The formation of the optic fibre projection after partial tectal removal in *Xenopus*

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**SUMMARY**

The formation of retinotectal connexions in *Xenopus* has been investigated by mapping the visual field projections of animals after various partial tectal removals.

Following removal of a sufficiently extensive piece of the left rostral, caudal, medial or lateral tectum with, in addition, section of the contralateral optic nerve, a consistent defect in the nasal, temporal, superior or inferior visual fields respectively was obtained. The various scotomas indicated that optic axons did not regenerate into the residual tectum from all regions of the retina. The conclusion drawn from the present experiments is that optic fibres tend to connect, in a residual half-tectum, only with appropriate tectal loci.

**INTRODUCTION**

Regeneration of the optic nerve in fish and in amphibians (Sperry, 1944; Attardi & Sperry, 1963; Gaze & Jacobson, 1963; Jacobson & Gaze, 1965) may result in restoration of the point-to-point retinotectal connexions and eventually normal vision of the operated eye may also be recovered. To account for the recovery of visual function Sperry (1951, 1965) put forward the hypothesis of neuronal specificity, according to which each of the ganglion cells and tectal neurons acquires a unique cytochemical character in neurogenesis, by means of which the cells are distinguished from each other. In otogeny or during regeneration retinal and tectal neurons with matching specificities would then link up, resulting in an ordered retinotectal connexion.

Gaze, Jacobson & Székely (1963, 1965) have reported embryological experiments on the connexions formed by surgically constructed compound eyes in *Xenopus*. They found that compound eyes (made of two nasal or two temporal hemiretinae) had a double projection to the contralateral tectum in that each half of the compound eye spread its fibres over the whole of the available tectal surface; and retinal points symmetrically disposed about the vertical midline of the retina projected to the same tectal locus. The authors (Gaze et al. 1963) concluded that the results suggested the existence of a system of crossed gradients rather than a rigid point-to-point specification for controlling the formation of retinotectal connexions.

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This suggestion has recently gained support from certain experimental results on goldfish (Gaze & Sharma, 1970; Yoon, 1971; Sharma, 1972), in which it was found that after removal of different tectal sectors an almost complete retinotectal projection was restored over the residual half-tectum, indicating a compression and concentration of optic fibre terminations in an ordered retinotopic manner. This experimental situation has been repeated in *Xenopus* embryos (Straznicky, Gaze & Keating, 1971a) where it was found that only the corresponding half of the retina linked to the residual half-tectum. In this particular case, the projection did not show evidence of plasticity in the formation of retinotectal connexions. These conflicting conclusions from complementary experiments in the one species (*Xenopus*), and the different results following the same experimental procedure (partial tectal removal) in fish and *Xenopus* larvae, seemed to justify investigation of whether compression of the retinotectal projection could also take place in adult *Xenopus*, as in adult fish. In the present experiment the retinotectal projection was mapped electrophysiologically in adult *Xenopus* that underwent an operation of partial tectum removal 6 weeks after metamorphosis.

**METHODS**

**Surgery**

Specimens of *Xenopus laevis* 6 weeks after metamorphosis were used. The animals were anaesthetized with an 0–1% MS 222 (tricaine methanesulphonate, Sandoz) solution. The left optic tectum was exposed by cutting open the skin as well as the hyaline cartilage and either the rostral, caudal, medial or lateral half-tectum was removed. The corresponding right optic nerve, near the optic chiasma, was simultaneously cut. The piece of hyaline cartilage and the flap of skin were then carefully replaced and the cut edges of the skin were approximated and sealed with Ethicon glue (isobutyl 2-cyanoacrylate monomer). The four different groups of operated animals with rostral, caudal, medial and lateral half-tectum removal were separately reared to maturity. After reaching a postmetamorphic size of 6–8 cm body length (4–7 months after the operation) they were used for electrophysiological mapping of the retinotectal projection.

**Electrophysiological mapping**

The animals were anaesthetized with ether and immobilized with 0·2–0·3 mg tubocurarine given intramuscularly. The cranium was opened and the dura mater removed from above the optic tecta. A drop of paraffin oil was used to protect the brain tissue from drying out. The left operated and right intact tecta were photographed with a final magnification of 50 and a 1 cm rectangular grid was superimposed on the print. The animals were positioned for recording at the centre of a Zeiss (Jena) perimeter arc. The right eye was centred on the fixation point of the perimeter and the left eye was covered with plasticine.
The visual projection was mapped according to the previous description of Gaze (1959) using a lacquer-insulated tungsten electrode with a naked tip of 1–2 μm. The electrode was regularly placed on predetermined tectal points as indicated by the intersections of the superimposed grid, and for each tectal electrode position the optimal point for a stimulus in the visual field was established. Usually at least 20–30 tectal points were tried and the corresponding visual field positions determined on each animal. The evoked action potentials were amplified by a transistorized preamplifier, displayed on an oscilloscope and monitored over a loudspeaker.

Histology

The heads of the successfully recorded animals were fixed in SUSA, their brains embedded in paraffin, sectioned serially at 15 μm and stained with Holmes's silver in order to determine the extension and the proper location of tectal lesions.

RESULTS

The results comprise the electrophysiological mapping of the visual field in 32 animals. The character of the retinotectal projections obtained in these animals will be described according to the type of the operation.

(A) Animals with left caudal tectal removal (10 animals)

The visual projection in normal Xenopus has been described by Gaze (1959). The nasal half of the visual field (temporal retina) is represented rostrally on the tectum, the temporal half (nasal retina) caudally, the superior pole of the visual field (inferior retina) projects to the medial surface of the tectum, whereas the inferior field (superior retina) is represented on the lateral edge of the tectum. In all but one of the animals of this group responses could be recorded from the tectum. In these animals an incomplete restoration of visual projection occurred in that responses were regularly absent from the temporal visual field. Histological examination of these brains confirmed that the caudal half-tectum, or the greater part of it, was successfully removed. The size of the tectal lesions corresponded with the parts of the visual fields from which responses were not obtained. The most demonstrative case of this group is shown in Fig. 1. There was an orderly projection from the part of the nasal half of the visual field to the residual rostral half-tectum. The visual responses from the temporal visual field, normally projecting to the removed caudal half-tectum, were absent. Similarly, no evoked potentials could be obtained from the caudal edge of the residual tectum, nor from its caudo-medial part (open circles). From the shape of the scotoma (shaded area) it appears that optic fibres from the naso-inferior hemiretina could not establish connexion with the residual tectum.
Fig. 1. The projection of the right visual field (Right eye) to the residual left optic tectum in animal with caudal tectal removal. In this and each of the following figures the numbers on the tectum represent electrode positions. The corresponding stimulus positions are indicated on the chart (lower part of the figure) of the visual field with the same numbers. Open circles on the tectum represent electrode positions from which no visual responses could be obtained. Shaded area in the chart indicates the region of visual field which would project to the missing part of the tectum.
Fig. 2. The representation of the right visual field (Right eye) to the residual left optic tectum in an animal with rostral tectal removal. Note that the size of scotoma in the visual field (shaded area) corresponds to the size of the tectal lesion.

(B) Animals with left rostral tectal removal (8 animals)

Three animals of this group gave no visual responses from the right eye, indicating a failure of optic nerve regeneration. In two cases insufficient tectum had been removed and an almost complete visual projection restored with the exception of the far nasal visual field. The remaining three animals showed
Fig. 3. The projection of the right visual field (Right eye) to the residual left optic tectum in an animal with medial tectal removal. The shaded area of the visual field indicates the size of scotoma.
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a deficit in the visual projection corresponding to the area of ablated tectum. The visual projection characteristic of this group of animals is shown in Fig. 2. The projection has been restored from the temporal visual field to the residual caudal half-tectum. It may be seen that the central part of the visual field is represented on the rostral part and the temporal pole of the visual field is on the caudal part of the residual tectum in a retinotopic order. Responses were absent, however, from the nasal and from the dorsal poles of the visual field, suggesting that the retinotectal connexion from the temporal hemiretina had not been restored.

(C) Animals with left medial tectal removal (8 animals)

In three animals of this group insufficient tectum had been removed and the visual projection from the right eye corresponded almost to that of a normal eye. In the other three animals a deficit of the visual projection was found. One of these is shown in Fig. 3. There is a regular projection from the central third and from part of the inferior visual field to the lateral half-tectum. The upper part of the nasal field (shaded area) from which responses could not be elicited, corresponded to the region of tectal ablation. It is worth mentioning that usually the most lateral part of the tectum is not accessible to an electrode because it curls down laterally. After removal of the medial half-tectum, the remaining lateral half moved slightly medially due to the healing process, making it possible to reach the lateral edge of the tectum with an electrode. Consequently some stimulus positions (for example field position 8) were established in the far ventral visual field. Responses from the temporal half of the visual field on the other hand seemed to be less regular along the medio-lateral axis of the tectum and the stimulus positions were concentrated near and below the horizontal meridian. The deficit in the visual field projection from the upper part of the temporal field corresponds to the region of the absent caudo-medial tectum. It is also seen that evoked potentials could not be picked up from the far caudal part of the tectum (open circles).

(D) Animals with left lateral tectal removal (8 animals)

Only three animals of this group gave a more or less retinotopically organized projection with the characteristic feature of an absent ventral visual field. In the other five animals only very few responses were obtained without much sign of retinotopic organization (Fig. 4). Histological examinations revealed a successful removal of the lateral half-tectum in all eight animals. Furthermore it was found that optic fibres of the lateral brachium of the optic tract, in the majority of the cases (Fig. 4), did not manage to grow back into the tectum as a result of the existence of a gap or scar tissue between the lateral surface of the diencephalon and the lateral edge of the residual tectum. The best preserved visual field projection is shown in Fig. 5. The projection is more or less retinotopically organized, in that the representation of stimulus positions is correct
Fig. 4. The projection of the right visual field (Right eye) to the residual left tectum in an animal with lateral tectal removal. No apparent order can be seen among the stimulus positions. Visual responses were absent from the caudal half of the residual tectum (open circles).
Fig. 5. The projection of the right visual field (Right eye) to the residual left tectum in an animal with lateral tectal removal. The shaded area in the visual field corresponds to the size of the ablated part of the tectum.
Table 1

<table>
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<th>Type of operation</th>
<th>No. of animals</th>
<th>Full-field visual projection</th>
<th>Presence of scotoma</th>
<th>No visual responses</th>
<th>Unorganized visual field</th>
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<tr>
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<td>—</td>
<td>3</td>
<td>—</td>
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* Insufficient tectum had been removed.

along the rostro-caudal and medio-lateral axes of the tectum. It can be seen that responses were absent from most of the central third of the visual field which normally projects to the removed lateral part of the tectum. The projection of the temporal visual field is also incomplete. Only three responses (11, 12, 13) were obtained from the caudal half-tectum, otherwise this part of the tectum was silent (open circles).

DISCUSSION

The aim of this study was to determine whether optic fibres from a normal eye could establish a full-field retinotectal projection to a residual rostral, caudal, medial or lateral half-tectum in adult *Xenopus*. It has been argued (Maturana, Lettvin, McCulloch & Pitts, 1960; Gaze & Jacobson, 1963; Gaze, 1970) that action potentials recorded from the superficial layers of the tectum, as in the present experiments, are due to the activity of the terminal arborizations of optic fibres, i.e. visual projection represents the distribution of optic terminals on the tectum. It is known (Gaze & Jacobson, 1963) that regeneration of the frog’s optic nerve may result in either a complete, or incomplete, as well as anomalous retinotectal projection. It is thus possible that some of our charts (for example Fig. 4) represent cases in which the regeneration process had not gone to completion. The majority of the cases, however, had properly arranged field positions along both tectal axes, suggesting that the regeneration process was complete. Gaze & Jacobson (1963) reported normal restoration of the retinotectal projection in the frog within 33–200 days of sectioning the optic nerve. The optic fibre regeneration may be faster in fish where the completion of the process is around 100 days (Gaze & Sharma, 1970; Yoon, 1971). In the present experiments approximately 120–200 days were allowed to elapse from the time of operation to the time of recording and this period may perhaps be expected to be sufficient for the completion of optic nerve regeneration.

Most of the visual field maps (Table 1) show a consistent projection deficit corresponding to the ablated part of the tectum, and in this sense the results are
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similar to those obtained on *Xenopus* after larval removal of rostral or caudal half-tectum (Straznicky *et al.* 1971a). As a rule, the larger the tectal lesion the larger the deficit in visual projection. Minor variations were obtained in the type of optic fibre regeneration pattern among animals with different types of tectal lesion. The least organized visual projections were seen in animals with lateral or rostral tectal removal. The medial and lateral brachia of the optic tract reached the tectum at its rostro-lateral pole so that both brachia were injured with these types of lesions. Caudal tectal removal left the optic pathway intact for regenerating axons and the nasal field projection in these cases was almost normal. In animals with medial tectal lesions the projection of the left brachium (ventral visual field) again showed normal distribution. In the latter two cases the operation did not involve injury of the brachia, and therefore the advancing fibres from all over the retina could reach either the rostral or the lateral half-tectum. The visual projections have convincingly shown, however, that optic fibres from the appropriate temporal (nasal field) or dorsal (ventral field) hemiretinae linked exclusively with the residual rostral or lateral half-tectum.

The situation revealed by previous experimental work on the retinotectal system in goldfish is confusing. Gaze & Sharma (1970) found that the projection from the whole visual field could compress onto the residual rostral half-tectum. Sharma (1972) was able to demonstrate a compression of the optic projection also onto a caudal half-tectum. Yoon (1971) has even found the compression of the visual projection along both the antero-posterior and medio-lateral tectal axes following excision of a caudo-medial sector of the tectum. These experiments appear to reveal some elastic properties in the retinotectal system in goldfish. Other experiments on the same species, however, involving decrease in the size of the retina or in the number of optic fibres, point in a different direction. The removal of a half-retina (Attardi & Sperry, 1963; Horder, 1971) or allowing only half the optic nerve fibres to regenerate into the tectum (Jacobson & Gaze, 1965) resulted in the establishment of a point to point retinotectal projection only to the appropriate half-tectum. These results might suggest that interference with the tectum in goldfish allows plasticity of the retinotectal projection to manifest itself, whereas interference with the retina does not. However, the situation may not be as simple as this, since Horder (1971) has found that half the retina may project, in an appropriate order, to the wrong half of the optic tectum.

Comparable experiments in *Xenopus* do not, up to the present, help us to clarify this problem. Surgically constructed compound eyes (double-nasal and double-temporal) appear to form a projection from each half retina across the entire dorsal tectal surface during neurogenesis (Gaze *et al*. 1963, 1965). A comparable result has been found in *Xenopus* after metamorphosis, where optic fibres from a compound double-nasal or double-temporal eye (Straznicky, Gaze & Keating, 1971b), were allowed to regenerate to a normal tectum. In this case the projection from each half of the compound eye spread again along the
antero-posterior axis across the whole tectum. In the present experiments involving operation on the tectum, optic fibres have been found to establish a point to point projection with the residual half-tectum without any sign of compression of the projection. Thus, the experiments on *Xenopus* suggest that plasticity of the retinotectal projection may be shown in some cases following operations on the retina in larvae, but not following operations on the tectum. It seems, therefore, that comparable operations on the retina and tectum in goldfish and *Xenopus* lead us to opposite conclusions.

The role of regulation in the retina and tectum has not yet been elucidated in any of the experiments so far mentioned. In the work on compound eyes in *Xenopus*, the operations were done at embryonic stage 32, and it is possible that a form of pattern regulation in the retina occurred following the operation. In the present experiments, however, the operations on the tectum were performed after metamorphosis and no suggestion of regulation was found. In the goldfish experiments the tectal operations and the eye operations were both done in adult fish, and if regulation is to be invoked to account for the results, then it has to be occurring long past embryonic life and, in addition, according to Yoon (1971), by the synaptic respecifications of individual tectal neurons.

Neurohistological observations seem to suggest that in mammals both expansion and compression of pre-synaptic terminals may be induced if the postsynaptic surface is mismatched. Liu & Chambers (1958) have demonstrated an intensive sprouting of collaterals of dorsal root fibres on ventral horn cells where the number of incoming fibres was decreased. Raisman (1969) has found expansion of the pre-synaptic terminals even in the septal nuclei of the adult rat. Recently Schneider (1971) has presented some data on the compression of the retinal projection on a diminished postsynaptic surface in hamsters with neonatal removal of one superior colliculus. In view of these results the mechanisms underlying the concentration (in fish) and the expansion (in amphibia) of optic terminals on the optic tectum, can hardly be understood. The differences between the results of pre- and post-synaptic lesions of the fish and amphibian retinotectal systems are very great and up to the present time we have inadequate information about the ultrastructural organization of these connexions. Further analytic experiments are needed to clarify the confusions in the field of retinotectal relations.

REFERENCES


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