

## **SOME DETERMINANTS OF OPTIC TERMINAL LOCALIZATION AND RETINOTOPIC POLARITY WITHIN FIBRE POPULATIONS IN THE TECTUM OF GOLDFISH**

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### **SUMMARY**

1. The reorganization of the retinotectal projection which results after partial ablation of tectal tissue was examined in goldfish using electrophysiological methods.

2. Regardless of the size of a unilateral ablation of caudal tectum, an orderly and virtually complete, 'compressed', visual projection re-formed on the remaining tectum after crushing the optic nerve and allowing it to regenerate.

3. If the optic nerve was left intact after ablations of caudal tectum, compressed projections were only found when the ablations were small. Large caudal ablations involving half or more of the dorsal tectum resulted in the cut fibres transposing onto the remaining tectum and forming an overlaid, 'duplicate', projection on the remaining intact projection.

4. In approximately one third of cases the duplicate projection lay in a reversed polarity along the rostrocaudal axis of the tectum. In the remaining cases the polarity of the duplicate projection was normal.

5. Transposed projections of reversed rostrocaudal polarity could be consistently obtained by ablating temporal retina and caudal tectum, leaving an intact strip of fibres terminals along the caudal edge of the tectal remnant.

6. Compression and duplication occurred in the same way if fish were maintained in constant light.

7. After ablations of lateral tectum, leaving the optic nerve intact, compression and some disorderly duplications were found.

8. Reversed projections could be induced across the mediolateral axis of dorsal tectum by denervating the medial tectum and ablating a strip of lateral tectum.

9. Projections of normal polarity were found after the optic nerve was allowed to regenerate into tecta which had previously supported reversed polarity projections.

### **INTRODUCTION**

A most fundamental, but still largely unresolved, problem in neurobiology is the question of how precise and orderly patterns of neuroanatomical structures and interconnexions are predictably established during development. Regeneration of visual projections in lower vertebrates has been extensively studied as a situation offering especially favourable opportunities to analyse mechanisms for the orderly

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selection of termination sites by growing axons. One experimental strategy which has been particularly revealing is to study the effects of the ablation of a portion of the retina or tectum or both.

Gaze & Sharma (1970) were the first to describe reorganization of the retinotectal projection following removal of the caudal tectum in goldfish which proves that, whatever mechanisms normally guide specific fibres to appropriate termination sites, these are not based on any unique invariant selective affinity. One of their observations, that the full retinotopic projection is reconstituted in a 'compressed' fashion on the remaining tectal fragment, has been confirmed in many similar studies (Yoon, 1971, 1972*a, b*, 1975, 1976; Meyer & Scott, 1977; Marotte, Wye-Dvorak & Mark, 1977; Cook, 1979; Wye-Dvorak, Marotte & Mark, 1979). However they also reported that in cases where the optic nerve was not undergoing regeneration a subpopulation of fibres which would normally terminate in the caudal tectum came to form a 'duplicate' ordered projection *superimposed* on the normal projection to the remaining rostral tectum. This observation was not replicated in any of the subsequent experiments mentioned above; only occasional duplicate field positions have been seen (Sharma, 1972; Meyer, 1977; Marotte *et al.* 1977, Marotte, Mark & Wye-Dvorak, 1981). Duplicate maps have not been reported even in cases where compression apparently failed to occur (Yoon, 1975; Marotte *et al.* 1977; Wye-Dvorak *et al.* 1979) while compression has been reported as the predominant modification observed in fish with intact optic nerves (Yoon, 1975; Marotte *et al.* 1977; Meyer, 1977; Cook, 1979).

The present series of experiments was initially undertaken in order to confirm the phenomenon of duplication and to identify the conditions which might explain its sporadic occurrence in the literature. In particular we hoped to resolve inconsistencies in the relationship of the phenomenon to non-regeneration as opposed to regeneration of the nerve as reported by Gaze & Sharma (1970). A source of variability between experiments might have been the amount of tectum ablated and this parameter we have systematically varied. We had to take into account a number of other extraneous factors which have been shown recently to affect the occurrence of compression and might therefore also have accounted for discrepancies in the appearance of duplication. Thus we compared fish kept in constant and diurnal lighting conditions since it has been reported that continuous illumination can prevent compression and other manifestations of plasticity (Yoon, 1975; Beazley & Humphrey, 1980; Marotte *et al.* 1981) although others have failed to replicate the effect (Arora & Grinnell, 1976; Marotte *et al.* 1977; Meyer, 1977; Meyer & Scott, 1977; Scott, 1977). Furthermore, Wye-Dvorak *et al.* (1979) have shown that compression (or duplication of fields) seldom occurs in fish operated in autumn although there are occasionally exceptions among large and small fish kept in constant light (Marotte *et al.* 1981). A final objective was to study whether these phenomena involve features peculiar to the rostrocaudal axis of the tectum and therefore the mediolateral axis has also been investigated.

An unexpected finding during the course of these experiments was the occurrence of maps in which the polarity of the superimposed projection was reversed. Since this observation is of some significance for our understanding of the mechanisms underlying map formation, we conducted further detailed investigations into the

conditions which generate polarity reversal. Some of these results have been briefly reported previously (Horder & Martin, 1977*a*; Bunt, Horder & Martin, 1979).

#### METHODS

##### *Animals*

Common goldfish (*Carassius auratus*) obtained from a local dealer were used throughout these experiments. The size of the fish was 4.8–6.4 cm, nose to tail-fin base (mean, 5.5; standard deviation, 0.4). They were kept in small groups in shallow opaque plastic tanks in natural lighting conditions or in constant light provided by fluorescent lamps. The luminance of the side walls and the bottoms of the tanks was 1390 lux. The water temperature of the fish maintained in constant light was  $21.2 \pm 0.7$  °C while that of fish kept in natural lighting was  $19.2 \pm 1.3$  °C. Two series of fish were maintained under the two lighting regimes at the same temperature of  $21.7 \pm 1.5$  °C and found to give exactly comparable results. Fish in which the optic nerve was left intact were operated on in November or January.

##### *Surgery*

The fish were anaesthetized by immersion in a saturated solution of ethyl 4-aminobenzoate (benzocaine, B.D.H.), and perfused with tap water for the duration of the operation. To reach the right optic nerve a small slit was made in the dorsal conjunctiva. When the nerve was clearly visible it was firmly and repeatedly crushed as close to the orbital wall as possible using watchmakers' forceps. For retinal ablations the retina was exposed by making a crescent-shaped cut in front of the limbus. The relevant piece of retina was separated using a sharp tungsten needle, taking care to avoid damage to the optic nerve head. The retinal fragment could then be removed as a single piece, taking with it as much of the underlying pigment epithelium as possible. Generally the cut sclera healed rapidly without further treatment, but if the cut was extensive fine sutures were inserted to keep the edges apposed. Subsequently the ablated area showed as a red patch within the darkly pigmented intact retina when the fish was transilluminated. The effectiveness of the lesion was confirmed by the extent of the scotoma on later electrophysiological mapping of the visual field and by direct observation together with histology after the terminal experiments.

In order to expose the left tectum for surgery or electrophysiological mapping, a flap of skull was removed by making a bevelled cut along the suture lines of the skull overlying the tecta. After operation or mapping, the skull flap was replaced and held in place by the bevelled edges without the use of adhesive, a method which could be repeated easily when the same fish was mapped a number of times. In ablating portions of the tectum, iridectomy scissors were used to make an incision to the level of the underlying ventricle, the incision being extended along the length or breadth of the tectum according to the nature of the lesion required. In ablations of the caudal tectum all but the most ventral tectum was removed; care was taken to ensure that no tectal tissue remained caudally. In ablations of the lateral portion of dorsal tectum only dorsal tectum was removed; lateral and ventral portions of the tectum were left intact.

In the first series of experiments the caudal portion of the left tectum was removed, with or without crushing the optic nerve supplying that tectum. An attempt was made to create three different sizes of residual rostral tectum. Since the actual size and shape of the tectum varies between fish it did not seem possible to remove a consistent, known volume of tissue. Therefore, following preliminary electrophysiological mapping (described below) of the intact tectum before the ablation, the position of the vertical meridian of the visual field projection on the tectum was established as a reference point. One of three positions was then marked using the micromanipulator. For the group of fish having a small rostral tectal remnant (s.r. group) the position was 200  $\mu\text{m}$  rostral to the reference point; a position 200  $\mu\text{m}$  caudal to the reference point was chosen for the intermediate sized group (i.r. group), and 600  $\mu\text{m}$  caudal to the reference point for the group having a large remnant of tectum (l.r. group). Using iridectomy scissors a cut was then made across the tectum at right angles to the mid-line of the fish at the appropriate rostrocaudal level.

##### *Electrophysiological recording*

In order to make the visuotectal maps comparable to those of most previous studies, conventional eye-in-air mapping was used, even though this method introduces refractive errors which result in an over-estimation of the extent of the field represented (Meyer, 1977).

Fish were anaesthetized and maintained by a continuous perfusion of a freshly made saturated solution of benzocaine. A fish was mounted with its right eye at the centre of an 'Aimark' perimeter. By transillumination the optic disk could be visualized and aligned on the centre of the pupil as viewed along the axis of the perimeter. The projection of the disk lies approximately 6° and 14° dorsal in the field with respect to the optic axis of the eye (Meyer, 1977; Schmidt, Cicerone & Easter, 1978) but it provided a reliable criterion for monitoring the eye position, which was checked regularly throughout the mapping session to exclude eye movement.

In order to relate the micromanipulator co-ordinates of the recording electrode to the dorsal surface of the tectum being mapped, the electrode was initially positioned to determine the co-ordinates of the boundaries of the exposed tectal surface and of the positions of prominent landmarks such as major blood vessels. A Polaroid photograph of the tectal surface was used to make a drawing in which recording positions were located in relation to these landmarks. In general as much of the dorsal tectal surface as possible was mapped by advancing the electrode through an evenly spaced (usually 200  $\mu\text{m}$ ) sequence of positions as determined by the vernier reading on the micromanipulator. The curved rostral pole of the tectum was not routinely mapped.

The electrodes were tungsten in glass, coated with gold and platinum (Merrill & Ainsworth, 1972) with 12–15  $\mu\text{m}$  of exposed tip, which made them suitable for recording multiple units and occasional single units. It is generally supposed that the source of electrical potentials so recorded in the tectum is the terminal arborizations of the optic nerve fibres (Maturana, Lettvin, McCulloch & Pitts, 1960; Gaze, 1970; George & Marks, 1974). Amplification was conventional. Responses were monitored aurally.

A large (15°) black or white disk was moved against a light grey background to determine the borders of the receptive fields. Generally the centre of a receptive field coincided with the region of maximum response to a small stimulus. Routinely all tectal layers were examined for visually evoked responses but responses could only be obtained consistently from layers B, C, D (the superficial layers) and F (the deep fibre layer) in the nomenclature of Jacobson & Gaze (1964). The receptive fields recorded in layer F tended to be more nasal than those recorded superficially, probably because of the tectal curvature. The centres of the receptive fields were noted at the time of mapping and subsequently plotted on a standard perimetric chart extending out to 100°. The visual field maps shown in this paper are plotted with the disk at the centre. The angle of rotation of the eye was monitored throughout the experiment by noting the position of the scar formed by closure of the embryonic choroid fissure. This mark was assumed to lie normally on a vertical line bisecting the eye. All maps were corrected subsequently for the degree of rotation of the eye, which could be as much as 30°. None of the optical and centring considerations mentioned above are of material significance for our results since we are concerned with the retinotopic ordering within the projection and with no more than the relative extent of partial field representations. Even in normal fish, and presumably due to the optics of mapping in air (Meyer, 1977), at the extreme periphery of the tectum it is sometimes difficult to locate a restricted receptive field consistent with the normal retinotectal projection; at such positions responses were distinctive in that they could only be obtained by using a large stimulus (30°–60°) presented centrally in the visual field.

Variations between fish were reduced as much as possible by multiple remapping of the individual fish throughout the duration of the experiment. Thus, although the numbers of fish in each group are small, a relatively large number of visuotectal maps were obtained. Most fish were mapped at least three times each, usually first within a month or two of the initial surgery, again after 1–2 months and then once more at the end of the experiment.

Magnification factors were calculated by dividing the length of the tectum in microns (measured along a rostrocaudal line in mid-dorsal tectum) by the degrees of visual field represented along that length. The significance of the differences in magnification factors between different experimental groups was computed using a *t* test and adopting a significance level of  $P \leq 0.01$ .

### *Histology*

Recording depths in the tectum were determined in a few representative fish by making lesions (10  $\mu\text{A}$  for 10 sec, electrode negative) at various positions where responses could be obtained. The brains were fixed in 10% formol saline, cut in 10  $\mu\text{m}$  wax sections and stained in thionine or with Holmes' silver method to determine the effectiveness of the lesion. In a sample of fish maintained under the two lighting conditions the eye was also cut separately in wax sections and stained with Masson's trichrome.

A histological examination of fish which had been maintained under constant light for long

periods (3 months or longer) showed that there was a marked reduction in the number of cells in the outer nuclear layer of the retina. The number of retinal ganglion cells however, was not obviously different from normal.

Standard methods (Rogers, 1967) were used for autoradiography. The eye was injected with 15  $\mu$ Ci of [<sup>3</sup>H]proline (specific activity, 1 mCi/ml.; concentration, 0.1  $\mu$ mol/ml.) after removing some vitreous humour. The fish were maintained for 24 hr before fixation in Carnoy's fixative for 6 hr followed by conventional wax embedding. Slides were dipped in emulsion (Ilford G5) and kept in light-proof boxes at 4 °C for 28–36 days before developing in Kodak D19 developer. The developed slides were then counter-stained in Harris' Haematoxylin.

RESULTS

I. Compression and duplication of projections after varying degrees of caudal tectal ablation

Table 1 details the number of fish subjected to particular operations and maintained under two different lighting conditions.

TABLE 1. Totals of numbers of fish used under each lighting regime. The numbers in brackets indicate the number of fish within each group showing duplications at more than one of the tectal positions mapped; s.r., i.r. and l.r. refer to the three sizes of tecta, made as described in the Methods. Fish in the two lighting regimes were maintained at different temperatures as described in Methods, with the exception of the groups with four fish which were maintained at the same temperature.

A. Optic nerve left intact			
Lighting conditions	Size of remaining tectum		
	s.r.	i.r.	l.r.
Constant light	7 (6)*	6 (5)*	7 (4)
Daylight lighting	—	6 (5)*	8 (4)

B. Optic nerve crushed			
Lighting conditions	Size of remaining tectum		
	s.r.	l.r.	
Constant light	6 (1)	4 (0)	6 (1) 4 (0)
Daylight lighting	6 (0)	6 (0)	4 (0)

\* One fish died before any duplications were detected in the group as a whole.

(a) Fish in which the optic nerve had been crushed

The phenomena reported in this series replicate those of previous studies (Gaze & Sharma, 1970; Yoon, 1971; Meyer, 1977; Cook, 1979). Regardless of the size of the remaining portion of the tectum, the earliest regenerated projections lacked temporal fields corresponding to the amount of tectum ablated, but later virtually the whole projection tended to 'compress' into the tectal remnant, as indicated by the magnification factor of  $10.6 \pm 2.0 \mu\text{m}/\text{deg}$  in the l.r. group (control value in normal fish  $17.9 \pm 3.2 \mu\text{m}/\text{deg}$ ). In the s.r. group the projections were significantly more compressed compared to the l.r. group (s.r. group magnification factor  $7.8 \pm 1.4 \mu\text{m}/\text{deg}$ ;  $P \leq 0.01$ ) but were not as complete as that found in the l.r. group. In the caudal tectal regions the receptive field sizes tended to be larger (20–30°, occasionally 50°) than those at more rostral positions (10–15°). Maintenance in constant light resulted in the first projections being recorded on day 24 post-operatively and first compressed maps on day 25, compared to day 33 and 37 respectively for fish maintained in daylight.

(b) *Classes of result obtained in fish in which the optic nerve had been left intact*

(i) *Compressed projections.* These were obtained only in fish of the l.r. group; in contrast to fish with crushed nerves, compression was restricted to caudal-most tectal regions.

(ii) *Mixed compression and duplication.* This second type of projection, which most closely resembles the duplicate maps obtained by Gaze & Sharma (1970), was found in three i.r. group fish, one l.r. group fish, but never in s.r. group fish. Compression had occurred in the most caudal tectum, but more rostrally two responses could be obtained at single recording sites, one from the normal field position and one corresponding to a part of the field normally represented in the ablated tectum. At the most rostral recording sites only single fields were recorded, corresponding to the normal projection. The bracketed numbers in Table 1 indicate the number of fish in which more than one duplicated position was found.

In seven fish it was also possible to obtain an almost complete map of the projection to the deep layer F of the tectum. Typically in this layer there were no duplicate positions: the fibres appeared to have either compressed or remained in their original positions.

(iii) *Duplicated maps of correct polarity.* The third and new type of projection is shown in Fig. 1. Here there is no evidence of compression. Instead a considerable area of tectum contains a well ordered map (points connected with dashed lines in Fig. 1) of fibres normally projecting to the ablated tectum *superimposed* on the intact normal projection (points connected with continuous lines in Fig. 1) to rostral tectum. Both projections have the same polarity, indicated here by arrows. These duplicate projections were found in the majority of fish in the s.r. and i.r. groups, but only in four out of fifteen fish of the l.r. group mapped more than 50 days post-operatively. The areas of tectum containing duplicate maps in the fish of the l.r. group were less extensive than those found in either the s.r. or i.r. groups.

(iv) *Duplicated maps of reversed polarity.* The fourth type of projection, also reported for the first time, resembles the third type except that the polarity of the superimposed projection is reversed along the rostrocaudal axis of the tectum (Fig. 2). Such projections were seen in about one third of the fish in which superimposed projections were found (Table 2). In contrast to tecta supporting duplicate maps of normal polarity, it was not possible to detect duplicated field positions along the caudal edge of tecta supporting reversed polarity projections. The reason for this is evident from Fig. 2: the map is mirror-imaged around field positions recorded along the caudal edge; thus any duplications would be so closely adjacent that they would appear as a single field. Although only one of the fifteen fish of the l.r. group showed this type of map it should be noted that only one other fish of this group showed extensive duplication at 95 days after operation. As with compressed projections and duplicate maps of normal polarity, the earliest reversed maps had a large scotoma in the temporal field. With time there was a sequential reduction of the scotoma, starting with most nasal parts of the unrepresented field, and a commensurate increase in the amount of tectum occupied by the superimposed projection. This can be taken to indicate a progressive but orderly addition of fibres to more rostral regions of the tectum. In common with duplicate projections of normal polarity, duplicate field

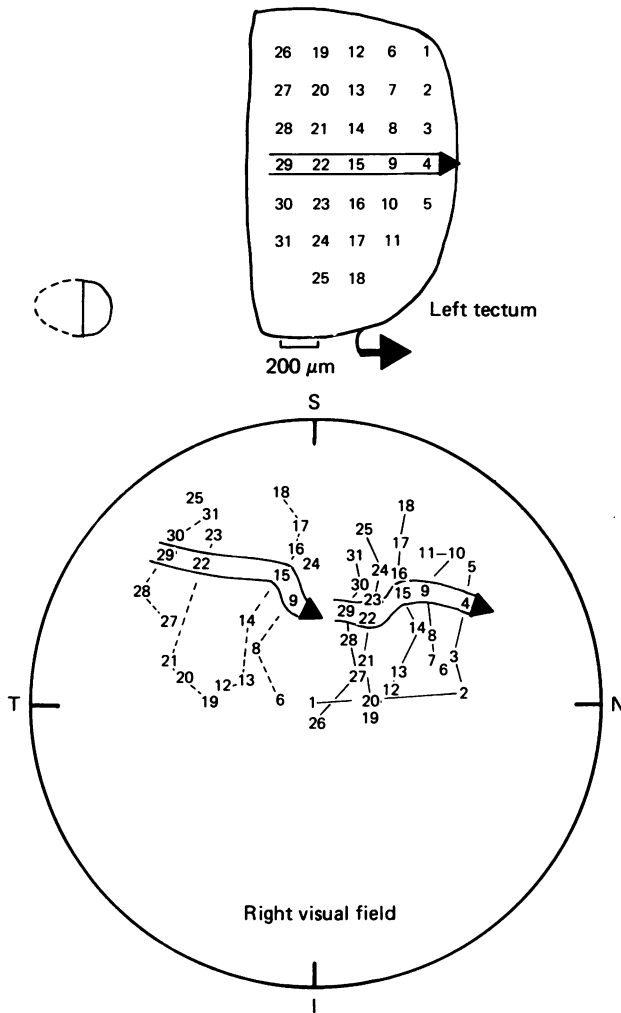


Fig. 1. The visuotectal projection mapped in a fish of the s.r. group 134 days after ablating the caudal tectum. The optic nerve was left intact and the fish was maintained in constant light. The numbers on the outline of the dorsal surface of the left tectum (above) indicate the positions of the recording electrode. The arrow is positioned along the medial edge of the tectum and points rostrally. The standard Aimark perimetric visual field chart extends out to 100° and the numbers indicate the positions of the receptive field centres recorded from the corresponding electrode positions. On the perimetric chart, S: superior, I: inferior, N: nasal, T: temporal. The sketch to the mid-left of the diagram summarizes the operation performed. Continuous lines join field positions appropriate to the normal intact projections. The dashed lines join coherent sets of duplicate field positions. The arrows containing numbers indicate the polarity of the projections along the rostrocaudal axis of the tectum. The same conventions are used in all the following visuotectal maps. This fish was previously mapped at 57 days post-operatively when less extensive duplications were present.

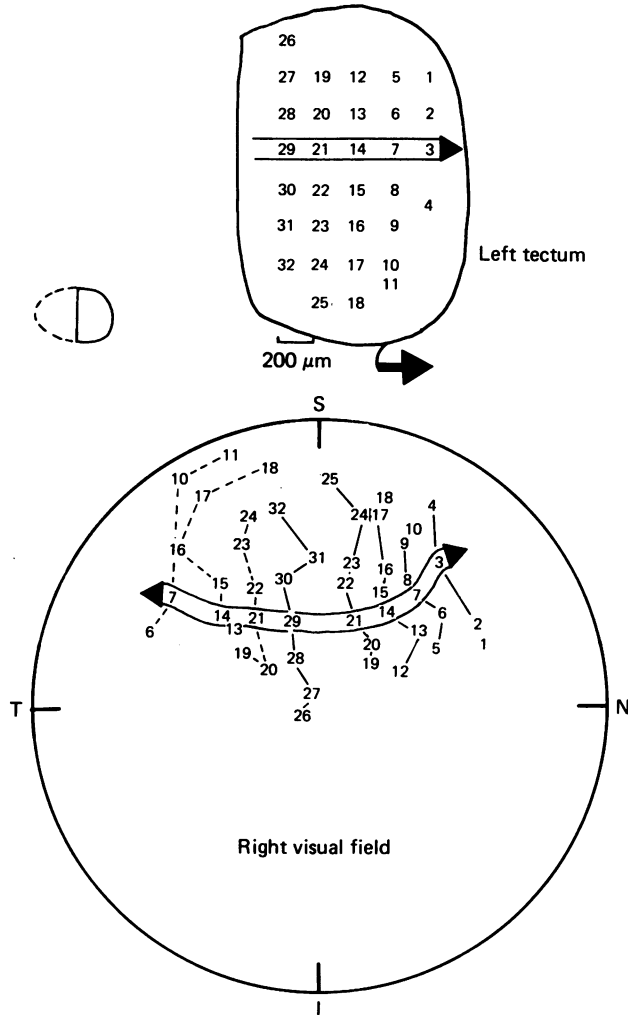


Fig. 2. The visuotectal projection mapped in a fish of the i.r. group 154 days after ablating the caudal tectum. The optic nerve was left intact and the fish was maintained in daylight conditions. The dashed lines join a coherent set of duplicate field positions. Note that the polarity of the duplicate map across the mediolateral axis of the tectum is normal, but is reversed along the rostrocaudal axis. Similar maps were obtained in this fish at 96 and 112 days post-operatively.

TABLE 2. The number of fish used and the incidence of reversed ordering of the projection along the rostrocaudal axis of the tectum (in brackets). Only fish with intact optic nerves are shown because there were too few duplicated field positions to form a map in fish with crushed optic nerves

Lighting conditions	Optic nerve left intact		
	Size of remaining tectum		
	s.r.	i.r.	l.r.
Constant light	7 (2)	6 (2)	7 (0)
Daylight lighting	—	6 (3)	8 (1)



positions were not obtained from the most rostral recording positions, even after the longest post-operative times (Fig. 2).

In the original study of Gaze & Sharma (1970) it was thought that a portion of the normal projection to rostral tectum had remained intact. However, since some compression had occurred in the caudal regions of their tecta, some reorganization of the projection in more rostral regions cannot automatically be ruled out. To examine whether any compression had occurred in the apparently intact projection, two tests were applied. First, the magnification factor of the assumed normal projection was calculated for seven fish and found to be  $16.9 \pm 2.0 \mu\text{m}/\text{deg}$  which does not differ significantly from that of controls ( $17.9 \pm 3.2 \mu\text{m}/\text{deg}$ ). The magnification factor for the assumed superimposed projection was  $15.4 \pm 3.1 \mu\text{m}/\text{deg}$  which does not differ significantly from the control value. Secondly, the maps obtained soon after the initial surgery were compared with those of the same fish after duplication had occurred. Fig. 3 shows the results of this analysis for positions mapped along the caudal edge of the remaining tectum where changes, if any, in the normal, intact projection would be most likely to occur. When two field positions were found for a particular electrode position, only the more nasal position was plotted since it would be more likely to form part of the normal projection. If the two sets of data are compared it can be seen (Fig. 3) that the positions are very similar for each size of tectal remnant. We conclude that the projection from nasal field is due to the original intact set of fibres uninfluenced in position by the duplicate map. The only evidence for any interaction between the two components of the duplicate projections was that, although responses could usually be recorded from both projections at the same depth in the tectum, the responses were seldom of the same strength. At most electrode positions stimulation of one receptive field would give a strong response but this would grow weaker as the electrode was advanced through layers B, C and D, while the response to stimulation of the other field would grow stronger. As the electrode was advanced further the process often reversed again. There was no consistency in the distribution in depth of this functional segregation of the two projections except for a tendency for optimum responses to be recorded in uppermost layers from the normal projection, then from the duplicated projection and then again from the normal projection. The receptive fields of the two projections were of approximately the same size ( $12\text{--}15^\circ$ ) except along the caudal edge of the tectal remnant where larger receptive fields ( $20\text{--}30^\circ$ ) with less distinct borders were sometimes recorded, which may correlate with the slight disorganization of the map sometimes found on this portion of the tectum. In projections showing elements of duplication and compression, or in projections with only occasional duplicated positions, the two receptive fields recorded at a single electrode position could sometimes overlap to form an oval or figure-eight shaped field.

The duplicate projections found in the fish of the s.r. and i.r. groups (without optic nerve crush) appeared to remain duplicate for the period over which they were examined (e.g. Fig. 2). The only exceptions were found in two fish of the l.r. group, in which initially duplicated projections were subsequently found to be compressed when the fish were remapped 95 days post-operatively.

Lighting conditions affected the rates of the processes responsible for the formation of duplicate projections but did not affect the frequency of their occurrence. Under

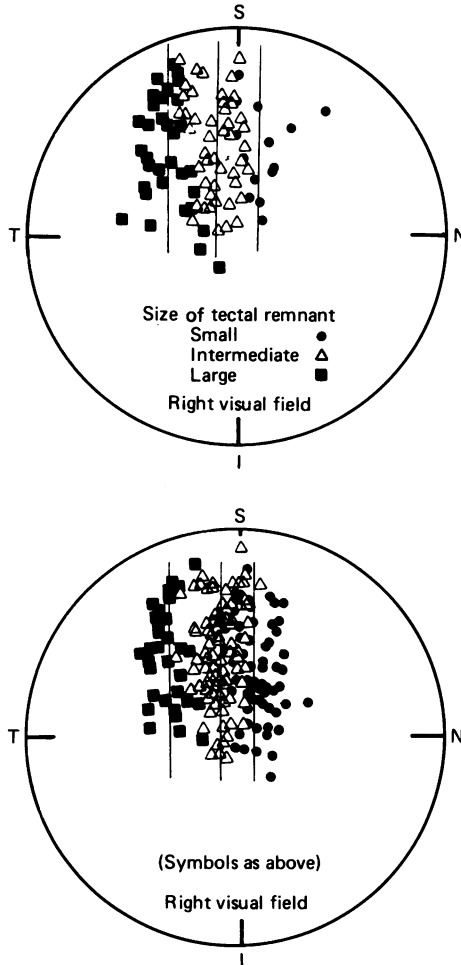


Fig. 3. Top: the receptive field positions corresponding to points mapped along the caudal edge of the remaining portion of tectum after varying amounts of the caudal tectum had been ablated. The optic nerve was left intact. All fish mapped 7-37 days post-operatively which *did not have* duplicate positions are included. The vertical lines indicate the mean horizontal extent of the visual field positions for each group. Fish from both lighting regimes were used. Bottom: the receptive field positions corresponding to points mapped along the caudal edge of the remaining portion of the tectum in the same groups of fish shown in the top diagram. Those fish which were mapped 30-154 days post-operatively and which *had* duplicate positions are included. When two fields were found at a recording site only the most nasal field position was plotted.

conditions of constant illumination the region in which duplications were detected expanded rapidly until about 60 days post-operatively and levelled off thereafter. By contrast this process was slower in fish maintained under daylight conditions and appeared to level off at about 90 days post-operatively. The time course of these events can be seen in Fig. 4 which also gives a graphical indication of the effect of the different tectal sizes on the occurrence of duplicate field positions for the various groups. This Figure shows the relationship between the number of duplicated

positions as a percentage of the total number of positions mapped in each tectum and the size of the tectal remnant. The projections in fish with intact optic nerves took longer to stabilize than those of fish in which the optic nerve had also been crushed. The l.r. group was slowest, and also showed more variability, in the time taken for disconnected fibres to re-form detectable terminals on the tectal remnant. Projections consisting of a normal map to the remaining tectum could be found as long as 82 days after the operation in l.r. fish.

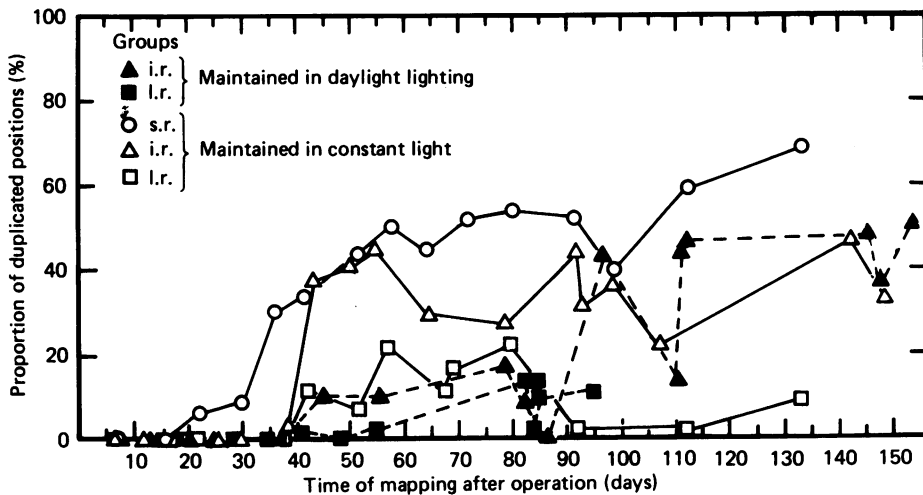


Fig. 4. Graph showing the proportion of duplicated field positions (number of duplicated positions found/total number of positions mapped), plotted against time after the initial operation (ablation of varying amounts of tectum); s.r., i.r. and l.r. = small, intermediate and large rostral remnants respectively. A graph of similar form is obtained if the total number of duplications found in each fish is plotted along the ordinate. Each point is the value obtained in a single fish, some of which were mapped several times. All fish from Table 1 A are included. An s.r. group was not examined under daylight conditions. Since the tectum was usually mapped in a grid with  $200\ \mu\text{m}$  spacings between the electrode positions this graph also gives a rough indication of the relative area of the dorsal tectal surface supporting a duplicate projection.

## II. Ablation of lateral tectum

Ablation of caudal tectum primarily affects the rostrocaudal tectal axis, which, approximately, is the direction of travel of fibre bundles crossing the tectum. It is therefore conceivable that some aspects of our results are dictated by this feature of the anatomy. There is some evidence (Jacobson & Gaze, 1965; Gaze & Sharma, 1970) that the rostrocaudal axis is different from the mediolateral. For this reason a parallel series of experiments were performed in the mediolateral axis. To avoid damaging all the fibres as they entered the tectum we ablated lateral tectum only.

In four fish the lateral third of the dorsal tectum was ablated and in a single fish the lateral half. The optic nerve was left intact and the fish were maintained in constant light. All fish were mapped 21–22 days after the operation to determine the extent of the ablation in terms of the visual field scotoma. Fig. 5 shows an example

of one of these. The same fish was remapped at 65 days and again at 157 days post-operatively. The last map (Fig. 6) shows a virtually complete restoration of the projection to the remaining tectum, indicating that the disconnected fibres had formed new terminations in a compressed projection with occasional duplications. These duplications were unlike those found after caudal tectal ablations, in that the

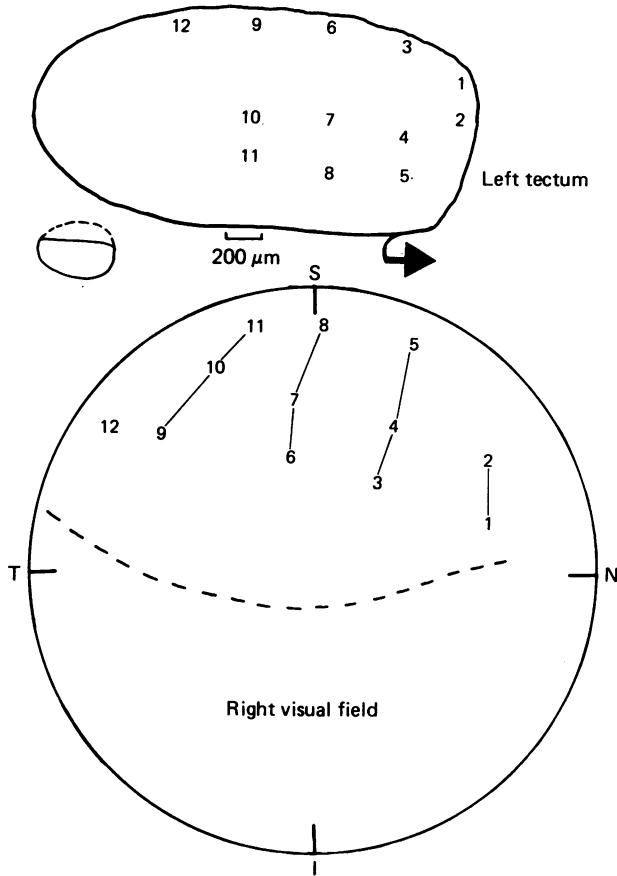


Fig. 5. The visuotectal projection mapped 21 days after ablating the lateral third of the dorsal tectum. The optic nerve was left intact and the fish was maintained in constant light. The dashed line on the field map shows the extent of the field which can be mapped on a normal dorsal tectum.

duplicating fibres did not form a well ordered projection and were restricted to the peripheral caudal and medial regions of the remaining tectum. These aberrant responses were first seen when the fish was mapped at 65 days post-operatively. Responses at similar aberrant positions were also seen in three other fish mapped at 65–66 days and were still present, though they were less extensive, in two of these fish re-mapped at 157 and 182 days. In the third fish, mapped at 181 days post-operatively, no duplications could be found but the responses in general were poor. Usually responses from the duplicate portion of the projection were weaker than those of the 'normal' projection. We are inclined to believe that these duplicate

responses are not explained by the difficulties associated with mapping extreme peripheral field (see Methods) on the grounds that the receptive fields could be well localized using small stimuli and because these responses only appeared after an interval of about 65 days. They were separable from normal responses in depth in the same way as duplicate responses following caudal ablations.

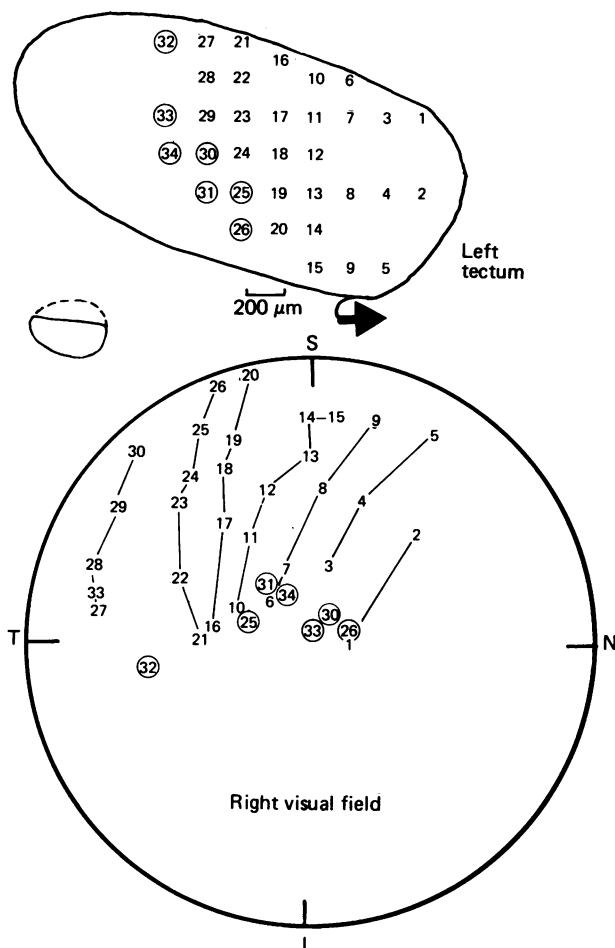


Fig. 6. The visuotectal projection mapped 157 days after ablating the lateral third of the dorsal tectum. The optic nerve was left intact and the fish was maintained in constant light. This fish was previously mapped 21 days post-operatively (Fig. 5). The circled numbers indicate aberrant terminations which did not form part of the coherent ordered projection (joined by lines).

### III. *An investigation of the factors responsible for the formation of projections of reversed polarity*

The novel finding of two maps of opposite polarity in some fish with duplicate projections seemed of sufficient importance that further experiments were conducted in an attempt to define some of the factors responsible for polarity reversal. The normal projection to the most caudal region of the tectal remnant was of particular

concern, since in all our reversed cases this projection remained intact whereas in other studies which did not observe reversal (Gaze & Sharma, 1970; Meyer, 1977; Marotte *et al.* 1981) the fibres in the caudal regions were displaced. Thus it appeared that the stability of the most caudal fibres may play a part in causing reversal of the transposed portion of the duplicate projection. We devised an experiment where, as in the case of duplication, fibres were induced to transpose onto rostral tectum by ablating caudal tectum, but with the difference that the only intact fibres remaining on the rostral tectum were those along the caudal edge. The method

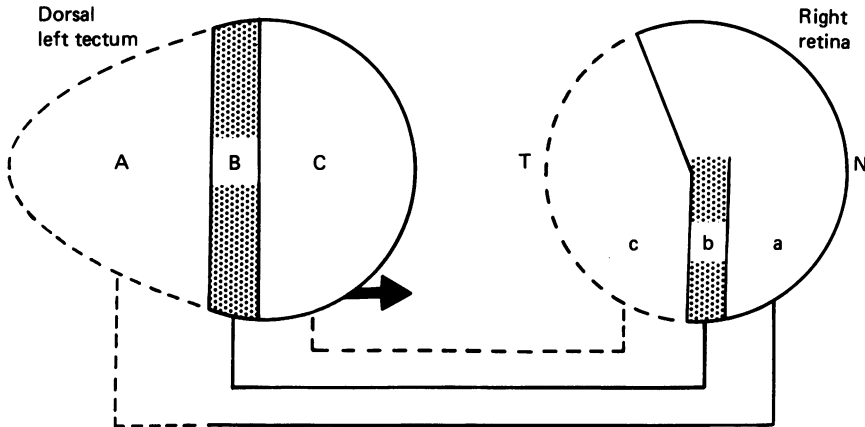


Fig. 7. Experiment to examine the effect of a caudally located strip of intact fibre terminations on the polarity of transposed projection. Fibres from nasal retina (area a) were induced to transpose onto rostral tectum (B and C) by ablating their normal termination sites (A), or by dividing caudal from rostral tectum with a Millipore filter barrier. All but the most caudal edge (region B) of the rostral tectum was denervated by ablating temporal retina (c). The optic nerve was left intact. It should be noted that only *ventral* retina projects to dorsal tectum: hence the partial retinal stippling.

of achieving this is shown in Fig. 7. An initial series of six fish was prepared as follows. The rostral tectum was denervated by ablating the temporal retina (areas C and c in Fig. 7). The presence of the remaining terminations was verified by mapping the fish 10–14 days later. The surviving retina innervated all but the most rostral 600–1000  $\mu\text{m}$  of the remaining dorsal tectum. Immediately after this the caudal portion of the tectum was ablated in four fish, leaving a strip of intact optic terminals approximately 400  $\mu\text{m}$  wide (area B in Fig. 7) at the caudal edge of the remaining tectum. In the remaining two fish the same effect was obtained by dividing rostral from caudal tectum with a 'Millipore' filter barrier to allow for possible re-expansion of the projection at a later time (Yoon, 1972*a, b*). All fish were maintained in diurnal lighting conditions. Of the six fish operated one gave a projection of reversed polarity when it was mapped 34 days after the caudal tectal ablation (Fig. 8). The intact strip of fibres with normal polarity was detected along the caudal edge but the remainder of the projection to the rostral tectum was reversed. The responses in rostral tectum were noticeably weaker than those normally obtained in duplicated projections but the fields were easily localized. The remaining five fish were mapped at various times

post-operatively and the ordering of the transposed projections were as follows: 33 days, predominantly normal ordering; 36 days, grossly disordered; 48 days, reversed polarity although irregularly ordered; 50 and 60 days (both fish had 'Millipore' barriers), disordered maps with some indication of polarity reversal in places. No responses were detected in the region caudal to the 'Millipore' barriers.

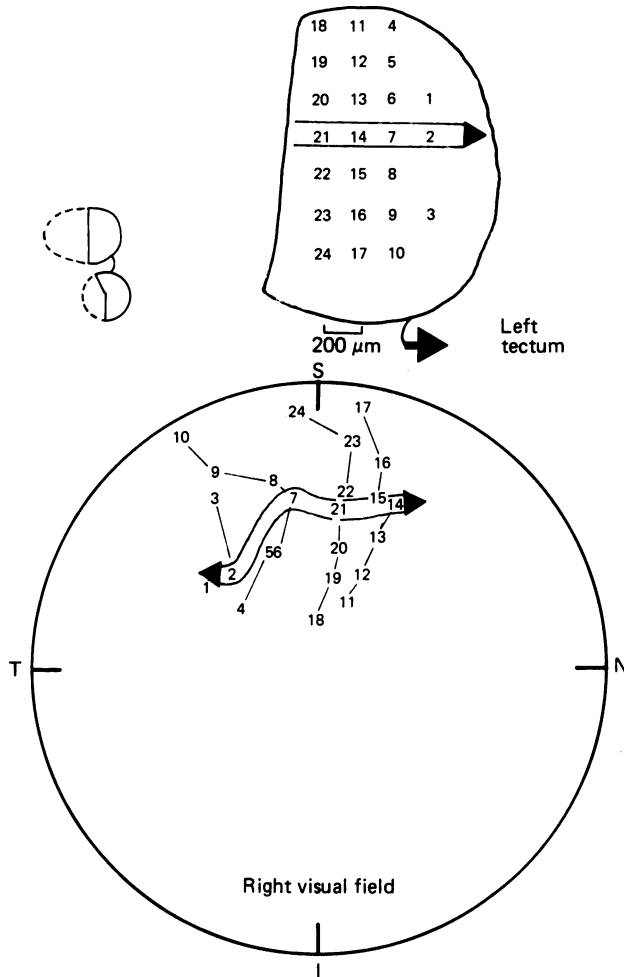


Fig. 8. The visuotectal projection mapped 34 days after the operative procedure shown in Fig. 7. The caudal tectum was ablated. The optic nerve was left intact and the fish was maintained in daylight lighting conditions.

In view of the partial success in producing reversals in this series a further thirteen fish were prepared in the same way, nine with 'Millipore' filter barriers to separate rostral from caudal tectum, and four with the caudal tectum ablated. In this series both the retinal ablations and the tectal operations were performed on the *same* day and the presence of the intact strip along the caudal edge could not be checked initially by mapping because of the condition of the operated eye. In the case shown in Fig. 9, mapped 64 days post-operatively, a narrow strip of the caudal tectum

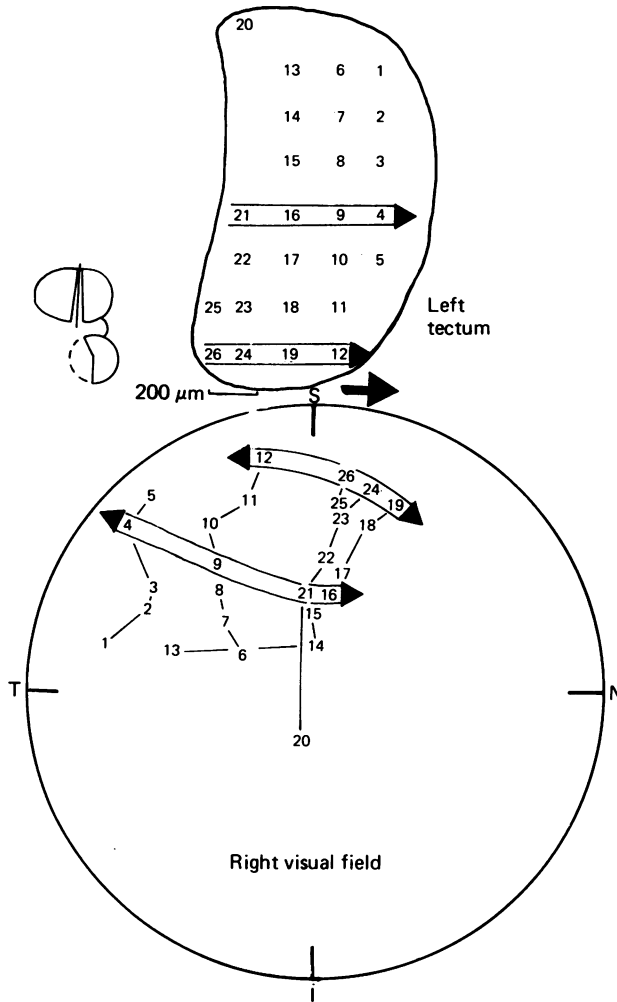


Fig. 9. The visuotectal projection mapped 64 days after the operative procedure shown in Fig. 7. The tectum was divided by a 'Millipore' filter barrier. Field positions 14–26 would be found in approximately the same locations in a normal visuotectal projection. The caudal tectal fragment, in which no responses were detected, is not drawn. The optic nerve was left intact and the fish was maintained in daylight lighting conditions.

received the normal retinal projection to that area while the remaining tectum received a projection of reversed polarity. Of the nine fish with 'Millipore' barriers, those mapped at 59 days, 60 days (three fish) and 105 days post-operatively gave similar projections to that shown in Fig. 9 as did one of the fish with a caudal tectal ablation mapped 104 days post-operatively. One of the four fish with a caudal tectal ablation could not be mapped due to a cataract. The remaining fish of both groups gave projections of predominantly reversed polarity with some minor irregularities. In general the responses obtained from these reversed maps in the second series of fish were good and had sharply localized, small receptive fields (10–15°).

One of this series of fish, mapped 105 days post-operatively, showed a particularly



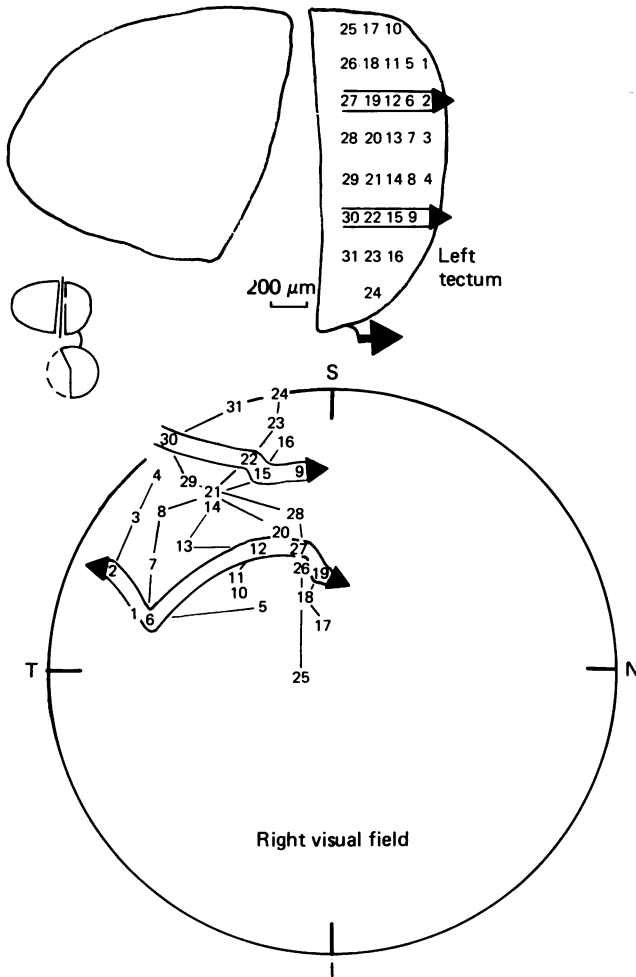


Fig. 10. The visuotectal projection in a fish mapped 105 days after having undergone the operative procedure shown in Fig. 7. The tectum was divided by 'Millipore' filter barrier. No responses could be detected on the remaining caudal portion of the tectum. Note that the lateral region of the rostral fragment contains a map of reversed polarity while in the medial region the polarity is normal. This is probably related to the fact that the intact band of fibres has only remained in lateral tectal regions (positions 17-19 and 25-27) while being displaced in medial regions. A similar projection was found when the same fish was remapped 425 days post-operatively. The optic nerve was left intact and the fish was maintained in daylight lighting conditions.

unusual map (Fig. 10). The transposed projection to the medial portion of the tectum was normally ordered but the projection to the lateral portion was reversed. The responses obtained from positions in the reversed portion of the transposed projection were generally stronger than those elsewhere. The barrier was removed and this fish was left for a further 320 days to see whether the projection remained stable over long periods. On remapping a similar projection was obtained on rostral tectum but a small projection of normal polarity was also found on the caudal fragment.

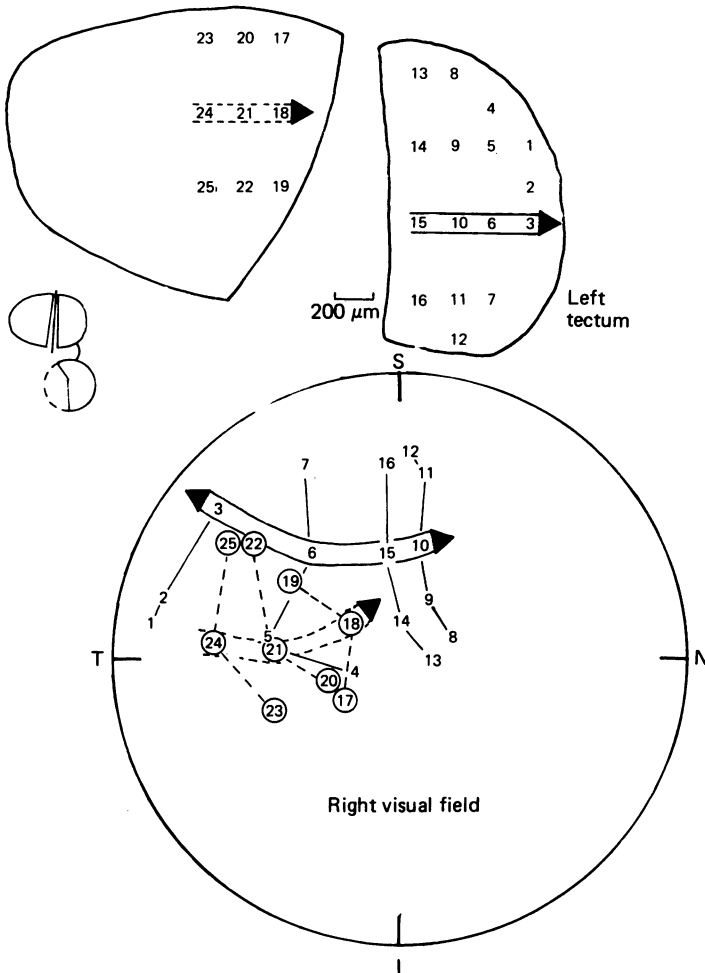


Fig. 11. The visuotectal projection in a fish mapped 206 days after having undergone the operative procedure shown in Fig. 7. The tectum was divided by 'Millipore' filter barrier. The barrier was removed after the first mapping at 60 days post-operatively when a similar projection was obtained on the rostral portion of the tectum. The polarity of the caudal projection (circled points and dashed lines) is normal. Note that the region of the retina stimulated from field positions 4-6 and 17-21 projects to quite separate tectal regions. This was seen in several of this series of fish when mapped long-term. The optic nerve was left intact and the fish was maintained in daylight lighting conditions.

In one of the fish with a 'Millipore' barrier, mapped at 105 days, weak responses could also be obtained in the region of the tectum caudal to the barrier. The projection to this caudal region was of normal polarity and came from the same area of the retina as the reversed polarity projection on the rostral tectum. In three of this series of fish the barrier was removed after the first mapping. When these fish were re-mapped at 206 days post-operatively the reversed projection was still present but an additional projection of normal polarity had formed in the caudal tectum (Fig. 11).

This suggests that fibres mediating the reverse projection lay near the cut edge and

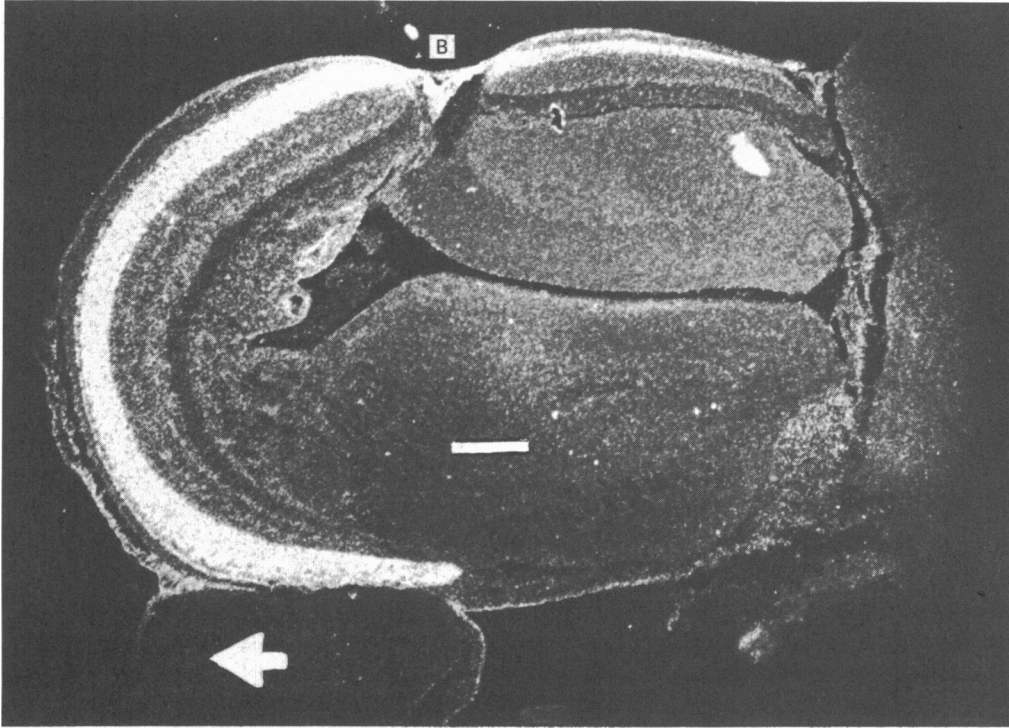


Fig. 12. A dark-field autoradiograph obtained from the fish whose visuotectal map is shown in Fig. 11. The fish was injected intraocularly with tritiated proline immediately after the electrophysiological recording and fixed 24 hr later. The arrow points rostrally on the parasagittal section taken through the mid line of the left tectum. B indicates the position of the 'Millipore' filter barrier which had initially separated rostral from the caudal tectum. Bar = 20  $\mu$ m.

were damaged by removal of the barrier; this is evidence that they may originally reach rostral tectum by growing from the cut tectal edge. One fish was injected intraocularly with tritiated proline immediately after mapping and one of the resultant autoradiographs is shown in Fig. 12. The superficial termination layer of the rostral tectum which supported the projection of reversed polarity was heavily labelled over its full extent. Thus the autoradiography shows that, in contrast to the reversed duplicate maps (e.g. Fig. 2), the reversed maps forming on denervated tecta extend to the rostral pole. The caudal bias in the density of the labelling seen in similar experiments by Meyer (1976) is not evident.

The two other fish mapped 206 days post-operatively were used in a further experiment to see whether the fibres forming the reversed projection, if cut, would again form a reversed projection. The optic nerve supplying the tectum was crushed and the tectum was re-mapped at regular intervals. The first localizable responses to be detected were near the caudal edge of the rostral portion of the tectum in a fish 25 days after crushing of the nerve. This area appeared to be innervated by fibres from the mid-nasal region of the retina, suggesting that the polarity of the projection was no longer reversed. Visually evoked responses could be recorded from more

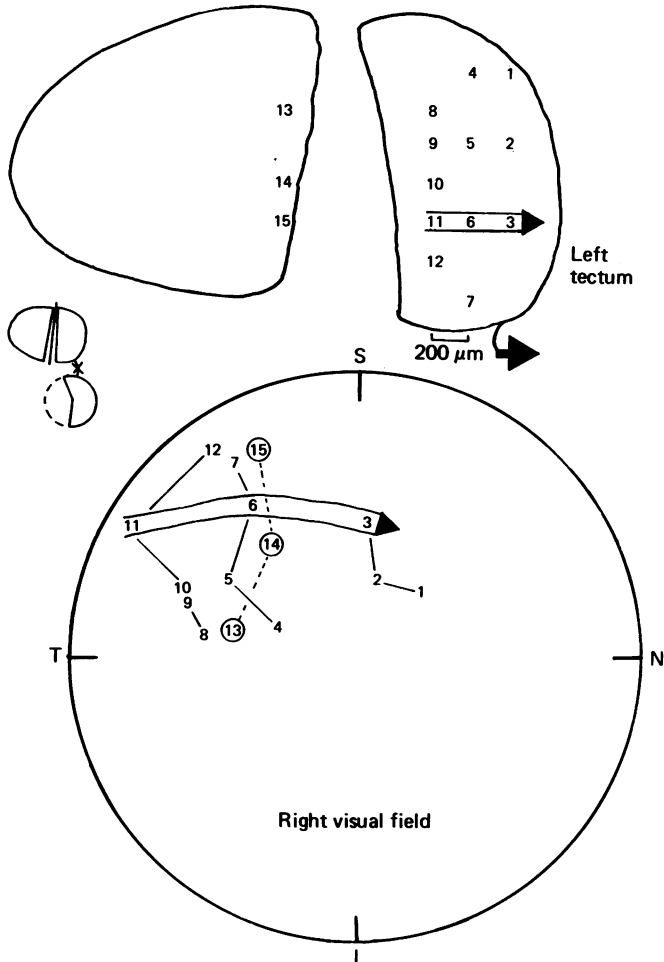


Fig. 13. The visuotectal projection mapped 59 days after crushing the optic nerve of a fish which had showed a projection of reversed order on the rostral portion of the tectum when mapped 206 days post-operatively, i.e. a similar projection to that shown in Fig. 11. The fish initially underwent the operative procedure shown in Fig. 7. The tectum was divided by a 'Millipore' filter barrier. The ordering of the projection shown here is normal. Note that the region of retina stimulated from field positions 4-7 and 13-15 projects to quite separate tectal regions. The fish was maintained in daylight lighting conditions.

rostral tectum but they could not be localized in the field. A further map obtained from this fish 57 days after crushing showed that the fibres had formed a projection of normal polarity on the rostral remnant. Unlike the reversed maps, the responses were weak and became progressively weaker more rostrally. A few diffuse responses were obtained in the caudal portion of the divided tectum. In the second fish diffuse responses could first be obtained at 32 days post-operatively, and a projection of normal polarity was obtained at a further mapping 59 days post-operatively (Fig. 13). Some responses could be detected in the caudal portion of the tectum on stimulation of the same retinal region.

In order to exclude the possibility that the reversal phenomenon was peculiar to

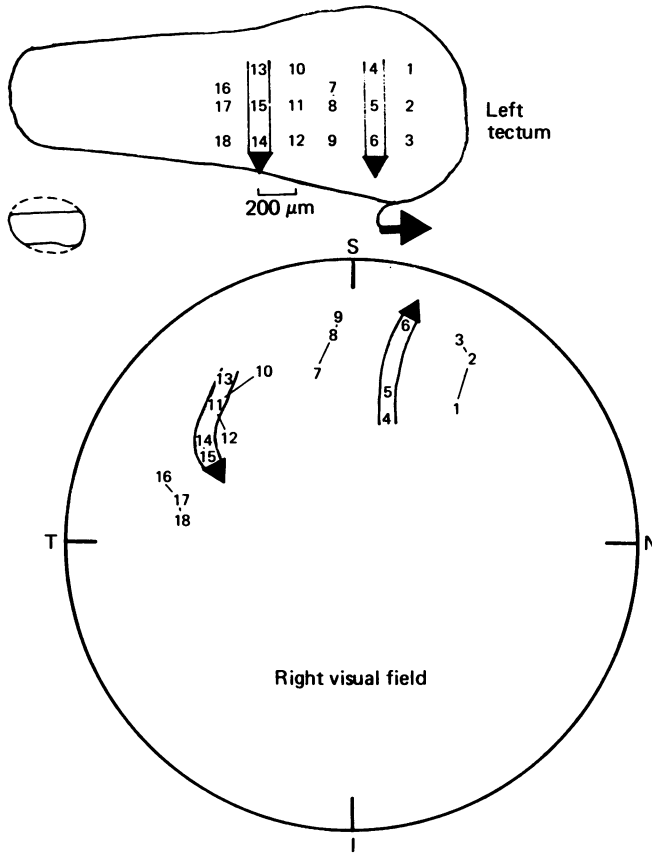


Fig. 14. The visuotectal projection mapped 72 days after ablations of the medial and lateral parts of the dorsal tectum. The optic nerve was left intact and the fish was maintained in constant light. Arrows indicate the polarity of the projection along the mediolateral axis of the tectum. Rows 10–12, 13–15 and 16–18 show a reversed polarity across this axis.

the rostrocaudal tectal axis, we used a similar strategy to force fibres to grow mediolaterally across an intact strip of fibre terminals. Between a third and a half of the lateral portion of dorsal tectum was ablated and additionally caudo-medial regions of the remaining dorsal tectum were denervated by making a small ablation in rostral tectum. This left a strip of intact terminations only along the lateral edge of the surviving tectal remnant, thus producing a situation analogous to that used to produce reversed projections along the rostrocaudal axis. This operation was performed in three fish which were also maintained under conditions of constant illumination. In two of the three fish mapped at 62 and 72 days post-operatively, the ordering and polarity of the projection to the rostral portion of the tectum was normal, but further caudally the polarity was reversed across the medio-lateral axis of the tectum (Fig. 14). The responses were good and had receptive fields of 15–20° in the region of the polarity reversal. Only poor responses could be obtained in the third fish mapped at 49 and 93 days post-operatively and the projection, although slightly disordered, was of normal polarity.

## DISCUSSION

Although reversed duplicate projections are the most novel aspect of our results their interpretation must rest heavily on our initial understanding of duplication itself and *its* relation to compression; it is with these questions that we begin the discussion. Although it is premature, in the limited state of our present knowledge, to seek any single explanatory mechanism for the phenomena described in the present paper, it is clear that our results cause grave difficulties for a number of the currently available theories of the specific nerve-fibre guidance underlying normal retinotectal projection formation, as will be made plain in the final section of the Discussion.

*Mechanisms underlying compression and duplication*

Our findings substantiate the trends in the evidence originally presented by Gaze & Sharma (1970) as regards the influence of regeneration of the optic nerve and resolve the inconsistencies in their results, e.g. one case of duplication after regeneration. We have confirmed that when the optic nerve is regenerated compression is the rule, while duplicate projections only occur to a significant extent when the nerve has been left intact, but only when sufficient amount of tectum is ablated. Although once started compression and duplication both tend to go to completion, it is clear that they are not absolute alternatives and that they do not reflect directly any categorical difference in conditions related to regeneration or non-regeneration of the nerve. Compression rather than duplication tended to occur in all l.r. cases regardless of whether the optic nerve had regenerated or not, whereas in the s.r. group only duplicate projections were obtained when the nerve was left intact. In the i.r. group either duplicate projections, or partially compressed, partially duplicated projections, were found. Thus, if the numerous studies which have failed to find duplication (Yoon, 1971, 1972*a, b*, 1975, 1976; Meyer & Scott, 1977; Marotte *et al.* 1977; Cook, 1979; Wye-Dvorak *et al.* 1979) had involved removal of less than half the caudal tectum, then duplicate responses are not to have been expected, even when the nerve was intact. Alternatively the possibility exists that in some cases of non-compression the occurrence of duplicate responses may have been overlooked, or, under certain conditions (Levine & Jacobson, 1975; Glastonbury & Straznicky, 1978), duplicate projections may have been unrecordable.

The phenomena described here document in consistent fashion and under controlled conditions two modes of fibre behaviour which are widely but confusingly described in the literature, namely overlay or mutual exclusion. Overlay occurs in normal binocular projections to the superior colliculus in mammals and is sometimes seen in lower vertebrates when both eyes are caused to innervate one tectum (Gaze & Jacobson, 1963; Sharma, 1973); one set of overlapping fibres may be arranged independently of the retinal origin of the second set and of tectal location (Cronly-Dillon & Glazner, 1974; Levine & Jacobson, 1975; Schmidt, 1978). There are numerous reports of mutual exclusion by sets of fibres from one or both eyes resulting in a situation in which neither subpopulation can occupy their normal tectal locations. Segregation of initially overlapping projections occurs normally in the development of mammalian binocular projections (Land & Lund, 1979; Frost, So & Schneider, 1979). In lower vertebrates a common finding is rostrocaudally running strips of

innervation from one or other eye (Levine & Jacobson, 1975; Straznicky & Glastonbury, 1979; Meyer 1979; Law & Constantine-Paton, 1980) which may also develop from initially homogeneously overlapping projections (Lo & Levine, 1980, Springer & Cohen, 1981). The fact that the strips run in approximately the line in which optic fibre fascicles normally cross the tectum suggests that they may result from intercalation of supernumerary fibres between normal fascicles and subsequent fasciculation by later arriving fibres in the same pattern. But on this interpretation it is hardly surprising, given the variability of the patterns of access of fibres and opportunities for secondary transposition likely to prevail in different experimental conditions, that a wide variety of different results have been reported, including large, non-intercalated displaced patches of innervation (Cronly-Dillon & Glaizner, 1974) and incompletely segregated patching (Levine & Jacobson, 1975). Our electrophysiology showed no indication of transverse patch-like segregation in duplicate projections: apart from segregation in depth, competitive fibre exclusion was only seen in the form of compression, presumably because of the broad and even pattern of arrival of transposing fibres.

Compression involves the progressive and sequential transposition of already established subsets of optic terminals; initially during regeneration of the optic nerve only the appropriate subset of fibres forms terminals in the rostral tectum (Cook, 1979), as the present results confirm. One assumes that compression is an expression of a propensity of optic fibres to compete for terminal space, an inference reinforced by the fact that displacement of the first established terminals does not occur autonomously after tectal lesions but only in the presence of fibres without alternative termination sites (Cook, 1979). The formation of duplicate projections is in principle a comparable phenomenon, but with the one difference that the pre-existing projection does not move: the disconnected fibres transpose sequentially onto the remaining tectum but independently of the pre-established fibres. Each successively added subset of fibres thus seems able to distinguish between terminals currently undergoing transposition and those of the pre-existing projection which are inert as regards the relevant competitive fibre/fibre interactions. Concerning the basis of this distinction, the greater stratification of intact as compared to regenerated projections (Attardi & Sperry, 1963) may favour transposition of regenerating fibre layers independent of intact fibre layers. Being more mature, fibres of the pre-existing projection may be less easily displaced than newly transposed terminals: compression of uncut fibres may occur after small caudal tectal ablations only because caudal fibres are laid down late in development (Meyer, 1978) and are therefore not fully mature. Alternatively, when the ablation has been large more fibres are disconnected and it may be the resulting increased pressure of fibre numbers which causes fibres to transpose rostrally at a rate which leaves no opportunity to displace intact fibres at the cut edge.

The only apparent effect of constant light on these phenomena was an increase in the rate at which the duplicate projections formed. The findings of Wye-Dvorak *et al.* (1979) help to explain why we, like many others, have failed to detect the non-compression of the optic projection described by Yoon (1975) in fish with intact nerves which were maintained in constant light after removal of the caudal tectum. Wye-Dvorak *et al.* (1979) found that this phenomenon only applies consistently to

fish of the size employed by Yoon (1975) which were larger than ours. Our fish were unaffected by the seasonal variation found by Wye-Dvorak *et al.* (1979) in that we found that duplications and compression occurred readily in winter-operated fish; this could be due to differences in rearing conditions (Horder & Martin, 1977*b*). Recently Marotte *et al.* (1981) have shown that thyroxine can induce compression or duplication under lighting and seasonal conditions in fish of a size in which they would not otherwise occur. The duplications they obtained in thyroxine-treated non-regenerated fish, though similar to our Type 2 maps, differed in that two separate but overlapping projections could not be distinguished and the maps included regions of the retina projecting to two separate regions of the tectum.

In general one must conclude that the mobility of optic terminals is subject to a wide variety of purely incidental variables and that many factors, including lighting conditions, have their effects only indirectly, perhaps endocrinologically. Variables, such as levels of hormones, should best be regarded as affecting the stability of terminals; they may determine whether or not fibres are free to express their mobility but say nothing whatsoever about mechanisms of translocation or terminal selection operating once this potential is released.

#### *Mechanisms of the control of polarity in duplicate projections*

Polarity reversal is the most surprising phenomenon to emerge from our experiments. The fact that reversed and non-reversed maps can occur in a single series of apparently identical halved tecta is hard to reconcile with any single existing theoretical framework, but the evidence available to us does not permit us to do more than speculate on the mechanisms of underlying patterns of fibre behaviour.

There are a number of situations in the literature in which fibres map without regard to the orientation of the tectal tissue: these are explicable as the direct reflexion of the pattern in which the fibres themselves are arranged as they enter the tectum (Cunningham & Speas, 1975; Bunt, Horder & Martin, 1977, 1979; Chung & Cooke, 1975; Thompson, 1979) as are cases of normal maps in rotated tectal grafts (Jacobson & Levine, 1975; Martin, 1978) and ordered maps established in non-visual regions of the central nervous system (Sharma, 1981). It is tempting to think that our reversed polarity maps are also a consequence of the direction of growth of fibres (e.g. from the caudal cut edge of the tectum rostrally), and termination on a first come, first served basis (Bunt *et al.* 1977). Although we have no direct evidence on the point, the experiments involving removal of the 'Millipore' barriers suggest that fibres of the duplicate projection remain at the caudal edge and therefore that they reach rostral tectum by growing rostrally rather than dying back along their original paths of transit through rostral tectum. However, in the case of non-reversed duplicate projections, assuming that the relevant fibres also follow caudo-rostral paths, pattern of arrival cannot be the only consideration.

In the light of our analytical experiments we propose additionally that reversal of polarity may be prompted by the presence of intact fibre terminations restricted to the region of the cut edge. No reversals occurred in similar experiments if intact fibres were deliberately not preserved in the rostral tectal fragment (Yoon, 1972*b*), and no reversals were seen by Gaze & Sharma (1970) or in the comparable maps which we obtained where the intact fibres along the caudal edge had been displaced



compressively by fibres which normally terminated on more caudal tectum (see also Fig. 10). Reversals were also less common in fish where retinal ablations were performed 1–2 weeks prior to tectal ablation, which may have allowed time for destabilization of the intact fibres.

It is very probable that in all situations where fibres are transposing onto foreign tectum, as in compression and duplication, the first fibres to move onto the tectal remnant are those which originally terminated nearest to the cut edge; early mapping in each series confirmed this as did long term residual gaps in the field which were usually temporal. Thus the two forms of duplicate map come to differ by virtue of the fact that in non-reversed projections, the first fibres to move continue moving rostrally as they would in compression, but in reversed projections these first fibres remain stable due, perhaps, to lack of competition, as would be the case when rostral tectum was denervated by retinal ablation, or due to some aspects of retinotopic relatedness of the fibres with intact fibres at the cut edge. The latter cannot be a powerful factor given that fibres in non-reversed duplications are not influenced by the intact fibres they are moving past. The difference in the results obtained when the rostral tectum is partially denervated compared to those obtained in wholly denervated tectum may be explained by the pathways provided by the axons of remaining intact terminals which allow the regenerating fibres to grow more rapidly onto the denervated areas of rostral tectum, thus further reducing inter-fibre competition. The fact that, once started, each form of duplication tends to go on to completion implies that conditions do not change during transposition of the fibre subsets, or that transposing fibres themselves maintain retinotopic relatedness in some way.

Other occasional instances of reversal in the literature (Meyer, 1979; Yoon, 1972*b*) may have involved chance creation of circumstances similar to ours. However, the notably contrasting results of superficially similar experiments by Cronly-Dillon & Glazner (1974) and Sharma & Tung (1979) suggest that precise conditions in time and space of arrival of fibres must influence the outcome considerably.

#### *Implications for theories of map formation*

At the level of individual optic fibres the evidence for the existence of tectal cues guiding fibres to appropriate termination sites is poor (Horner & Martin, 1978). Most evidence for 'tectal specificity' is based on the matching of map orientation to tectal orientation or to tectal laterality and on fibre selection of transplanted tectal grafts. Such evidence involves large numbers of fibres and gross regional distinctions in the tectum (reviewed by Horner & Martin, 1978, Bunt *et al.* 1979). These effects could be mediated by few and diffuse tectal regional markers; their diffuseness is indicated by the ability of one tectal region to correct the polarity of entirely foreign sets of fibres (Bunt *et al.* 1979).

The findings we have reported here are yet further examples of results incompatible with hypotheses of map formation based on chemoaffinity mechanisms (Sperry, 1943*a, b*), or its variants (Meyer & Sperry, 1976), founded on a specific matching of individual nerve fibres to their termination sites. Duplicate maps are the most obvious example of this incompatibility because if specific tectal cues are to be held responsible for locating the fibres of the normal map on rostral tectum they cannot

simultaneously be responsible for organizing the second, transposed map; the original tectal cues cannot have altered because the normal map remains in position. Fibre self-ordering models (Hope, Hammond & Gaze, 1976; Willshaw & von der Malsburg, 1976, Cook & Horder, 1977; Cook, 1979), by definition, cannot explain retinotopic discontinuities in experimental projections. Thus to account for duplicate maps these models would have to make further arbitrary assumptions about the autonomy of the fibre sub-populations involved. Our results showing segregation of terminals in depth in animals with duplicate maps and maps with mixed compression and duplication show that there is interaction between the two subsets of fibres, and on these models this might have been expected to lead to a coherent continuous projection. The map showing part-normal, part-reversed polarity (Fig. 10) is particularly relevant in this regard since the respective subpopulations of fibres must both have regenerated into the tectum at approximately the same time and yet did not form a coherent map.

Models involving induction of cues on the tectum by the retinal fibres themselves (Meyer & Sperry, 1976; Schmidt, 1978; Gaze, 1978; Gaze & Straznicky, 1980) also invoke specific matching between retinal and tectal cues to account for the maps formed during regeneration and thus are subject to the same difficulties mentioned above for the chemoaffinity models. A further difficulty for the cue-induction hypothesis is our finding that regenerating fibres re-form projections of normal polarity on tecta which had hitherto supported reversed polarity projections of the same fibres for lengthy periods; this is consistent with other instances in which the regenerated maps differed from those previously existing on the tectum (Martin, 1978; Cook, 1979).

Irrespective of their tectal location, which is determined largely by patterns of access of fibres, and even in the face of a non-correspondence in polarity of fibre projection and tectum, fibres are remarkably resistant to experimental disruption of retinotopic ordering. The ease with which a fibre population can switch its polarity while maintaining retinotopicity suggests that the phenomenon reflects two alternative patterns of termination intrinsic to an ingrowing fibre population. In mammalian binocular projections to the tectum the ipsilateral retina has to map with a naso-temporal ordering opposite to that of its contralateral tectal projection in order to bring about correspondence of binocular visual field representation, and in lower vertebrates a similar polarity reversal occurs at the mid-brain-diencephalic border (Scalia & Fite, 1974), so map reversal is an essential feature of normal development. Ipsilateral projections can readily be caused to display these two forms of polarity under experimental conditions in mammals (Cunningham & Speas, 1975; Thompson, 1979) and lower vertebrates (Bunt *et al.* 1977). Regenerating fibres travelling by aberrant routes from caudal tectum are known to be able to form projections of normal polarity (Horder, 1974). The present results suggest that tectal cues, being perhaps of a simple indirect kind, may operate only by triggering modes of behaviour intrinsic to fibre populations.

It is a pleasure to thank Mrs C. Edgar for help with the histology and Miss D. Weedon for assistance with the drawings.

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