.3 a and b.

In some animals after injection of HRP into the nerve a few, well filled apparently very large fibres were seen scattered through the nerve away from the main region of labelling (fig. 4.2b and 4.2c). These may be the result of a very irregular injection lesion due to the distortion of the nerve fibre arrangement by the tungsten needle or they may represent a small atypical population of fibres not constrained by the order seen in the main group of fibres. They were however more often seen and more prominent in the nerve-labelled animals, supporting the former explanation.

Fig. 4.4 gives a schematic summary of the observed (in the case of nerve labelled animals) or believed (in the case of retinal labelling) position of retinal labelling and the appearance of a typical nerve section from the corresponding animal taken between $1000\mu\text{m}$ and $2000\mu\text{m}$ behind the eye.

Because of uncertainty about the orientation of the nerve sections - there was a tendency for the nerve to twist during fixation and no satisfactory method was found to prevent this - no firm conclusions could be drawn about the exact nature of the retinal representation within the nerve. There was also some disparity between animals apparently labelled in the same way over the region of nerve stained. However the most likely representation seems to be a direct map without any transformation. There was certainly no evidence for the sort of annular 'chronotropic' representation of the retina seen in the fish optic nerve (Dawney, 1979b; Rusoff and Easter, 1980).

No attempt was made to study in detail the arrangement of fibres in the optic chiasm as in the majority of animals the staining became very granular and faint at this point. However in occasional animals the interdigitation of large fascicles from the two eyes, about 5-10 fascicles from each eye visible in each section, was very striking (fig. 4.5). This confirmed the chiasmatic

anatomy outlined by Ramon y Cajal (1960) and Polyak (1957). Fibres passing ipsilaterally remained in a dorsal and lateral position while crossing fibres passed through the chiasm in an oblique dorsoventral direction.

Discussion

The results presented here strongly support the idea of a high degree of order being present in the arrangement of fibres in the mouse optic nerve. The good agreement between the percentage area of retinal staining and the mean percentage area of nerve staining (in animals labelled into the nerve), and the constancy of the proportion of the nerve cross section stained over a considerable length of the pathway (in both retinal and nerve labelled animals) is not consistent with the idea that the order is of only a very crude sort with considerable intermixing of fibres from different retinal areas. In that case one would expect a progressive increase in the proportion of the nerve stained as one moved away from the site of labelling.

The method used here makes the assumption that the staining is due to HRP within the axons. Though electronmicroscopic confirmation of this is not available there are several strong reasons for believing this to be so. Firstly in the available retinae ganglion cells and their dendritic trees were extremely well stained and showed their characteristic morphologies, indicating the HRP to be travelling within the neurons. Secondly the high power light microscopic appearance of staining in obliquely cut nerve sections was consistent with intraaxonal labelling, being reminiscent of a packet of spaghetti. Thirdly, in some preparations, for example M4N (fig. 4.2b) and M7N (fig. 4.2c) scattered points of staining occurred outside the main area and formed a fairly consistent pattern from section to section suggesting that those were scattered stained axons. Finally if the HRP were spreading between rather than in fibres this would have to be by a passive mechanism, diffusion. One would then expect the stain to fade out

gradually both towards the edges of the patch within each section, and as one moved along the nerve. In fact the edges of the labelled area were quite sharp, if occasionally rather ragged.

These results are consistent with those of Polyak (1957) and many of his predecessors, disagreeing only with two recent studies. The first of these is the electrophysiological study of the spider monkey optic nerve (Hubel and Wiesel, 1960) and the second the combined electrophysiological and HRP study of the cat (Horton et al., 1979) published after this study began. The wide separation of receptive fields of successive fibres encountered in an electrode penetration of the optic nerve might occur despite the presence of a high degree of order within the nerve for two reasons. Firstly the passage of the electrode through the nerve may cause major distortions of the fibre arrangement so that successive fibres recorded do not normally lie close together in the undisturbed nerve. Secondly, particularly in the cat, the length of nerve present within the skull is so small that stereotaxic electrode positioning is likely to lead to recording not from the nerve itself but from the anterior edge of the chiasm where fibre ordering is bound to be undergoing considerable change.

In the anatomical investigation of the cat the conclusion of little order is based upon the tracing of a very small number of fibres, 8 in the published example, which happened to stain particularly well, between the lateral geniculate nucleus and the retina. As the fibres could not be traced through either the chiasm or the optic nerve head it was not possible to determine which fibre belonged to which cell body. The fibres appeared to undergo considerable alteration of their relative positions, and to spread out, between eye and chiasm though this assumes that each fibre could be identified with certainty from one section to the next. The area of retina labelled lay between the area centralis and the optic nerve head in that region most likely to

undergo some distortion in an otherwise highly ordered, retinotopically organized nerve because of the need to accommodate a large number of foveal fibres into the map. It is thus possible that Horton's study revealed a distortion of the ordering of the nerve not present in mammals such as the mouse which lack a fovea, and not present in other regions of the cat nerve carrying fibres from retinal regions further from the fovea. Alternatively the 8 fibres which stained so much better than the background majority might form part of a small population not possessing the same overall order seen among the vast majority, perhaps because they arose at a different time in development.

The finding here of a high degree of order in the mouse optic nerve has important implications for passive models of the formation of nervous connections. Obviously it does not provide proof that no active processes perhaps of a chemospecific nature, are involved in the formation of specific synapses but it does make an at least contributory role for passive mechanisms a tenable hypothesis. How such an ordered representation, probably a direct map, of the retina arises within the nerve is not clear. The little available data on the pattern of growth of the mammalian retina suggests that ganglion cells are added concentrically at the retinal margin (Sidman, 1960) though the analysis is complicated by the presence of displaced amacrine cells in the ganglion cell layer (Eayrs, 1952; Bunt et al., 1974). Whether the timing of origin of the ganglion cells is reflected in the timing of axon outgrowth is unknown though an orderly sequence of centre to periphery fibre outgrowth combined with substrate guidance by channels (Silver and Sidman, 1980) might well produce a retinotopic map within the nerve without need for any "active" guidance process.

Order within the optic nerve might well contribute to the generation of retinotopic connections in the primary visual centres. However, as discussed in chapter one, phenomena such as bunching of axons (Cunningham and

Seagraves, 1976), and the differing behaviour of the various ganglion cell classes at the chiasm (Stone and Fukada, 1974) make it unlikely that an orderly optic nerve can provide the complete explanation for the formation of the primary visual projections.

The organization of optic fibres within the primary visual pathway of the normal goldfish

Introduction

Much of the experimental work aimed at detecting the operation of chemospecific mechanisms in the formation of specific nervous connections has been performed using fish, and particularly the goldfish, Carassius auratus. Fish therefore form an important group in which to attempt a complete description of fibre arrangement throughout the visual pathway for such a description would be of considerable help when interpreting the results of experimental manipulation of the pathway.

Extensive evidence that fibres tend to have the same neighbours in the pathway as the retina has come from the tracing with electron microscopy (Horder, 1974a), and at the light microscopic level (Anders and Hibbard, 1974; Roth, 1974; Scholes, 1979; Dawney, 1979a, 1979b) of degeneration caused by a retinal lesion. Labelling of fibres by injection of HRP into the tectum (Dawney, 1979a), optic nerve (Dawney, 1979b; Rusoff and Easter, 1980), and retina (Rusoff, 1979), have confirmed this orderliness.

Two attempts have been made to explain the arrangement of optic fibres between eye and tectum in fish (goldfish - Bunt and Horder, 1977; cichlid fish - Scholes, 1979). However the former was a purely theoretical model which does not conform with recently adduced data suggesting that fibres are grouped together according to their radial location in the retina. The latter model, dealing with the ribbon nerve of certain cichlid fishes, seems, for the reasons discussed in chapter one, not to be wholly satisfactory. It was therefore decided to reinvestigate the arrangement of fibres in the normal goldfish optic nerve as a good description of this would be of use in the interpretation of the effects of surgical manipulations of the system to be described later in this thesis.

Methods:

HRP was used to follow small groups of axons through the visual pathway of small adult goldfish (45-65mm, nose to base of tail). HRP was introduced into small groups of fibres at three sites.

1. The optic tectum - this was exposed as described in chapter 2, a small lesion was made using a sharpened tungsten needle and the lesion packed with HRP solution (30% in 0.1M phosphate buffer at pH 7.4) absorbed into a small piece of gelatine foam (Steirispon).
2. The optic nerve in the orbit - the nerve was exposed as described in chapter 2, a few fibres were torn through using a sharpened tungsten needle and packed with HRP on Steirispon.
3. The retina - a small incision was made in the conjunctiva and the eye deflected forwards. A small retinal lesion was then made through the sclera with a tungsten needle and HRP on a small piece of Steirispon was inserted through the incision into the eye. The eye was then pushed gently back into place.

In each case a survival time of 18-48 hours was allowed. Fibres were then traced through serial thick (40 μ m) frozen sections. The ganglion cells stained were determined from the retinal wholemount after processing as described in chapter 2.

Results:

Arrangement of fibres within the optic nerve

1. Evidence from tectal introduction of HRP

Adequate staining of optic fibres rostral to the chiasm was obtained in only a small number of cases after introduction of HRP at the tectal level.

Moreover there was some uncertainty as to the number and nature of the fibres labelled as the HRP did not give good staining of the ganglion cell bodies in the retina. However after an HRP injection at the medial edge of the tectum

about midway along its rostro-caudal extent, presumably filling fibres from peripheral ventronasal retina, several fish were produced in which the label extended anterior to the chiasm. In each case a single compact area of the nerve was labelled.

2. Evidence from introduction of HRP into the optic nerve

This proved by far the best site for HRP administration as the label could be traced forward into the retina and back to the far extent of the tectum. The intraaxonal location of the HRP was strongly suggested by the way in which ganglion cells showed a very characteristic morphology with good visualization of their dendrites (fig. 5.1) and by the filling of terminals in the tectum (illustrated in chapter 6).

HRP was introduced into lesions just behind the eye. These lesions were classified into four groups, dorsal, ventral, nasal and temporal. Greater precision was not attempted as it was not possible to control the eye rotation completely during surgery nor was it clear exactly where in the nerve most damage had been produced. In all cases the typical pattern of labelling in the retina was of one or more partial or complete annuli of filled ganglion cells (fig. 5.2a). In no case was a complete sector of the retina filled.

Dorsal lesion. $n = 16$

In the majority of animals (14/16) the HRP labelled cells formed 1 or 2 complete or partial annuli located in the middle third (in terms of radial position) of the retina. Two of these also had label in the central part of the retina while two others were labelled only in the peripheral third.

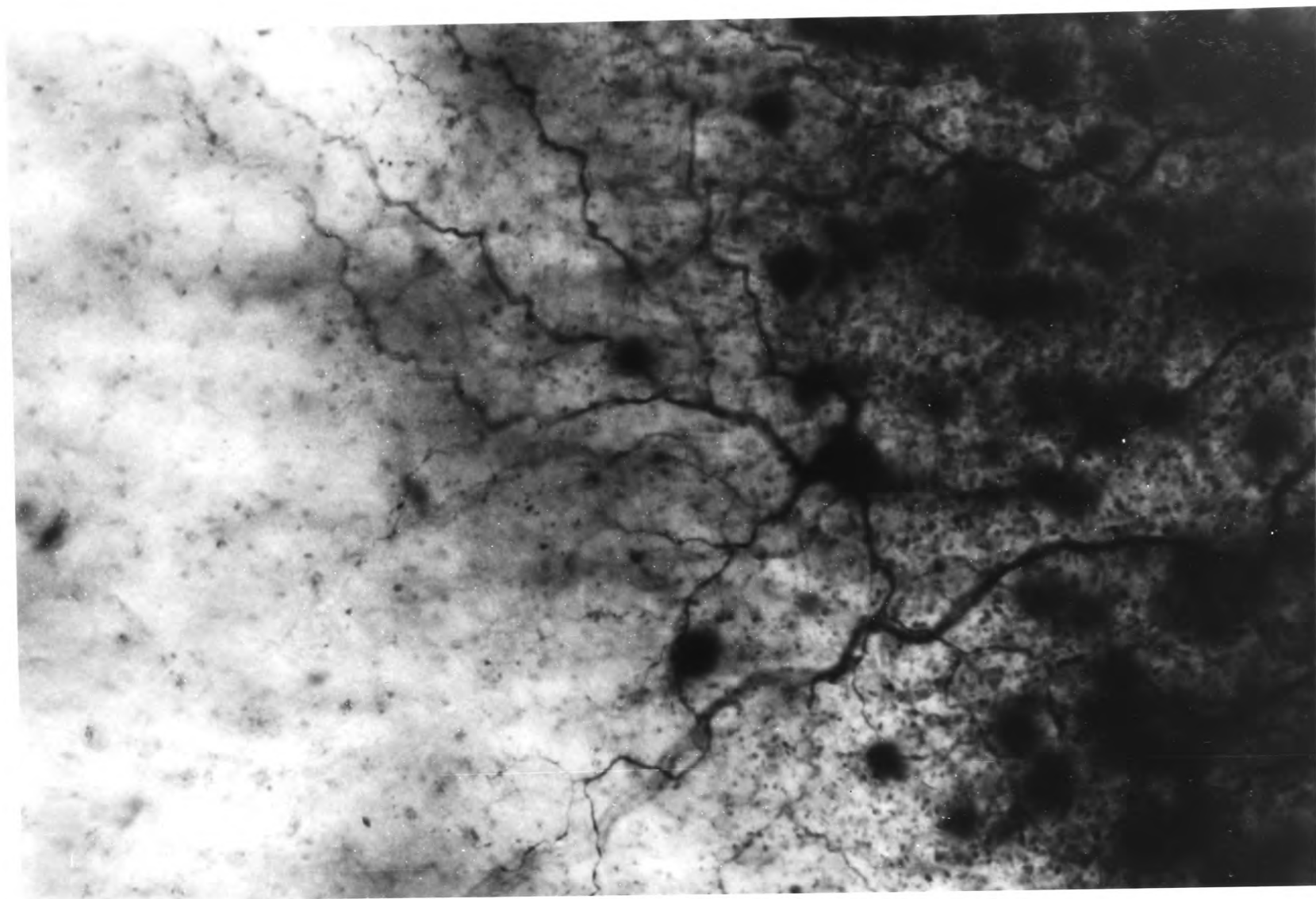
Ventral lesion. $n = 14$

Twelve of the 13 animals showing any retinal labelling had that label confined to the peripheral third of the retina, and in 10 of these the labelling actually lay at the peripheral retinal edge.

Fig. 5.1. Photomicrographs of retinal ganglion cells in goldfish retinal wholemounts labelled by introduction of HRP into a nerve lesion. The good filling of the soma and dendrites, with their characteristic morphology, strongly suggests an intracellular location for the HRP.

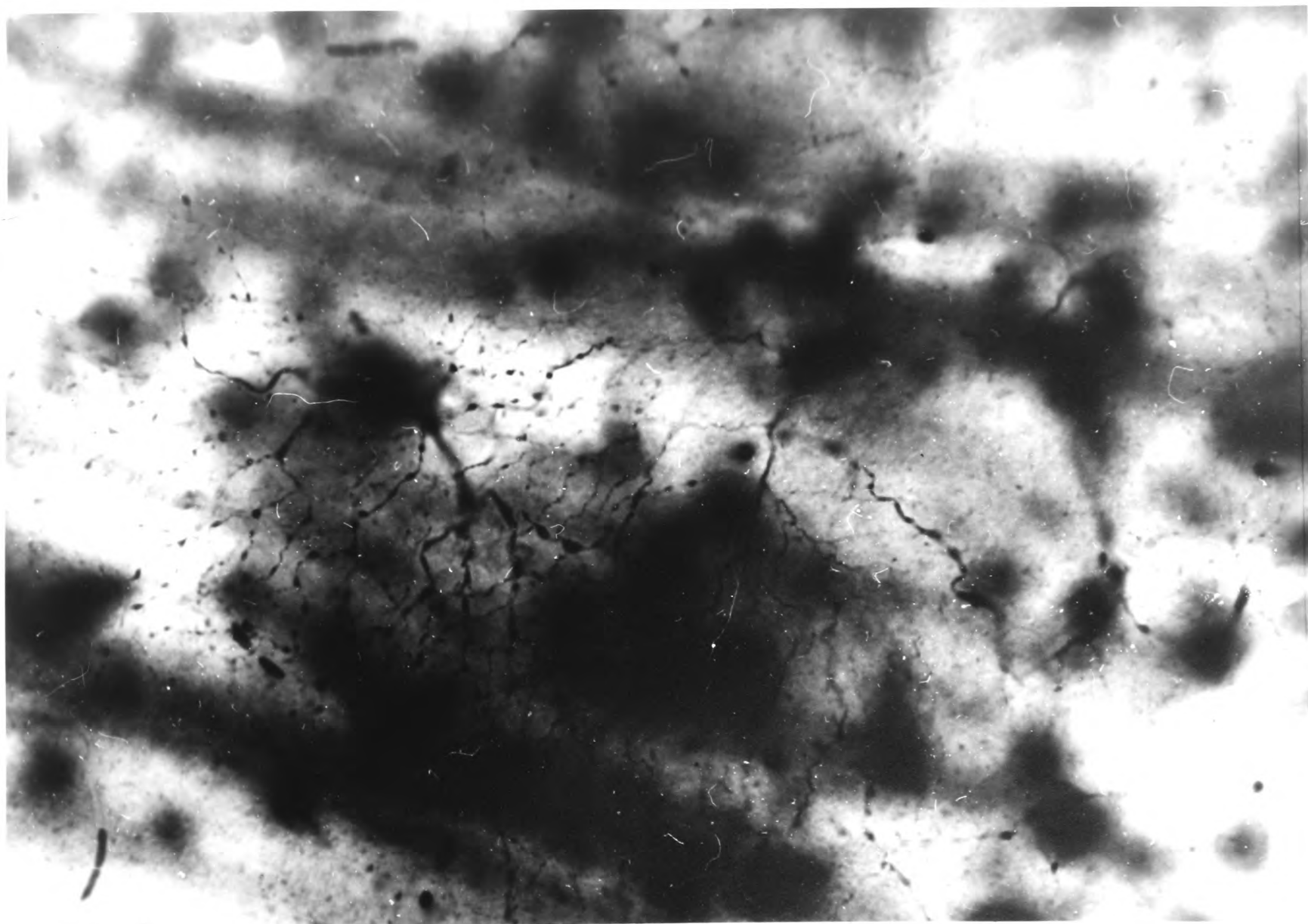
fig 5.1

a



b

30 μ m



Nasal lesion. n = 21

This lesion site produced a great variety of results, 3 animals being labelled only in central retina, 7 only in the middle region, and 11 only peripherally. Two animals were labelled in both the centre and middle regions. Thus staining tended to occur in the outer half of the retina.

Temporal lesion. n = 21

Again a variety of labelling positions occurred, with 13 animals labelled centrally, 3 in the middle third, 1 peripherally and 4 in both the central and middle thirds. Thus in this case the tendency was for staining to be more central.

In many of these animals the HRP filled one or more partial annuli of ganglion cells. In all, 37 such partial annuli were noted. The circumferential locations of the edges of these part rings were allocated to one of four groups, dorsal, ventral, nasal or temporal. This was possible because the boundaries of the partial annuli were usually very sharply defined (fig. 5.2b).

Data on both the radial and circumferential locations of the partial and complete annuli are summarized in table 5.1. Regardless of the position of the nerve lesion the most common position for a circumferential boundary of a part ring was ventrally (35/74 boundaries), usually coinciding closely with central incision made in the retina to define the position of the choroid fissure (fig. 5.2b).

3. Evidence from retinal introduction of HRP

Satisfactory staining within the optic nerve after retinal administration of HRP proved difficult to obtain though it was achieved in a small number of cases. However this staining never extended caudal to the chiasm at a useful density. The site and extent of labelling was in each case checked

Fig. 5.2a. Photomicrograph of a wholemount of a left retina after HRP had been introduced into a ~~nasal~~ retinal lesion. Two annuli of stained ganglion cells, in the centre and middle thirds of the retina are separated by an unstained ring.

Fig. 5.2b. Photomicrograph of wholemount of a left retina after HRP had been introduced into a dorsal optic nerve lesion. A partial annulus of ganglion cells lying in the middle third of the retina has been stained. The circumferential boundaries of this annulus lie ventrally (almost coinciding with the ventral cut) and dorsally.

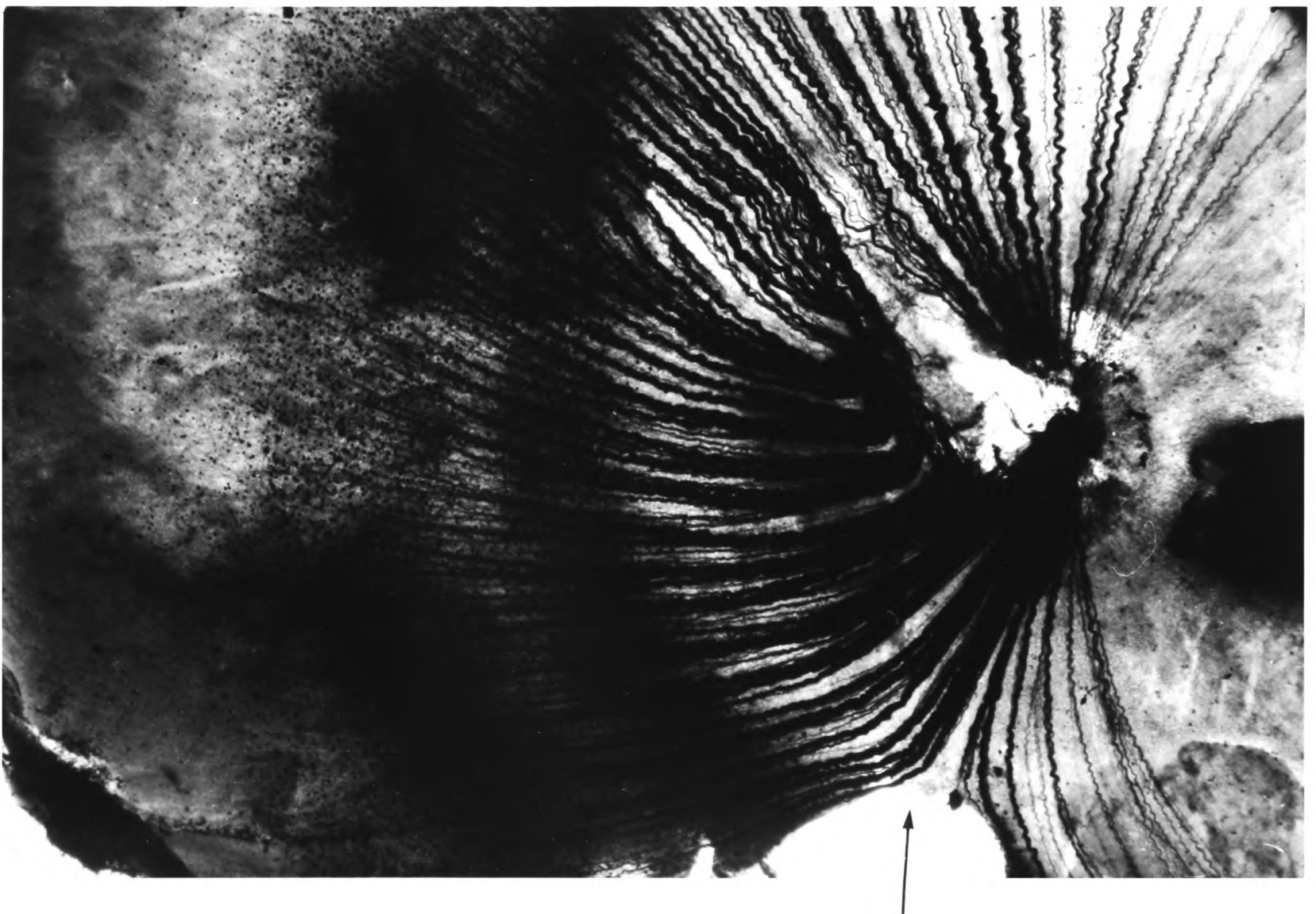
fig 5.2

a



b

500 μm



from the retinal wholemount, the appearance of which was strongly suggestive of intraaxonal transport of the HRP.

Because each retinal lesion necessarily interrupted not only ganglion cells but also fibres of passage from ganglion cells located more peripherally it was not possible to label central retinal cells without also labelling more peripheral cells in that sector. Injection sites were, therefore, confined to the edges of the retina in order to limit the size of the filled fibre population.

The characteristic appearance of HRP within the nerve after injection at most retinal locations was of a single irregular patch, occupying several of the large fascicles into which the optic nerve is partitioned by connective tissue. However in the case of ventral lesions spanning the choroid fissure (as indicated by the ventral radial mark on the iris) two separate patches of label were occasionally seen in the optic nerve (fig. 5.3).

Thus between eye and chiasm the above evidence clearly supports a highly ordered arrangement of fibres in the goldfish optic nerve. Moreover this ordering seems to be in the form of a ribbon with fibres situated at a particular radial distance from the optic nerve head grouped together in the nerve. Thus nerve lesions gave annuli of labelled ganglion cells. In view of the circumferential growth of the retina (Johns and Easter, 1977) this probably reflects a chronotopic ordering of fibres.

Such a ribbon arrangement would be formed by the opening out of the retina along one radius. Two pieces of evidence support a ventral position, corresponding to the choroid fissure, for that division point. The first is the high incidence of a sharp ventral boundary in partial annuli of ganglion cells stained by nerve injection, suggesting that the ventral most part of

Fig. 5.3. Photomicrograph of a cross section of the optic nerve of a fish after introduction of HRP into a ventral peripheral retinal lesion. The section is taken about 400 μ m behind the eye. Note the two small separate patches of staining (arrows).

Fig. 5.4. Photomicrograph showing the dorsomedial area of HRP staining in the medial brachium of the optic tract after HRP injection into the caudomedial edge of the dorsal left tectum. The arrow indicates the HRP.

fig 5.3



200 μm

500 μm

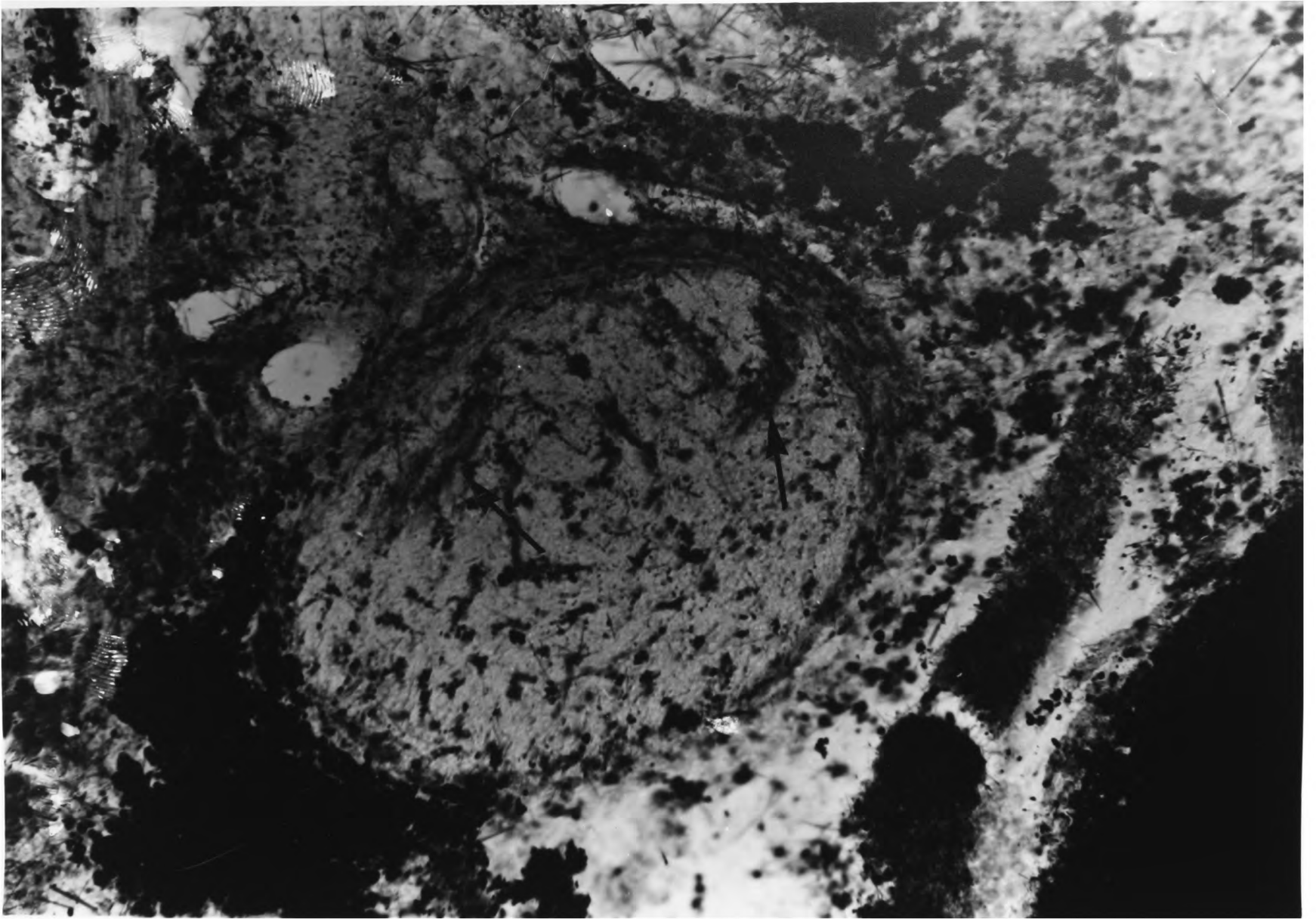
fig 5.4



Fig. 5.3. Photomicrograph of a cross section of the optic nerve of a fish after introduction of HRP into a ventral peripheral retinal lesion. The section is taken about 400 μ m behind the eye. Note the two small separate patches of staining (arrows).

Fig. 5.4. Photomicrograph showing the dorsomedial area of HRP staining in the medial brachium of the optic tract after HRP injection into the caudomedial edge of the dorsal left tectum. The arrow indicates the HRP.

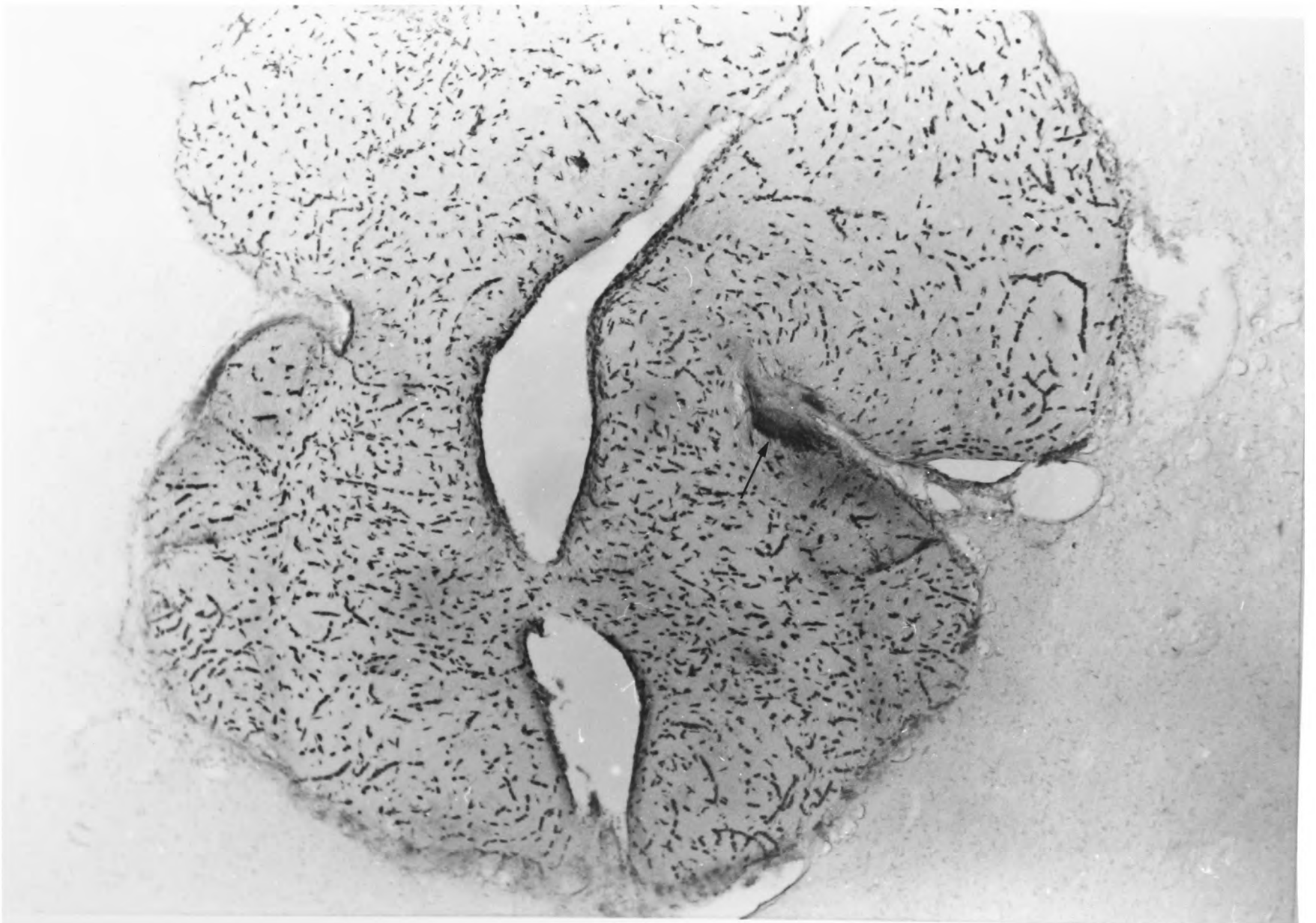
fig 5.3



200 μm

500 μm

fig 5.4



the retina is located at either edge of the ribbon. The second is the occasional production of two, widely separated regions of staining in the nerve after a ventral retinal HRP injection.

The high incidence of complete annular staining, and of two annuli separated by an unlabelled ring (fig. 5.2a) may be explained by glial partitioning of the nerve which has served to group together fibres formed at a similar time. Slight folding of the ribbon could then bring together two such bundles while displacing the intervening fibre bundle to one side so that it is not damaged by a lesion severing the other two.

Arrangement of fibres between chiasm and tectum

The above data indicate that when fibres reach the optic chiasm they are in the form of a ribbon with central retinal fibres at one end, peripheral fibres at the other, and ventral fibres at either edge. Further reorganization is however required to bring them into a pattern whereby they can form the observed projection on to the tectum.

1. Evidence from labelling of fibres in the tectum with HRP.

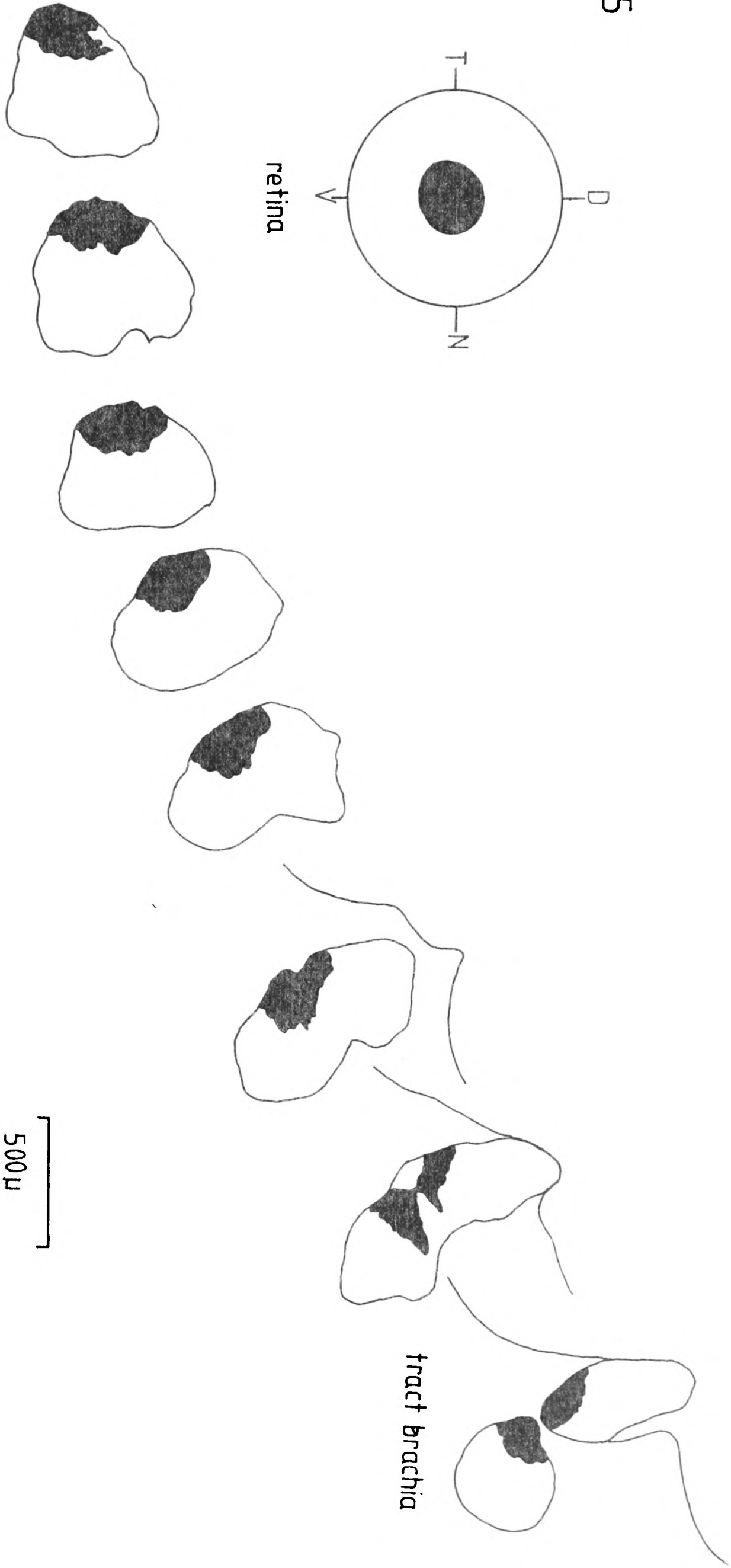
Due to uncertainty about the exact ganglion cells whose axons had been filled, arising because no successfully stained retinal wholemounts were obtained after tectal labelling, this group of animals did not provide any precise data on fibre organization in the optic tract. However small injections of HRP into the caudomedial edge of the tectum gave small compact patches of label at the dorsomedial edge of the medial tract brachium supporting the idea of a dorsal position in the tract for peripheral fibres (fig. 5.4).

Fig. 5.5. Camera lucida tracing of the pattern of labelling seen in the left optic tract after HRP injection into the right optic nerve just behind the eye. The right retina is shown schematically - central fibres were labelled. The injection was into the temporal edge of the nerve.

Fig. 5.6. Camera lucida tracing of the pattern of labelling in the left optic tract after HRP injection into the right optic nerve. The right retina is shown schematically - peripheral fibres are labelled. The injection was into the nasal edge of the nerve.

fig 5.5

chiasm



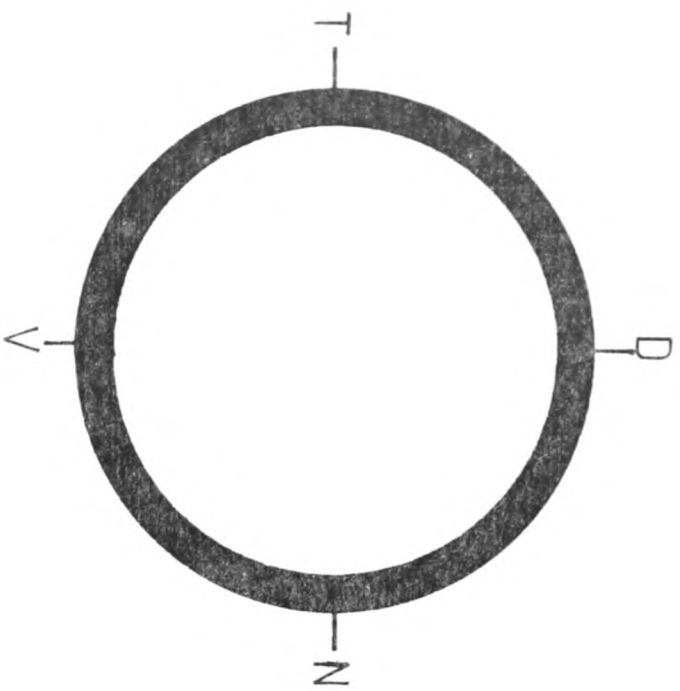
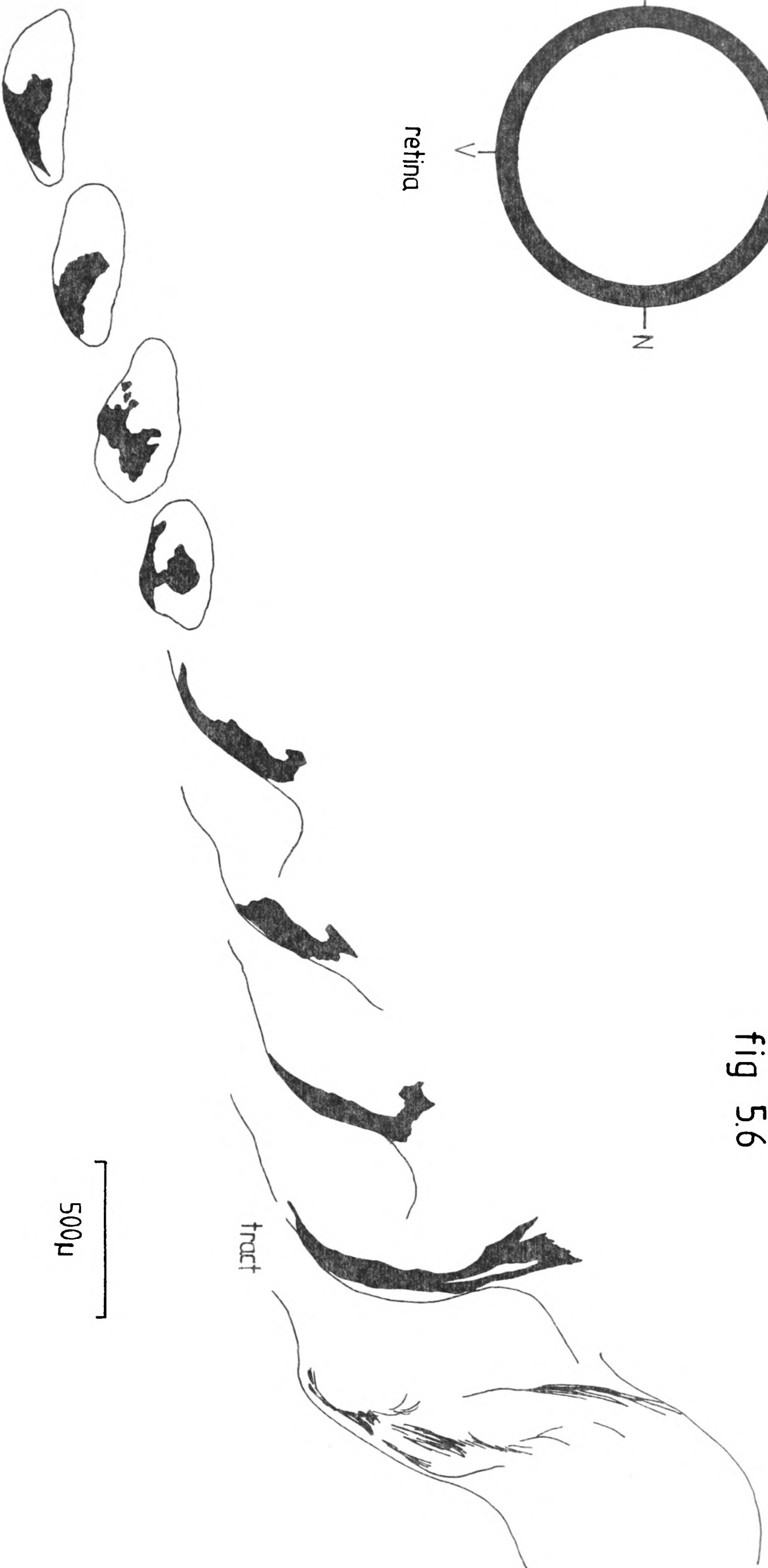


fig 5.6



chiasm

2. Evidence from HRP introduction into nerve lesions.

This proved the best method for gaining information about fibres in the optic tract as it gave both good staining in the post chiasmatic pathway and good labelling of ganglion cells.

When nerve injection of HRP produced labelling of only the most central part of the retina, the typical pattern of staining in the tract was of a single discrete patch at one edge, usually dorsomedial, which continued apparently unchanged to the point of tract division. Thereafter the brachia each carried half of the labelled fibres on their adjacent ventral edges round the diencephalon and towards the tectum. As the brachia reach the tectum they seem to rotate apart slightly so that central retinal fibres come to lie on the medial edge of the lateral brachium and the lateral edge of the medial brachium (fig. 5.5, 5.7).

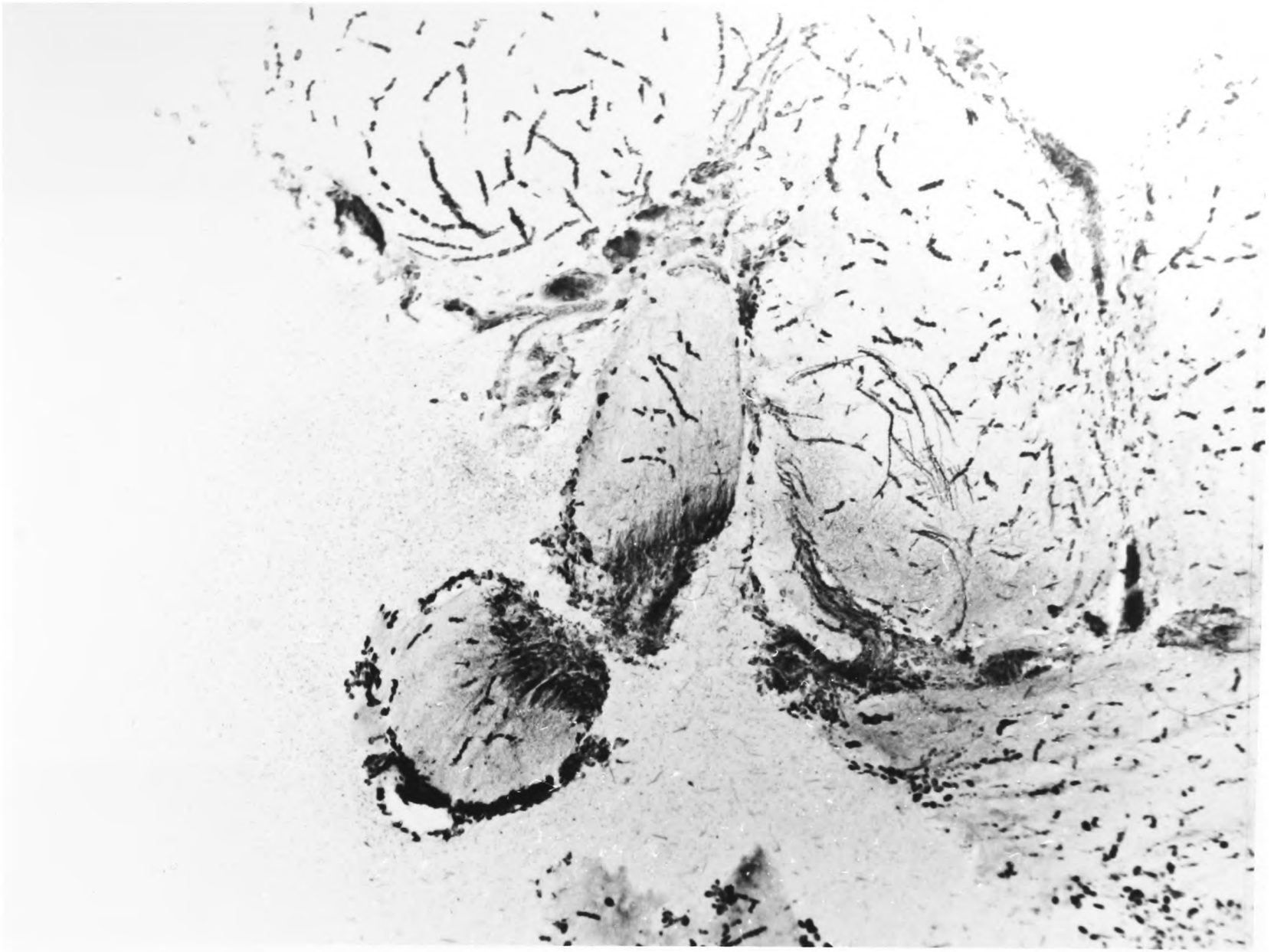
Labelling of peripheral most ganglion cells again gave a single compact area of staining at the chiasm though usually at the ventral edge of the nerve. However the fibres then passed laterally round the edge of the tract to occupy a peripheral lateral position. By the point of tract division the fibres had reached a laterodorsal position on top of the tract. Tract division and rotation then served to move apart the two groups of peripheral fibres so that they came to lie on the outermost edges of the brachia (fig. 5.6, 5.8).

Thus the tract just prior to the division into the two brachia seems also to form a chronotopic representation of the retina with central retina sending fibres into the ventromedial part while more peripheral retinal fibres pass laterally and dorsally to lie on the outside of the tract. With regard to the circumferential axis of the retina some reorganization is required to transform the ribbon from one opened along the dorsoventral axis to one spread out along a nasotemporal axis as the tract division splits the dorsal

Fig. 5.7. Photomicrograph showing the pattern of labelling seen in the right optic tract after HRP filling of central fibres from the left retina by injection into the nerve just behind the eye at the temporal edge.

Fig. 5.8. Photomicrograph showing the pattern of labelling seen in the right optic tract (on the left of the photograph) after HRP labelling of peripheral fibres from the left retina by injection into the nerve just behind the eye at the nasal edge.

fig 5.7



500 μ m

fig 5.8

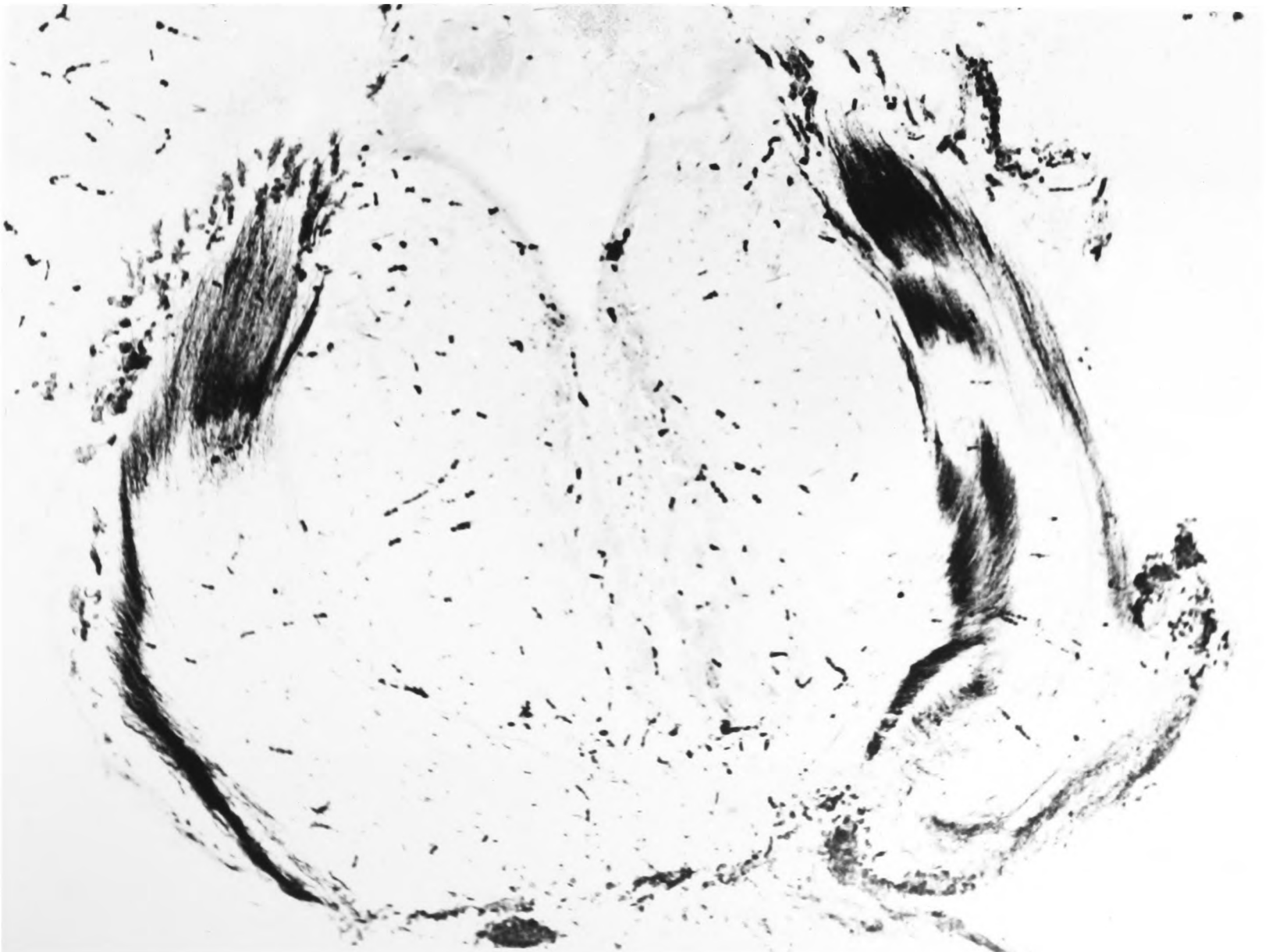
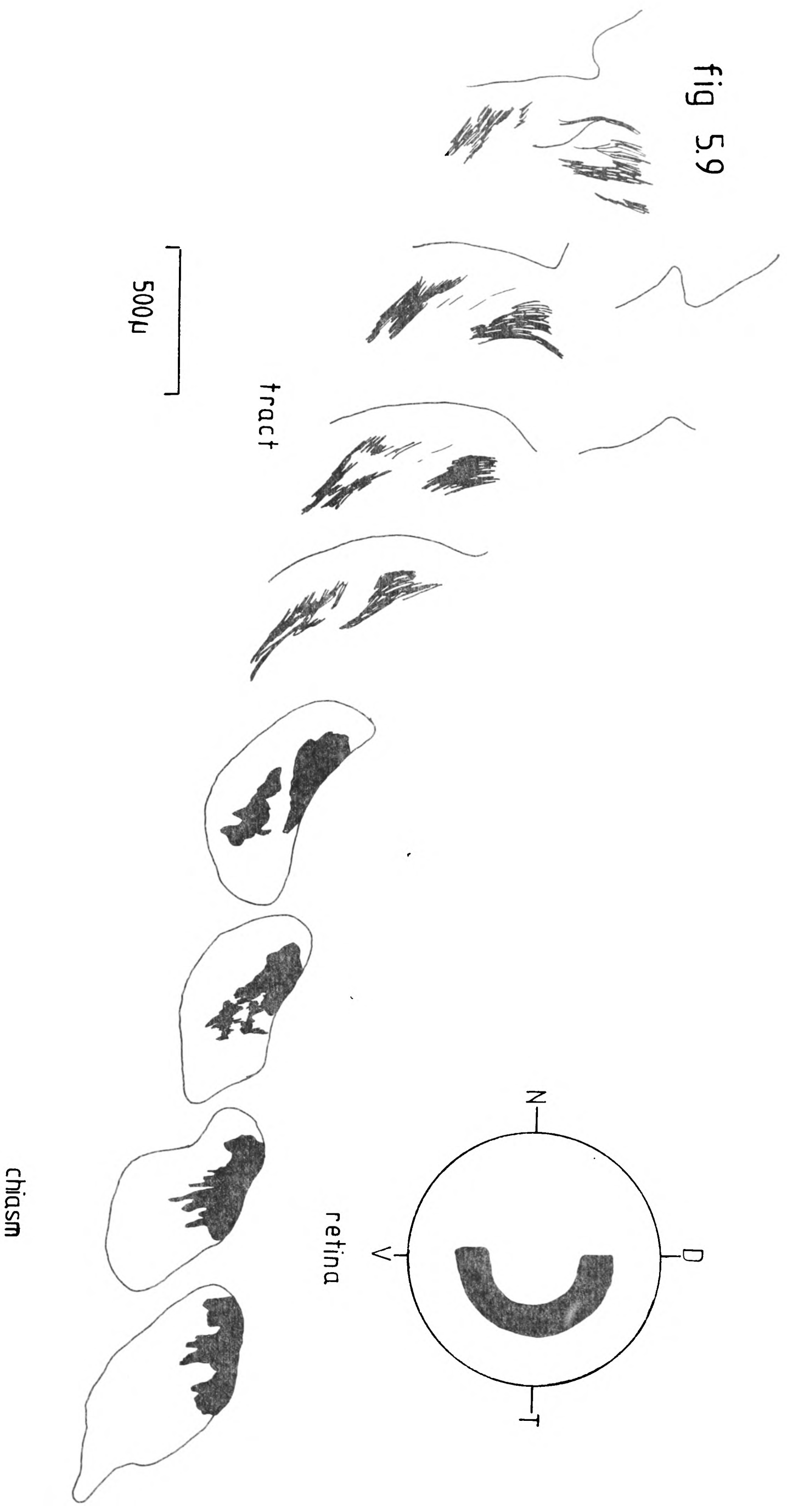


Fig. 5.9. Camera lucida tracing of the area of staining in the right optic tract after HRP had been injected into the left optic nerve just behind the eye. The retina is shown schematically - a partial annulus of cells on the temporal side of the retina in the mid-region of the retina was stained.

fig 5.9



fibres from the ventral ones. That this happens along a temporal radius is suggested by the appearance of staining in animals in which a partial temporal annulus of cells has been filled. Initially one patch of label is seen prior to the chiasm but more caudally this is seen to divide and form two patches on opposite edges of the tract prior to its division (fig. 5.9).

A model for the reorganization of optic fibres within the goldfish visual pathway

As indicated in fig. 5.10. it is not possible to explain the production of the observed retinotectal projection in the goldfish without some reordering of the fibres including a reversal of one axis independent of the other. The following model is therefore proposed in order to explain both the observed patterns of staining seen after HRP labelling and the need for this reorganization.

Firstly, on passage through the optic nerve head fibres become divided along a ventral radius to form a "ribbon" in which fibres from central retina lie dorsally, or perhaps, after glial partitioning of the nerve, more temporally, and fibres from peripheral retina lie ventrally (fig. 5.11, step 1). This arrangement would agree well with the annular patterns of staining seen after nerve injection of HRP. It's developmental origin might, as proposed by Bunt and Horder (1977), lie in the guidance of fibres through the choroid fissure in the optic cup by mechanical forces. Later added fibres would then join the pathway in an orderly manner by fasciculation with preexisting fibres originating on the same retinal radius.

The final consequence of guidance over the optic stalk proposed by Bunt would be the reclosing of the ribbon to form a polar representation of the retina with one axis, the dorsoventral, reversed compared with the retina (step 2). In the adult goldfish this process appears to be delayed until after the chiasm with the ribbon arrangement maintained through the

chiasmatic region. However this may simply be the consequence of pathway elongation as the fish grows. The distance between the formation and dissolution of the ribbon may obscure a close developmental relationship.

The growing tectum is then postulated to split this array along a temporal radius and to separate the fibres into two groups (steps 3 and 4). This would leave central and early formed fibres lying against the diencephalon with more recent fibres added at the edge of the brain, as has been observed above. Within the chronotopic organization of the tract divisions temporal fibres would then lie at the far edges with nasal fibres at the adjacent borders of the two brachia. Slight rotation apart of the two brachia would orientate fibres correctly in order to produce the observed retinotectal map (steps 5 and 6).

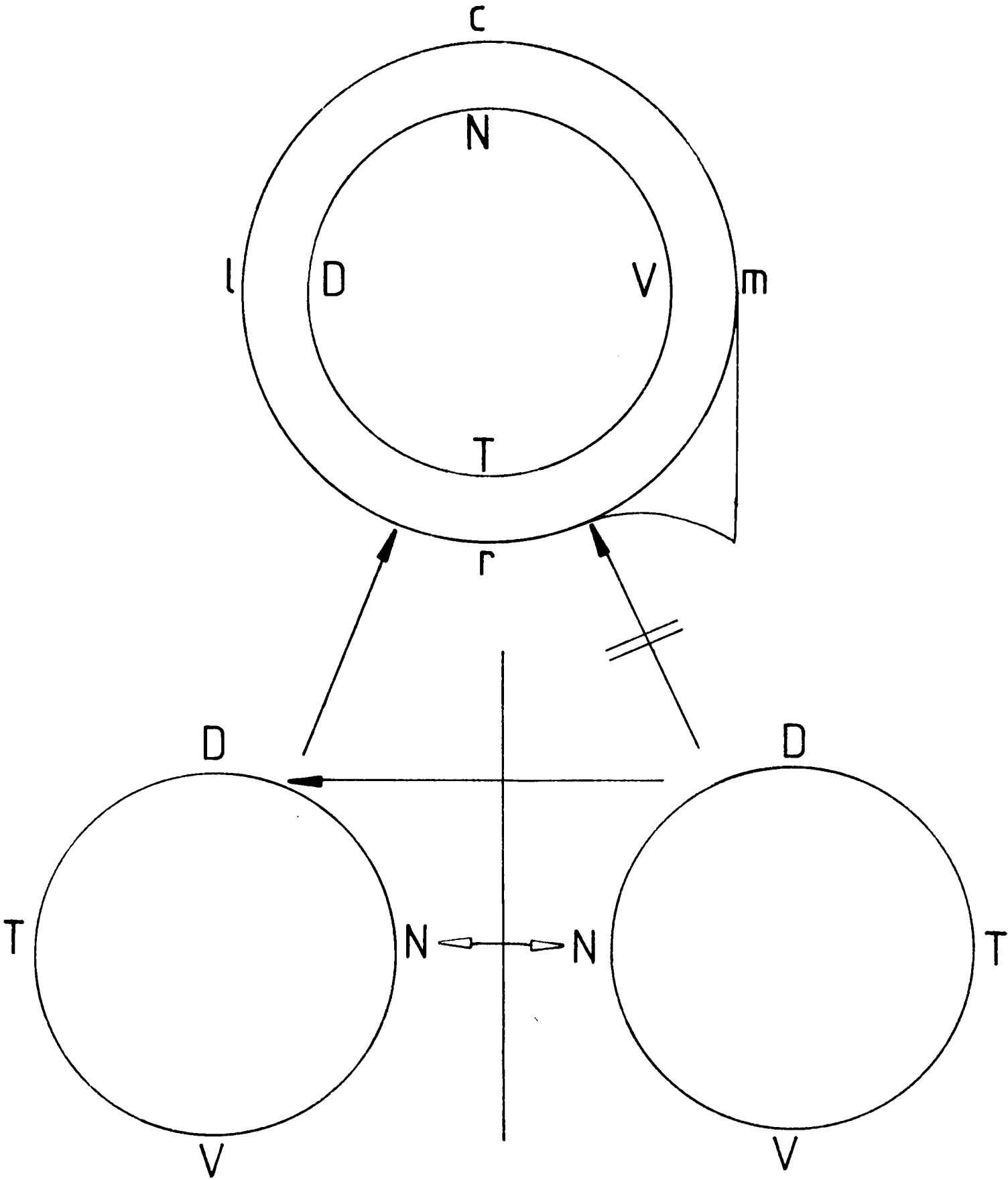
Discussion

The results described above confirm the long-held view that within the optic pathway of the goldfish fibres are arranged in a highly ordered manner reflecting the retinal positions of their cell bodies (Stroer, 1940; Horder, 1974a; Roth, 1974). However in contrast to the degeneration study of Roth, which suggested a retinotopic ordering, they strongly support the idea of a ribbon arrangement in which fibres of a similar radial position in the retina are grouped together. Thus the radial position in the retina would be expressed along one axis of the ribbon and circumferential position along the other axis. Such an arrangement has been interpreted as "chronotopic" (Dawnay, 1979b; Rusoff and Easter, 1980) as the growth of the retina by addition of cells around its margin (Johns, 1977) means that ganglion cells situated at the same radial distance from the optic nerve head were generated at about the same time. Such a model is consistent with the presence of unmyelinated fibres, presumably derived from the most recently generated ganglion cells, at one edge of the nerve in a compact group (Dawnay, 1979a).

Fig. 5.10. The arrangement of fibres in the left retina will not, even after rotation, give the observed projection to the right tectum without reordering. The required reordering is given by a mirror-image inversion of the ordering - in the diagram this is shown as a N-T reversal though theoretically a D-V reversal would also suffice.

fig 5.10

Right tectum



Left retina

Fig. 5.11. Diagram summarizing the proposed model for the organization of the goldfish retinotectal pathway.

Step 1: formation of a ribbon nerve by guidance of fibres over the optic cup rudiment.

Step 2: closure of the ribbon to give a mirror image of the retinal arrangement of fibres.

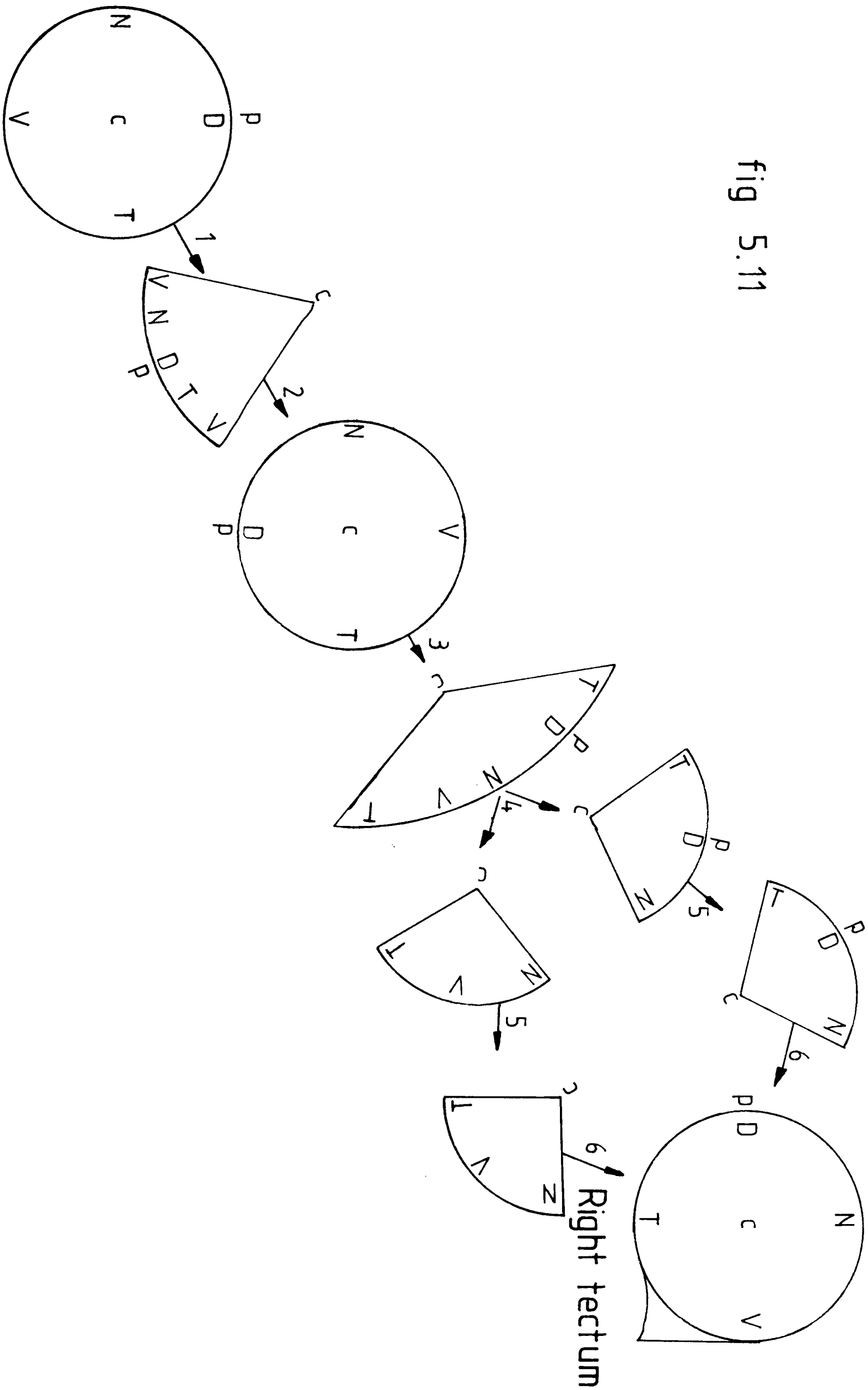
Step 3: splitting of this array along a temporal radius.

Step 4: pulling apart of the two tract brachia.

Step 5: rotation apart of the brachia.

Step 6: projection on to the optic tectum.

fig 5.11



Left retina

The opening of the retina along a ventral axis, indicated by the positions of the boundaries of partial annuli of filled cells, agrees with Scholes' (1979) suggesting of the arrangement of fibres within the cichlid fish optic nerve and indicates an important role for the choroid fissure in the developmental origin of the optic nerve. This was proposed by Bunt and Horder (1977) whose model employed simple mechanical guidance of first formed fibres over the optic cup and through the choroid fissure into the optic stalk. This would generate a ribbon nerve as a transitory step in the refolding of the fibre array into a retinotopic pattern reversed along one axis only. Unlike Bunt and Horder's model, the model outlined above involves delay of the final part of this refolding until the chiasm is reached for a ribbon arrangement seems to be present throughout the optic nerve. However refolding may still reflect the sort of guidance of optic fibres round the surface of the optic stalk envisaged by Bunt and Horder.

Reopening of the folded, mirror-reversed, array along a temporal radius then reforms a chronotopic pattern in the optic tract, into which new fibres can easily be fitted as the retina continues to grow. Such an arrangement of fibres in the optic tract accords both with the experimental data described above and the arrangement seen in the optic tract of *Xenopus laevis* (Gaze and Grant, 1978).

The overall rearrangement of the optic fibres as they pass from retina to tectum in the goldfish proposed in this chapter resembles the arrangement seen within one subunit of fibres in the cichlid fish model (Scholes, 1979). However as the whole fibre array undergoes reorganization as one unit there are no discontinuities generated, as at the boundaries between subunits in Scholes' model. Furthermore this model allows easy incorporation of new fibres into the correct place in the array as the most peripheral fibres are envisaged as remaining at the edge of the fibre array throughout the pathway.

A mechanical explanation of the mechanism for the proposed reorganization of fibres seems feasible when considering just the retinotectal fibres. However there remains a considerable problem in explaining how retinotopic projections to other nuclei, such as the nucleus rotundus and the lateral geniculate nucleus (Sharma, 1972a) are derived from the same fibre array without invoking more specific guidance signals than contact guidance and fasciculation.

Chapter 6

The morphology of optic terminal arborizations in the goldfish tectum

Introduction

Though investigations of the formation of neuronal connections in the goldfish visual system have made extensive use of electrophysiological recordings from the presynaptic terminals of retinal ganglion cell axons (see Gaze, 1970 for a review of this method) there exists remarkably little information about the size and shape of these-terminal arborizations in either the normal or the experimental animal. This may reflect a lack of a suitable method to stain selectively a few relatively extensive, by histological standards, terminals and allow their delineation. Golgi studies, while providing selective staining, on some unknown basis, of a small sample of cells and their processes, have been hampered by the need to use tissue sections. The reconstruction of individual terminals is thus extremely tedious. The Golgi investigations of Sharma and colleagues (Sharma, 1972a ; Romeskie and Sharma, 1977a, 1979) and Potter (1976) have furnished excellent accounts of the morphology of cells in the fish tectum but provided little data on the optic fibre terminals beyond their lamination pattern. A similar reconstruction problem arises with electronmicroscopy and Ito's work (1970) had described the synaptic terminations but not the overall anatomy of the terminals.

With regard to amphibia slightly more information is available. Golgi stains have facilitated description of the optic fibre arborizations in the tecta of Rana catesbiana (Potter, 1968, 1969, 1972), Rana esculenta (Lazar and Szekely, 1967; Szekely et al., 1973), and Xenopus laevis (Lazar, 1973). In each case the pronounced lamination of fibre terminals has been stressed. A variety of arborization forms have been described, some showing extensive branching over an area extending up to 150 μ m in length while others seem to consist of beaded axons ending freely without branching (Potter, 1969). Considerable overlap was observed between

neighbouring branched arbors.

Lazar has investigated the change in terminal arborization size and shape in the developing Xenopus laevis larva. He recorded a gradual evolution from the flat elongated terminals ($20\text{-}30\mu\text{m}$ by $150\text{-}180\mu\text{m}$) which first appeared at stage 50 (Nieuwkoop and Faber, 1956) to a longer arbor at stage 57 which still lacked many secondary and tertiary branches, to the adult arbor which was finely branched and had a maximum length of $150\mu\text{m}$. Similar results have been obtained in larval Xenopus laevis using cobalt staining after large retinal lesions (Piper, et al., 1980).

An attempt has also been made to determine the extent of optic fibre arborizations in the tectum of adult Rana pipiens using electrophysiological techniques (George and Marks, 1974). Employing two electrodes they examined the extent of a single arborization by looking for coincident spike activity after stimulation of its ganglion cell. The results were consistent with the histological data, indicating the terminals to be elongated in the direction of the ingrowing axon and to extend up to $150\mu\text{m}$ across the tectum.

Method

Small injections of HRP solution were made into the optic nerves of normal goldfish and of goldfish in which the nerve had been cut behind the eye 18-41 days previously and allowed to regenerate at room temperature ($18\text{-}22^{\circ}\text{C}$). After 20-24 hours the animals were killed and the tecta processed as described in chapter 2 using the method extensively modified from that of Hanker et al (1977).

Relatively isolated terminals at the edge of the main area of staining were located and drawn using a camera lucida attachment on a binocular microscope.

For each camera lucida drawing of an axon terminal arborization four measurements were made:

1. the axon axis - this was the length of the arborization measured from the first branch point in a direction parallel to the parent axon.
2. the axis orthogonal to the axon axis.
3. the area of the arborization - this was measured by plotting the terminal envelope (drawn to join the distal ends of the branches) on to graph paper.
4. the number of branch points.

These possible limitations exist for this method of determining tectal arborization morphology:

1. It is not possible to be sure that the entire extent of the arborization is filled with HRP and is thus visible. This would require electron-microscopic confirmation which is not available. Thus the terminals drawn with camera lucida show the minimum extent of the arborizations.
2. The observed arbor may be derived from more than one axon. To minimize the risk of drawing composite arbors the parent axon was traced back as far as possible for at least 500 μ m and often to the rostral pole of the tectum. If at any point along its often tortuous course there was any indication that two or more axons were running together that arbor was rejected.
3. The HRP filled arborizations may not form a representative sample of the complete range of tectal terminals. There is unfortunately no Golgi study with which these results may be compared but they do show arborizations fed by a range of axon sizes, as judged by eye.

Results:

Normal Fish

Typical normal optic fibre terminals are shown in figures 6.1 and 6.2. Nineteen terminals were analysed and found to be slightly elongated in the direction of axon ingrowth (axon axis = $177 \pm 47 \mu\text{m}$, mean $\pm$ standard deviation: orthogonal axis $145 \pm 40 \mu\text{m}$). They covered an average area of about $16 \times 10^3 \mu\text{m}^2$ ($15.7 \pm 5.1 \times 10^3 \mu\text{m}^2$). Each terminal showed extensive branching with a mean of $26.1 (\pm 10.4)$ branch points at a density of 1.79 ± 0.63 branch points per $1000 \mu\text{m}^2$. The distributions of terminal size, area and branching are shown in the histograms of fig. 6.3 and 6.4 and 6.5.

An interesting morphological feature seen in several of the normal terminals is the presence of swellings on the main axon and its branches, occurring predominantly at branching points. These swellings resemble the boutons en passant described by Potter in the bullfrog (Potter, 1969). One such terminal is shown in fig. 6.1b.

Optic nerve regeneration

Twenty three terminals were analysed from fish 18-41 days after optic nerve cut and regeneration. Typical terminals are shown in figures 6.6 and 6.7. The areas of the regenerated terminals and their branch point numbers and densities were plotted against duration of regeneration (figs. 6.8 and 6.9). As no significant trends were observed with time in any of the three graphs all the data on regenerated terminals were pooled.

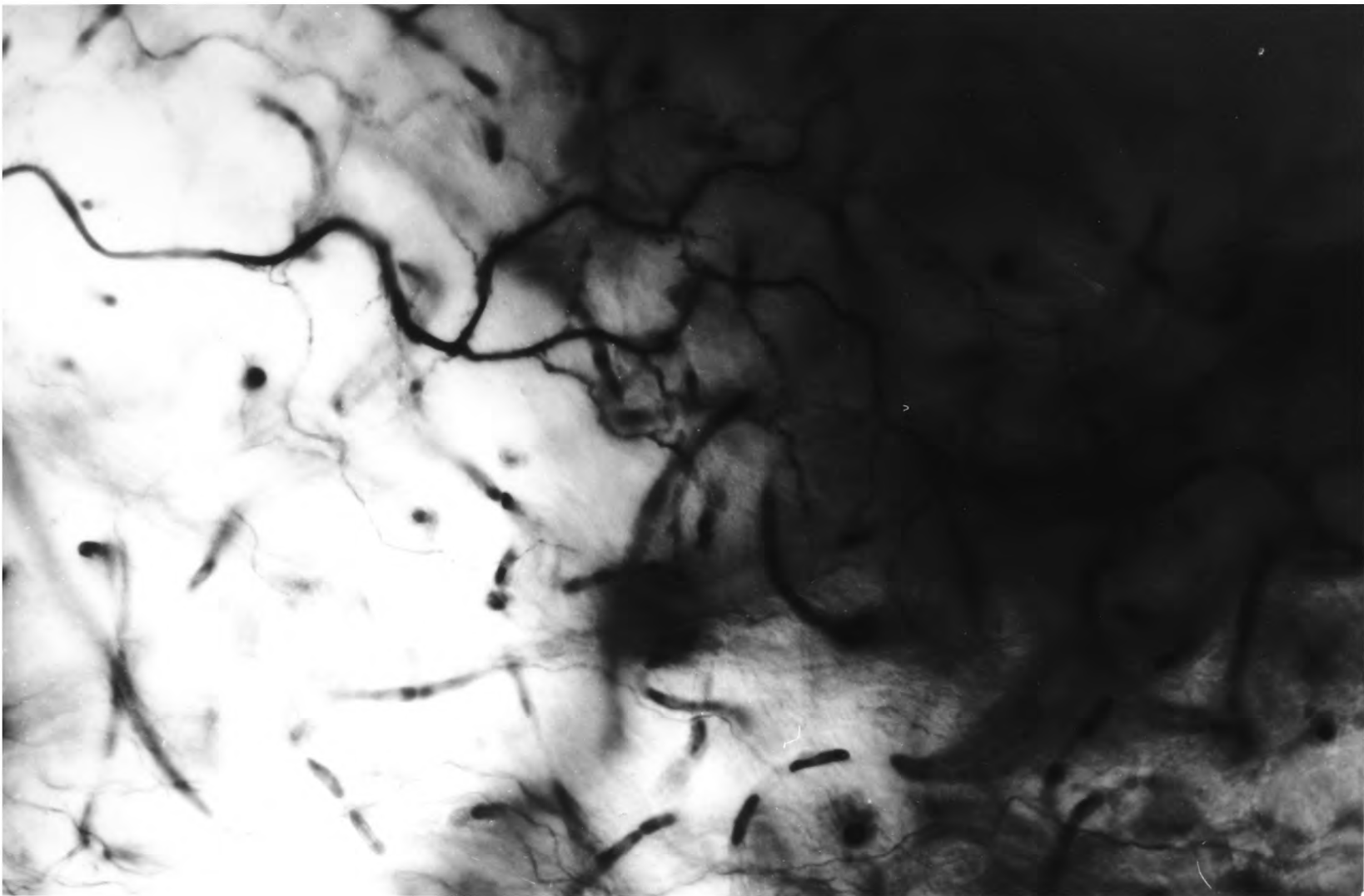
Again the terminals were elongated in the direction of axon ingrowth (axon axis = $164 \pm 56 \mu\text{m}$; orthogonal axis = $122 \pm 46 \mu\text{m}$). Though neither dimension was significantly different from the normal values the terminal area was significantly reduced ($p = 0.014$, Mann-Whitney "U" test - two tailed). The number of branch points per terminal was also reduced ($p = 0.0014$, Mann-Whitney "U" test - two tailed) though the branch point density was not

Fig. 6.1. Photomicrographs showing HRP filled terminals in wholemounts of the normal goldfish optic tectum. Note the axonal swellings resembling "boutons en passant" in the lower photograph. Photomicrography gives a poor impression of the extent of the arborizations due to variations in the depth of focus required to visualize various parts of the terminal and to the great technical difficulties encountered in illuminating these wholemount preparations for photography.

Fig. 6.2. Camera lucida tracings of the branching patterns of terminals in the normal goldfish optic tectum as revealed by HRP filling. No attempt was made to represent the width of the branches accurately.

fig 6.1

a



50 μm

b

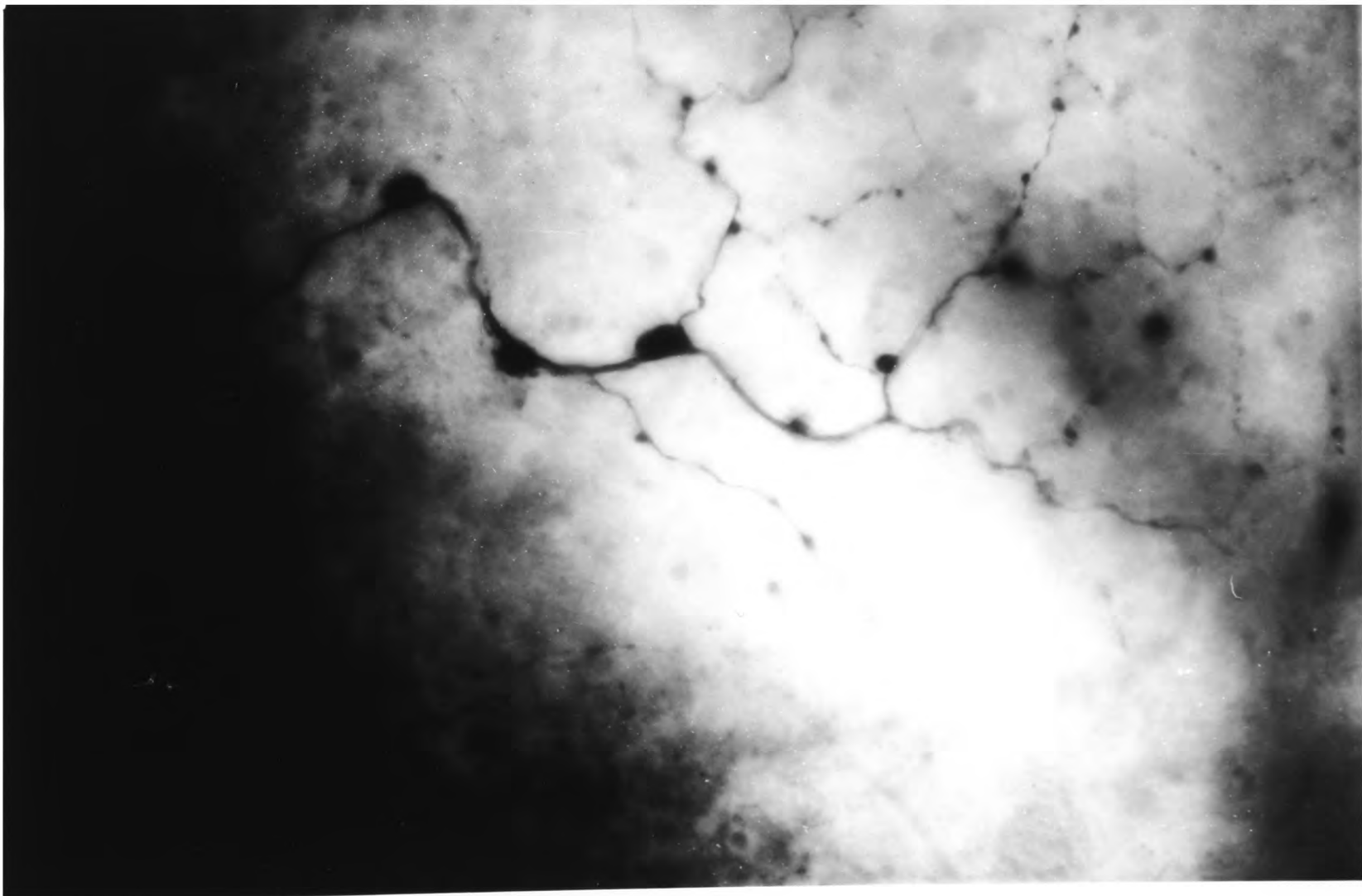


fig 6.2

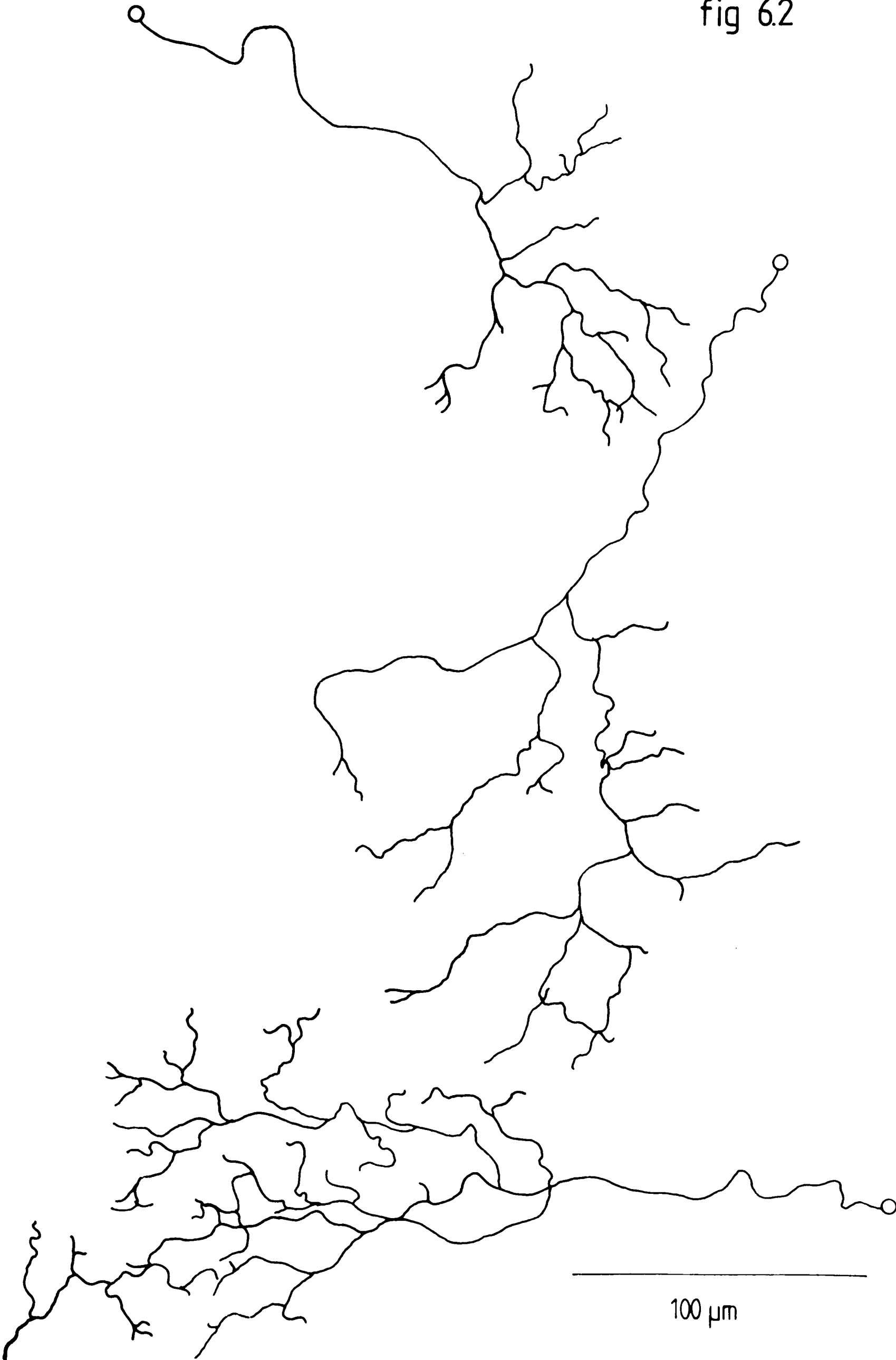


fig 6.2

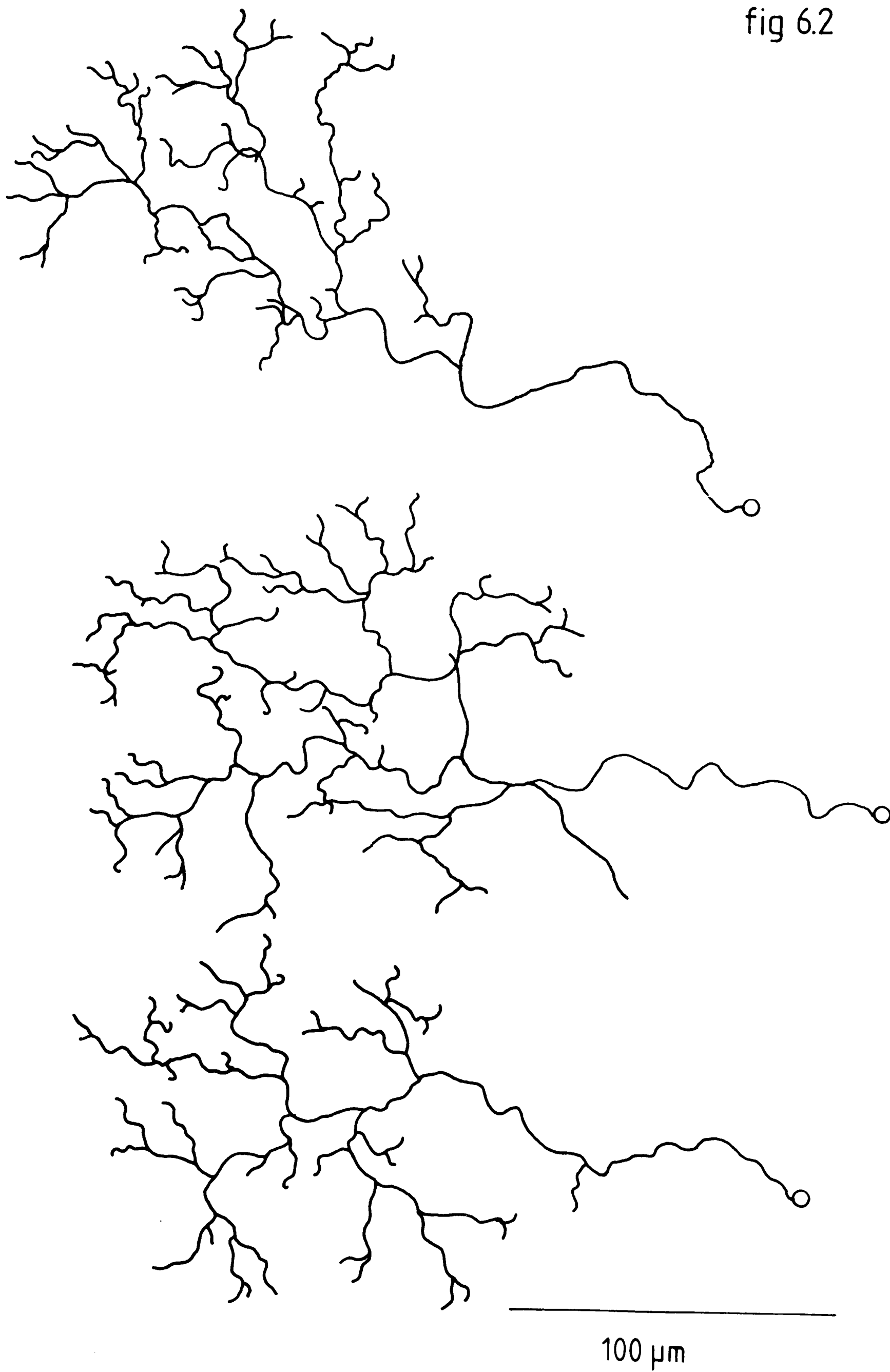


Fig. 6.3. Histograms showing the distribution of terminal arborization sizes.

top left - normal terminals, axonal axis

top right - normal terminals, orthogonal axis

bottom left - regenerate terminals, axonal axis

bottom right - regenerate terminals, orthogonal axis

Fig. 6.4. Histogram showing the distribution of terminal arborization areas (define as the area within an envelope linking distal branch ends).

top - normal terminals

bottom - regenerate terminals

Fig. 6.5. Histograms showing the distribution of branching parameters.

top left - normal terminals, number of branch points

top right - normal terminals, branch point density

bottom left - regenerate terminals, number of branch points

bottom right - regenerate terminals, branch point density

fig 6.3 Size

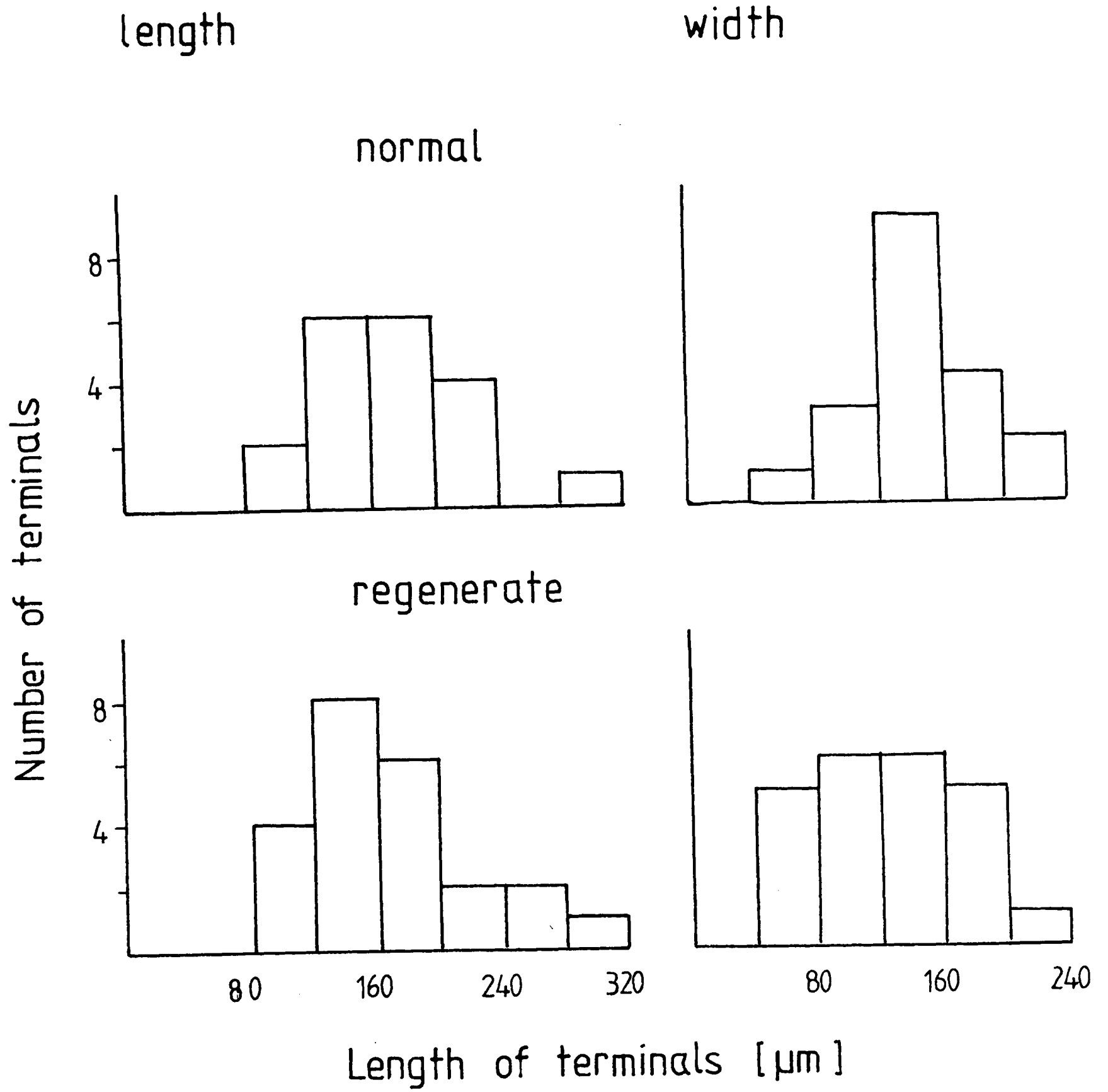


fig 6.4 Area

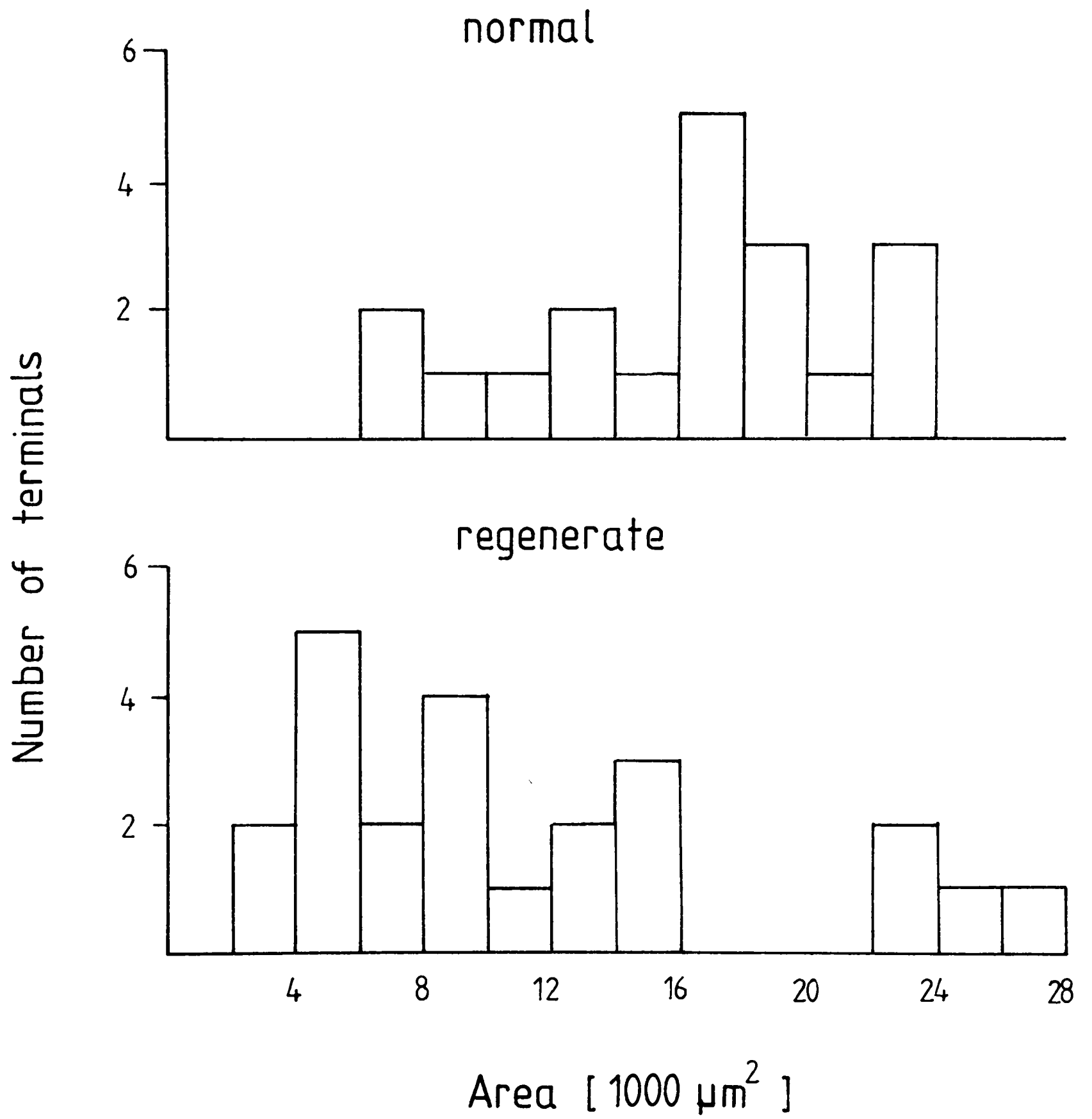


fig 6.5 Branches

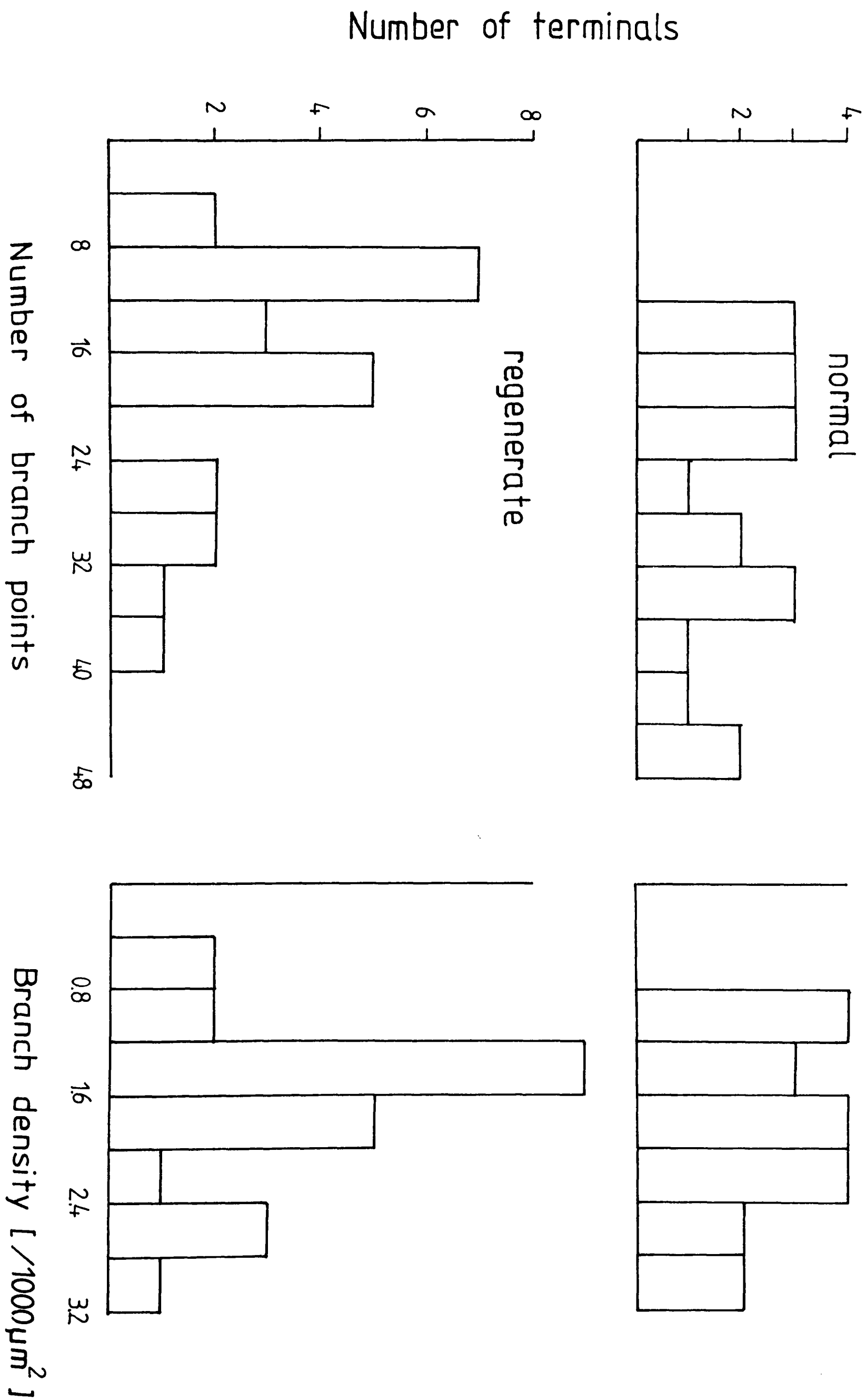
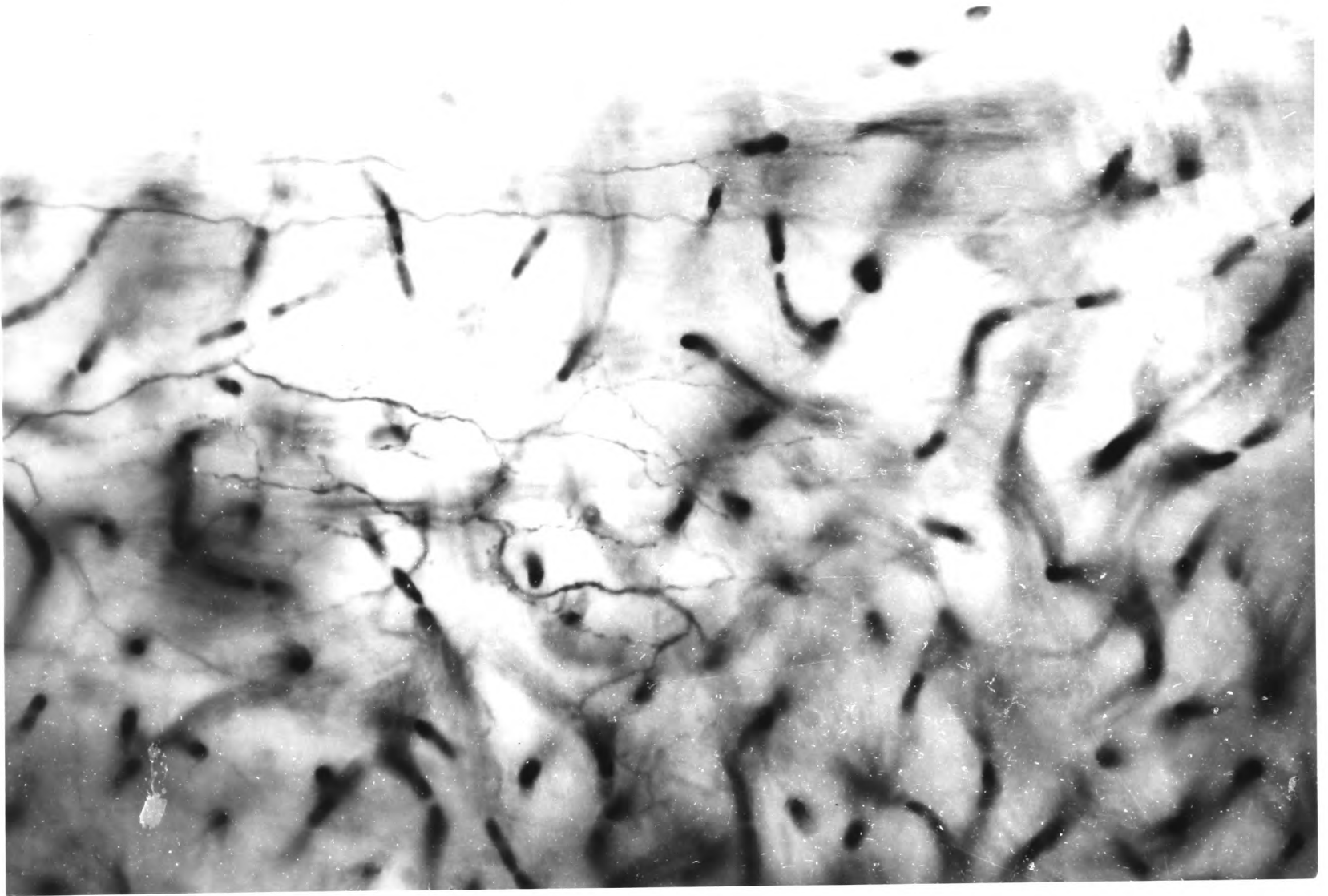


Fig. 6.6. Photomicrographs of HRP filled terminals in the tecta of goldfish (a) 28 and (b) 33d after cutting the optic nerve and allowing regeneration at room temperature (18-22°C). These give a poor impression of the arborization as the whole terminal does not lie at the same depth of focus in the preparations. Furthermore it proved very difficult to illuminate these whole-mounts adequately for photography.

Fig. 6.7. Camera lucida tracings of the branch patterns of regenerated optic terminals of various ages as revealed by HRP filling in wholemount preparations. No attempt has been made to represent accurately the thickness of the branches.

fig 6.6

a



b

50 μ m

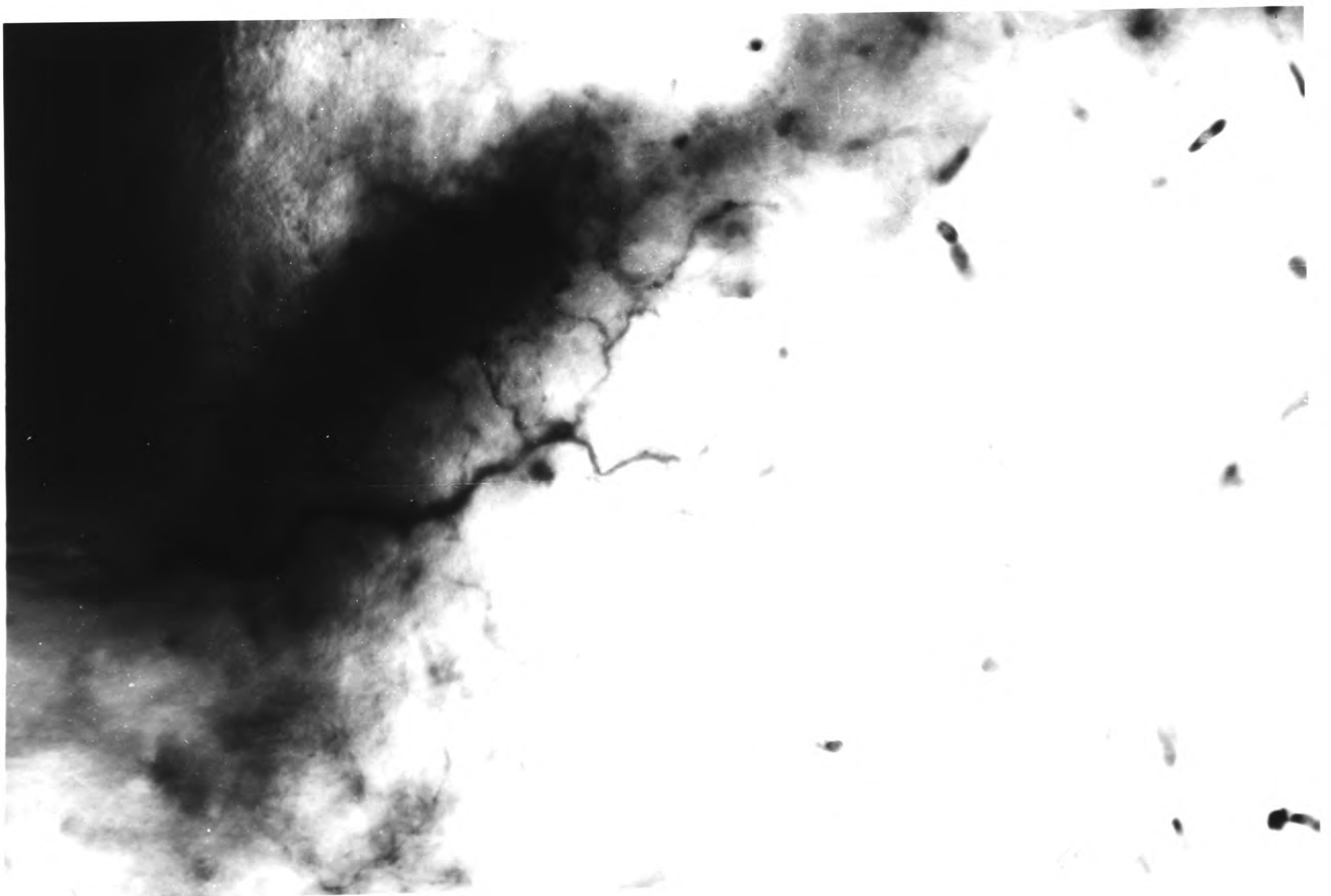


fig 6.7

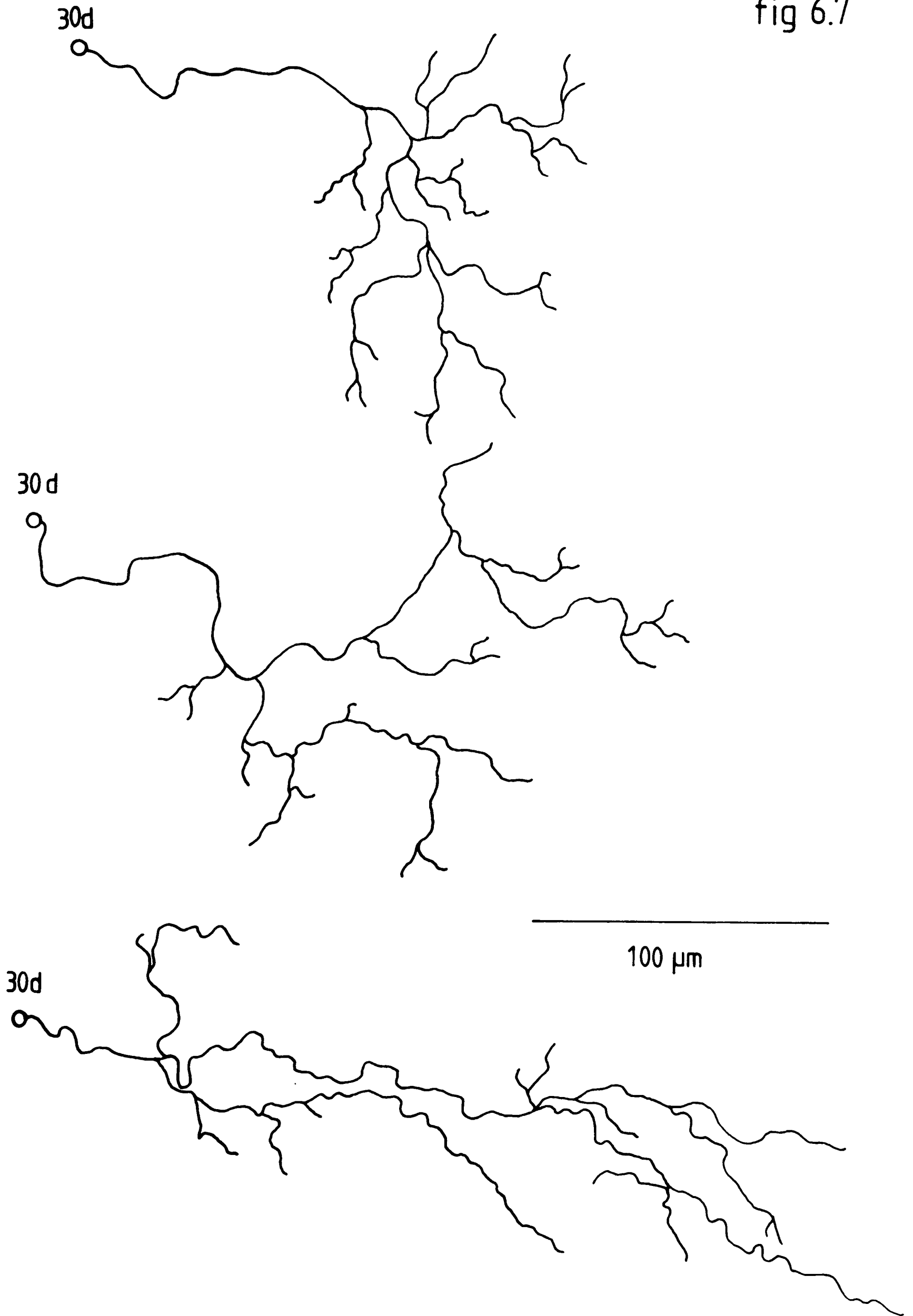


fig 6.7

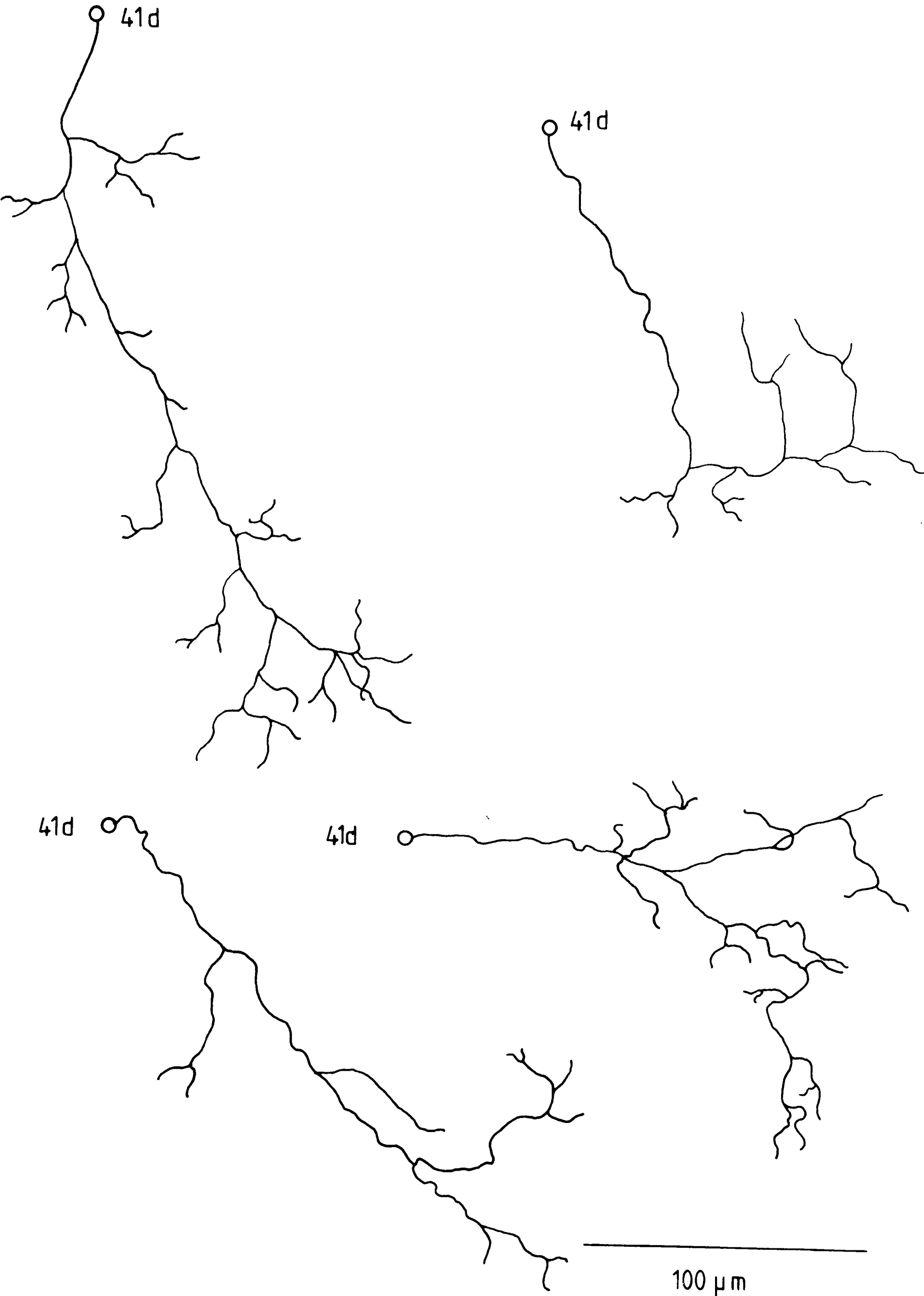


Fig. 6.8. Graph of terminal arborization area (in $1000\mu\text{m}^2$) against time after intraorbital optic nerve cut. N shows the mean ($\pm$ standard deviation) of the value for normal terminals.

Fig. 6.9. Graphs of branching parameters against time after intraorbital optic nerve cut. N shows the mean ($\pm$ standard deviation) values for normal terminals.
top - branch point density
bottom - branch point number

Fig. 6.10. Graph of total number of branches of each terminal arborization against its area for both normal (O) and regenerate (●) terminals.

fig 6.8 Area

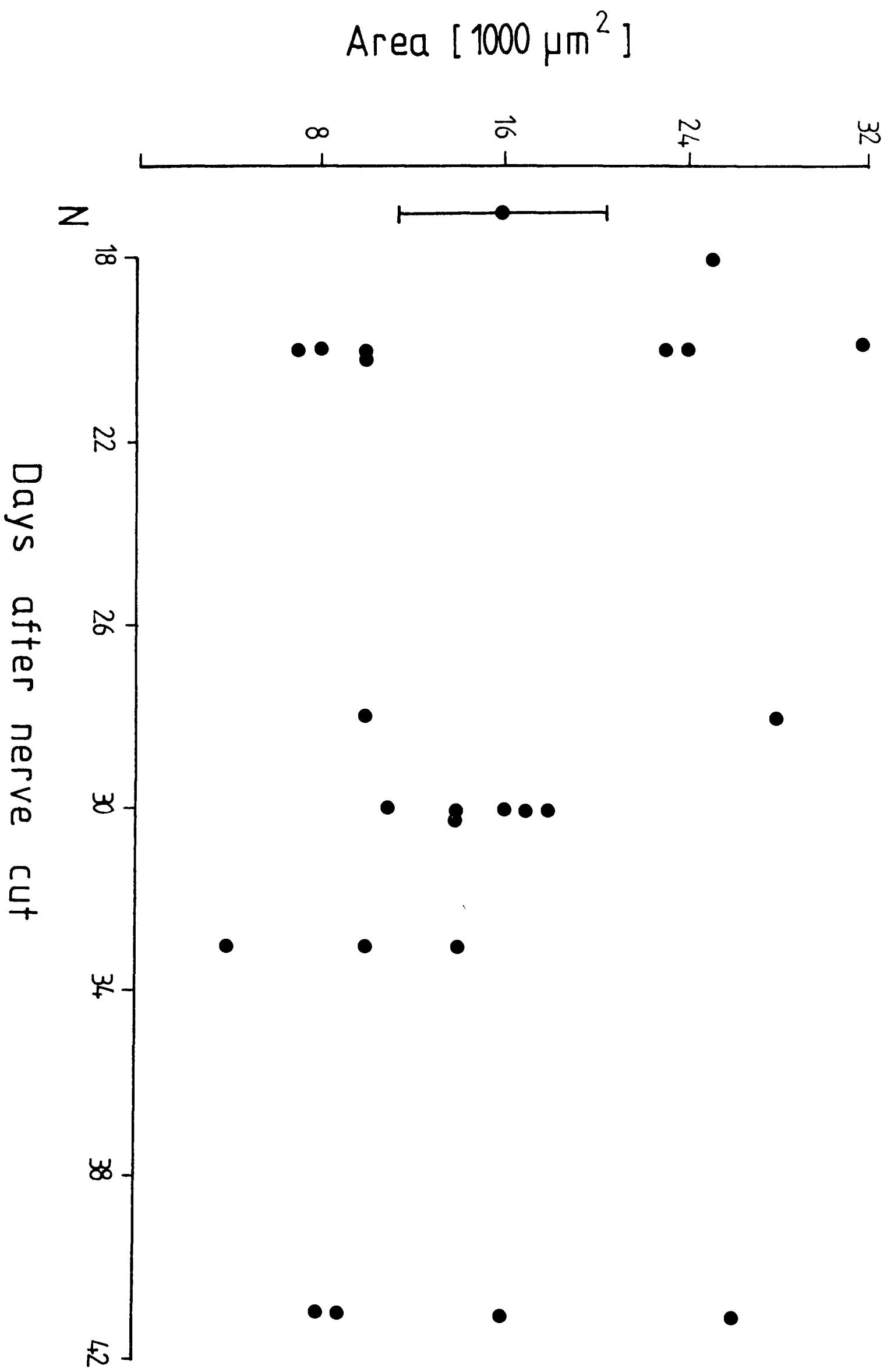


fig 6.9 Branches

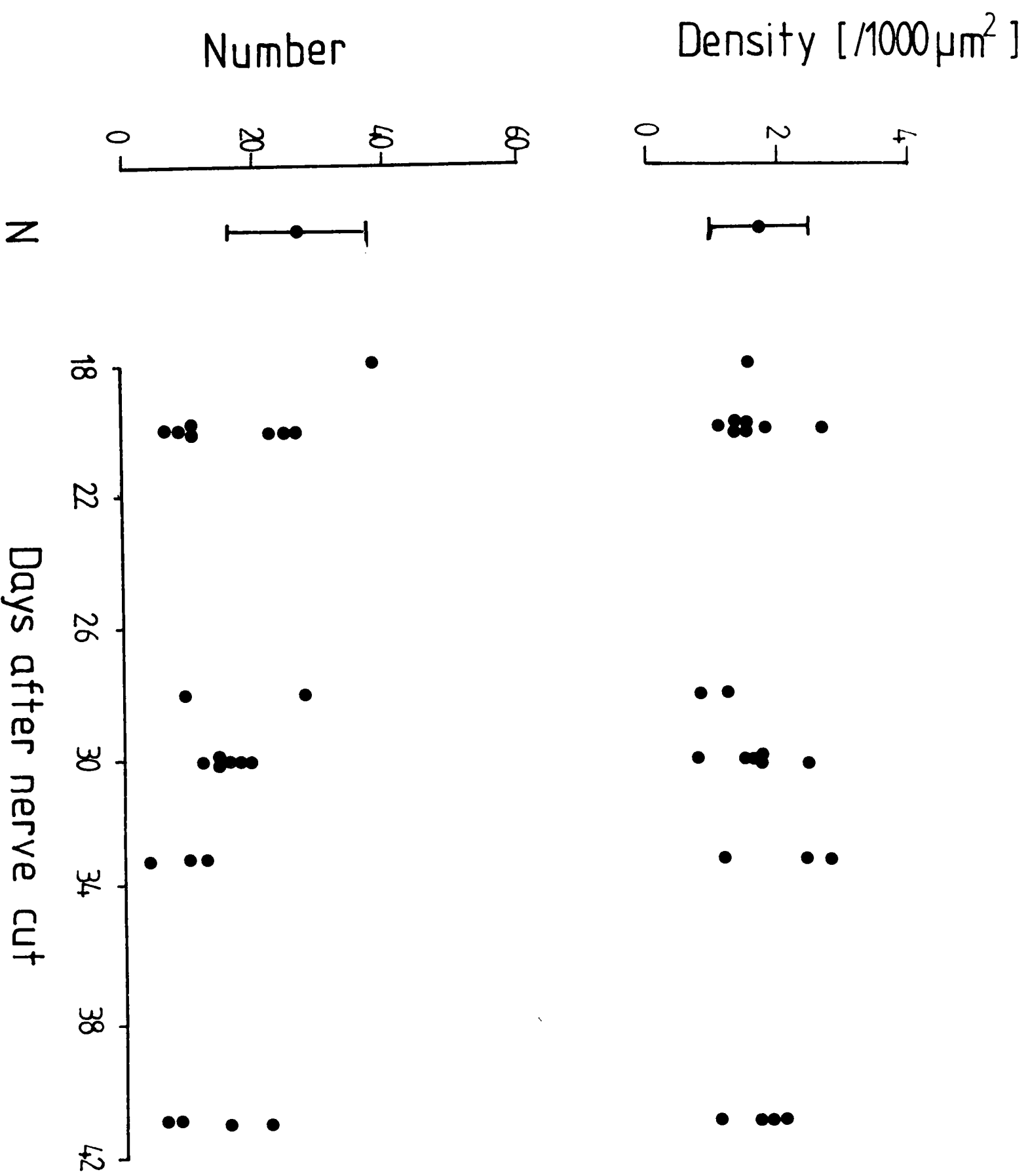
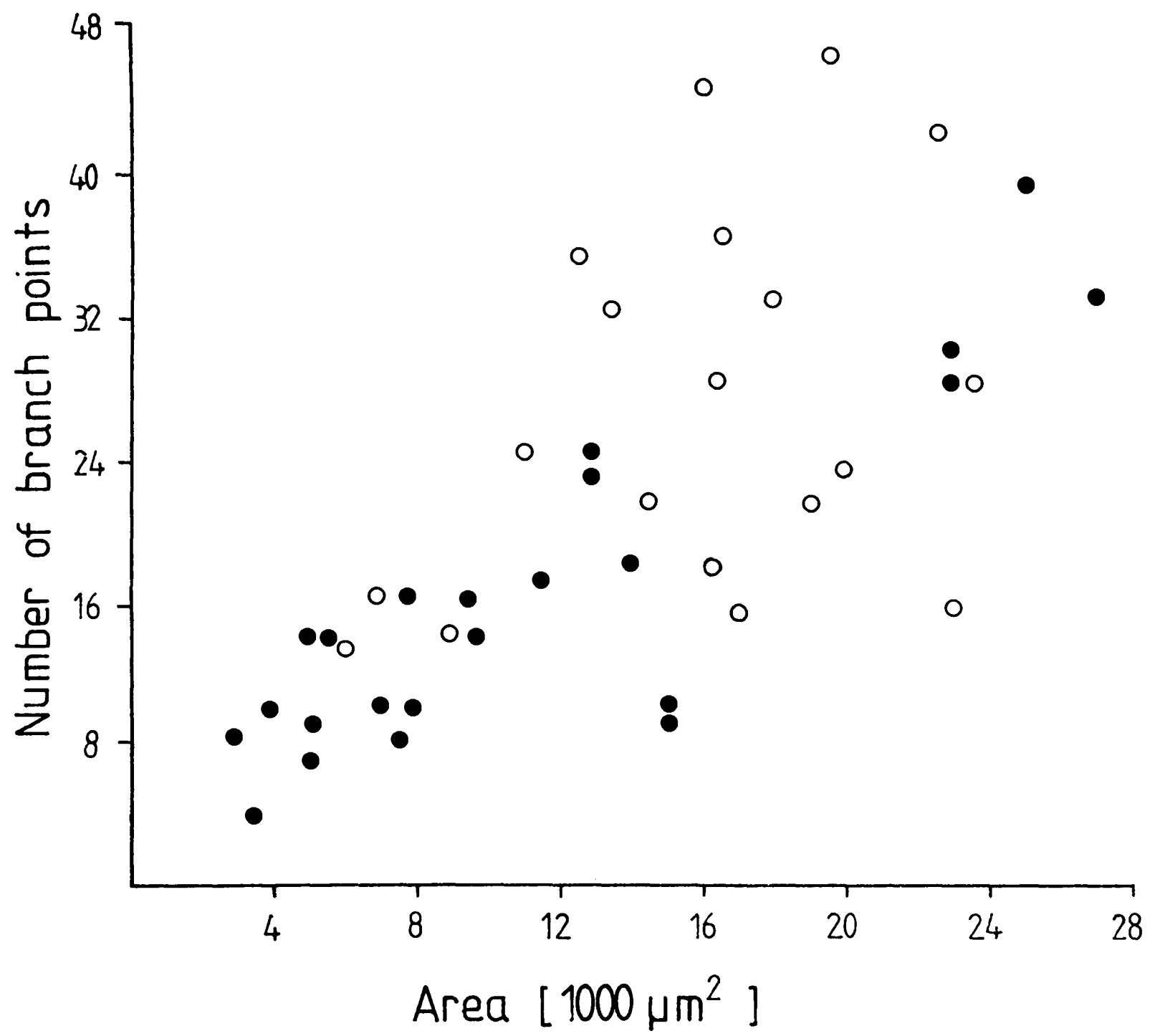


fig 6.10 Branches v Area



○ normal

● regenerate

significantly changed (1.61 ± 0.58 per $1000 \mu m^2$). The distributions of terminal size, shape and branching are shown in the histograms of fig. 6.3, 6.4 and 6.5. Fig. 6.10 shows the number of branch points plotted against terminal area for both normal (O) and regenerated (●) terminals.

Particularly striking in the wholemounts of regenerated fibres were the devious paths followed by the fibres towards their termination sites. Frequently two fibres were seen to enter the tectum in close apposition but follow markedly different courses, repeatedly converging and diverging, only to form terminals in adjacent, and even overlapping, areas of tectum.

Discussion

The size and morphology of the terminal arborizations observed above in the normal goldfish tectum resemble those seen in Golgi studies of the frog. A variety of sizes and branch patterns were found though the histograms of terminal areas and of branch numbers suggests a continuum rather than the existence of a small number of distinct terminal classes. The mean terminal area of $16000 \mu m^2$ means that for 50,000 optic fibres (as in a typical 50mm goldfish, Johns and Easter, 1977) to be accommodated in a tectal area of $5-10 \times 10^6 \mu m^2$ there must be considerable terminal overlap - to the extent of 80:1 or more. With such a high degree of overlap it is difficult to envisage chemospecificity cues which could be read with sufficient accuracy to ensure that the terminals are arranged in precisely the correct sequence. It may be that a considerable degree of "noise" is tolerated in the formation of retinotectal connections, either inaccuracies in the reading of labels or slight mechanical disordering of fibres.

The dimensions of the terminals, $177 \mu m$ long by $145 \mu m$ wide, are similar to those detected electrophysiologically by George and Marks (1974) in the frog, suggesting that the arborization visualized in this wholemount study may represent that part of the fibre detected using low impedance electrodes.

With regard to regeneration no significant trends were observed with increasing age of the terminals over the period studied, 18-41d. Two differences from normal terminals were noted, a reduction in the mean terminal area and in the total number of branchpoints per terminal. However the branch point density was unchanged. This indicates that the reduced area was not due to failure of penetration of HRP into fine terminal branches as the branching pattern of the terminals was not geometrical but consisted of fine short side branches coming from main branches. If failure of penetration of HRP were occurring a large reduction in branchpoint number would be expected with a proportionately much smaller reduction in area so reducing the branching density.

After optic nerve regeneration an increase in receptive field size, as detected electrophysiologically, has been recorded (Horder, 1971b) as has a reduced degree of order measured using an autoradiographical technique (Meyer, 1980). Two explanations are possible for these findings, either that terminal arborization size is increased early in regeneration so that there is a greater amount of overlap between terminals or that early in regeneration fibres form terminals with less accuracy. The findings here support the latter hypothesis though it is possible that though the actual histological size of the terminals is reduced in regeneration the proportion of the terminal detectable with electrophysiological methods is increased as it is not certain that the whole of the normal terminal is accessible to microelectrode recording.

Chapter 7

Do distortions of the normal pathway from retina to contralateral optic tectum have any influence on the projection?

Introduction

As reviewed in chapter 1 there is considerable evidence for the existence of an orderly arrangement of fibres within the visual pathway of lower vertebrates and in chapter 5 a model was proposed, based upon pre-existing and new data, for the nature of the fibre organization within the goldfish optic nerve and tract. However the existence of order within the pathway of a form appropriate to the production of the observed projection does not preclude the existence of more complex and specific guidance signals.

Attempts to detect such signals require the disruption of the ordering of optic fibres relative to the tectum by manipulation of either the pathway or the tectum itself. The simplest manipulation of the pathway has been to cut it and allow regeneration, though the extent of the disordering produced at the lesion is unclear. However Horder (1974b) and Meyer (1980) in the goldfish, and Udin (1978a) and Fujisawa (1981) in the amphibian, have shown that simple regeneration does lead some fibres to attain approximately the correct tectal termination sites by abnormal routes. Arora attempted a more severe deviation of optic fibres to inappropriate tectal regions and then studied histologically the routes taken by those fibres. However, his techniques did not allow him to be certain that fibres were either going back to the correct site or forming functional connections there (Arora and Sperry, 1962; Arora, 1963, 1966).

An alternative experimental strategem has been to rotate part of the tectum relative to the normal ingrowing fibre array, a paradigm employed extensively in the goldfish. Though Yoon (1975b) claims that fibres then entered the graft in the normal rostrocaudal direction there is a strong suggestion that many fibres may have been diverted by the scar tissue around

the graft so that their ordering on entering the graft was disrupted (Martin, 1978a, 1978b). Furthermore interpretation of these experiments requires a careful distinction to be made between cues which might control polarity of the map and cues controlling actual place of termination of a particular fibre.

In view of the uncertainty concerning the effect of pathway manipulations on the normal contralateral retinotectal projection in the goldfish it was decided to employ both anatomical and physiological methods to reinvestigate the system. The first experimental design employed was to divert a small fascicle of fibres within the orbit into an abnormal position in the nerve. In a second series of animals the medial and lateral brachia of the optic tract were dissected free and crossed over, a paradigm first used by Arora and Sperry (1962) though without the use of electrophysiological or selective anatomical means of analysis. Finally a small number of fish whose optic nerve had been cut in the orbit and allowed to regenerate were analysed in the same way as the mediolateral tract cross animals to check the much underplayed results of Horder (1974b) suggesting that some fibres regenerate to the correct tectal region by abnormal routes.

Methods:

Surgery

1a) Optic nerve fascicle transplants.

The right optic nerve of the anaesthetized fish was exposed within the orbit as described in chapter 2. Using sharpened tungsten needles a small part of the nerve was cut through at the orbital wall and freed from the rest of the nerve as far forward as possible. This bundle comprised 5-20% of the nerve. The free end of the fascicle was then diverted to the opposite side of the nerve and inserted into a small lesion made with a tungsten needle.

In all 15 fish later undergoing electrophysiological mapping the fascicle was taken from the nasal side of the nerve and inserted temporally. This operation was less hazardous than the converse deviation as it involved less dissection around the blood vessels lying temporal to the nerve.

1b) Partial optic nerve cuts.

As controls for the optic nerve fascicle transplants a number of fish were operated as above except that the freed fascicle was not diverted to an abnormal site in the nerve.

2) Medial - lateral tract cross.

The optic tecta of the anaesthetized fish were exposed as in chapter 2. The forebrain was deflected rostrally using small swabs, leaving the brachia of the left optic tract visible. Using sharpened tungsten needles these brachia were dissected free from the tectum as far caudally as possible and then cut. The free ends were then crossed and inserted without rotation into the opposite sides of the tectum. After removal of excess blood clot the skull was closed.

3) Optic nerve regeneration;

The right optic nerve of the anaesthetized fish was exposed in the orbit and cut with iridectomy scissors as described in chapter 2.

Electrophysiology

1a) Optic nerve fascicle transplants and

1b) Partial optic nerve cuts.

A single map of the projection from the right eye to the left tectum was obtained as described in chapter 2. These two groups of animals were mapped blind - the mapping operator did not know from which group a particular animal was taken. Tectal positions receiving a projection from peripheral retina were not mapped in order to avoid the "anomalous" responses described

in appendix II. Any animal showing responses similar to this anomaly - a radial displacement of field position or a radial elongation of the field - was disregarded.

- 2) Medial-lateral tract crosses and
- 3) Optic nerve regeneration.

Those animals could not be mapped blind as the tectal deformations occurring in the tract cross group (group 2) made those animals readily identifiable. For some animals a complete map of 30 or more responses was obtained of the projection of the right eye to the left tectum. A cut was then made across rostral tectum in front of the first mapping positions, and extended caudally at its lateral extremity in some animals to form an L. The area behind the cut was then remapped at the same mapping session using the same photograph. This gave electrode positioning to within $50\mu\text{m}$ of the original positions. For both the first and second maps the quality of each response, whether from a few or many units, was noted as was its position.

Some of the medial-lateral cross animals were mapped only once at the first mapping session and received no tectal cut at that time. They were then allowed to recover and remapped after a period of 130-169 days using the original photograph to place the electrode. Any change in the projection was then assessed by eye and by determining the mean change in response positions at the same electrode locations. The response position change was taken as the great circle angle between the original visual field response position to a given electrode placing at the first mapping and the position of the response to that electrode position (defined photographically) at the second mapping.

In order to assess the magnitude of the response shift arising from errors in electrode positioning due to operator error and to changes in the tectal vasculature over a period of months 3 normal fish were mapped and then remapped 130-133 days later using the original photograph. An example of

the two maps obtained 133 days apart is shown in fig. 7.1. This animal showed a field response position shift of $5.55 \pm 2.60^\circ$ (mean $\pm$ standard deviation). Other animals gave values of $8.03 \pm 3.92^\circ$ and $6.25 \pm 3.55^\circ$.

Anatomy

1a) Optic nerve fascicle transplants.

After electrophysiological mapping the right optic nerve was exposed in the orbit. The diverted fascicle was identified, usually with ease, cut using a tungsten needle, and the lesion packed with Sterispon soaked in HRP solution. After 20-24 hours the animal was killed and the brain with optic nerves attached removed. The brain was then processed, cut into frozen sections and developed for HRP as described in chapter 2. O-dianisidine was used as the developing chromagen.

1b) Partial optic nerve cuts.

After electrophysiological mapping the right optic nerve was exposed and a small fascicle of fibres, at about the same position as was cut originally, was lesioned. The cut was packed with Sterispon soaked with HRP solution. After 20-24 hours the animal was processed in the same way as an optic nerve fascicle transplant animal.

2) Medial-lateral tract cross and 3) optic nerve regeneration.

After the second mapping the right optic nerve was exposed in the orbit, and cut and filled with HRP on Sterispon. After 20-24 hours the animal was killed and the left tectum processed for HRP as a wholemount by the method described in chapter 2. This method was designed to demonstrate any fibres entering the area of tectum behind the rostral cut by an abnormal route. In order to check that HRP could not be transported across the cut in a significant quantity six normal goldfish were subjected to the same anatomical analysis. A cut was made across the rostral edge of the tectum through the full thickness. Two hours later, the minimum time required

for the second mapping, the optic nerve was cut in the orbit and filled with HRP on Sterispon. In none of the six controls was any HRP subsequently detected in the tectum caudal to the cut.

Results

The normal goldfish retinotectal projection

For reference the electrophysiological map of the projection of the visual field seen by the right eye on to the left tectum is shown in figure 7.1. The polarity of the projection is of dorsal field to medial tectum and nasal field to rostral tectum. Throughout the experimental studies described in this thesis the visuotectal projection is taken to be a direct representation of the retinotectal projection after allowance has been made for the inversion due to the optics of the goldfish eye. N, T, D and V on the visuotectal maps refer to nasal, temporal, dorsal and ventral poles of the visual field of the mapped eye.

As can be seen from figure 7.1. the projection is highly ordered. Though there is some irregularity in the spacing of the field positions, probably a result of small errors in the positioning of the electrode, the receptive fields always lie in the correct relative positions. The rows of response positions corresponding to rows of electrode penetrations are spread approximately evenly across the visual field.

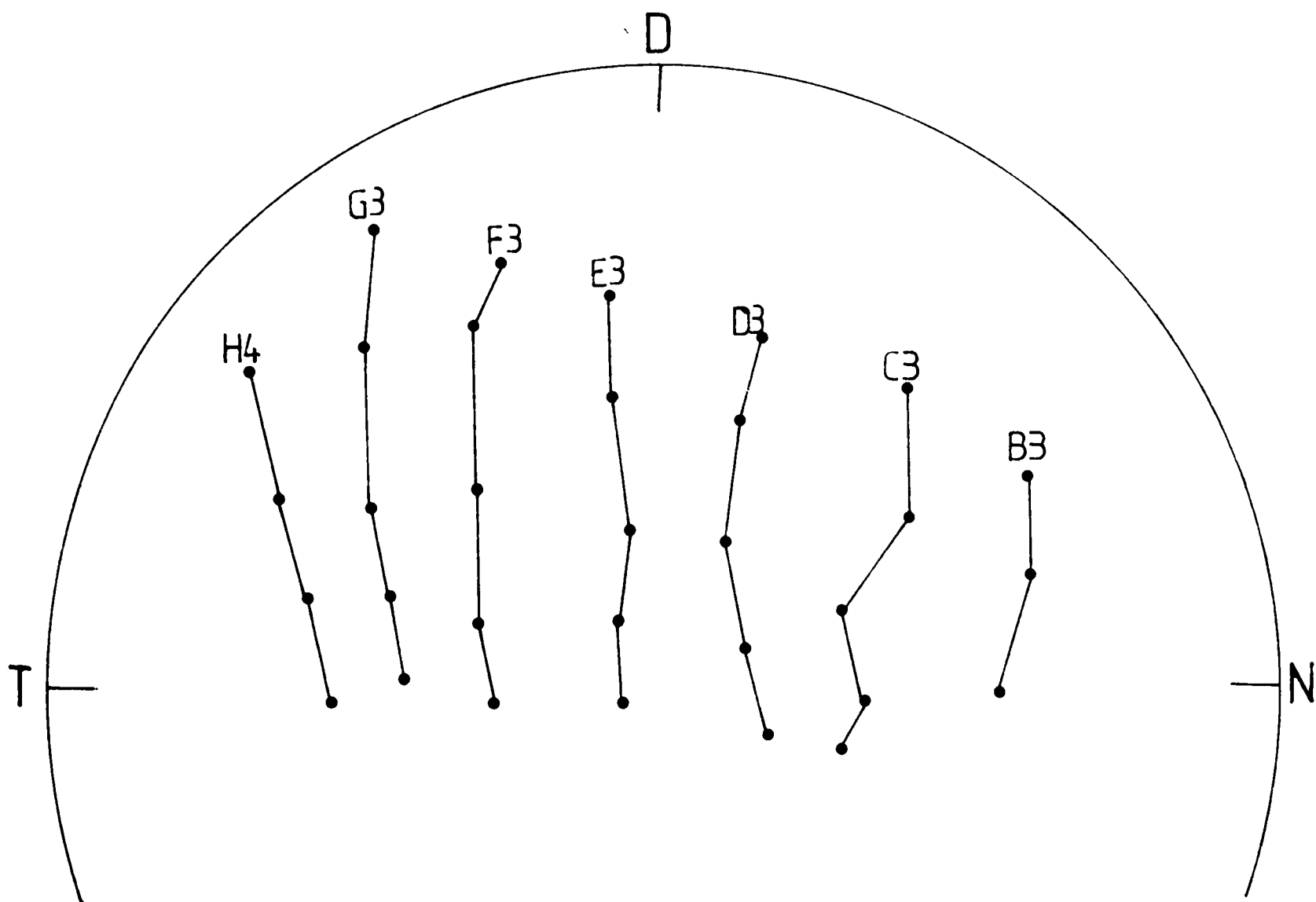
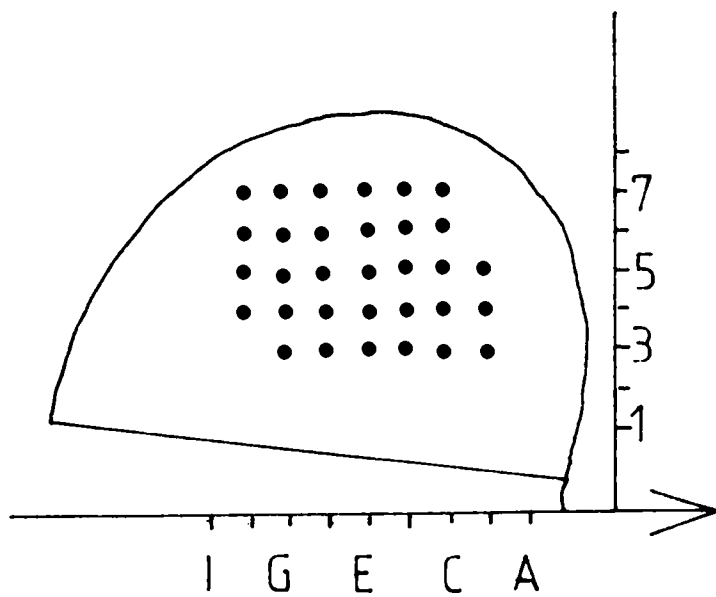
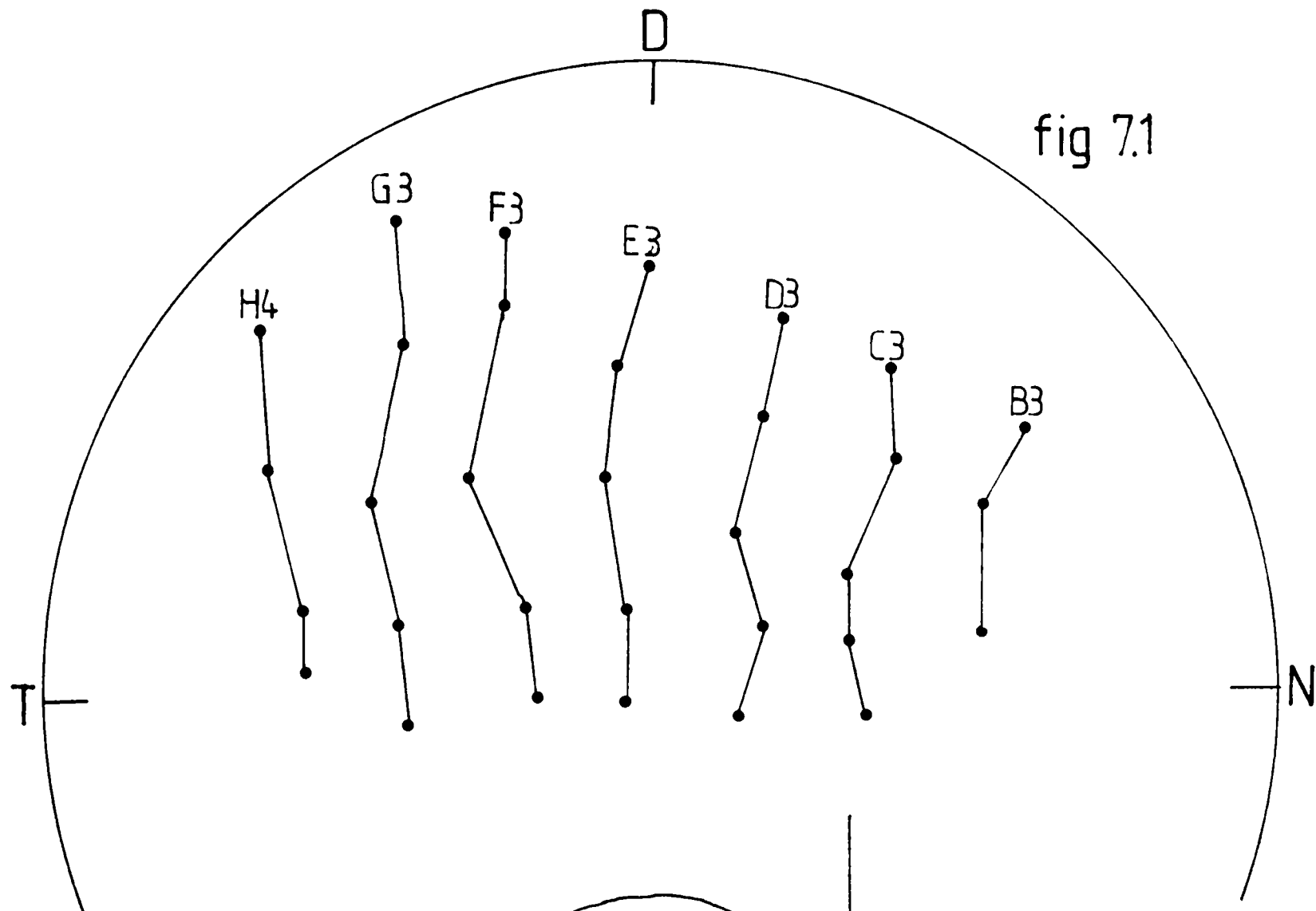
Optic Nerve Fascicle Transplants

Fifteen fish having nasal to temporal fascicle transplants were mapped electrophysiologically at 83-194 days after operation. Of these, 7 were rejected because any abnormal responses were not clearly distinguishable from the anomalous responses of peripheral tectum described in appendix II.

Of the remaining 8 fish one gave an apparently normal map. The other 7 maps were all clearly abnormal, the most common abnormality being the presence of

Fig. 7.1 Electrophysiological maps of the visuotectal projection of a normal goldfish. The top diagram shows the initial map, while the bottom diagram shows the map obtained 133 days later using the first photograph to define the electrode positions. Both maps are corrected for eye rotation, the first by 26° and the second by 14° .

fig 7.1



double responses. This meant that at one electrode position responses could be elicited by stimulation at two distinct field positions not lying on the same radius (to avoid confusion with the anomalies described in appendix II).

In the earliest two fish (83d and 92d) no double points were detected but the maps were distorted, a number of response positions being located far more temporally than normal. The map obtained at 83 days is shown in figure 7.2. In the five animals mapped later (103 - 294 d) a total of 15 double responses were obtained. In each animal these double responses occurred in small clusters at immediately adjacent electrode positions, so that the distortions were well localized in the tectum, and 13 of the 15 points had a component located more temporally in the visual field than normal. This suggests that the temporal displacement of some responses in the earlier maps may be equivalent to the abnormal component of double responses obtained in other animals. An example of a map obtained at 260 days is shown in figure 7.3.

The HRP labelled sections showed that a considerable number of optic fibres (5-20%) had been diverted into a new position in the nerve and continued in that position towards the tectum. However it was not possible to determine the paths of such fibres beyond the division of the optic tract brachia. Thus the anatomical results are only able to confirm that the fascicle diversion was substantial and effective.

Partial optic nerve cuts

Three animals having partial nasal optic nerve cuts were mapped electrophysiologically at 78-85 days. All three maps were essentially normal, no major distortions or double points being detected. A typical map obtained 79 days after surgery is shown in figure 7.4.

Fig. 7.2 Electrophysiological map of the visuotectal projection of an optic nerve fascicle transplant fish at 83 days after surgery. The map is corrected for an eye angle of 29° .

fig 7.2

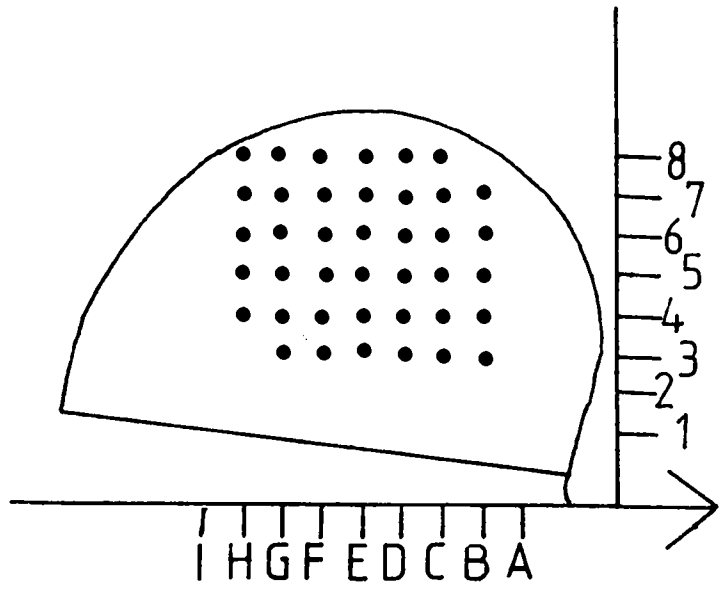
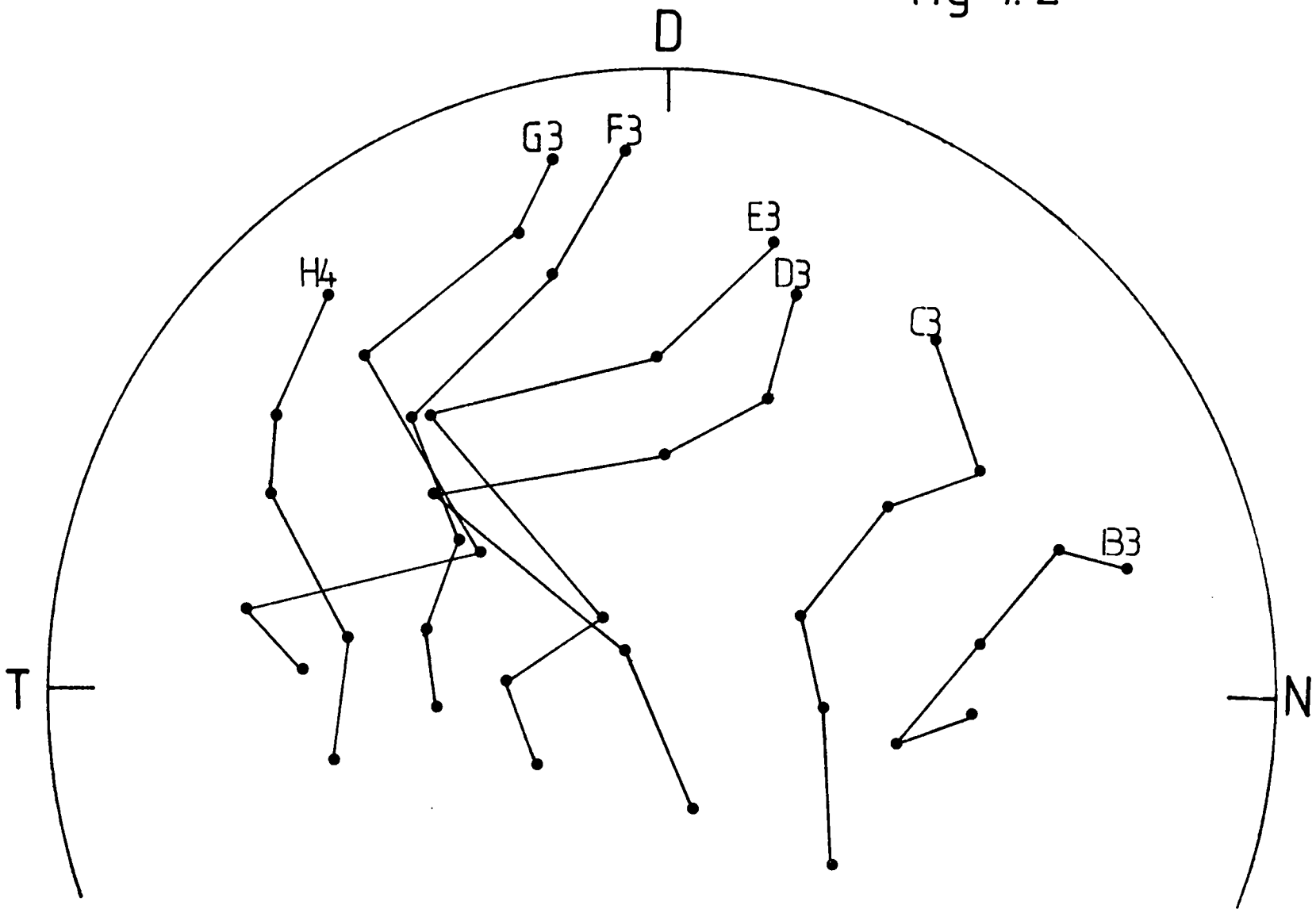


Fig. 7.3 Electrophysiological map of the visuotectal projection of an optic nerve fascicle transplant fish at 260 days after surgery. The map is corrected for an eye angle of 12° . Double response are shown as ●—○. Dotted lines linking two responses indicate that they do not come from adjacent electrode positions.

fig 7.3

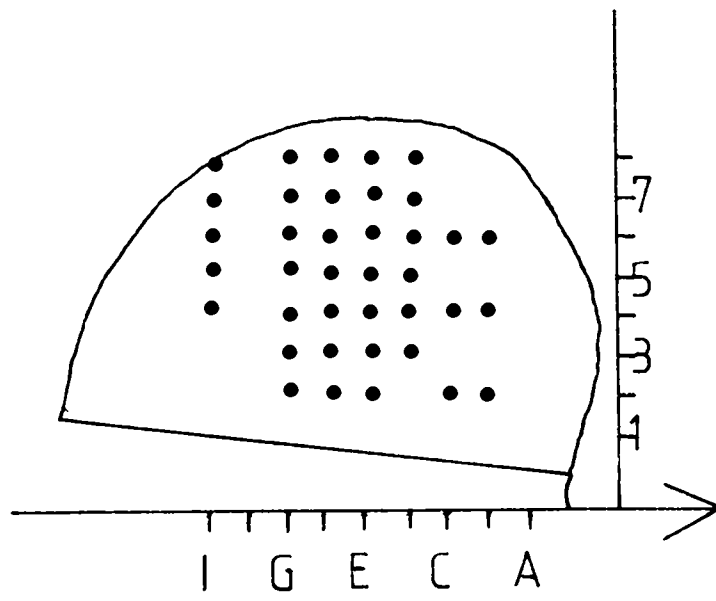
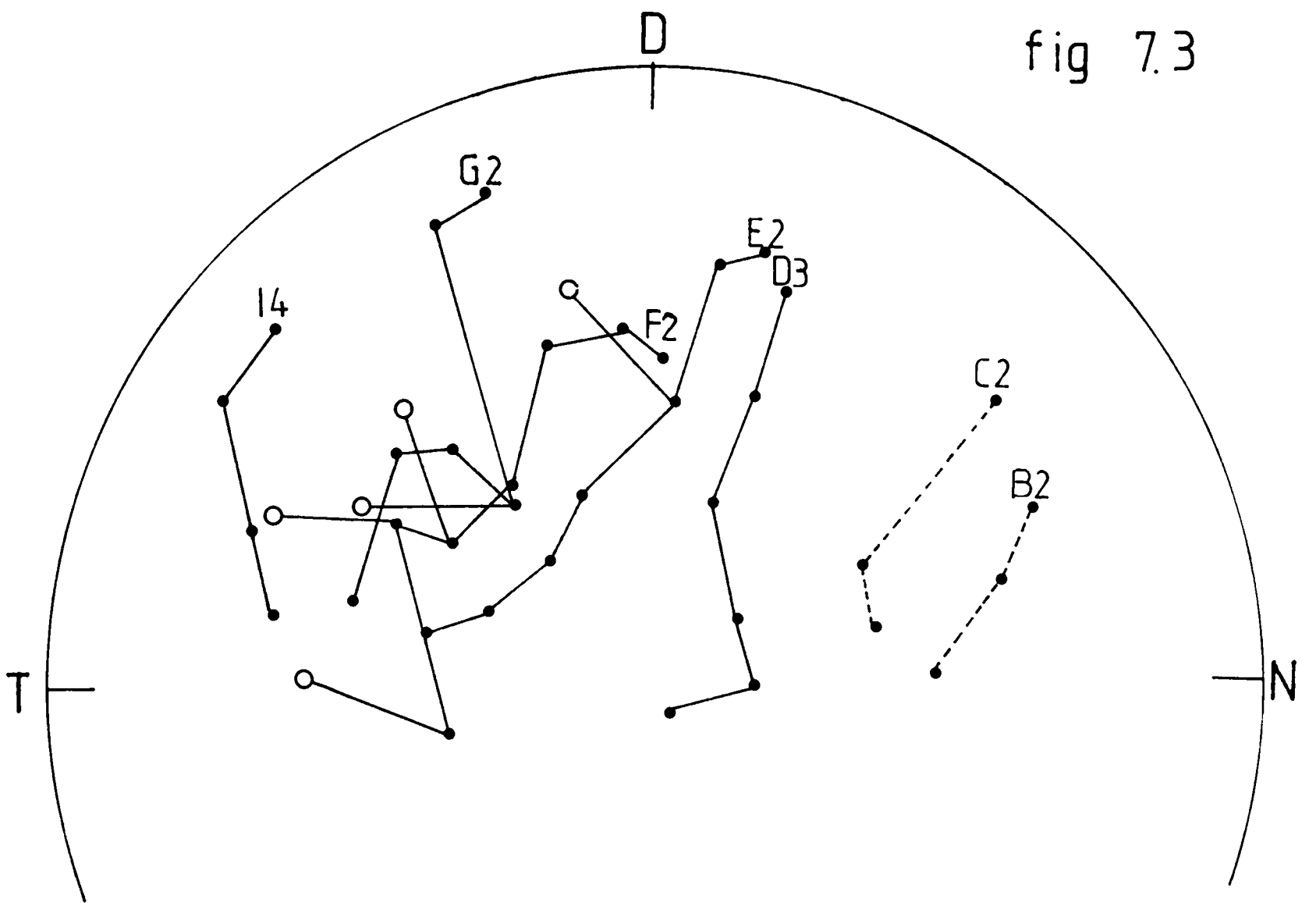
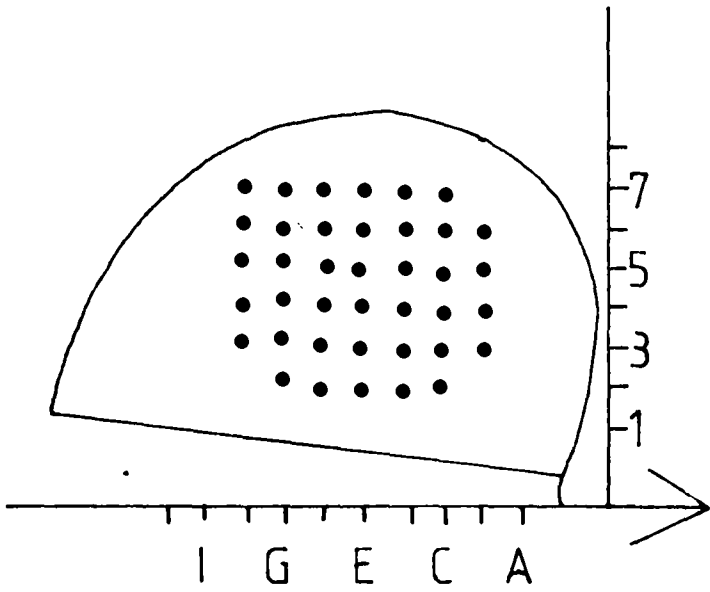
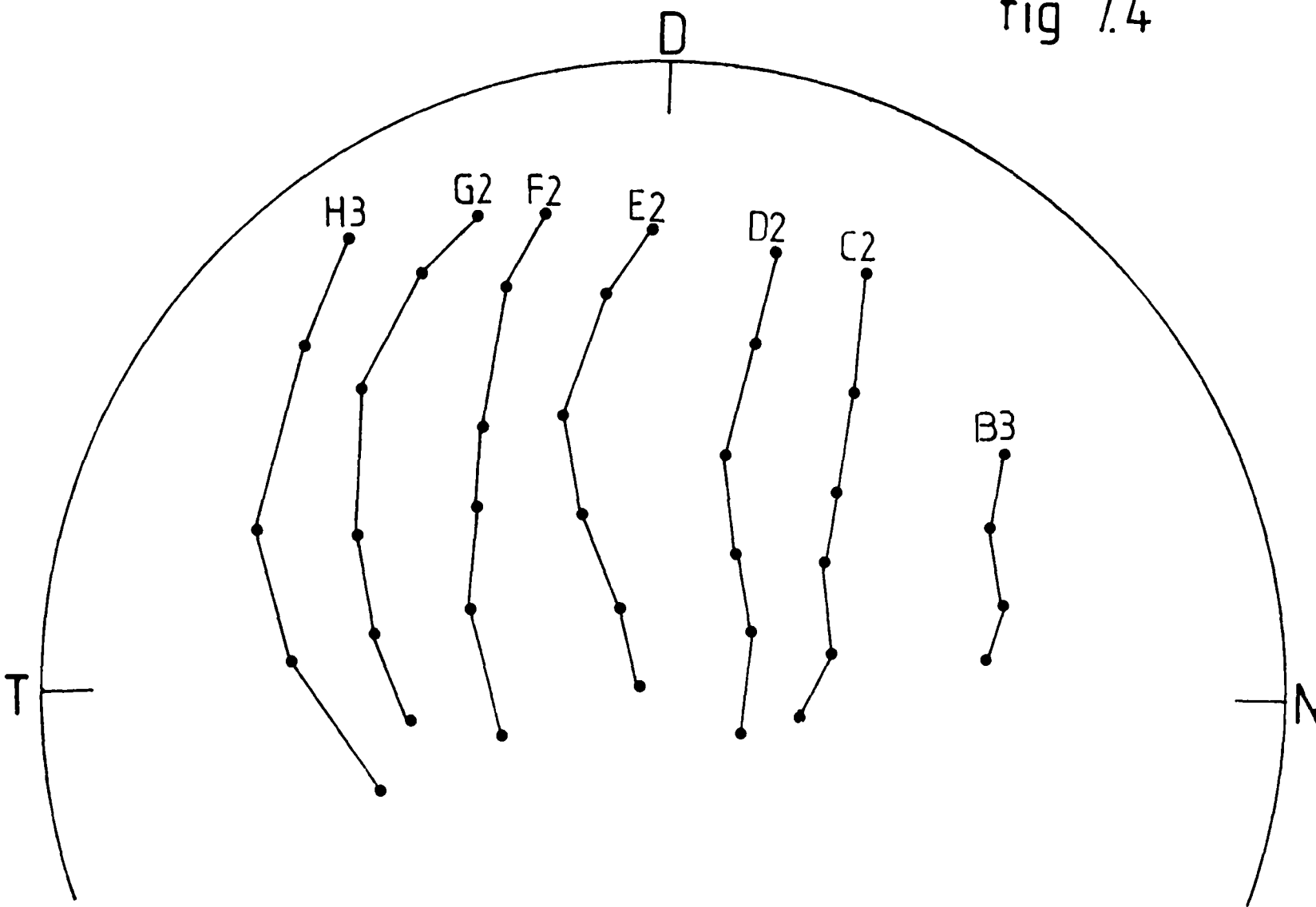


Fig. 7.4 Electrophysiological map of the visuotectal projection
of a partial optic nerve cut animal at 79 days after
surgery. The map is corrected for an eye angle of 29° .

fig 7.4



Medial-lateral tract cross

Nine animals were mapped, had a rostral tectal incision made and were then immediately remapped at between 50 and 287 days after surgery. There was considerable variation in the orderliness of the first map, from essentially normal, with no major distortions, to much less well ordered with a number of grossly misplaced points. However in every case the overall order and polarity of the projection was correct. In this series of animals there was no obvious tendency for the first map to improve with time after operation normal maps occurring both early (78 days, fig. 7.5) and later (261 days, fig. 7.6) as did maps showing some disordering (271 days, fig. 7.7).

After a rostral incision had been made in the tectum the projections of these animals were remapped using the same electrode positions ($\pm 50\mu\text{m}$). Between 12 and 26 (mean = 16.3) positions lying directly behind the cut and whose fibres would in a normal animal have been severed by the cut were sampled in each animal and responses were obtained at between 3 and 14 of these (mean = 9.5). Most of these responses occurred at approximately the same field positions as in the first mapping. Quantification of the displacements was not attempted as bleeding from the tectal lesion often made electrode positioning difficult, and some distortion of the tectal shape resulted from the cut. However where sufficient responses could be obtained in the second mapping to allow assessment of ordering this was found to be correct (fig. 7.5, 7.6) and in one case (fig. 7.7) the second map was considerably more ordered than the first.

Responses obtained during the first mapping were almost always from multi-units though occasionally with a prominent single unit as well. On remapping 26 of the 73 localizable responses were from single units - distinguished on the basis of their response to a flashing stimulus. Only 6 of these electrode positions had given a purely single unit response during the first mapping.

Fig. 7.5. Electrophysiological maps of the visuotectal projection of a medial-lateral tract cross animal 78 days after surgery. The top diagram shows the first mapping. The tectal cut is indicated on the tectal outline. The bottom diagram shows the responses persisting after the cut. Both maps are corrected for an eye angle of 15° .

fig 75

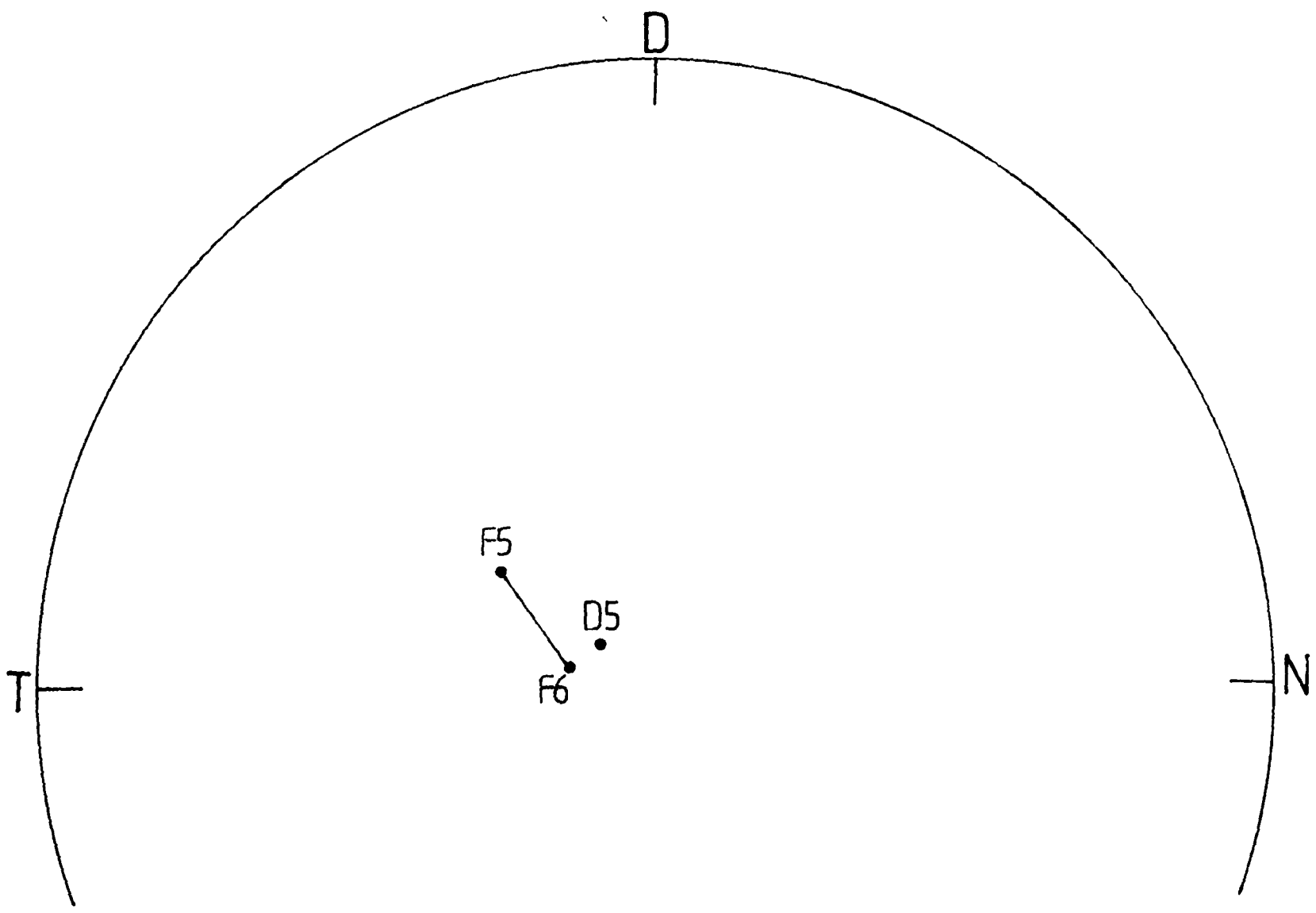
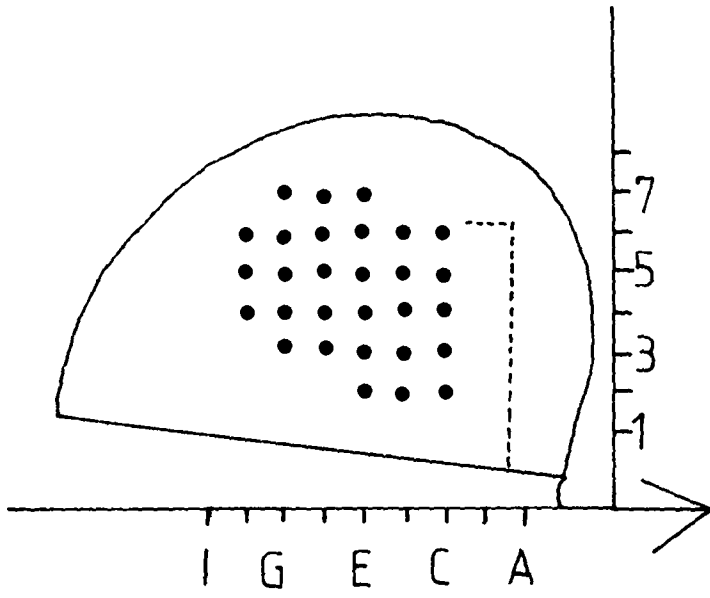
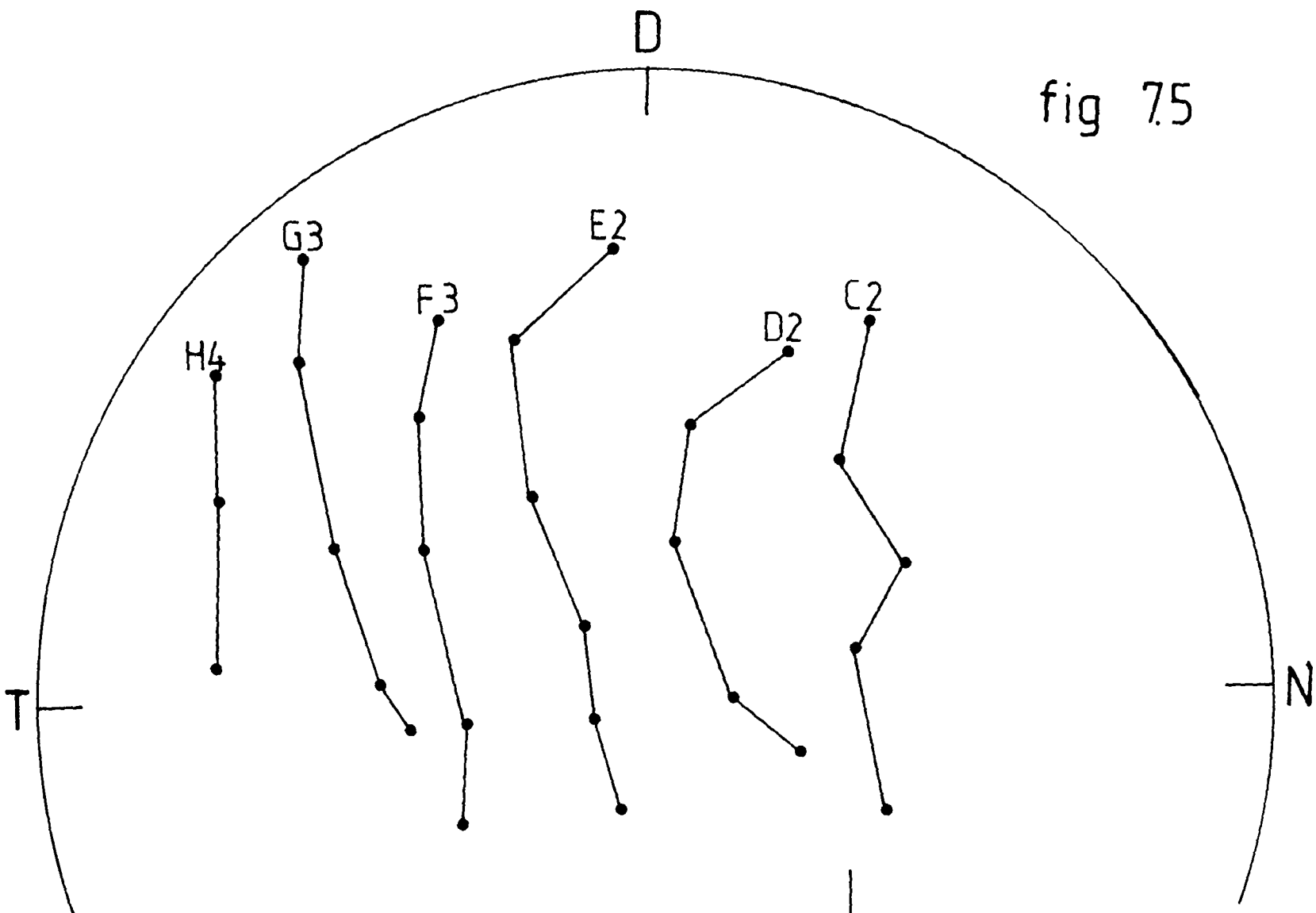
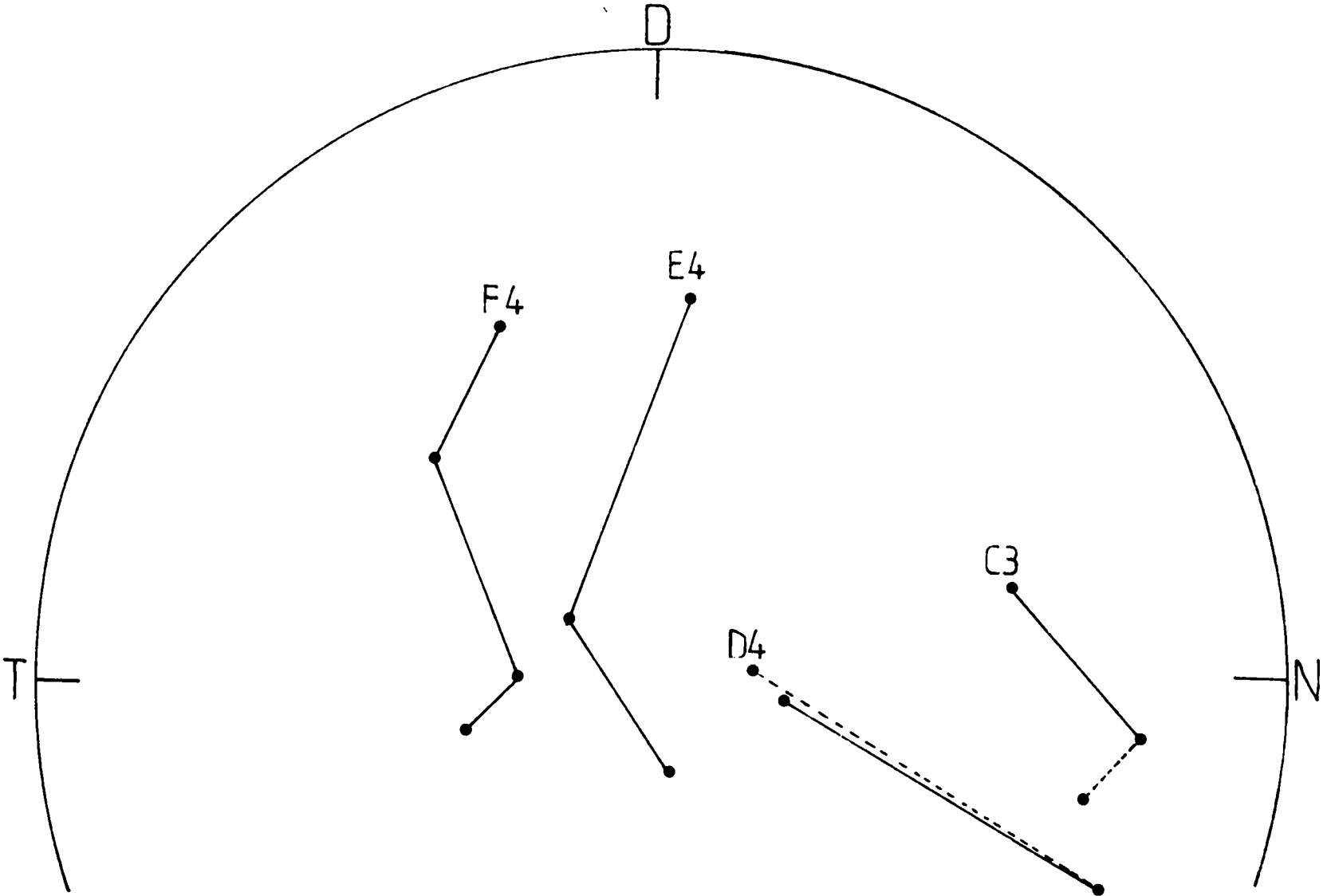
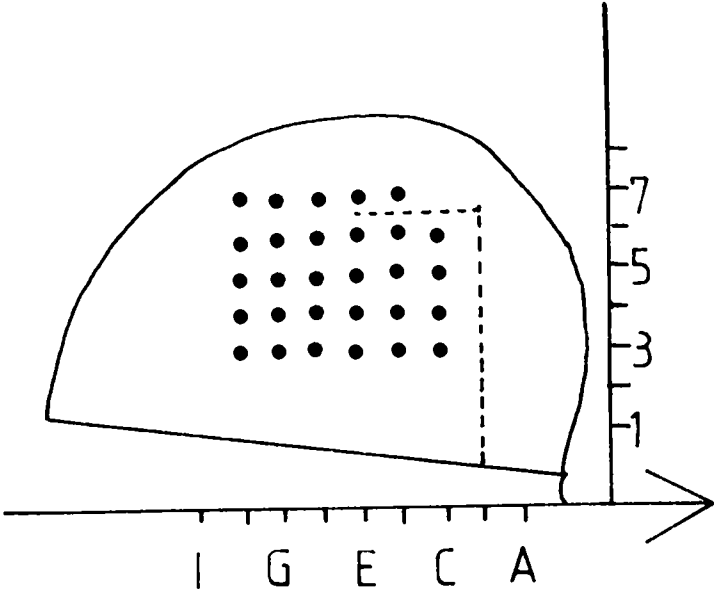
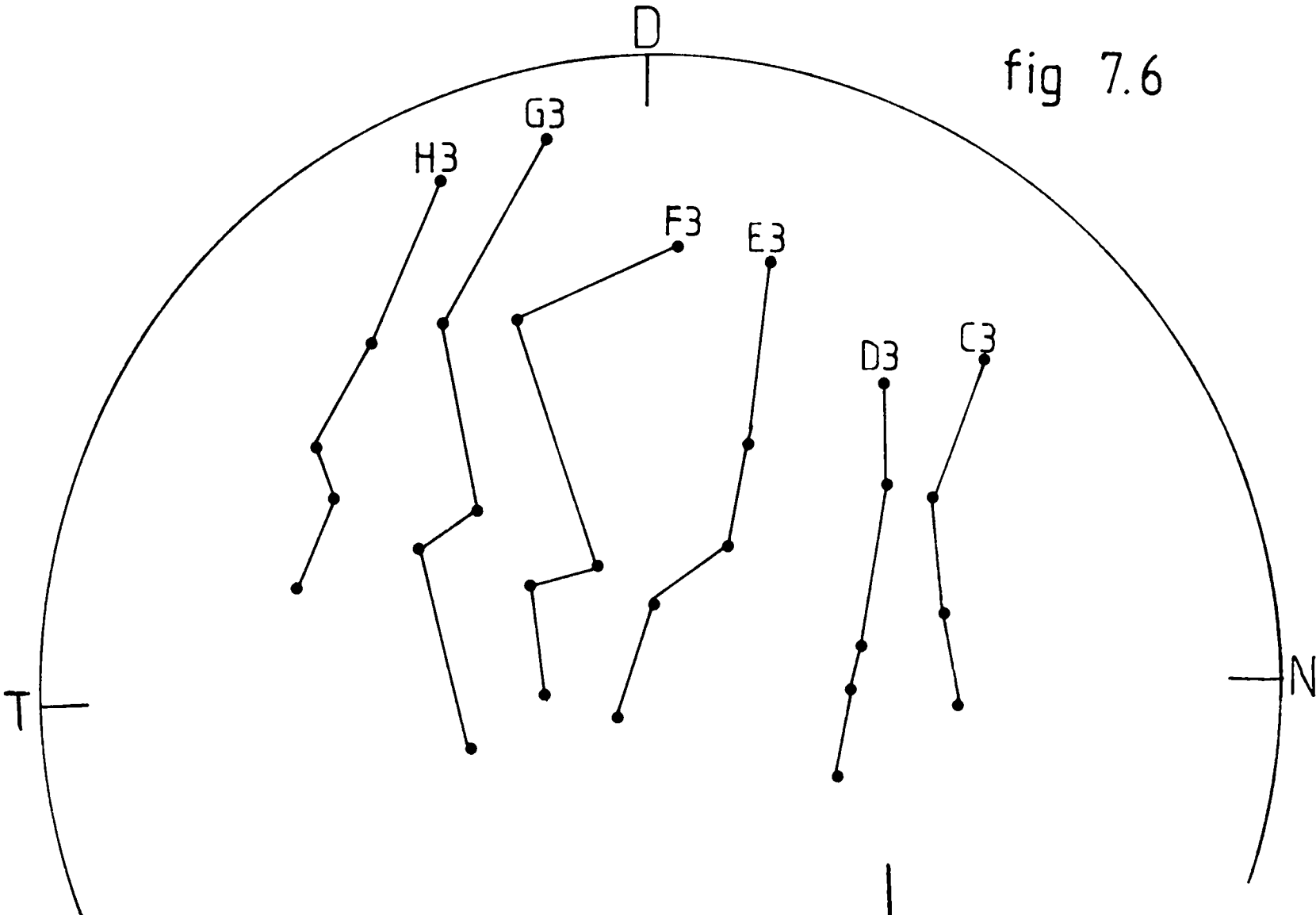


Fig. 7.6 Electrophysiological maps of the visuotectal projection of a medial lateral tract cross animal obtained 261 days after surgery. The top diagram shows the first mapping and the lower diagram the second, obtained after making a cut as marked with dotted lines on the tectal outline. Both maps are corrected for an eye angle of 25° .

fig 7.6



In each of the above nine fish the right optic nerve was filled with HRP after the second mapping and the left tectum was processed as a wholemount. The presence of many darkly stained blood vessels and a high background level of staining meant that the wholemounts were difficult to illuminate for examination and photography. However in all cases the quality was adequate to confirm that the rostral tectal lesion had interrupted all fibres entering the medial part of the dorsal tectum in a rostrocaudal direction, the normal direction. In some cases it was possible to trace small groups of fibres passing into the region behind the cut by abnormal routes, usually in a latero-medial direction (fig. 7.8).

In order to determine whether the ordering of the retinotectal projection increased with time after the medial-lateral tract cross surgery seven fish were mapped at short times after operation (35-80d) and then allowed to recover for subsequent remapping. In only two of these was the tectal vasculature sufficiently well visualized in the first mapping photograph to allow remapping using the same electrode positions. One fish was first mapped 66 days after surgery and remapped 235 days after surgery (fig. 7.9) while the other was first mapped 74 days after surgery and remapped at 204 days after surgery (fig. 7.10).

In the first case (fig. 7.9) the ordering of the map 66 days after surgery, though far from normal, was roughly correct and there was no major change on remapping at 235 days. Though a number of responses showed substantial shifts, two major ordering defects the inappropriately central position of the response at E_3 , and the dog leg in row F which caused it to intersect both row E and row G, were present in both maps. In the second case (fig. 7.10) a considerable improvement in ordering between the two mappings is discernible though certain errors, notably a dog leg in row F which caused it to cross rows E, G and H were again retained in the second map. In this case the great circle shift in response positions was $16.45 \pm 12.15^\circ$

Fig. 7.8. Camera lucida tracing of a tectal wholemount of a medial-lateral tract cross animal mapped 184 days after surgery. The position of the rostral cut is marked with an arrow. The only fibres passing to the area behind the cut are following an abnormal latero-medial route. Inset is an enlargement of the circled area just behind the cut illustrating the devious paths followed by fibres. Fissures in the tectum resulting from flattening of the wholemount during mounting are marked O.

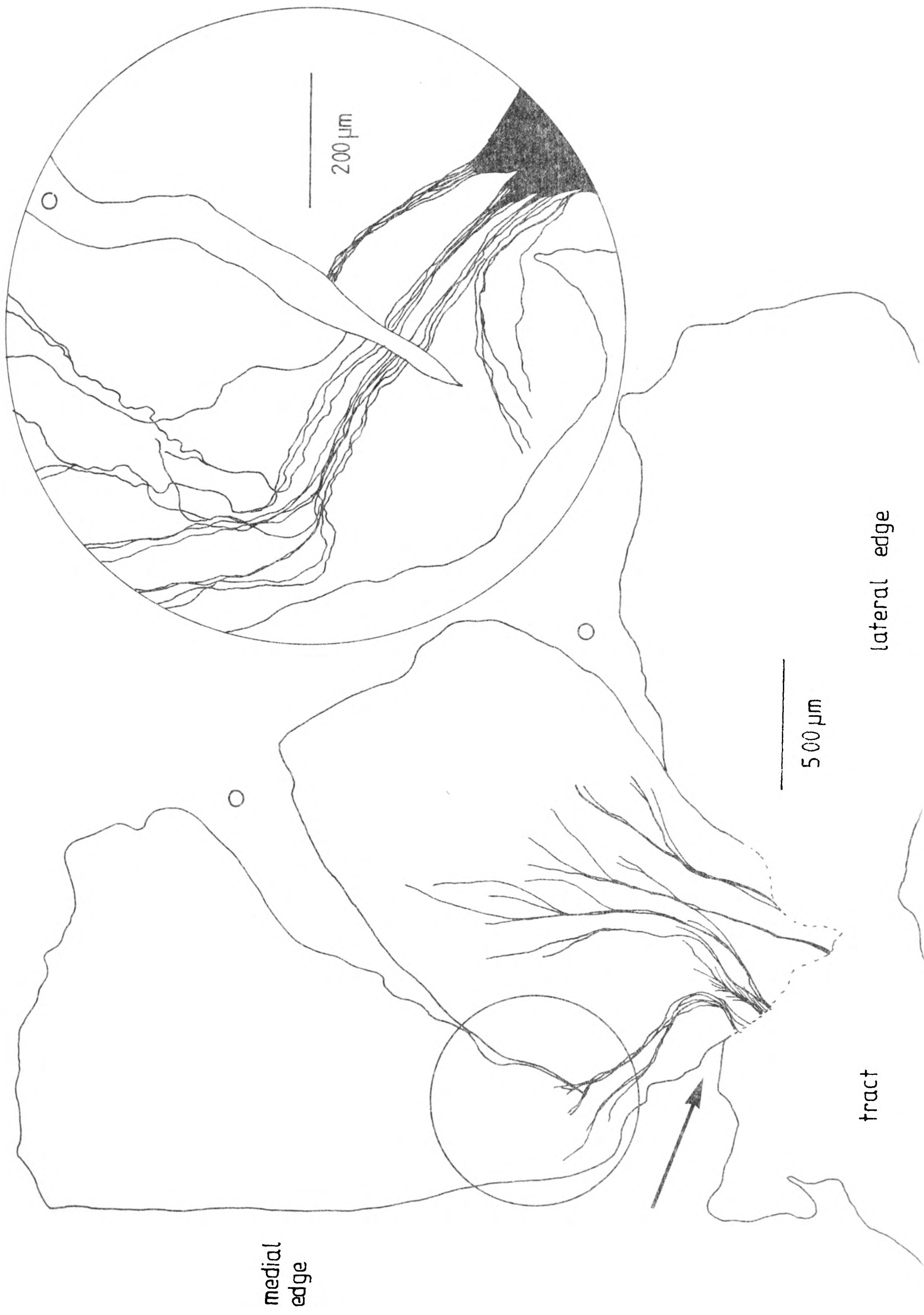
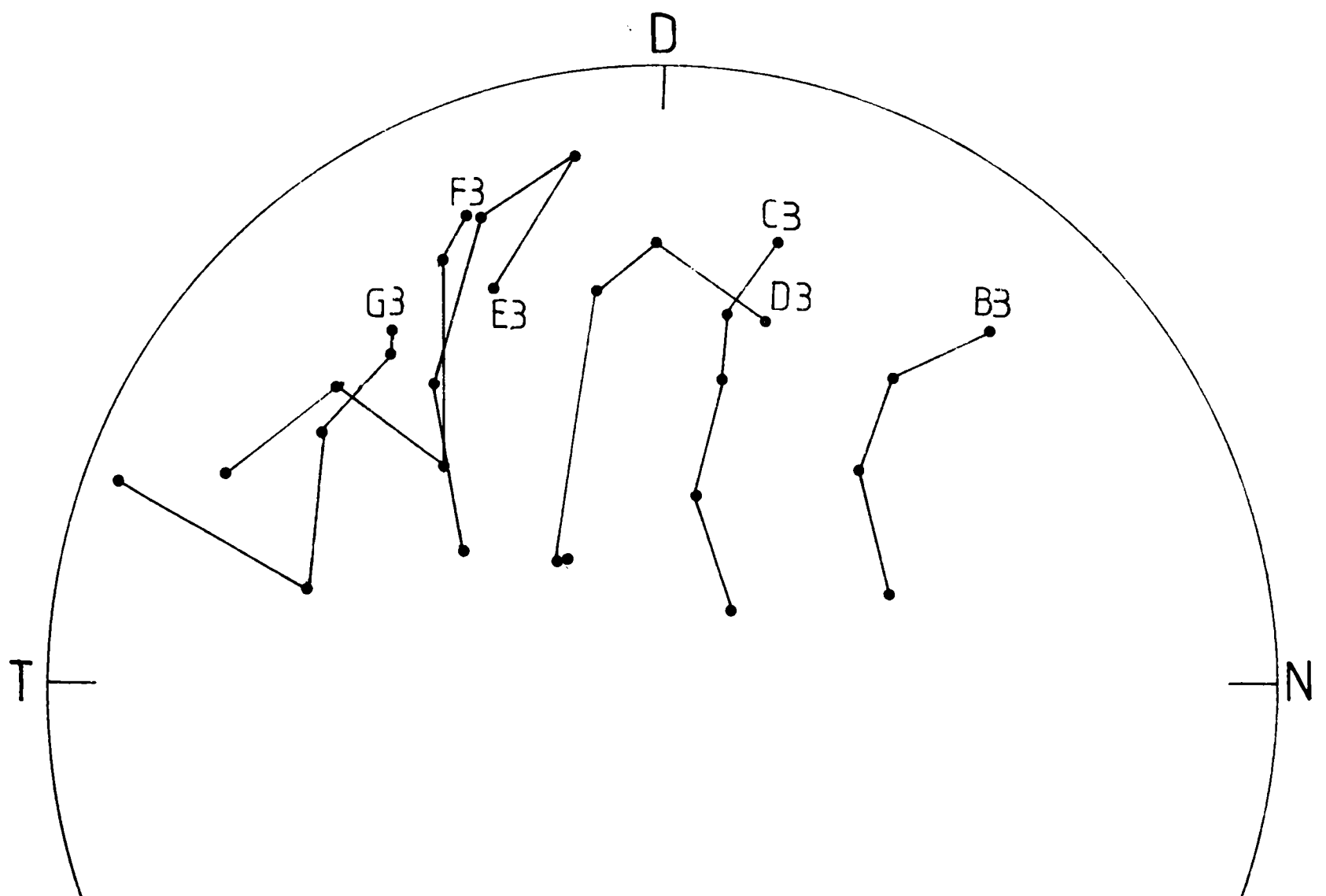
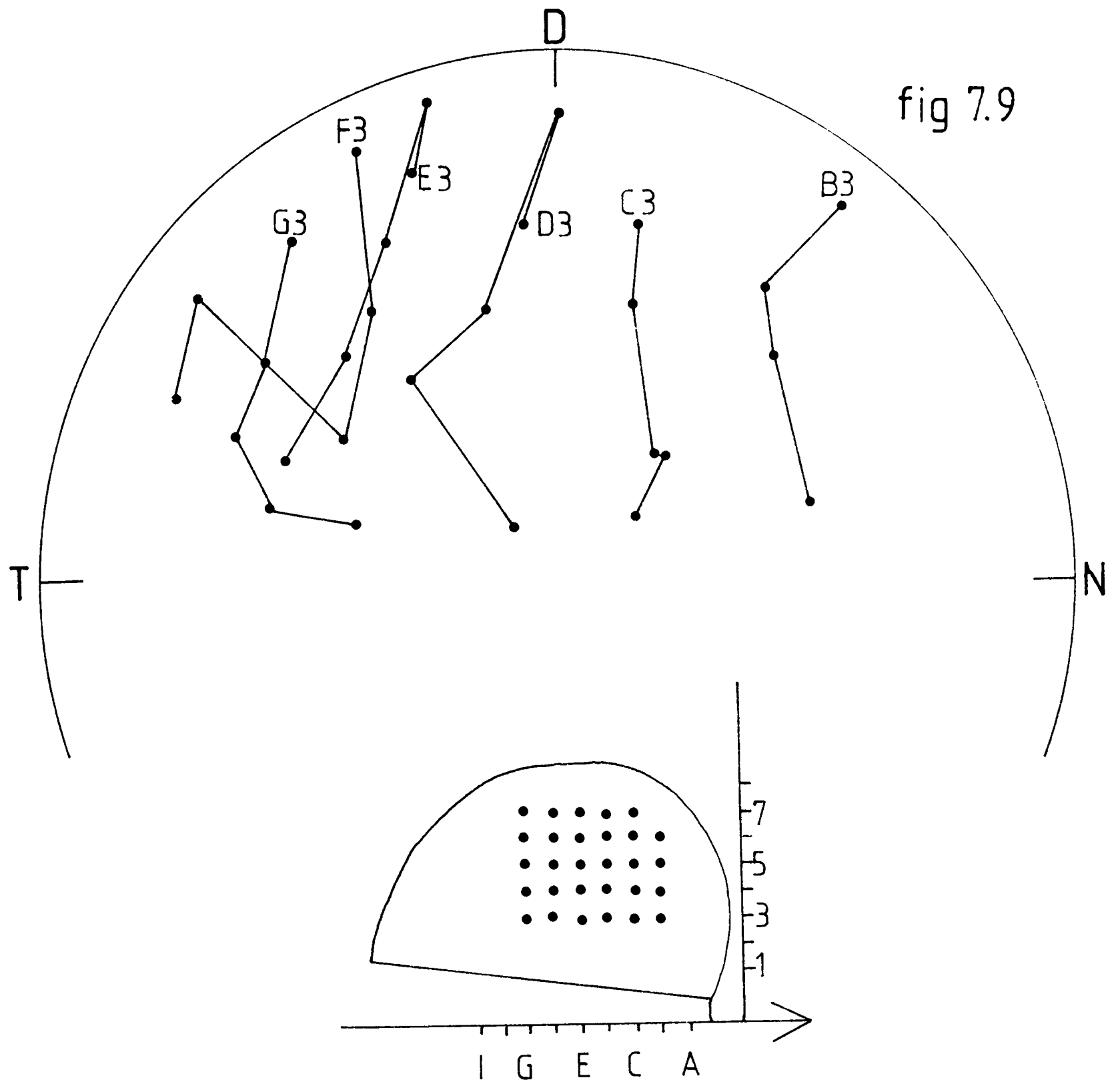


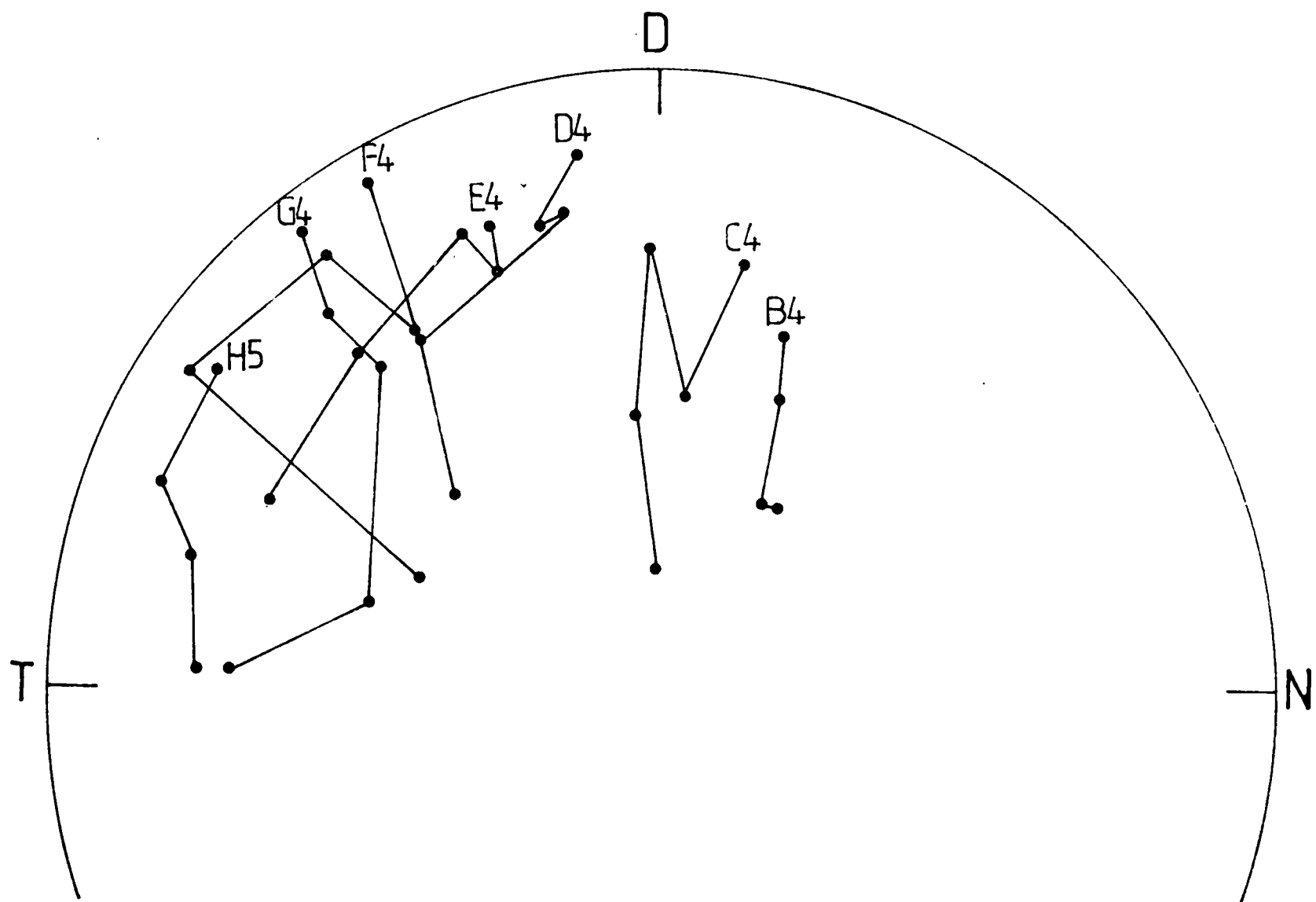
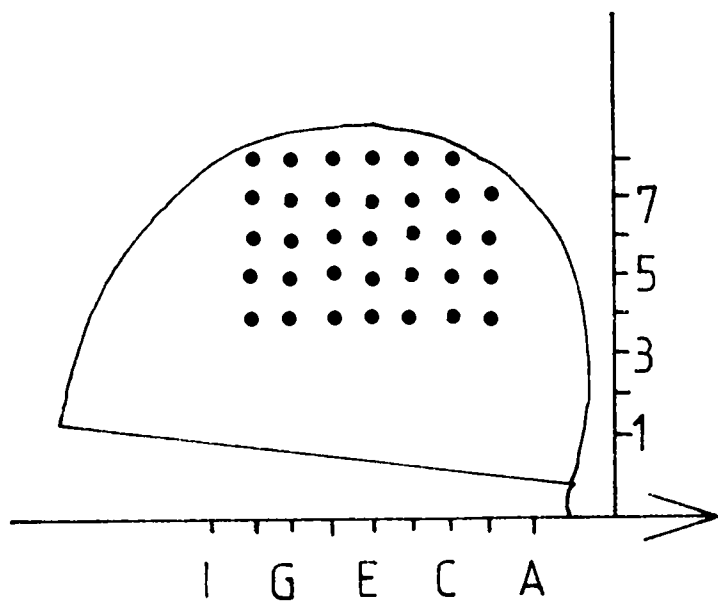
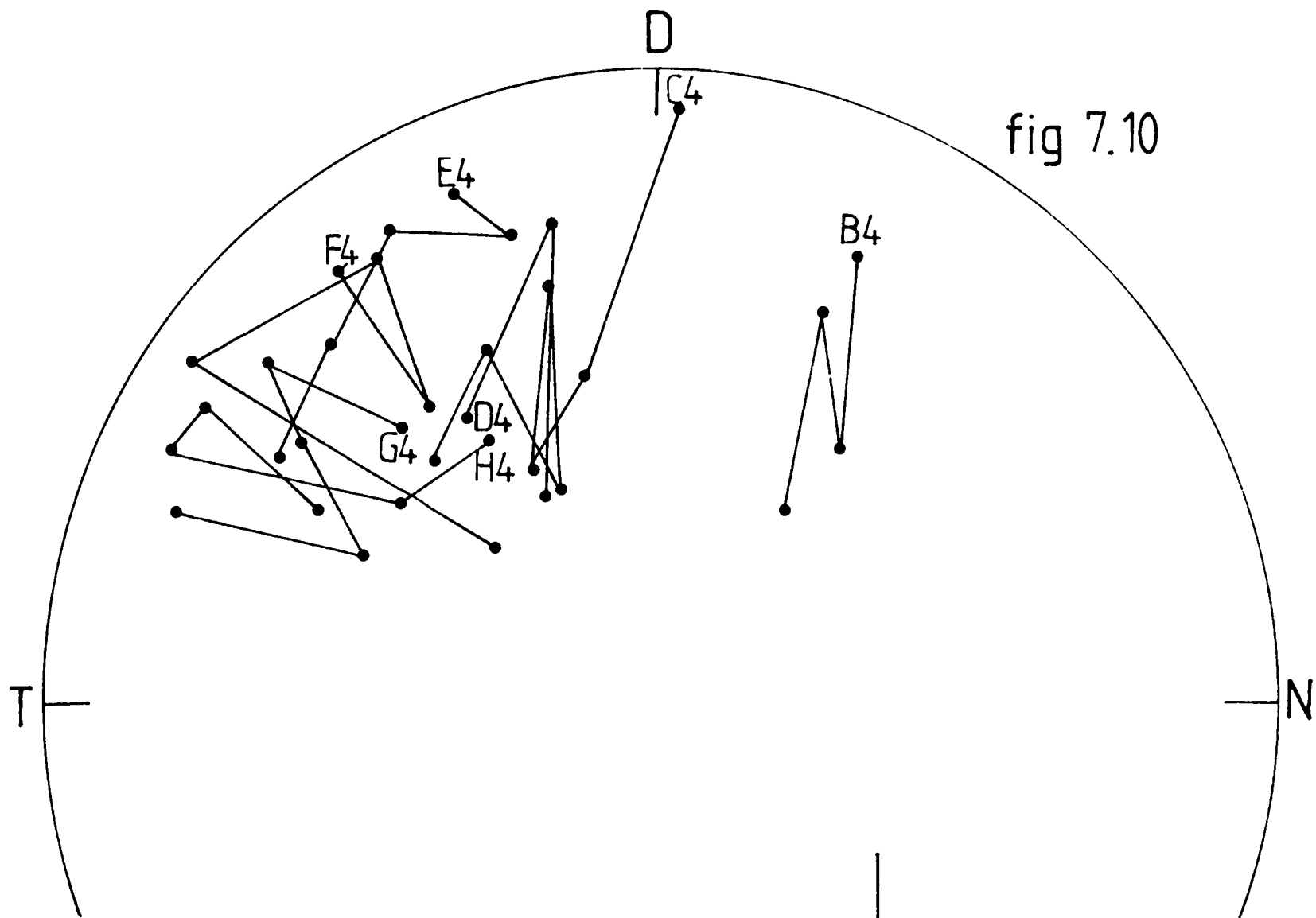
Fig. 7.9 Electrophysiological maps of the visuotectal projection of a medial-lateral tract cross animal. The top map was obtained at 66 days after surgery and is corrected for an eye angle of 20° . The lower map was obtained at 235 days after surgery and is corrected for an eye angle of 21° . The same photograph was used for the location of electrode positions at both mappings.

fig 7.9



Fig, 7.10 Electrophysiological maps of the visuotectal projection of a medial-lateral tract cross animal. The top map was obtained 74 days after surgery and is corrected for an eye angle of 16° . The lower map was obtained at 204 days after surgery and is corrected for an eye angle of 16° . The same photograph was used for the location of electrode positions at both mapping sessions.

fig 7.10



(mean $\pm$ standard deviation), considerably greater than that occurring on remapping a normal animal supporting the idea of a significant shifting of retinotectal connections. For the first of these remapped animals no great circle shift was computed as it was felt that electrode positioning was not as accurate as in the normal controls due to tectal bleeding.

The earliest time after optic nerve sections at which localizable responses were detected from the majority of positions sampled in dorsal tectum was 42 days in this series. However as the optic fibres were lesioned near the tectum regeneration might be expected into the tectum much earlier than this - Horder (1971b) reported regeneration of fibres from the orbit to the tectum in 3 weeks using comparably sized fish maintained at room temperature. In order to investigate the paths of early regenerated fibres at times before electrophysiological responses were detectable a number of medial-lateral tract cross animals had their right optic nerve filled with HRP at 33 or 35 days after surgery.

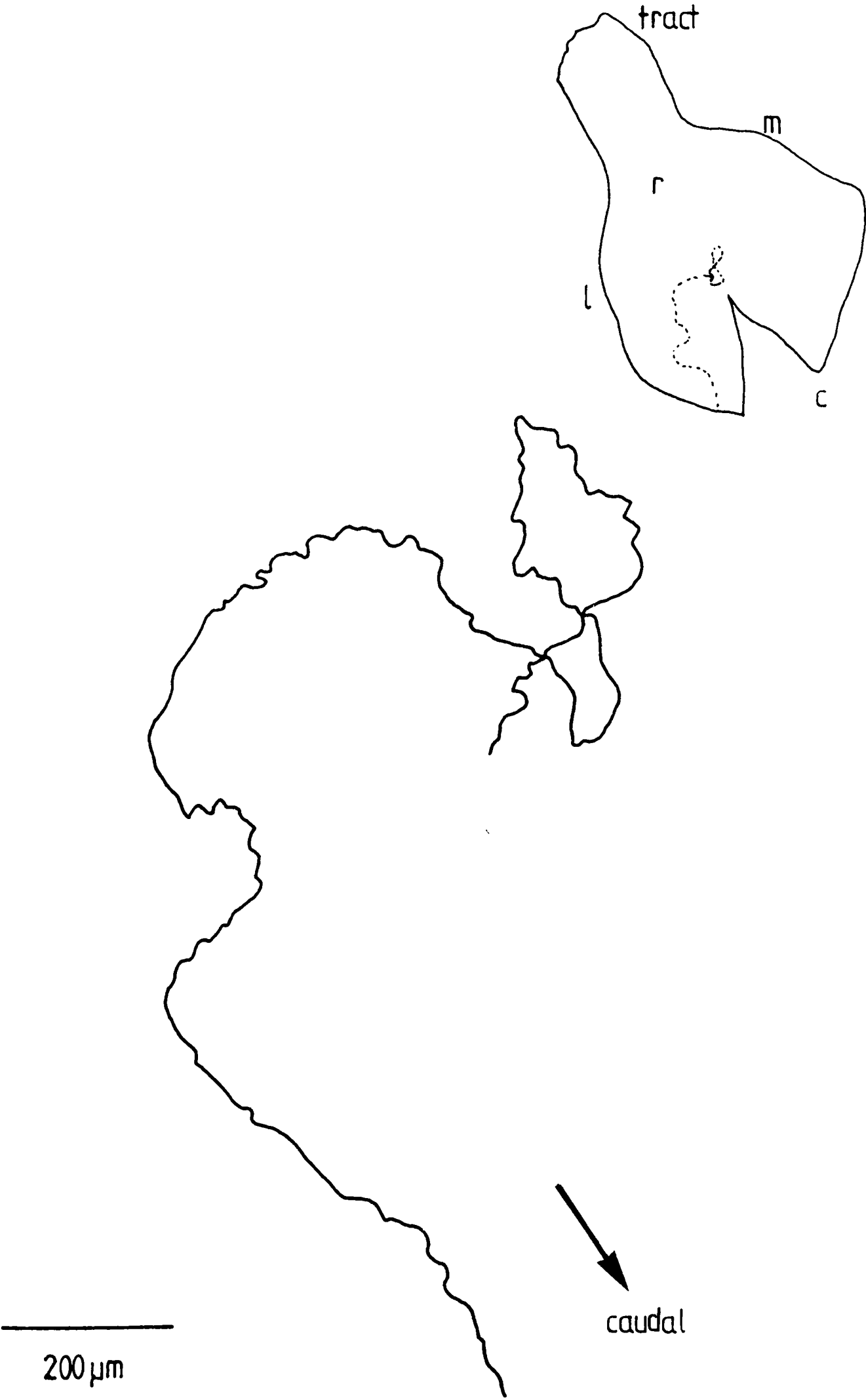
The wholemounts showed extensive growth of fibres over the tectum with little overall order beyond a tendency to sweep in large fascicles back towards their original side of the tectum. Many fibres were seen to pursue extremely varied and tortuous paths towards their termination sites (fig. 7.11).

Optic nerve regeneration

Four goldfish were mapped electrophysiologically 85-147 days after section of the right optic nerve in the orbit. Though some ordering defects were present in the maps the overall order and polarity was correct and in three of the four it approached normal. A cut was then made through the entire tectal thickness across the rostral pole of normal tectum and then extended caudally at its lateral edge (as an "L") for a variable distance. The area behind the cut was remapped using the same electrode placements. Between 16 and 20 positions directly behind the cut, and whose fibres would normally

Fig. 7.11 Camera lucida tracing of an HRP filled fibre pursuing a typically tortuous path across the tectum of a medial-lateral tract cross animal 33 days after surgery. The relationship of this fibre to the tectal boundaries is shown in the small tectal diagram (top right).

fig 7.11



be expected to have been severed by the cut, and 3-16 (mean = 9) of these gave localizable responses. On initial mapping all 36 of the responses obtained at these positions had been multiunit whereas on remapping at least 16 were clearly identifiable as due to isolated single units.

In most cases there was close correspondance between the visual field positions of the responses of the first and second map, the second map even retaining distortions of ordering present initially. Figures 7.12 and 7.13 show the maps obtained after 91 and 142 days of regeneration, respectively. As with the medial-lateral tract cross animals no quantification of the response position shifts was attempted as a variable error in electrode positioning arose due to tectal bleeding and tectal distortion after making a rostral cut.

It is interesting to note that the few responses persisting after the cut in the 142 day animal lie on a rostromedial-caudolateral line across the tectum. The fibres producing these responses must have entered the mapped area from a caudal direction and may have done so by following one of the fibre fascicles (or its debris) which run rostromedial to caudolateral across normal dorsal tectum, though in the reverse of the normal direction.

In each of these 4 animals HRP was introduced into the cut optic nerve in the orbit on completion of the mapping. The tectum was then processed as a wholemount after 20-24 hours survival. These wholemounts clearly confirmed that all fibres entering the mapped region in a rostrocaudal direction had been interrupted. Thus the responses persisting after the cut were due to fibres which had followed aberrant routes to (roughly) the correct tectal region.

Fig. 7.12 Electrophysiological maps of the visuotectal projection of an optic nerve regenerate fish obtained 91 days after surgery. The top diagram shows the initial mapping while the lower one shows the responses remaining after a rostral cut (dotted line on tectal outline). Both maps are corrected for eye rotation of 20° .

fig 7.12

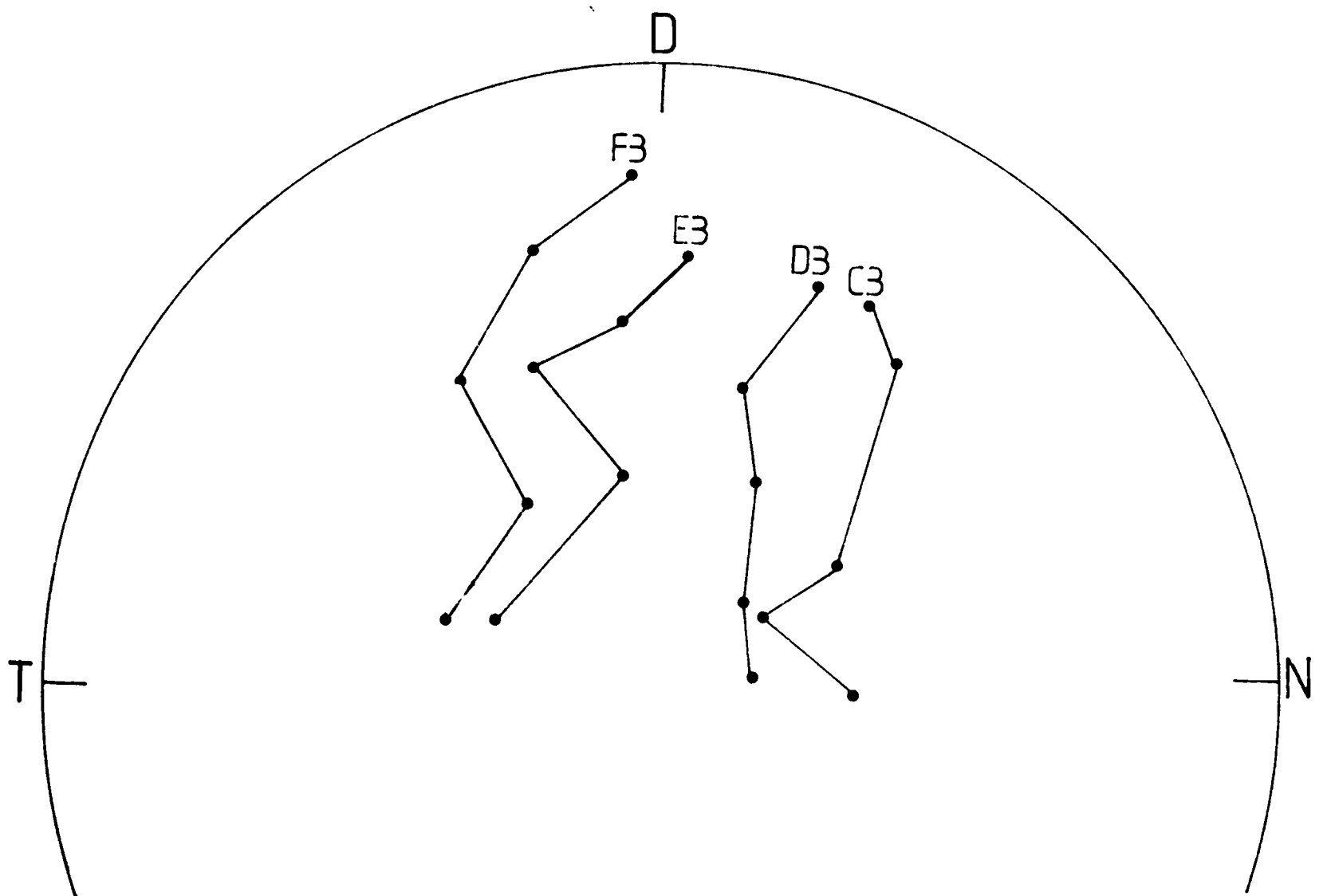
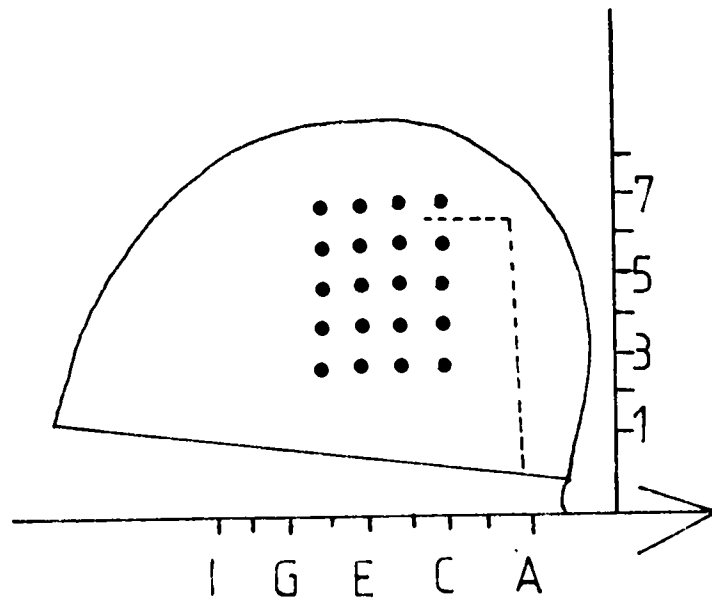
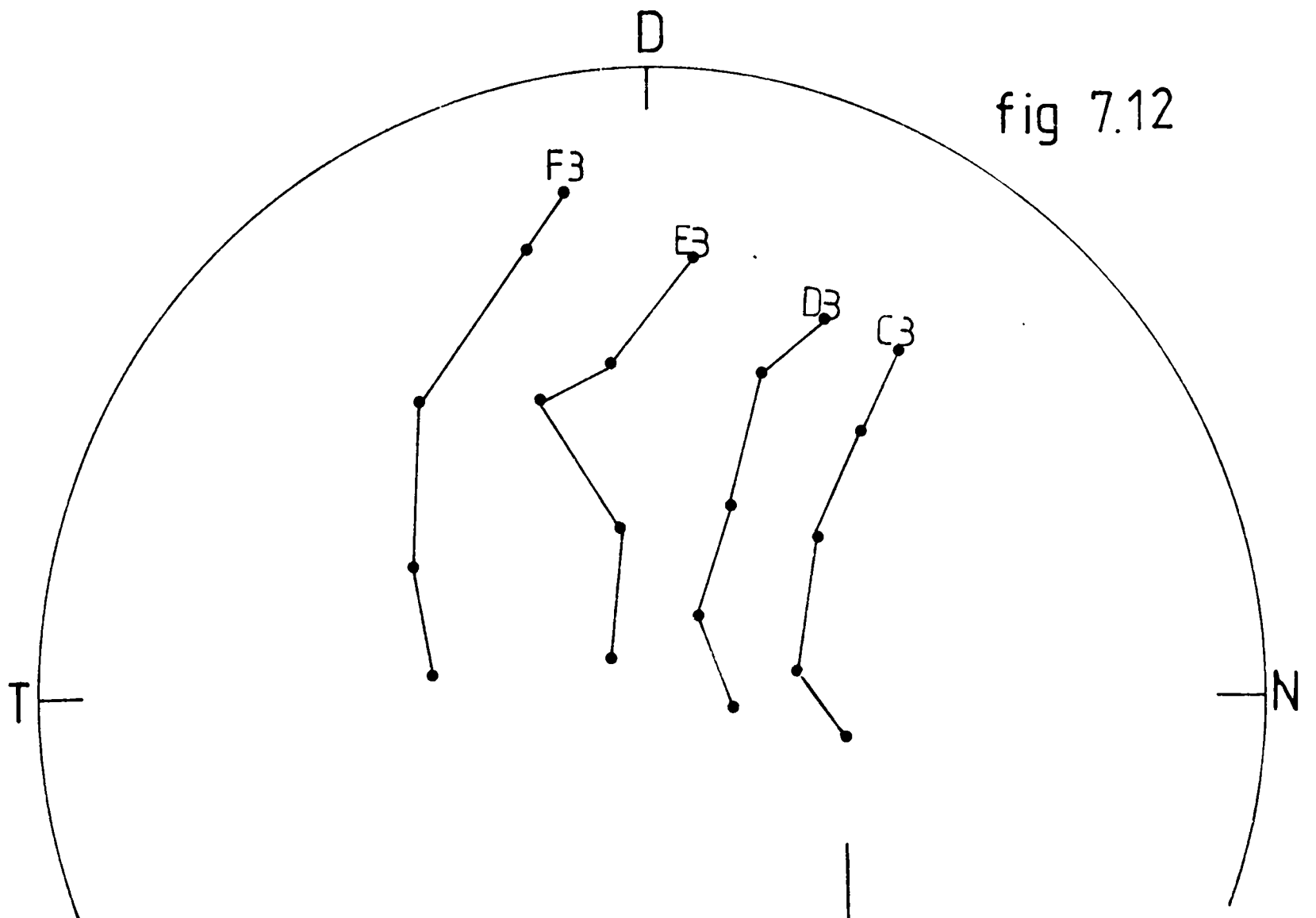
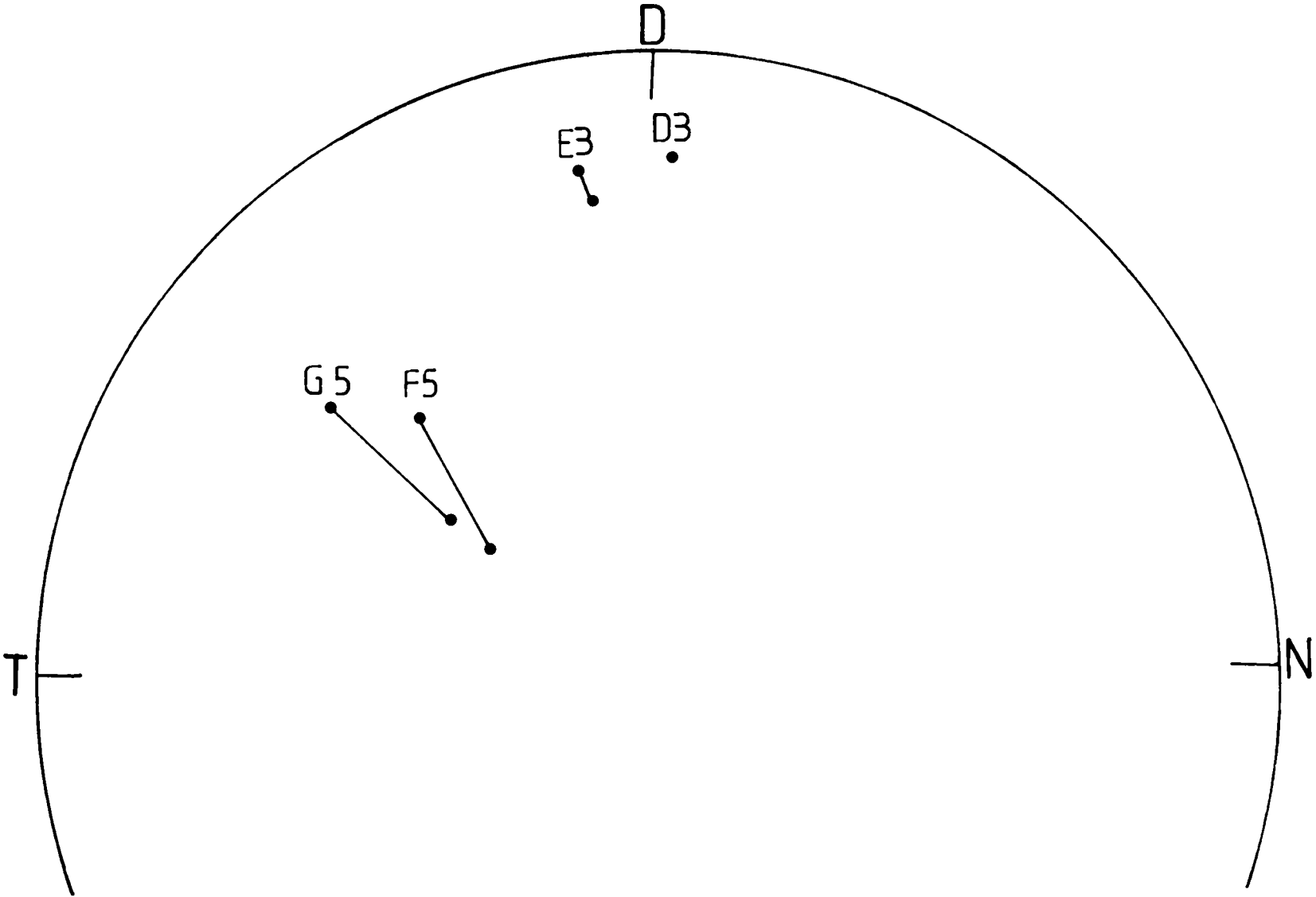
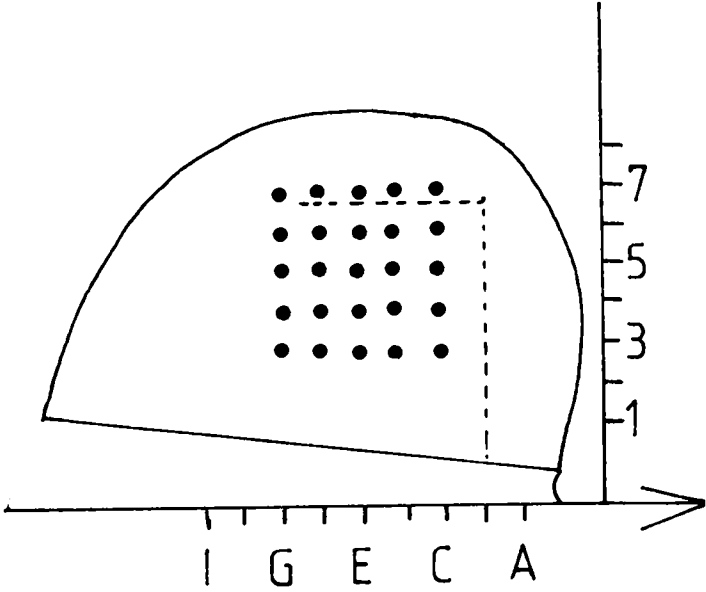
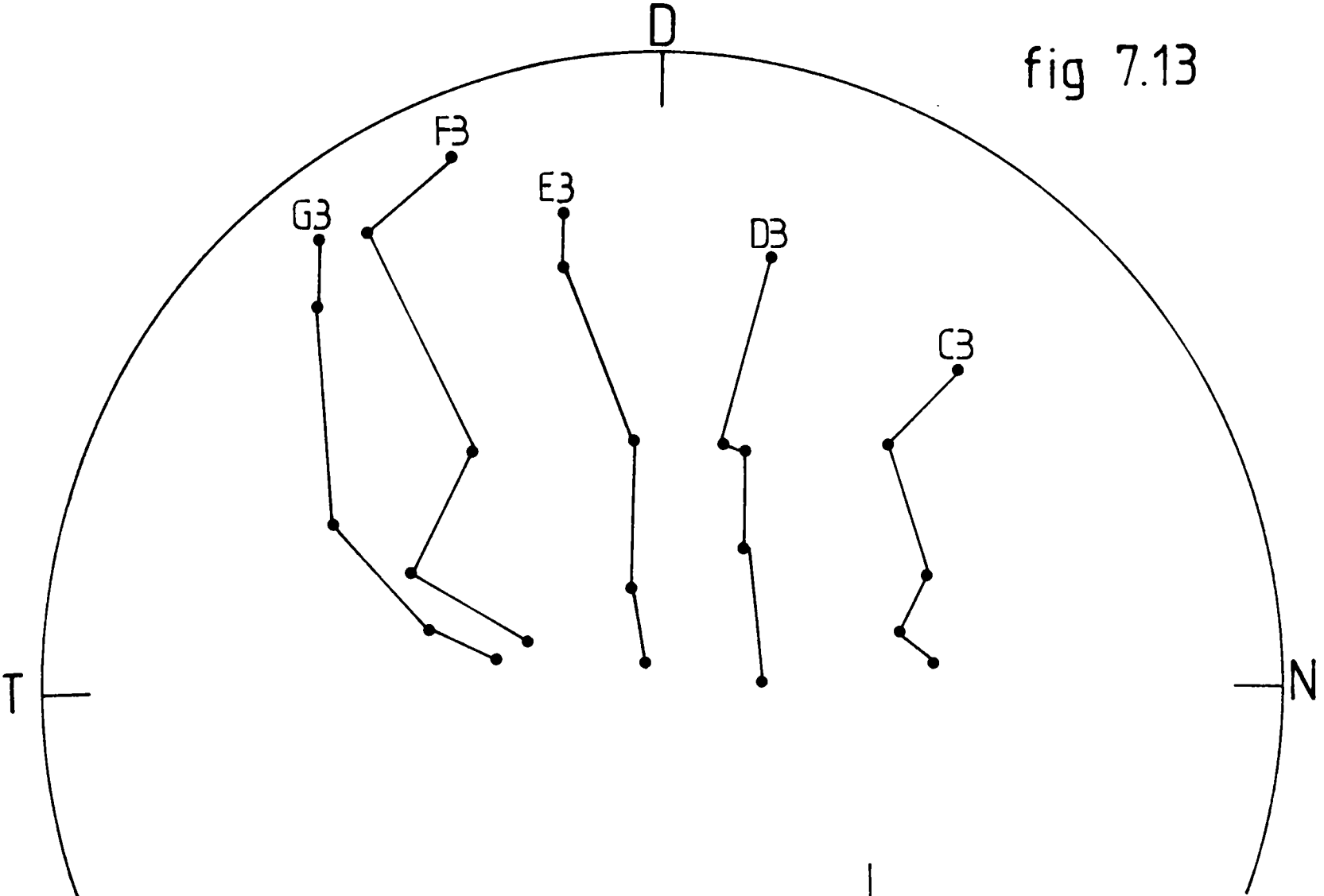


Fig. 7.13 Electrophysiological maps of the visuotectal projection of an optic nerve regenerate fish 142 days after surgery. The first map is shown at the top while the second map, obtained after making the tectal cut shown on the outline (dotted line) is shown below. Both maps are corrected for eye rotation of 15° .

fig 7.13



Discussion

The aim of the experiments was to test the effects of distortions of the ordering with which optic fibres grow into the tectum on the projection which they then form. If the morphogenetic model of Horder and Martin (1978) were correct a considerable disruption of the map might be expected whereas models involving precise chemospecific matching of optic fibres with their "correct" termination sites (Sperry, 1963) would predict that such pathway disordering would be overcome and a normal map produced.

After diversion of a fascicle of optic fibres from one part of the nerve to another in fifteen fish only one gave a normal electrophysiological map. Seven fish had to be disregarded because of possible confusion with anomalous responses described in appendix II. However the remaining seven fish showed very characteristic abnormalities with some electrode positions yielding responses located more temporally than normal. In the earliest mapped fish (83 and 92 days) only the abnormal responses were detected but in later fish (103 - 294 days) both the expected and a more temporal abnormal response were detected at some electrode positions. This strongly suggests that some of the deviated fibres were forced to grow into abnormal tectal regions and terminated there. That only abnormal single responses were obtained in the early fish but that these were accompanied by the correct response (double responses) in later fish indicates that the appropriate fibres to that tectal region may have initially been displaced from it and grew back only slowly. However the time series of fish is not extensive enough to draw any firm conclusions about the time course of events.

Thus this series of fish gave results suggesting that as a result of the pathway distortion some fibres became trapped in incorrect tectal regions and remained there long term (294 days). No such abnormally located fibres were detected in the cases where a fascicle of fibres was dissected free from the nerve but not deviated to the far side of the nerve.

The number of abnormal responses obtained in the fascicle transplant fish was small (2-5 double responses in 5 fish mapped at 103-294 days). Though the exact number of fibres deviated by the surgery is not known it probably represented 5-15% of the total. Thus many of these fibres probably overcame the surgical diversion, either in the pathway or at the tectum, to reach the correct tectal region. Certainly no obvious gaps were found in the maps even though the area of tectum to which the aberrant fibres should have projected was well within that sampled electrophysiologically.

The second experimental strategem employed to study the effect of pathway distortion was that of Arora and Sperry (1962), of crossing the medial and lateral tract brachia of the optic tract. Electrophysiological confirmation was obtained of their anatomical finding that many of the fibres do grow back to the correct tectal region, so that the projection is of the correct order and polarity. However the route taken by the fibres was not always directly back to the correct side across rostral tectum as Arora and Sperry had indicated. Remapping after a rostral cut, particularly when the cut had been extended caudally for up to $1200\mu\text{m}$ demonstrated that some fibres reached the correct tectal area by very unusual paths for such a cut would, in the normal animal, abolish all responses within the tectal region caudal to it. (Normally dorsal tectum is supplied by a fan of fibres running rostro-caudally and rostro-caudo-laterally across its surface). That the majority of fibres do, however, correct their paths early and then adopt a rostro-caudal route is suggested by the relatively small number of HRP filled fibres seen in the region caudal to the cut. Furthermore, many responses which were initially from multiple units changed to a single unit pattern of firing after cutting.

The accuracy with which fibres attain their correct tectal sites despite an abnormal route seems to be high. There was a good correspondence between response positions at a particular electrode position before and after making a rostral lesion (Fig. 7.5, 7.6, 7.7), though for reasons discussed above

quantification of this was not attempted.

Remapping of two medial-lateral cross animals after intervals of several months revealed that some distortions of ordering in the visuotectal projection persisted long term though in one case a considerable improvement in ordering at the second mapping was noted (fig. 7.10). Thus again it seems that if disruption of the fibre pathway is sufficiently gross some of the irregularities produced in the projection are not resolved even though many fibres do, as Sperry and his colleagues suggested (Arora and Sperry, 1962; Arora, 1963, 1966) overcome the deviations to reach the correct tectal sites.

In the case of optic nerve regeneration the degree of disturbance of the pathway ordering is not clear. Horder (1974b) thought it to be minor after tracing a small number of fibres with the electron microscope though recent HRP studies have indicated that many fibres do regenerate along abnormal routes (Bunt and Dawney, personal communication). That fibres manage to reach the correct place in the tectum via these abnormal routes was first suggested by Horder (1974b) who detected a few such fibres in the goldfish. Subsequently Udin (1978a) and Fujisawa (1981) have reported similar findings in amphibia though Fujisawa's method was unsuitable for detecting more than a crude level of accuracy in the termination sites.

In this study such aberrantlyrouted but correctly terminating fibres were found to exist at relatively long survival times (85-147 days) in numbers that varied greatly between fish. 19-100% of positions directly behind the rostral cut, and hence normally denervated by such a cut, were found to retain a response. To reach these positions the fibres must have followed very abnormal pathways, as also happened in the medial-lateral tract cross group. Meyer (1980) has also detected aberrantly-routed fibres in the goldfish tectum after regeneration though his anatomical method did not allow

him to determine whether they attained the appropriate termination sites.

In summary these results suggest that the ordering of fibres as they arrive at the tectum is important in determining the nature of the final projection in that gross disturbances of order seemed to cause long term entrapment of fibres at incorrect sites. However, in support of earlier anatomical work (Arora and Sperry, 1962; Arora, 1963, 1966) substantial diversion of fibres at the tectum could be overcome to a large extent to produce a grossly normal map, though a number of fibres (a fairly small proportion is indicated by the HRP wholemounts) follow very unusual paths to achieve this correction. Furthermore a minority of fibres were found to adopt such abnormal paths in simple regeneration thus emphasizing the much underplayed results of Horder (1974b), and supporting those of Meyer (1980). Electrophysiological methods employed here allowed a much higher degree of accuracy in detecting how precisely fibres got back to the right place than the anatomical study of Fujisawa (1981) and showed it to be surprisingly precise.

Chapter 8

How do optic fibres behave when diverted into a denervated tectum whose polarity is inconsistent with their internal ordering?

Introduction

The presence of a high degree of ordering within the retinotectal pathway of the goldfish may certainly contribute to the formation of a highly ordered projection on to the tectum (Horder and Martin, 1978). This projection has been found to occur in both the normal and the optic nerve - regenerated animal with a consistent orientation, nasal retina connecting with caudal tectum and dorsal retina with lateral tectum (Jacobson and Gaze, 1965). Is this consistent orientation, or polarity, merely a consequence of the direction of ingrowth of an ordered optic fibre population or does it reflect a controlling influence of the tectal tissue upon the optic fibres?

In an attempt to resolve this question a variety of experimntal stratagems have been employed, including rotation of the eye, rotation of all or part of the tectum or diversion of optic fibres into the ipsilateral tectum. As discussed in chapter 1, very few of these approaches have yielded any information concerning the relative ordering of fibres as they approach the inappropriately orientated tectal tissue. In the case of rotation of a small area of tectum in an adult goldfish, where an appropriately rotated projection was seen within the graft (Yoon, 1972a, 1973, 1975b, 1975c, 1977; Romeskie and Sharma, 1977, 1980), it has been assumed that fibres entered the graft in their normal rostrocaudal direction of growth and hence possessed an internal ordering the reverse of that required for the rotated projection. This was supported by Yoon's observation (1975b) that responses within the graft were unaffected by lesion of the medial, lateral and caudal edges of the graft but abolished by lesion of the rostral edge. However in a series of graft translocation experiments Martin (1978b) observed the deflection of fibres by scar tissue at the edges of the graft so that the projection seen within rotated grafts may have been due to few fibres entering the graft in considerable disorder albeit in the normal rostrocaudal direction.

Within the graft itself there was also the possibility of nonspecific guidance of fibres by degenerating debris from the previous fibre fan. This was particularly likely in cases where the rotation was of less than 180° . However this was not a vital factor in the formation of a rotated projection as such rotation has been observed in the projection to a graft in a long-term denervated tectum (Romeskie and Sharma, 1977b, 1980).

In the case of eye rotation experiments (Sperry, 1943b) again little was known of the arrangement of optic fibres as they approached the tectum and extensive reordering within the pathway, rather than at the tectum, was not excluded.

One experimental design in which some prediction can be made of a relative arrangement of optic fibres as they enter the inappropriately orientated tectal region was the case where a small strip of optic fibres are dissected with their underlying tectal tissue from one tectum and diverted into the other, ipsilateral tectum. This stratagem, developed by Meyer (1978b, 1979a, 1979b), posed a particularly great problem for the deviated fibres as the rearrangement of ordering required in order to conform to the polarity of the recipient tectum, could not be achieved by simple rotation of the fibre array but required reversal of only one of its orthogonal axes. This is because the tecta are mirror images of each other about the midline of the animal.

Meyer has employed a number of variants of this technique in which the fibre strip was inserted into a permanently, temporarily or partially denervated host tectum. In the latter two situations the behaviour of the deviated fibres may have been modified by interactions with the normal fibre population of the host tectum (1979a, 1979b). Only in the case where the host tectum is permanently denervated by enucleation might a polarity-controlling influence by the host tectum be distinguished. Bunt (Bunt, Horder and Martin, 1978;

Bunt 1980) has investigated the behaviour of such fibres electrophysiologically after inserting them at various sites and in various orientations into the host tectum while Meyer (1978b) used both anatomical and electrophysiological methods to study the behaviour of fibre strips taken from a variety of sites on the donor tectum but inserted at approximately the same site on the host tectum. In both cases the aim was to detect both polarity and position specificity. In both cases a slight preference of fibres for the appropriate tectal termination site was observed though there was also considerable expansion into other tectal regions, particularly those lying between the point of insertion and the correct tectal region. Meyer also detected a very crude polarity within the projection that was appropriate to the host tectum though Bunt claimed the polarity to be more heavily influenced by the ordering of the incoming fibre array. However in both studies the area of visual field represented on the ipsilateral tectum was very small, making determination of polarity difficult. Furthermore any distortion of fibre ordering at the edges of the strip due to the surgery could have concealed an effect of that ordering on the projection.

It was therefore decided to repeat and extend these studies using wider fibre strips, subserving a greater area of visual field, and employing both anatomical and electrophysiological methods to define the polarity of the resulting ipsilateral projection and to study the paths taken by fibres in any reordering process.

Methods

Surgery

Under anaesthesia the left eye was removed from each fish as described in chapter 2. The optic tecta were then exposed and one of the following operations performed on each animal.

1. A narrow strip of tissue was dissected free, except at its rostral end, from the left tectum using tungsten needles and iridectomy scissors. This strip was about $1000\mu\text{m}$ long and $100\text{--}200\mu\text{m}$ wide, representing about 5-15% of the tectal area, and was taken from the medial edge of the left tectum. The strip was deflected towards the right tectum and its distal end was inserted into a small lesion in the centre of the dorsal aspect of that tectum. Great care was taken to avoid inversion of the strip during deflection.

2. A similar operation was performed except that the strip was of a triangular shape with its apex located rostrally on the left tectum. The medial edge of the strip was taken as the medial tectal margin while the lateral edge ran obliquely across the dorsal tectum from rostromedial to caudolateral, along the line of the fibre fan. This strip extended about $1000\mu\text{m}$ caudally on the tectum, was $400\text{--}600\mu\text{m}$ wide at its caudal end, and represented 10-20% of the tectal surface. The strip was deflected, again without inversion, and its caudal edge inserted into a small lesion in the centre of the dorsal aspect of the right tectum.

3. A triangular strip as in (2) was freed, except for its rostral pole, from the left tectum and inserted into the right tectum. This strip had a similar shape and dimensions as in (2) but was located with its medial edge at least $200\text{--}300\mu\text{m}$ lateral to the medial edge of the left tectum.

In each case the skull was then closed and the animals allowed to recover. All three operations are illustrated in figure 8.1.

Electrophysiological mapping

A single electrophysiological map was obtained of the projection from the right eye to the right (ipsilateral) tectum using the methods described in chapter 2.

Fig. 8.1. Diagram illustrating the three surgical operations employed to divert a small population of optic fibres in their original order into the ipsilateral tectum.

1

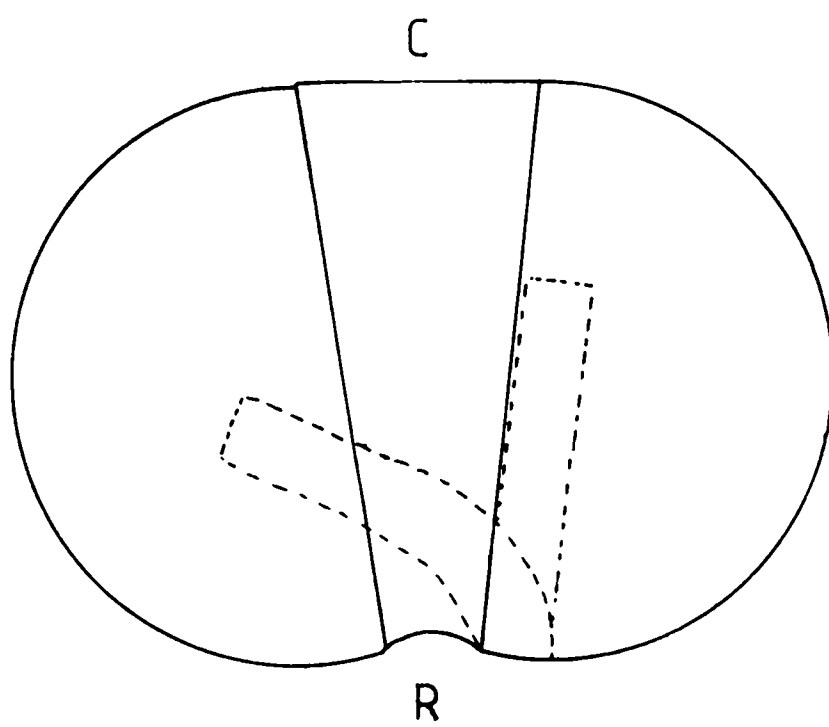
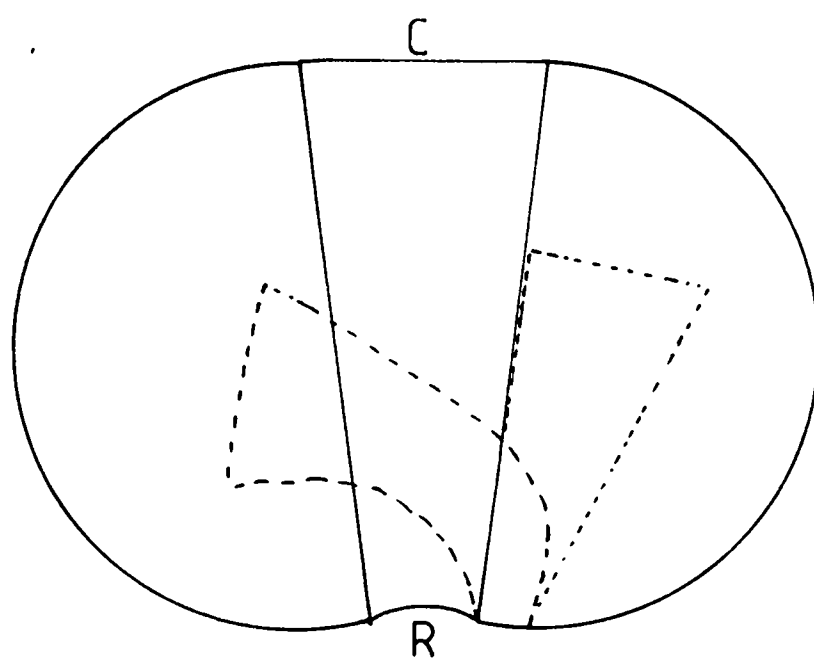
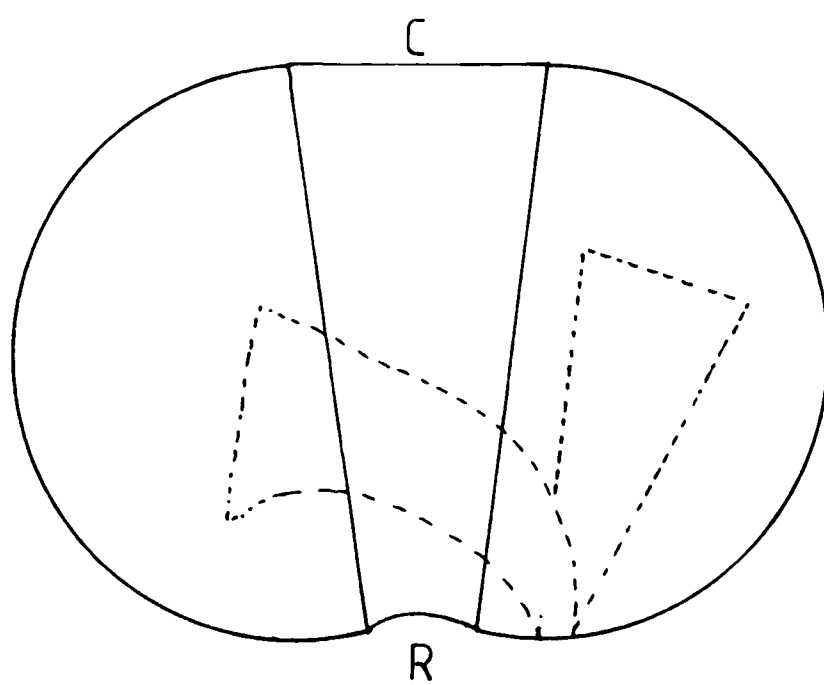


fig 8.1

2



3



Anatomy

After completion of mapping the right optic nerve was cut in the orbit and filled with HRP solution. After 20-28 hours survival the tecta were processed for HRP as a wholemount.

Some animals which had not undergone mapping were also analysed anatomically as above.

Results

1. Small narrow fascicles

Three fish were mapped at 99-266 days after operation. In each case visual responses were detected over almost the entire extent of dorsal tectum sampled but were confined to a small region of the dorsotemporal visual field.

Because the area of field represented was small it was extremely difficult to detect whether the ordering of fibres as they reached the ipsilateral tectum had contributed to their final projection. A typical map, obtained 266 days post operatively is shown in figure 8.2 and illustrates the difficulty in interpreting the polarity and ordering of the projection.

Because the quality of HRP labelled wholemounts obtained after electrophysiological mapping was poor a number of animals were analysed using only anatomical techniques. As can be seen from the camera lucida diagram of such an animal labelled 222 days after surgery fibres leaving the bridge spread out extensively over the vacant tectum (figure 8.3). Some crossing of fibres was observed near the base of the bridge but no systematic reordering was detected.

2. Medial fan-shaped fascicles

Two animals were mapped 38 and 44 days post operatively. As with the first group of small narrow fascicle animals only extreme temporal visual field

Fig. 8.2 Electrophysiological map of the projection from the right eye to the right tectum of a small fascicle animal 266 days after surgery. Electrode positions are indicated on the small tectal diagram. O = point at which a localizable response was detected. . = no localizable response. The map is corrected for an eye angle of -4° . Note that this map is reversed compared with the usual form of representation. The nasal and temporal labels are correct.

fig 8.2

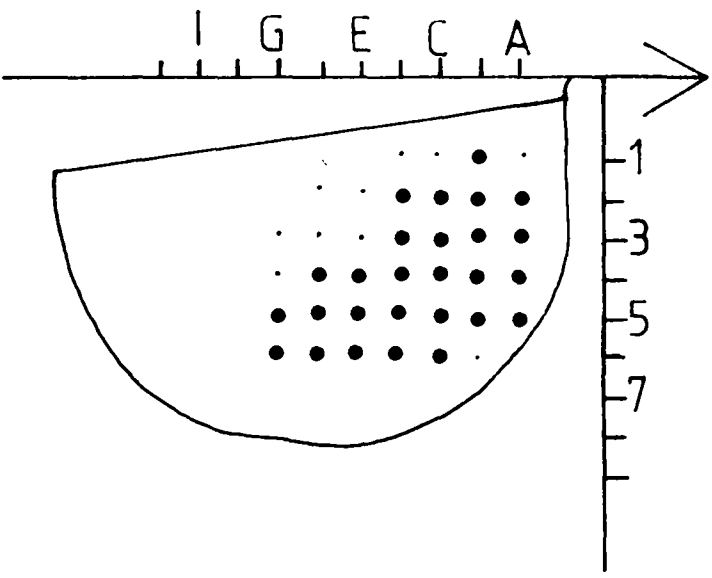
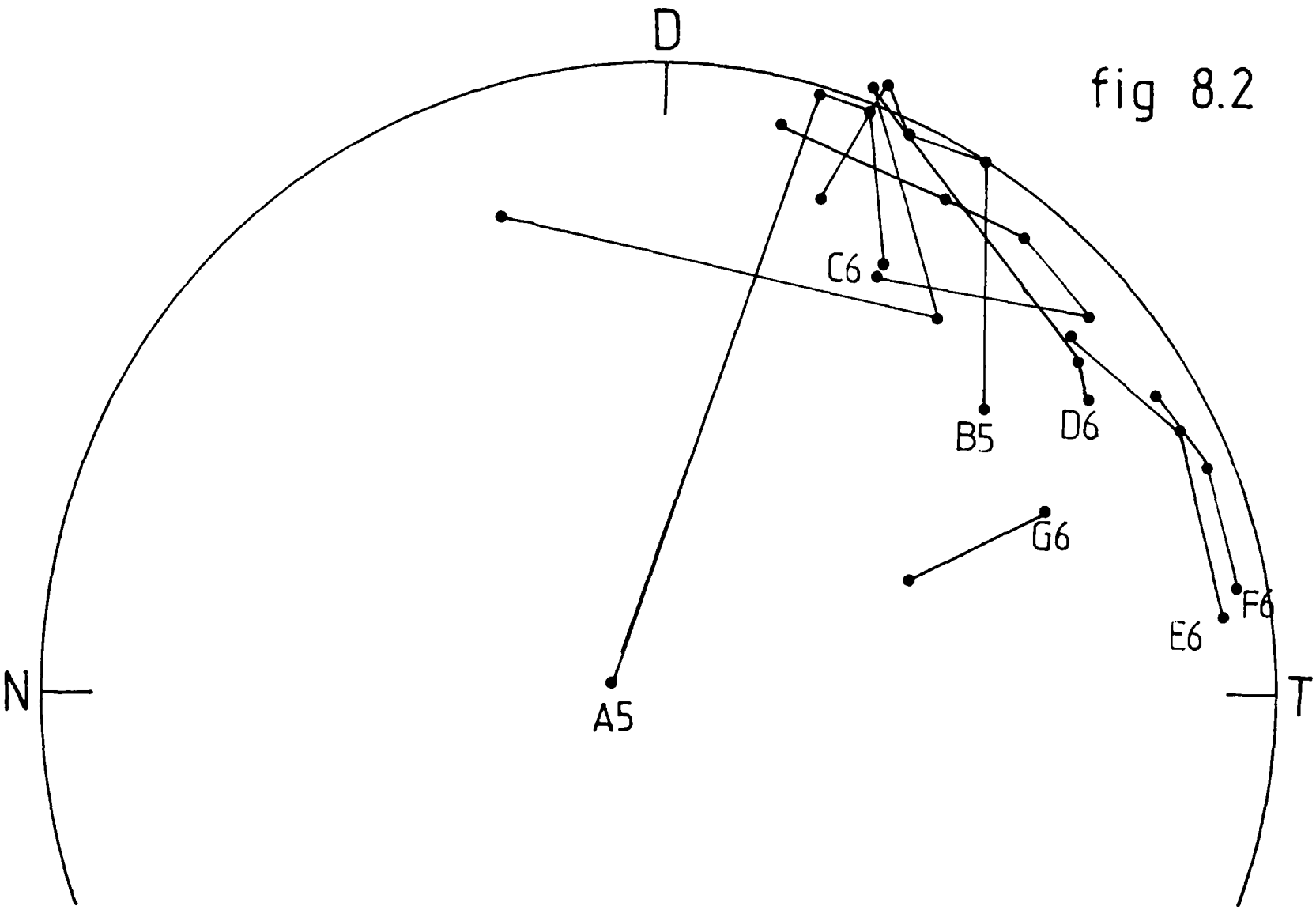


Fig. 8.3 Camera lucida drawing of fibres from the right eye of a small narrow fascicle animal spreading out over the right tectum on leaving the bridge. This animal was labelled 222 days after surgery.

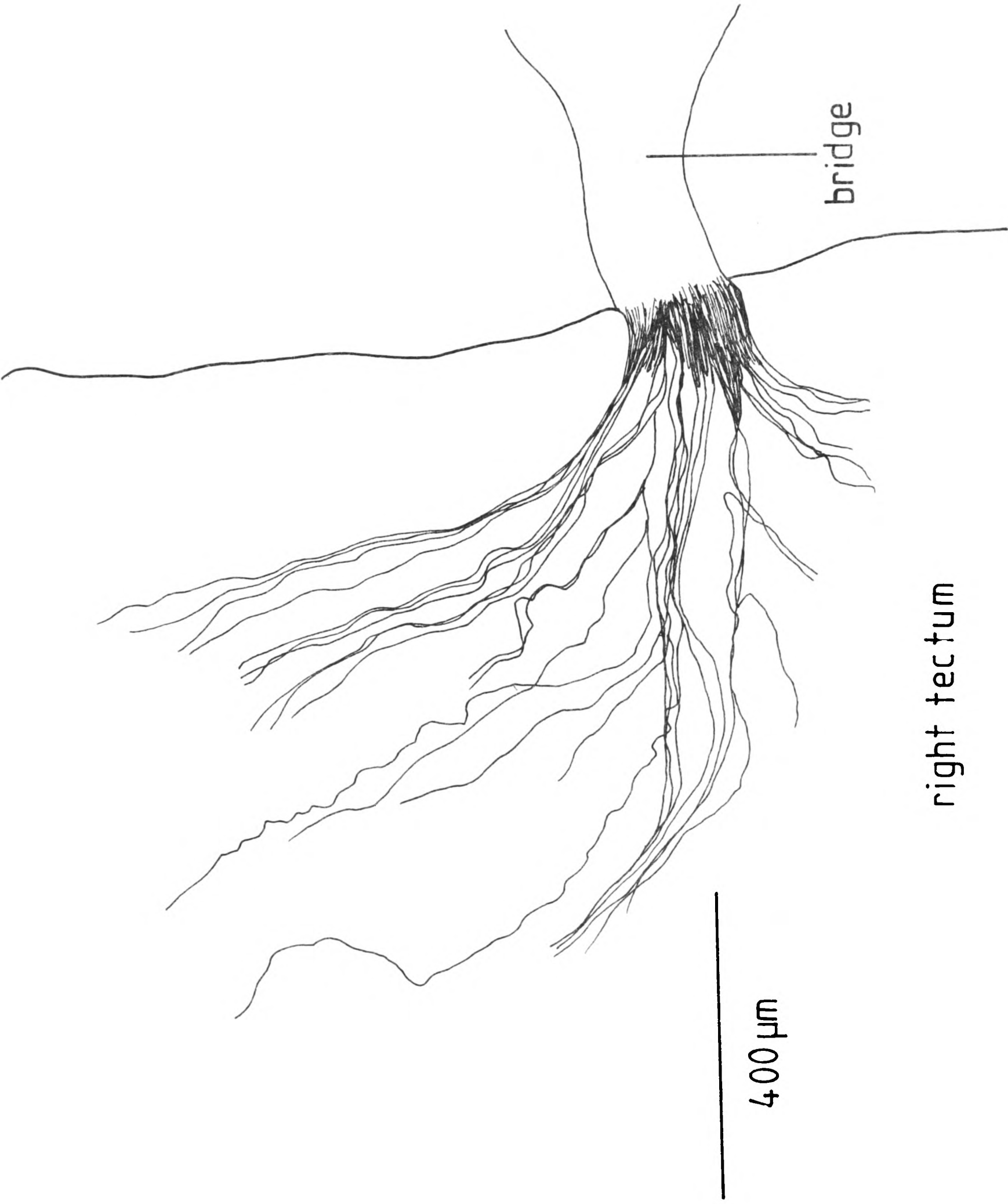


fig 8.3

was represented on the ipsilateral tectum even though most of the dorsal extent of that tectum was found to be responsive to visual stimulation. Because of the problems involved in determining the order and polarity of such a grossly expanded projection from a difficult-to-map area of field electrophysiological analysis of this series of animals was not continued. The remaining animals were studied using HRP labelling only.

Wholemounds of the tecta of two animals whose right optic nerve had been filled with HRP 71 days after operation revealed some disorganization in the pattern of outgrowth of fibres from the end of the bridge. Figure 8.4. shows a camera lucida drawing of one such preparation.

3. Fan shaped fascicles from more lateral tectum

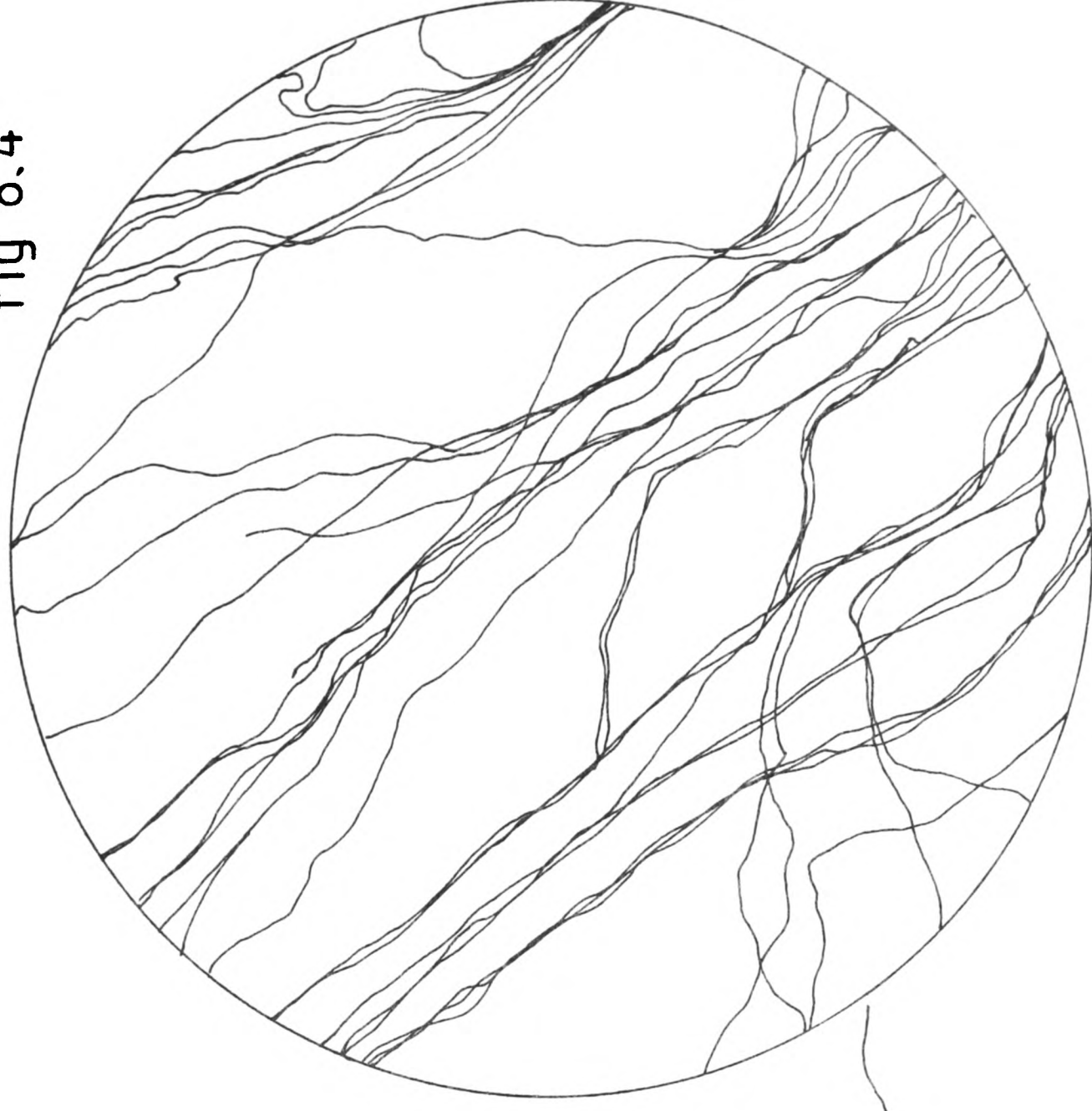
Five animals were mapped electrophysiologically between 43 and 105 days after surgery and in each case a much larger area of visual field, the majority of dorsotemporal field, was represented on the ipsilateral tectum than in the previous two series of animals. In each case the projection was extremely disordered with frequent crossing over of lines of responses. Hence firm conclusions about the ordering and polarity of the projection were difficult.

However in three of the five animals the polarity of the projection did appear to be approximately that expected from the relative ordering of fibres in the bridge and the position of their insertion into the host tectum. Fibres were initially directed in a medial to lateral direction across the ipsilateral tectum. Figure 8.5. shows such a map obtained 50 days after surgery, with an explanation of the projection in terms of extant fibre ordering on entry to the ipsilateral tectum.

In two cases the maps obtained were disordered to such an extent that no firm conclusion could be reached concerning the polarity of the projection though

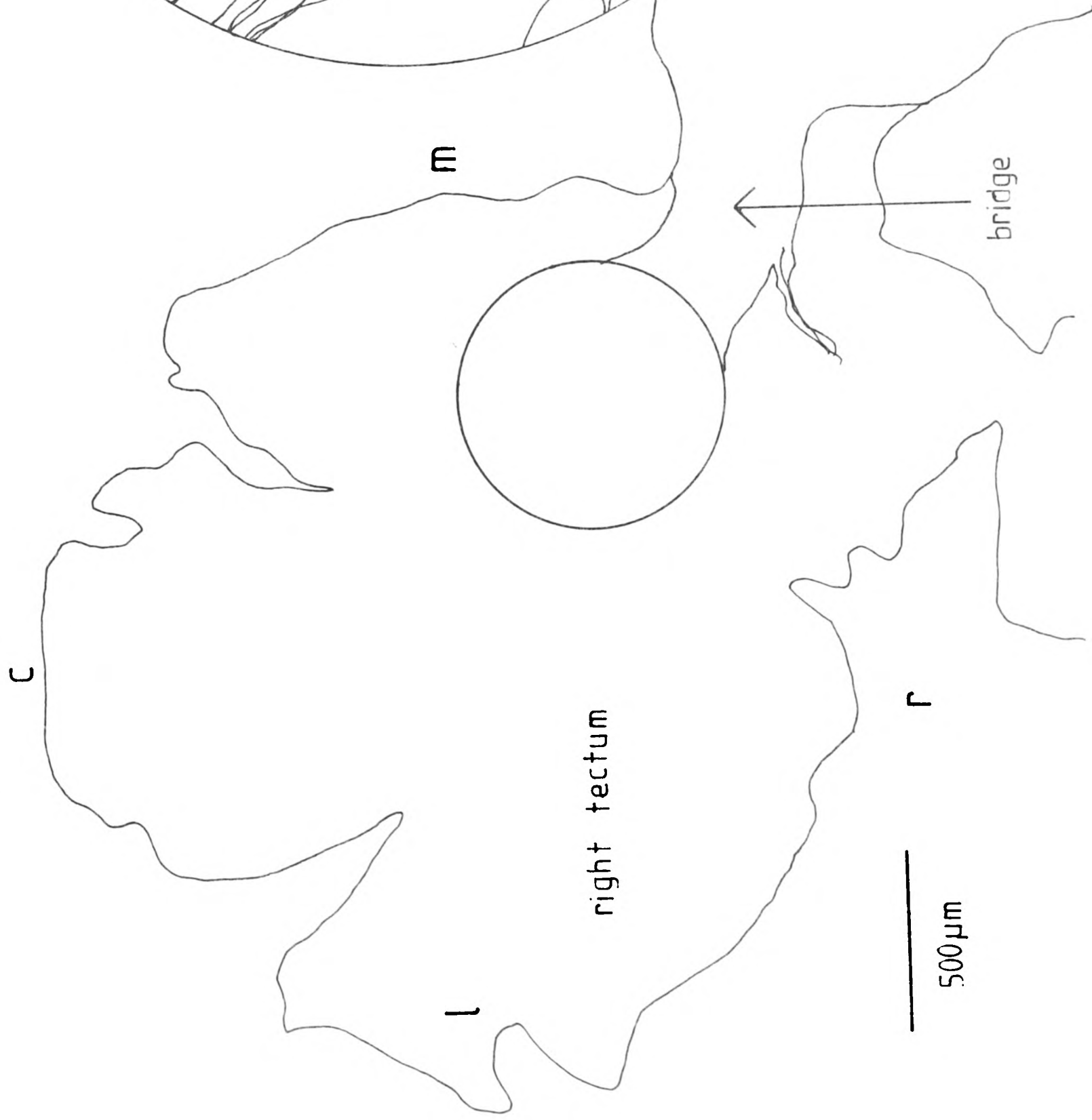
Fig. 8.4. Camera lucida drawing of the fibres from the right eye of a medial fan-shaped fascicle animal spreading out over the right tectum on leaving the bridge. Some crossing over of fibres was observed but no systematic reordering was detected.

fig 8.4



200 μm

left tectum



500 μm

bridge

right tectum

m

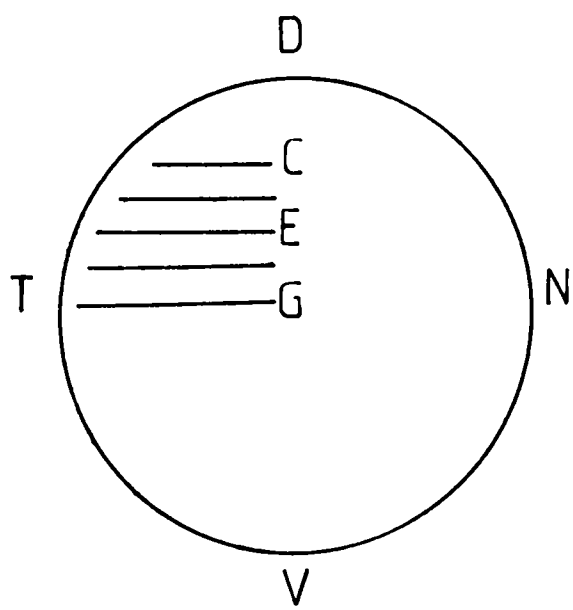
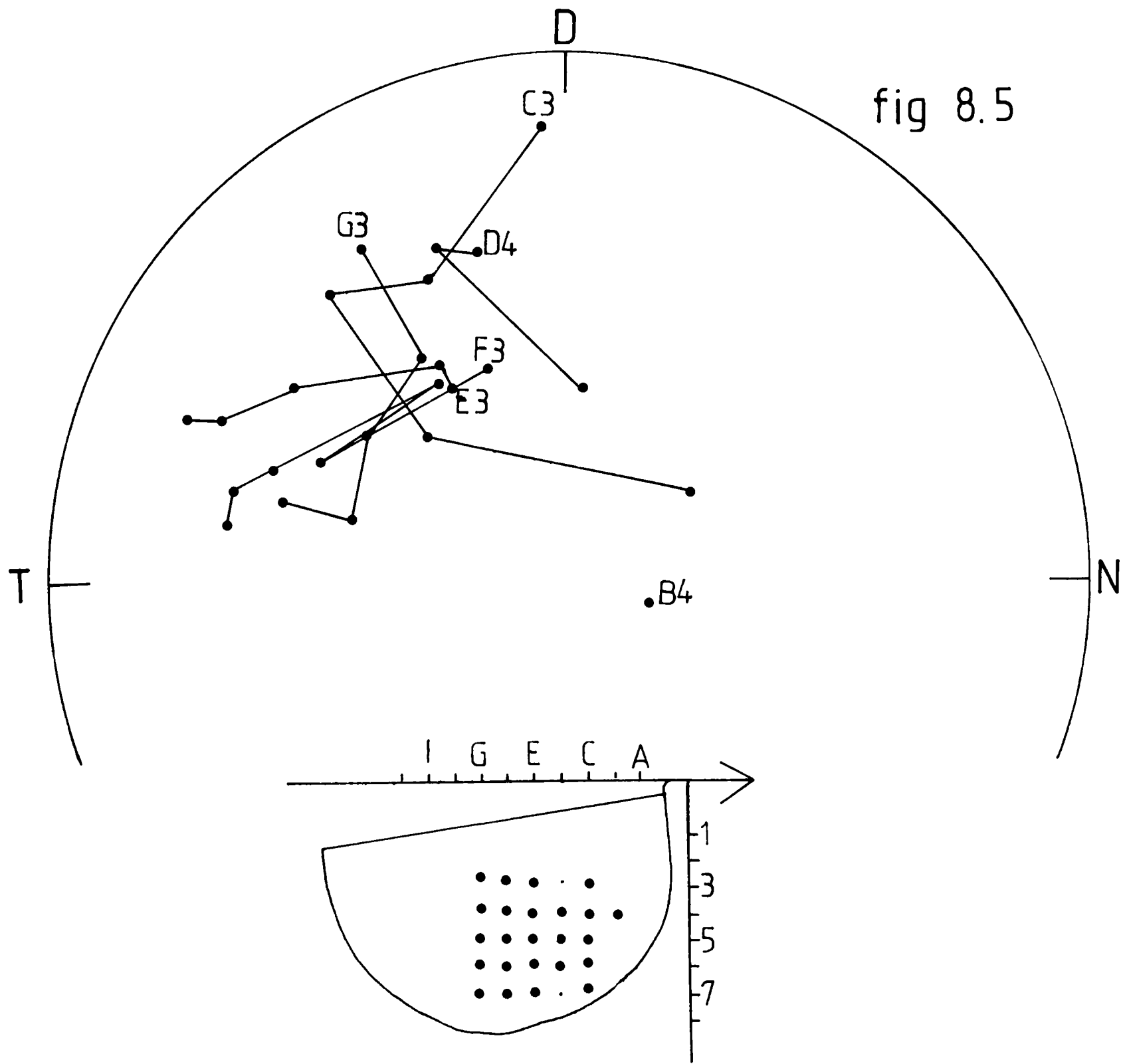
r

l

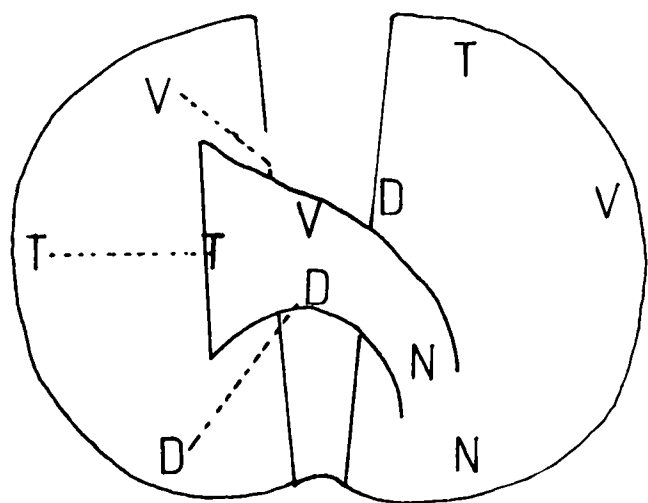
c

Fig. 8.5 At the top is shown the electrophysiological map of the projection from the right eye to the right tectum, in a lateral fan-shaped fascicle animal mapped 50 days post-operatively. The map is corrected for an eye angle of 27° (● = responding points. . = points at which no localizable response was detected).

Below is a diagram indicating how this projection may be the consequence of the ordering possessed by the fibres as they arrive in the host tectum. On the left is a diagrammatic representation of the map and on the right a sketch of the tectal bridge showing how the observed map is derived from the fibre arrangement in the bridge. In contrast to the normal map electrode position rows C-G gave predominantly horizontally orientated lines of responses corresponding to the 90° rotation of the fibre array produced by swinging the fan-fascicle across the midline. The dorso-ventral ordering of these rows was that expected from the relative arrangement of the fibres on entering the host tectum.



right field



tecta

there was certainly no evidence of a major reorganization of the fibres to fit the host tectum polarity. It is possible that again the maps were formed by the operation of morphogenetic forces on the fibre arrangement in the bridge - possibly surgical manipulation caused more disruption in these particular cases.

Figure 8.6. shows such a map obtained at 94 days after surgery. No systematic change in the nature of the projection produced in this series of animals was observed with increasing survival time.

Again mapping was found to reduce greatly the quality of the HRP wholemounts. However the wholemounts were adequate to confirm that the fibres entering the ipsilateral tectum were arranged in a parallel manner in the bridge with only minor distortions at the bridge edge.

Discussion

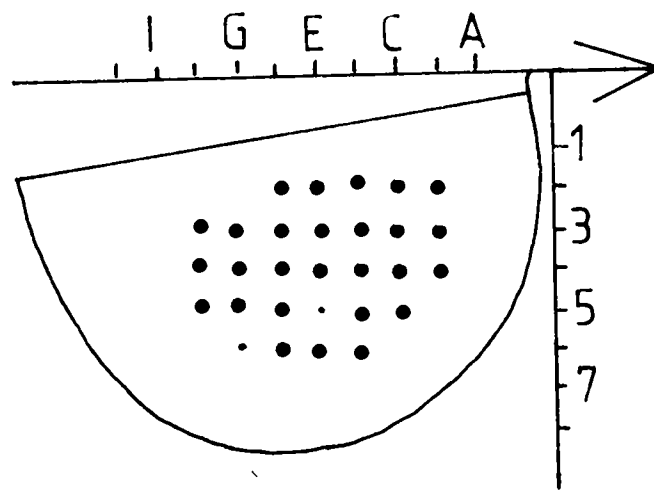
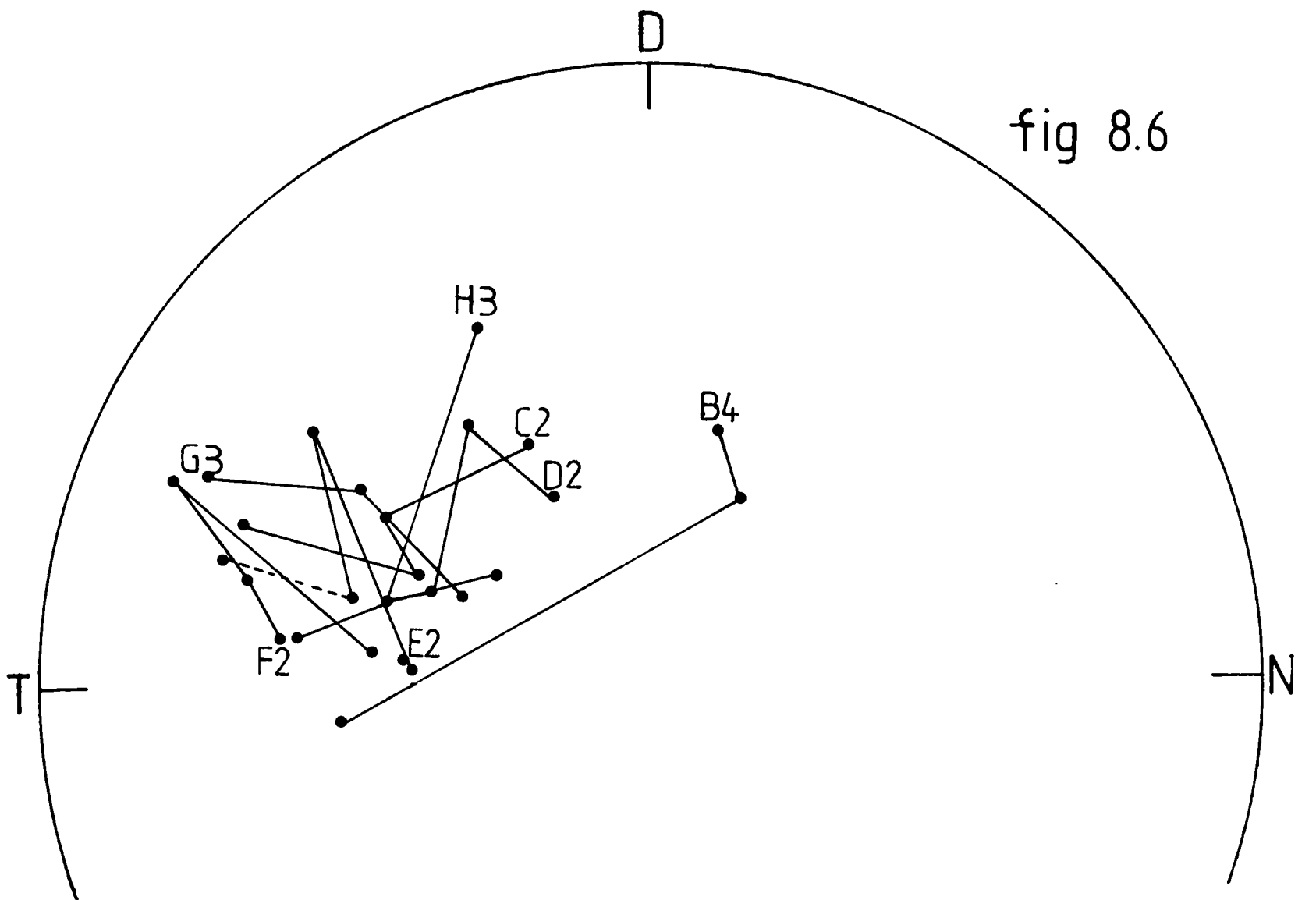
The first series of animals in which a narrow, parallel-sided strip of tectum was deflected into the permanently denervated ipsilateral tectum served to confirm the results of Meyer (1978b) and Bunt (1980). A grossly expanded projection from those fibres which would normally transverse the strip was found in the ipsilateral tectum. Very little order was detected in this projection. However the determination of order and polarity in the projection was hampered by the extreme peripheral temporal location of the responses. Peripheral field positions proved difficult to locate accurately as they frequently extended beyond the perimeter. Furthermore because of the distortion of the projection caused by mapping in air the most peripherally located ganglion cells could not be stimulated (Meyer, 1977), and thus many peripheral response positions mapped may have corresponded to the edges rather than the true centres of those particular fields. This is discussed in chapter 2. This limitation of eye-in-air mapping may explain why the

Fig. 8.6. Electrophysiological map of the projection from the right eye to the right tectum in a lateral fan-fascicle animal 90 days after surgery. The map is corrected for an eye angle of 27° .

This map shows no clear evidence of adjustment of polarity to fit the host tectum.

(O = localizable response obtained . = no localizable response).

fig 8.6



projections observed in this series of animals failed to show even the crude polarity detected by Meyer using eye-in-water mapping (Meyer, 1978b).

A similar problem of mapping accuracy in the far temporal field was encountered in the second series of animals in which a medial, fan-shaped area of tectum was deflected ipsilaterally. Again the area of field was so small and the map so disorderly that the polarity of the projection was in considerable doubt. In both series HRP staining of the fibres in tectal wholemounts illustrated the great expansion of the fibre array to occupy most of the host tectum. It demonstrated some disordering or crossing over of fibres as they left the bridge though in no case was an systematic reordering detected. Frequently fibres which occupied neighbouring and parallel positions in the bridge pursued widely divergent paths on entering the ipsilateral tectum.

The third series of animals in which a fan-shaped tectal strip was taken from a more lateral location presented the best opportunity for the detection of ordering in the resultant projection. Fibres were entering the host tectum in a wide parallel array and represented an area of field in an easily mapped region and of a sufficient size to make determination of projection polarity and ordering possible. Thus the most striking aspect of the resulting electrophysiological maps was their lack of order. All five animals gave very messy maps whose interpretation was very difficult. There was certainly no clear indication that the host tectum was able to control the polarity of the projection regardless of the incoming fibre ordering. Three animals gave maps very crudely appropriate to the fibre ordering in the bridge (fig. 8.5). Even where no polarity was obvious it seemed likely that fibres had reached their final positions by means of purely mechanical guidance (fig. 8.6). No systematic reordering of fibres on entry into the host tectum was seen anatomically.

An explanation for the disorder of the projections in terms of the massive distortion caused to the brain by surgery seems unlikely as fibres were observed to cross the bridge in an orderly parallel array. Very little damage was caused surgically to the host tectum.

These results confirm the ability of optic fibres to project to inappropriate tectal regions, particularly those encountered first as they grew into a vacant area of tectum (Bunt, Horder and Martin, 1978), and to expand to cover a much larger area than normal (Horder, 1971a; Meyer, 1978b). However they fail to demonstrate any polarity-controlling ability of the host tectum able to override completely the pre-existing inappropriate ordering of ingrowing fibres. Nor, however, can the ordering of the ingrowing fibres be considered to be the absolute determinant of projection polarity for in that case one would expect the projection to exhibit a clear polarity corresponding to the pattern of ingrowth. It therefore seems likely that several factors are operating to control the formation of nerve connections and these will be discussed further in chapter 10.

What are the effects on an ipsilateral retinotectal projection, induced by removal of the other tectum, of the pathways followed by the optic fibres?
How is such a projection affected by the presence, absence or regeneration of a contralateral projection to that tectum?

Introduction

In the normal adult goldfish no direct projection has been detected from the retina to the ipsilateral optic tectum using either autoradiographic tracing after injection of a radioactive label into one eye (Springer and Landreth, 1977) or by staining for degeneration after unilateral enucleation (Sharma, 1972a). However the autoradiographic study did detect a direct ipsilateral projection to other nuclei via either an ipsilateral path at the optic chiasm or a contralateral path at the chiasm followed by recrossing in the posterior or minor commissures. Similarly in the frog, both Rana ssp and Xenopus laevis, a direct projection from retina to ipsilateral tectum has not been detected though there is one to other centres, particularly in the thalamus (Lazar and Szekely, 1969; Szekely, 1971; Currie and Cowan, 1974; Scalia and Fite, 1974; Scalia, 1976; Steedman et al., 1979).

The ipsilateral tectum provides a target for optic fibres of a polarity which they would not normally encounter, the medial-lateral axis being the reverse of that encountered in the contralateral tectum, the rostral-caudal axis being the same. Thus if fibres could be induced to project to the ipsilateral tectum the polarity of the resulting retinotectal connections and the relationship of this polarity to the ordering of the fibre array as it approached the tectum would be of considerable interest in any attempt to elucidate the mechanisms controlling the formation of the retinotectal projection. Similarly a study of the influence of a partial, complete or regenerating direct contralateral projection on the nature of the induced ipsilateral projection might be expected to provide information on the existence and nature of interactions between optic fibres.

A considerable body of experimental work has been published on the production and nature of an ipsilateral retinotectal projection in both goldfish and frog and this will be reviewed below. However there is usually little, if any, information available as to the paths followed by the fibres and their relative ordering as they entered the ipsilateral tectum. Thus it is not possible to determine whether the polarity of the projection results from information available to optic fibres within the tectum or whether it is the consequence of active reordering of the fibres by cues present in the pathway prior to the tectum. Furthermore conflicting results have been obtained on the behaviour of the ipsilateral projection in the presence of a contralateral projection.

In the goldfish simple crush of one optic nerve led to the regeneration of a few fibres into the ipsilateral tectum and these were detected autoradiographically at early times after the crush. However by 16 days the level of labelling in the ipsilateral tectum started to decline and none was detectable by 31 days. Thus an ipsilateral projection did not persist in the presence of an intact contralateral projection if the normal target of these fibres remained intact (Springer, 1980b). Nor was a persisting ipsilateral projection produced if both optic nerves were crushed simultaneously though it did occur if the contralateral projection to that tectum was removed completely or caused to regenerate two days or more after the ipsilateral fibres (Springer and Agranoff, 1977; Springer et al., 1977). Such a projection was, however, extremely small comprising perhaps 5% of the fibres from that eye and was not functional (as determined by behavioural testing) even though it extended over most or all of the ipsilateral tectum and could be shown electrophysiologically to have the polarity appropriate to the tectum. Nothing is known of the ordering of these fibres as they approached the tectum.

A more substantial ipsilateral projection was produced, as in the goldfish, by removal of one tectum and the ipsilateral eye. The remaining eye then projected, via ill-defined pathways, to its ipsilateral tectum giving a projection which was shown both behaviourally and electrophysiologically to have a polarity appropriate to the host tectum (Misantone and Stelzner, 1974a, 1974b; Straznicky et al., 1980). Thus in neither frog nor goldfish is it clear which routes were taken by optic fibres which were forced to project to an ipsilateral denervated tectum by removal of their normal target, or how fibre ordering in that pathway may have contributed to the mirror-image projection so formed.

In a number of experiments on goldfish an ipsilateral projection has been induced to a tectum retaining a contralateral projection. In its simplest form this was achieved by ablating one tectum though in other cases the disconnected fibres were deflected towards the remaining tectum. In every case the ipsilateral projection had a polarity appropriate to the host tectum (Sharma, 1973; Cronly-Dillon and Glaizner, 1974; Levine and Jacobson, 1975; Easter and Schmidt, 1977). However in some cases the ipsilateral projection completely overlay the contralateral projection in a uniform manner (Sharma, 1973) as detected by electrophysiological mapping and by autoradiography whereas in other cases there was partial segregation of the two fibre groups so that some areas of tectum were innervated exclusively by one or other eye, and some areas by both (Cronly-Dillon and Glaizner, 1974; Levine and Jacobson, 1975). Lo and Levine (1980) found that this "patching", as seen using autoradiography, developed with time, only becoming clear at 8-12 weeks after unilateral tectalablation. However Sharma's failure to detect "patching" is unlikely to be due to this as his survival times were long, 12-25 weeks. Furthermore Lo and Levine maintained their fish, which were of a similar size to those used by Sharma, at a temperature which was not unusually high and therefore not likely to have provoked particularly rapid neuronal regeneration.

With regard to pathways Sharma (1973) found that fibres reached the ipsilateral tectum via either the ipsilateral optic tract or the inter-tectal commissures. Schmidt et al., (1978) found that fibres followed the same commissures to the ipsilateral tectum whether the contralateral projection to that tectum was present or absent. In neither study was the ordering of fibres within the pathways investigated.

In a further variation of this experimental stratagem Cronly-Dillon and Glaizner (1974) diverted an optic tract into the ipsilateral tectum which had been partially denervated by ablation of the temporal hemiretina of the contralateral eye. The normal target of the diverted fibres was also partially ablated to prevent them returning to it. The two eyes tended to project to separate areas of the tectum though there was some overlap. The extent of this varied between fish. The polarity of the projections was appropriate to the host tectum. In contrast when Meyer produced an abnormal ipsilateral projection by deflecting fibres which normally supplied posterior tectum into the anterior end of the other tectum there was very little overlap between the two eyes. Moreover the ipsilateral projection was appropriately polarized with respect to the medial-lateral axis of the recipient tectum but reversed with respect to the rostrocaudal axis! (Meyer, 1979b). An ability of ipsilaterally deflected fibres to exclude contralateral fibres from their normal termination sites was noted when fibres were deflected into a tectum whose contralateral projection was forced to regenerate (Meyer, 1979a).

In adult Rana pipiens ipsilateral projections have been induced by tectal ablation in the presence of either an intact or a regenerating contralateral projection (Misantone and Stelzner, 1974a, 1974b). These projections were shown behaviourally to have the correct polarity for the host tectum though they formed less rapidly than in the case where the host tectum had been permanently denervated. No information was available in this study as to the paths of the fibres or the occurrence of patching.

However in a similar study, again involving simple tectal ablation in adult Rana ssp, the two fibre groups were seen anatomically to form bands on the remaining tectum resembling the banding seen when a third eye was forced to project to an already innervated tectum (Constantine-Paton and Law, 1978; Law and Constantine-Paton, 1980). Similar patching was observed anatomically after unilateral tectal ablation in larval Xenopus (Straznicky and Glastonbury, 1979). In neither case could the segregation be detected electrophysiologically and the pathways followed by fibres was not studied in detail.

Thus very little is known of the paths taken by fibres when forming an abnormal ipsilateral retinotectal projection in either goldfish or frog. The way in which the projection becomes appropriately reordered to fit the host tectal polarity, which it does with a few exceptions, is not clear. Furthermore there is some controversy about the behaviour of the ipsilateral projection when fibres from the contralateral tectum are also present. Do they overlies each other, or do they interact in such a way as to separate out into almost exclusively one-eyed zones? There is also surprisingly little correlation between the anatomically observed patching and the results of electrophysiological analysis (Levine and Jacobson, 1975; Constantine-Paton and Law, 1978; Straznicky and Glastonbury, 1979; Law and Constantine-Paton, 1980).

It was therefore decided to investigate the nature of the ipsilateral projection formed after various tectal ablations in the presence, absence or regeneration of the contralateral projections. Particular points of interest were the fibre pathways followed by the ipsilateral fibres, the fibre ordering within the pathways and the effect of this upon the final ipsilateral projection, and the anatomical and electrophysiological expressions of interactions between the ipsilateral and contralateral projections to one tectum.

4. Whole left tectal removal with right optic nerve cut (WTRON)

This group was set up as group (1) except that the projection from the left eye to the right tectum was left intact.

5. Partial left tectal removal (7/8 only)

This group was set up as group (2) except that the projection from the left eye to the right tectum was left intact.

6. Partial left tectal removal with construction of a rostral intertectal bridge (Bridge)

This group was set up as group (3) except that the projection from the left eye to the right tectum was left intact apart from the small tectal lesion required for bridge insertion.

7. Whole left tectal removal with right and left optic nerve cut (WTRON + LON cut).

This group was set up as group (1) except that instead of removing the left eye completely the left optic nerve was cut in the orbit.

8. Partial left tectal removal with left optic nerve cut (7/9 + LON cut)

This group was set up as group (2) except that instead of removing the left eye the left optic nerve was cut in the orbit.

9. Whole left tectal removal with left optic nerve cut (8/8 + LON cut)

In this group the whole of the left tectum was removed using iridectomy scissors, forceps and suction. The left optic nerve was then cut in the orbit.

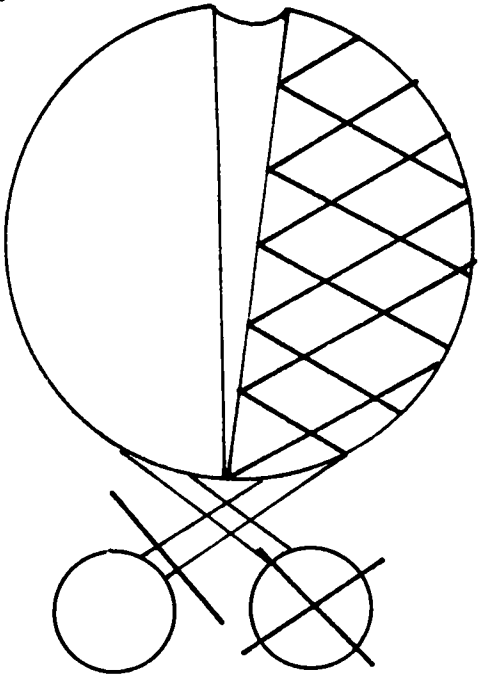
These experimental groups are summarized in figure 9.1.

Fig. 9.1. This diagram summarizes the various surgical manipulations of the retinotectal pathway produced in the nine groups of fish.

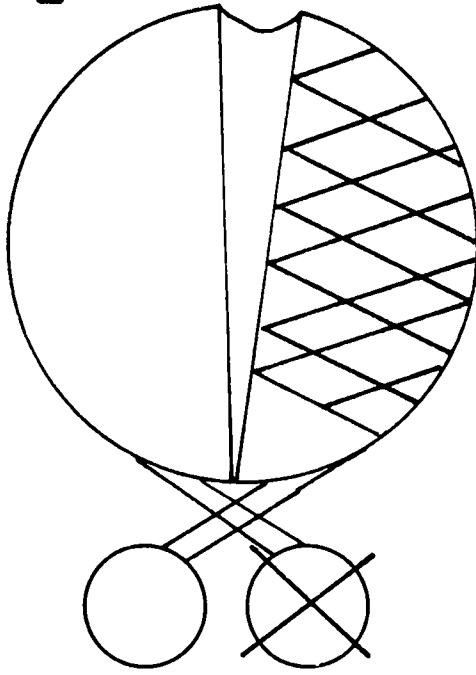
1. WTRON + LER
2. 7/8 + LER
3. Bridge + LER
4. WTRON
5. 7/8 only
6. Bridge
7. WTRON + LON cut
8. 7/8 + LON cut
9. 8/8 + LON cut

fig 9.1

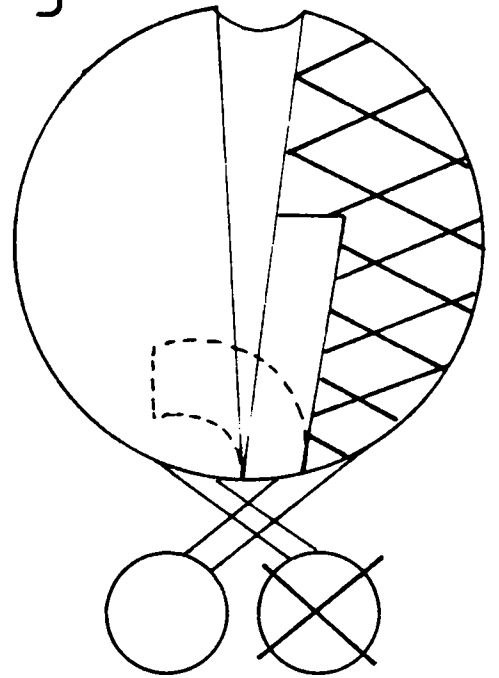
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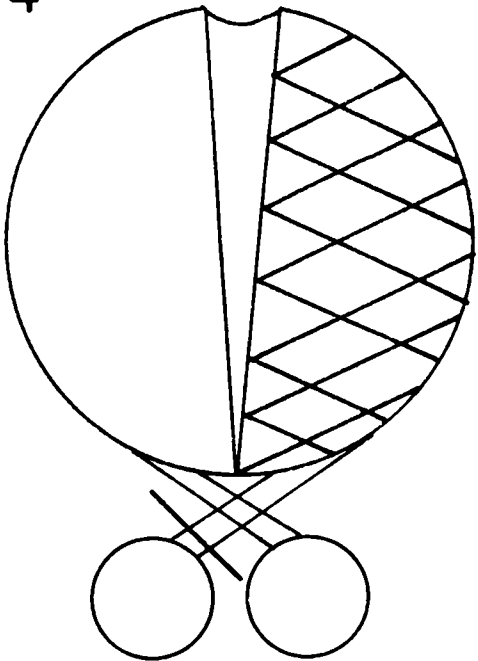
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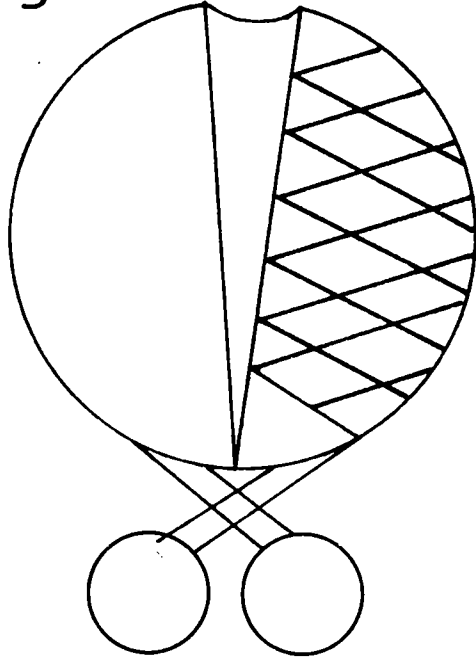
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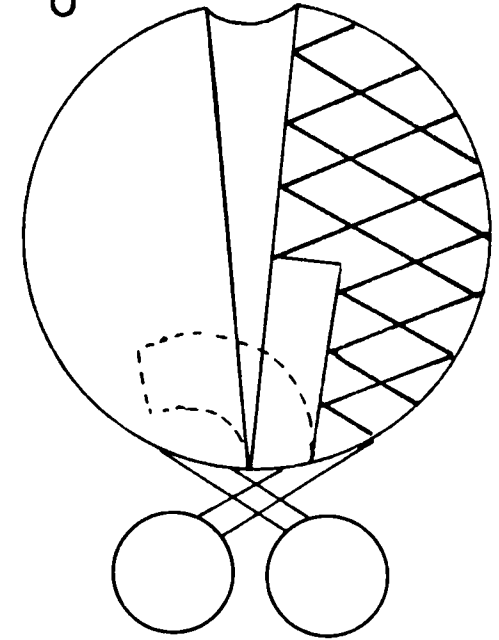
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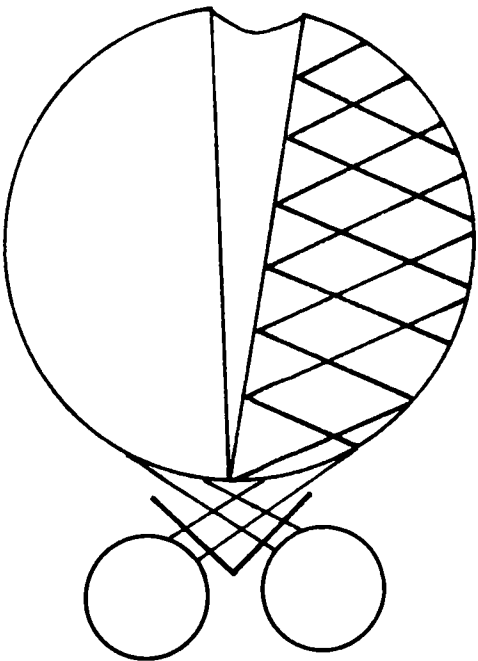
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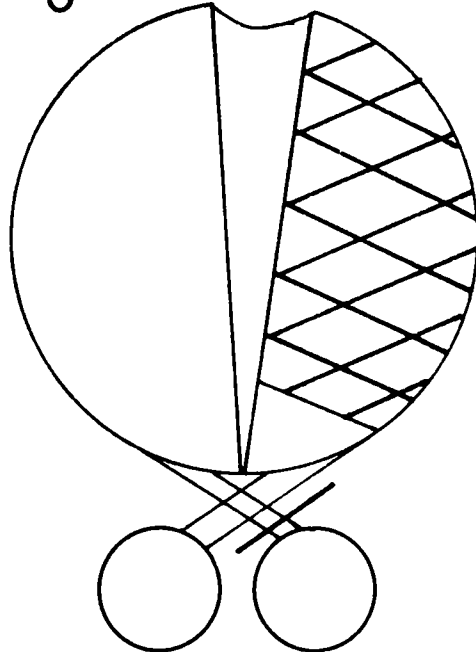
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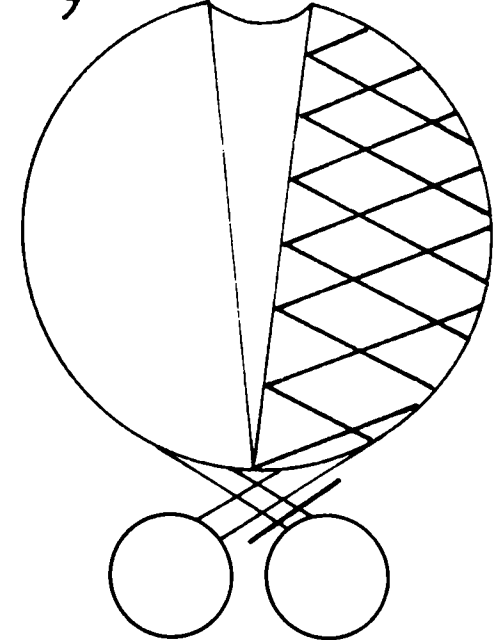
7



8



9



Electrophysiological mapping

General electrophysiological mapping techniques were as described in chapter 2. Position localization in the right tectum, the only remaining tectum, was always by photographic means to allow accurate remapping. Mapping of the first three groups of animals in which only the ipsilateral eye, the right eye, remained, presented no particular problems and was performed as in chapter 2. However mapping the remaining six groups in which both eyes projected to one tectum posed a number of technical problems which are discussed below.

1. Occlusion of the eye not being mapped.

One of the major points of interest in the two-eyed fish was the nature and possible interactions between the two projections to the remaining tectum. Separate maps of the two projections were required. It was therefore necessary to suppress the responses from one eye temporarily. Injection of 2% Marcaine in saline into the eye or around the optic nerve was employed initially but proved insufficiently long lasting. More satisfactory was the occlusion of one eye using an opaque black rubber "contact lens" attached to the eye using a mixture of carbon powder and Vaseline. This did not eliminate the spontaneous activity from the occluded eye but this was rarely sufficiently strong to obscure responses from the mapped eye.

2. Repeat mapping.

In all these fish repeat maps via one eye (groups 1-3) or maps from the two eyes (groups 4-9) were obtained at the same mapping session which lasted 4-7 hours. Tectal electrode position localization was from a tectal photograph and was accurate to within 50 μ m in most animals. Tectal positions were always mapped in a different sequence during the second mapping to reduce observer bias.

3. Response strengths.

At each electrode position the depth of the electrode tip was adjusted to obtain the maximum response. This response was then rated on a scale 0-6.

- 0 no localizable response
- 1 receptive field centre could just be defined
- 2 receptive field limits could just be defined
- 3 field readily defined but response submerged in thermal noise
- 4 response stands out from thermal noise
- 5 response strength typical of normal fish - very clear and easily defined
- 6 response exceptionally clear and brisk.

This rating system was subjective and inevitably the criteria used to classify responses varied slightly from one mapping session to the next, as did the sensitivity of the electrode and the level of anaesthesia.

However its purpose was to allow comparison of responses from the two eyes at one tectal position. As both maps were obtained during one mapping session with the fish in a stable state of anaesthesia the error in response strength assessment was probably never more than 1 group. To check this some positions were sampled twice through one eye with a gap of several hours, and only rarely did the assessed response strength differ, and never by greater than one unit. There was also good agreement between the response strength assessments of the mapping operator and the observer.

4. Correlation of response strengths via the two eyes.

When both maps had been completed a scattergram was plotted of the response strength via the left eye against that via the right eye for all tectal positions. A correlation between the response strengths was then sought using a non-parametric statistical method - the Spearman rank correlation test (Seigel, 1956). This calculation was corrected for ties arising because a small finite number of response classes was employed. The coefficient was then tested for significance using a one-tailed test, as a negative correlation was predicted.

5. Expression of the tectal distribution of response strengths via the two eyes.

In those areas where a significant negative correlation was obtained between the response strengths of the two eyes a means was sought of depicting the tectal distribution of the response strengths. It was thus hoped to detect areas of tectum supplied predominantly by one or other eye.

To do this each tectal position was allocated a dominance value corresponding to the relative strengths of the ipsilateral and contralateral inputs. The criteria for allocation of these groupings were taken from the ocular dominance groupings of Hubel and Wiesel (1962) as it was felt that this would help to instil some uniformity into the literature. However it must be stressed that Hubel and Wiesel's criteria applied to the binocular inputs to single cortical cells whereas in this study there was no way of knowing, using extracellular presynaptic multiunit recording techniques, whether the retinal inputs from the two eyes connected to the same population of tectal cells.

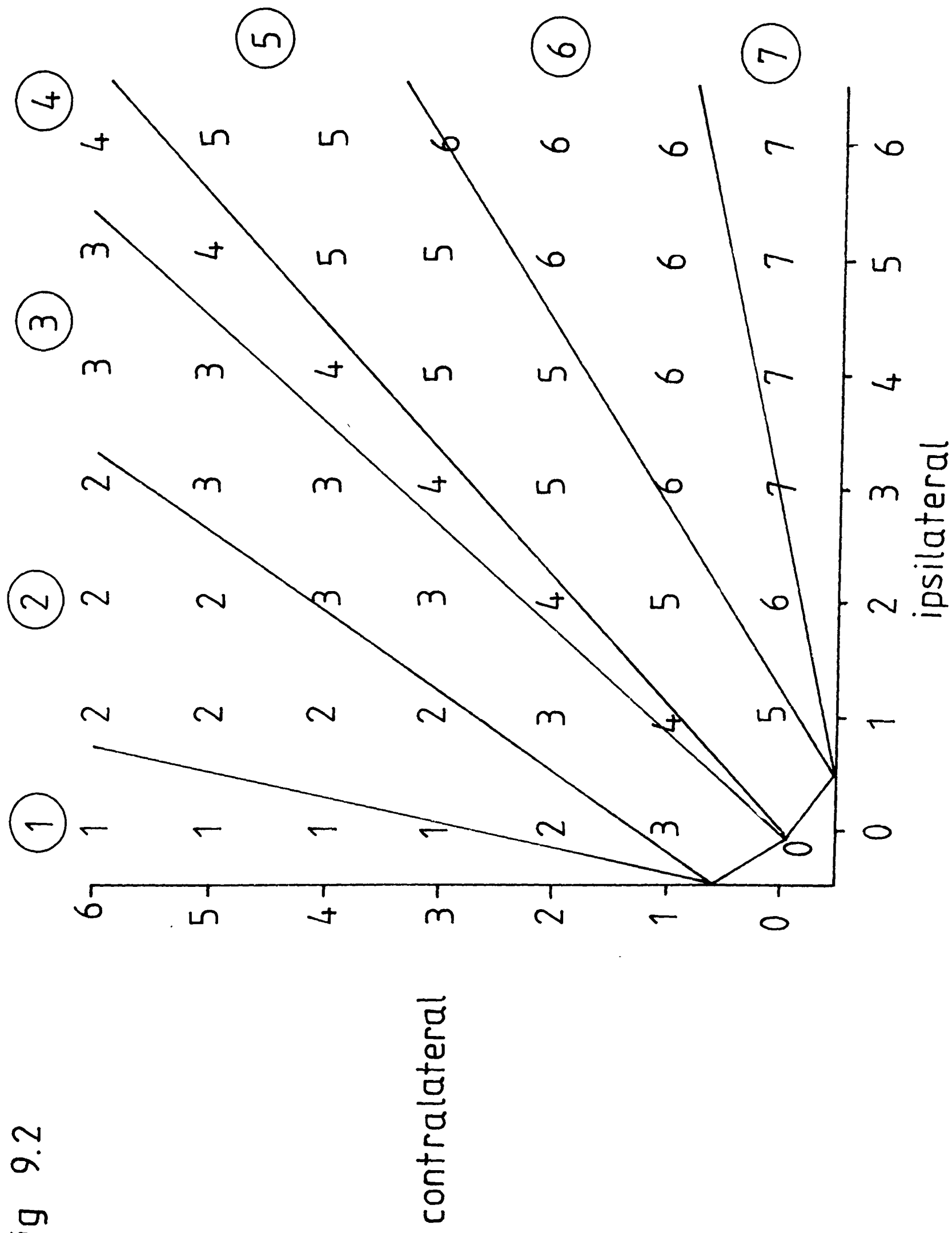
The criteria are:

1. exclusively contralateral
2. contralateral eye much more effective than ipsilateral
3. contralateral eye slightly more effective than ipsilateral
4. no obvious difference between eyes
5. ipsilateral eye slightly more effective than contralateral
6. ipsilateral eye much more effective than contralateral
7. exclusively ipsilateral.

The important factors in assigning a dominance value were the relative strengths of the two eyes rather than their absolute values as the latter varied with the state of the animal, equipment and observer. The application of the criteria is shown in figure 9.2. Certain points must be noted:

Fig. 9.2 Allocation of dominance categories on the basis of relative response strengths via the two eyes. On each axis is shown the response strength (classified 0-6) while between the axes lie the ocular dominance groupings (classified 1-7 using the criteria of Hubel and Wiesel (1962)).

fig 9.2



- a) 0-0 could not be classified and was designated 0;
- b) categories 1, 4 and 7 were "absolute" and were less common than categories 2, 3, 5 and 6;
- c) category 0 for response strength (i.e. no mappable response) represented a detection threshold. A three point difference in response strength was considered necessary to define an absolute dominance on the assumption that a one point error was possible on each axis. Thus parts of strength 0 in one eye and 1 or 2 in the other eye were not classified as being absolutely dominated by the second eye.

Anatomy

Two anatomical methods, HRP and ^3H proline autoradiography, were used to investigate the paths taken by the optic fibres and the sites at which they terminated.

1. HRP

Horseradish peroxidase was employed as described in chapter 2. It was introduced into the visual pathway either by cutting one optic nerve in the orbit or by injection into a small area of tectum. In both cases the HRP was studied in serial frozen sections after development by the method of Mesulam (1978). Reconstructions of the pathway were done using a projector microscope or a camera lucida attachment on a binocular microscope.

2. Autoradiography

For autoradiography 6-8 μl of a solution of tritiated proline were injected into the eye over a period of 1-2 minutes using a screw driven micropipette.

The survival period post-injection varied according to the part of the pathway of interest. For labelling of terminals the animal was sacrificed 20-26 hours after injection whereas for labelling of the fibres in the chiasmatic region a survival period of 24-49 days was allowed. These survival

periods were designed to give labelling by fast and slow axoplasmic transport mechanisms respectively (Grafstein, 1977).

Subsequent preparation of the tissue varied depending on whether HRP had been injected into the animal. With dual labelling the autoradiographic processing was performed on an alternate section series of $40\mu\text{m}$ thick frozen sections. Where only proline had been used the material was processed in $10\mu\text{m}$ thick wax sections. General autoradiographic methods were as in chapter 2.

Results

Preliminary anatomical investigations

There is evidence in the literature that simple optic nerve section in the goldfish will produce a transient ipsilateral projection (Springer, 1980b) and that in the frog such nerve section will produce a more persistent projection (Glastonbury and Straznicky, 1978; Tay and Straznicky, 1980) as will removal of one eye (Stelzner, 1979). A preliminary check was therefore made to ensure that a substantial ipsilateral projection could not be produced in the goldfish without ablation of all or part of the contralateral tectum.

Three groups of animals were set up

- a) section of the right optic nerve only. Four animals were studied 50-104 days after surgery;
- b) removal of the left eye only. Four animals were studied 56-104 days after surgery;
- c) removal of the left eye followed by right optic nerve section 133 days later. Four animals were studied 50-104 days after the second surgery.

In each case an ipsilateral projection from the right eye was sought by

filling the right optic nerve with HRP in the orbit and then processing the material, after a survival period of 20-28 hours, as frozen sections by the method of Mesulam (1978). In no case was a projection to the ipsilateral tectum detected. All subsequent groups of animals employed at least partial ablation of the left tectum to induce an ipsilateral projection from the right eye to the right tectum.

WTRON + LER

Twelve animals were mapped electrophysiologically at 92-198 days after operation. In all cases the projection from the right eye to the right tectum was found to be appropriately orientated for the host tectum with nasal retina projecting caudally and dorsal retina laterally. The projection was thus the mirror image of that normally occurring from the right eye to the left tectum. Dorsotemporal field was well represented in the mapped region of the right tectum though the extent of the dorsonasal representation varied from virtually nil to over half of the quadrant. It was usually substantially less than would be expected in the same tectal region of a normal fish. Ordering of the projection was also variable though usually fairly good. Neither the extent of the field representation nor the degree of ordering showed any systematic variation with time after surgery. A typical map, obtained at 119 days after operation is shown in figure 9.3.

In five fish HRP was introduced into the cut right optic nerve at the end of the mapping session in order to trace the paths taken by the ipsilaterally projecting fibres. In each case the label was seen to pass both ipsilaterally and contralaterally at the chiasmatic region and to fill both optic tracts (fig. 9.4a). HRP was not observed crossing the midline anywhere except in the region of the chiasm. It was not clear from these animals whether the fibres were separating into two populations at the chiasm with one group passing ipsilaterally and the other contralaterally or whether the majority of fibres were passing contralaterally, as in the

Fig. 9.3 Electrophysiological map of the projection from the right eye to the right tectum of a WTRON + LER fish mapped 119 days after surgery. The map is corrected for an eye angle of 9° .

fig 9.3

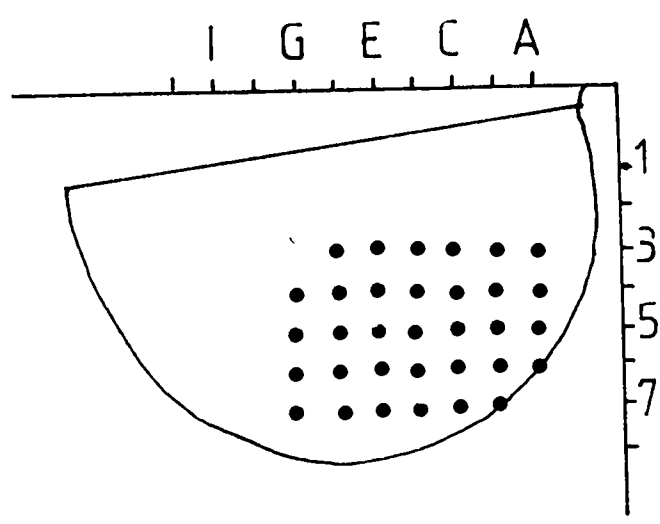
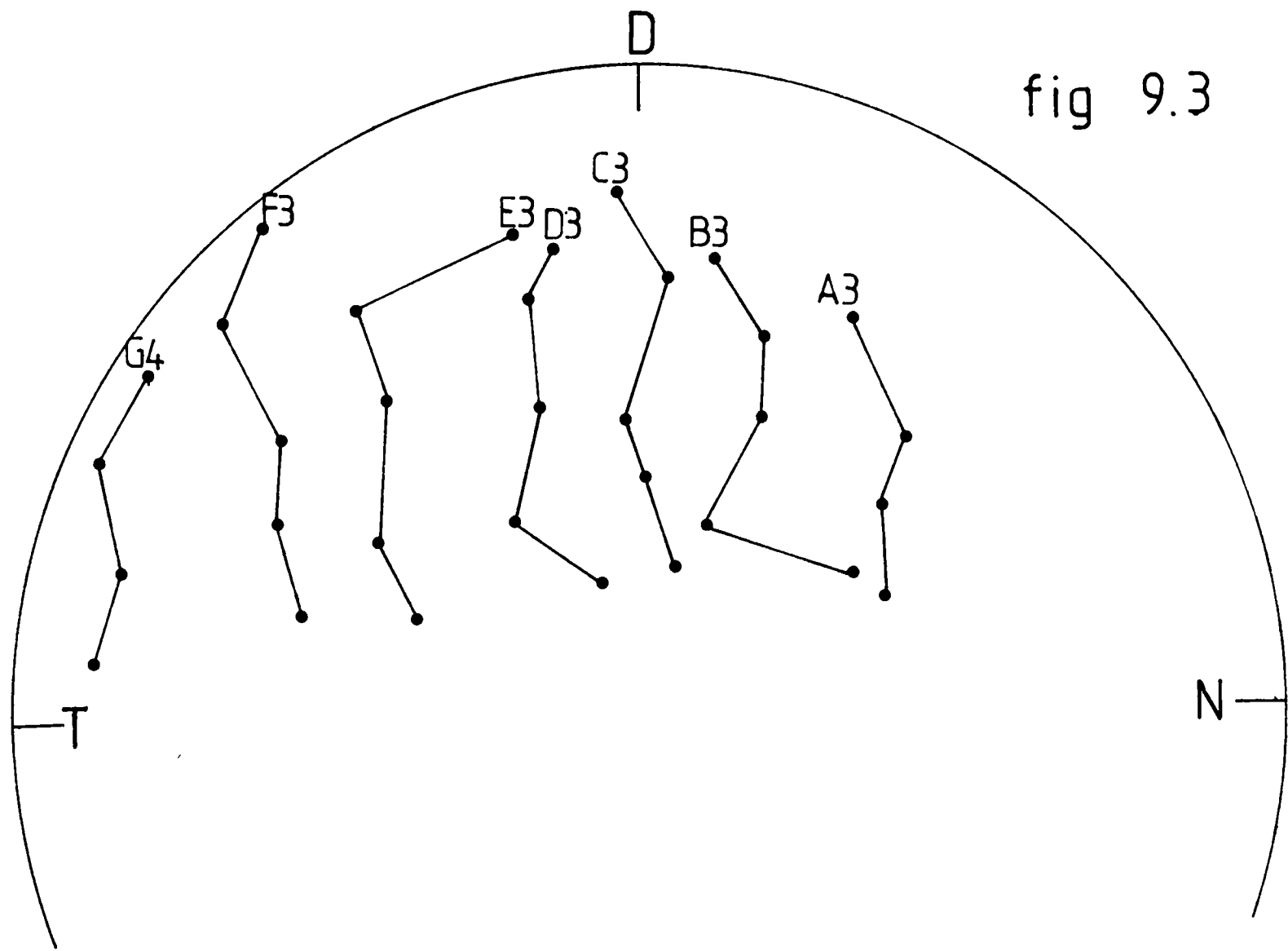
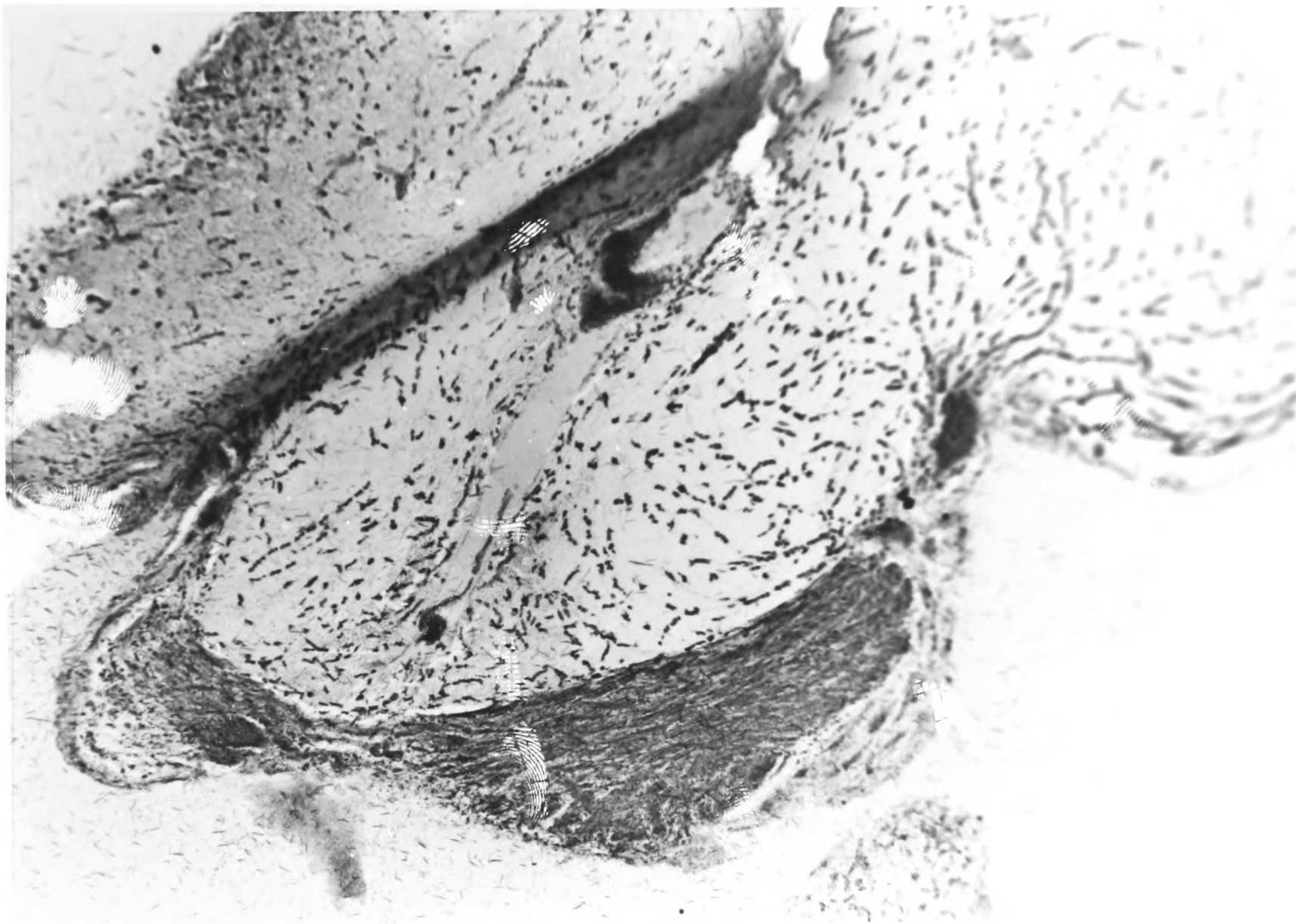


Fig. 9.4a Low power photomicrograph of a section from the brain of a WTRON + LER fish labelled by HRP injection into the cut right optic nerve 92 days after surgery. Labelled fibres can be seen passing into both optic tracts in this section taken $120\mu\text{m}$ behind the caudal edge of the chiasm. The right optic tract lies on the left of the photograph.

Fig. 9.4b High power photomicrograph of the chiasmatic region of a WTRON + LER fish labelled by HRP injection into the medial edge of the right tectum 196 days after surgery. Labelled fibres can be seen crossing the midline from the ipsilateral optic tract towards the contralateral side of the brain. The right optic tract lies on the left of the photograph.

fig 9.4

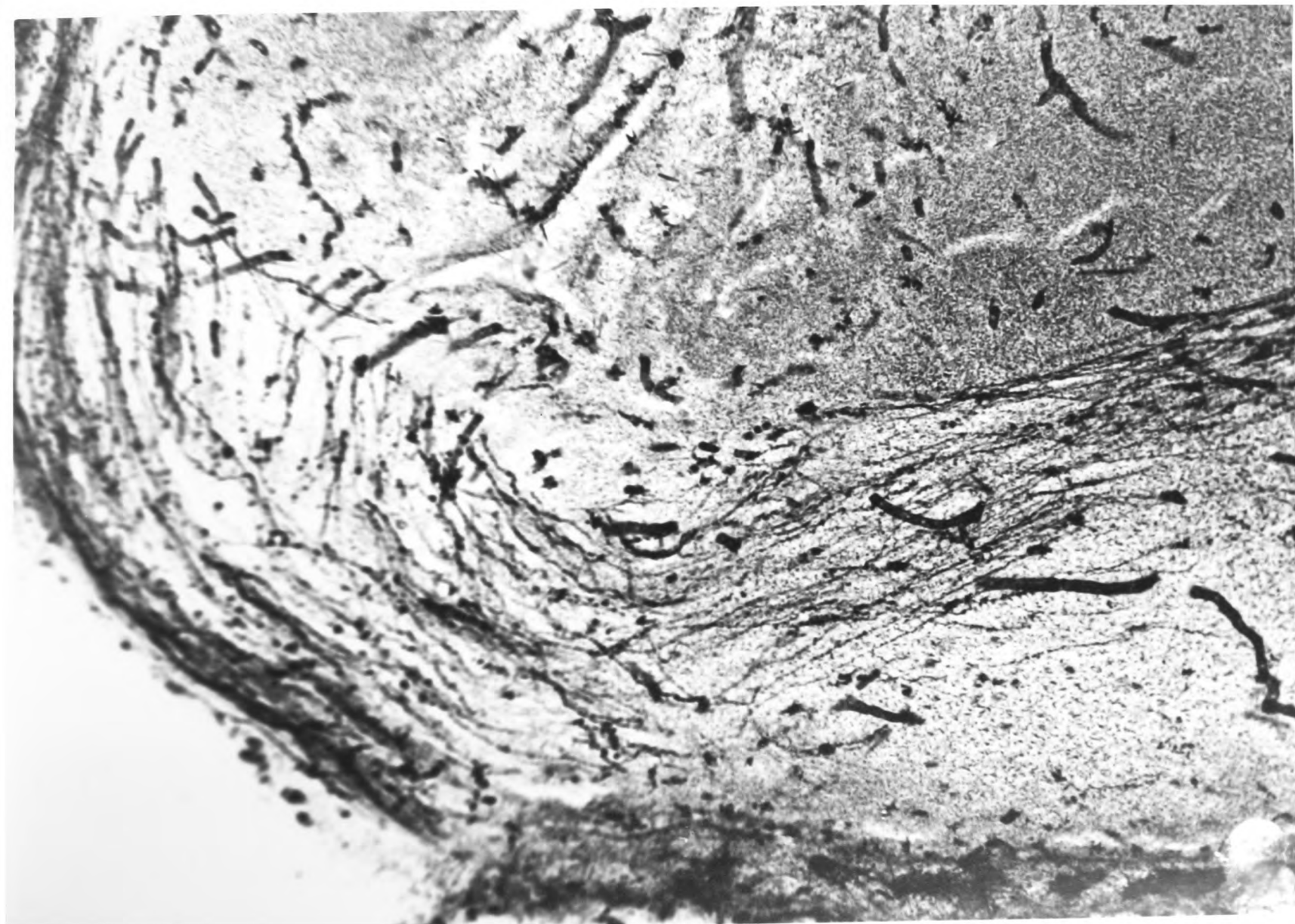
a



500 μ m

100 μ m

b



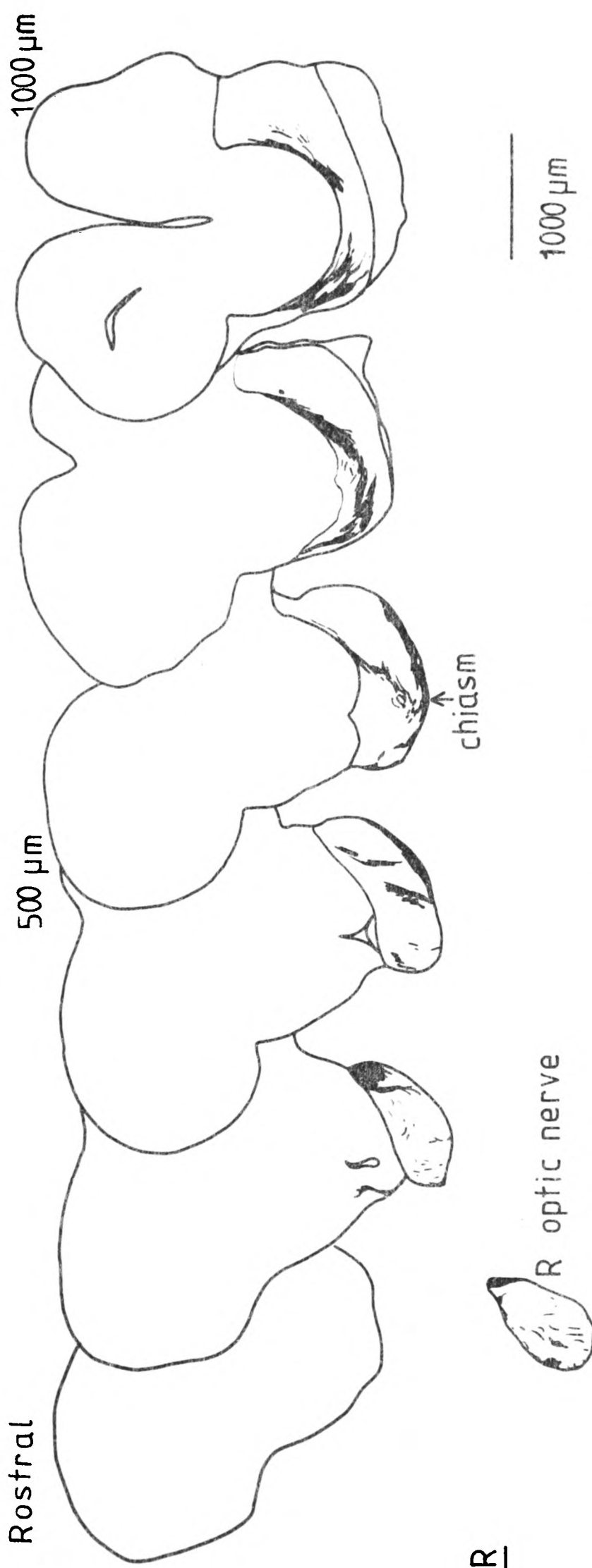
normal animal, and then retracing their paths back to the chiasm and then passing ipsilaterally in a manner akin to that suggested by Sharma (1973). Nor was it possible to conclude anything about the relative ordering of fibres within the pathway.

In an attempt to resolve this issue a small tectal injection of HRP was made in one fish immediately after mapping. Subsequent development showed that after the medial injection HRP-filled fibres occupied a small region in the correct dorsomedial position of the medial optic tract brachium indicating the ordering of fibres as they approached the ipsilateral tectum to be crudely correct. Labelled fibres were then observed to cross the midline in the region of the chiasm (fig. 9.4b) and pass into the contralateral tract. A small area of filled fibres was also found in the ipsilateral optic nerve. Thus at least some of the fibres which terminated ipsilaterally initially followed a route towards the contralateral tectum and then turned back to recross the midline near the chiasm. The proportion of fibres which followed such a route was not clear as only one fish was successfully labelled but it appeared to be large. Accidental direct injection of HRP into the contralateral side of the brain was unlikely and would not explain the observation of fibres passing across the midline from the ipsilateral tract. A camera lucida reconstruction of the pattern of HRP labelling in this fish is shown in figure 9.5.

In 10 of the 12 fish, including the six analysed with HRP above, there was no sign at mapping of any fibres reaching the ipsilateral tectum via the intertectal commissure. In two fish, however, a few fibres were seen so crossing the midline. The electrophysiological maps obtained from these fish were less well ordered than those from the other ten fish. One of the two fish was remapped after cutting the fascicle of fibres seen on the intertectal commissure. The second map was considerably better ordered than the first. This result, illustrated in figure 9.6. suggests that the small group of fibres crossing the intertectal commissure was unable to

Fig. 9.5. Camera lucida reconstruction of the visual pathway of a WTRON + LER fish labelled by HRP injection into the medial edge of the right tectum 196 days after surgery. Labelled fibres could be traced rostrally from the right tectum as a discrete group within the right optic tract. At the chiasm some labelled fibres crossed the midline towards the contralateral side of the brain. These results suggested that at least some of the fibres from the right eye initially passed contralaterally at the chiasm towards the left side of the brain but then retraced their paths to the chiasm and then passed ipsilaterally towards the right tectum.

fig 9.5



WTRON & LER

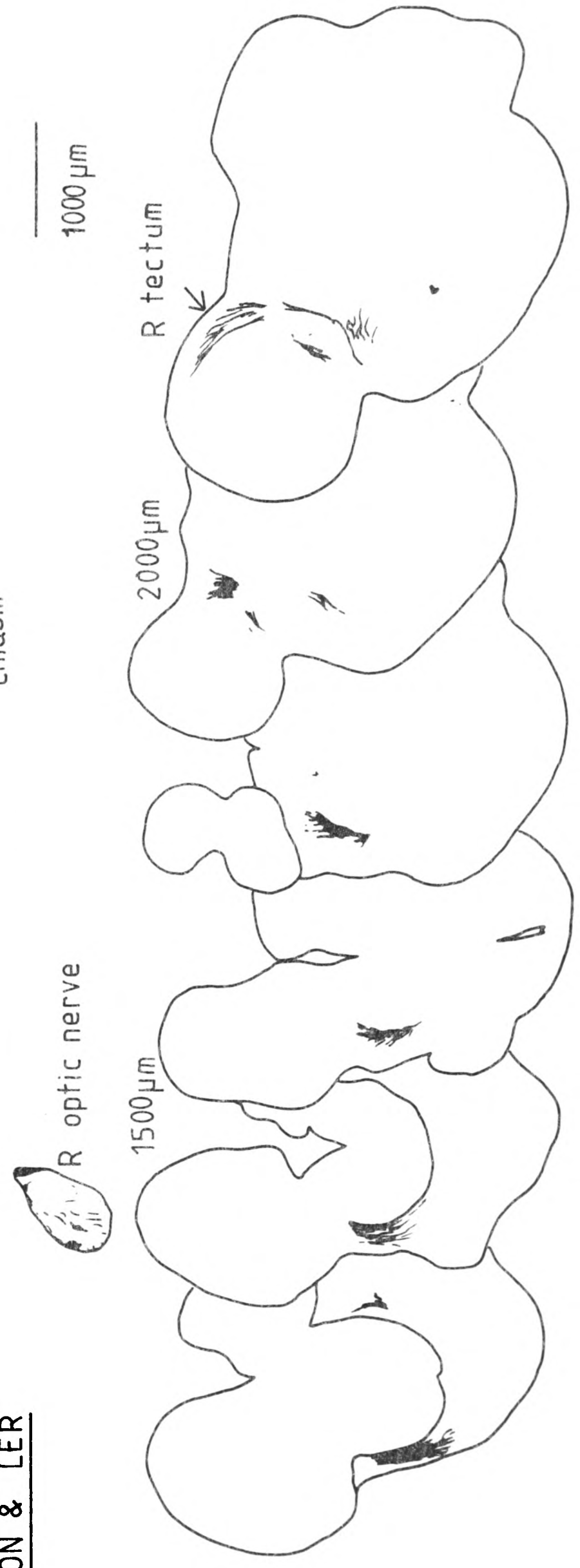
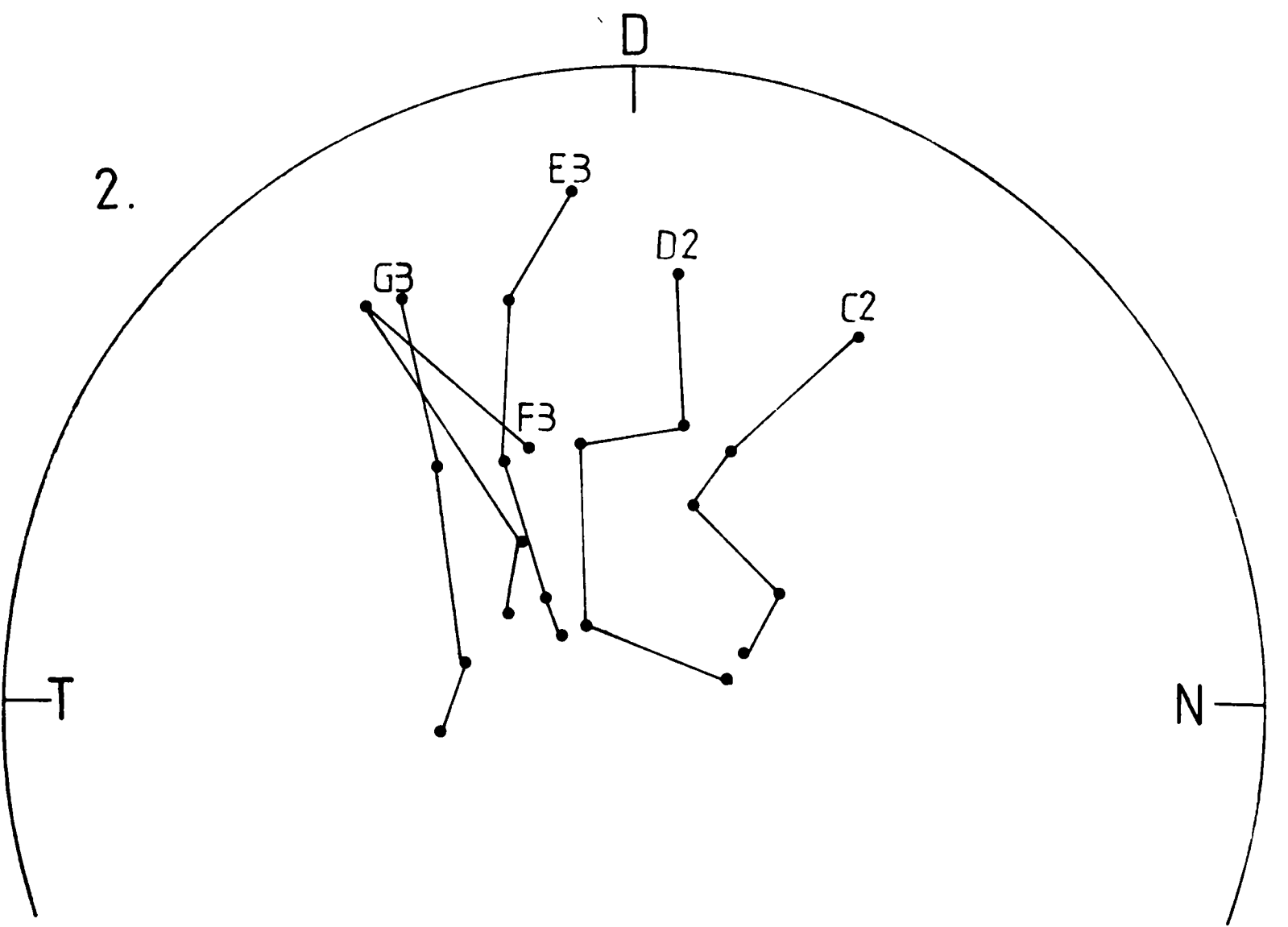
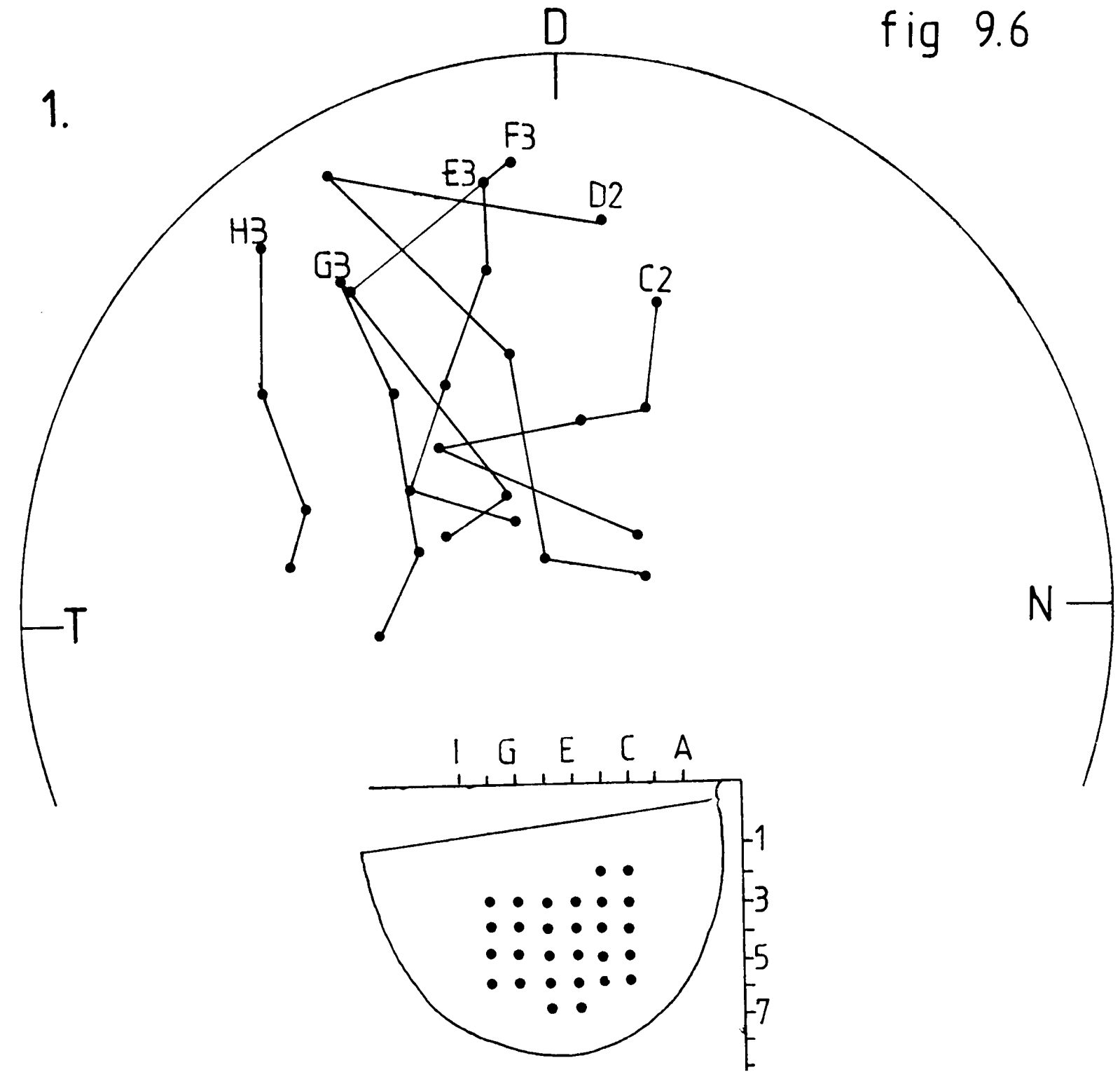


Fig. 9.6 Electrophysiological maps of the visuotectal projection from the right eye to the ipsilateral tectum of a WTRON + LER fish at 173 days after surgery. The first map is shown at the top. Below is the map obtained after cutting fibres crossing to the right tectum via the intertectal commissure. This lesion produced a considerable improvement in the orderliness of the map. Both maps are corrected for an eye angle of 20° .

fig 9.6



interact with and become integrated into the projection from fibres reaching the ipsilateral tectum via its optic tract.

7/8 + LER

Five fish were analysed electrophysiologically at 118-217 days after operation. As with the previous group every animal gave a projection whose polarity accorded with that of the host tectum. However in only one animal did the projection extend significantly into the dorsonasal visual field. In the remaining 4 fish there was an expanded representation of dorsotemporal field occupying the mapped area of tectum. The ordering of the projections was fairly good though a number of crossovers of projection lines did occur. A typical map obtained at 203 days is shown in figure 9.7.

In none of these animals were fibres seen to cross the midline in the inter-tectal commissure. To investigate the paths taken by the fibres to the ipsilateral tectum HRP was introduced into the cut right optic nerve of one fish immediately after mapping and 124 days after surgery. Labelled fibres were seen to pass to both the ipsilateral tectum and the contralateral remnant. The majority of optic fibres were observed to cross the midline at the chiasm into the contralateral optic tract and then to recross in the retrochiasmatic area below the diencephalon, in the region of the transverse commissure. Considerable interweaving of fibres occurred as they crossed the midline. A camera lucida reconstruction of this animal is shown in figure 9.8 and photomicrographs of the recrossing fibres in figure 9.9.

In two further animals a small injection of HRP was made into the caudomedial edge of the right tectum 203 and 207 days after surgery. The labelled fibres were then followed back to the eye. They were found to form a discrete patch in the appropriate (for their termination site) place in the ipsilateral optic tract at its dorsomedial edge. Fibres then crossed the

Fig. 9.7 Electrophysiological map of the visuotectal projection
from the right eye to the right tectum in a 7/8 + LER
animal at 203 days after surgery. The map is corrected
for an eye angle of 6° .

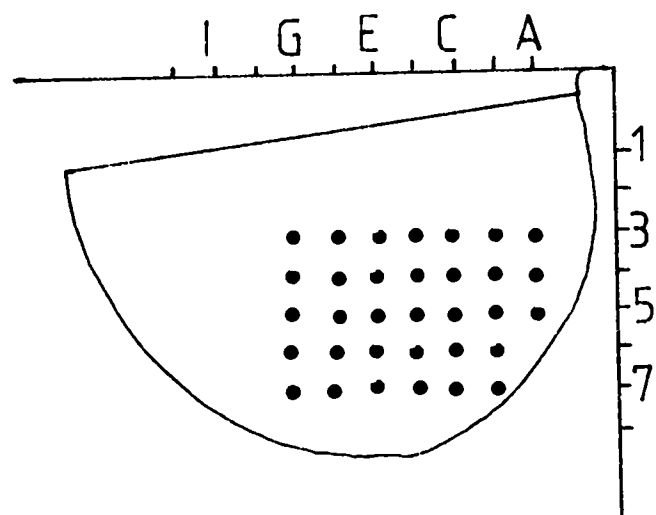
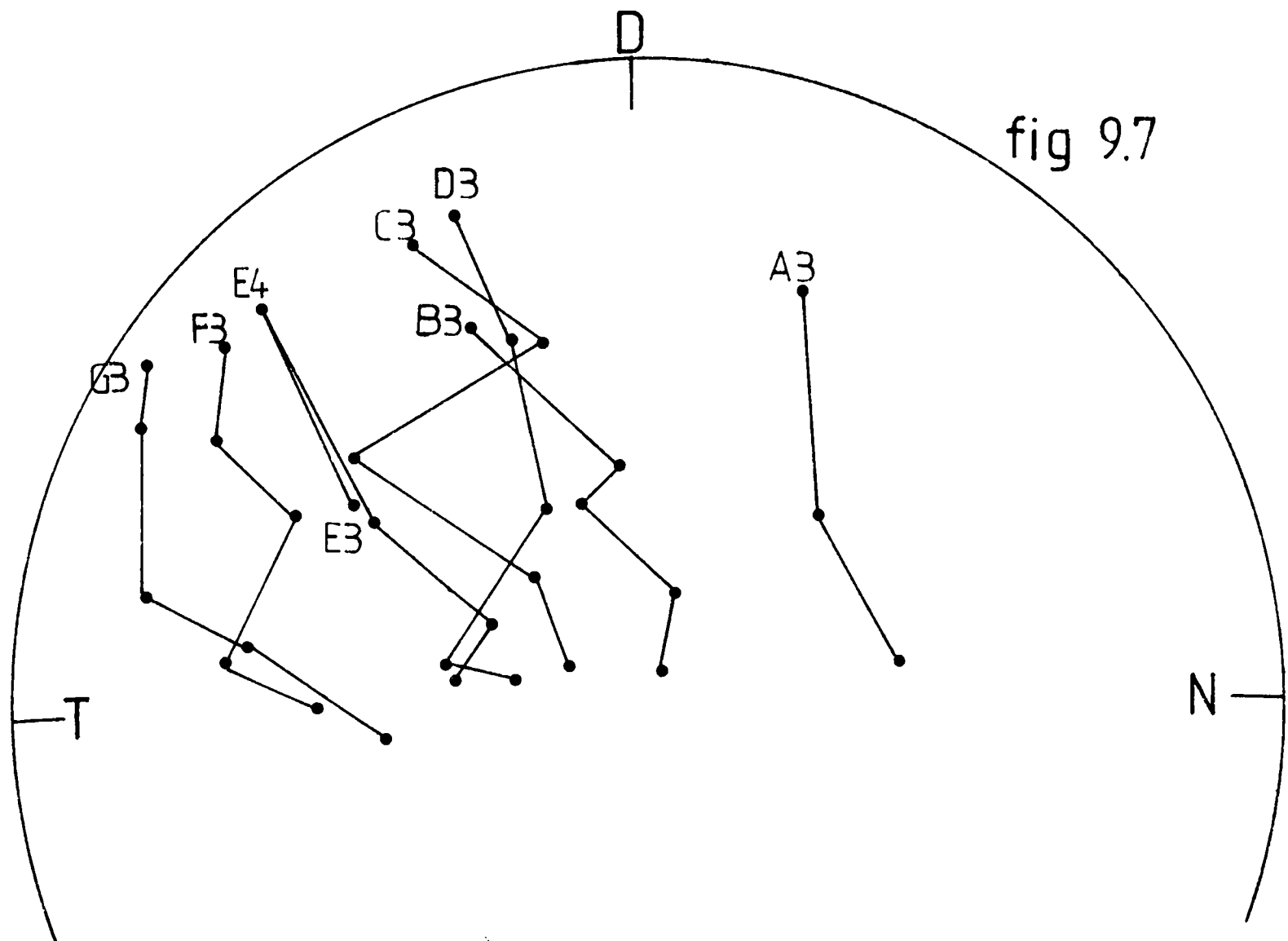


Fig. 9.8 Camera lucida reconstruction of the brain of a 7/8 + LER animal labelled by introduction of HRP into the cut right optic nerve 124 days after surgery. Labelled fibres can be seen crossing towards the left optic tract at the chiasm, some fibres then recross the midline in the retrochiasmatic region, near the transverse commissure to reach the right tectum via the right optic tract.

fig 9.8

ROSTRAL

7/8 & LER

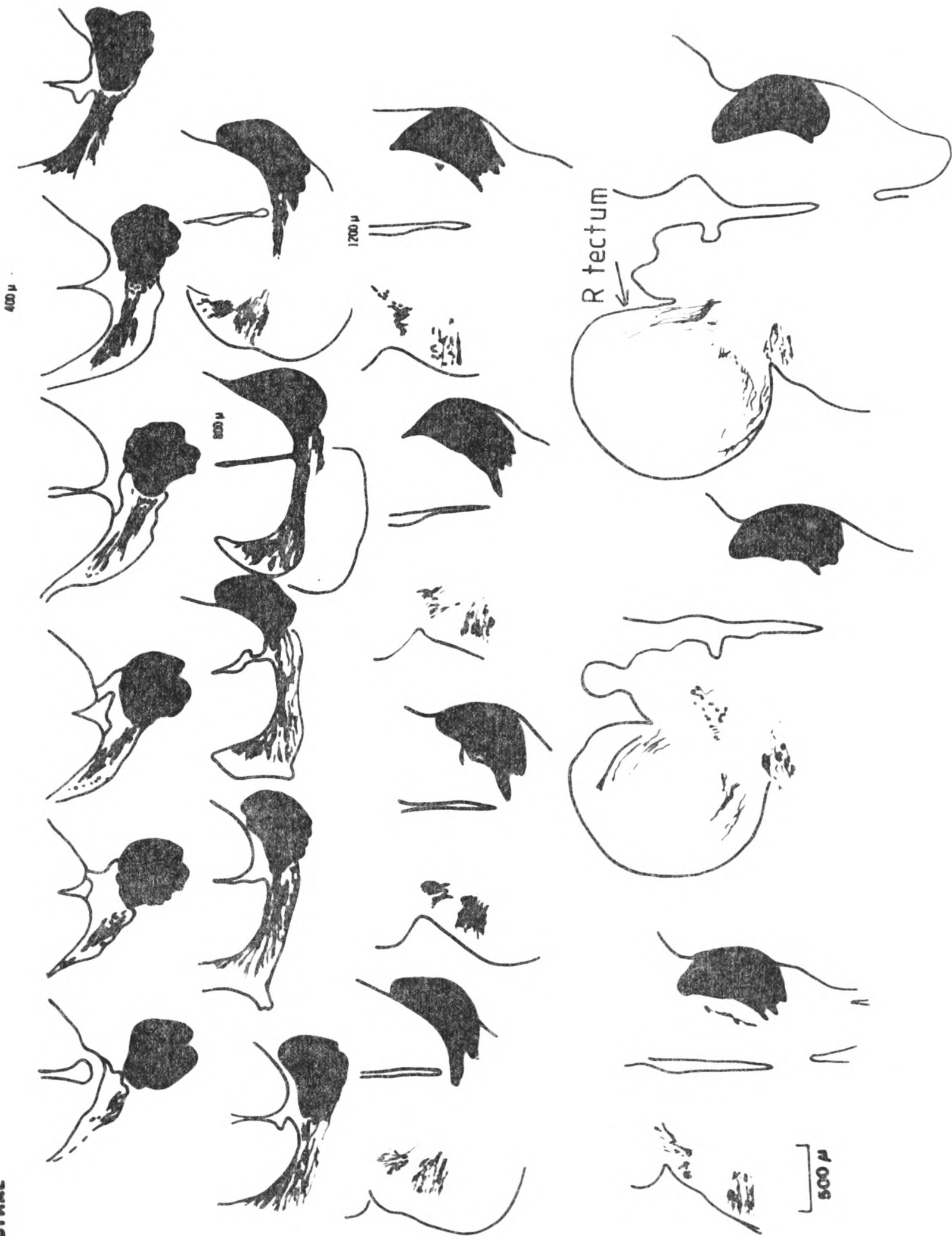


Fig.9.9a Low power photomicrograph of the retrochiasmatic region of a 7/8 + LER animal labelled by introduction of HRP into the cut right optic nerve 124 days after surgery.

Fig.9.9b High power photomicrograph of the retrochiasmatic region of the same animal illustrating the considerable interdigititation of fibres in this area. In both photomicrographs the right optic tract is shown at the right edge of the picture.

fig 9.9

a



b

500 μ m

100 μ m



midline in the region of the transverse commissure and passed up the contralateral tract where they were widely scattered. The quality of staining was not sufficiently high to allow tracing of fibres back to the contralateral tectal remnant or determination of how reordering was achieved. However it seems likely that the optic fibres in these 7/8 + LER animals were travelling from the right eye into the left optic tract and towards the left tectum. While some temporal retinal fibres were able to terminate on the tectal remnant the remainder seemed to turn back either before or on reaching the tectum, return towards the chiasm and recross the midline in the retrochiasmatic region below the diencephalon. Reorganization of fibre ordering seemed to occur in the pathway, probably in the region of the midline recrossing, so that by the time the fibres reached the ipsilateral tract they were crudely correctly ordered.

Bridge + LER

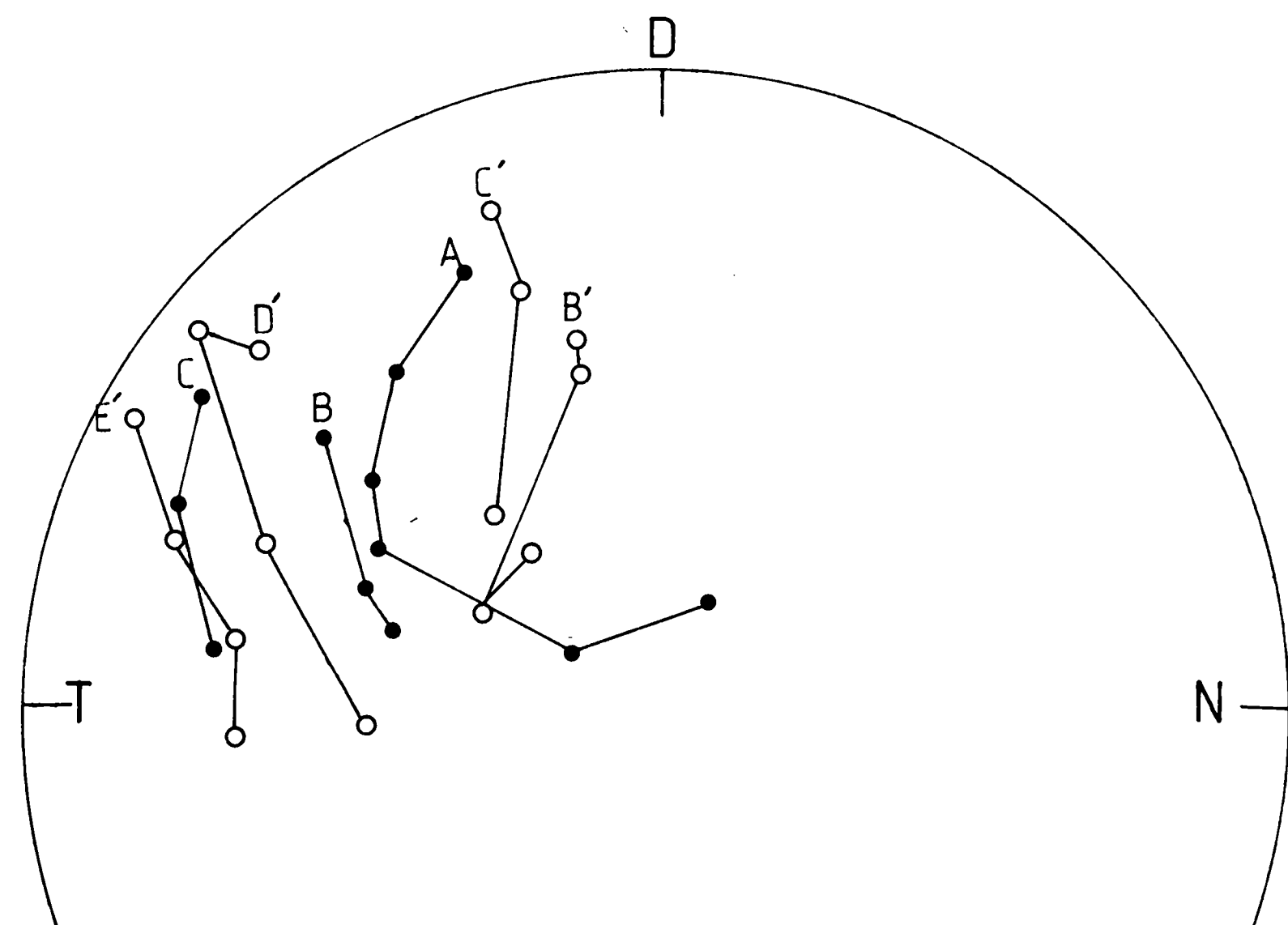
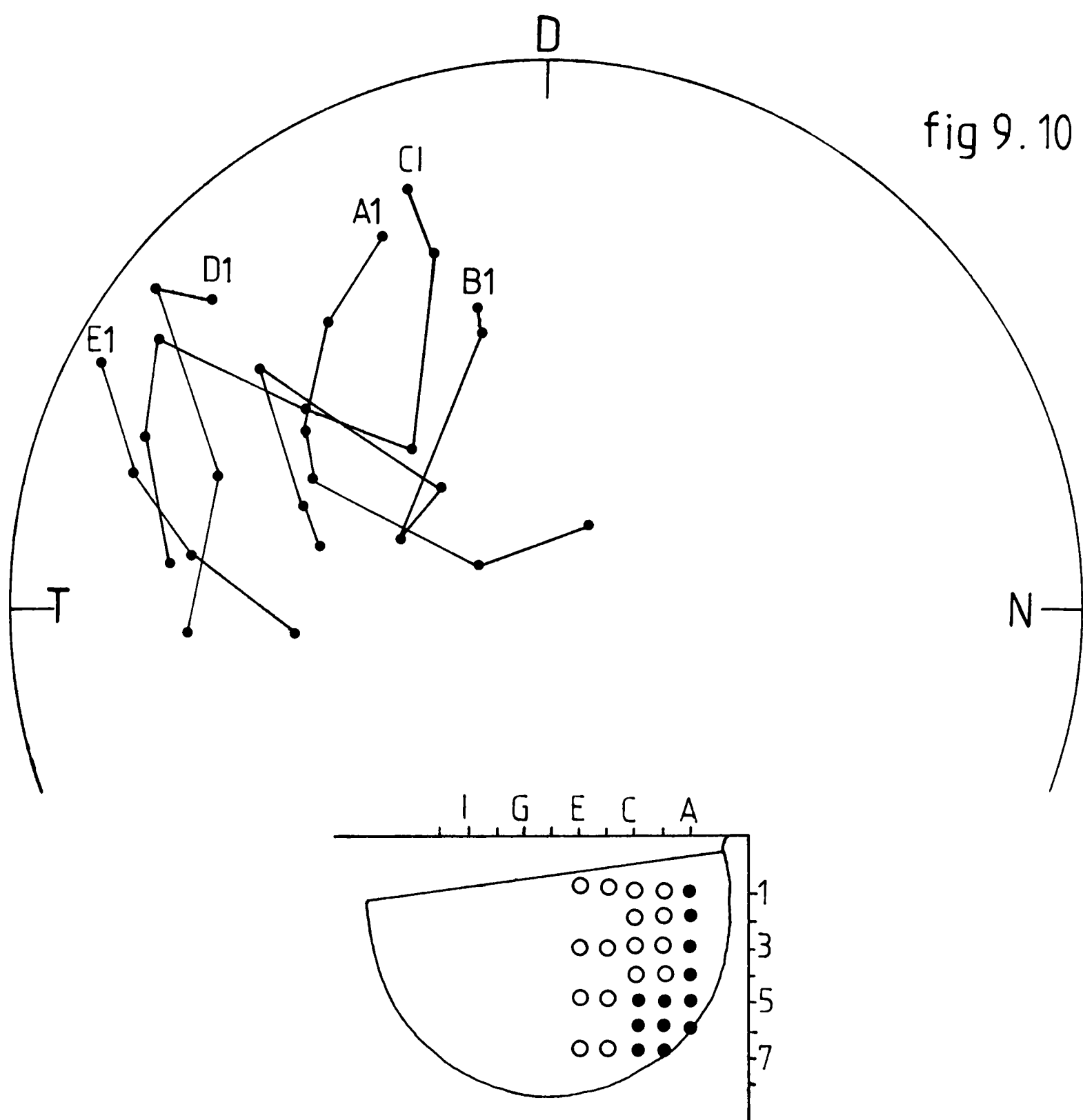
Seven animals were mapped electrophysiologically at 68-125 days after surgery. Though the polarity of all the maps was grossly appropriate to the host tectum the ordering was extremely poor. However on detailed inspection it was found that in four of the seven fish the tectum could be divided into two regions each of which contained a projection from most of the dorsotemporal ipsilateral visual field. Each of these maps was considerably better ordered than the composite map. An example of such a map, obtained at 68 days after surgery, and its resolution into two components is shown in figure 9.10. It is interesting to note that on mapping from the ipsilateral tectum across the bridge to the contralateral remnant the projection in this animal showed a reversal of medial-lateral polarity at the midline.

In the remaining three animals no such resolution of the map into two components was possible. Possibly these maps had three or more components.

Fig. 9.10 Electrophysiological map of the visuotectal projection from the right eye to the left tectum of a Bridge + LER animal at 68 days after surgery. The map is corrected for an eye angle of 7° .

Above is shown the composite map while below the map is redrawn to indicate the two postulated component projections shown by the solid and the empty circles

fig 9.10



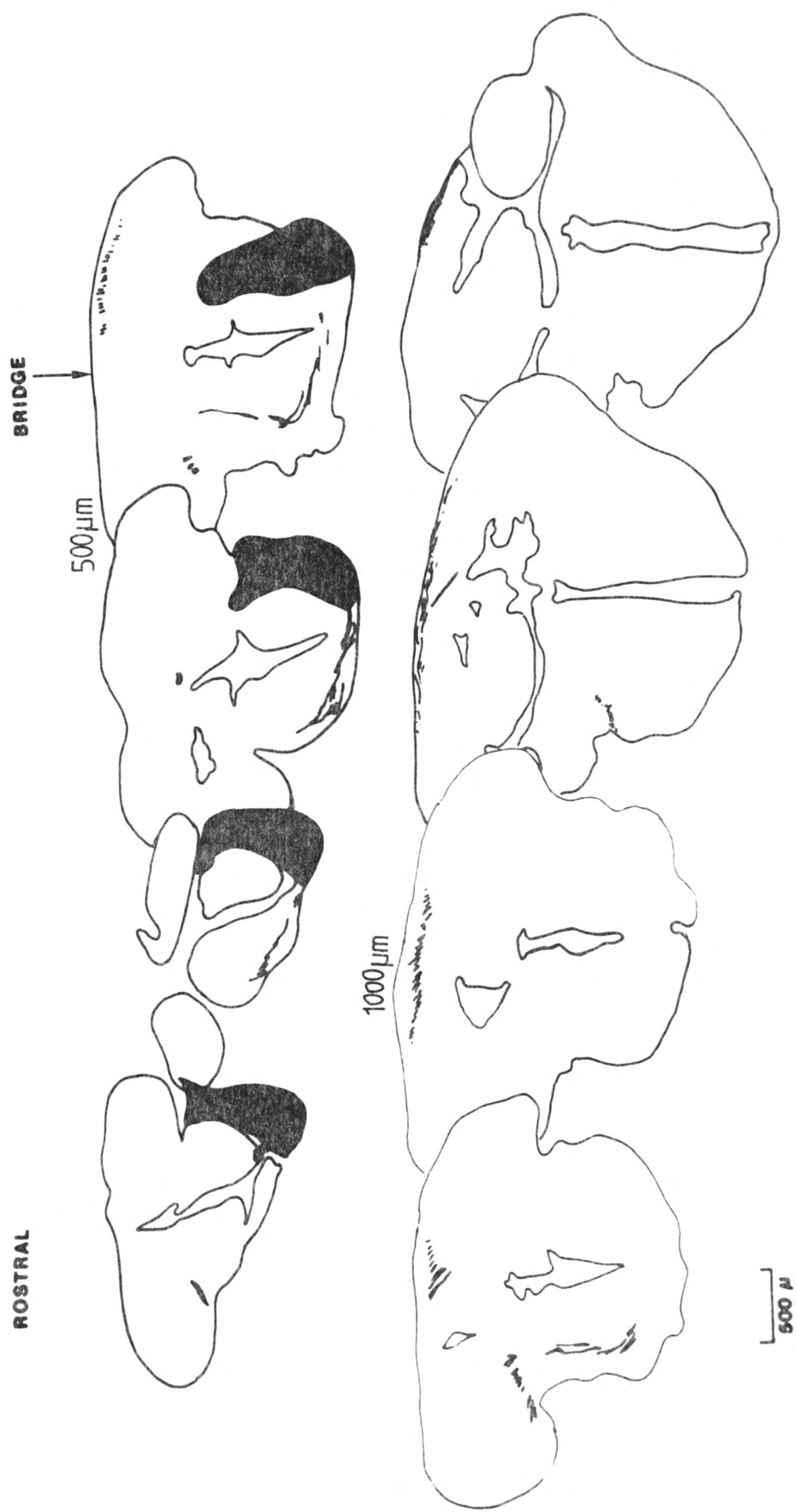
Introduction of HRP into the cut right optic nerve of the animal whose map is given in figure 9.10, and in another animal without a resolvable map revealed fibres reaching the ipsilateral tectum both via the ipsilateral tract and via the contralateral tract and over the bridge. The majority of fibres followed the latter pathway. A camera lucida reconstruction of an animal labelled 68 days after surgery is shown in figure 9.11. Injection of HRP into the caudomedial edge of the ipsilateral tectum gave a patch of labelled fibres in the appropriate position in the contralateral optic tract. Again this indicated that the majority of fibres were reaching the right tectum via the left optic tract and the bridge.

In view of the two pathways revealed by HRP labelling of the right optic nerve (figure 9.11) it was possible that the two components seen in four of the seven fish might represent the independent projections of two fibre groups following separate routes to the tectum. The separate points of arrival of the two groups of fibres may have prevented interactions between them. To test this hypothesis two animals having a 2-component map were remapped after cutting through the bridge. In one case all responses, and in the other all but three, were abolished by this manoeuvre. In a further two fish mapping was followed by making a cut across the front of the ipsilateral tectum to sever all fibres entering that tectum via its optic tract. In both cases remapping at the original electrode positions gave maps virtually identical to those obtained initially. Thus the bridge formed the major route into the ipsilateral tectum and it was not possible to separate out the various components of the map on the basis of fibre pathway.

An alternative hypothesis is that the various components of the map represented groups of fibres arriving at the tectum at different times but by the same route. However both maps which could be analysed in terms of overlapping components and those which could not were obtained early and later in the experimental series, at 68-119 days and 71-125 days after surgery respectively.

Fig. 9.11 Camera lucida reconstruction of part of the brain of a Bridge + LER animal labelled by HRP introduction into the cut right optic nerve at 68 days after surgery. A few labelled fibres can be seen crossing into the right optic tract in the retro-chiasmatic region while the majority pass up the left optic tract from which some enter the right tectum over the bridge.

fig 9.11 Bridge & LER



WTRON

Eleven animals from this group were analysed electrophysiologically at 148-305 days after surgery. Eight were mapped through the right eye only while three were mapped through both eyes at the same mapping session and using the same electrode positions.

In each case the projection from the right eye to the ipsilateral right tectum was of the appropriate polarity for that tectum and represented, in the mapped region, the majority of the dorsal visual field. Ordering was not as good as in the normal goldfish, there being considerable variation in the spacing of visual field responses obtained at regularly spaced electrode positions. However there were very few crossings over of response lines. The projection from the left eye to the right tectum, the pathway of which had not been disturbed surgically, was essentially normal, of correct polarity and high order. Maps of the projections from the right and left eyes of a typical animal analysed at 270 days after surgery are shown in figure 9.12.

In the three cases in which the projections of both eyes to the right tectum were mapped an attempt was made, using the statistical method outlined at the beginning of this chapter, to detect a correlation between the response strengths of the two eyes at each tectal position. Figure 9.13 shows a plot of response strengths via the right eye against those via the left for the same animal whose maps are shown in figure 9.12. No correlation was apparent using the Spearman rank correlation test corrected for ties. Table 9.1 gives the correlation coefficients and their significances for the three animals.

Fig. 9.12 Electrophysiological maps of the visual field projections from the right (above) and left (below) eyes to the right tectum of a WTRON animal 270 days after surgery. The right eye map is corrected for an eye angle of 8° and the left eye map for -17° .

fig 9.12

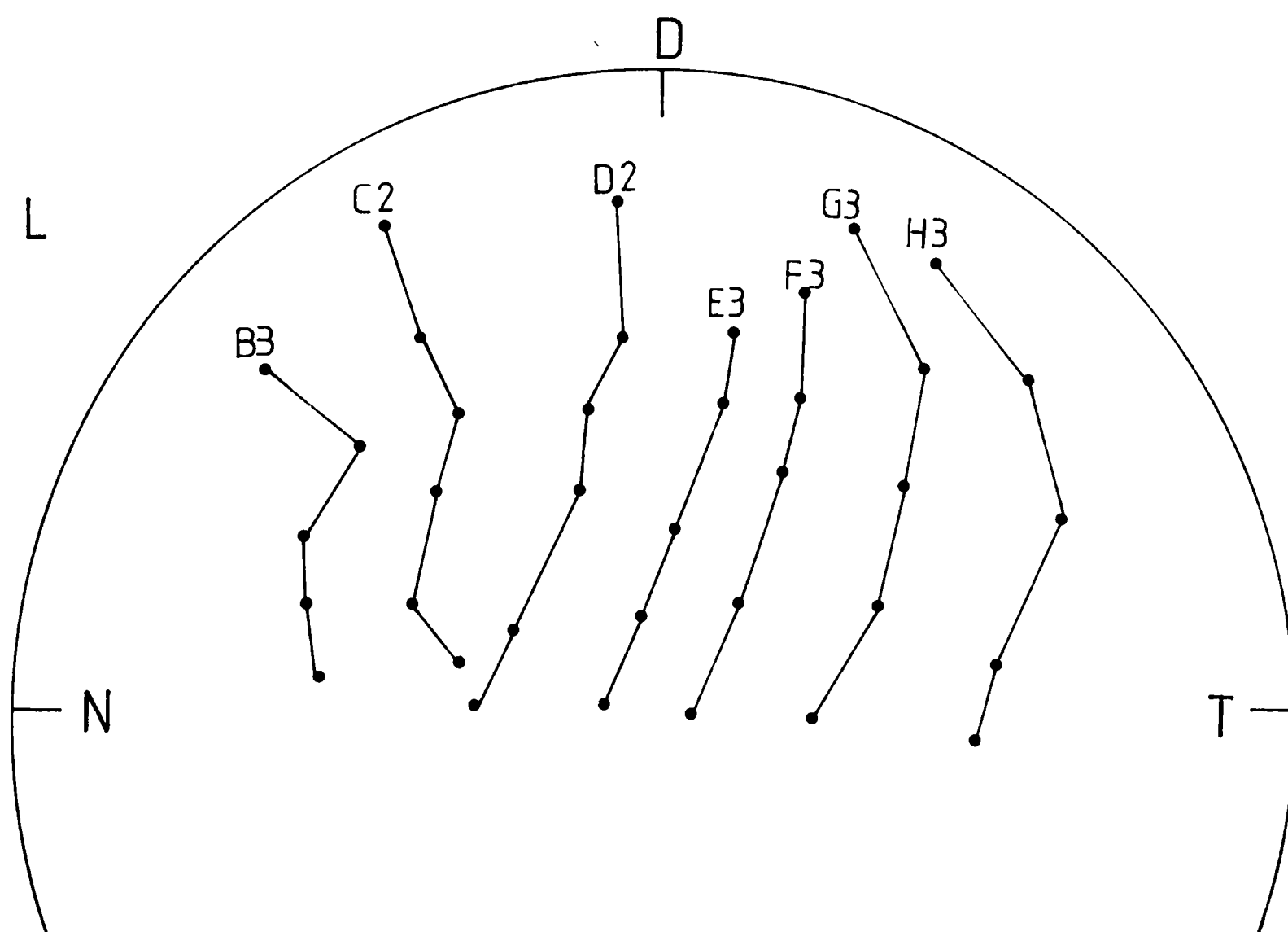
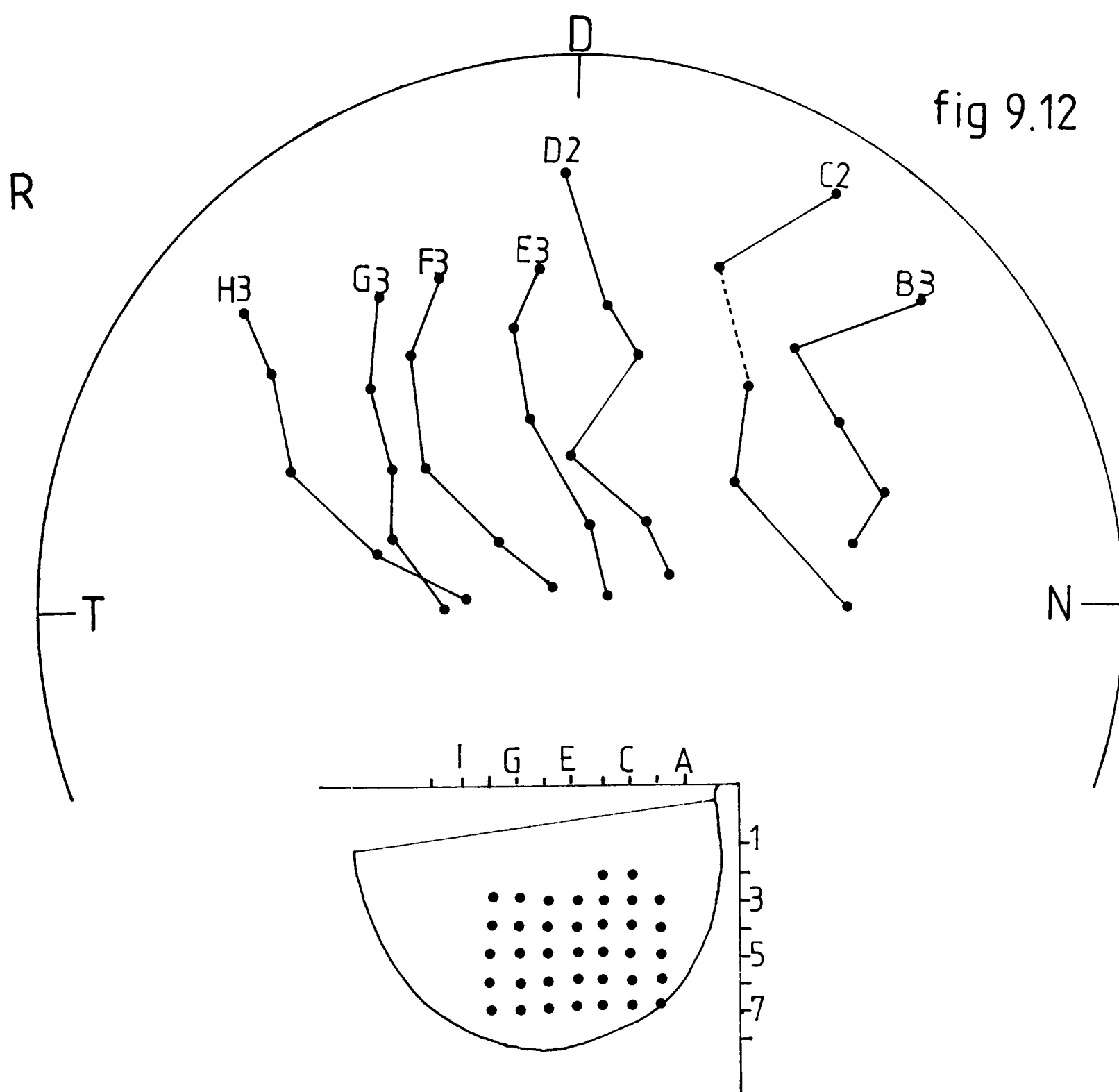


Fig. 9.13 Plot of response strengths via the right eye against these
via the left for a WTRON animal mapped 270 days after surgery.
The maps for this animal are shown in figure 9.12.

$$n = 37$$

$$R_s = -0.015$$

$$p = \text{N.S.}$$

fig 9.13

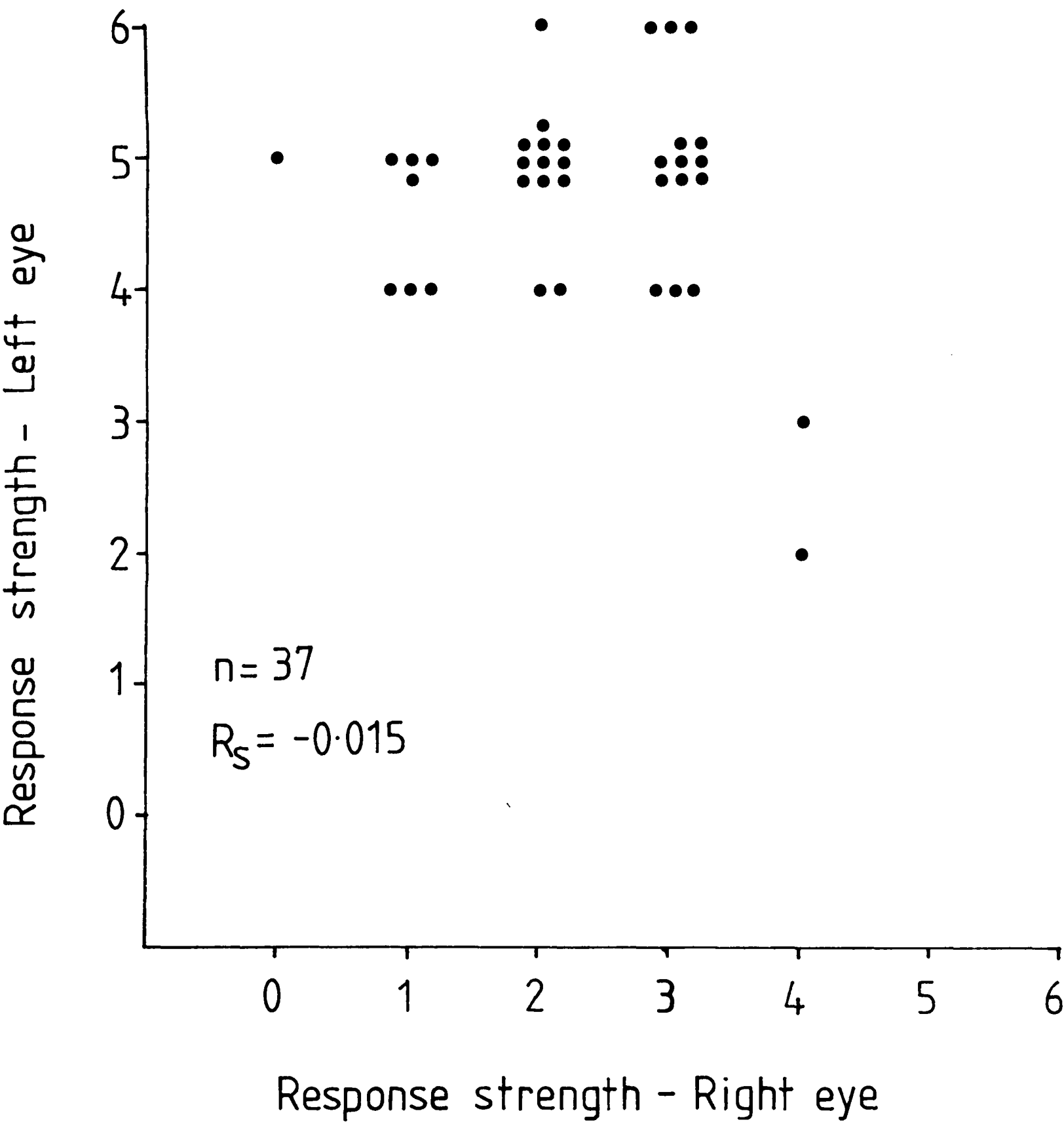


Table 9.1.

Animal	No. of points mapped	Correlation coefficient (Spearman rank correlation test)	Significance (one-tailed t-test)
26.6.80	37	-0.015	N.S.
28.7.80	34	+0.01	N.S.
31.7.80	35	+0.06	N.S.

There was thus no electrophysiological evidence of an interaction between the two sets of fibres to produce patches of tectum dominated by one or other eye.

In the three cases where both eyes were mapped an attempt was made visually to detect any correspondance between the visual field positions of the responses via the two eyes at any particular tectal position. Initially the maps were inspected after correction for eye rotation but no close correspondance was seen. The maps were then rotated about their centres (representing the field projection of the optic nerve heads) but no position was found at which the two projections coincided within the limits of the mapping technique - which should have imposed an error no greater than half the average separation in the visual field of responses from two adjacent electrode positions.

A number of anatomical approaches were employed in an attempt to determine the paths of the ipsilaterally-projecting fibres. In four animals the right optic nerve was cut and filled with HRP immediately after mapping. After processing it was found that at the chiasm a considerable number of labelled fibres passed contralaterally and ramified throughout a large area of brain underlying the removed tectum. However a number of labelled fibres peeled off from the right optic nerve in the chiasmatic region and passed in large fascicles into the right optic tract where they interdigitated with fascicles from the left eye. Towards the tectum the fascicles tended to

split into smaller units which became widely distributed through the tract brachia. The labelled fibres did however remain in small clumps and never became uniformly mixed with the unlabelled fibres from the left eye. A few labelled fibres were also seen crossing the midline in the horizontal commissure though their number was tiny in comparison with the number passing ipsilaterally in the tract. A camera lucida reconstruction of the paths of fibres from the right eye in an animal labelled 179 days after surgery is shown in figure 9.14. The quality of labelling in the right tectum was poor but the distribution of HRP filled terminals appeared to be uniform. Photomicrographs of the interdigitation of fibre fascicles from the two eyes in the chiasmatic region are shown in figure 9.15.

Labelling of a complete optic nerve does not provide information about the ordering of fibres within the ipsilateral pathway. Therefore in two animals a small injection of HRP was made into the mediocaudal edge of the right tectum. In both cases a small bundle of labelled fibres was seen in the appropriate (with regard to their final ipsilateral termination sites) dorsomedial position in the medial tract brachium of the right optic tract. The labelled fibres could be traced as a discrete bundle back to the chiasm where some fibres passed together into the left optic nerve while others crossed ventrally between the two nerves to form a discrete patch in the right optic nerve. A camera lucida reconstruction of one of these fish, labelled at 241 days after surgery is shown in figure 9.16. No labelled fibres were detected in the left optic tract though a path to the ipsilateral tectum via the contralateral tract for some fibres could not be excluded.

In three further animals an injection of ³H-proline was made into the right eye 24-27 days before mapping. Immediately after mapping a small injection of HRP was made into the caudomedial edge of the right tectum. These animals were mapped 302-305 days after surgery. The purpose of this combination of HRP and ARG was to label all the ipsilaterally projecting fibres with slowly

Fig. 9.14 Camera lucida reconstruction of the paths of HRP labelled fibres from the right eye of a WTRON fish 179 days after surgery. The fish was labelled by packing the cut right optic nerve with HRP soaked Sterispon. The majority of filled fibres can be seen passing to the contralateral side of the brain. However in the chiasmatic region a number of fascicles peel off and pass into the ipsilateral tract where they become widely distributed. A few fibres cross the midline in the horizontal commissure.

fig 9.14

WTRON



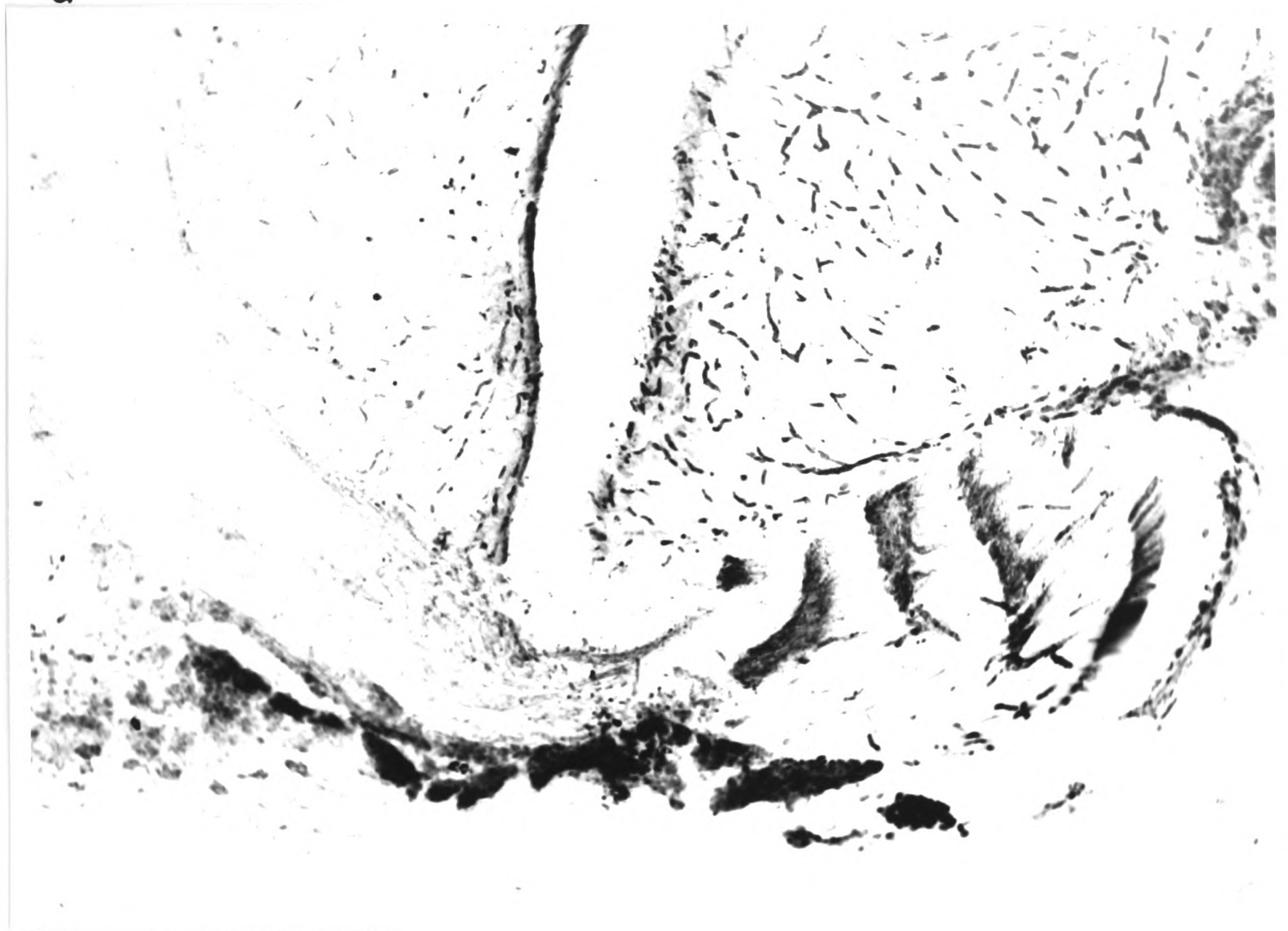
Fig. 9.15a Low power photomicrograph of the chiasmatic region of a WTRON fish labelled by HRP introduction into the right optic nerve at 179 days after surgery.

Fig. 9.15b High power photomicrograph showing the interdigitation of fibre fascicles from the two eyes in the chiasmatic region of the same fish.

In both photographs the right optic tract is seen on the right side of the photograph.

fig 9.15

a



300 μm

100 μm

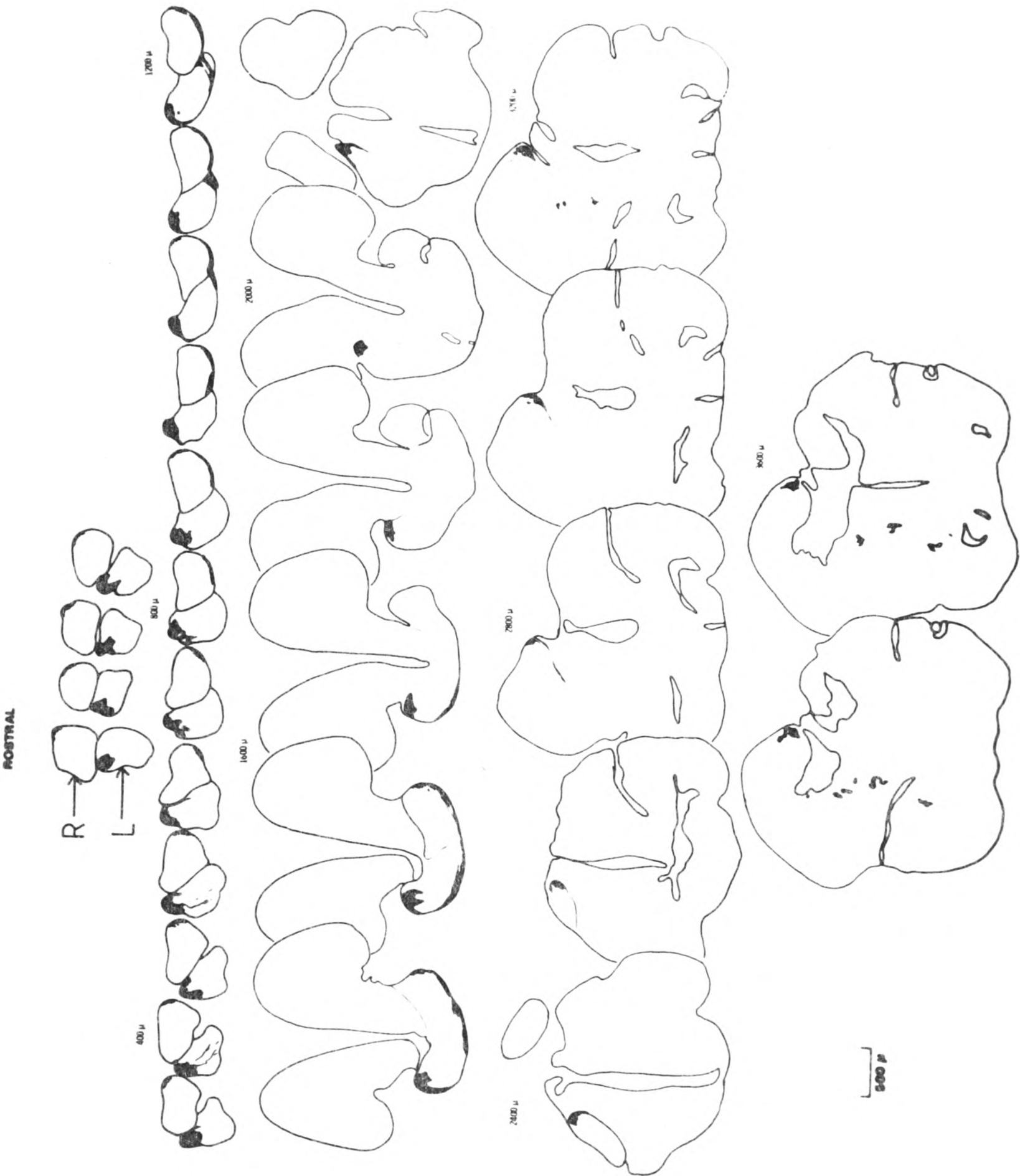
b



Fig. 9.16 Camera lucida reconstruction of the retinotectal pathway of a WTRON fish labelled by injection of HRP into the caudomedial edge of the right tectum 270 days after surgery. The injection filled fibres from both eyes as labelled fibres could be traced into both optic nerves. Fibres from the right eye were seen to join the ipsilateral optic tract by passing ventrally between the two nerves in the chiasmatic area. Ipsilateral and contralateral labelled fibres formed a single discrete patch in the correct (for their termination sites) region of the tract.

fig 9.16

WTRON



transported ^3H -proline in order to detect the ipsilateral fibres within the small group of HRP filled fibres. In these animals alternate $40\mu\text{m}$ frozen sections were processed for HRP and for ARG.

The ipsilateral fibres in these animals were heavily labelled by the radioactive proline and gave a pattern of staining very closely resembling that seen when the ipsilateral eye was filled by HRP. Broad fascicles of fibres were seen to pass ventrally between the two optic nerves in the chiasmatic region and the ipsilateral fibres formed a number of discrete patches within the ipsilateral tract, never becoming intimately intermixed with fibres from the contralateral eye (fig. 9.17). When adjacent ARG and HRP sections were compared it was found that the small group of fibres labelled with HRP from the tectum included fibres from both eyes. However these two fibre groups remained in separate fascicles, and did not become intimately intermixed.

To seek anatomical confirmation for the lack of patching seen electrophysiologically in this group one animal, which was mapped at 270 days after surgery and shown not to be patched, was injected into the right eye with ^3H -proline. After 24 hours survival, by which time tectal terminals should be well filled with fast-transported proline, the brain was processed for autoradiography as $10\mu\text{m}$ wax sections. In these sections visual inspection revealed no gross variations in the density of labelling throughout the extent of the optic fibre terminal layers of the tectum (figure 9.18).

7/8 only

Eight animals from this group were analysed electrophysiologically including three which were set up as bridge only animals but which showed no evidence of a persistent bridge when prepared for mapping. Mapping was performed at 185-359 days after operation. The projection from the right eye to its ipsilateral tectum was very variable in its extent, ranging from just a few

Fig. 9.17 Photomicrograph of the optic tract of a WTRON animal labelled by injection of ^3H -proline into the right eye 24 days before mapping. The animal was sacrificed 304 days after surgery. In this autoradiograph bands of labelled ipsilateral fibres can be seen interdigitating with fibres from the left eye in the right optic tract.

Fig. 9.18 Photomicrograph of part of the tectum of a WTRON animal labelled by injection of ^3H -proline into the right eye 1 day before sacrifice at 270 days after surgery. The radioactive label was uniformly distributed across the tectal extent though this is obscured in this photomicrograph due to focusing problems.

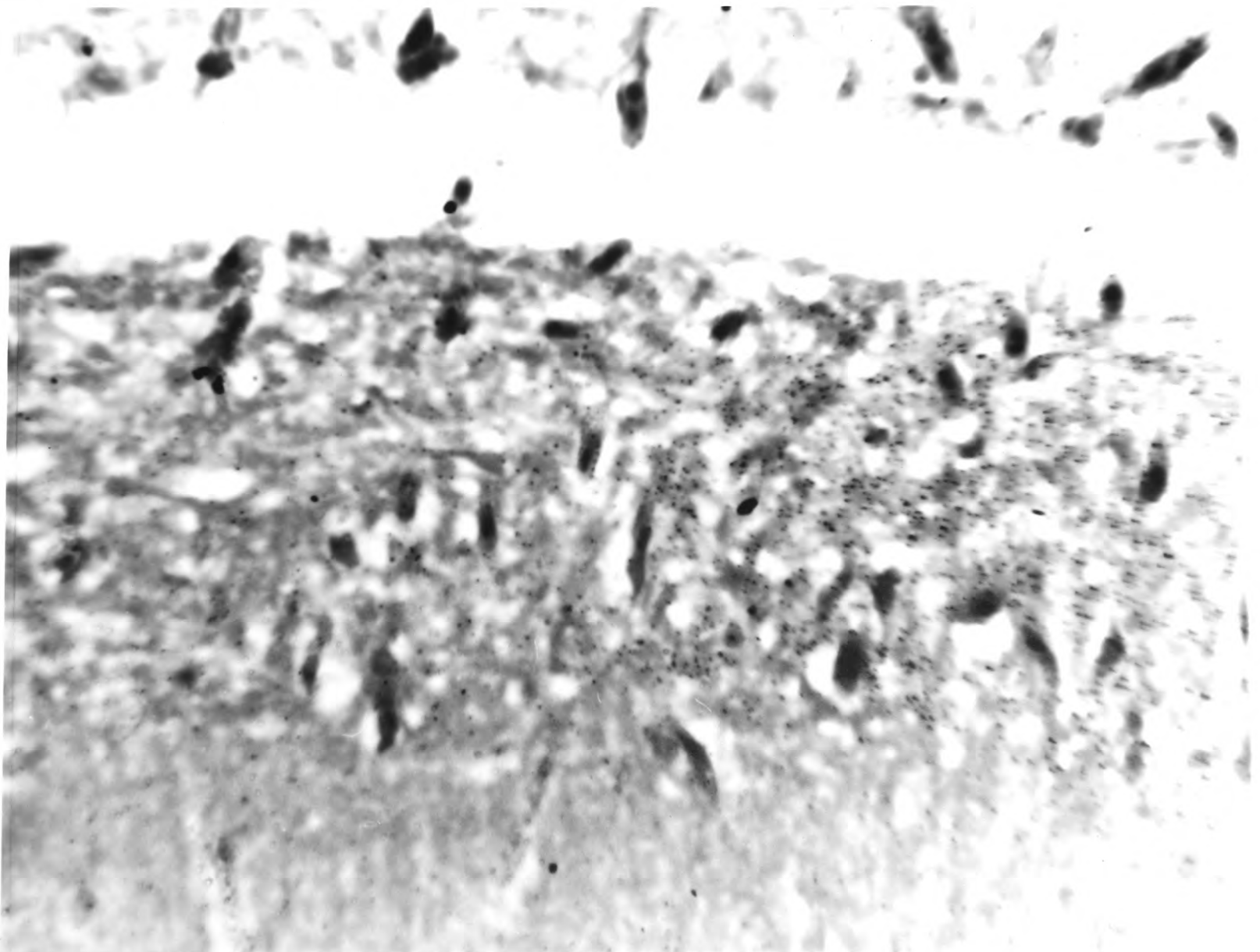
fig 9.17



100 μm

50 μm

fig 9.18



responses to temporal visual field stimulation obtained at the caudal part of the tectum to an almost complete map with responses obtainable throughout most of the mapped area of tectum. The orderliness of the projection also varied from almost normal with only rare crossings over of response lines, to very poor. Whenever sufficient responses were obtained to allow its determination the polarity of the projection was found to be appropriate to the host tectum.

In the seven fish in which the projection from the left eye to the right tectum was mapped it was found to be normal in five and almost normally ordered in the remaining two, both of which were "failed bridge" animals and had thus sustained slight damage to the normal contralateral pathway on insertion of the bridge. Electrophysiological maps obtained at 317 days and 332 days after surgery are shown in figures 9.19 and 9.21.

In the five animals in which both eyes were mapped and sufficient responses obtained from the right eye to make the exercise worthwhile the correlation between the response strengths from the two eyes was analysed using the statistical methods already described. Figures 9.20 and 9.22 show the response strength plots for the fish whose maps are given in figures 9.19 and 9.21 respectively. Table 9.2. summarizes the results of the statistical analysis.

Table 9.2.

Fish	Points mapped	Correlation coefficient (Spearman rank correlation)	Significance (one-tailed t-test)
30.6.80	25	-0.47	p < 0.01
15.7.80	34	-0.11	N.S.
8.8.80	34	-0.435	p < 0.01
2.7.80*	30	-0.235	N.S.
14.8.80*	31	-0.279	p < 0.1

* both "failed bridge" animals.

Fig. 9.19 Electrophysiological maps of the visuotectal projections from the two eyes to the right tectum of a 7/8 only animal mapped 317 days after surgery. At the top is the map from the right eye, corrected for an eye angle of 30° . At the bottom is the map from the left eye corrected for an eye angle of -10° . The tectal diagram on the left shows the mapping positions while the plot on the right shows the relative response strengths of the two eyes at those positions using the classification described at the beginning of this chapter.

fig 9.19

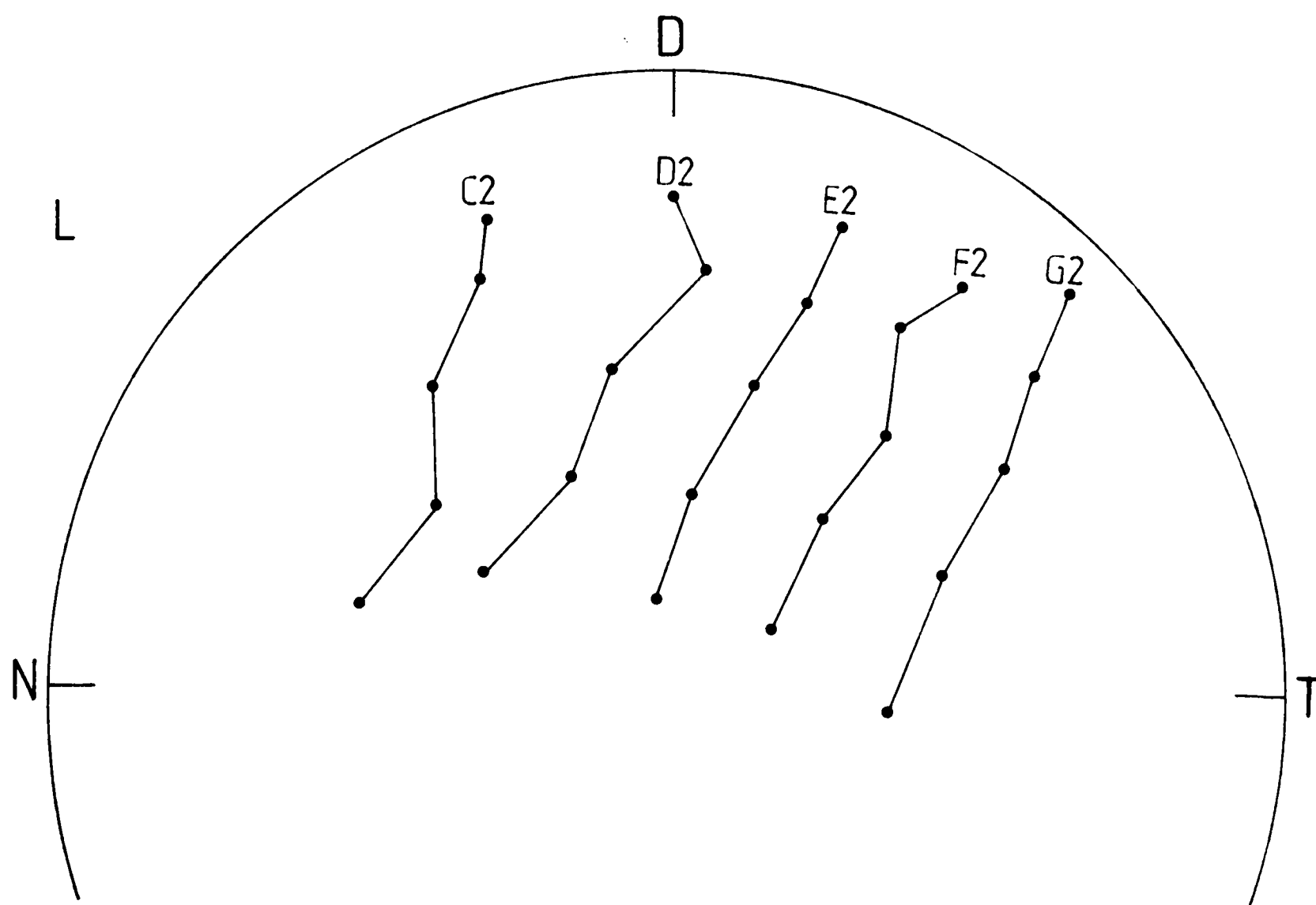
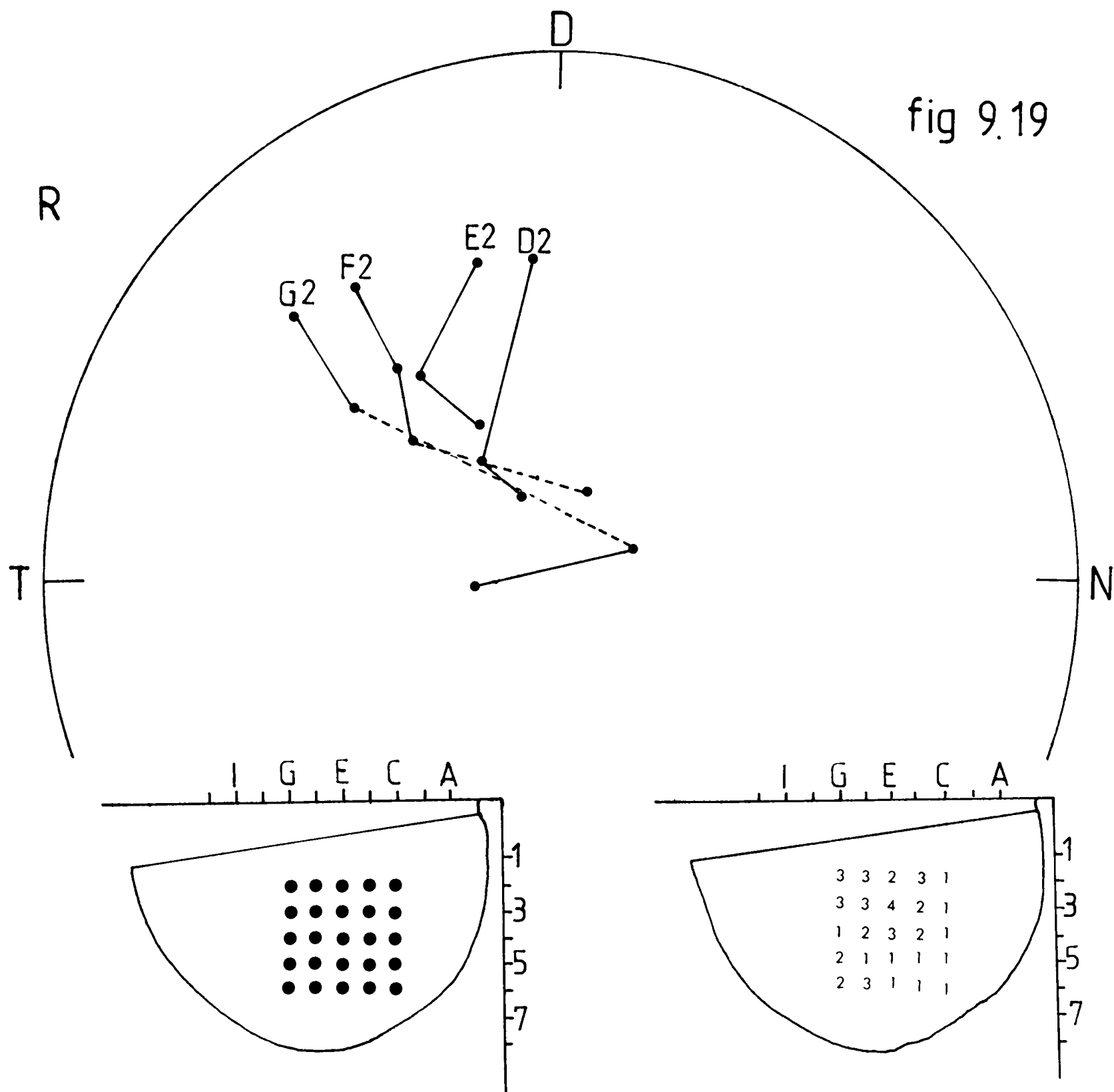


Fig. 9.20 Response strength plot for 7/8 only fish mapped 317 days after surgery. (The maps of this fish are shown in figure 9.19). A significant negative correlation between response strengths in the two eyes was detected ($p = 0.01$).

fig 9. 20

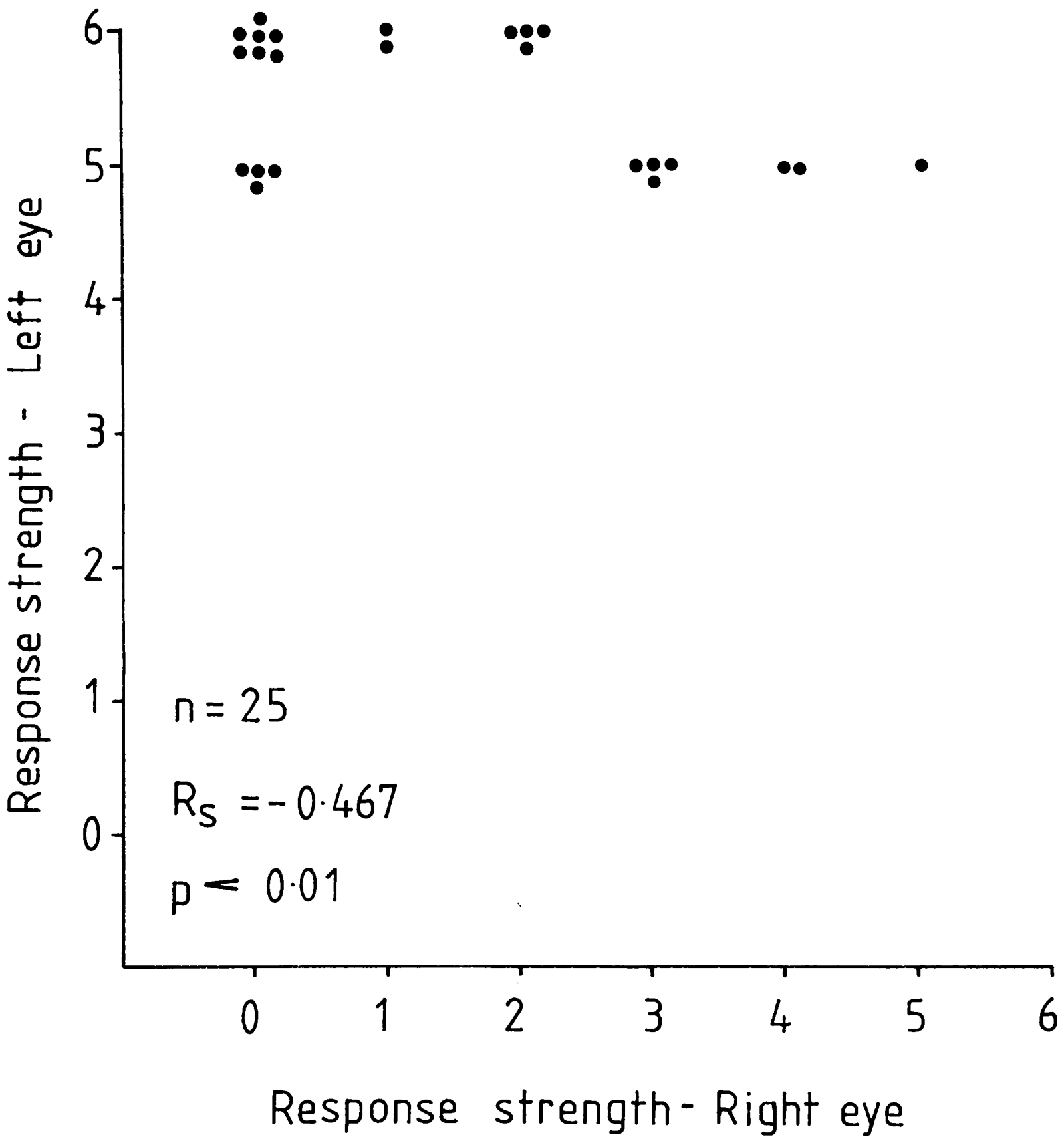


Fig. 9.21 Electrophysiological maps of the visuotectal projections from both eyes to the right tectum of a 7/8 only fish mapped 332 days after surgery. The map from the right eye is shown at the top, corrected for an eye angle of 28° . The left eye map is shown at the bottom, corrected for an eye angle of -10° . Mapping positions are shown on the tectal diagram.

fig 9.21

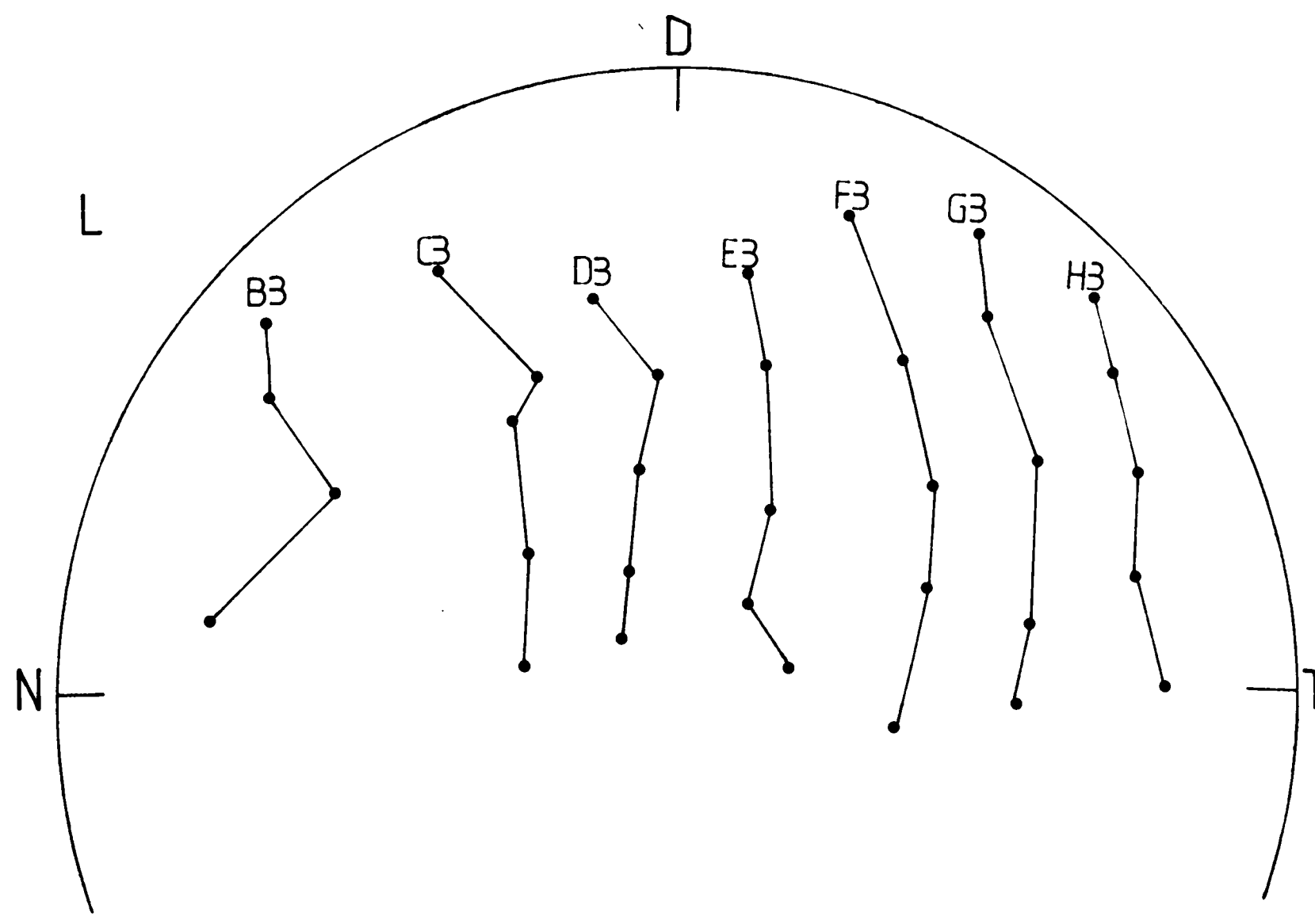
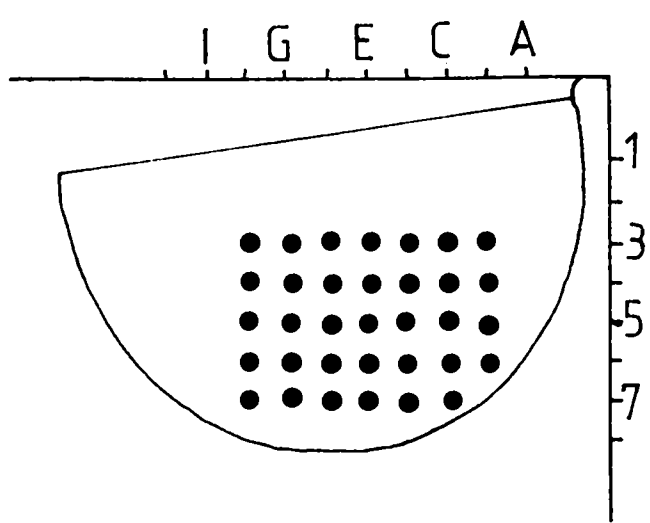
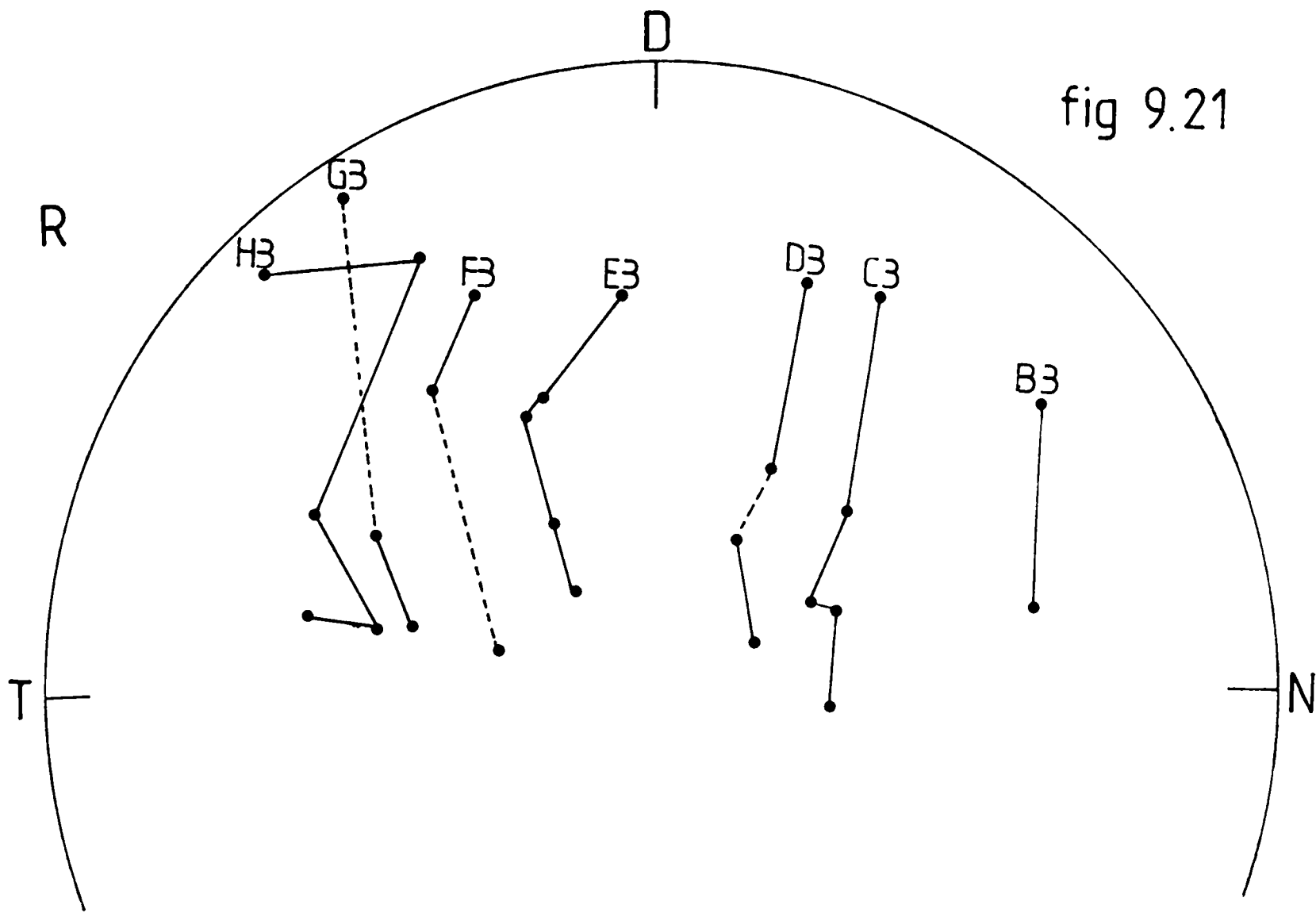
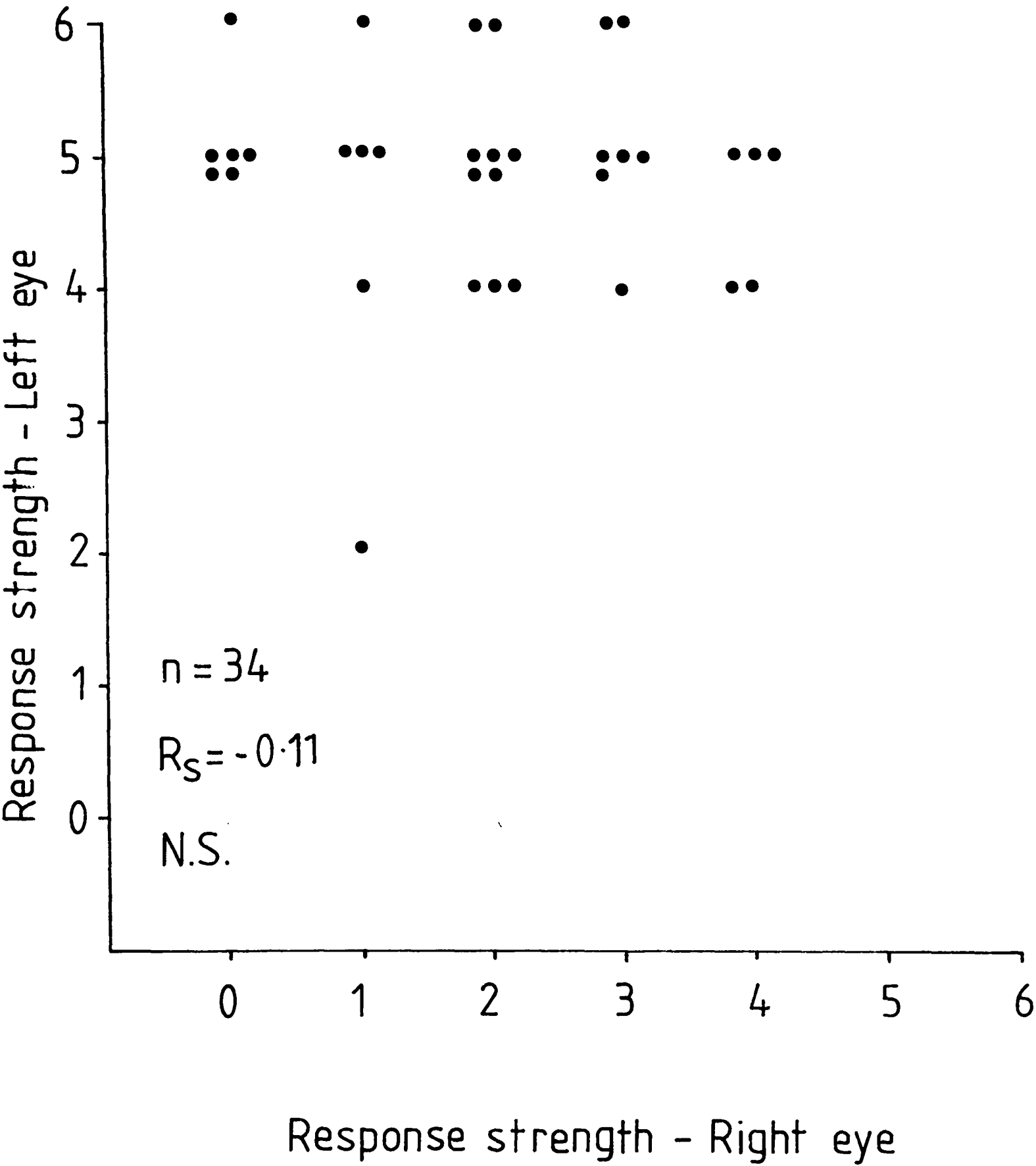


Fig. 9.22 Response strength plot for a 7/8 only fish mapped 332 days after surgery. The maps from this fish are shown in figure 9.21. The negative correlation between the response strengths via the two eyes was not significant.

fig 9.22



Electrophysiological evidence for interactions between the two fibre populations is thus equivocal. Three correlation coefficients were significant and two not, the distinction not being explicable in terms of the two series of fish, the original 7/8 only and the "failed bridge" animals. Visual inspection did not reveal any close correspondance between the response positions in the visual field obtained at each electrode position via the two eyes. There was thus no evidence of interaction between the two fibre populations in terms of their retinal loci of origin.

In an attempt to elucidate the paths taken by fibres from the ipsilateral eye to the tectum HRP was introduced into the cut right optic nerves of five fish immediately after mapping. Good labelling was obtained in three of these, all of which showed labelled fibres crossing as a band into the ipsilateral optic tract in the chiasmatic region. In the ipsilateral tract the labelled fibres became fairly widely scattered though with a concentration at the ventromedial edge. The fibres remained in small clusters rather than becoming uniformly intermixed with the contralateral fibres, **and the number of ipsilaterally-projecting fibres, though not** determined accurately, seemed smaller than in the WTRON series, probably reflecting the existence of a small tectal remnant on the left side of the brain on which some of the right eye fibres terminated. From the tract the labelled fibres spread out over the tectum though the quality of staining of the terminals was not sufficient to allow any firm conclusions to be drawn concerning the uniformity of the terminals. A camera lucida reconstruction of the visual pathway of one of these animals, labelled 267 days after surgery is given in figure 9.23. Photomicrographs of the passage of ipsilateral fibres into the ipsilateral tract at the chiasm and their subsequent distribution in the tract are shown in figure 9.24.

To investigate the ordering of fibres within the pathway two animals were given injections of ^3H -proline into the right eye, 30 days before mapping.

Fig. 9.23 Camera lucida reconstruction of the visual pathway of a 7/8 only fish labelled by introduction of HRP into the cut right optic nerve 267 days after surgery. Labelled fibres can be seen passing into the right optic tract in the chiasm. They become widely scattered as small clumps throughout the ipsilateral optic tract.

fig 9.23
7/8 only

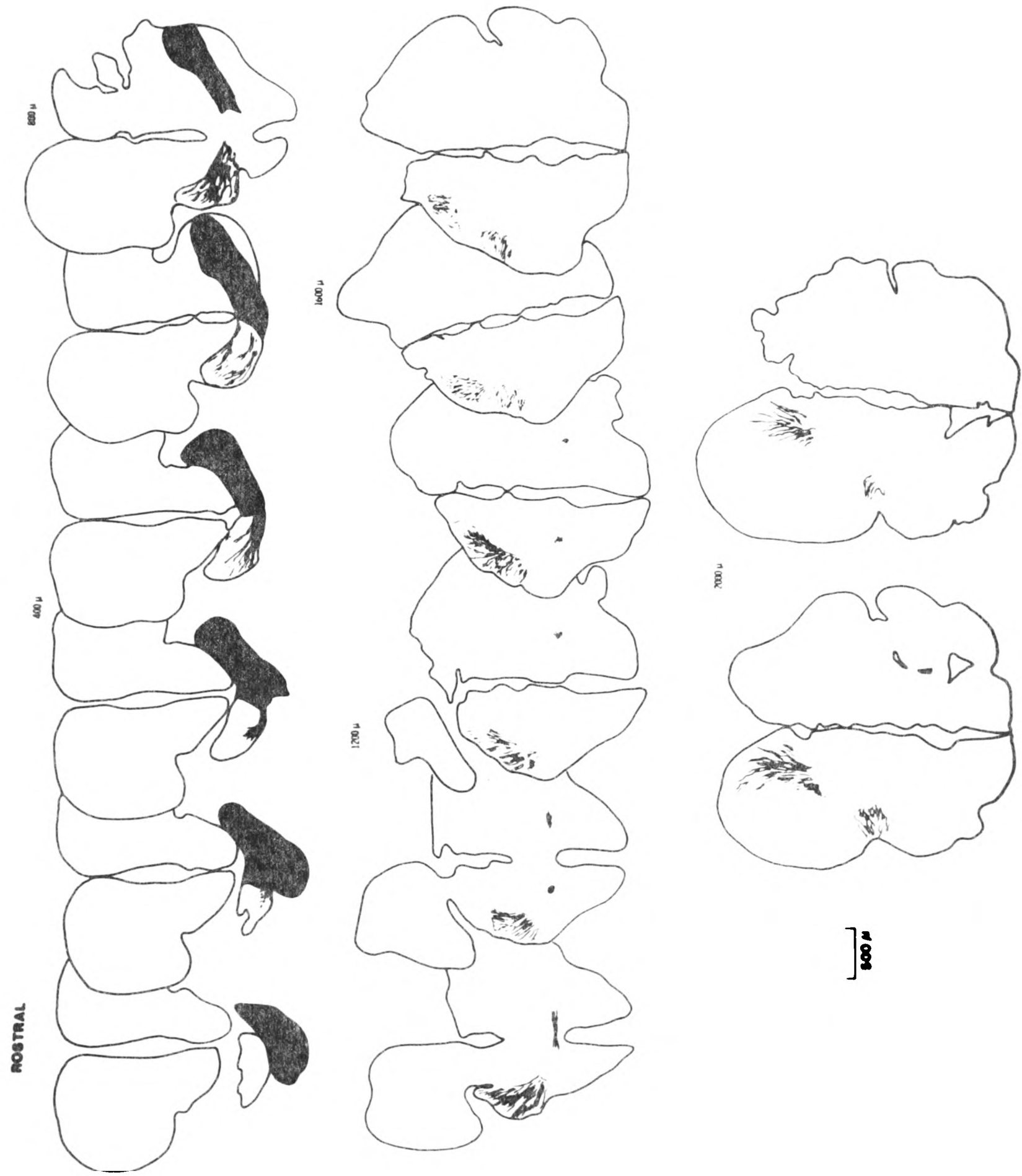


Fig. 9.24a Low power photomicrograph of the chiasmatic region of a 7/8 only animal labelled 317 days after surgery, by HRP introduction into the cut right optic nerve. Labelled fibres can be seen passing into the right optic tract at the chiasm (The right side of the brain is shown on the right side of both these photomicrographs).

Fig. 9.24b Low power photomicrograph of the optic tract of the same animal. Small clumps of labelled fibres can be seen scattered within the right optic tract. These are indicated by the arrows.

fig 9.24

a



b

500 μ m



Immediately after mapping a small amount of HRP was injected into the caudomedial region of the right tectum. After 1 day survival alternate frozen sections were taken for ARG and HRP development. HRP labelling was found in a small group of fibres in the correct dorsomedial area of the medial brachium of the optic tract and remained as a compact group into the contralateral optic nerve. However no labelled fibres could be identified in the ipsilateral optic nerve, probably because of the relatively sparse innervation of the right tectum by the right eye so that no conclusions can be drawn about the ordering of those ipsilateral fibres in the visual pathway. Slow transport of ^3H -proline within the ipsilateral fibres of these animals also indicated that no fibres from the ipsilateral eye lay within the region of the tract labelled with HRP by tectal injection. The radioactively labelled ipsilateral fibres appeared to form small clumps within the ipsilateral tract rather than being uniformly distributed, confirming the HRP findings.

To determine whether the ipsilateral projection had influenced the normally uniform distribution of contralateral fibres across the right tectum three animals (mapped at 267-332 days after surgery) were given injections of ^3H -proline into the left eye immediately after mapping. After 24 hours survival, to allow fast transport of the radioactive label into the terminals, frozen sections of these animals were processed for ARG. Slight variations in the density of labelling of the optic terminal layers of the tectum were seen in two of the three animals though these were not pronounced. In the third animal the labelling appeared uniform across the tectum (fig. 9.25). One animal received an injection of ^3H -proline into the right ipsilateral eye, just after mapping and was processed for ARG 24 hours later. In this case the density of labelling appeared uniform across the tectum. There was thus no clear anatomical evidence for the occurrence of patching in this group of animals. Slight variations in terminal density across the tectum were indicated in two animals but the quality of the frozen section autoradiography was not sufficient to allow quantification of this.

Fig. 9.25 Photomicrograph of part of the tectum of a 7/8 only labelled by injection of ^3H -proline into the right eye 24 hours before sacrifice at 317 days after surgery. The label is almost uniform across the tectal extent - only small variations in intensity were noted. However focusing problems obscure this uniformity to some extent in this photomicrograph.

fig 9.25



100 μ m

Bridge

This series of animals proved to have a very poor operative success rate, only three animals having a persistent bridge at mapping. On mapping one animal gave no detectable responses to right eye stimulation (119 days after surgery) and one gave only a few responses from a small area of temporal field projecting to the caudomedial right tectal edge (121 days after surgery). In the third animal, mapped 317 days after surgery, the projection from the right eye to the mapped area ^{of} right tectum covered most of the temporal field quadrant and was mainly to caudomedial tectum. The polarity of this projection was appropriate to the host tectum though the ordering was poor. In contrast the projection from the left eye was normal. These maps are shown in figure 9.26.

There was certainly no close correspondance between the visual field response positions via the two eyes at a given tectal location. Statistical analysis of the response strengths did however suggest an interaction between the two sets of fibres as the correlation coefficient (Spearman rank correlation test) of $R_s = -0.242$ was significant at $0.05 > p > 0.1$ (one-tailed t-test). The response strength plot of this animal is shown in figure 9.27.

Injection of HRP into the right optic nerve of this animal immediately after mapping revealed the path to the ipsilateral tectum to be entirely via the contralateral optic tract and the bridge. No HRP labelled fibres were detected in the ipsilateral optic tract. Due to the lack of surgically successful animals it was not possible to investigate the ordering of fibres as they passed from the right eye to the right tectum. However as that part of the pathway from eye to left tectum had not been disrupted there is no reason to believe that any reordering of fibres occurred prior to entry into the bridge.

Fig. 9.26 Electrophysiological maps of the visuotectal projections from both eyes to the right tectum of a Bridge fish mapped 317 days after surgery. At the top is shown the sparse projection from the right eye, corrected for an eye angle of 2° . At the bottom is the normal looking left eye map, corrected for an eye angle of -2° . On the left is a tectal diagram indicating the electrode positions while on the right is a tectal plot of relative response strengths via the two eyes, determined using the criteria described at the beginning of this chapter.

fig 9.26

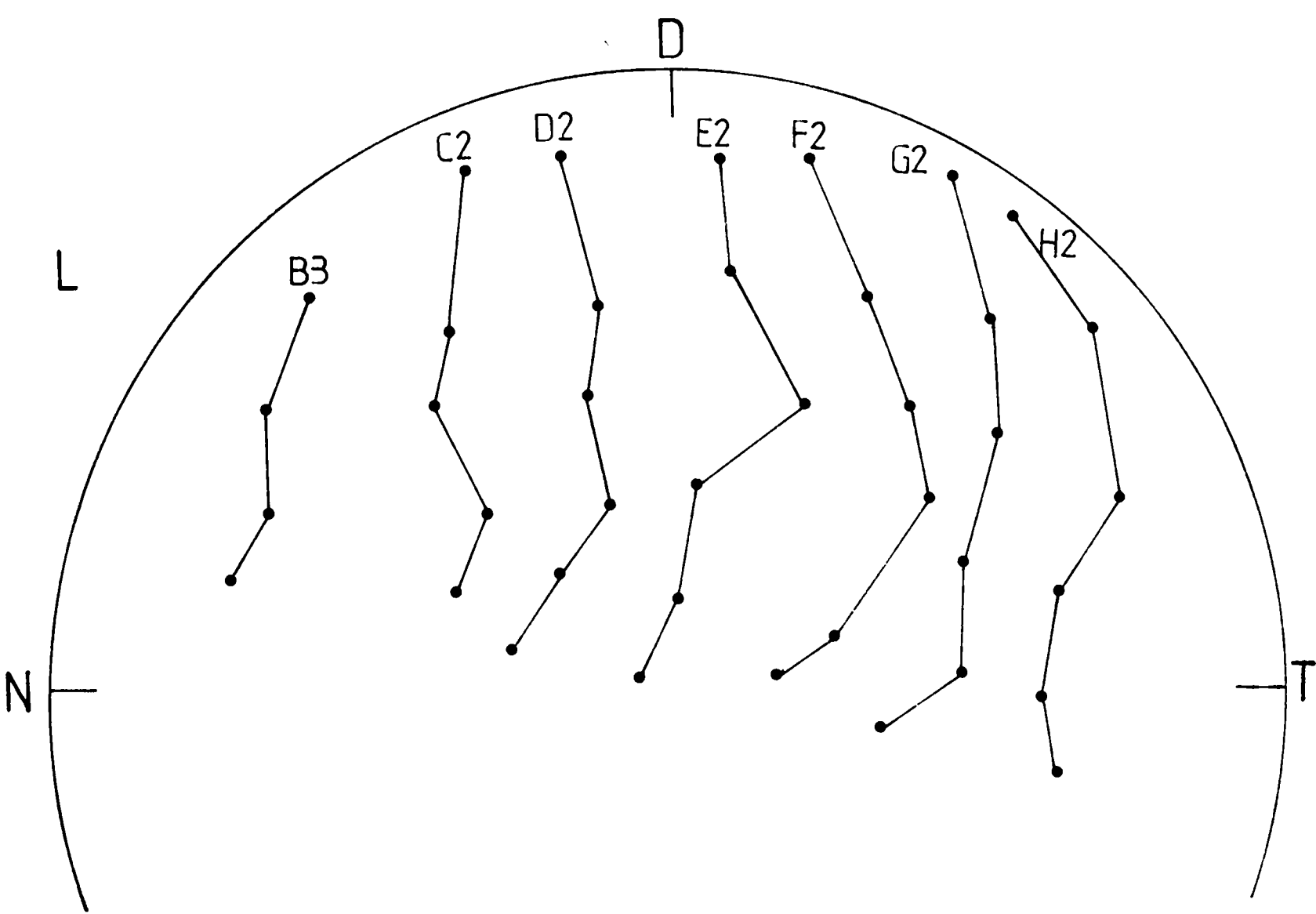
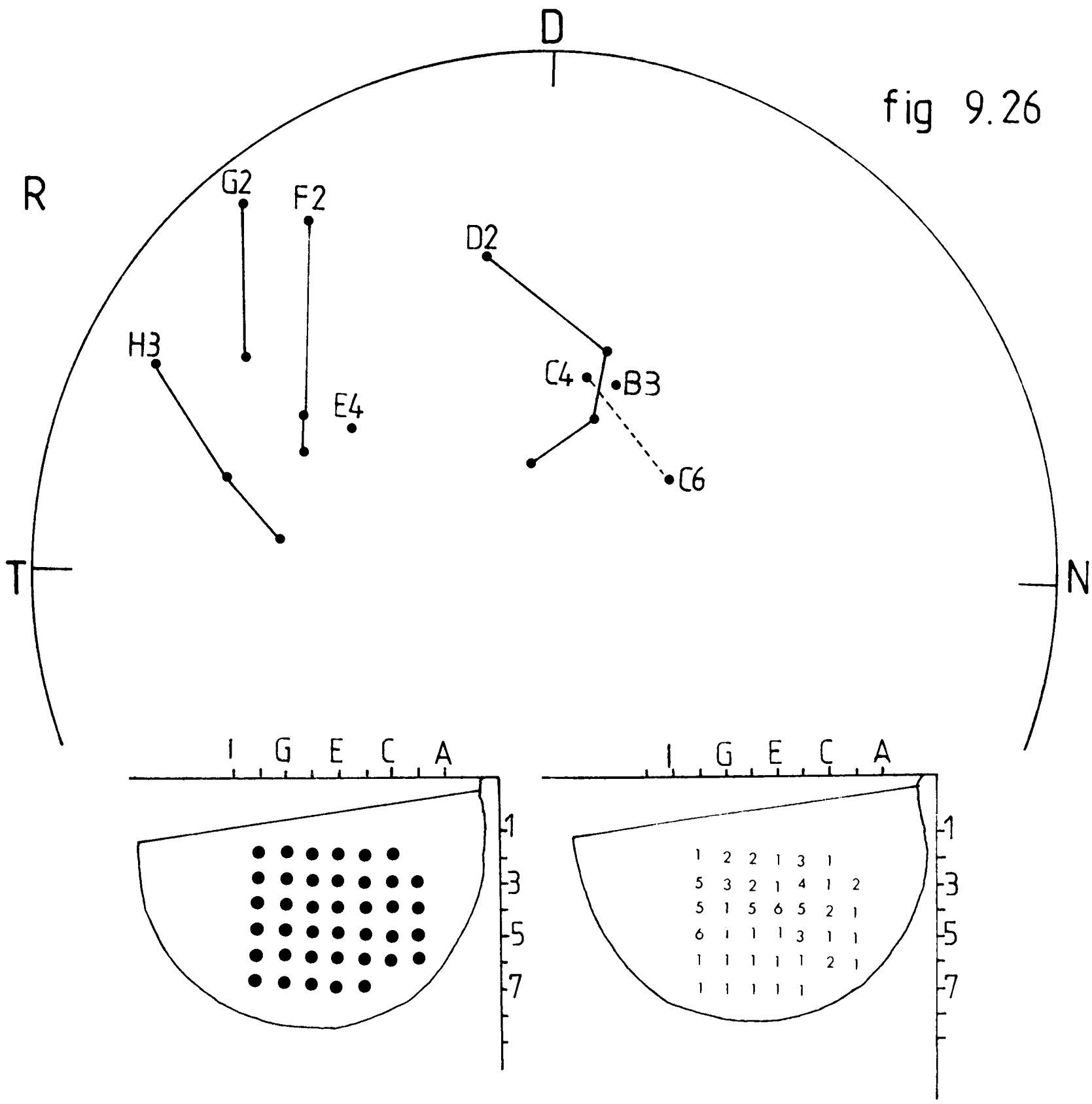
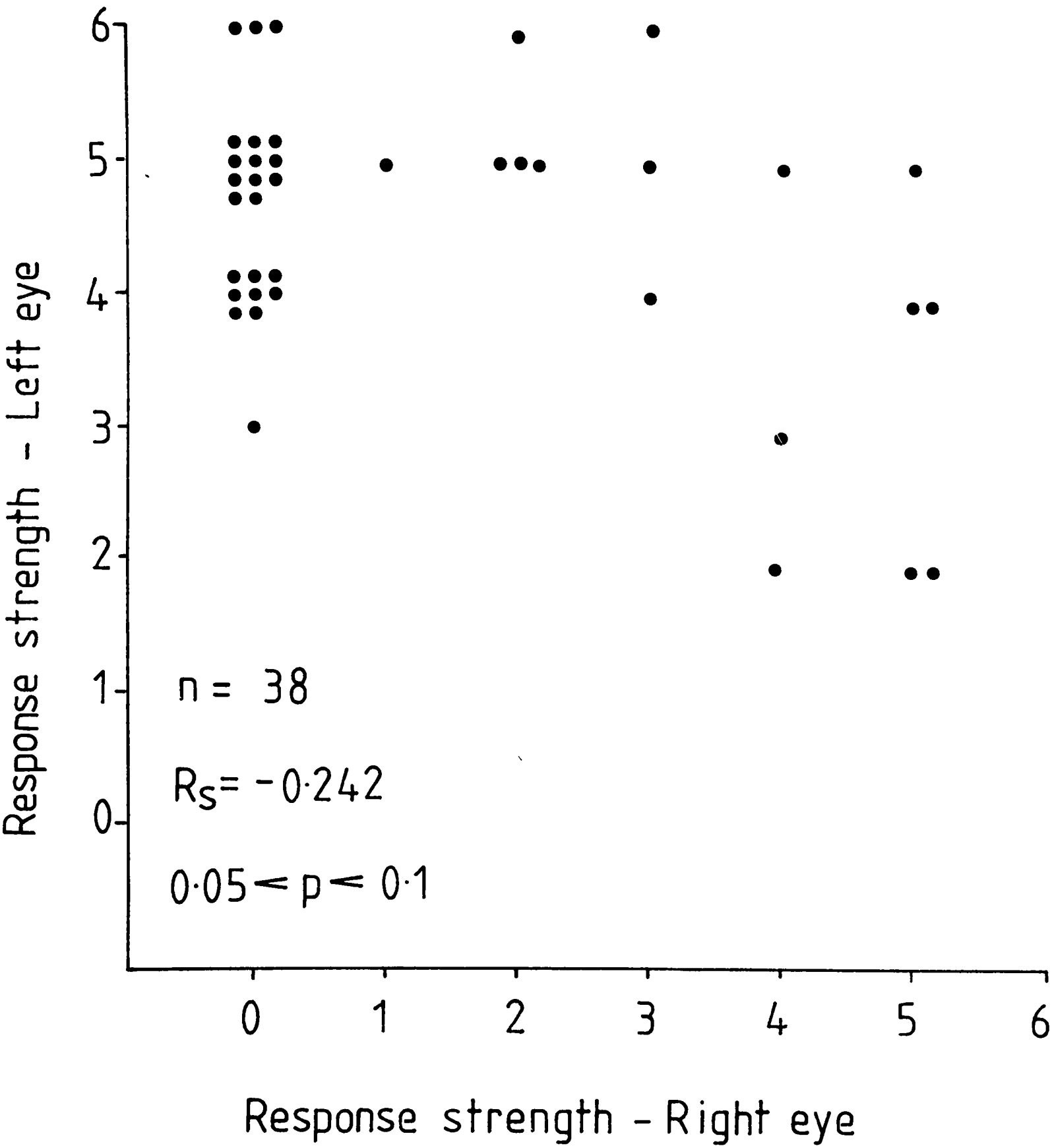


Fig. 9.27 Response strength plot for the same animal whose maps are shown in figure 9.26, a Bridge animal 317 days after surgery. The inverse correlation detected using the Spearman rank correlation test was significant at 0.05 $p \leq 0.1$ using a one-tailed t-test.

fig 9. 27



The same animal also received an injection of ^3H -proline into the left eye immediately after mapping. Autoradiography of alternate frozen sections showed some variation in the intensity of labelling from place to place across the tectal extent indicating an anatomical basis for the patching detected electrophysiologically. However insufficient sections were obtained after processing to allow reconstruction of the tectum in an attempt to correlate anatomy and electrophysiology at any particular tectal position.

WTRON and LON cut

Eight fish from this series were analysed electrophysiologically at 44-101 days after surgery. In the earliest fish (44 days) no localizable response was obtained from either eye, and very few responses were obtained at 55 or 63 days. Thereafter enough responses were detected to allow the polarity of the projection to be assessed. In every case the projections from both eyes were appropriate in polarity to the host right tectum. The degree of order in these projections did however vary from the very messy to the almost normal. There was no clear tendency for the degree of order to improve with time after surgery or for the projection from the left eye to be better than that from the right. Typical maps obtained at 78 and 101 days are shown in figures 9.28 and 9.30 respectively. In the first of these both left and right eye projections were well ordered and extensive. In contrast the later fish possessed an almost complete and well ordered projection from the right eye but only gave detectable responses to left eye stimulation at the medial part of the tectum. The results of a statistical analysis of the response strengths of these two fish is given in table 9.3.

Fig. 9.28 Electrophysiological maps of the visuotectal projections from both eyes to the right tectum of a WTRON + LON cut fish 78 days after mapping. The right eye projection, shown at the top, is corrected for an eye angle of 16° . The left eye map, shown below, is corrected for an eye angle of -9° .

fig 9.28

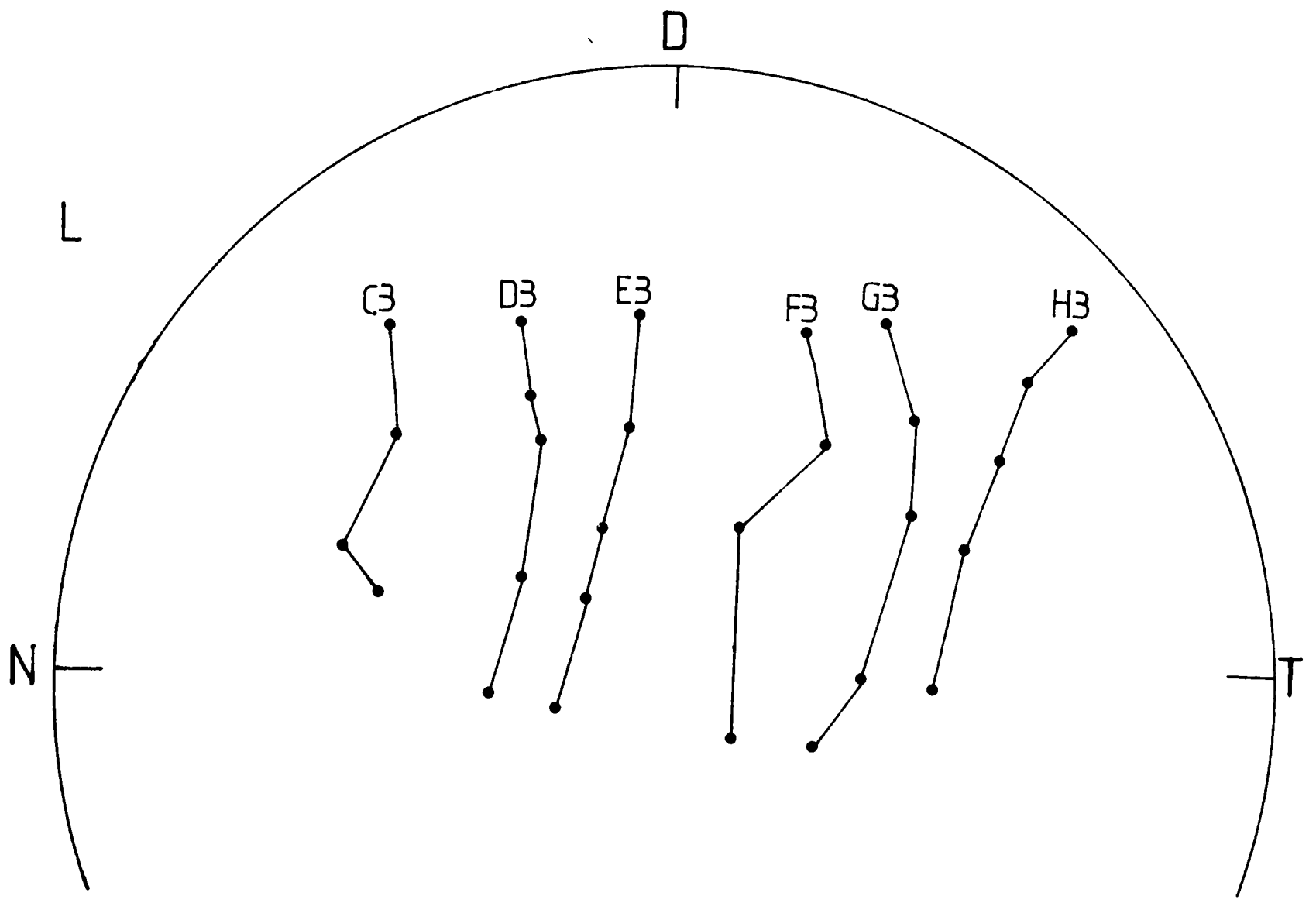
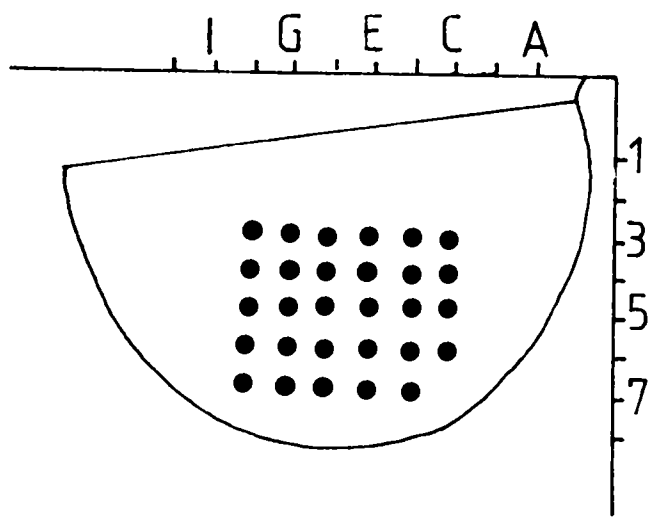
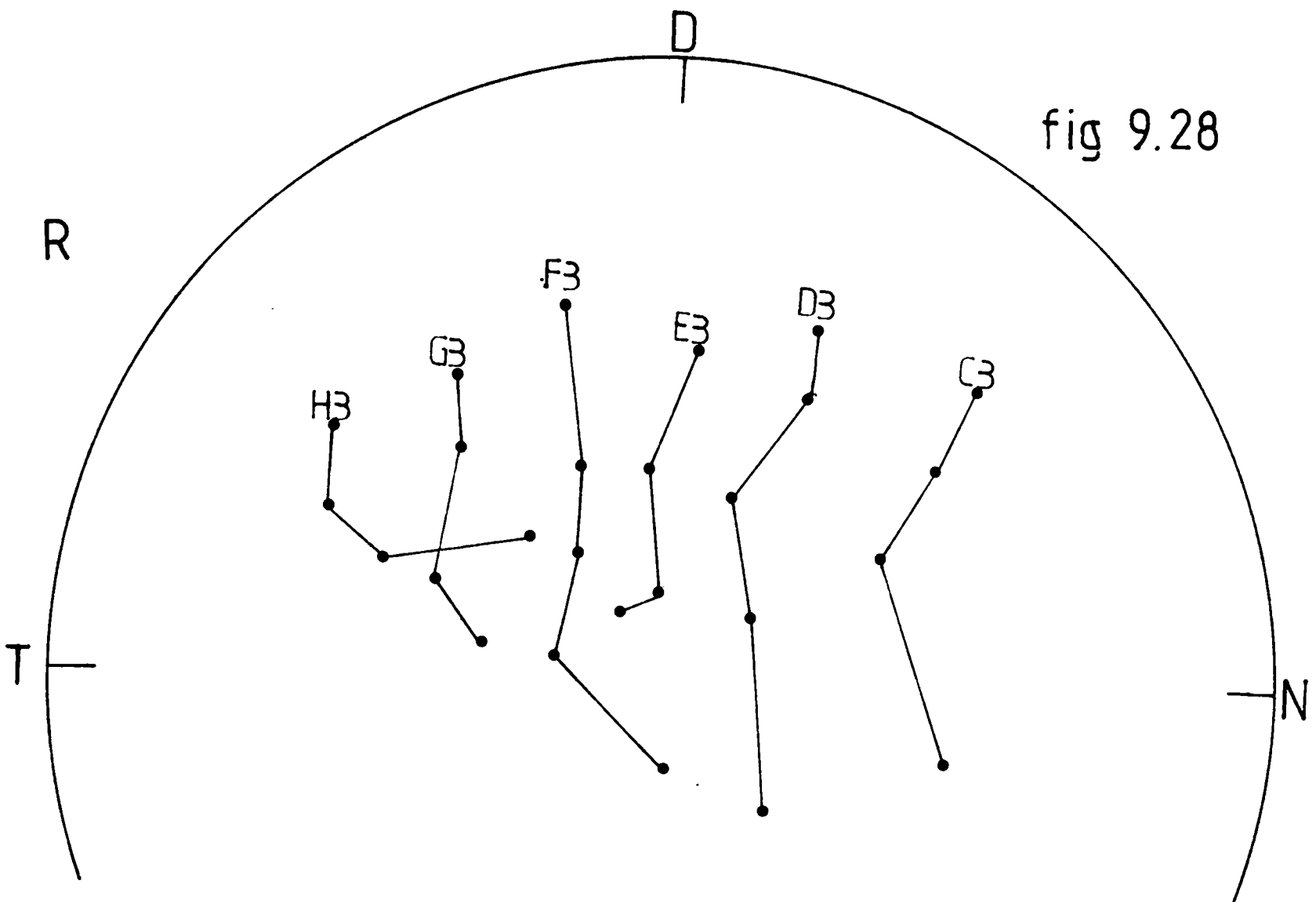


Fig. 9.29 Response strength plot for a WTRON + LON cut animal mapped 78 days after surgery. The maps of this animal are shown in figure 9.28. The very small positive correlation coefficient was not statistically significant.

fig 9.29

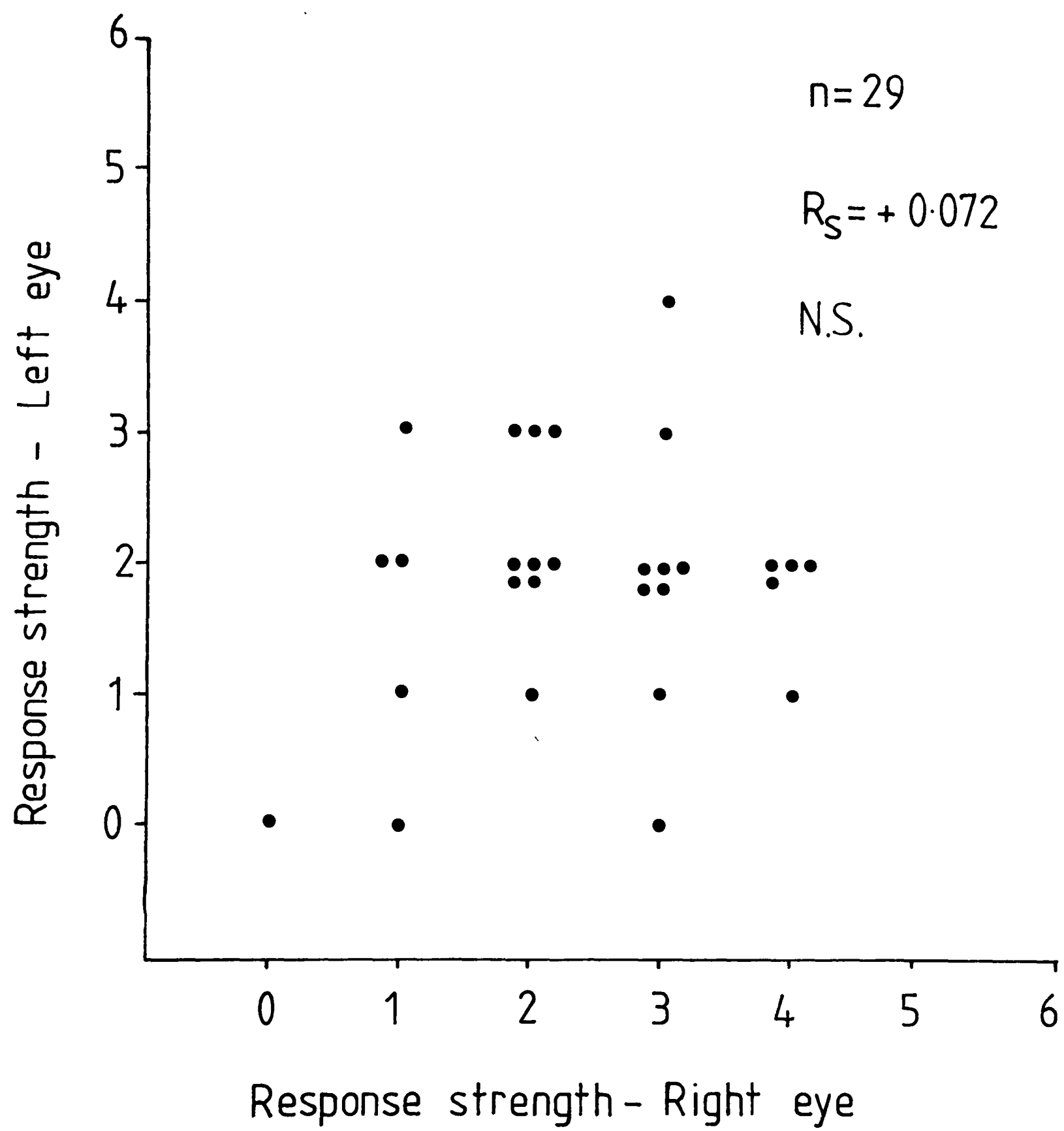


Fig. 9.30 Electrophysiological maps of the visuotectal projections from both eyes to the right tectum of a WTRON + LON cut animal mapped 101 days after surgery. The right eye map, shown above, is corrected for an eye angle of 22° , and the left eye map, shown below, for an eye angle of -12° .

fig 9.30

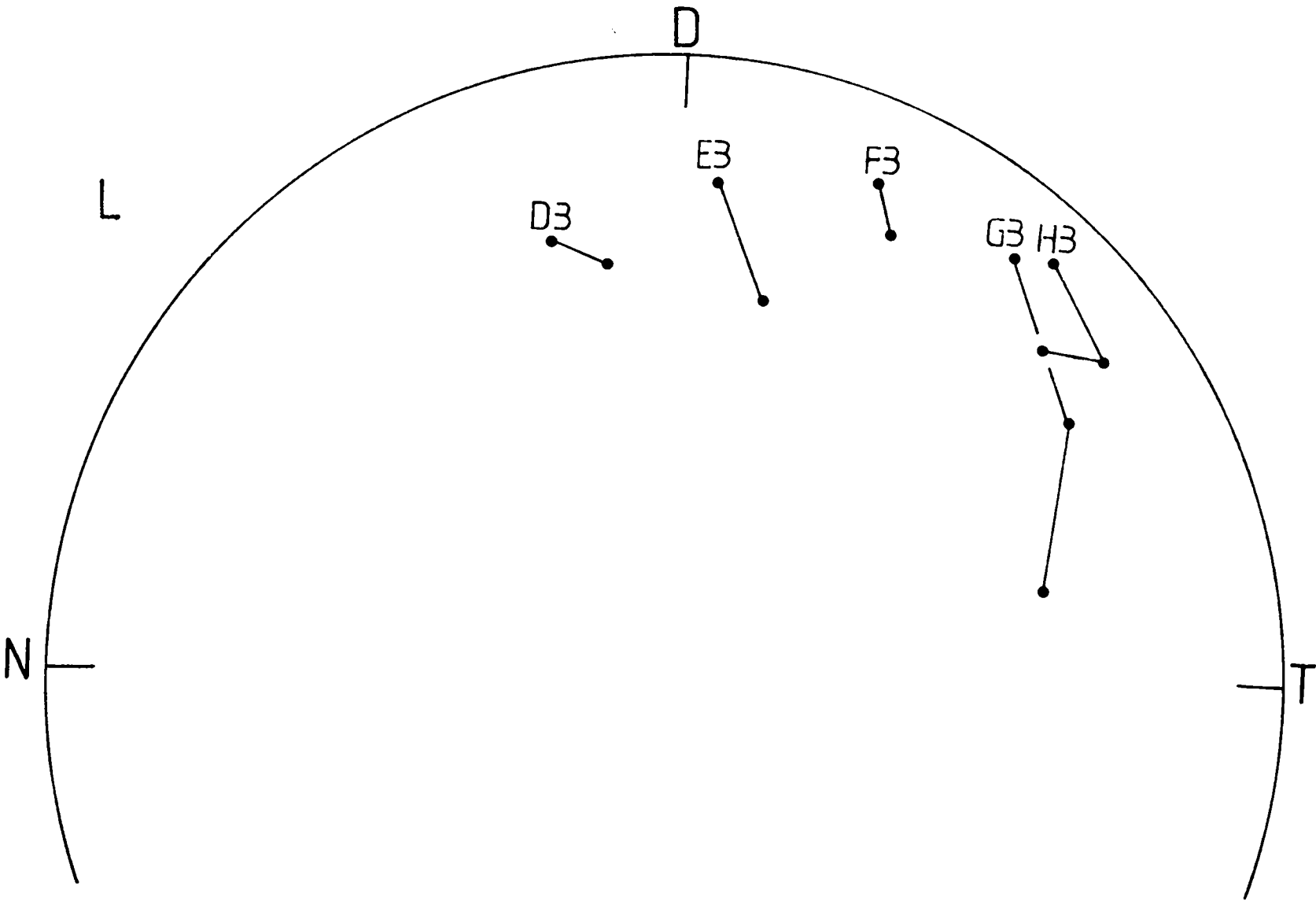
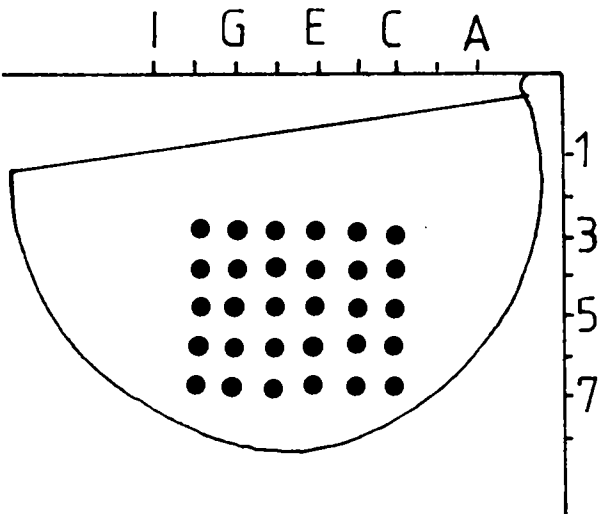
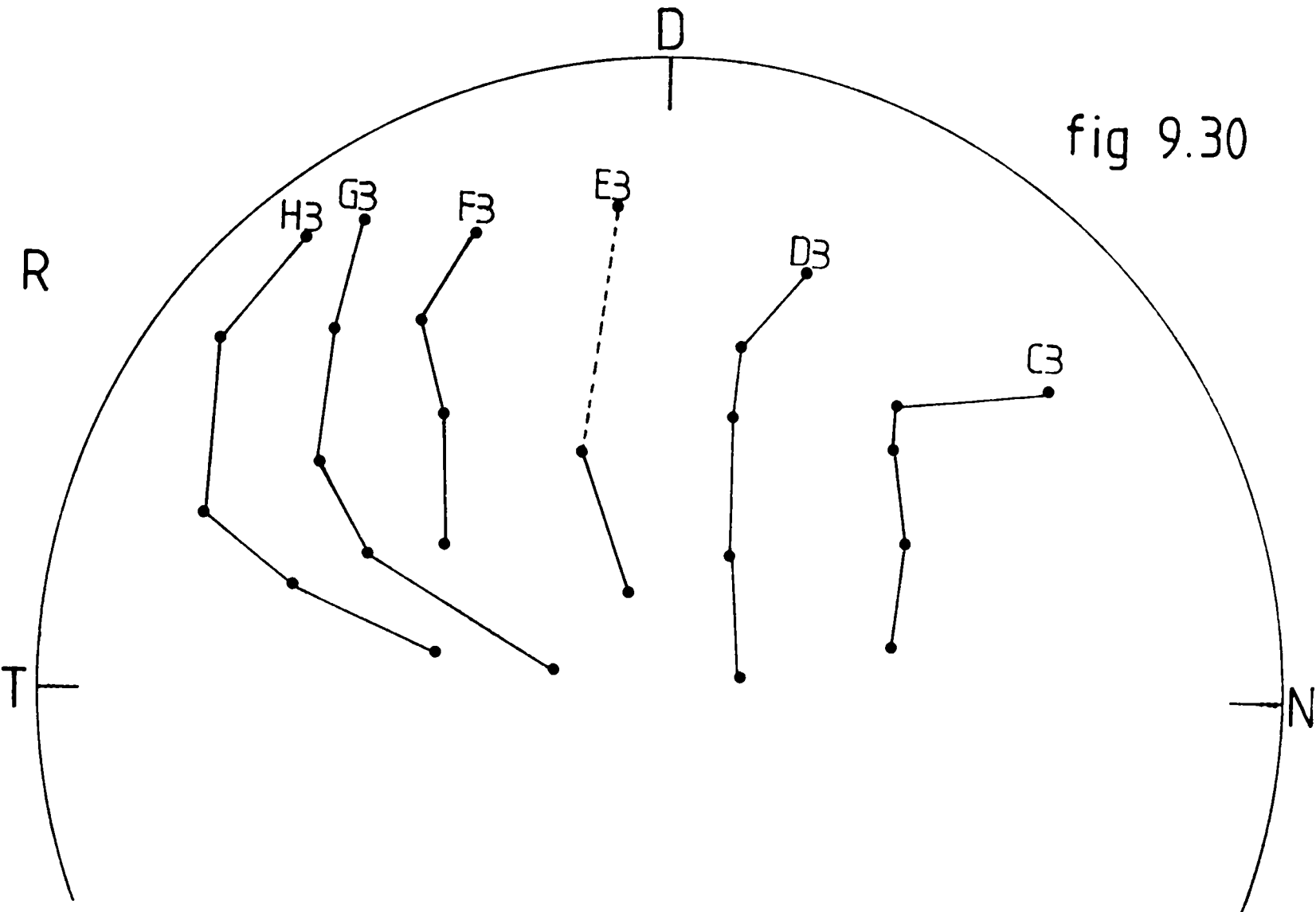


Table 9.3.

Fish	Points mapped	Correlation coefficient (Spearman rank test)	Significance (one-tailed t-test)
5.8.80	29	+0.072	N.S.
28.8.80	30	-0.086	N.S.

The pathway taken by the ipsilaterally projecting fibres was investigated in three fish by introducing HRP into the cut right optic nerve immediately after mapping. Good staining was achieved in two of these and showed fibres crossing in a band from the right optic nerve into the right optic tract ventrally under the chiasm as a large fascicle. Within the right optic tract fibres became widely scattered as small clumps and passed into both tract brachia. This is illustrated in figure 9.31 showing a camera lucida reconstruction of a fish labelled 63 days after surgery. Figure 9.32 shows a photomicrograph of the chiasmatic region of the same fish.

In two further animals ³H-proline was injected into the right eye 27 and 44 days before mapping. Immediately after mapping a small amount of HRP was injected into the caudomedial border of the right tectum. After a further 1 day survival the animals were killed and alternate 40 μ m frozen sections processed for HRP and ARG. The HRP staining showed a small discrete patch of labelled fibres occupying an appropriate position in the dorsomedial part of the medial tract brachium. However on tracing these fibres rostrally towards the eyes they became widely dispersed as bands throughout the tract. In the chiasmatic region most of these fascicles joined together to form a ventral band of fibres running into the left optic tract and then caudally towards the left tectum. Thus it appears that at least some of the fibres from the right eye which projected to the ipsilateral tectum in this series of fish did so via a pathway into the left tract and then back to the chiasm.

Fig. 9.31 Camera lucida reconstruction of the visual pathway of a WTRON + LON cut animal labelled by introduction of HRP into the cut right optic nerve 61 days after surgery.

fig 9.31

ROSTRAL

WTRON & LON cut

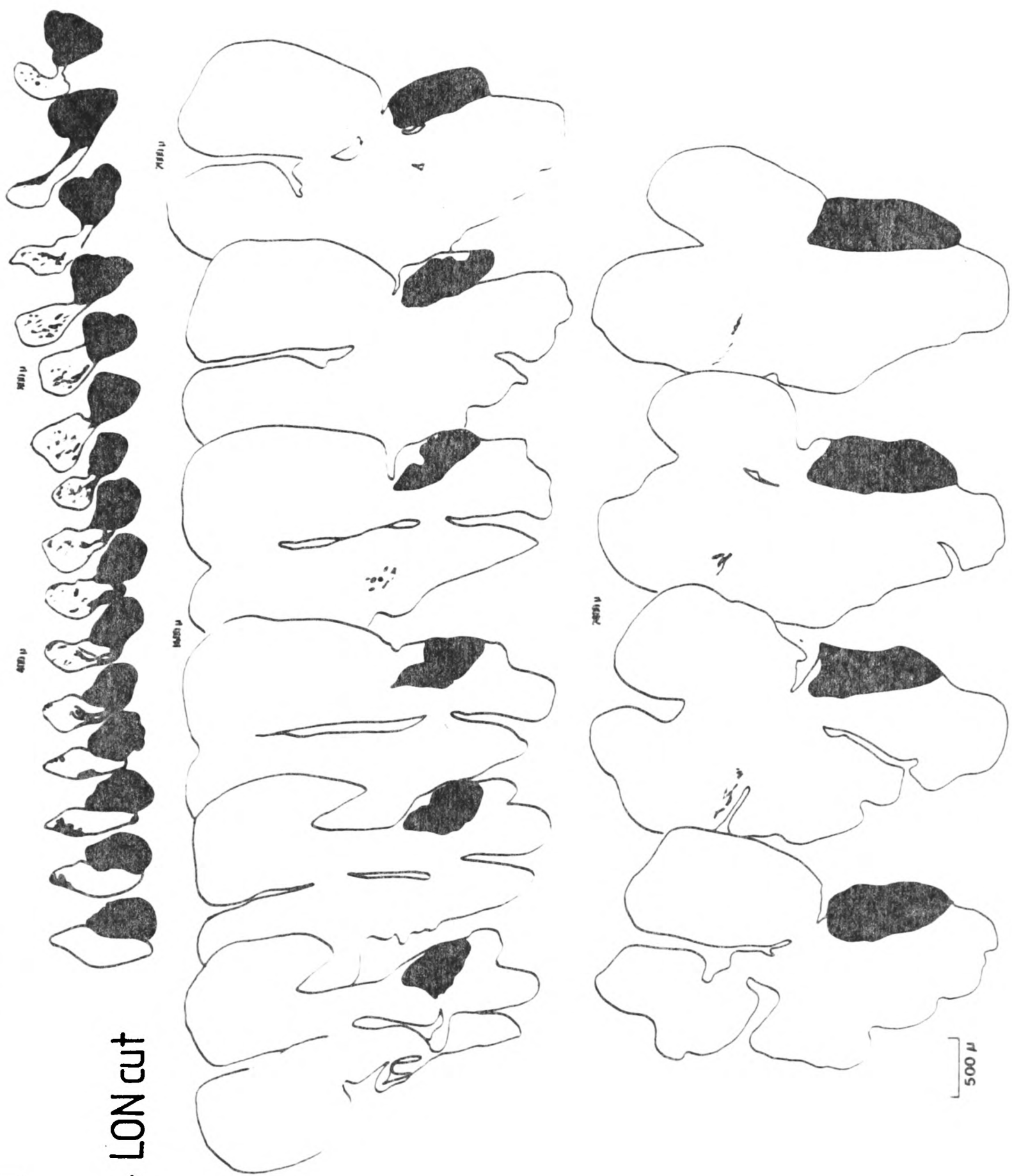
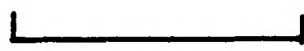
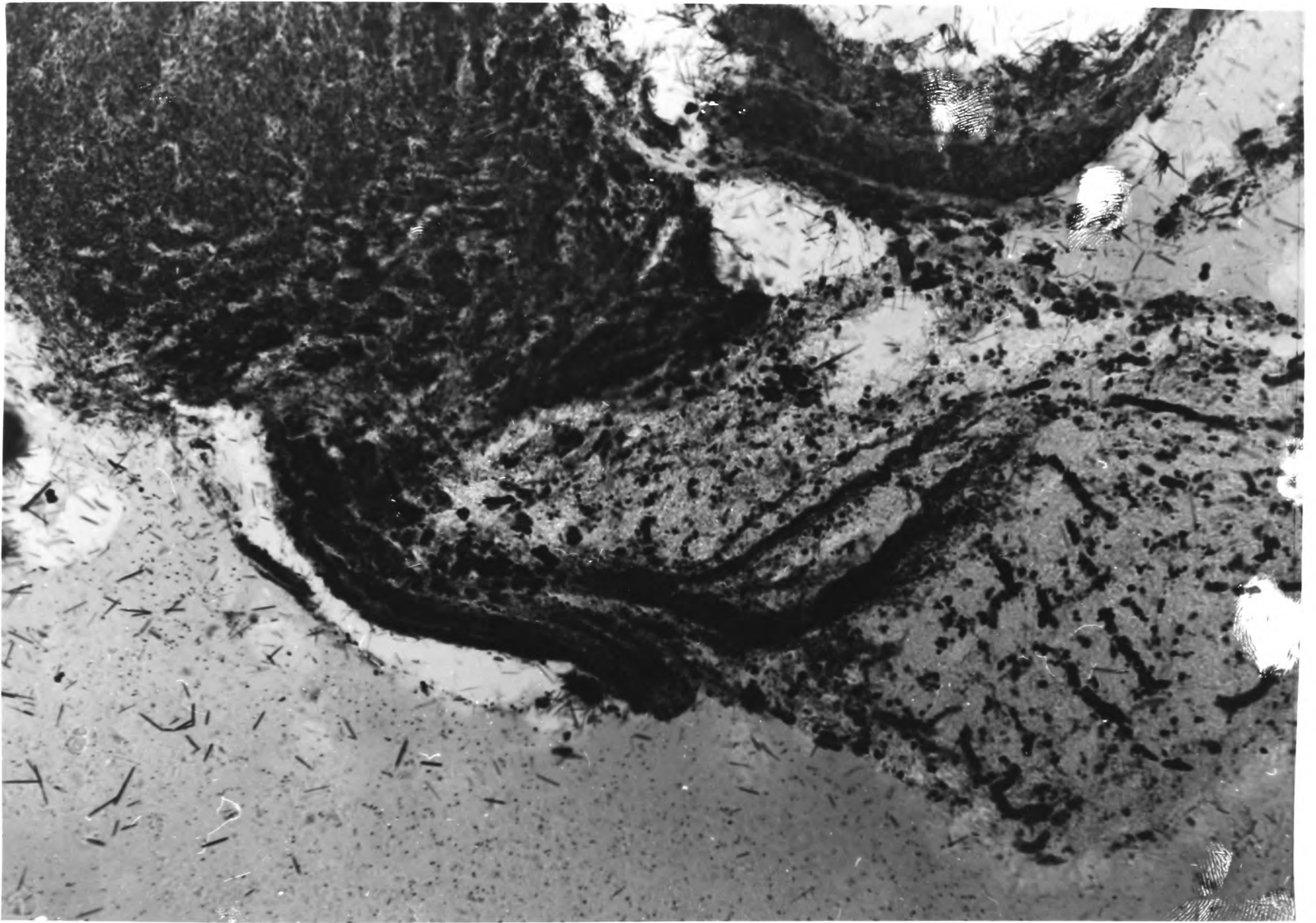


Fig. 9.32 High power photomicrograph of the chiasmatic region of a WTRON + LON cut animal labelled by introduction of HRP into the cut right optic nerve 63 days after surgery. Labelled fibres can be seen passing in a broad fascicle from the right optic nerve (on the left) into the right tract (on the right) by a ventral path under the chiasm.

fig 9.32



100 μm

Moving rostrally labelled fibres occupied a small discrete area of the left optic nerve. Thus the tectal HRP injection filled both ipsilateral and contralateral fibres which were closely related in the tract brachia.

Figure 9.33a shows a photomicrograph of the chiasmatic region of an animal labelled by tectal HRP injection 78 days after surgery.

Slow-labelling of the ipsilateral fibres by injection of ^3H -proline into the right eye of one animal 27 days before mapping gave good visualization of the fibres in the visual pathway. The appearance confirmed the findings using HRP. Fibres passed into the ipsilateral tract via a ventral path under the chiasm and penetrated that tract as discrete fascicles. However as they progressed up the tract these fascicles became divided into smaller clumps. Complete mixing of fibres from the two eyes was never achieved. The chiasm of this animal is shown in figure 9.33b.

Comparison of figures 9.33a and 9.33b, which are adjacent sections from this WTRON + LOW cut animal mapped 78 days after surgery but 9.33a is stained for HRP and 9.33b for ARG, shows that tectal injection of HRP labelled fibres from both eyes but that these fibres did not become completely mixed in the pathway. Instead they remained separated in fairly large fascicles.

7/8 + LON cut

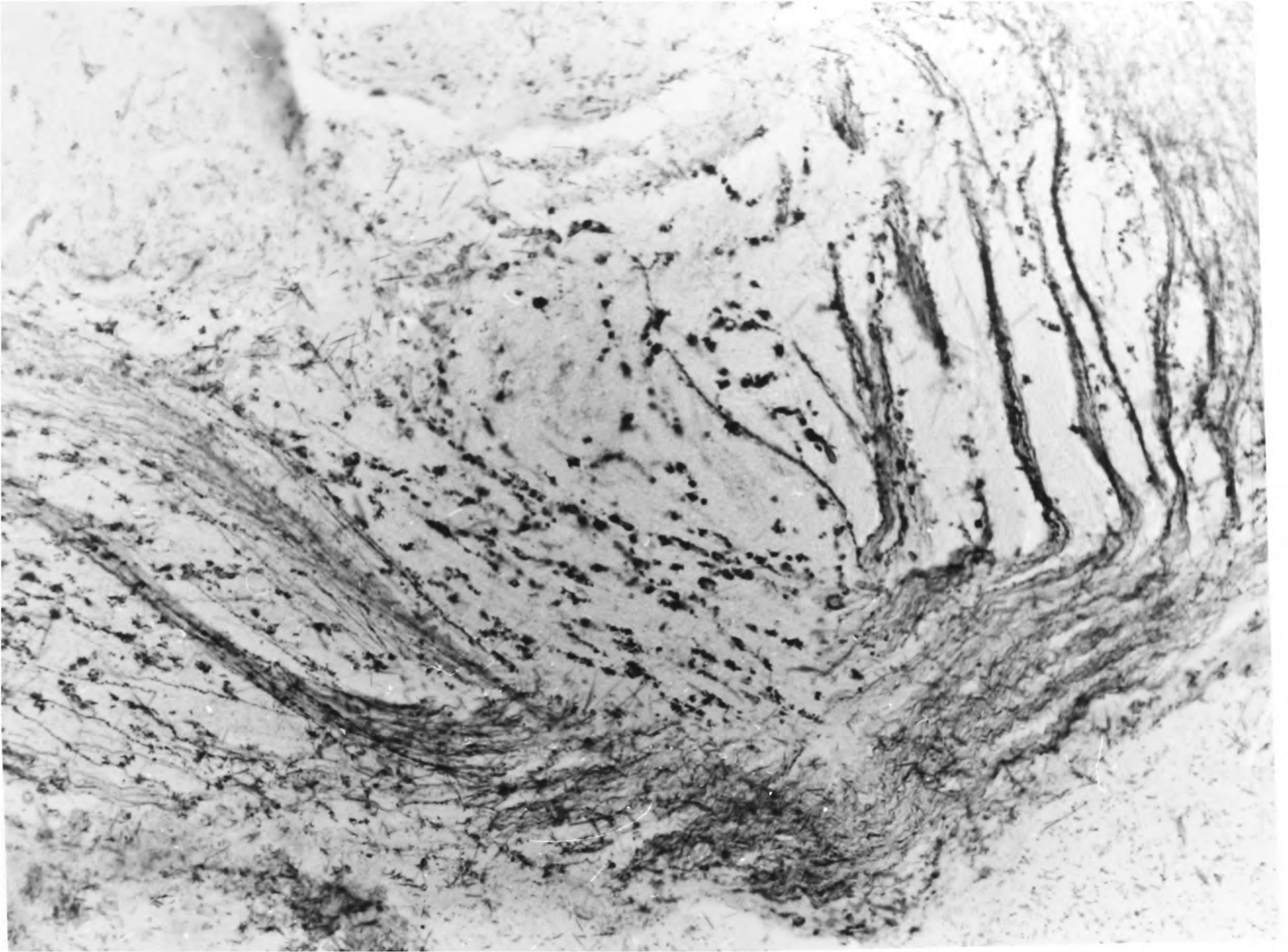
Nine animals from this series were mapped at 142-271 days after operation. In every case the projection from the right eye to the right tectum was of the appropriate polarity for that tectum and was reasonably orderly though with occasional crossings over of response lines and some non-responding tectal positions, particularly towards the rostral pole of the tectum. This latter finding was surprising in view of the rostral to caudal path of fibre ingrowth, which meant that the rostral tectum should be the first to be

Fig.9.33a High power photomicrograph of the chiasmatic region of a WTRON and LON cut animal labelled by tectal injection of HRP 78 days after surgery. Labelled fibres can be seen in the right optic tract (to the right of the picture) and passing across the midline and up into the left tract (to the left of the picture).

Fig.9.33b High power photomicrograph of the chiasmatic region of the same animal labelled by injection of ^3H -proline into the right eye 27 days before mapping. This section is adjacent to that shown above. It can thus be seen that the tectal HRP injection labelled fibres from both the ipsilateral and contralateral eye.

fig 9.33

a



b

200 μm

100 μm



encountered. However a rostral remnant of tectum remained on the left side of the brain and may have formed a target for fibres which preferred a rostral termination site, both from the right and the left eye as both fibre populations were regenerating. In all nine animals considerable variations were seen in response strengths at adjacent tectal positions, a finding highly suggestive of patching.

In seven animals the projection from the left eye to the right tectum was also mapped and was found to be of normal polarity. However, these contralateral projections were certainly not as well ordered as in the normal animal. Furthermore the contralateral projection to the right tectum was not consistently better than the ipsilateral projection in the same animal. Again marked variations in response strengths were detected. Statistical analyses of the response strengths were performed for all seven animals mapped through both eyes and the results are shown in table 9.4.

Table 9.4

Animal	Points mapped	Correlation coefficient (Spearman rank test)	Significance (one-tailed t-test)
24.6.80	33	-0.506	$p < 0.005$
1.7.80	32	-0.413	$p < 0.01$
7.7.80	33	-0.731	$p < 0.0005$
29.7.80	33	-0.779	$p < 0.0005$
4.8.80	27	-0.680	$p < 0.0005$
13.8.80	34	-0.553	$p < 0.0005$
18.8.80	33	-0.705	$p < 0.0005$

There is thus extremely strong electrophysiological evidence for an interaction between the fibres from the two eyes with the production of patches of tectum dominated by one or other eye.

Maps obtained from a typical animal are shown in figure 9.34 and the response strength plot for the same animal, mapped 216 days after surgery, is given in figure 9.35. In every animal mapped through both eyes an attempt was made, by visual inspection, to correlate the positions in the right and left visual fields of responses obtained via the two eyes at each electrode position. However in no case was a good correlation detected.

The ipsilateral pathway was investigated in five animals by introducing HRP into the cut right optic nerve immediately after mapping. In each case labelled fibres were seen to pass caudally towards the chiasm where some continued towards the left tectal remnant and others, or perhaps branches of the same fibres passed ventrally under and behind the chiasm to cross the midline over a rostrocaudal extent of up to $1200\mu\text{m}$. These labelled fibres then penetrated the ipsilateral tract where they became widely spread out, particularly within the lateral tract brachium. Tectal distribution of the fibres seemed to be widespread though the quality of staining was inadequate to allow a quantitative description of the distribution of terminals. Figure 9.36 shows a camera lucida reconstruction of the pathway of an animal labelled 229 days after surgery. Figure 9.37 shows photomicrographs of the chiasm and rostral tectum of the same animal.

In an attempt to determine the ordering of fibres within the pathway of this series of animals three fish received an injection of ^3H -proline into the right eye 25-45 days before mapping. Immediately after mapping (which took place 251-271 days after surgery) a small amount of HRP was injected into the caudomedial border of the right tectum and after 24 hours alternate $40\mu\text{m}$ frozen sections were processed for ARG and HRP. Fairly widely scattered HRP labelling was seen in the medial brachium of the right optic tract and labelled fibres crossed the midline in a wide band below the diencephalon, some passing into the left tract. More rostrally, filled fibres were evident in both optic nerves and were widely scattered - much more so than in any

Fig. 9.34 Electrophysiological maps of the visuotectal projections from the right and left eyes to the right tectum of a 7/8 + LON cut animal mapped 216 days after surgery. The map via the right eye, shown above, is corrected for an eye angle of 22° . The left eye map, below, is corrected for an eye angle of -10° . Mapping positions are indicated on the tectal diagram to the right while on the tectal diagram to the left is an ocular dominance plot indicating relative response strengths via the two eyes.

fig 9.34

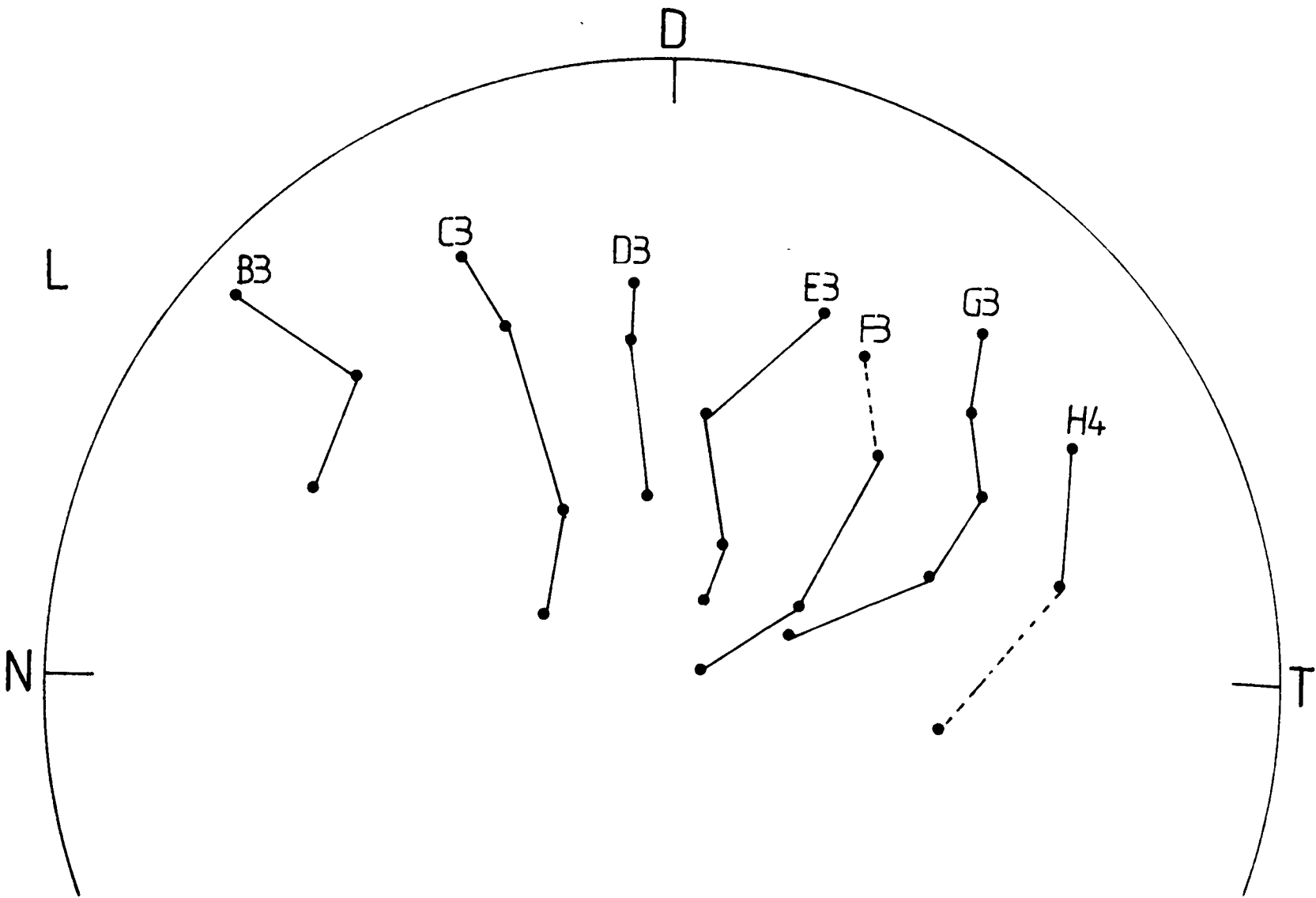
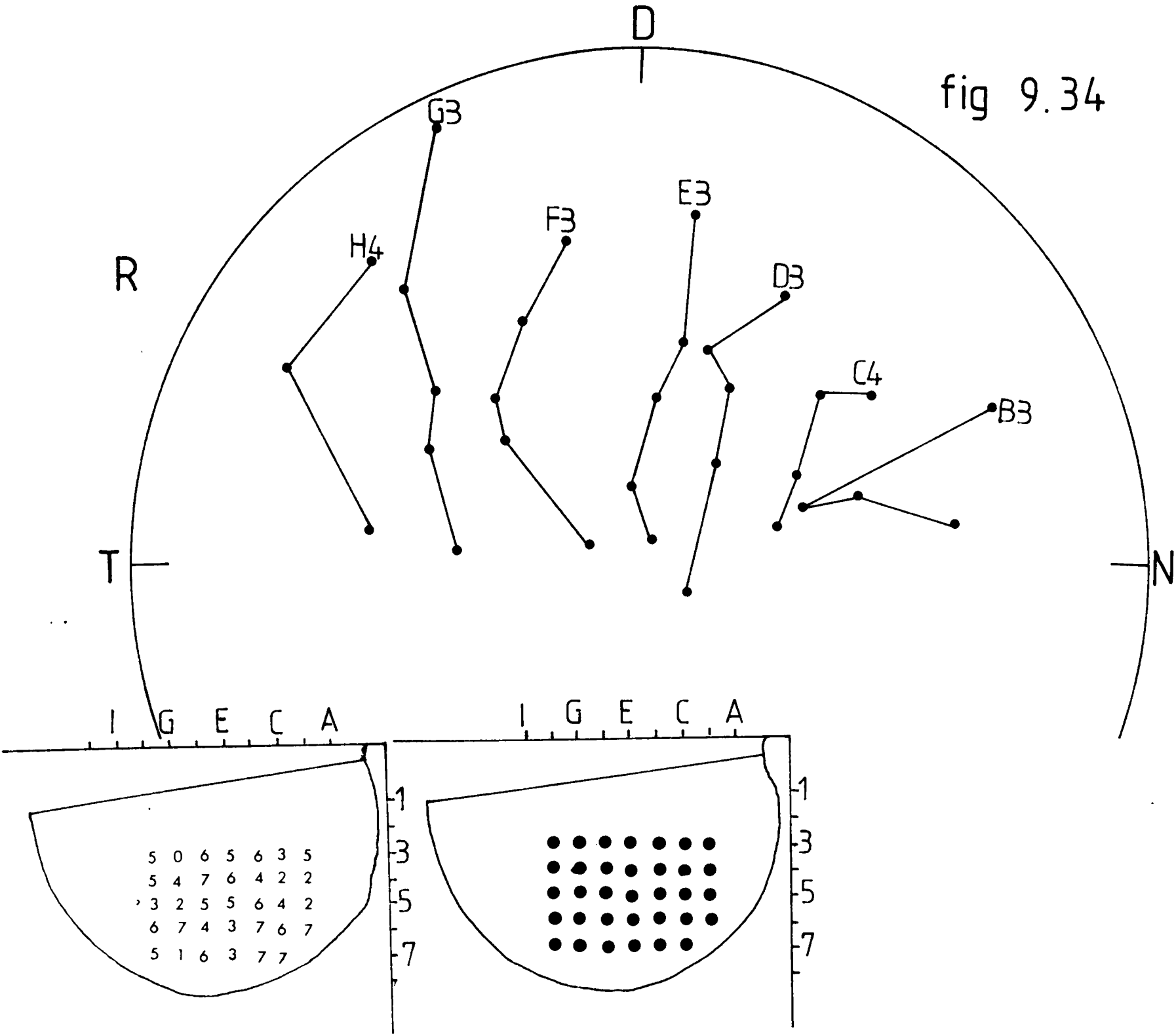
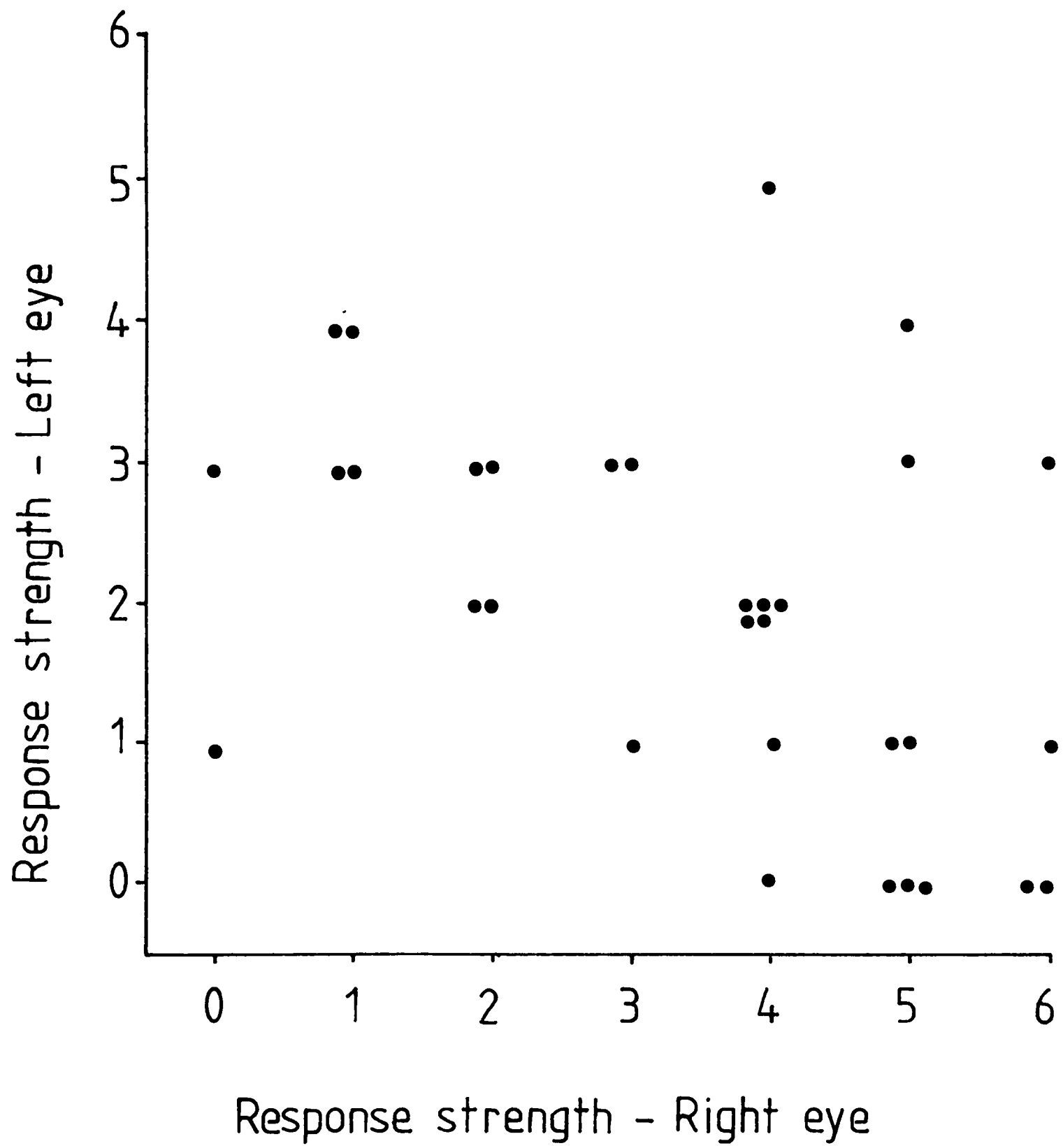


Fig. 9.35 Response strength plot for the 7/8 + LON cut animal whose maps are shown in figure 9.34. A highly significant inverse relationship between the response strengths in the two eyes was detected.

fig 9.35



$n = 32$

$R_s = -0.506$

$p < 0.005$

Fig. 9.36 Camera lucida reconstruction of the visual pathway of a 7/8 + LON cut animal labelled 229 days after surgery by introduction of HRP into the cut right optic nerve immediately after mapping. Labelled fibres can be seen crossing the midline into the right, ipsilateral optic tract, over a large rostrocaudal extent in the chiasmatic and retrochiasmatic region.

ROSTRAL

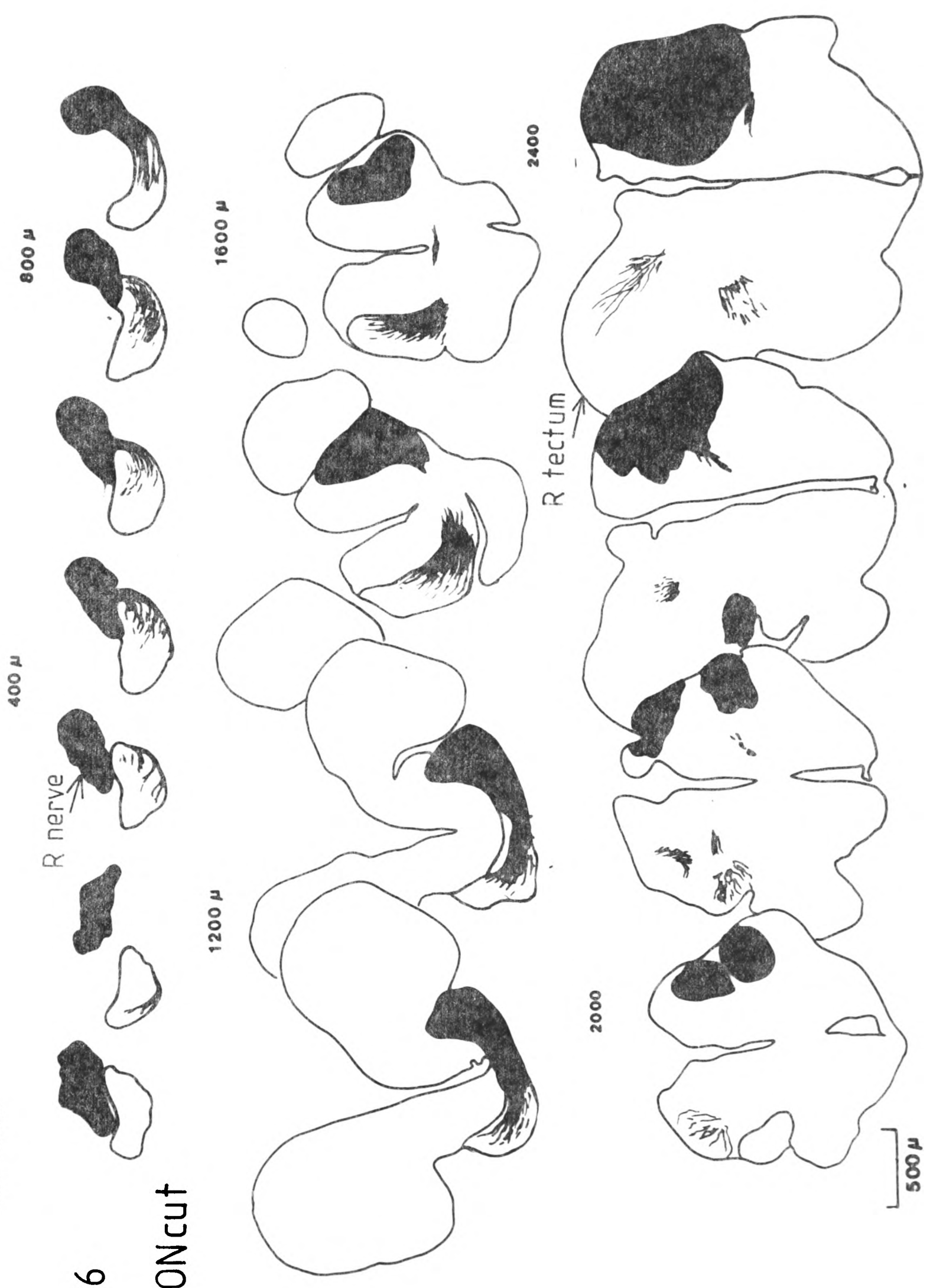


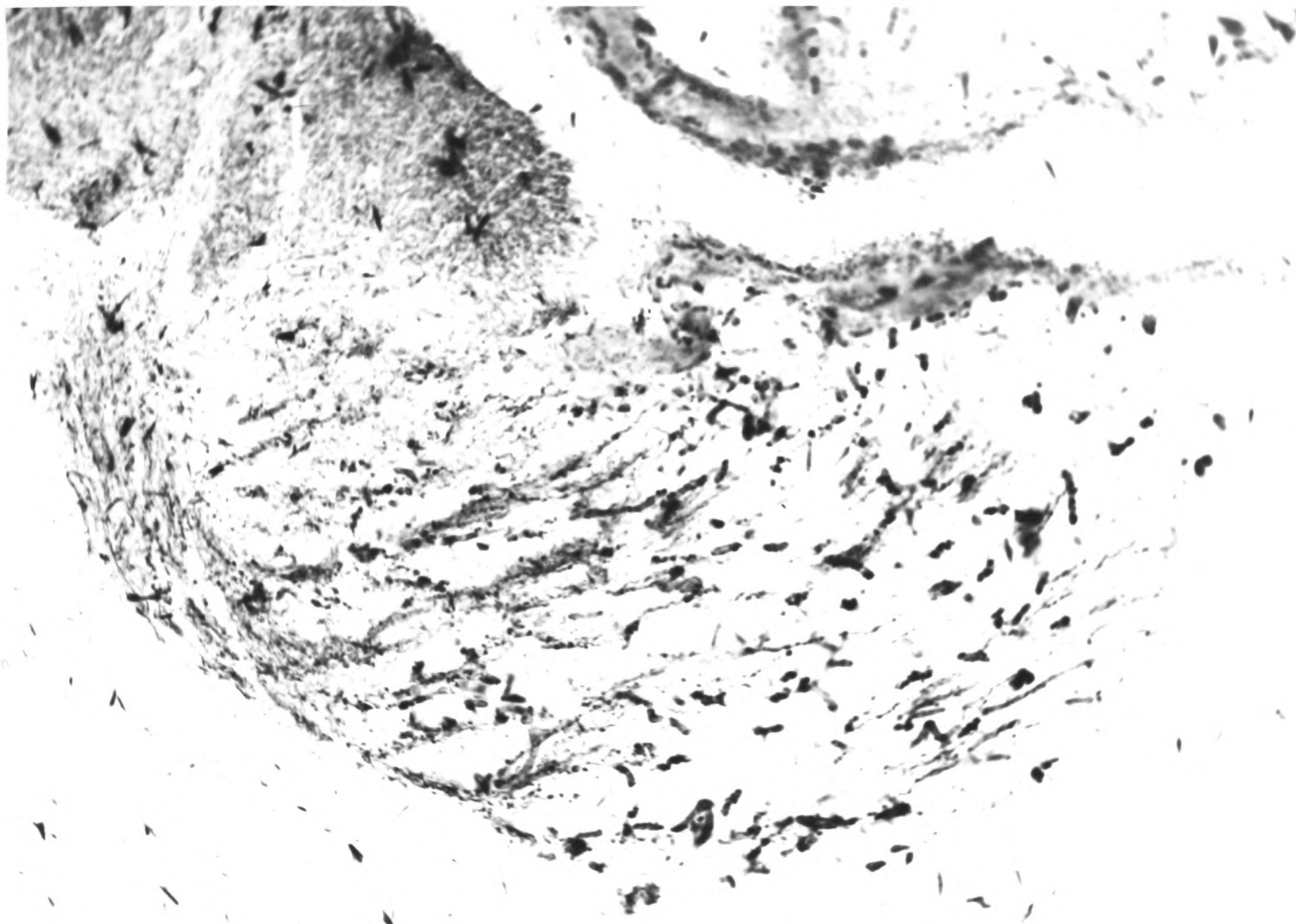
fig 9.36
7/8 & LONcut

Fig.9.37a High power photomicrograph of the chiasmatic region of a 7/8 + LON cut animal labelled 229 days after surgery by HRP introduction into the right optic nerve. Labelled fibres can be seen spreading throughout the right optic tract (to the right in the photograph).

Fig.9.37b Low power photomicrograph of the rostral tectal pole of the same animal. Labelled fibres can be seen spreading out across the right tectum from the tract brachia. The right tectum is on the right of this photomicrograph.

fig 9.37

a



b

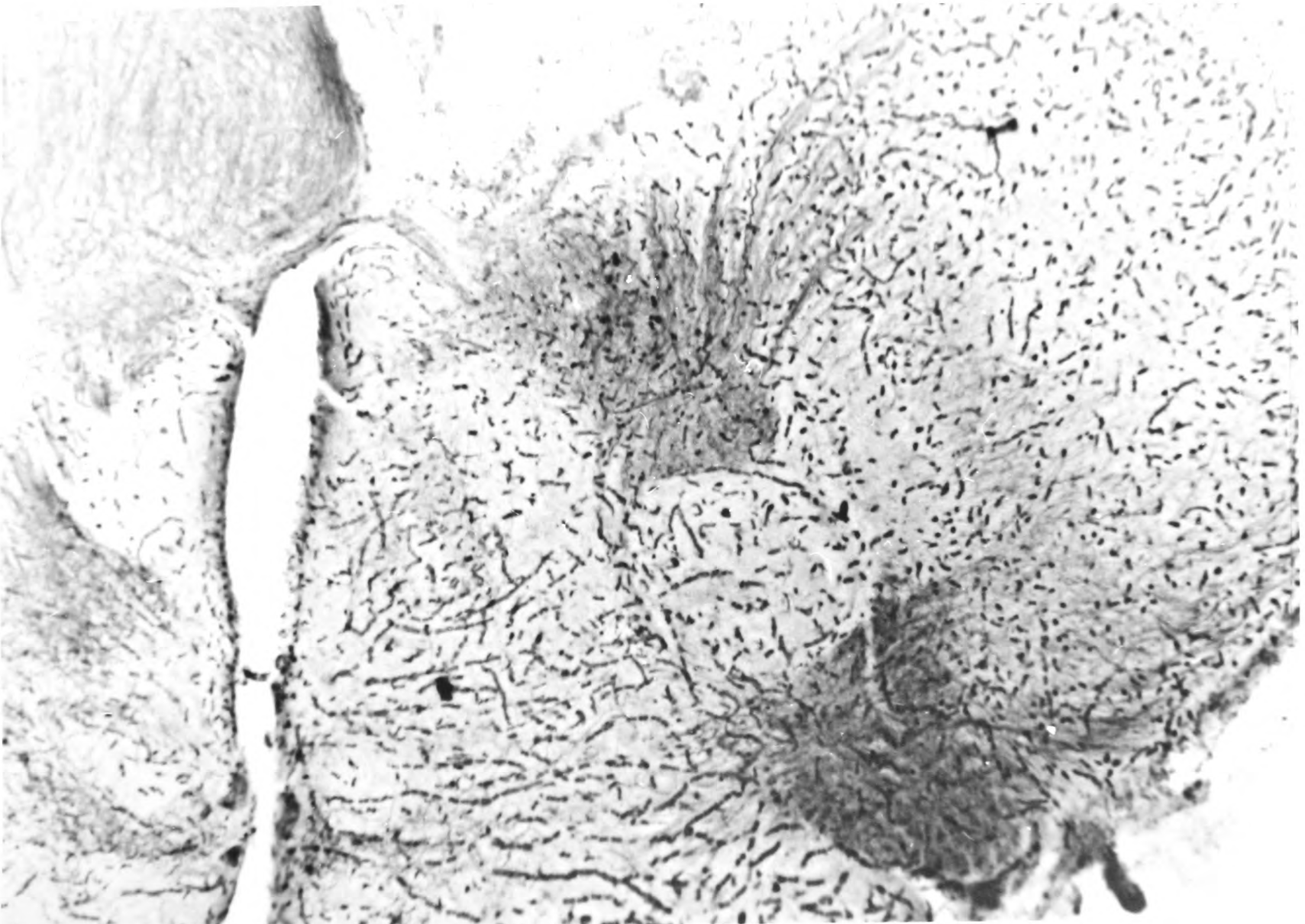
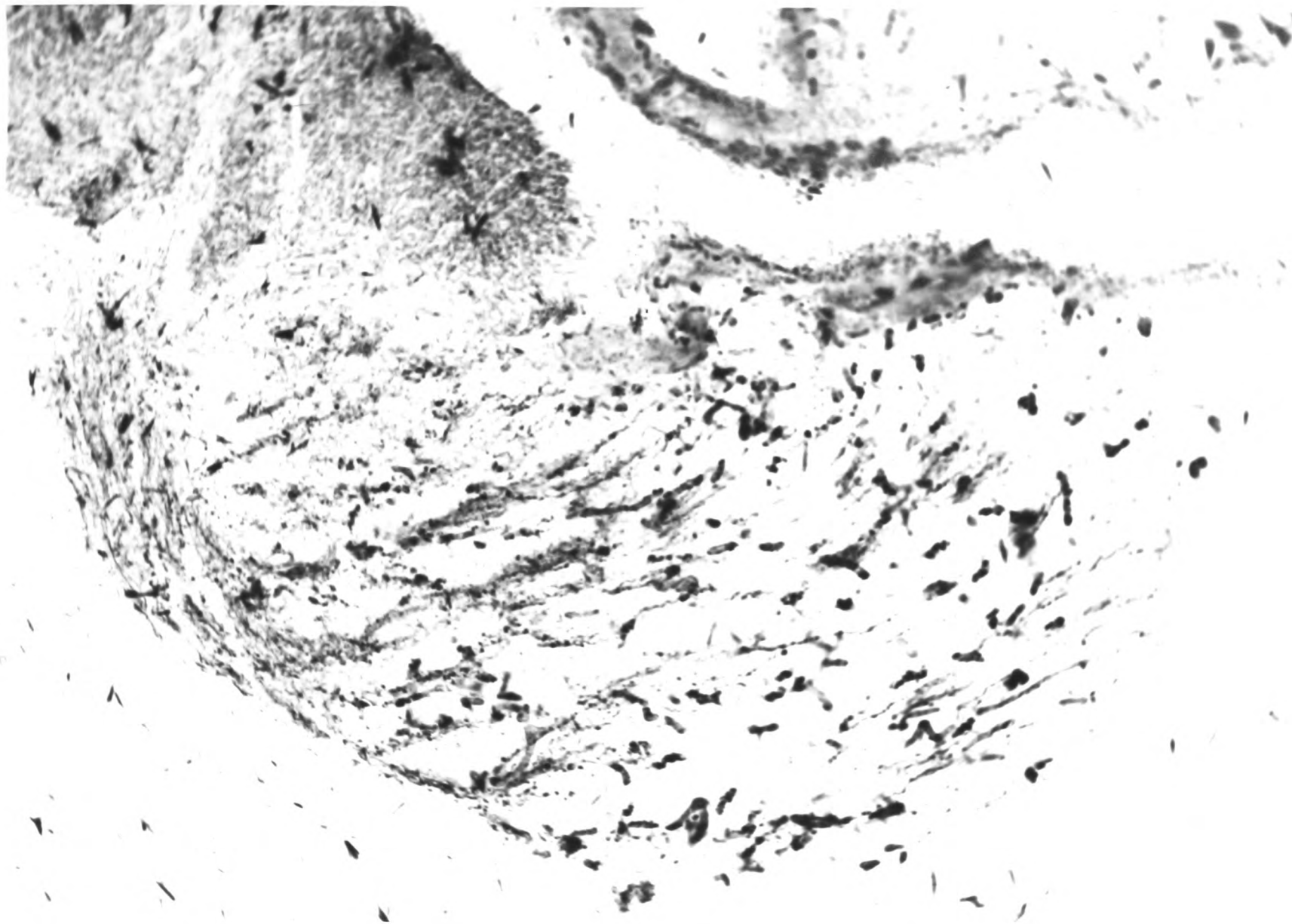


Fig.9.37a High power photomicrograph of the chiasmatic region of a 7/8 + LON cut animal labelled 229 days after surgery by HRP introduction into the right optic nerve. Labelled fibres can be seen spreading throughout the right optic tract (to the right in the photograph).

Fig.9.37b Low power photomicrograph of the rostral tectal pole of the same animal. Labelled fibres can be seen spreading out across the right tectum from the tract brachia. The right tectum is on the right of this photomicrograph.

fig 9.37

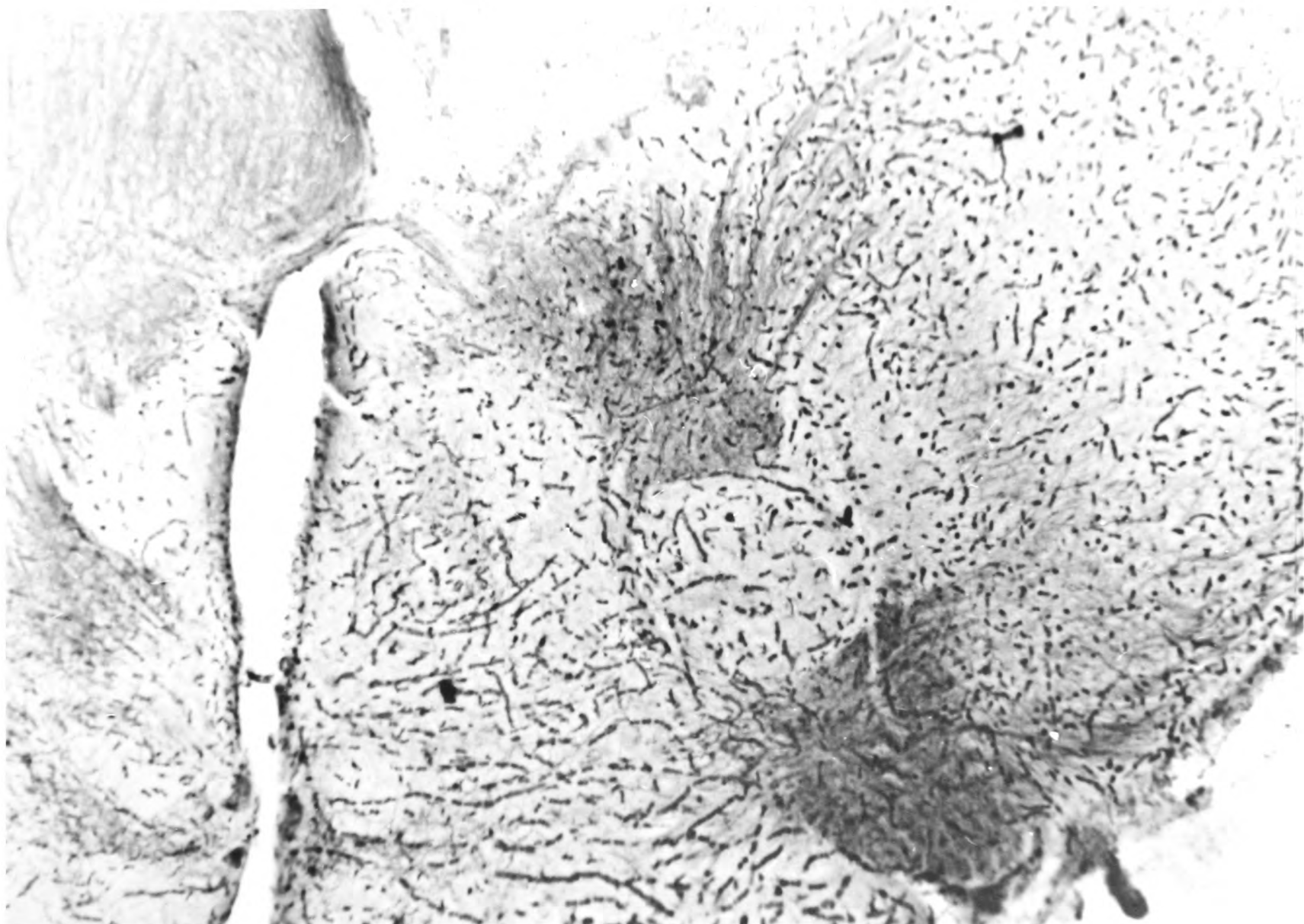
a



b

100 μ m

200 μ m



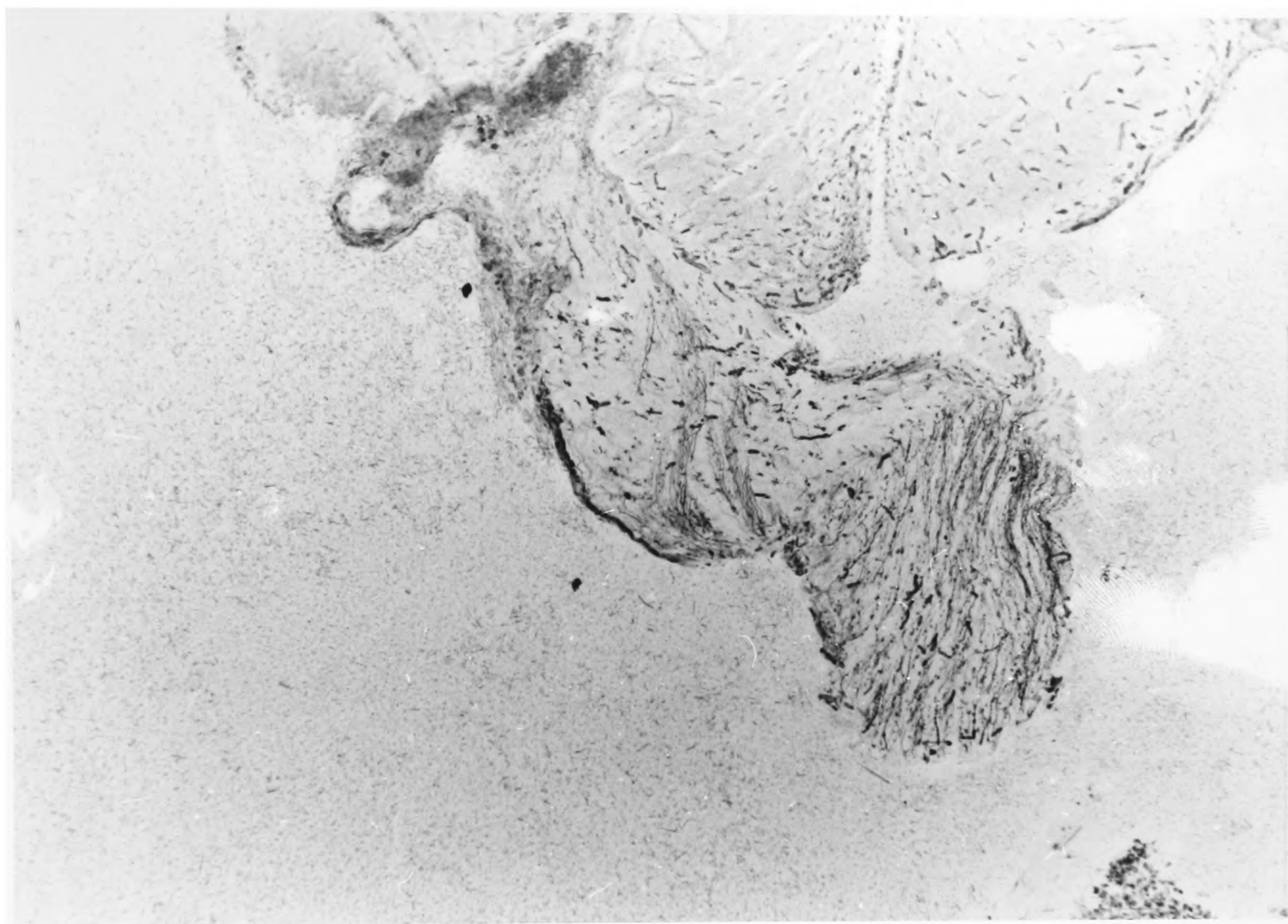
other group of fish labelled by tectal injection of HRP. This HRP labelling is illustrated in figure 9.38. The autoradiography was generally unsatisfactory though the silver grains did seem to be evenly distributed throughout the ipsilateral tract, suggesting that fibres from the two eyes were poorly segregated.

In contrast good quality autoradiographs were obtained of one 7/8 + LON cut animal which received a ^3H -proline injection into the right eye 266 days after surgery. This animal was processed for wax sections 1 day later. In the tectum marked variations of labelling intensity were seen in the optic fibre terminal layers, providing anatomical support for the electrophysiological finding of patching (figure 9.39).

Dr. J. Cook has reconstructed the tectal surface of this animal from camera lucida tracings of the $10\mu\text{m}$ sections and has rated the radioactive labelling intensity at $50\mu\text{m}$ intervals across the tectum using a five point scale. He then matched this reconstruction to the mapping positions using the tectal photograph and attempted to correlate the labelling intensity directly with the response strength via the right eye and inversely with the response strength via the left eye. For the right eye $R_s = +0.717$ (Spearman rank correlation test) $p \leq 0.0005$ (one tailed t-test). For the left eye $R_s = -0.424$ (Spearman rank correlation test) $p < 0.01$ (one tailed t-test). This provides strong evidence of an anatomical basis for the electrophysiologically detected patching in contrast with the findings of Levine and Jacobson (1975).

Fig. 9.38 Low power photomicrographs of the chiasmatic (a) and retro-chiasmatic region (b), of a 7/8 + LON cut animal labelled by tectal injection of HRP 257 days after surgery. Labelled fibres can be seen to be widely distributed in the right optic tract (to the right of the photographs), though with a bias towards the dorsal edge. Some labelled fibres cross the midline into the left tract and can be seen passing caudally. (b) is taken 240 μ m caudal to (a).

a



500 μ m

b

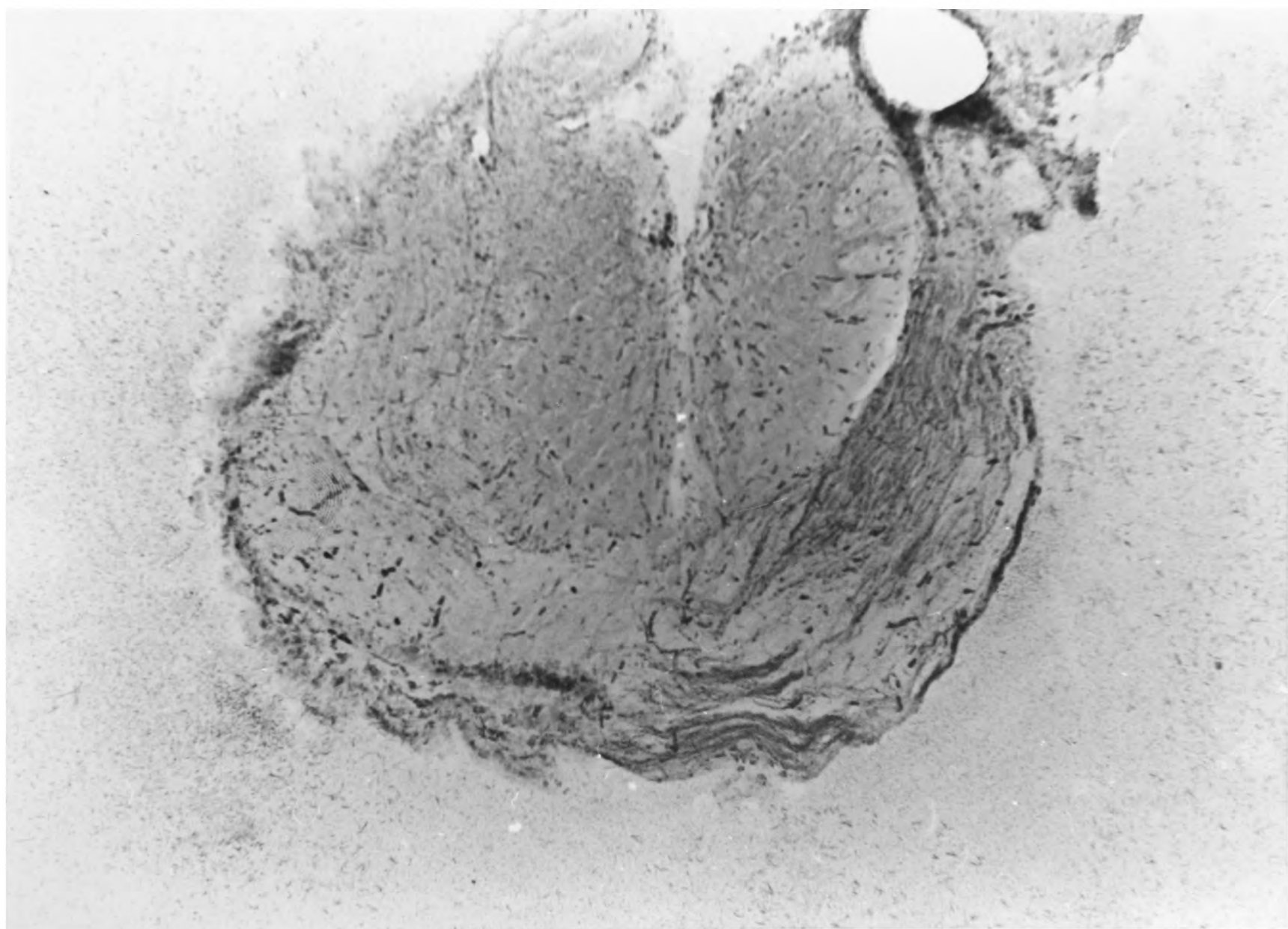
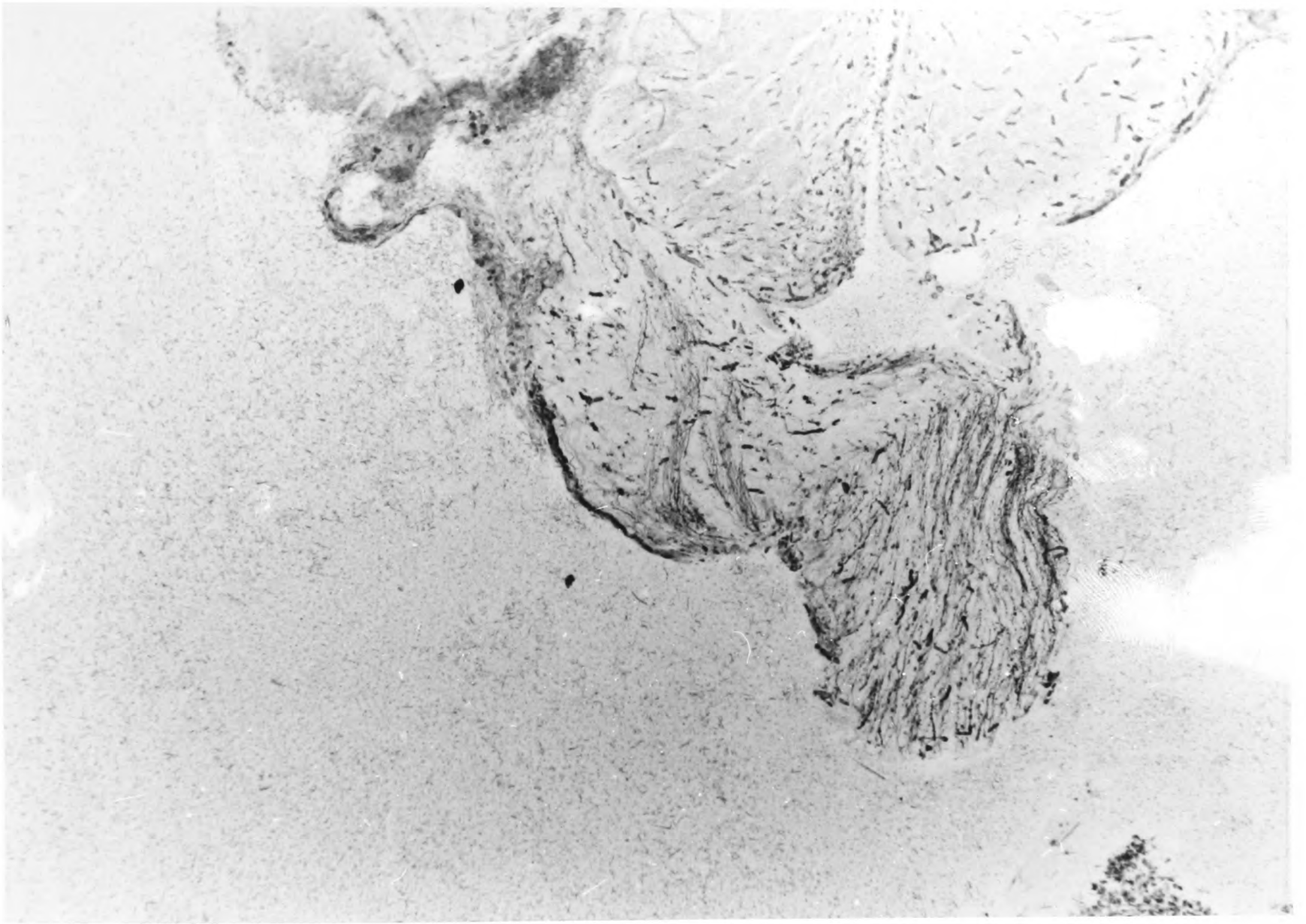


Fig. 9.38 Low power photomicrographs of the chiasmatic (a) and retro-chiasmatic region (b), of a 7/8 + LON cut animal labelled by tectal injection of HRP 257 days after surgery. Labelled fibres can be seen to be widely distributed in the right optic tract (to the right of the photographs), though with a bias towards the dorsal edge. Some labelled fibres cross the midline into the left tract and can be seen passing caudally. (b) is taken 240 μ m caudal to (a).

fig 9.38

a



500 μm

b

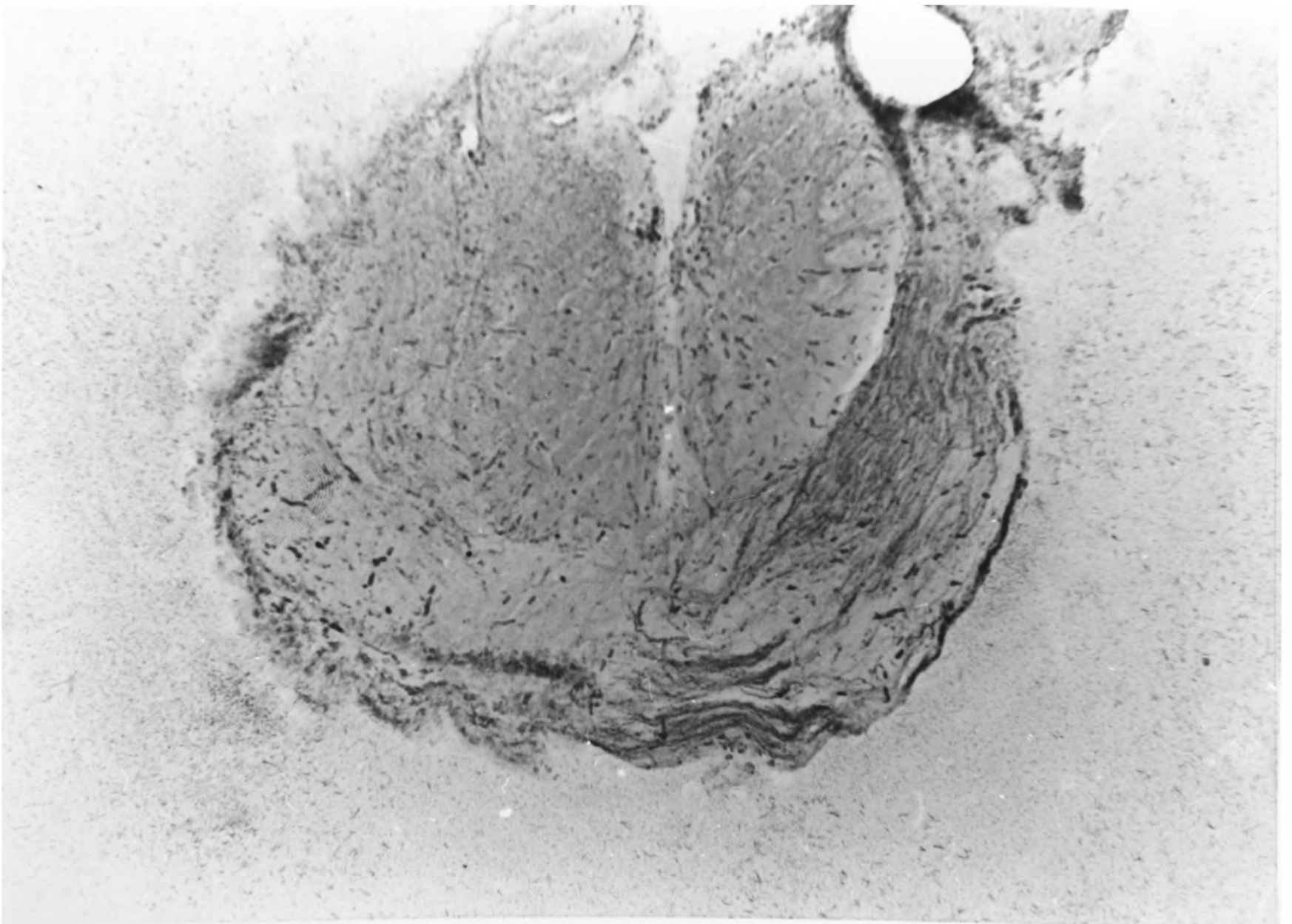
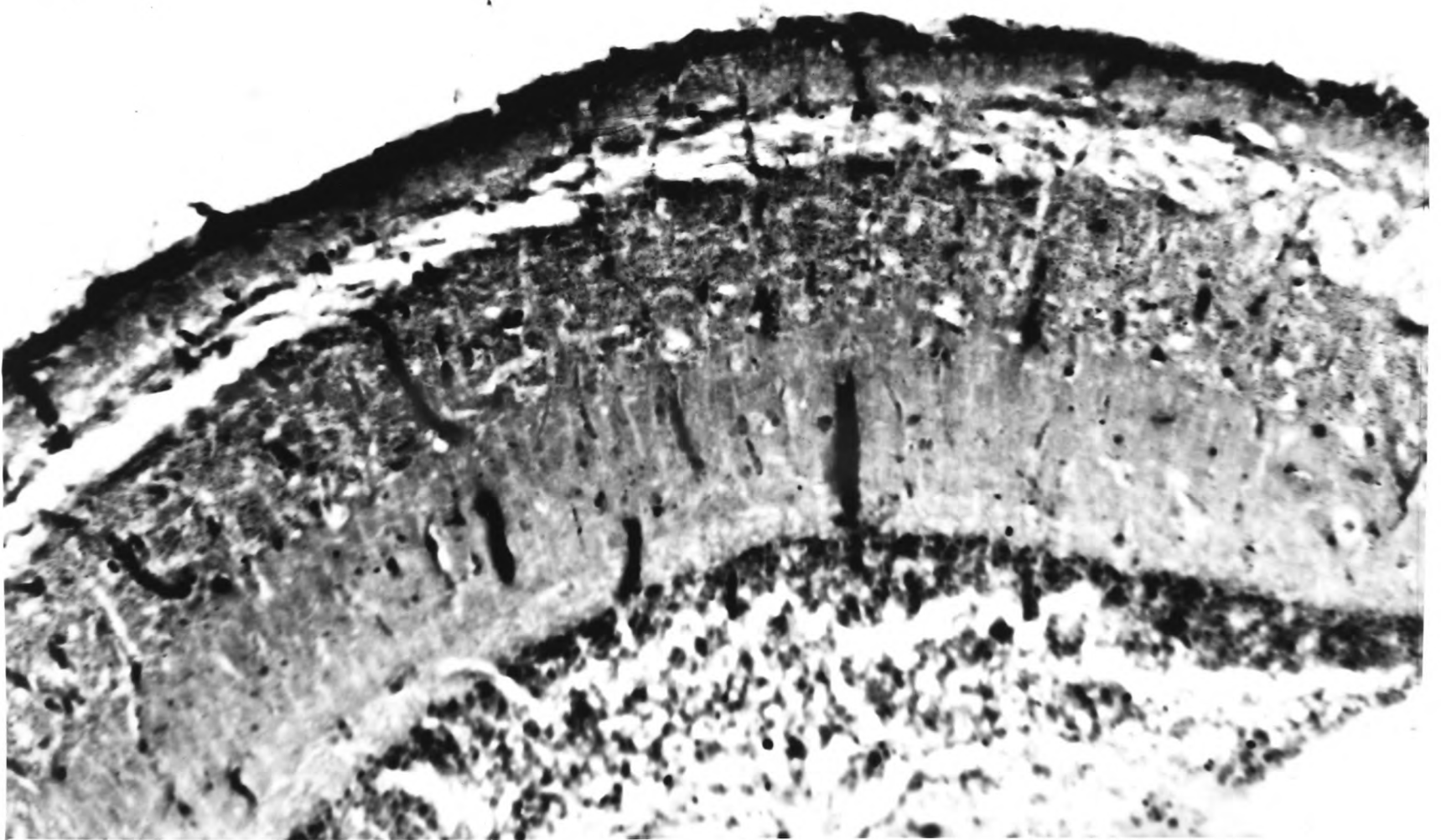


Fig. 9.39 Photomicrograph of part of the right tectum of a 7/8 and LON cut animal labelled by injection of ^3H -proline into the right eye one day before sacrifice at 266 days after surgery. Marked variations in the density of labelling across the tectal extent were noted. These variations are, however, difficult to see in photomicrographs because of the small size of the silver grains.

fig 9.39



100μm

8/8 and LON cut

Five animals from this series were mapped electrophysiologically at 64-71 days after surgery. Of these two gave no responses to stimulation of the left eye and in at least one of these extensive retinal degeneration had occurred. The right eye to right tectum projections of these animals were discounted because of the probable absence of a regenerating projection from the left eye. In the remaining three animals the projections from the left and right eyes to the right tectum were of appropriate polarity and were moderately well ordered with frequent distortions and occasional crossings over of the response lines. They represented the majority of the dorsal visual field and there was no clear tendency for the right eye projection to be less well ordered or less extensive than that from the left eye. The maps obtained from a typical animal 65 days after surgery are shown in figure 9.40.

As with the 7/8 and LON cut series the wide variation in response strengths was highly suggestive of patching and a correlation between the two eyes was sought using statistical methods. The results of this analysis are shown in table 9.5. A response strength plot from a typical animal, the same animal illustrated in figure 9.40, is shown in figure 9.41.

Table 9.5

Animal	Points mapped	Correlation coefficient (Spearman rank test)	Significance (one tailed t-test)
21.8.80	33	-0.712	p < 0.0005
26.8.80	29	-0.251	p < 0.1
27.8.80	28	-0.599	p < 0.0005

Thus as with the 7/8 + LON cut group there is strong electrophysiological evidence for patching.

Fig. 9.40 Electrophysiological map of the visuotectal projections from the two eyes of an 8/8 + LON cut animal mapped 65 days after surgery. The right eye map shown above is corrected for an eye angle of 15° and the left eye map below, for -5° . Electrode positions are shown on the tectal diagram to the left while on the right is an ocular dominance plot.

fig 9.40

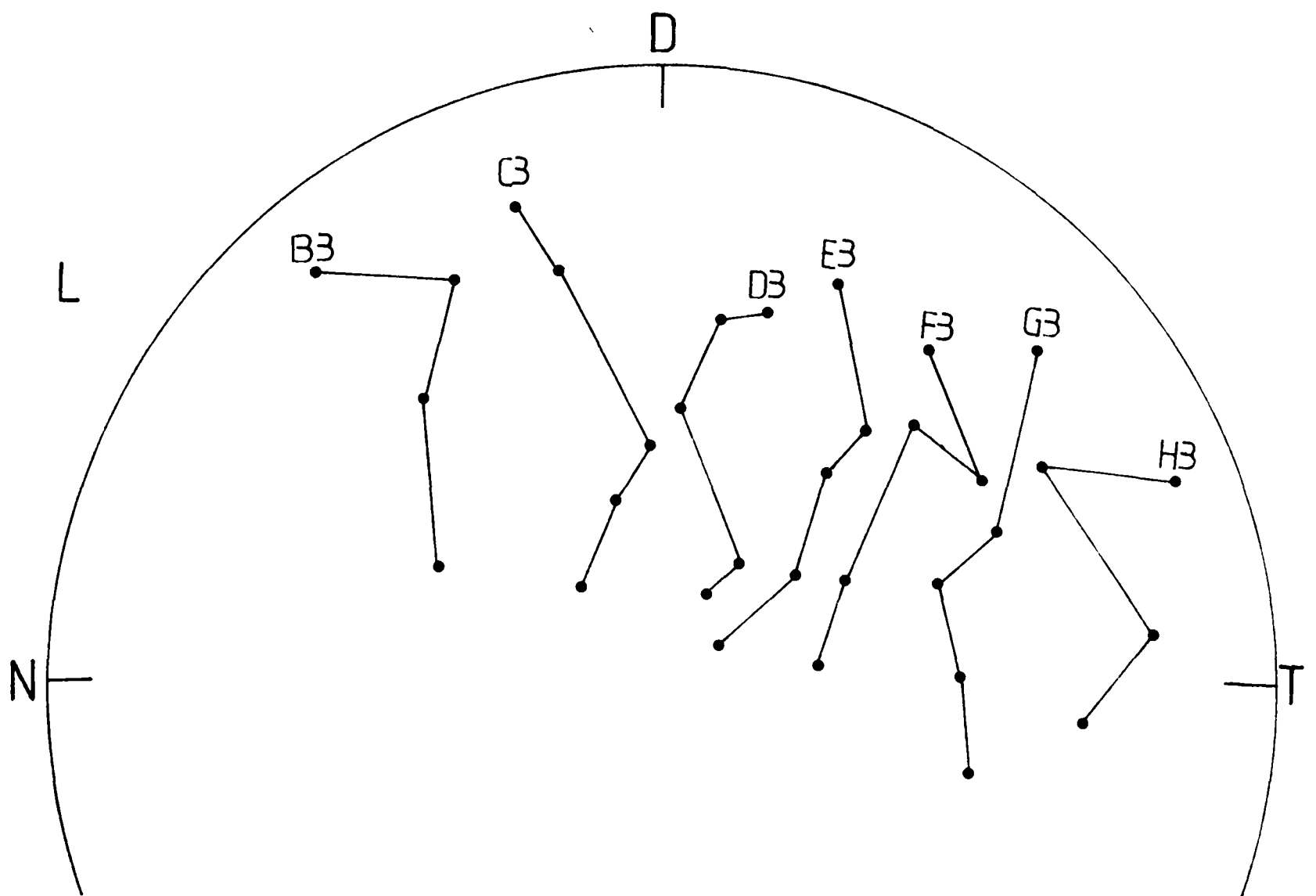
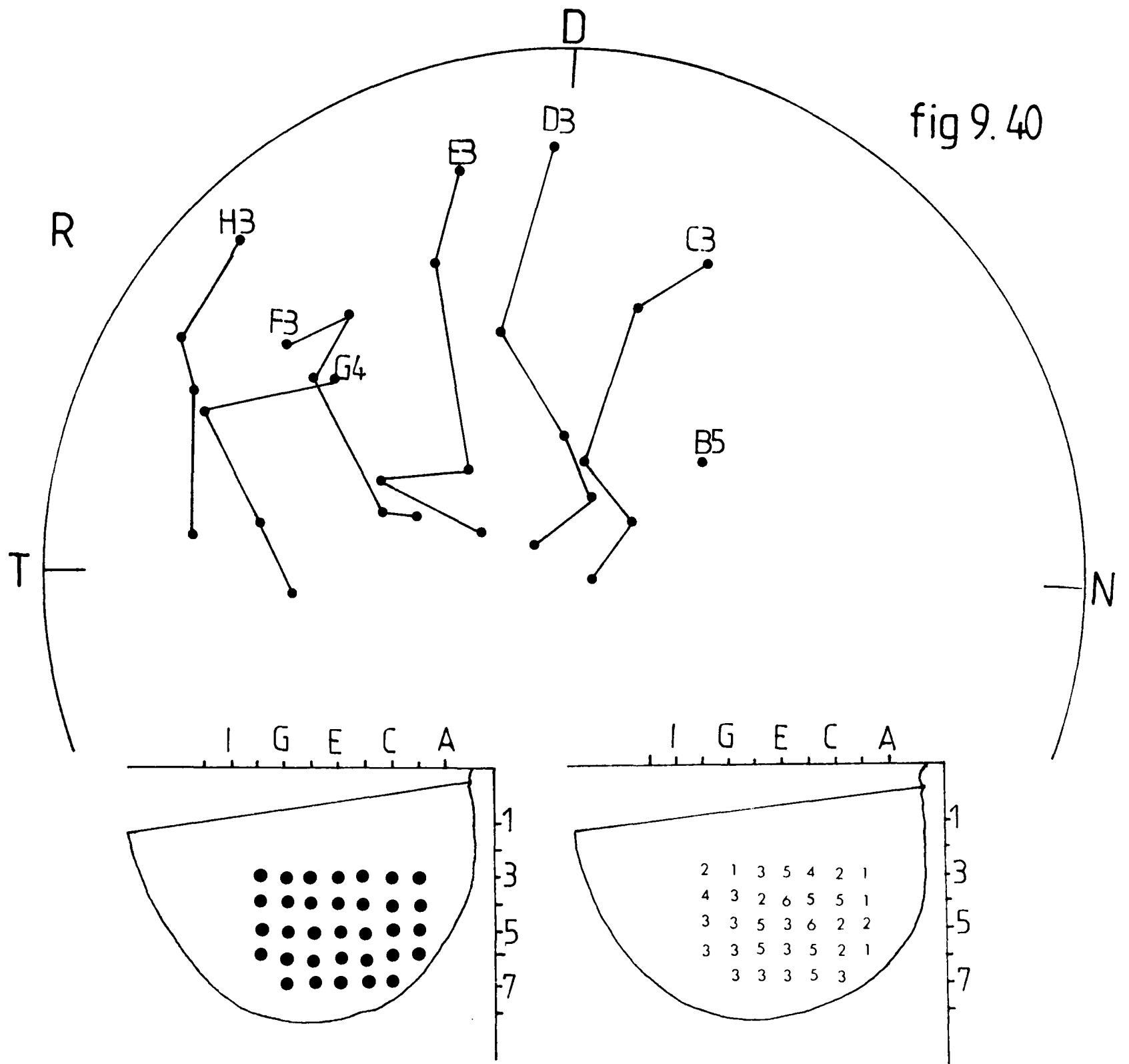
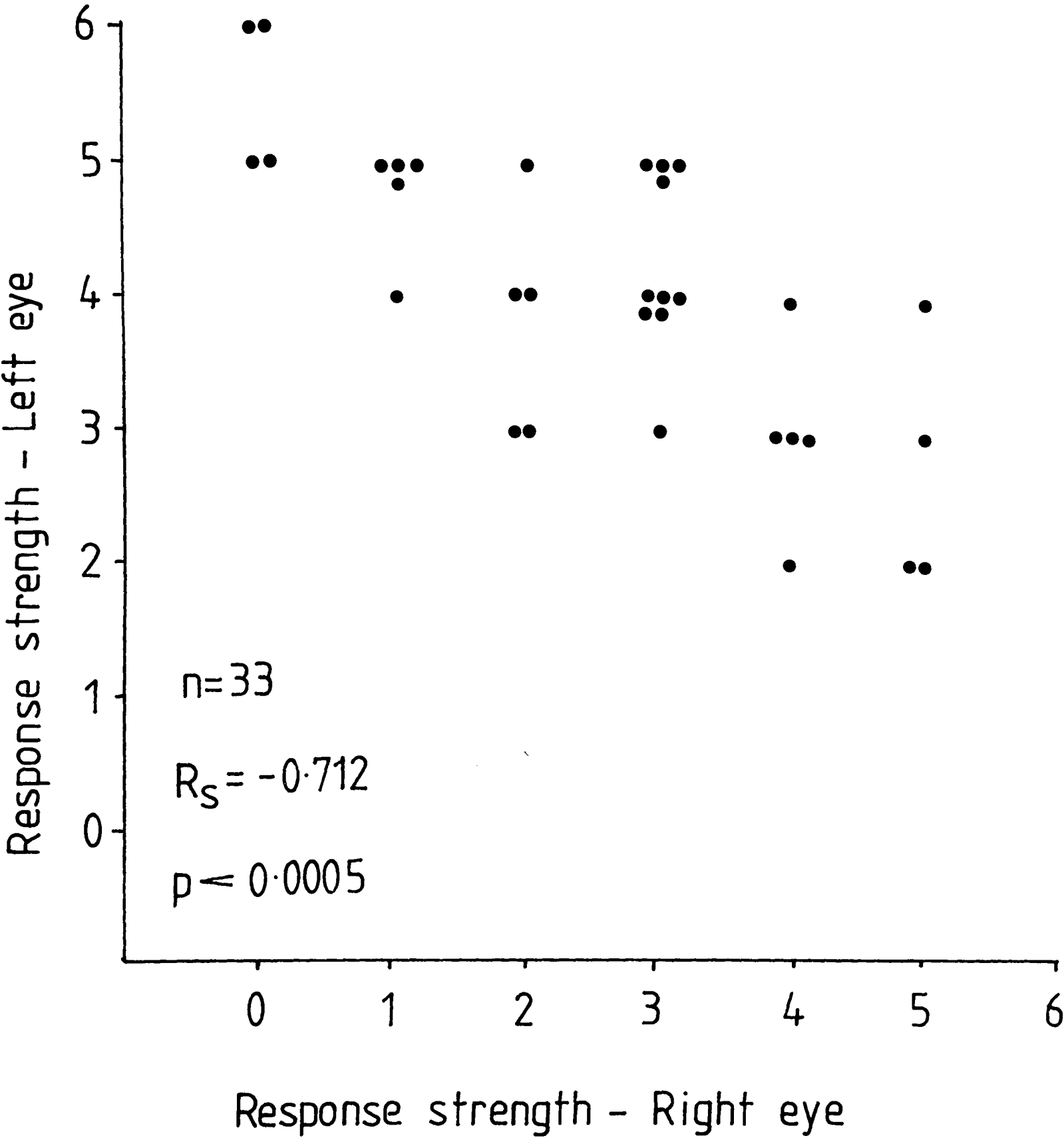


Fig. 9.41 Response strength plot for an 8/8 + LON cut animal mapped 65 days after surgery. The maps obtained from this animal are shown in figure 9.40. The response strengths from the two eyes showed a highly significant negative correlation ($R_s = -0.712$ using the Spearman rank correlation test. $p \leq 0.0005$ using a one-tailed t-test).

fig 9. 41



Filling the right optic nerve with HRP immediately after mapping in two animals revealed that the majority of labelled fibres passed into the left optic tract and up into the area of brain underlying the ablated tectum. However a few fibres were seen to cross into the right optic tract by either a ventral path under the chiasm or in the retrochiasmatic region in the vicinity of the transverse and minor commissures. Small groups of fibres were then seen to be widely scattered throughout the right optic tract, though with a particular concentration ventromedially, and to pass on to the tectum via both tract brachia. This is illustrated in the photomicrographs of figure 9.42.

In one other animal the left nerve was filled with HRP after mapping but in neither this nor the above two animals was the quality of tectal staining high enough to allow the distribution of labelled terminals to be mapped out.

Two animals received intraocular injections of ^3H -proline, one into the right eye and one into the left eye, 70 and 71 days after surgery respectively, in order to investigate the distribution of tectal terminals. These animals were processed for autoradiography one day after injection of label. In both cases the density of radioactive labelling in the optic fibre layers of the tectum showed marked variations across the tectal extent supporting the electrophysiological finding of patching. Reconstruction of the tectum was not however attempted as insufficient sections were of an adequate quality.

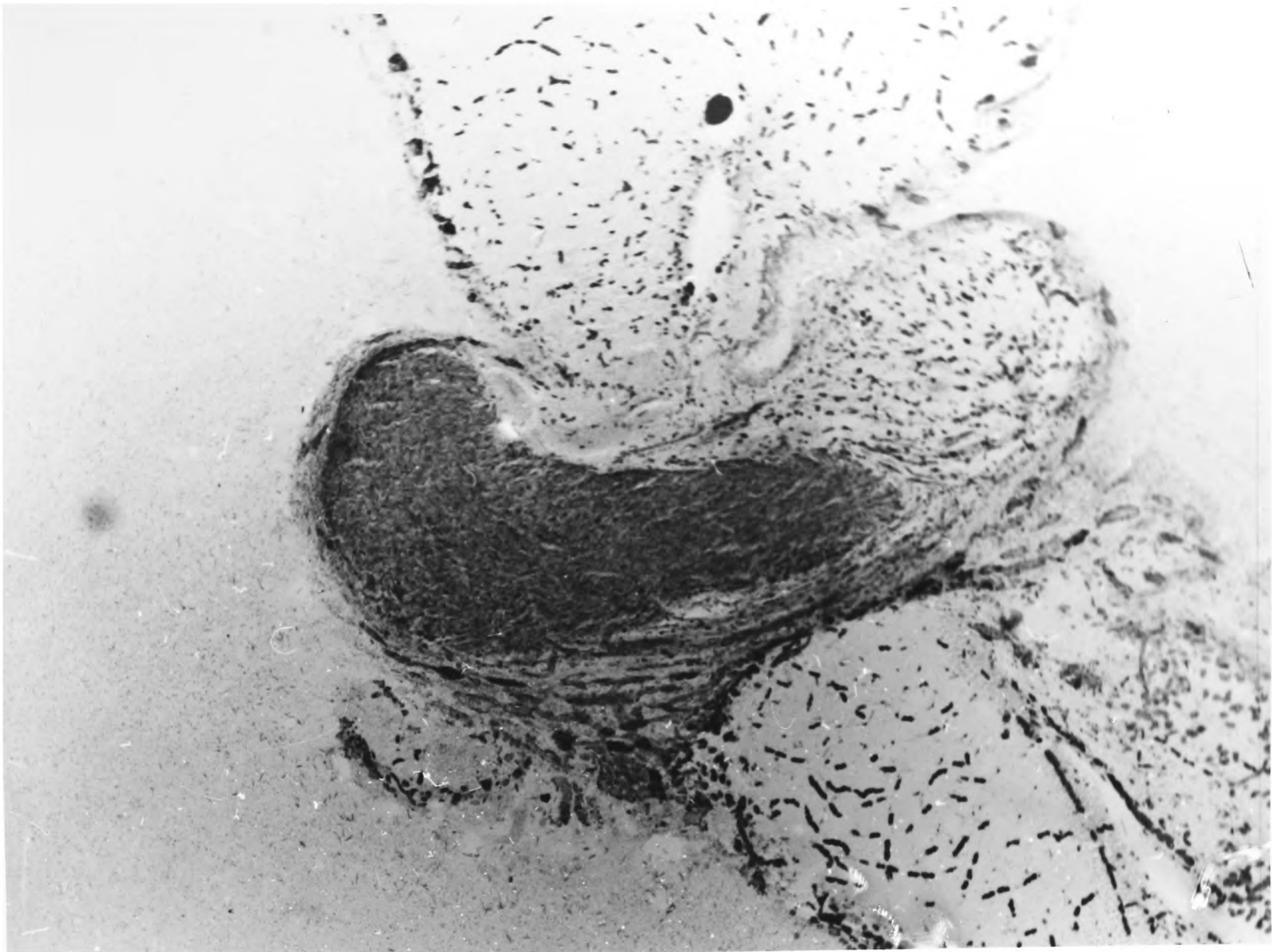
Slow labelling of the visual pathway of one animal by injection of ^3H -proline into the right eye 49 days before mapping (at 69 days after surgery) confirmed the HRP finding of widely scattered fibres from the right eye within the ipsilateral optic tract.

Tectal injection of HRP in order to investigate ordering within the visual pathway of this series of animals was unsuccessful in the one fish in which it was attempted.

Fig. 9.42 Low power photomicrographs of the visual pathway of an 8/8 + LON cut animal labelled 65 days after surgery. The right optic tract is shown at the right of each photograph. The sections are taken at 240μ m intervals from the chiasm (a) caudally to the tract brachia (d).

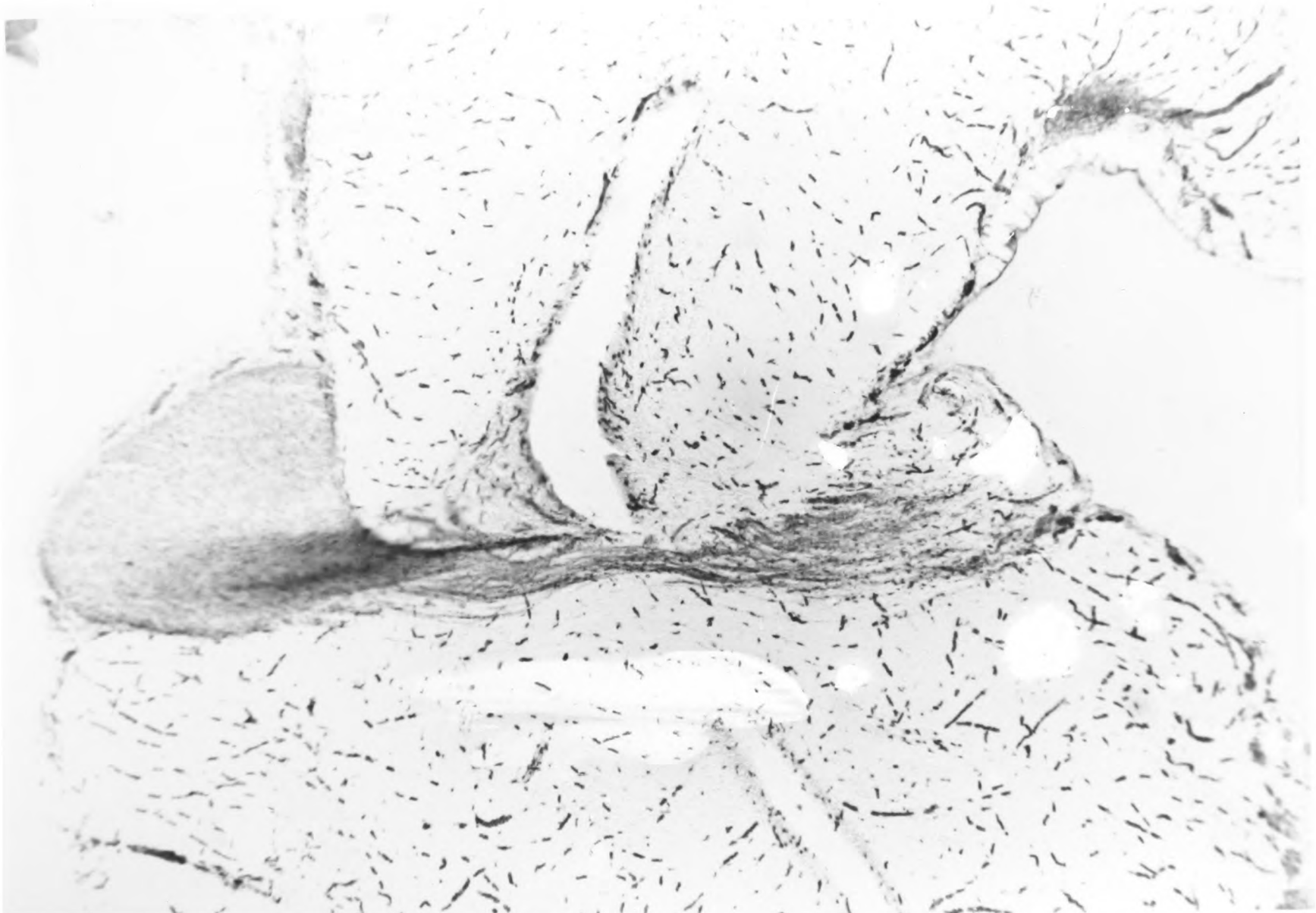
fig 9.42

a

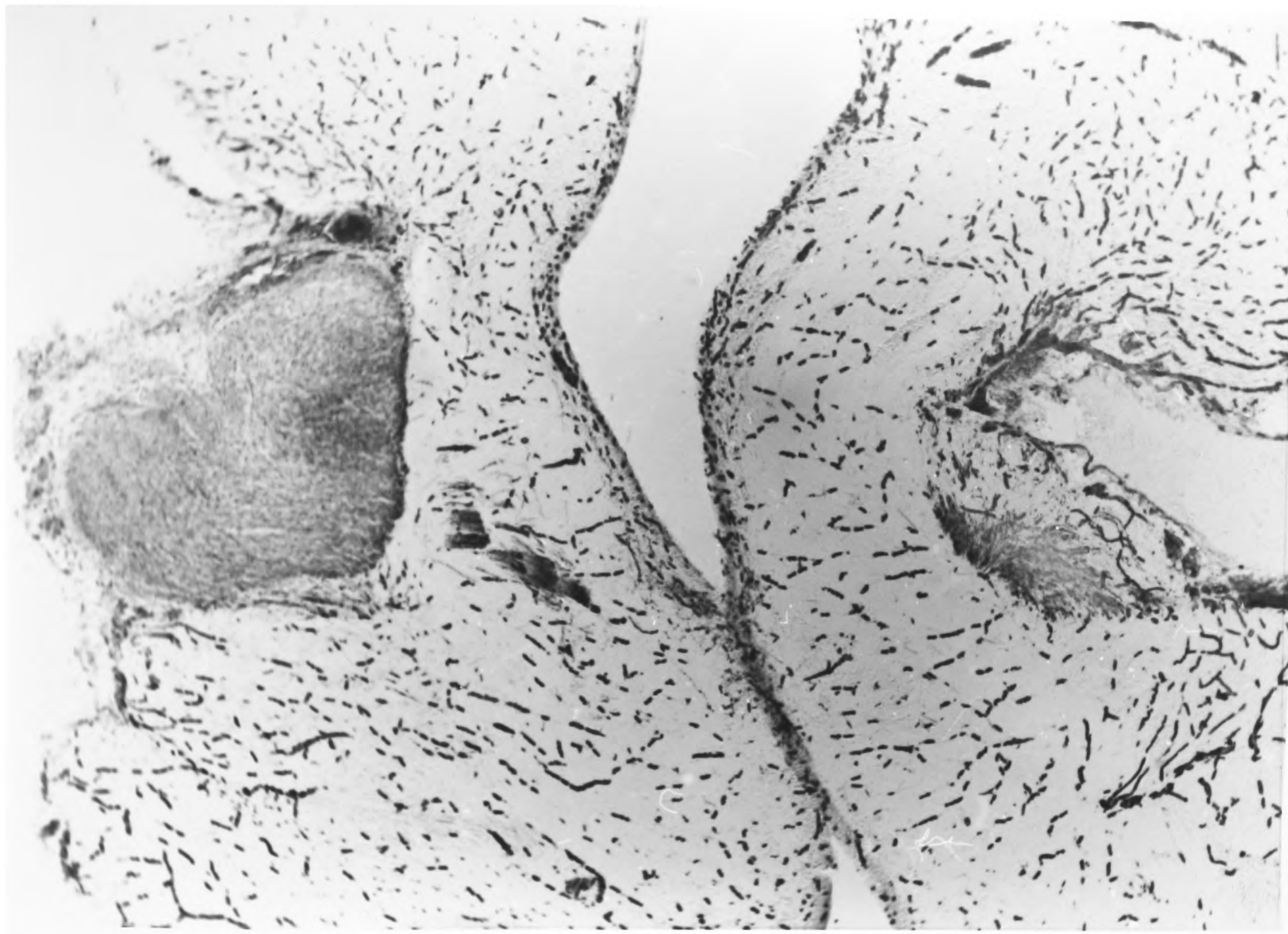


b

500 μ m

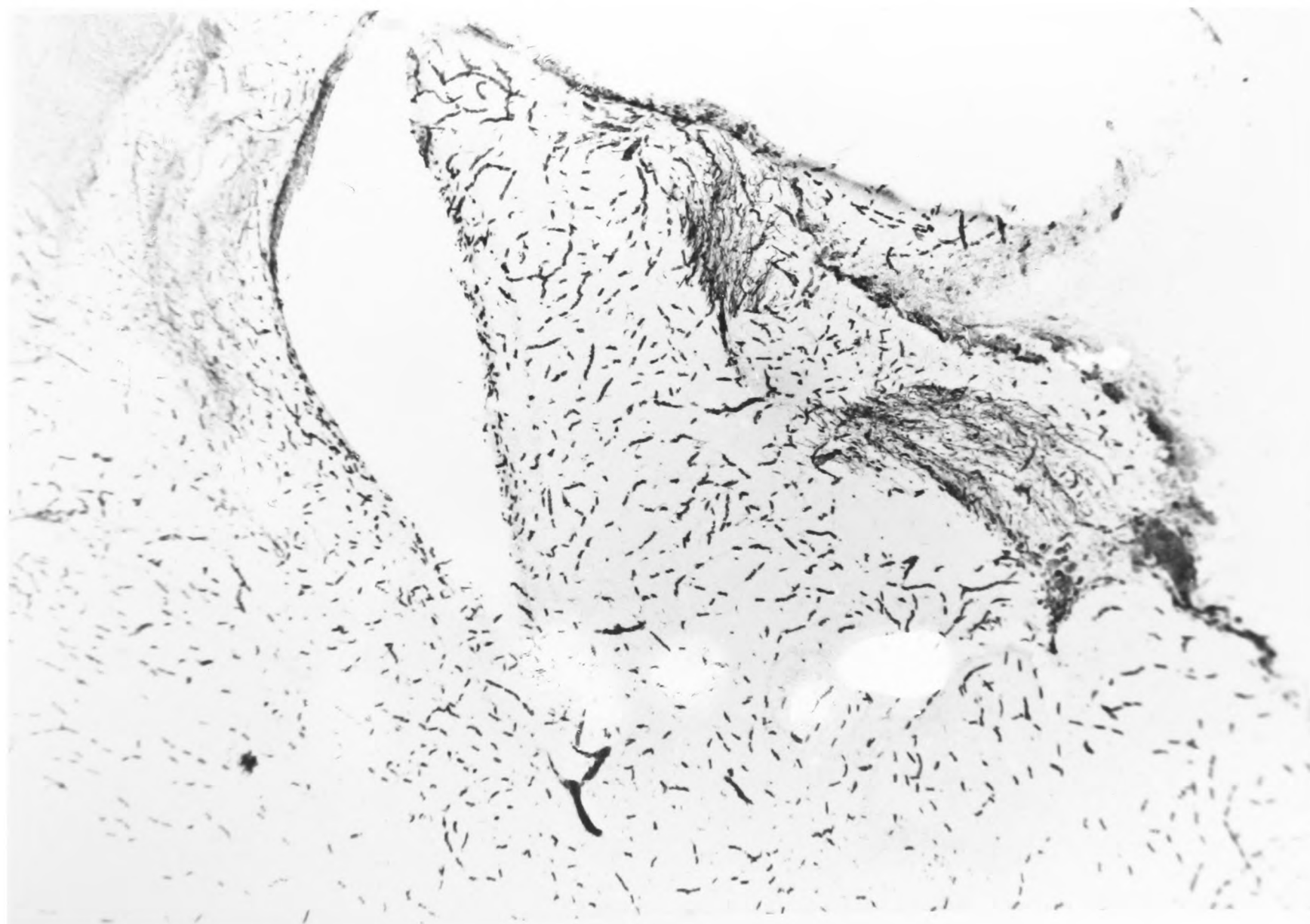


c



500 μ m

d



Discussion

The aims of the experiments described in this chapter were threefold: to investigate the projection formed by optic fibres on a region of tectum of a polarity different to that which they would normally encounter; to study the pathways followed by those fibres in the hope of determining how ordering in the pathways contributes to the final projection; and to study the effect on that projection of the presence of another intact or regenerating fibre population to the same target. To achieve this use was made of the abnormal projection which can be induced from one retina to the ipsilateral tectum. Thus a target tissue was provided of mirror image polarity to that normally seen by the fibres without need for the extensive tectal surgery, and hence the problem of diversion of fibres by scar tissue, which bedevils the interpretation of tectal graft experiments (Martin, 1978a; 1978b) was avoided. Furthermore the normal contralateral projection to that tectum from the left eye could easily be removed, left intact or forced to regenerate in order to allow study of the effects of a second group of fibres.

Preliminary anatomical studies looked at the conditions necessary for the induction of a substantial and persistent ipsilateral projection and confirmed Springer's (1980b) observation that no such projection persists after simple unilateral optic nerve section. This is in contrast to *Xenopus* where section of the optic nerve in a larval or post-metamorphic animal produces a persistent and extensive, anatomically if not electrophysiologically detectable, ipsilateral projection (Glastonbury and Straznicky, 1978; Tay and Straznicky, 1980). Nor, unlike Rana (Stelzner, 1979) did unilateral enucleation give a significant ipsilateral projection. Unilateral enucleation with simultaneous or subsequent optic nerve section also failed to give a projection to the ipsilateral tectum detectable with the method of HRP labelling employed here though other workers have detected a small ipsilateral projection under similar circumstances

(Springer et al., 1977). However this latter projection was not detectable electrophysiologically until the normal tectal target of these fibres, the contralateral tectum, had been ablated (Springer, 1980a). Therefore the paradigm adopted to produce an ipsilateral projection sufficient to be detected electrophysiologically as well as anatomically was that of partial or complete removal of the left tectum, which thus induced fibres from the right eye to project to the right tectum.

Several surgical manipulations were employed when setting up the groups of fish in order to vary the pathways followed by fibres towards the ipsilateral tectum. In the whole tectal ablation with orbital right optic nerve cut (WTRON) groups of fibres were found to regenerate from just behind the eye and thus had a potential choice of routes on reaching the chiasm. In the 7/8 groups the path of the fibres from the right eye to the left tectum was preserved as was a small proportion of their termination sites. The disconnected fibres had thus either to die back to the chiasm in which case their choice resembled that of the WTRON groups, or must find a path between the two tecta. In the 8/8 group a similar situation should exist except that all the right eye fibres were deprived of a contralateral tectal target. Finally the Bridge groups had a ready-made path available between the two tecta.

A number of experimental techniques were employed in these investigations and it is worth considering their adequacy in resolving the issues raised. The polarity and ordering of the projections were assessed using extra-cellular multiunit electrophysiological recording of the response to visual field stimulation. The validity of this technique is discussed in chapter 2 and appendix I and seems adequate to allow the polarity of the projection to be determined. Orderliness was judged qualitatively on the basis of the regularity of spacing of the visual field response positions mapped at regularly spaced tectal positions and on the presence or absence of crossings over of adjacent response position lines. No quantification of order was attempted because of the many sources of error in the mapping procedure,

the non-uniformity of the projection mapped in air and the difficulty of correcting for the variation in error with the visual field location of each response. Such an involved procedure seemed likely to contribute little more than a subjective assessment of ordering for the purposes of these studies. Similarly a correlation between the projections from the two eyes to one tectum was sought by eye rather than mathematically.

When investigating the interaction of the two projections within one tectum a method was required to allow comparison of electrophysiological and anatomical results. This was particularly important as a lack of anatomical and electrophysiological correlation has been reported previously in both goldfish (Levine and Jacobson, 1975) and frog (Constantine-Paton and Law, 1978; Law and Constantine-Paton, 1980). In the series of animals described in this chapter very few tectal positions were found at which a response could be elicited from only one eye. Patching would therefore, if present, have to take the form of dominance of a tectal region rather than the exclusive occupation seen by other workers (Cronly-Dillon and Glaizner, 1974; Levine and Jacobson, 1975). The method adopted here was to rate all responses on a seven point scale of strength using the criteria described at the beginning of this chapter. This method is strongly subjective. However remapping of particular points at the start and end of a mapping session revealed these judgements to be highly consistent within one session, rarely differing by even one category and never by more than one. Moreover as these ratings were used to compare two projections mapped at the same session day-to-day variation in subjective assessment and fish-to-fish variation in depth of anaesthesia had no effect on the results.

The relationship of the strength of a response to the nature, density and extent of the tectal innervation at that point is as yet unknown for there is no clear evidence about which parts of the terminal arborization give rise to the electrical signals recorded with a tungsten-in-glass extracellular microelectrode (Gaze, 1970; Chung et al., 1974).

However it seems a reasonable assumption that an increased response strength reflects an increased density of innervation either in terms of numbers of fibres or in the complexity of the terminal arborizations for in a regenerating projection the response strengths have been observed to increase with time as more fibres arrive and perhaps as their terminals mature (Horder, 1971b). Strong support for this assumption is provided by the high positive correlation detected by Cook between the response strengths from the right eye and the density of tectal labelling with ^3H -proline after injection into the right eye of one 7/8 + LON cut animal taken from this study. Reconstruction of the tectal surface showed that a good response detected electrophysiologically was almost always accompanied by dense radioactive labelling at the same tectal location. A Spearman rank correlation test gave a correlation coefficient of +0.717 significant at $p < 0.0005$ (one tailed t-test) (Cook - personal communication).

A correlation was then sought between the strengths of responses via the two eyes at each tectal position again using the Spearman rank correlation test. A non-parametric method was chosen as the data were not normally distributed, and the calculations were corrected for ties as the data were not continuously variable. The finding of an inverse correlation was taken to indicate an interaction of some sort between the two fibre populations the possible nature of which will be discussed later.

The first three series of fish were designed to investigate the formation of an ipsilateral projection in a denervated tectum. In the first two groups WTRON and LER and 7/8 + LER an ipsilateral projection was formed of a polarity appropriate to the tectum and hence a mirror image of that normally formed by the fibres on the contralateral tectum. These projections were well ordered though not as good as normal and in the 7/8 + LER group were certainly less extensive than normal, lacking a dorsonasal field projection

to the mapped area of dorsal tectum. This undoubtedly reflects the presence in this group of a small rostral tectal remnant on the contralateral side of the brain upon which fibres from ventrotemporal retina were able to retain a connection. These electrophysiological results confirm those of Sharma (1973) on fish in which one tectum and the ipsilateral eye had been removed but in which, unlike the WTRONs, the remaining optic nerve had been left intact in the orbit. Such a projection as observed here would also be expected to provide the physiological basis of the overtly reversed visuomotor responses observed in a similar class of fish by Easter and Schmidt (1977).

The pathways followed by the ipsilaterally projecting fibres were traced using HRP in both classes of fish and in both cases the major route was found to be contralateral at the chiasm with recrossing of some fibres, or branches, in the chiasmatic and retrochiasmatic regions (possibly corresponding to the transverse and minor commissures). Such a pathway was observed, using autoradiographic methods, by Easter et al (1978) though in contrast to their results no labelled fibres were observed in the more caudally placed horizontal, ansate and posterior commissures in this study. The autoradiographic methods used by both Sharma (1973) and Easter et al (1978) did not provide any information on the ordering of fibres as they passed into the ipsilateral tectum. Nor could they determine whether the label seen contralaterally in the optic tract and in tissues underlying the ablated tectum represented fibres passing towards the missing tectum and then doubling back or whether the fibre population split into two groups with some fibres going directly back into the ipsilateral tract from the chiasm and some passing through the contralateral tract to terminate in neighbouring structures.

In an attempt to resolve both these problems small injections of HRP into the ipsilateral tectum were employed. These were always made caudomedially in the tectum to avoid hitting large numbers of fibres en passage.

The results are therefore open to the objection that they concern an atypical group of fibres as other groups were not investigated. However there is no reason to believe that this tectal position was supplied by an atypical class of fibres. In both groups of animals the labelled fibres were seen to occupy a compact area in the correct location (for their tectal termination sites) of the ipsilateral tract. This suggests that fibres forming the ipsilateral projection become at least crudely correctly ordered in the tract to generate that projection. There is thus some form of fibre guidance within the visual pathway of a form even more precise than the crude preference for one or other tract brachium detected by Attardi and Sperry (1963) and Horder, (1971a).

Judgement of the precision of the ordering from the compactness of the labelled fascicle was not possible due to variation in the size of the HRP injection and hence of the number of labelled fibres. Nor was it possible to determine whether unlabelled fibres were intermixed with the labelled fibres.

In both groups of animals, WTRON + LER and 7/8 + LER, labelled fibres could be traced back across the midline from the ipsilateral to the contralateral tract and then towards the contralateral tectum. This indicates that the fibres projecting to the ipsilateral tectum either initially grow towards the missing tectum and then turn back to the chiasm on failing to find a target, or that they are actually branches of fibres which send a second branch to the contralateral side of the brain. It was not possible to distinguish between these alternatives.

In only two fish, both of the WTRON + LER group, were fibres observed crossing in the intertectal commissure, a path observed by Sharma (1973). It is interesting that lesions of these fibres considerably improved the ordering of the electrophysiological maps in these cases. Possibly the few

fibres following this route to the ipsilateral tectum were unable to integrate into the majority projection of fibres reaching the tectum via the tract.

In the Bridge + LER group the electrophysiological maps were, in contrast to the first two groups of animals, far from straightforward. Polarity was, as always, grossly correct for the host tectum. However in four of the seven animals the tectum could be divided into two areas having projections which overlapped substantially in the visual field. In the remaining three animals the maps were so disorderly that no such resolution was possible. Possibly these were composed of three or more overlapping components. HRP studies showed the majority of fibres to reach the ipsilateral tectum via the bridge though some label could be detected in the ipsilateral tract. The possibility was therefore considered that, as with the two WTRON + LER animals discussed above, there might be failure of interaction and hence independent mapping of fibre populations following separate routes to the tectum. However lesions of one or other route failed to resolve the maps. An alternative explanation, applicable also to the WTRON + LER animals, is that the failure of interaction between the two or more fibre populations occurs because they arrive at the tectum at different times. In these fish with two component maps the tectum could be divided into one area around the bridge giving one map component, and a more distant area giving the other. These would thus represent an early and a later arriving set of fibres.

In the remaining six groups of fish both eyes came to innervate the remaining tectum, the contralateral projection being left intact in three groups and forced to regenerate in three. Three aspects of the resulting visual projections of these series were of particular interest: the nature of the retinotectal projections from the two eyes; the pathways followed by the ipsilateral fibres; and whether any interactions occurred between the two fibre populations.

Considering first the three groups of animals in which the contralateral projection was left intact. In every case this contralateral projection was apparently unaffected by the later addition of an ipsilateral projection to the tectum, the electrophysiological map being of normal polarity and ordering. There was however some variation between the groups as to the orderliness and extent of the ipsilateral projection. The WTRON group gave maps of normal extent though with a subnormal degree of ordering. The 7/8 group gave a less extensive map, always lacking the most dorsonasal field representation, with a very variable degree of order. Only the Bridge animal gave a substantial ipsilateral map which was less well ordered than normal. These groups of animals all confirm the finding of Sharma (1973) and many others that an ipsilateral projection can be formed in the presence of an intact contralateral projection.

Differences were found between the three groups in the pathways adopted by the ipsilateral fibres. In both the WTRONs and 7/8s a route via the ipsilateral tract was adopted. However in the former group entry into this tract seemed to occur in a small region below the chiasm and involved the penetration of large fascicles of fibres into an intact tract. In the case of 7/8s the recrossing of the midline occurred over a much more extensive region of brain at and behind the chiasm. Again fibres penetrated deep into the ipsilateral tract though generally in much smaller fascicles than in the WTRON group. In neither case did complete intermixing of ipsilateral and contralateral fibres occur though even the partial mixing of fibre bundles is surprising in view of the absence of any surgical interference with the contralateral pathway, particularly as in Xenopus laevis there is evidence that later added fibres join the outside of the tract and do not become distributed throughout its extent (Gaze and Grant, 1978).

The most striking feature of the pathways of the WTRON group of fish, is the finding that the ipsilateral fibres become appropriately ordered within the pathway for the ipsilateral projection which they will generate. Thus tectal injection of HRP in this group gave a single discrete patch of labelling in the nerve containing both ipsilateral and contralateral fibres. This matching of fibres from similar retinal regions of the two eyes within the tract is a strong indicator for extrinsic guidance of fibres within the visual pathway for there is no fibre debris present which might have explained fibre reordering within the one eyed groups of fish (Schmidt, 1978). It is not possible to determine whether similar fibre reordering occurred within the pathway of the 7/8 fish as tectal labelling of ipsilateral fibres was unsuccessful. The only likely alternative explanation for the reordering seen in the pathway of the WTRON fish is exchange of retinal origin information between the intact contralateral axons and the regenerating ipsilateral fibres, an explanation which obviously could not apply also to the one-eyed fish.

In the Bridge group the pathway to the ipsilateral tectum was via the bridge, at least for the vast majority of fibres. Ordering of the fibres within the bridge could not be determined but, as with the experimental groups involving smaller bridges which were described in chapter 8, there is no reason to believe that there was any disruption of normal fibre ordering until the fibres actually entered the tectal bridge.

None of these three groups of animals showed any correlation between the visual field response positions detected via the two eyes at a particular tectal location. The distance between these response pairs almost invariably greatly exceeded the error in placement expected with the mapping methods used. There was thus no evidence that the two fibre populations matched up on the tectum on the basis of their precise retinal loci of origin. Nor did any of these animals show complete segregation of fibres from the two eyes into

separate tectal regions as seen by Cronly-Dillon and Glaizner (1974) and Levine and Jacobson (1975). Instead the majority of tectal positions responded to stimulation of both eyes though the responses varied greatly in strength.

In order to detect incomplete segregation of the two fibre populations the response strengths via the two eyes at each tectal position were compared and an inverse correlation sought using the Spearman rank correlation test. Lack of a correlation would indicate that the two fibre populations were acting completely independently with no limitation upon the total number of synaptic contacts made at a particular tectal location.

The significance of any correlation so detected was then determined using a one-tailed t-test (as the sign of the correlation had been predicted). The finding of such a correlation, particularly when it occurred consistently throughout a series of animals, was taken to indicate an interaction between the two groups of fibres to produce uniform tectal innervation density though with particular areas being dominated by one or other eye. It might be argued that such a correlation was merely the consequence of an absolute limit, imposed by the tectum, on the density of synaptic inputs which it would support. Normally the projections would act independently but when this absolute density limit was approached some constraint would occur on the distribution of fibres and a correlation would be produced. Thus the occurrence of a correlation would depend only on the total number of fibres innervating the tectum. Two points may be raised against this interpretation of the correlations observed experimentally. Firstly although the upper limit of tectal innervation density is not known the literature on "compression" suggests that at least double (Jacobson and Gaze, 1965; Marotto et al., 1979) and on occasions over three times (Meyer and Scott, 1977) the normal density may be achieved. Therefore even assuming that all the optic fibres reach the remaining tectum it is unlikely that the tectal limit for innervation is achieved.

Secondly in these series of animals the WTRON group was consistently uncorrelated while the single Bridge animal and three of the 7/8 animals were significantly correlated. Yet in the two latter groups one small remnant of the contralateral tectum remained to provide a target for some fibres from the right eye. The highest tectal innervation density might therefore be predicted to occur in the WTRON group and the lowest in the Bridge group, the reverse of that which would explain the response strength correlation results.

These three series of animals thus furnished electrophysiological evidence for some form of interaction between the existing intact contralateral projection to the tectum and the experimentally induced ipsilateral projection. This did not, however, take the extreme form of segregation of the two fibre populations into separate, or nearly separate, tectal regions seen by Cronly-Dillon and Glaizner (1974) or Levine and Jacobson (1975) using electrophysiological methods. Segregation of the two fibre groups was also detected anatomically by the latter workers though they were unable to match the anatomical and electrophysiological patches in the one animal in which this was attempted. In this WTRON series both autoradiography and electrophysiology supported the lack of any patching. Unfortunately the autoradiography of the three patched 7/8 animals was inadequate to allow reconstruction of the tectum.

Anatomical patching in the presence of an intact contralateral projection to the remaining tectum has also been reported using autoradiographic methods by Lo and Levine (1980) in the fish and by Straznicky and Glastonbury (1979) and Law and Constantine-Paton (1980) in the frog. Law and Constantine-Paton also employed electrophysiological methods but failed to detect patching with them. Interestingly Lo and Levine (1981) have also reported patchy tectal labelling after injecting radioactive label into the remaining eye of a fish which had suffered the removal of one eye

and the ipsilateral tectum. They proposed that this represented the interaction of visual fibres with other non-visual, and presumably intact, tectal inputs.

The literature contains very few studies on the formation of an induced ipsilateral projection in the presence of a regenerating contralateral projection to that tectum. In the goldfish only the experiment of Meyer (1979a) in which a small fascicle of fibres was diverted from one tectum to the other employed simultaneous regeneration of the contralateral projection, to the recipient tectum. In that study the small group of ipsilateral fibres was able to exclude contralateral fibres from small areas of tectum as shown both anatomically and electrophysiologically. In the frog Misantone and Stelzner (1974a; 1974b) utilized a paradigm involving simultaneous regeneration of both an ipsilateral and a contralateral projection to a single tectum but neither electrophysiological nor anatomical evidence was presented as to the distribution of the two fibre populations within the tectum. There is thus very little existing experimental work with which to compare the results obtained in the last three series of animals.

With regard to the nature of the visuotectal maps obtained from the WTRON + LON cut, 7/8 + LON cut and 8/8 + LON cut animals, all projections, from right or left eyes, were appropriately polarized to fit the host tectum. However the degree of order was very variable and did not show any overall tendency to improve with time after surgery, though the number of animals was too low to be certain that no gradual improvement occurred. Surprisingly the projection from the left eye to the right tectum was not always better ordered than that from the right eye indicating that the normal supplying fibres had no great advantage when forced to regenerate alongside an abnormal ipsilateral fibre population.

The pathways followed by the ipsilateral fibres were traced with HRP.

The WTRON and LON cut group resembled the WTRON group in that fibres crossed into the ipsilateral tract near the chiasm as a fairly compact bundle and then penetrated deep into the ipsilateral tract as a number of fascicles. These fascicles did however appear to break up and become intermixed with the contralateral fibres to a greater extent than was seen in the WTRON group. The 7/8 and LON cut and 8/8 and LON cut group resembled the 7/8 group in that fibres from the ipsilateral eye crossed the midline into the ipsilateral tract over a much greater rostrocaudal extent of the brain than in the WTRON group and mixing with the contralateral fibres seemed more extensive. It was however difficult to draw any firm division between the pathways taken by ipsilateral fibres in the three groups with a regenerating contralateral projection.

In both the WTRON and LON cut and 7/8 and LON cut group tectal injection of HRP again gave staining patterns indicative of reordering of the ipsilateral fibres before they reached the tectum. This again supports the concept of guidance of fibres on the basis of their retinal origins within the visual pathway. However the labelled fibres formed a less compact group than in the WTRON and 7/8 groups so that regeneration of both fibre populations seems to involve some loss of precision in pathway ordering.

Interactions between the ipsilateral and contralateral fibre populations were again sought using two methods. Firstly the left and right eye maps from each animal were compared in a search for matching of fibres from the two eyes within the tectum on the basis of their retinal origins. In the vast majority of animals in these three groups no such matching was detected. However in one WTRON and LON cut animal, illustrated in figure 9.31, distortions were detected in one projection which served to fill in a gap left by absent responses in the other projection. Furthermore correspondence between the two projections seemed good. However because this result was obtained in only one animal its significance is difficult to assess.

It may represent a chance occurrence as with two eyes projecting with the same polarity to one tectum the maps are bound not to show vast disparities. Alternatively it could represent matching of the two fibre populations on the basis of retinal origins, either direct interactions between the two fibre groups or between them and the tectum though in that case it is surprising that such a close matching of the maps was seen only in one animal.

The second method adopted in a search for interactions between the two fibre populations was to assess statistically the correspondence between the response strengths via the two eyes at each tectal position. This method indicated a complete absence of a correlation in any of the WTRON + LON cut animals and the presence of a highly significant negative correlation in the remaining two groups, 7/8 + LON cut and 8/8 + LON cut. Again these results are the reverse of those expected if the correlation were simply a consequence of an absolute limit to tectal innervation density. Instead they indicate a strong interaction between the two fibre populations in the 7/8 and LON cut and 8/8 and LON cut groups, with some areas of tectum dominated, though not exclusively, by one or other eye.

The finding of partial patching in the 7/8 + LON cut group using electrophysiological methods was strongly supported by the patchy, uneven distribution of radioactive label across the tectum seen in the autoradiographs. In one animal a positional correspondence between high density radioactive labelling of ipsilateral terminals and strong electrophysiological responses to stimulation of the ipsilateral eye was achieved (Cook - personal communication). In other animals a response strength correlation of statistical significance was accompanied by patchy label in the autoradiographs while a lack of a significant response strength correlation was accompanied by uniform radioactive labelling in the tectum. Thus in contrast to Levine and Jacobson (1975) and Law and Constantine-Paton (1980) the

electrophysiological patching seemed to have an anatomical basis in the non-uniform distribution of terminals from the two eyes.

The important findings to emerge from this series of experiments are three. Firstly, in confirmation of many other workers the formation of an ipsilateral projection which was appropriately polarized for the host tectum was demonstrated. Surprisingly however when the contralateral projection was forced to regenerate with the experimental ipsilateral projection the contralateral projection was not always better ordered than the experimental projection.

Secondly, the projection from the ipsilateral eye was generally less well ordered in those groups, Bridge + LER, and Bridge, where the pathway to the ipsilateral tectum was highly abnormal, than in those groups involving a path via the ipsilateral tract. Furthermore reordering of the ipsilateral fibres seemed to occur within the pathway in these latter groups so that they come to lie in the correct place in the tract for their eventual tectal termination sites. This supports the concept of active guidance of fibres within the pathway on the basis of their retinal origins. An explanation in terms of fibre debris in the pathway is not tenable as this reorganization was observed in these groups, WTRON + 7/8, where the ipsilateral tract was already occupied by intact fibres from the contralateral eye. Guidance seems therefore to come from some external source. ^{~ from external source} This reordering in the pathway may contribute to the ordering of the final projection so explaining the reduced order of the two Bridge groups.

Finally electrophysiological and anatomical evidence was found for the occurrence of interactions between the two fibre populations, in some of the two-eyed groups of animals with the production of regions of tectum dominated by one or other eye. The occurrence or not of such an interaction was not easily explained. It did not require that both groups of fibres be

regenerating and did not always occur when they were so regenerating. It therefore seemed unlikely to be dependent on the lability or newness of retinotectal synapses.

The implications of these findings for theories concerning the formation of specific nervous connections will be discussed in the next chapter.

Discussion

This thesis has been concerned with the formation of specific, highly ordered, connections between groups of neurons and their targets, either in the periphery or within the central nervous system. In the first chapter the historical development of theories concerning the mechanisms involved in the formation of such connections was reviewed. The theory which has dominated the field has been the chemospecificity hypothesis of Sperry (1963) which postulated active matching of precisely labelled neurons with targets bearing similar labels. More recently an alternative model, the "Morphogenetic" model, has been put forward (Horder and Martin, 1978) which challenged the need for such precise and active matching mechanisms. Instead less specific phenomena such as contact guidance and fasciculation were invoked as the major factors controlling the formation of neuronal connections.

Even at the time it was first formulated it was clear that the "Morphogenetic" model was inadequate to explain many of the results obtained in studies of neuronal development and regeneration. However the model does provide a useful starting point in any discussion of the mechanisms involved in the formation of specific nervous connections. It provokes an examination of how much non-specific forces can contribute. Active processes can then be called upon only to the extent actually required to explain experimental observations. The resulting model will therefore contain the minimum of active mechanisms, in contrast to the chemospecificity model in which connections were formed entirely as a result of active matching of each neuron to its specific target.

This concluding chapter will therefore provide a brief summary of the main points which emerged from the experimental work described in chapters 3 to 9 followed by an attempt to incorporate the results into a coherent model of

the development of patterned nervous connections consistent with other published findings.

The experimental work described in this thesis can conveniently be divided into two parts. Firstly there are anatomical studies of the ordering of fibres in several nervous pathways of normal adult animals. These studies were stimulated by the realization that in the absence of ordering of fibres within nerves passive morphogenetic forces would be unable to generate ordered connections. Secondly there is a group of experiments in which the regenerating goldfish retinotectal pathway was employed as a model of development. The responses of this system to surgical manipulation were studied with particular emphasis upon the pathways followed by fibres and the effects that these paths had upon the resulting projection. Evidence was sought for active guidance of optic fibres within the pathway for active direction of fibres to "correct" termination sites within the tectum, and for interactions between and within fibre populations.

Evidence for order within normal neuronal pathways

Two neuronal pathways were investigated in an attempt to demonstrate an orderly arrangement of fibres. These were the peripheral nerves of frogs, voles and mice and the optic nerves of mice. In both systems considerable controversy had existed as to the occurrence of order. Cobalt filling of axons was employed in the study of peripheral nerves, and in every case examined, the filled fibres were found to remain as a discrete group, travelling parallel to each other as they approached the spinal cord.

These results support the concept of a "cable" structure within peripheral nerves in which fibres supplying a particular target remain as a compact group throughout their course from spinal cord to periphery (Stoffel, 1913). The results fit well with Veyama's (1978) finding that fibres from a particular spinal route remain together as they pass out into the limb in the dog. Thus models of limb innervation such as that of Horder (1978) in which

orderly outgrowth of fibres from the cord play a major part in determining the pattern of nervous connections receive some support. However, as discussed in chapter 1 and 3, certain more subtle details of the innervation pattern are less easily amenable to such a mechanical explanation (Burke et al, 1977).

The mouse optic nerve was investigated because despite a considerable body of anatomical evidence (reviewed by Polyak, 1957) some doubt had arisen (Hubel and Wiesel, 1960; Horton et al., 1979) about the existence of order within the mammalian visual system. HRP was used to label part of the optic nerve and the proportion of the nerve occupied by labelled fibres was studied at a number of points between eye and chiasm. Analysis of the staining patterns revealed that the vast majority of the optic fibres retained within the nerve the same neighbours as their ganglion cells possessed in the retina. This was in marked contrast to the results of Hubel and coworkers, obtained in cats and monkeys.

It is possible that this discrepancy reflects species differences as, unlike the cat and monkey, the mouse has no fovea (Chievitz, 1891). More likely is that the large fibres traced by Horton which showed disruptions of neighbourhood relationships represented an atypical fibre population, perhaps equivalent to the few stained fibres seen in this study lying outside the main region of labelling, which for some reason, possibly related to their time of fibre outgrowth, become incorporated into the nerve at unusual locations.

Evidence concerning the nature of fibre ordering within the goldfish retinotectal pathway

The final anatomical work concerning the normal neuronal pathway was directed at determining the nature of the ordering of fibres in the goldfish retinotectal pathway. Considerable evidence exists in the literature that this pathway is highly ordered (Roth, 1974; Dawmay, 1979a, 1979b; Rusoff, 1979; Rusoff and Easter, 1980). However the nature of that ordering had not been

satisfactorily explained despite the importance of a knowledge of the arrangement of optic fibres in any attempt to discuss the influence of fibre pathway on the pattern of fibre terminations. In chapter 5 the consistent finding of annular patterns of ganglion cell staining after nerve injection agreed well with Rusoff (1979; Rusoff and Easter, 1980) and Dawney's (1979b) interpretation of the fibre organization within the optic nerve being on the basis of radial location of the ganglion cells. This may reflect the annular pattern of growth of the retina (Johns, 1977), a chronotopic organization.

Furthermore analysis of the boundary positions of partial annuli of labelled cells provided strong evidence that this "ribbon" arrangement of fibres was achieved by opening the retina along a ventral radius corresponding to the embryonic ventral fissure of the eye cup.

A model was put forward, supported by the results of HRP, of the organization of retinal fibres within the pathway. The retina was opened along a ventral radius, as in the model of Bunt and Horder (1977), as a consequence of fibre contact guidance across the developing optic cup. However unlike Bunt's model reclosing the fibre array was postponed to a point behind a ribbon with central fibres at one edge (defined on the basis of their retinal origin), peripheral fibres at the opposite edge and ventral fibres on either side of the array. Some folding of this ribbon, as a consequence of glial partitioning of the nerve, was envisaged. Beyond the chiasm refolding the fibre array occurred to give a polar representation of the retina though with one axis reversed. This refolding would be the consequence of completion of the process started in the central fissure though it is proposed that stretching of the optic stalk during growth delays this refolding until beyond the chiasm. The observed arrangement of fibres in the optic tracts could then be generated by separating the fibre array along a nasotemporal axis with rotation of the two halves, due to growth of the intervening tectum.

This model thus explains how the observed pattern of retinotectal connections may be the consequence of the organization of the fibres within the pathway, itself a result of contact guidance of optic fibres across the surface of the optic cup and stalk. No active guidance of fibres within either the pathway or tectum need be invoked to explain fibre order, and it's apparently complex reorganization.

Evidence that "Morphogenetic" forces alone are inadequate to explain some aspects of the regeneration of the goldfish retinotectal system

Though the results summarized above do confirm the existence of order in neuronal pathways about which some controversy had reigned, and the vast body of evidence showing order within neuronal pathways which has been reviewed in chapter 1, cannot prove that other more active mechanisms are not in operation in the formation of specific nervous connections. The second group of experiments presented in this thesis was designed to seek other mechanisms than passive maintenance of fibre order acting in the regenerating retinotectal pathway. Though some evidence was produced which indicated that the ordering of fibres as they reached the tectum was important in the production of the resulting projection, explanation of a number of results required the operation of mechanisms over and above passive morphogenetic forces.

Active guidance of fibres within the visual pathway

In chapter 1 the evidence for active guidance of fibres within the lower vertebrate visual pathway prior to the tectum was reviewed. Little evidence for guidance within the optic nerve was found as these experiments in which fibres were forced into abnormal positions within the pathway (the eye rotation experiments) gave little information as to the site of reordering of the fibres (Sperry, 1943a, b, 1944, 1945, 1948). Deviation of fibres outside the normal pathway did not provide a fair test as active

cues might be confined to the normal anatomical limits of the pathway. Similarly little convincing evidence was found for a preference of fibres for one or other side of the brain (Szentagothai and Szekely, 1956; Beazley, 1975) and the decussation at the chiasm may have a purely mechanical basis.

However at the optic tract division slightly more evidence for active selection of one or other brachium was found. Selection of the appropriate tract branch has been seen in regeneration alone (Rotn, 1972, 1974), regeneration after retinal lesion (Horder, 1974b) and after cross union of the brachia (Arora and Sperry, 1962) in goldfish, and after construction of double ventral eyes in Xenopus (Straznicky et al., 1979). Yet this guidance was not absolute, some fibres reaching the tectum via the "wrong" tract (Horder, 1974b; Udin, 1976; Fujisawa, 1981).

In this study it was found that mistakes could be produced within the optic tract by forcing the fibres to regenerate en masse, by diverting a small fascicle of fibres to an incorrect position in the nerve, and by cross union of the tract brachia. In each case some fibres were found to reach the tectum by unusual routes. However, more striking than these errors were the results of pathway studies on the groups of fish having an induced ipsilateral projection (chapter 9). In these animals the organization of the ipsilateral pathway was investigated by injecting a small amount of HRP into the caudomedial edge of the tectum and then tracing the labelled fibres rostrally. In all groups having an ipsilateral pathway via the ipsilateral tract (all groups from chapter 9, except the two Bridge groups) which were successfully labelled in this way, the HRP filled fibres from the ipsilateral eye, and where present the contralateral eye, were located at the dorsomedial edge of the medial tract brachium. This is the correct tract position for fibres terminating caudomedially in the tectum and derived from nasoventral retina.

To explain this correct positioning of ipsilateral fibres within the ipsilateral tract (and here one is assuming that the whole ipsilateral fibre population was similarly appropriately reordered in the tract) a number of mechanisms can be proposed. Firstly, this might result from a passive rearrangement of fibres as they grow across the midline. Such an inversion of the normal fibre ordering could be a consequence of each fibre doubling back along itself from the contralateral tract into the ipsilateral tract. This could give an ipsilateral tract the mirror image of that seen contralaterally and hence correctly arranged for termination on the ipsilateral tectum. However it is difficult to see why, if the fibre reorganization arises passively thus, the ipsilateral fibres become so closely apposed to the corresponding contralateral fibres in the two eyed fish. Instead fasciculation of the ipsilateral fibres on to the lateral edge of the ipsilateral tract would be expected, at least in those animals in which the contralateral pathway remained intact (Gaze and Grant, 1978).

The alternatives are either guidance of the fibres by extrinsic signals, which would act on both the ipsilateral and contralateral fibres, or guidance of the ipsilateral fibres by the contralateral fibres or their debris, the normal arrangement of contralateral fibres being a consequence of mechanical factors operating in the pathway as proposed in the model above.

The results obtained here do not allow a firm distinction to be made between these two possibilities. If guidance of the ipsilateral fibres is by intrinsic cues then these must take the form of a chemical or similar property of the contralateral axons present also in the debris of these axons, since the correct positioning of ipsilateral fibres in the tract of the WTRON + LER group precludes guidance by active exchange of information (for example in terms of impulse patterns) between intact axons. The property of the axons implicated in this pathway guidance may be the same as that proposed by Schmidt (1978) to confer positional markers upon the tectum.

The guidance within the tract seemed to be less accurate in some, at least, of these animals in which both ipsilateral and contralateral fibre populations were regenerating (particularly the 7/8 + LON cut group) and most accurate where the contralateral pathway was intact. This can be interpreted as slight support for intrinsic rather than extrinsic guidance though the combination of these results with those on developing double ventral eyes (Straznicky et al, 1979) would favour an extrinsic guidance system.

Evidence for guidance of optic fibres within the tectum to the correct termination sites.

In the optic nerve regeneration and medial-lateral tract cross animals described in chapter 7 remapping of the retinotectal projection after lesion of the normal route of entry of fibres into dorsal tectum revealed a number of fibres entering the tectum by abnormal routes. Furthermore many of these fibres were shown electrophysiologically to be terminating at the correct tectal site despite their abnormal routes. Such fibres had been detected previously in the frog (Udin, 1978) and the goldfish (Horder, 1974b) though in the latter case their number and importance had been understated. More recently similar aberrant regenerative pathways have been demonstrated in the newt (Fujisawa, 1981) and the goldfish (Meyer, 1980) though in the former case the accuracy of their termination sites could be determined only crudely and in the latter case not at all. The results obtained here serve to emphasize the accuracy with which fibres can reach their tectal terminations despite arriving at the tectum in an inappropriate position in the fibre array. The ordering of that array cannot therefore be the sole determinant of the pattern of fibre terminations.

A second result which indirectly supports the presence of some means of fibre guidance within the tectum responsible for producing an orderly projection in regeneration comes from the study of optic fibre terminals in normal and optic nerve regenerated animals (chapter 6). Apart from

providing a description of certain aspects of the morphology of the terminal arborizations from which electrophysiological recordings have been made in a vast range of retinotectal experiments, this study also revealed a reduction in terminal size during early regeneration. If this reduced terminal size in regeneration is real and not an artefact of the staining method (for electronmicroscopic confirmation of the completeness of filling of the terminal arborizations by HRP in the normal and regenerated situation is not available) then it has important implications for theories concerning the formation of specific nervous connections. Early in regeneration of the goldfish optic nerve Horder (1971b) reported a substantial enlargement of the multiunit receptive field size as detected electrophysiologically. With time these fields gradually shrank to a normal size. Previously it had been thought that this shrinkage could represent either gradual reduction of initially greatly enlarged tectal terminals so that their overlap decreased progressively and fewer became detectable at any one electrode position or that it might represent an improvement in the accuracy of termination of the regenerated fibres either by movement of terminals or by dying back of those terminating at incorrect sites. The enlarged terminal arborization size seen in the *Xenopus* larva during development of the retinotectal projection (Piper et al., 1980) indicated that the first of these possibilities was likely. However the findings here, that regenerated terminals are smaller than normal early in development, render this hypothesis untenable. Instead the reduction in multiunit field size must represent a gradual improvement in the ordering of the projection. In that case some mechanism is required for the recognition and correction of errors in the early projection.

The nature of the mechanisms involved in this active guidance of fibres to the correct termination sites is unclear. It may reflect either some form of labelling on the tectum or represent the effects of self ordering of the fibre population. In the former case the labelling could have been

present throughout development as in the chemospecificity model (Sperry, 1963) or might be the result of previous innervation of the tectum by a normal contralateral retinotectal projection (Schmidt, 1978) for each of the results discussed above involved regeneration of a contralateral projection to a tectum which had received a normal projection until just before regeneration and reinnervation. The evidence for such labelling of the tectum has been reviewed in chapter 1 and is extremely poor. Only the translocated tectal graft experiments (Hope et al., 1976; Martin, 1978a, 1978b) provide clear evidence for tectal place differentiation as opposed to polarity control and even then the differentiation need only be of a very crude nature. Furthermore their experiments were performed on the regenerating retinotectal system of the goldfish and hence could not distinguish between labels predating and those resulting from innervation.

Self-ordering of the fibre array without need for extrinsic cytochemical cues has been proposed by a number of workers. Its basis could be either chemospecific interactions between fibres (Meyer, 1975a; Cook and Horder, 1977) in which case a highly differentiated retina is required, or similarities in the pattern of activity of fibres from adjacent retinal loci (Willshaw and von der Marlsburg, 1976; Chung and Cooke, 1978). The latter case seems to be contraindicated as an ordered projection can be formed in complete darkness (Yoon, 1975a; Cook and Horder, 1977) though in fact spontaneous activity in the goldfish retina maintained in darkness has been shown to be similar in adjacent ganglion cells (Arnett, 1978). Better evidence against such a mechanism comes from the regeneration of "normal" projections in Xenopus maintained in stroboscopic lighting (Chung et al., 1973) where all ganglion cells should have shown the same activity patterns though it is not clear that absolutely synchronous firing of ganglion cells occurred during this study. Self-ordering would therefore, if it occurred, seem likely to be on the basis of precise labelling of the ganglion cells or

the basis of their retinal origins. Evidence for specification of the retinal axes during development has come from the work of Hunt and Jacobson (reviewed 1974) though some controversy reigns as to the details of this process (Gaze et al., 1979b). However it is not clear that this axis specification is sufficiently precise to define retinal loci within the accuracy required for a self-ordering process capable of correcting small errors in the regenerated projection.

Active self-ordering of the optic fibres has a number of attractions in attempting to explain some of the results obtained during this work. It could explain the guidance of fibres to the correct tectal location via an abnormal route in the regenerate and medial-lateral tract cross fish. It could provide a means for correction of errors during regeneration. Furthermore it could account for the formation of a projection of good internal ordering but inappropriately orientated on the tectum. Such a projection was detected in a number of the fan-shaped fascicle animals of chapter 8 where the projection was rotated and inverted with respect to the host tectum.

However as explained in chapter 8 these inappropriately polarized projections could also be the consequence of the internal ordering of the fibre populations as it grew across the bridge - a result consistent with the action of passive morphogenetic forces. It is difficult to explain the persistence of inappropriately placed fibres in the tecta of optic nerve fascicle fish (chapter 7) and the poorly ordered projections obtained in many of the small fascicle animals (chapter 8) if a self-ordering mechanism were active. In both cases the explanation put forward by Cook and Horder (1977) to explain disordered projections (Gaze and Jacobson, 1963; Gaze and Keating, 1970; Hunt, 1977; Martin, 1978b) and projections of the same or equivalent parts of the retinae to different tectal positions (Cronly-Dillon and Glaizner, 1974; Levine and Jacobson, 1975; Meyer, 1975a; Schmidt, 1978) may

apply. This postulated that such active self ordering of fibres could only occur when neighbouring fibres arriving at the tectum came from nearby retinal regions. Greatly disparate fibres would be unable to interact successfully. Thus in the optic nerve fascicle animals the mechanical deviation of the fascicle would be too great for sorting out of the projection to occur. Similarly in the small bridge fascicle animals the mechanical disruption of the fibre arrangement due to surgery might be too great to correct. In this latter case the efficacy of any active self ordering process might also be reduced by the great dispersal of a small number of fibres over a large area of tectum.

Two further groups of results need to be discussed in terms of the mechanisms involved in the formation of the retinotectal projections. These are the Bridge + LER animals in which there is strong evidence for the production of two or more independent representations of the whole retina in separate regions of the tectum, and the six groups of two-eye one-tectum animals in which the significance of the presence or absence of inverse correlations between the response strengths from the two eyes, and of inhomogeneity in the distribution of tectal terminals, must be explained.

In the Bridge + LER group of animals several of the maps obtained could be resolved into two separate components and it was possible that in the remaining cases the map had three or more components. Attempts to separate the various components of the map on the basis of routes into the tectum failed and it was postulated that the separation might have occurred due to temporal differences in the ingrowth of the two fibre groups. No means was available to test this hypothesis. Self ordering of the two fibre populations arriving at different times into separate maps of the visual field rather than one integrated projection could have occurred for the reasons quoted above by Cook and Horder (1977). Alternatively the area around the bridge base may be occupied by the first group of fibres to arrive.

These could then produce an ordered projection using tectal position and polarity cues - for these projections, unlike those seen in some of the fan-bridge animals, were appropriately orientated for the host tectum. Stability of these terminals upon their first occupied region of the tectum might then force later fibres to grow over them into more distant unoccupied areas. Again tectal polarity-defining mechanisms could generate an orderly map.

Further evidence for the occurrence of interactions within and between optic fibre populations comes from the results of the experiments in which two eyes were forced to project to one tectum. In certain groups (7/8 + LON cut, 8/8 + LON cut and Bridge, as well as some of the 7/8 only group) a highly significant negative correlation was obtained between the response strengths to visual stimulation of the two eyes at each tectal position. Other groups consistently lacked such a correlation (WTRON, WTRON + LON cut). In those groups showing a significant correlation there was considerable variation across the tectum in the strengths of the responses to stimulation of one eye. This indicated considerable variations in the density of optic fibre terminals, a conclusion supported by the results of autoradiographic analysis using rapidly transported ^3H -proline. In contrast much less variation was seen between the response strengths across the tecta of the non-correlating groups and this was reflected in a much more uniform pattern of autoradiographic labelling.

Two facets of these results are of particular interest, firstly the presence of a correlation between response strengths from the two eyes in some cases but not in others, and secondly the patchy distribution of terminals from the two eyes in the same cases. The negative correlation between the response strengths could have an explanation in an absolute limit imposed by the tectum on the innervation density as discussed in chapter 8. In that case the theoretical importance of the occurrence of a correlation is minimal as it does not reflect an active interaction between the two fibre populations.

However there are no reasons to believe that the groups in which a correlation occurred had a higher number of fibres projecting to the remaining tectum than those in which it did not.

Assuming therefore that a correlation does reflect an active interaction between the two fibre populations the effect of which is to even out the distribution of terminals across the tectum, why then does such a correlation not occur in certain groups of animals? Three explanations are possible. There could be no interaction between the fibre populations so that total terminal density varied greatly across the tectum. There could be no interaction but because each population independently spreads evenly across the tectum the overall tectal innervation density is uniform. Finally, there could be an interaction between the two populations but because there is no patching the response strengths via the two eyes do not vary enough for the negative correlation between the two eyes to be detectable statistically. The uniformity of the autoradiographic labelling in these groups makes the latter two hypotheses more likely. Which of the two is correct is more difficult to determine though the existence in the literature of many cases in which terminals spread out or compress together to fill all available tectum (Gaze and Sharma, 1968; Horder, 1971a) does suggest that in the WTRON and WTRON + LON cut groups an interaction was occurring but was obscured by the absence of patchy terminal distribution.

It therefore remains to consider why only some groups of animals showed patchy distribution of terminals. Such a disparity is reflected in the literature, with some workers detecting patching (Cronly-Dillon and Glaizner, 1974; Levine and Jacobson, 1975; Constantine-Paton and Law, 1978; Meyer, 1979a; Straznicky and Glastonbury, 1979; Law and Constantine-Paton, 1980; Lo and Levine, 1980, 1981) whereas others have not (Sharma, 1973; Misantone and Stelzner, 1974; Easter and Schmidt, 1977). It is worth noting that the patching seen in this study was much less extreme than that seen elsewhere, in that tectal regions were dominated by one or other eye rather than

exclusively occupied by them.

The consistency with which patching was seen in some groups but not others, only the 7/8 group being equivocal, indicates that its occurrence was some function of the surgical manipulation of the system. However it cannot depend upon the presence of two labile terminal populations as it occurred in some 7/8 fish with an intact contralateral projection, yet not in the WTRON + LON cut group in which both optic nerves were regenerating.

Furthermore Lo and Levine have observed patchiness in the termination pattern of one eye projecting alone to the ipsilateral tectum, a phenomenon which they interpreted as due to interactions of the optic fibres with other intact non-visual projections to that tectum (Lo and Levine, 1981). The explanation may lie in the pathway adopted by the ipsilateral fibres and their intermixing with the fibres from the contralateral eye. Surprisingly though, those groups in which the ipsilateral fibres became most thoroughly intermixed with the contralateral fibres in the pathway, the 7/8 + LON cut and 8/8 + LON cut were those which demonstrated the clearest patching. An entirely satisfactory explanation for the patching of optic fibres in some of these experimental groups but not others is thus not obvious. Its, occurrence, combined with the failure in all groups (with the exception of one WTRON + LON cut animal) to demonstrate any good positional relationship between the two retinotectal projections, does indicate that internal ordering of fibres within each population was more important than precise matching of the optic fibres to tectal labels.

A model for the development and regeneration of specific nervous connections

The results presented in this thesis which have the greatest significance in any discussion of the mechanisms involved in the formation of specific nervous connections in development, are the findings of order within peripheral nerves and the mouse optic nerve. If any neuronal pathway were

found to be disorderly then that would indicate that order elsewhere was purely coincidental and that some active process was present to sort out the fibres and form a highly ordered array of connections. However the only pathways in which the literature had indicated a possible lack of order were here shown to consist of fibres arranged in a very precise manner reflecting the arrangement of their cells or origin. Thus there is strong evidence that this order could make a substantial contribution towards the formation of precise connections in development as suggested in the "Morphogenetic" model (Horder and Martin, 1978; Horder, 1979).

Further support for this simple concept of development comes from the proposal in chapter 5 of a model for the goldfish visual pathway in which an, at first sight, extremely complex, reorganization of the fibre population was explained in terms of the patterns of growth of fibres over the optic cup and stalk and on to an expanding tectum. Indeed it is much more satisfying to believe that the retinal projection upon the tectum is orientated as it is as a consequence of the way in which fibres are arranged as they reach the tectum, than that they have to be actively rearranged to conform with some sort of labelling within the tectum.

The experiments on the regeneration of retinotectal connections in the goldfish also provided evidence that the pathway followed by fibres to the tectum could influence the way in which they terminated there. Thus distortions of the normal pathway led to mistakes persisting within the map. Indeed in the case of some Bridge animals even the map orientation seemed to be controlled by the internal and inappropriate ordering of fibres as they arrived at the host tectum, rather than by the polarity of that tectum.

As to guidance within the pathway to the tectum the regeneration results obtained here support its existence. It could however be provided by labels left in the pathway by the previous fibres. Only the results of Straznicky

et al (1979) on Xenopus with double ventral eyes have clearly indicated external guidance of fibres in development and then on a very crude basis. Certainly the greater precision seen here may be a consequence of regeneration and not present in development (Schmidt, 1978).

Evidence has also been presented here for some form of interaction between fibres so that groups of fibres attain considerable internal ordering. Whether this was achieved by use of labels borne by each ganglion cell and its axon, defining precisely its retinal position of origin or whether by means of activity patterns carried by the fibres could not be determined. However the most economical explanation is that both ordering within the regenerating ipsilateral pathway and within the fibre populations on the tectum is achieved using labels carried on the cells, activity patterns not being available in some of the ipsilateral pathways.

Is such a fibre self-ordering mechanism at work during development? On this question there is no firm evidence. However there is no reason to believe that it could not operate in development. Furthermore it would provide a means for correcting small mistakes in the projection due to fibres which accidentally become incorporated into the pathway in the "wrong" place.

In summary therefore the development of precise neuronal connections can be explained by the growth of neuronal fibres in an orderly manner due to contact guidance and fasciculation. Guidance signals of a very crude level ensure that the fibres reach the correct target. Fine tuning of the projection could then be achieved by self-ordering of the fibre array on the basis of some expression of the retinal origins of each fibre, probably a physicochemical label though possibly in terms of activity patterns. In regeneration these same mechanisms would normally operate though with the added influence of modification of the pathway and target structure by pre-existing or co-existing fibres.

APPENDIX 1The origin of the electrical activity detected during electrophysiological mapping

Electrophysiological maps have been taken throughout this study to reflect the tectal positions of the terminal arborizations of retinal ganglion cells. There is now considerable evidence that the recorded responses to visual stimulation do originate in the presynaptic arborizations, though of course the map could equally represent terminal positions if it were obtained by recording from the tectal cells underlying those terminals. In the latter case the effect of tectal processing of the incoming visual information would have to be assessed when interpreting the maps. Both Maturana et al. (1960), and Gaze (1970) have summarized most of the evidence for a pre-synaptic origin for the responses recorded with the sort of tungsten electrode of impedance 50-250 K Ω used in this study. Essentially this is that:

- (1) at a given tectal position a microelectrode responds to stimulation of a small region of the visual field. A small shift in electrode position across the tectal surface causes an appropriate shift in the visual field position to which it responds. Thus the electrode is unlikely to be detecting activity in fibres passing by;
- (2) when single units are detected they have the same receptive field characteristics as units recorded within the optic nerve. This implies that in the tectum one is recording from the terminations of those optic axons;
- (3) deeper in the tectum activity can be detected which has different receptive field characteristics from those of optic nerve axons. This presumably originates in the tectal cells and their processes;
- (4) electrical characteristics of the superficial units resemble those of optic nerve axons but not those of deeper units.

For these reasons Gaze (1970) concluded that:

"the probable site of origin of the signals is the terminal arborization of the fibre since here branching would be expected to increase the signal."

Further evidence of a presynaptic origin for the responses detected during electrophysiological mapping comes from George and Marks (1974) who found that in the frog those responses were resistant to synaptic blocking agents. Furthermore when recordings were made of retinal activity and activity at the appropriate tectal location exact correspondance was obtained between the spike patterns. Thus the recordings were almost certainly of the retinal and tectal ends of the same fibres.

Similarly Chung et al. (1974) were able to detect, in the frog tectum and using low impedance electrodes, a fast response to optic nerve stimulation which was resistant to sodium pentobarbitone applied to the tectal surface and which had a latency appropriate for a presynaptic origin.

Extrapolation of these conclusions to the goldfish seems valid as the electrodes used are of a suitable low impedance and also Konishi (1960) has recorded spoke activity in the goldfish tectum resistant to the tectal application of procaine.

APPENDIX 2

Possible mapping artefacts

When mapping the tecta of normal goldfish it was discovered that electrode positions near the caudal and medial edges of dorsal tectum would respond to stimulation not only of the small region in the peripheral visual field expected, but also to an elongated region extending radially from the expected position up to $40-50^{\circ}$ towards the centre of the visual field. These radial response bands were particularly easily detected when mapping the fish in darkness using the flashing LED stimulus. They only occurred when mapping at the edges of the tectum where the predicted response would be more than 70° from the field centre and were always directed approximately radially towards that centre, never circumferentially. An example of a map from a normal fish showing such bands is given in figure A2.1.

The cause of these anomalous responses is unclear though an optical basis seems likely, possibly as a consequence of the optical activity of the cornea in air. Such responses were not noted by Meyer (1977) in his extensive investigation of the goldfish retinotectal projection using eye-in-water mapping.

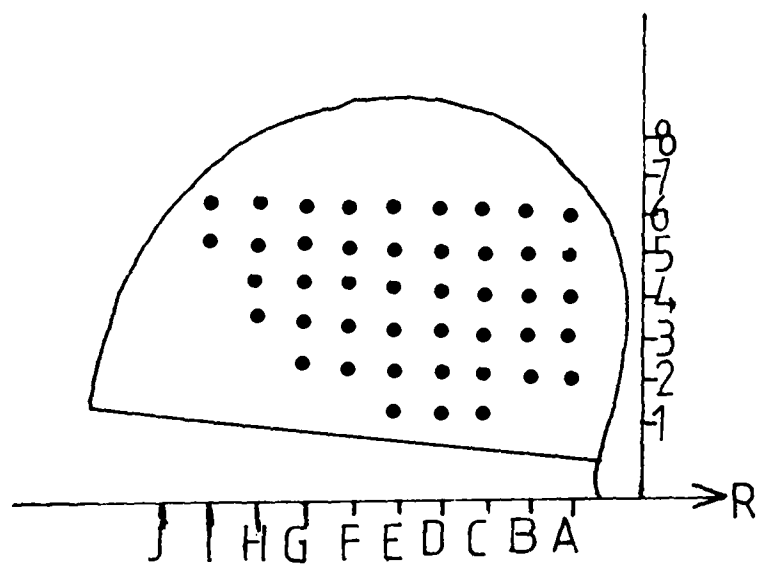
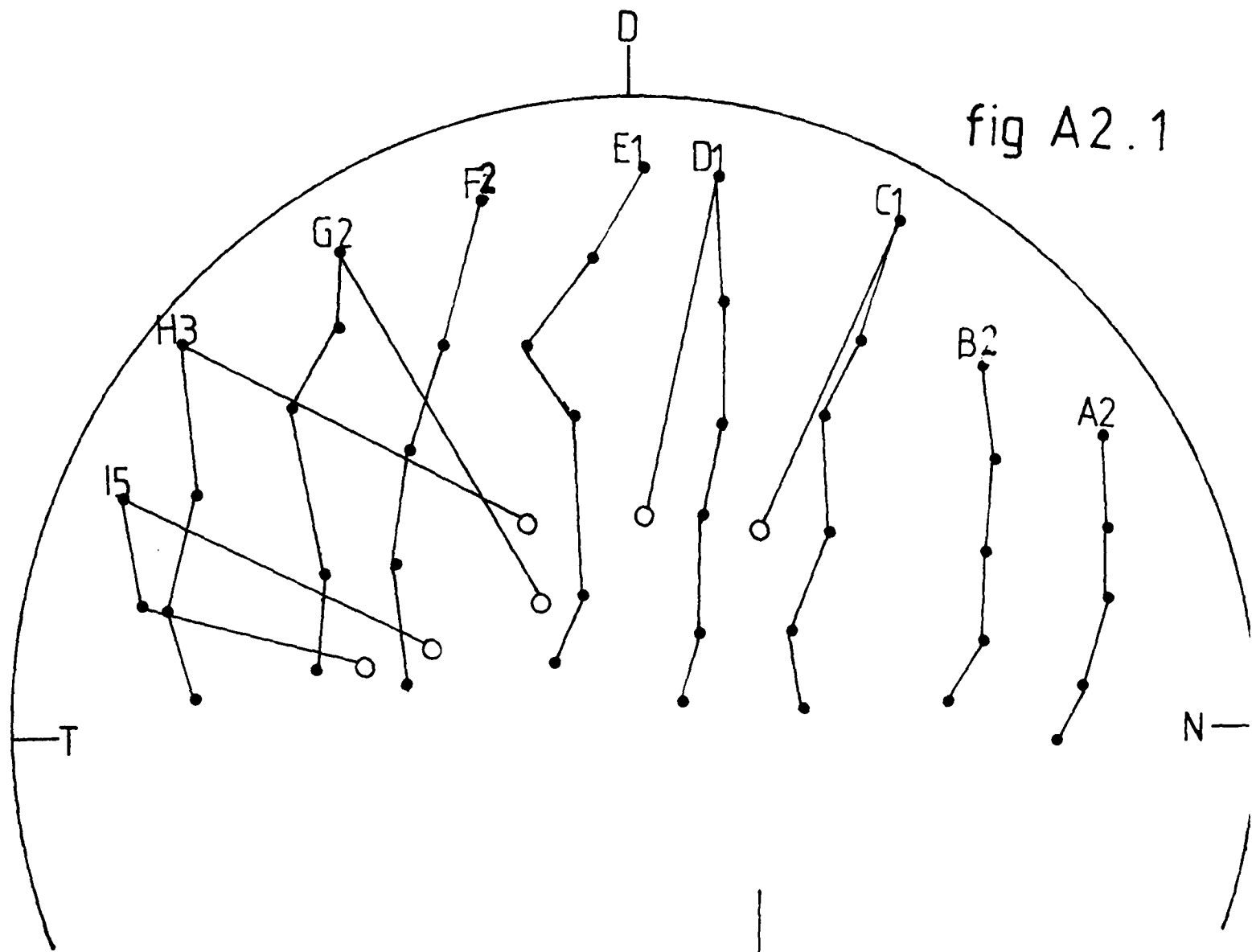
In order to avoid any confusion of those anomalous responses with real displacements of visual response positions due to experimental manipulation, a number of precautions were taken.

- (1) In experimental animals the area of tectum mapped excluded the most medial and caudal regions. The electrode position was placed at least $400\mu\text{m}$ from the medial tectal edge and somewhat more than this from the caudal edge.
- (2) Any responses obtained from peripheral tectal electrode positions were disregarded if they showed a radial displacement from the expected location towards the field centre.

In practise by limiting the area of dorsal tectum mapped electrophysiologically very few responses were detected which might have resulted from this anomaly.

Fig. A2.1. Electrophysiological map of the visuotectal projection of a normal goldfish. The response fields for electrode positions at the caudal and medial edges of the tectum showed a radial elongation towards the centre of the field. (●—○). The map is corrected for an eye rotation of 23° nasal.

fig A2.1



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Fig. 7.7 Electrophysiological maps of the visuotectal projection of a medial-lateral tract cross animal obtained 271 days after surgery. The top diagram shows the first mapping while the lower diagram shows the map obtained after making the tectal lesion shown in dotted lines on the tectal outline. Both maps are corrected for an eye angle of 23° .

fig 7.7

