Interactions between compound and normal eye projections in dually innervated tectum: a study of optic nerve regeneration in *Xenopus*

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SUMMARY

Right compound eyes were formed in *Xenopus* embryos at tailbud stages by the fusion of two nasal (NN), two temporal (TT) or two ventral (VV) halves. The left eye was kept intact. Two to four weeks after metamorphosis the optic nerve from the intact eye was severed to induce bilateral optic nerve regeneration. The contralateral retinotectal projections from the compound eye and the induced ipsilateral projections from the intact eye to the same (dually innervated) tectum were studied by [³H]proline autoradiography and visuotectal mapping from 3 to 6 months after the postmetamorphic surgery. The results showed that the NN, TT and VV projections, in the presence of optic fibres from the intact eye failed to spread across the whole extent of the dually innervated tectum. Unexpectedly the bulk of the regenerating projection from the intact eye was confined to the previously uninnervated parts of the dually innervated tecta, the caudomedial region in TT, the rostrolateral region in NN and the lateral region in VV eye animals. The partial segregation of the two populations of optic fibres in the dually innervated tectum has been taken as a further indication of the role of fibre–fibre and fibre–tectum interactions in retinotectal map formation.

INTRODUCTION

The orderly point-to-point correspondence between the retina and the contralateral tectum has been well documented in frogs both by morphological (Lázár, 1971; Scalia & Fite, 1974) and electrophysiological (Gaze, 1958) methods. The nasotemporal axis of the retina is represented along the caudorostral tectal axis and the dorsoventral axis of the retina along the lateromedial tectal axis. In larval and adult amphibia and in fish when the retinotectal connexions are severed by optic nerve section, optic fibres are able to regenerate and eventually they restore their spatial correspondence with the tectum to a remarkable degree of normality (for review, see Gaze, 1978).

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Although the formation or the reformation of the retinotectal map has recently received particular attention, many aspects of the underlying mechanisms remain obscure. The most comprehensive hypothesis to date to account for the formation of connexions between the eye and the tectum has been formulated by Sperry (1951, 1963). He proposed that during development retinal ganglion cells and tectal neurons acquire matching cytochemical labels denoting their positions within the populations of retinal and tectal cells. Each ingrowing ganglion cell axon, carrying the position-related cytochemical label, seeks out a neuron in the tectum with a corresponding label. The sequential connexions between matching pairs of retinal and tectal cells result in the formation of the orderly topographic map.

It is likely that at least the reformation of the retinotectal map involves selective affinities between the retinal ganglion cell axons and tectal neurons (Sperry, 1963; Gaze, 1978). The existence of retinal positional markers has been demonstrated from early embryogenesis in *Xenopus* (Gaze, Feldman, Cooke & Chung, 1979; Sharma & Hollyfield, 1980). Similarly tectal positional markers which serve as targets for incoming optic fibres have also been reported (Levine & Jacobson, 1974). Experiments with fused eye fragments in *Xenopus* suggest furthermore that there is a stable programme for generating retinal positional markers (Straznicky & Gaze, 1980; Gaze & Straznicky, 1980a, b). On the other hand, retinotectal size-disparity experiments in frogs have shown that the retinotectal spatial relationship is modifiable. Following partial retinal removal expansion of the hemiretinal projection occurs across the whole tectum (Gaze, Jacobson & Székely, 1963; Straznicky, Gaze & Keating, 1974; Straznicky, Tay & Lunam, 1978). Similarly partial tectal lesion brings about a compression of the whole retinal projection on to a half tectum (Udin, 1978). On account of these results and of recent observations in goldfish it has been suggested that the observed modifiability of the retinotectal spatial correspondence is due to changes in tectal positional markers (Schmidt, 1978). Willshaw & von der Malsburg (1979) have subsequently proposed an elegant model for a possible mechanism of tectal marker induction. According to the model the tectum is initially devoid of markers which are later induced in the tectum by incoming optic fibres. It is also presumed that tectal markers decay after the removal of the resident optic fibre projection.

The present paper is one of a series in which we study optic nerve regeneration in the compound eye paradigm in *Xenopus* in an attempt to obtain further information about the mechanisms of retinotectal map formation. A right compound eye is formed by fusing two similar half-eye fragments into one eye blastema, whilst the left eye is kept intact. According to the nature of the half-eye fragment, double nasal (NN), double temporal (TT) or double ventral (VV) compound eyes can be formed besides other combinations which will not be considered in this paper.

Following section of the optic nerve of such compound eyes, regeneration
Optic nerve regeneration in Xenopus occurs to the contralateral tectum and an abnormal direct projection is established to the ipsilateral tectum, which is also innervated by the normal eye (Gaze & Straznicky, 1980b). In this bilateral regeneration the tectal coverage of the compound eye projection is strikingly different on the contralateral and ipsilateral tecta. The compound eye projection to the ipsilateral tectum is restricted to the corresponding half, i.e. from a TT eye to the rostrolateral part, from a NN eye to the caudomedial part and from a VV eye to the medial part of the ipsilateral tectum. In contrast the projection to the contralateral tectum is not restricted but extends across the whole tectum as it does in adult animals with one compound eye without optic nerve section (Gaze et al. 1963; Straznicky et al. 1974).

Recent observations have shown that optic fibres from a TT or VV eye initially innervate the appropriate half of the growing tectum, followed later by a gradual expansion of the projection across the whole tectum (Straznicky, Gaze & Keating, 1981). However, many months after metamorphosis, there is still a projection deficit, autoradiographically detectable in the caudomedial part of the tectum from TT eyes (Straznicky & Tay, 1981) and in the lateral part of the tectum from VV eyes (Straznicky, Gaze & Horder, 1979). In contrast to the slow expansions of TT and VV projections, the NN eye projection appears to cover the whole tectum by metamorphosis, although autoradiographic analysis has shown consistently less dense innervation in the inappropriate rostrolateral part (Straznicky et al. 1981).

Since the tectum receiving the compound eye projection is not yet fully innervated in postmetamorphic animals, we thought that the interactions between two populations of optic fibres could be studied in the tectum contralateral to the compound eye. In animals with right compound eye, the left optic nerve was sectioned after metamorphosis to induce regeneration from the left eye to the contralateral and to the ipsilateral tecta. The latter tectum is also innervated by compound eye optic fibres. The results reported here demonstrate that the two populations of optic fibres partially segregate in the dually innervated tectum with a clear preference by optic fibres from the normal eye to innervate the unoccupied parts of the tectum.

METHODS

Laboratory-bred Xenopus laevis were used in this study. Since the applied microsurgical, electrophysiological and autoradiographic methods have been described in detail in recent papers (Gaze & Straznicky, 1980b; Straznicky & Tay, 1981) only a very brief account is given here.

The experimental arrangement included early embryonic right eye operations to obtain double-nasal (NN) (made by the fusion of two nasal half eye anlagen), double-temporal (TT) and double-ventral (VV) eyes. Animals with NN, TT and VV eyes were kept separately and reared past metamorphosis. Two to
four weeks after metamorphosis the left optic nerve was cut close to the chiasma in order to facilitate bilateral regeneration to the tecta from the optic nerve of the normal eye. Autoradiographic and electrophysiological studies were performed from 89 to 189 days after optic nerve section to show optic fibre distribution in the left dually innervated tectum. The right eye (REi) or the left eye (LEi) was injected with 10–20 μCi L-[5-3H]proline (Amersham, specific activity 21 μCi/mmol), depending on the age of the animal. Twenty-four hours later the visual projections from the compound and intact eyes were recorded electrophysiologically and the animal then killed and prepared for autoradiographic examination. The distribution of the optic fibre projections from the compound and intact eyes was established on the autoradiographs. Every tenth section of the transverse serial sections through the tectum was photographed and the size of the tectum and the extent of the optic fibre projection, as revealed by the heavy silver grain deposition, was measured on the prints and the area of the whole optic fibre projections reconstructed using a Hewlett-Packard digitizer (Model 9876 A)

RESULTS

Twenty-seven animals with successful bilateral optic nerve regeneration from the left normal eye were included in this report. All but two of those animals gave compound eye projections through the right eye verifying the success of the embryonic operation (Table 1). Electrophysiological and autoradiographic mappings showed in all animals that the visual field and retinal projections from the left normal eye to the right tectum (Fig. 1, projection ‘c’) were restored with a normal orientation, extent and orderliness. Since these findings are comparable with observations previously reported in detail (Glastonbury & Straznicky, 1978; Straznicky, Tay & Glastonbury, 1980), here we consider only the projections formed between the left intact eye and left tectum (Fig. 1, projection ‘b’) and between the right compound eye and left tectum (Fig. 1, projection ‘a’).

(A) The patterns of dual tectal innervation from TT and intact eyes

Of the eleven animals in this group (Table 1), five had isotope injected into the right compound eye (REi) and six into the left normal eye (LEi). In all REi animals the area reconstructions on the autoradiographs shows that silver grains were deposited over the rostrolateral half to two-thirds of the tectum (Fig. 2). The boundary between the labelled and unlabelled tectum is marked by an abrupt decrease of silver grain density (Fig. 3b), suggesting that the TT eye projects to the rostrolateral portion of the tectum, which normally receives optic fibres from the temporal hemiretina. The reconstruction of the autoradiographs in one of the six LEi animals showed a whole projection across the entire extent of the tectum, although a higher grain density was found caudomedially (Table 1). In the other five animals the autoradiography
Table 1. Retinotectal and visuotectal projections from right compound and left normal eyes to right and left tecta in animals with left optic nerve section.

<table>
<thead>
<tr>
<th>Frog</th>
<th>Survival in days from left optic nerve section</th>
<th>Autoradiography</th>
<th>Visuotectal projections</th>
<th>Right eye to left tectum</th>
<th>Left eye to left tectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTREi1</td>
<td>92</td>
<td>—</td>
<td>Rostrolateral two-thirds</td>
<td>TT*</td>
<td>No recordable response</td>
</tr>
<tr>
<td>REi2</td>
<td>92</td>
<td>—</td>
<td>Rostrolateral half</td>
<td>TT</td>
<td>No recordable response</td>
</tr>
<tr>
<td>REi3</td>
<td>112</td>
<td>—</td>
<td>Rostrolateral two-thirds</td>
<td>TT</td>
<td>Whole visual field</td>
</tr>
<tr>
<td>REi4</td>
<td>165</td>
<td>—</td>
<td>Rostrolateral two-thirds</td>
<td>Few doubled field positions only</td>
<td>Unsuccessful recording</td>
</tr>
<tr>
<td>REi5</td>
<td>179</td>
<td>—</td>
<td>Rostrolateral three-quarters</td>
<td>TT</td>
<td>Temporal field only</td>
</tr>
<tr>
<td>TTLEi1</td>
<td>89</td>
<td>Whole extent</td>
<td>Caudomedial one-fourth</td>
<td>TT</td>
<td>No recordable response</td>
</tr>
<tr>
<td>LEi2</td>
<td>92</td>
<td>Whole extent</td>
<td>Caudomedial one-fourth</td>
<td>TT</td>
<td>Temporal field only</td>
</tr>
<tr>
<td>LEi3</td>
<td>131</td>
<td>Whole extent</td>
<td>Caudomedial half</td>
<td>Unsuccessful recording</td>
<td>Unsuccessful recording</td>
</tr>
<tr>
<td>LEi4</td>
<td>140</td>
<td>Whole extent</td>
<td>Whole extent higher grain density caudomedially</td>
<td>TT</td>
<td>Whole visual field</td>
</tr>
<tr>
<td>LEi5</td>
<td>166</td>
<td>Whole extent</td>
<td>Caudomedial two-thirds</td>
<td>TT</td>
<td>Temporal field only</td>
</tr>
<tr>
<td>LEi6</td>
<td>167</td>
<td>Whole extent</td>
<td>Caudomedial two-thirds</td>
<td>TT</td>
<td>Whole visual field</td>
</tr>
<tr>
<td>NNREi1</td>
<td>90</td>
<td>Whole extent</td>
<td>Caudomedial two-thirds</td>
<td>NN†</td>
<td>Not tested</td>
</tr>
<tr>
<td>REi2</td>
<td>125</td>
<td>—</td>
<td>Caudomedial three-quarters</td>
<td>NN</td>
<td>Whole visual field</td>
</tr>
<tr>
<td>REi3</td>
<td>141</td>
<td>—</td>
<td>Caudomedial half</td>
<td>NN</td>
<td>No recordable response</td>
</tr>
<tr>
<td>REi4</td>
<td>155</td>
<td>—</td>
<td>Whole extent</td>
<td>NN</td>
<td>Few points from the nasal field</td>
</tr>
<tr>
<td>NNLEi1</td>
<td>150</td>
<td>Whole extent</td>
<td>Whole extent</td>
<td>NN</td>
<td>Nasal field only</td>
</tr>
<tr>
<td>LEi2</td>
<td>150</td>
<td>Whole extent</td>
<td>Rostrolateral half</td>
<td>NN</td>
<td>No recordable response</td>
</tr>
<tr>
<td>LEi3</td>
<td>172</td>
<td>Whole extent</td>
<td>Rostrolateral one-third</td>
<td>NN</td>
<td>No recordable response</td>
</tr>
<tr>
<td>LEi4</td>
<td>188</td>
<td>Whole extent</td>
<td>Rostrolateral one-third</td>
<td>NN</td>
<td>Nasal field only</td>
</tr>
<tr>
<td>LEi5</td>
<td>189</td>
<td>Whole extent</td>
<td>Rostrolateral half</td>
<td>NN</td>
<td>No recordable response</td>
</tr>
<tr>
<td>VVREi1</td>
<td>123</td>
<td>—</td>
<td>Medial half</td>
<td>VV†</td>
<td>No recordable response</td>
</tr>
<tr>
<td>REi2</td>
<td>129</td>
<td>—</td>
<td>Medial half</td>
<td>VV</td>
<td>No recordable response</td>
</tr>
<tr>
<td>VVLEi1</td>
<td>130</td>
<td>Whole extent</td>
<td>Lateral half</td>
<td>VV</td>
<td>No recordable response</td>
</tr>
<tr>
<td>LEi2</td>
<td>145</td>
<td>Whole extent</td>
<td>Lateral half</td>
<td>VV</td>
<td>No recordable response</td>
</tr>
<tr>
<td>LEi3</td>
<td>146</td>
<td>Whole extent</td>
<td>Lateral half</td>
<td>VV</td>
<td>Few points from the centre of the field</td>
</tr>
<tr>
<td>LEi4</td>
<td>170</td>
<td>Whole extent</td>
<td>Whole extent</td>
<td>VV</td>
<td>Unsuccessful recording</td>
</tr>
</tbody>
</table>

* † ‡ Denote double temporal, double nasal and double ventral field projections, respectively.
Fig. 1. Diagram of the experimental plans. NN denotes right double nasal compound eye and NT left normal eye. (a) Uninterrupted right NN eye to left tectum projection. (b) Regenerated left NT eye to left tectum projection. (c) Regenerated left NT eye to right tectum projection. The same symbols apply to TT and VV eye animals.

Fig. 2. Drawings from tectal autoradiography of animal TTREI1. Sections are 100 μm apart starting from rostral (R), top left to caudal (C) bottom right. The black areas in the tectal drawing correspond to autoradiographic TT eye projection. In this and in each of the following figures right tectum is on the left and left tectum is on the right. Arrowheads indicate the caudomedial extent of a normal eye projection. Note that the TT projection does not extend to the midline, hence there is an apparent caudomedial projection deficit. Scale 1000 μm.
clearly showed a partial projection from the left normal eye to the right dually innervated tectum. The bulk of the silver grain deposition was confined to the caudomedial part of the tectum (Fig. 3a), extending to a varying degree rostro-laterally with a steadily decreasing grain density (Table 1). Consideration of the autoradiographic results from REi and LEi animals showed that the caudomedial part of the tectum is innervated exclusively by the normal eye and the rostrolateral part by the TT eye with substantial overlap along the boundaries (Fig. 8a, b). This division of tectal area is brought about by competitive interactions between fibres from the two eyes.
Fig. 4. Visuotectal recordings in animal TTREi5 through the right TT (A) and left intact (B) eyes. In this and each of the other visuotectal maps shown, the upper drawing is of the dorsal surface of the left dually innervated tectum, arrowhead projecting rostrally. Rows of numbered electrode positions on the tectum correspond to rows of field positions in the chart of the visual field below. The visual field chart extends out from the centre of the field for 100°. When recording through one eye the other eye was covered with an opaque shield. N, Nasal; T, temporal; S, superior; I, inferior. Dots correspond to recordings through right TT eye only, filled triangles to recordings through left normal eye only and large circles to recordings through both eyes. Small open circles in the tectum denote position from which no recordable visual responses were obtained. The large open arrows indicate the orientation of the temporonasal axis of the visual field in relation to the rostrocaudal tectal axis. Map (A) shows the duplication of field positions characteristic of TT eyes. Note in (B) that only the temporal field of the intact eye projects to the tectum.

In nine out of eleven animals, the visuotectal projection from the TT eye confirmed the success of the operation to form the compound eye. The projections were reduplicated about the vertical meridian of the field (Fig. 4), with both temporal and nasal poles of the field projecting to the rostral tectum. The central field mapped onto the caudal third of the tectum, in contrast to adult TT eye projection without left optic nerve section where it would extend across the whole tectum (Gaze et al. 1963). The visuotectal maps tally well with the autoradiographic results from such eyes, which show that TT eye projections, in the presence of an introduced subpopulation of fibres from the normal eye, fail to spread across the whole tectum.

Electrophysiological assessment of the projections from the left eye to the
Optic nerve regeneration in *Xenopus*

Fig. 5. Drawings from tectal autoradiography of animal NNLEi3. Note that in the dually innervated tectum (right on the drawings) the intact eye projection is restricted to the rostrolateral part, in contrast the whole projection to the right tectum (left on the drawings). Scale 1000 μm.

left tectum showed whole visual field projections in three animals and partial temporal field projections to the caudal tectum in another three animals (Fig. 4). No recordable direct left eye to left tectum visual responses were obtained in three animals with shorter postmetamorphic survival (Table 1). In two animals, recordings through the left eye to the left tectum were unsuccessful for technical reasons.

(B) The patterns of dual tectal innervation from NN and intact eyes

Of the nine animals comprising this group, four were injected in the right compound eye (REi) and five in the left normal eye (LEi; see Table 1). Reconstructions of the autoradiographs in the four REi animals showed either a partial caudomedial tectal silver grain distribution (2) or a projection across the whole tectum (2) with consistently heavier grain density in the caudomedial two thirds (Table 1). In the LEi animals the extent of the autoradiographic projections was complementary to the REi subgroup such that in four animals the rostrolateral part of the tectum was the only region covered with silver grains (Figs. 3c, d and 5) or in one case a whole projection was found with higher silver-grain density in the rostrolateral tectum (Table 1). Comparison of the autoradiographic projections suggests partitioning of dually innervated tectum where its rostrolateral part is innervated by the left normal eye and its caudomedial part by the right NN eye (Fig. 8c, d). It should be noted, however, that there is a substantial overlap between the two projections and this appears to be more marked than in the TT cases.
Electrophysiological recordings through right NN eyes yielded double nasal field maps in all nine animals investigated (Table 1). Both the temporal and nasal poles of the visual field were represented in the caudal tectum, whereas the centre of the field projected on to the rostral tectum. Most of the tectal electrode positions had two visual receptive fields, disposed as mirror images about the vertical meridian of the visual field. Consequently, each temporal and nasal hemifield projected to the tectum in an overlapping fashion. In six of the nine animals, there was a slight but detectable deficit of the compound eye projections in the rostral tectum (Fig. 6).

Although in each animal the presence of optic fibres from the left eye to the left tectum was confirmed, a full field visual projection was obtained in only one animal. In three animals visual responses were recorded from the rostral tectum in response to stimulation in the nasal hemifield of the intact eye (Fig. 6). No recordable responses through left eye to left tectum were obtained in four animals.

(C) The patterns of dual tectal innervation from VV and intact eyes

There were two REi and four LEi animals in this group. Following right (compound) eye injections silver grains were distributed over the medial half of the tectum. The boundary of the innervated and uninnervated tectum was sharply marked by the abrupt decrease of silver grains. The localization of the
optic fibres from the VV eye in the left dually innervated tectum was almost identical with that which has been previously demonstrated in animals with ipsilateral VV eye regeneration (Straznicky et al. 1979; Gaze & Straznicky, 1980b). Isotope injected into the left (normal) eye resulted in a heavy silver-grain deposition in the lateral half of the tectum from whence it steadily decreased medially (Fig. 3e, f). The dually innervated tectum, as in the previous two groups, became partitioned between the left normal and right VV eyes, the former occupying the lateral and the latter the medial parts of the tectum respectively (Fig. 8e, f).

All six animals gave characteristic VV projections through the operated right eye. Visual responses were reduplicated about the horizontal meridian of the field. The dorsal and ventral poles of the visual field projected medially and the centre of the field to more lateral parts of the tectum (Fig. 7). Although recording through left eyes was attempted in every case we succeeded in only one animal in obtaining visual responses, and these were from about the centre of the field (Fig. 7). The unsuccessful attempts to record a projection from the left eye to the left tectum are not surprising in view of the autoradiographic results which show optic fibres from the left eye only in the lateral part of the tectum which is not normally accessible for recording. It is worth while pointing
out here that the same left eyes projected in an orderly fashion to the right tectum.

**DISCUSSION**

The retinotectal map in adult normal *Xenopus* extends across the entire contralateral tectum in a topographical order and in a particular orientation in relation to the retinal and tectal axes. In the foregoing study we are concerned with the question of retinotectal map formation when two sets of optic fibres, one from the normal and one from the compound eye, are deployed in the same tectum. In particular, we consider the extent of such projections and the manner in which they interact.

Previous results on optic nerve regeneration from a compound eye have shown that the two component hemiretinae project across the *entire* contralateral tectum and only to the *corresponding half* of the ipsilateral tectum which also accommodates optic fibre projection from the normal eye (Gaze & Straznicky, 1980b). On the basis of these results we could predict a similar pattern and extent of normal and compound eye maps in the present experiments. Unexpectedly, neither the autoradiographic nor the visuotectal mapping results confirm such predictions. The normal eyes, following optic nerve regeneration, predominantly projected to the rostrolateral, lateral and caudomedial portions of the ipsilateral (dually innervated) tectum in NN, VV and TT animals,
respectively. The tectum contralateral to the normal eye, on the other hand, received whole projection, confirming the assumption that the optic nerve regeneration was complete at the time of the assays. Although the representation of the compound eyes was somewhat larger than those of the normal nasal, temporal and ventral hemiretinal projections, they were confined to the appropriate part of the dually innervated tectum.

The visuotectal mapping involves single or multiunit recordings from the optic fibre terminals. Because the curved rostrolateral and lateral parts of the tectum are inaccessible for electrode penetration, only its dorsal surface is available for recording. Thus neither the extent nor the density of terminations of the two sets of optic fibres can be established by visuotectal recordings. Autoradiography is complementary to visuotectal mapping and it enables us to determine the extent and the relative density of termination of one set of optic fibres. However, the relationship between the two fibre populations could only be inferred since either the right or the left eye of the animal was assayed autoradiographically.

_Fibre-fibre interactions in the dually innervated tectum_

We have previously reported that compound eye projections gradually expand over the entire tectum during development (Straznicky et al. 1981). This process is still incomplete after metamorphosis in the case of TT (Straznicky & Tay, 1981) and VV eyes (Straznicky et al. 1979). Although in postmetamorphic toadlets the NN projections cover the entire extent of the tectum, autoradiography shows a decrease of silver grain density on the rostrolateral tectum (Straznicky et al. 1981). The introduction of a set of normal fibres, soon after metamorphosis appears to have an effect on the process of expansion of the compound eye projections. Both autoradiographic and visuotectal mapping results showed partial cover of the compound eye projection in the dually innervated tectum many months after metamorphosis. Thus the presence of a set of normal fibres prevents further expansion of the compound eye projection which otherwise would have occurred. The observed partial NN projections furthermore suggest that not only was the expansion of the compound eye projections halted by regenerated fibres, but in NN animals the extent of the existing projection was reduced or compressed by regenerating fibres. We propose that the eventual expansion or the confinement of a compound eye projection in a dually innervated tectum depends on fibre competition and interactions between the two fibre populations. This assumption is compatible with recent experimental results (Schmidt, 1978; Straznicky, 1981) and with proposed models on the mechanisms of retinotectal map formation (Gaze, 1978; Willshaw & Von der Malsburg, 1979).

Autoradiographic mapping of the projection from the left normal eye to the left (dually innervated) tectum in the present experiments clearly indicated that the majority of regenerated fibres innervated one part of the tectum, whilst
relatively few fibres managed to innervate the other part, which is also supplied by compound eye fibres. The presence of high- and low-grain density areas in the dually innervated tectum, representing the distribution of normal fibres, does not offer any clues as to the fibre composition of these areas. Because of the limitations of the electrophysiological technique, only a few reliable recordings were obtained in NN and VV animals. These successful recordings, along with the more coherent results in TT animals (Table 1), show either partial, or in four animals, whole visual field projections across the dually innervated tectum. Thus there is an apparent discrepancy between the autoradiographic and visuotectal mapping results in some animals. Since the autoradiography gives a reliable picture of the extent and the relative density of the fibre projection we are compelled to put more weight on the autoradiographic than on the visuotectal mapping results. These results therefore, permit us to suggest that the high silver-grain density represents optic fibres from the normal retinal half which has a composition (N or T) opposite to that of the compound eye. Consequently, nasal fibres of the normal eye in TT, temporal fibres in NN and dorsal fibres in VV animals occupy previously vacant or sparcely innervated portions of the tectum. In contrast, the low grain density areas appear to correspond to the location of a limited number of optic fibres from the normal retinal half which has a composition similar to that of the compound eye (Fig. 8a–f). Indeed, these fibres were detected electrophysiologically in some animals but not in others. Both morphological and electrophysiological assays indicate a partitioning of the dually innervated tectum into nasal and temporal, or dorsal and ventral, regions of normal and compound eye projections with a considerable overlap between the two fibre populations. A similar kind of segregation and retinotopic sharing of the dually innervated tectum by temporal fibres of one eye and nasal fibres of the other eye has recently been reported in fish (Sharma & Tung, 1979) and in Xenopus (Straznicky, 1981).

Do incumbent optic fibres exclude regenerating fibres from occupying the same area?

The present results, which suggest that fibres from an existing innervation somehow exclude regenerating fibres from occupying the same area, stand in contrast to our recent observations on the nature of superimposed optic fibre projections. Fibres from a normal eye (Straznicky et al. 1980) or from a compound eye (Gaze & Straznicky, 1980b) readily regenerate to a tectum occupied by a complete projection from the normal eye. The patterns of the incumbent fibre projection, at the time of arrival of the regenerating fibres, are apparently different in the previous and in the present experimental arrangements. In the present experiments the tectum was partially innervated, in contrast to the previous experiments where the entire tectum was innervated. In TT animals, for example, the rostrolateral tectum has twice as many temporal fibres as normal and the caudomedial tectum has none. It is conceivable that regenerating fibres from the appropriate retinal sector (nasal fibres) bind firmly to the
unoccupied tectal area (which is their natural target). In contrast, regenerating temporal fibres destined for the innervated tectum are double disadvantaged: (i) incumbent fibres occupy their tectal target and (ii) this part of the tectum is overpopulated. Apparently for the above reasons, they fail to compete successfully for termination with incumbent fibres and eventually they are repelled. If the offered explanation is correct then during early regeneration, fibres from the normal eye should project to the entire dually innervated tectum and they should gradually withdraw to parts of the tectum as shown autoradiographically in the present study. Recent, relevant observations appear to support our speculation. Mutually exclusive interactions between optic fibres of identical retinal origin from right and left eyes in Rana pipiens bring about the formation of eye-specific termination zones in a dually innervated tectum by repelling one set of optic fibres from the termination area of the other (Constantine-Paton & Law, 1978). Results on short-time optic nerve regeneration suggest that at least grossly abnormal projections, present during early regeneration, disappear later (Bohn & Stelzner, 1979; Springer, 1980).

CONCLUSION
In summary, the present work showed that two sets of optic fibres from a normal and from a compound eye established topographical projections in the dually innervated tectum. Each projection covered only part of the tectum such that nasal and temporal or dorsal and ventral fibres from the compound eye and normal eye divided the tectum into complementary areas. The evidence we have obtained is consistent with prevailing assumptions that fibre–fibre and fibre–tectum interactions are instrumental in retinotectal map formation. It is proposed that the underlying mechanism is a detailed retinal marker system at the cell-to-cell level based on a cytochemical gradient.

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REFERENCES


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