J. Physiol. (1975), **252**, *pp.* 137–158
With 6 plates and 6 text-figures
Printed in Great Britain

READJUSTMENT OF RETINOTECTAL PROJECTION FOLLOWING REIMPLANTATION OF A ROTATED OR INVERTED TECTAL TISSUE IN ADULT GOLDFISH

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(*Received 13 March 1975*)

SUMMARY

1. The pattern of visual projection from the retina on to the optic tectum following reimplantation of a piece of the tectal tissue was studied with neurophysiological mapping methods in adult goldfish.

2. When a rectangular piece of the tectum was dissected, lifted free, and then reimplanted to the same tectum after rotation by 180° around the dorsoventral axis, the re-established visual projection later showed a complete reversal of retinotopic order within the reimplanted area with reference to the normal projection on to the intact surrounding area of the same tectum. The localized reversal was observed as early as 65 days, and also as late as 721 days after the 180° rotated reimplantation.

3. If a square piece of the tectal tissue was reimplanted after rotation by 90° anticlockwise around the dorsoventral axis, the restored visual projection later showed a corresponding localized 90° rotation within the reimplanted area.

4. When the entire laminar structure of a dissected tectal tissue was inverted, and then reimplanted upside-down along the same rostrocaudal axis of the tectum, the restored visual projection on to the inverted tectal reimplant was found to be organized in a reverse retinotopic order along only the mediolateral axis within the reimplanted area. The restored visual projection retained a correct retinotopic order along the rostrocaudal axis. The same trends were also observed after regeneration of the optic fibres following section of the contralateral optic nerve.

5. If the inverted tectal tissue was reimplanted along the same mediolateral axis of the tectum, the re-established visual projection showed a localized reversal of retinotopic order along only the rostrocaudal axis within the reimplanted area. Sectioning the contralateral optic nerve made no difference to the result.

6. These results suggest that a piece of adult tectal tissue retains its original topographic polarity regardless of the orientation of reimplantation after either a rotation or an inversion. Furthermore the retention is not a short-lived transitory phenomenon. It persisted as long as the reimplanted tissue survived.

7. Histological examination of the operated tecta revealed that the reimplanted tectal tissues underwent a severe derangement in their laminar structures. It was impossible to identify the main target zone of retinotectal projection (the *stratum fibrosum et griseum superficiale*) or the central cellular layer (the *stratum griseum centrale*) in the reimplants. The prominent feature of the deranged tectal tissue was irregular vortices of tangled fibre bundles. Sparse tectal neurones of bipolar and granular types were irregularly scattered in the deranged structure of the reimplant.

8. Thus, the retention of original topographic polarity did not require an integrity of the cytoarchitectonic structure of the reimplanted tectal tissue.

INTRODUCTION

The visual projections from the retina on to the mid-brain optic tectum develop in a consistent topographic pattern. This orderly development of specific neural connexions in the visual pathways was interpreted by Sperry (1943*a*, *b*, 1945, 1965) as follows: during neurogenesis the ganglion cells in the eye anlage undergo a polarized field or gradient type of differentiation along the nasotemporal and the dorsoventral axes. The retinal ganglion cells acquire axially graded differential affinities (of cytochemical nature) for intercellular recognitions according to their relative locations in the retinal field. The corresponding visual neurones in the optic tectum also undergo congruent embryonic differentiation and acquire matching or complementary affinities according to their relative positions in the tectal field. The ingrowing axons of the retinal ganglion cells are guided chemotactically to appropriate zones in the tectum, and preferentially form synaptic connexions with selected tectal neurones according to their mutually matching or complementary cytochemical affinities.

The topographical polarization of the retina has been investigated in a number of experiments involving rotation or transplantation of the optic cup at different stages of embryonic development in lower vertebrates (Stone, 1944, 1947, 1960; Székely, 1954; Jacobson, 1968*a*, *b*; Hunt & Jacobson, 1972, 1973, 1974; Sharma & Hollyfield, 1974). When the optic cup is excised, rotated 180° and then reimplanted or transplanted to a host before a critical embryonic stage, the host later attains a normal visual projection regardless of the eye rotation. If the optic cup

is rotated 180° and reimplanted after the critical stage, however, the host later develops an inverted visual projection from the rotated eye. The topographical polarity of the retinal tissue becomes irreversibly fixed at an early embryonic stage in development. At this critical stage, the neural elements of the rapidly proliferating retina have not completed morphological differentiation as yet.

Experimental evidence for the topographic polarization of the optic tectum was recently found in adult goldfish (Sharma & Gaze, 1971; Yoon, 1973) and also in post-metamorphic *Xenopus* froglets (Levine & Jacobson, 1974). When a piece of adult tectal tissue is dissected out, lifted free, and then reimplanted to the same tectum after 180° rotation, the tectal reimplant survives occasionally, and then becomes reinnervated by regenerating optic fibres. The re-established visual projection on to the 180° rotated tectal reimplant shows a complete reversal of retinotopic order in contrast to the visual projection on to the intact surrounding area of the same tectum. This indicates that a piece of adult tectal tissue retains its original topographic polarity (acquired sometime during its embryonic development) regardless of the orientation of reimplantation. The retention of original topographic polarity by a reimplanted adult tectal tissue suggests that the optic tectum is not a passive receiver of incoming optic fibres but an active accommodator: it selects appropriate optic fibres to make proper neural connexions in a consistent topographic order (Yoon, 1973).

In the present experiments, a piece of tectal tissue was dissected from an adult goldfish, and its laminar structure was lifted intact. The entire laminar structure of the dissected tectal tissue was inverted and then reimplanted to the same tectum upside-down. The pattern of re-established visual projection on to the inverted tectal reimplant was studied at various post-operative intervals with neurophysiological mapping methods. A preliminary account of the present results was presented elsewhere (Yoon, 1975*a*).

METHODS

Common goldfish (*Carassius auratus*) used in the present experiments weighed 12–18 g and were about 72–80 mm long from the nose to the base of the tail at the time of surgery. Individual fish were anaesthetized by immersion in 0·03 % ethyl-*m*-aminobenzonate methanesulphonate (MS 222, Sandoz) for 2–5 min and then placed between two soft sponge pads in a holder that restrained the fish to a desired position for surgery or neurophysiological recording. The gills were continually infused with aerated water (about 0·5 l./min) through a tube in the mouth.

The optic tectum was exposed by opening a single cranial bone flap that was restored at the completion of surgery. The experiments involved the following types of surgical operations; a rectangular or square piece of the tectal tissue was dissected by making four sharp vertical incisions down to the level of the optic ventricle (Pl. 1*A*, and Pl. 4*A*). The dissected tectal tissue was lifted intact, and

then reimplanted to the same tectum after rotation around the dorsoventral axis (Pl. 1*B*) or after inversion of its laminar structure upside-down (Pl. 4*B*). In some cases the contralateral optic fibres were also sectioned at a distance about 0·5–1 mm from the posterior pole of the eyeball. All surgery and neurophysiological recordings were performed with the aid of a Zeiss operating microscope at magnification of 6–40 times. The operated fish were kept under a regular daily cycle of 12 hr in light and 12 hr in darkness. The mean water temperature of their aquaria was about 22° C.

Standard neurophysiological methods were used for mapping retinotectal projections as described in previous reports (Yoon 1971, 1972, 1975*b*). Action potentials were recorded at different levels along the vertical axis of the tectal layers by advancing a metal micro-electrode (tungsten plated at the tip with gold and platinum). In a normal tectum or in the intact area of the operated tectum, it was usually possible to distinguish two classes of visual responses: when visual responses were recorded from the outer plexiform layers (the *stratum opticum* or the *stratum fibrosum et griseum superficiale*) the size of their receptive fields was remarkably small. It ranged from about 3° to 5° in diameter. The luminance of grey background of the perimeter was about 1·1 cd/m^2 and that of the test stimuli was either 6·8 cd/m^2 (for a white spot) or 0·34 cd/m^2 (for a black spot). The visual units with small receptive fields gave phasic, brisk responses, and resolved a relatively high frequency of visual flickering. When the recording micro-electrode was advanced to the deeper cellular layer (the *stratum griseum centrale*), a new class of visual units emerged. These deeper units had larger receptive fields, which ranged from 10° to 25° in diameter. The locations of receptive fields for the two classes of visual units recorded during a given vertical penetration did not differ from each other significantly. The small receptive field of an upper unit was located within the larger receptive field of a deeper unit on the perimetric chart of the contralateral visual field. Most of the deeper units showed sustained visual responses. When a small amount (0·3–0·5 mg) of (D-)-tubocurarine chloride (Squibb) was topically applied on to the exposed tectum, the visual responses from these deep tectal units diminished, and then disappeared. If the micro-electrode was moved back to the upper plexiform layer level, however, the previous class of phasic visual responses (with small receptive fields) usually reappeared even in the presence of (D-)-tubocurarine chloride. The results suggest indirectly that the phasic visual responses with small receptive fields originated from presynaptic elements of incoming optic fibres in the upper plexiform layer, whereas the sustained visual responses with large receptive fields arose from post-synaptic visual neurones in the inner cellular layer of the intact optic tectum. Occasionally non-visual spontaneous neural activities were also recorded in the deeper layers of a normal tectum.

Visual responses recorded from the reimplanted tectal tissue were very sluggish. Three types of visual responses, 'on', 'off' and 'on–off' were observed in tectal reimplants. It was impossible, however, to distinguish different classes of responses along the vertical axis of the reimplanted tectal layers. The visual responses were transient and became easily fatigued. Their receptive fields were somewhat vague and large (about 20° in diameter). When (D-)-tubocurarine chloride (0·3 mg) was topically applied, the visual responses from the tectal reimplant deteriorated rapidly. The sluggish and capricious nature of visual responses recorded from the tectal reimplant made it impossible even to guess about their proper neural origins with the present extracellular recording technique.

The locations of the recording micro-electrodes on the dorsal surface of the tectum were marked on polaroid photographs of the tectum at magnifications of 21–33 times. The corresponding receptive fields for the tectal units were marked on the perimetric chart of the contralateral visual field. The cornea of the fish's eye,

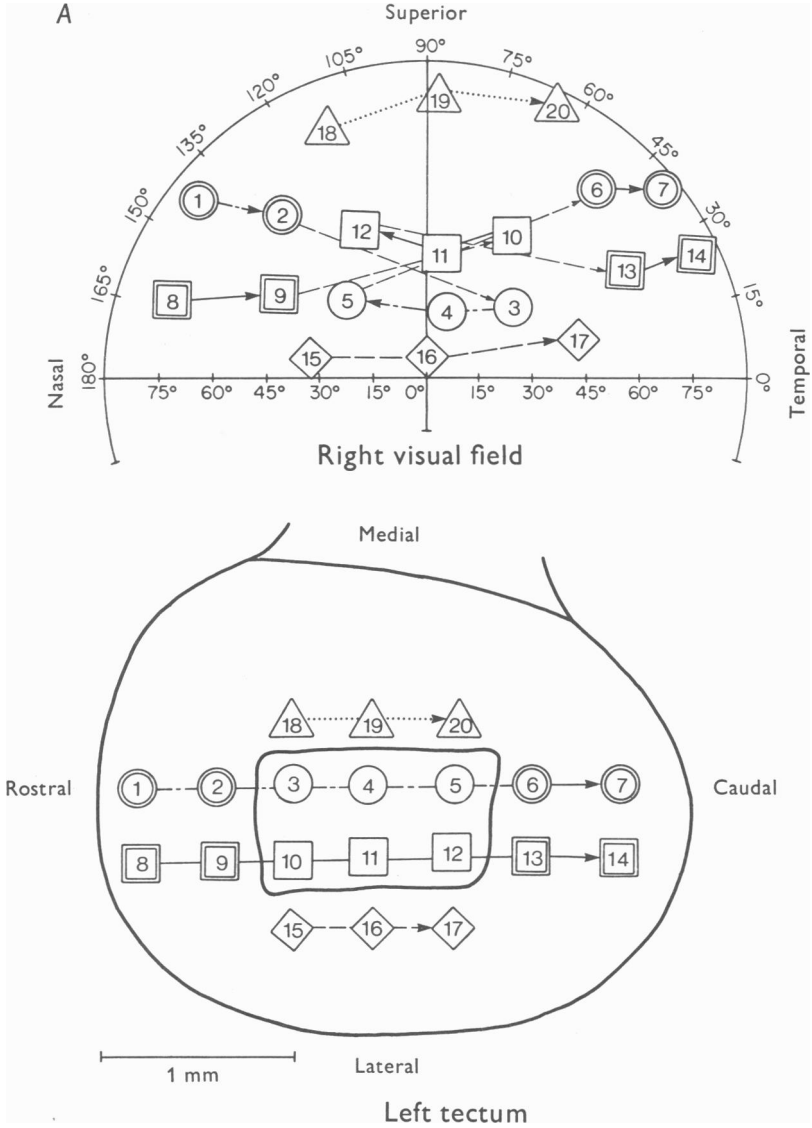
exposed in the air, was continually bathed with a uniform flow of water infused from the tip of a fine cannula. In some cases, the extraocular muscles were cut in order to immobilize the eyeball.

At the end of a terminal mapping experiment, the fish head was immersed in a mixture of 18 parts of 80% ethanol, 1 part glacial acetic acid and 1 part formalin. The brain was dissected free, embedded in paraffin and serially sectioned at 10–15 μm. These sections of the brain were stained by a modified Bodian protargol method (Bodian, 1936; Davenport & Kline, 1938; Attardi & Sperry, 1963).

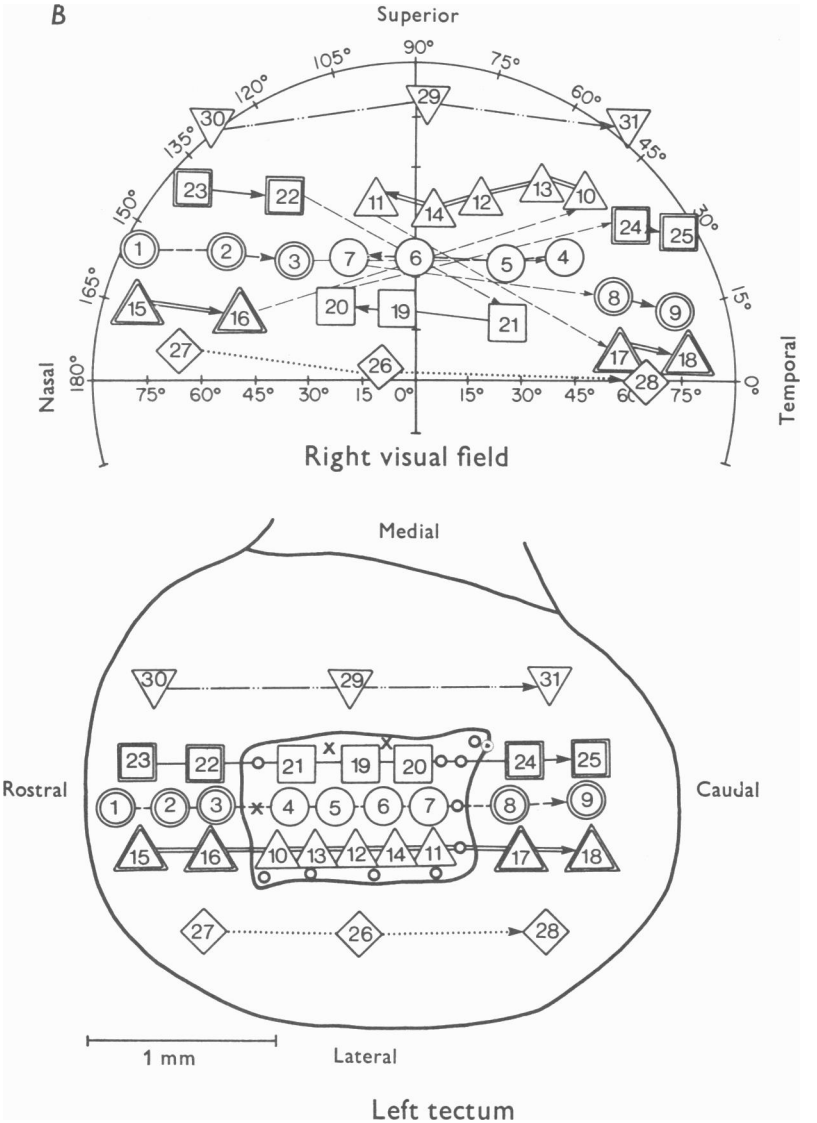
RESULTS

Visual projection on to uninverted tectal reimplant after rotation by 180° or 90° anticlockwise

Experiment 1. In thirty-six adult goldfish a rectangular piece of the tectal tissue was dissected by making sharp vertical incisions down to the level of the optic ventricle in the left tectum (Pl. 1*A*). The laminar structure of the dissected tissue was lifted intact, and then reimplanted to the same tectum after rotation by 180° around the dorsoventral axis (Pl. 1*B*). The optic nerves were left intact in all fish. Two fish died during recovery. Visual projections on to the operated tectum were mapped at various post-operative intervals between 62 and 721 days. Eleven fish showed a complete degeneration of the reimplanted tectal tissue. Visual responses were recorded from the tectal reimplant in the other twenty-three fish. In general the visual responses from the tectal reimplant were very sluggish compared with those from the intact surrounding tissue of the same tectum (see Methods). In four of the twenty-three fish only a small portion of the reimplanted area gave visual responses. The other nineteen fish yielded consistent results, one of which is shown in Text-fig. 1. The first map in Text-fig. 1*A* was obtained 167 days after the tectal reimplantation. It showed that the visual projection from the part of the retina which reinnervated the 180° rotated tectal reimplant was organized in a completely reverse retinotopic order in contrast to the normal projection from the other part of the retina on to the intact surrounding area of the same tectum. The fish was successfully revived. The second map shown in Text-fig. 1*B* was obtained from the same fish 408 days after the first mapping experiment. The later map also showed the same reversal of retinotopic order within the reimplanted tectal area. The result suggests that retention of the original topographic polarity by a piece of adult tectal tissue is not a short-lived transitory phenomenon. The retention persisted at least 575 days in this fish. At the end of the second mapping experiment, the brain of the same fish was preserved for histological examination. The tectal reimplant was well revascularized (Pl. 1*C* and *D*). It showed partial degeneration along its lateral and caudal margins (Pl. 1*D*). A parasagittal section of the operated tectum (stained by a



Text-fig. 1. Projection of the visual field on to the contralateral optic tectum in an adult goldfish following reimplantation of a rectangular piece of the tectal tissue after 180° rotation around the dorsoventral axis as shown in Pl. 1*A* and *B*. The numbers marked on the enlarged drawing of the tectum indicate the loci of micro-electrodes on the dorsal surface of the tectum in the order of recording visual responses. The numbers marked on the perimetric chart show the positions of the corresponding receptive fields in the contralateral visual field for the experimental points on the dorsal tectum. No attempts were made to record from the curled ventrolateral surface of the tectum, which is known to receive projections from the lower half of the visual field.



A shows the restored visual projection, mapped 167 days after the 180° rotated tectal reimplantation. The optic nerves were left intact. The map shows that the receptive fields for the tectal units recorded from the reimplanted area are distributed in a completely reverse retinotopic order.

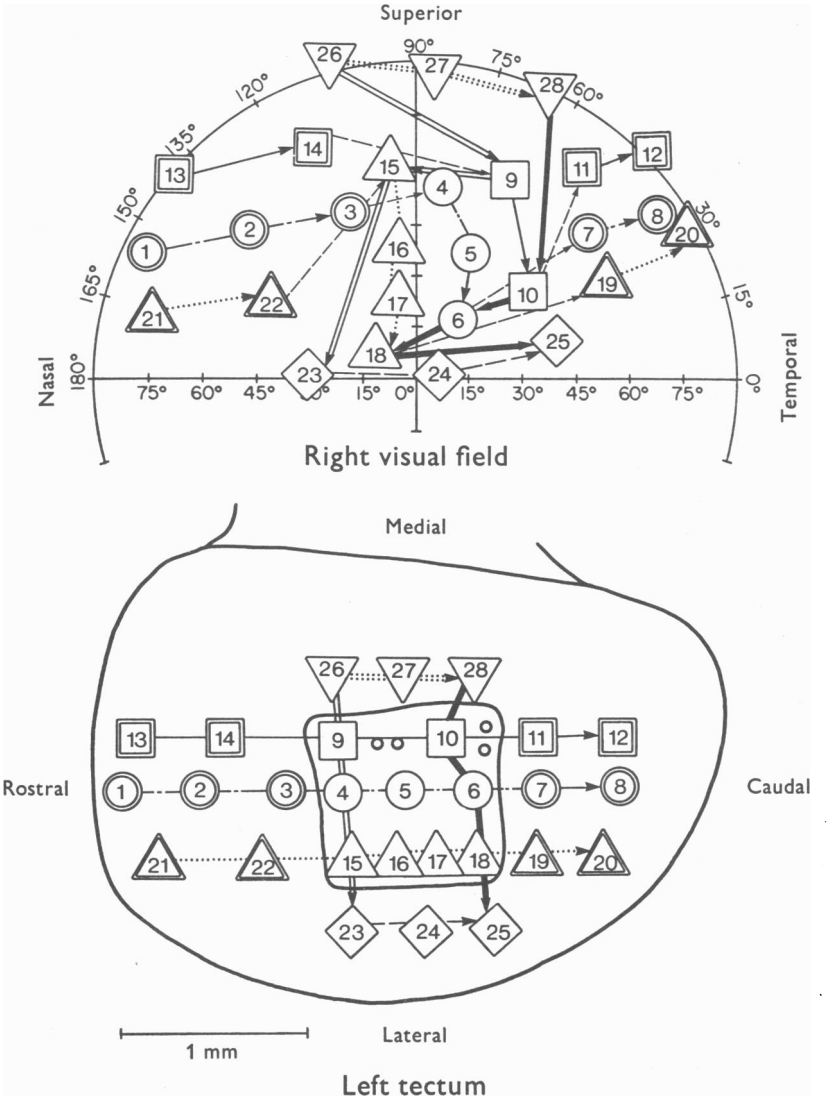
B shows a second map, obtained from the same fish 575 days after the tectal reimplantation. The same trend of the localized reversal of retinotopic order within the reimplanted area persisted in the latter map. The points marked by crosses (×) on the reimplanted area gave too sluggish visual responses to map their receptive fields. The points marked by open circles (○) gave no responses.

modified Bodian's protargol method) is shown in Pl. 2. The over-all view shows the 180° rotated reimplant in the middle zone between the rostral and the caudal parts of the tectum. Details of the same section reveal the following changes in the laminar structure of the reimplanted tectal tissue. The intact rostral part of the tectum (shown in the bottom right side of Pl. 2) retained a normal laminar structure with six distinguishable horizontal layers: (*a*) *startum fibrosum marginale*; (*b*) *stratum opticum*; (*c*) *stratum fibrosum et griseum superficiale*; (*d*) *stratum griseum centrale*; (*e*) *stratum album centrale*; and (*f*) *stratum periventriculare*. On the other hand, the reimplanted tectal tissue (shown in the upper half of Pl. 2) underwent a severe derangement of its laminar structure. Only the outermost layer (*a*) and the innermost layer (*f*) seem to remain. The main target zone of retinotectal projection (*stratum fibrosum et griseum superficiale*) and the cellular layer (*stratum griseum centrale*) in the central zone of the tectal tissue underwent a drastic disintegration after the reimplantation. The prominent feature of the deranged tectal structure was irregular vortices of tangled fibre bundles. Sparse tectal neurones of bipolar and granular types were dispersed in the deranged central zone of the reimplanted tissue.

The same trends were also found in the other eighteen experimental fish. The localized reversal of retinotopic order in the restored visual projection on to the 180° rotated tectal reimplant was observed in one fish as early as 65 days after the tectal reimplantation and also in another as late as 721 days.

Experiment 2. In twelve fish, a square piece of the tectal tissue was dissected out, lifted free and then reimplanted to the same left tectum after rotation by 90° anticlockwise around the dorsoventral axis. The optic nerves were left intact. Two fish died prematurely. Retinotectal projections were mapped at post-operative intervals between 95 and 452 days. Three fish showed an extensive degeneration of the reimplanted tectal tissue. The other seven fish yielded consistent results, one of which is shown in Text-fig. 2. The retinotectal projection was mapped 435 days after the tectal reimplantation (rotated 90° anticlockwise). The restored visual projection on to the tectal reimplant showed a corresponding localized 90° rotation with reference to the normal projection on to the intact surrounding area of the same tectum. At the end of the mapping experiment, the fish brain was processed for histology. Pl. 3 shows a parasagittal section of the operated tectum. It reveals the characteristic changes in the laminar structure of the reimplanted tissue; the outer plexiform layer, the cellular layer and the two fibrous layers disappeared from the central zone of its laminar structure. The reimplanted tissue was filled mainly with irregular vortices of tangled fibre bundles and a few scattered tectal neurones.

The other six experimental fish showed the same trend: a piece of adult tectal tissue reimplanted after rotation by 90° anticlockwise retains its original topographic polarity, in spite of the severe derangement in its laminar structure.



Text-fig. 2. Retinotectal projection mapped 435 days after reimplantation of a part of the tectum following rotation by 90° anticlockwise. The optic nerves were left intact. The restored visual projection shows a corresponding localized 90° rotation within the reimplanted area of the operated tectum.

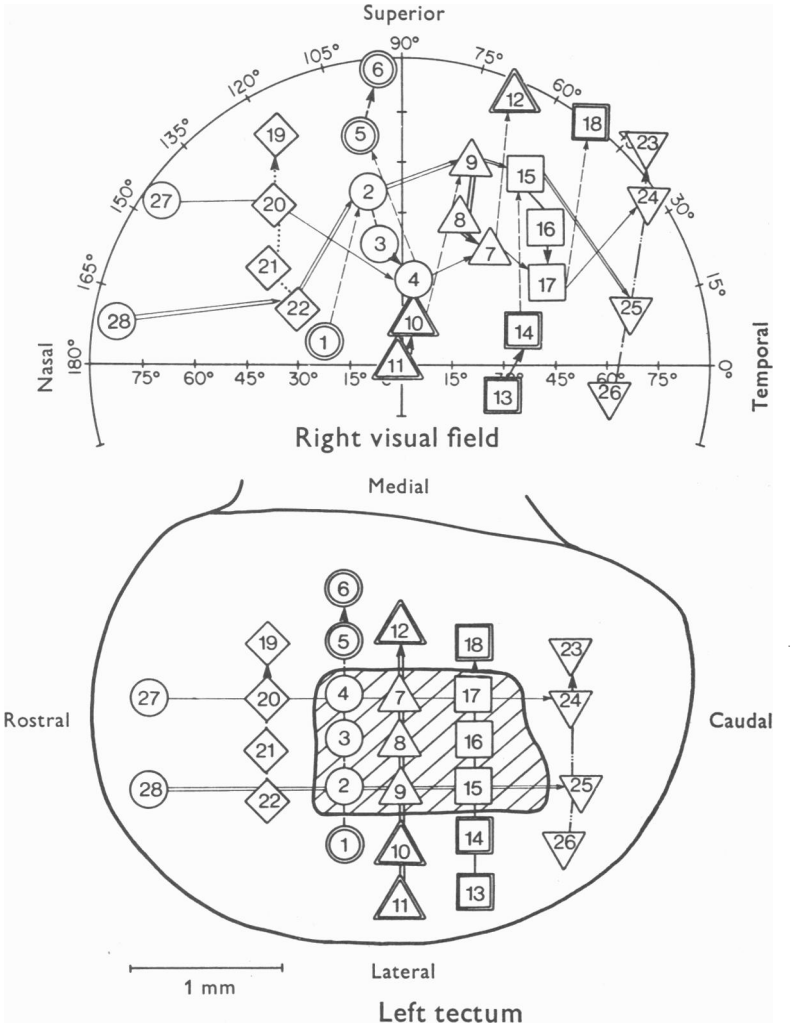
Visual projection on to tectal reimplant after inversion of the tectal layers

Suppose that the laminar structure of a tectal tissue is inverted, and then reimplanted to the same tectum upside-down. Would the inverted tectal reimplant become reinnervated by regenerating optic fibres? If so, would it retain its original topographic polarity? Would the tectal tissue reorganize its laminar structures following inversion?

Experiment 3. In twenty-four adult goldfish, a rectangular piece of the tectal tissue was dissected by making sharp vertical incisions down to the level of the optic ventricle in the left tectum (Pl. 4*A*). The laminar structure of the dissected tissue was lifted intact, and then turned upside-down around the rostrocaudal axis. The inverted tectal tissue was reimplanted along the same rostrocaudal axis of the tectum (Pl. 4*B*). In twelve fish, the right optic nerve was also sectioned near the posterior pole of the right eyeball. The optic nerves were left intact in the other twelve fish. Three fish died prematurely. Visual projections on to the operated tectum were mapped at various post-operative intervals between 102 and 507 days. Thirteen fish showed a complete or extensive degeneration of the inverted tectal tissue. Visual responses were recorded from the inverted tectal reimplant in the other eight fish. Five fish, in which the optic nerves were left intact, yielded consistent results, one of which is shown in Text-fig. 3. The map was obtained 477 days after reimplantation of the inverted tectal tissue. It shows that the restored visual projection on to the inverted tectal reimplant is organized in a reverse retinotopic order only along the mediolateral axis within the reimplanted area. Along the rostrocaudal axis, on the other hand, the restored visual projection retained a correct retinotopic order.

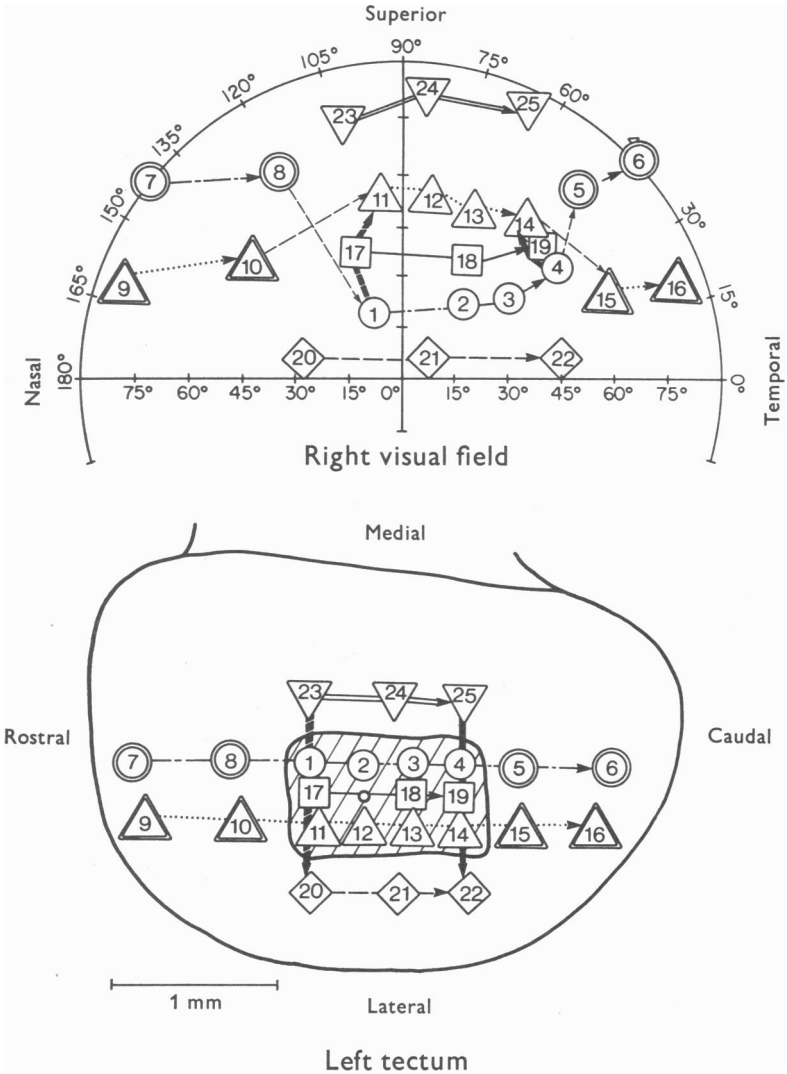
The same trends were also found in the other three experimental fish, in which the right optic nerve had been sectioned. Text-fig. 4 shows one of these results, obtained 335 days after reimplantation of the inverted tectal tissue and section of the optic nerve. The newly re-established visual projection on to the inverted tectal reimplant shows a localized reversal of retinotopic order only along the mediolateral axis within the reimplanted area. At the end of mapping experiment, the brain of the same fish was preserved for histological examination. In general, the inverted tectal reimplants (see Pl. 4*C* and *D*) were not so well revascularized as the uninverted reimplants (Pl. 1*C* and *D*). A parasagittal section of the operated tectum is shown in Pl. 5. The inverted tectal tissue remained well in place between the intact rostral and the caudal parts of the tectum. Details of the same section reveal the following changes in the laminar structure of the inverted tectal reimplant. Virtually all six horizontal layers disappeared from the inverted tissue. The plexiform

layer (*stratum fibrosum et griseum superficiale*), the cellular layer (*stratum griseum centrale*) and the two fibrous layers (*stratum opticum* and *stratum album centrale*) in the central zone of the tectal tissue underwent a complete disintegration. Even the outermost layer (*stratum fibrosum marginale*),



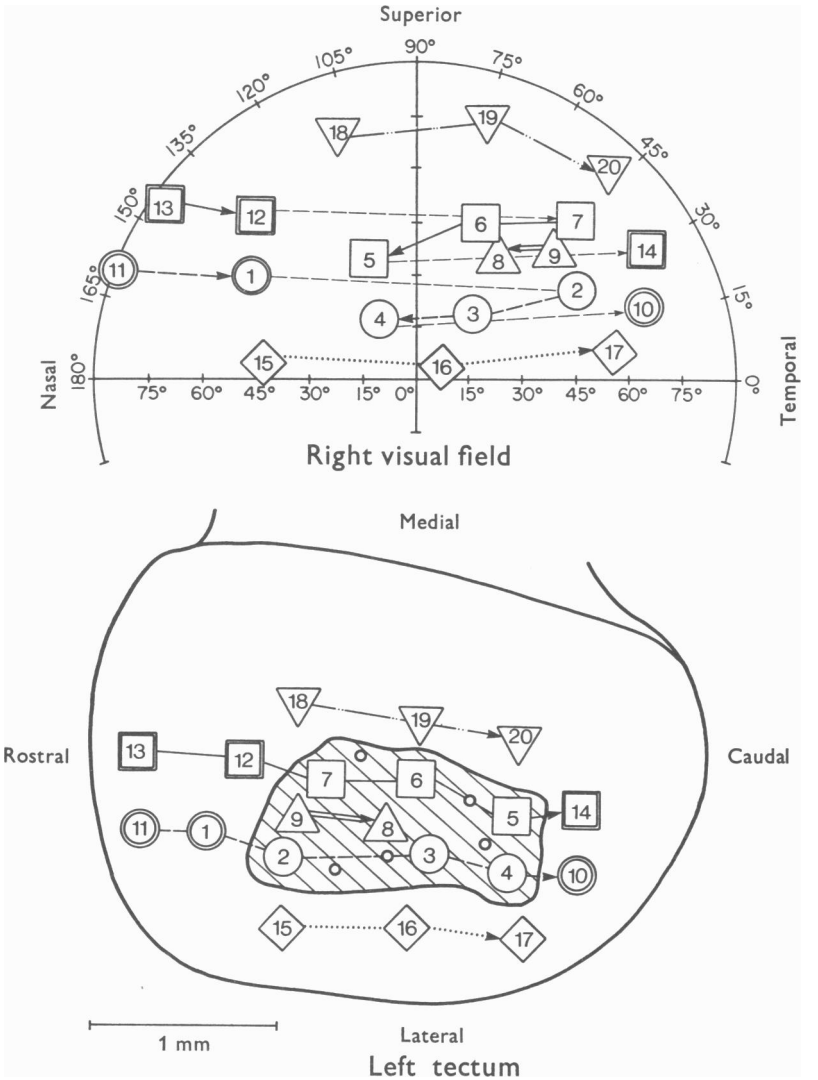
Text-fig. 3. Retention of the original topographic polarity by an inverted tectal reimplant. The retinotectal projection map was obtained 477 days after reimplantation of the inverted tectal tissue along the same rostrocaudal axis of the tectum (for example, see Pl. 4*A* and *B*). The optic nerves were left intact. The restored visual projection shows a localized reversal of retinotopic order along only the mediolateral axis within the reimplanted area of the operated tectum.

turned upside-down to face the optic ventricle, disintegrated, and became partially invaded by glial cells migrating presumably from the ependymal layer. The innermost layer (*stratum periventriculare*), exposed to the dorsal surface of the inverted tissue, also disappeared. The whole extent



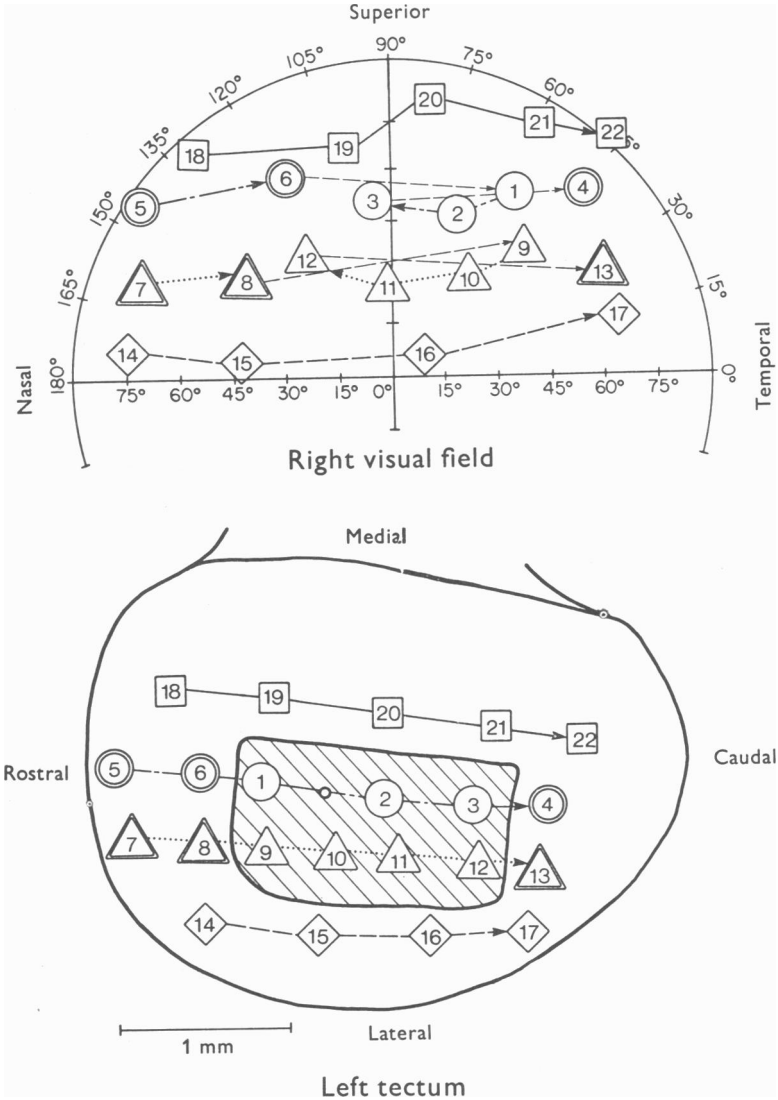
Text-fig. 4. Re-established retinotectal projection mapped 335 days after reimplantation of an inverted tectal tissue along the same rostrocaudal axis of the tectum as shown in Pl. 4 *A* and *B*. The contralateral optic nerve was sectioned, and then allowed to regenerate into the operated tectum. The re-established visual projection shows a localized reversal of retinotopic order along only the mediolateral axis within the reimplanted area.

of the severely deranged structure of the inverted tectal tissue was filled mainly with irregular vortices of spiralling bundles of tangled fibres. Sparse tectal neurones of bipolar and granular types were irregularly scattered in the deranged structure of the inverted reimplant.



Text-fig. 5. Retention of the original topographic polarity by an inverted tectal reimplant. The map was obtained 336 days after reimplantation of the inverted tectal tissue along the same mediolateral axis of the tectum. The optic nerves were left intact. The restored visual projection shows a localized reversal of retinotopic order along only the rostrocaudal axis within the reimplanted area of the operated tectum.

The aforementioned results, found in all eight experimental fish, suggest a dramatic fact: a piece of adult tectal tissue reimplanted after inversion of its entire laminar structure is able to accommodate re-



Text-fig. 6. Re-established retinotectal projection mapped 501 days after reimplantation of an inverted tectal tissue along the same mediolateral axis of the tectum. The contralateral optic nerve was cut and then allowed to regenerate into the operated tectum. The restored visual projection shows a localized reversal of retinotopic order along only the rostrocaudal axis within the reimplanted area of the operated tectum.

generating optic fibres. Furthermore the inverted tectal tissue retains its original topographic polarity, in spite of the chaotic derangement in its cytoarchitectonic structures.

Experiment 4. In twenty-four fish, a rectangular piece of tectal tissue was dissected in the left tectum. The laminar structure of the dissected tissue was lifted intact, and then turned upside-down around the mediolateral axis. The inverted tectal tissue was then reimplanted along the same mediolateral axis of the tectum. In twelve fish, the right optic nerve was also sectioned near the posterior pole of the right eyeball. The optic nerves were left intact in the other twelve fish. Two fish died during recovery. Retinotectal projections were mapped at various post-operative intervals between 103 and 509 days. Fifteen of the operated fish showed a complete or extensive degeneration of the inverted tectal tissue. In the other seven fish, however, visual responses were recorded from the reimplanted area as well as from its surrounding intact area of the tectum. Four of these fish, in which the optic nerves were left intact, yielded consistent results. Text-fig. 5 shows one of these maps, obtained 336 days after reimplantation of the inverted tectal tissue. The map shows that the newly restored visual projection on to the inverted tectal reimplant is organized in reverse retinotopic order only along the rostrocaudal axis within the reimplanted area. The visual projection is in a correct retinotopic order along the mediolateral axis. At the end of mapping experiment, the brain of the same fish was processed for histology. Pl. 6 shows a parasagittal section of the operated tectum. The inverted tectal tissue was found to have rejoined with the intact rostral and caudal parts of the tectum. Details of the same section show the drastic derangement in the laminar structure of the inverted tectal tissue. Virtually all six horizontal layers disintegrated. Irregular vortices of tangled fibre bundles were predominant features in the chaotic structure of the inverted tectal tissue. Sparse tectal neurones were dispersed in the severely deranged reimplant.

The same trends were also found in the other three fish, in which the right optic nerve had been sectioned. Text-fig. 6 shows one of these maps, obtained 501 days after reimplantation of the inverted tectal tissue and section of the optic nerve. The newly re-established visual projection on to the inverted tectal tissue showed a localized reversal of retinotopic order only along the rostrocaudal axis within the reimplanted area.

DISCUSSION

The present experiments show that a piece of the tectal tissue in adult goldfish may survive reimplantation after either a rotation or even an inversion of its entire laminar structures, and that the reimplanted tectal

tissue eventually becomes reinnervated by regenerating optic fibres. Furthermore the tectal reimplants were found to have retained their original topographic polarity regardless of the orientation of reimplantation. When the inverted tectal tissue was reimplanted along the same rostrocaudal axis of the tectum, the restored visual projection showed a localized reversal of retinotopic order only along the mediolateral axis within the reimplanted area. The restored visual projection retained a correct retinotopic order along the rostrocaudal axis. On the other hand, if the inverted tectal tissue was reimplanted along the same mediolateral axis of the tectum, the re-established visual projection on to the inverted reimplant was found to be organized in a reverse retinotopic order along the rostrocaudal axis within the reimplanted area, and in a correct retinotopic order along the mediolateral axis. The same trends were also observed after regeneration of optic fibres following section of the contralateral optic nerve. Furthermore, the retention of original topographic polarity was not a short-lived transitory phenomenon. It persisted as long as the reimplanted tectal tissue survived (at least for about 2 years).

The present results are compatible with those of previous reimplantation experiments, involving 90° clockwise rotation (Sharma & Gaze, 1971) or 180° rotation (Yoon, 1973) in adult goldfish tectum and also in the optic tectum of post-metamorphic *Xenopus* froglets (Levine & Jacobson, 1974). The retention of original topographic polarity by a small fraction of the adult tectal tissue (regardless of the orientation of its reimplantation after either a rotation or an inversion) suggests that the optic tectum is not a passive receiver of incoming optic fibres. Instead, the tectal tissue should be regarded as an active accommodator, which selects appropriate optic fibres to make proper synaptic connexions in a consistent topographic order according to its original polarity (Yoon, 1973). This interpretation is consistent with Sperry's (1943*a*, *b*, 1944, 1945, 1948, 1951) hypothesis that not only the retina but also the optic tectum undergoes congruent topographic polarization during neurogenesis. It is not known at what stage of embryonic development the presumptive optic tectum becomes topographically polarized for the first time, and when the topographic polarity of the embryonic tectal tissue eventually becomes irreversibly fixed. In *Ambystoma punctatum* embryos, Piatt (1949) reversed the rostrocaudal axis of the di-mesencephalic region by making a homoplastic substitution of a left for a right half at early embryonic stages (Harrison stages 21 and 22). The operated animals later showed negative results for visual tests. No optic fibres were observed to reach the inverted tectum. Crelin (1952) found that when the presumptive optic tectum in *Ambystoma* embryo was rotated 180° at stages between 22 and 30, before the topographic polarity of the embryonic retinal tissue became fixed at

stage 36 (Stone, 1944, 1947, 1960), the operated animal later developed a normal vision. Therefore the topographic polarity of the presumptive tectal tissue had not been irreversibly fixed as yet when the embryo reached the Harrison stage 30. If the tectum was reimplanted at a later stage, the grafted tectal tissue did not survive. This technical difficulty made it impossible for Crelin to determine the critical stage at which the topographic polarity of the embryonic tectal tissue becomes fixed. On the other hand, the pioneering experiments of Spemann (1906, 1912), involving reimplantation of the anterior part of the neural plate after 180° rotation in amphibian embryos, showed that the anteroposterior polarity of the neuroepithelium was already determined in the prenurula stage. In general, the polarity becomes fixed along the anteroposterior axis first, and then along the mediolateral axis (Spemann, 1912, 1938; Harrison, 1921; Weiss, 1939; Roach, 1945).

The present results show a puzzling fact that the retention of original topographic polarity does not require an integrity of the cytoarchitectonic structures of the reimplanted tectal tissue. Histological examination of the reimplanted tissue revealed a severe derangement in its laminar structures. Virtually all six horizontal layers underwent a drastic disintegration in the inverted tectal tissue. It was impossible to identify either the outer plexiform layer (*stratum fibrosum et griseum superficiale*), which was the main target zone of retinotectal projection, or the central neuropil (*startum griseum centrale*), which contained cell bodies of the central tectal neurones. The whole extent of the drastically deranged tectal tissue was filled mainly with irregular vortices of tangled fibre bundles. Sparse tectal neurones of bipolar and granular types were irregularly dispersed within the severely deranged structure of the reimplanted tectal tissue. Then, what factors of the reimplanted tectal tissue would be responsible for the orderly re-distribution of incoming optic fibres within the reimplanted tissue according to its original topographic polarity, in spite of the apparent chaos in its deranged structures? Did the structural derangement occur before the regenerating optic fibres entered the tectal reimplant, or only after they had reinnervated the reimplanted tissue? Further experiments are in progress to resolve the latter aspect of the perplexing problems.

I thank F. Baker for technical assistance. The research was supported by grants from the Medical Research Council (MA 4994) and the National Research Council (A 9756) of Canada, while the author held a Killam research professorship.

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EXPLANATION OF PLATES

Plate 1

Microphotographs of the dorsal view of the optic tectum after surgical operations in an adult goldfish.

A shows that a rectangular piece of the tectal tissue was dissected from the central zone of the dorsal tectum by making four sharp vertical incisions down to the level of the optic ventricle.

B shows that the dissected tissue was lifted intact, and then reimplanted to the same tectum after rotation by 180° around the dorsoventral axis.

C shows the operated tectum, re-exposed for a second mapping experiment, 575 days after the tectal reimplantation (see Text-fig. 1*B*). It also shows the intact right tectum, part of the two forebrains (right side), and part of the cerebellum (left side).

D shows the same operated tectum at a higher magnification. The reimplanted area became well revascularized. The tectal reimplant underwent partial degeneration along its lateral and caudal margins.

The calibration bar is equivalent to 1 mm for *A*, *B*, and *D*, and 1·6 mm for *C*.

Plate 2

Micrographs of a parasagittal section of the operated tectum shown in Pl. 1. The brain was fixed 575 days after the 180° rotated reimplantation, embedded in paraffin, serially sectioned at 15 μm, and stained by a modified Bodian protargol method.

An over-all view of the mid-brain structures is shown in the centre of the montage. A portion of the intact rostral zone of the operated tectum is shown at a higher magnification in the bottom right side of the montage. It retained a normal laminar structure with six distinguishable horizontal layers. *a*, *stratum fibrosum marginale*; *b*, *stratum opticum*; *c*, *stratum fibrosum et griseum superficiale*; *d*, *stratum griseum centrale*; *e*, *stratum album centrale*; and *f*, *stratum periventriculare*.

The 180° rotated tectal reimplant is shown in the upper half of the montage. The reimplanted tissue underwent a severe derangement of its laminar structure. The prominent feature of the deranged tissue was irregular vortices of tangled fibre bundles. Sparse tectal neurones of bipolar and granular types were scattered in the deranged central zone of the reimplanted tissue.

A portion of the caudal zone of the same tectum is shown in the bottom left side of the montage.

PLATE 3

Micrographs of a parasagittal section of the operated tectum shown in Text-fig. 2. The brain was fixed 435 days after reimplantation of the tectal tissue following rotation by 90° anticlockwise.

The entire extent of the reimplanted tissue is shown in the upper half of the montage. Note that the central layers of the reimplanted tissue disintegrated and were replaced by irregular vortices of tangled fibre bundles. Sparse tectal neurones of granular and bipolar types were dispersed in the deranged structure of the reimplant.

Portions of the intact rostral and the caudal areas of the same tectum are shown in the lower right side and the lower left side of the montage, respectively. They retained more or less normal laminar structures.

PLATE 4

Micrographs of the optic tectum after surgical operations.

A, shows that a rectangular piece of the tectal tissue was dissected from the central zone of the dorsal tectum by making four sharp vertical incisions down to the level of the optic ventricle.

B, shows that the dissected tissue was lifted intact, turned upside-down around the rostrocaudal axis, and then reimplanted along the same rostrocaudal axis of the tectum. The ventral surface of the inverted tectal reimplant was exposed to the dorsal view of the operated tectum.

C and *D* show the operated tectum, re-exposed for a mapping experiment, 335 days after the inversion of the tectal layers (see Text-fig. 4). The contralateral optic nerve was sectioned, and then allowed to regenerate into the operated tectum. The calibration bar is 1 mm long for *A*, *B*, *D*, and 1·6 mm long for *C*.

PLATE 5

Micrographs of a parasaggital section of the operated tectum shown in Pl. 4. The brain was fixed 335 days after reimplantation of the inverted tectal tissue along the same rostrocaudal axis of the tectum. The right optic nerve was sectioned at the same time, and then allowed to regenerate into the operated tectum.

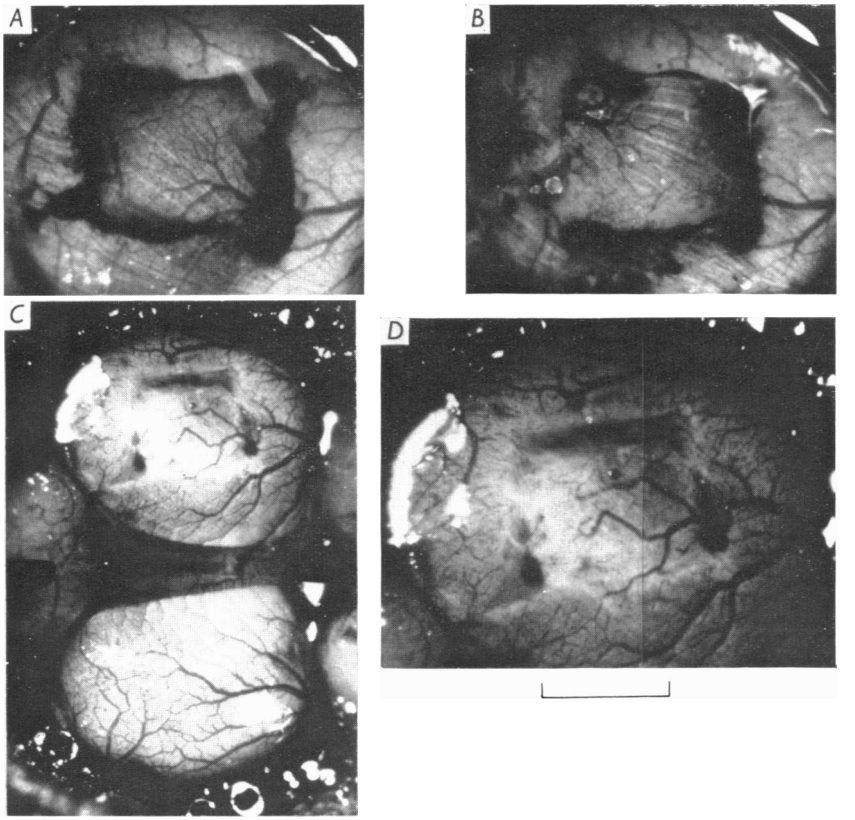
The inverted tectal reimplant is shown in the upper half of the montage. Note the severe derangement of the inverted tissue. Virtually all six layers underwent drastic disintegration. The prominent feature in the deranged tectal tissue is irregular vortices of tangled fibre bundles. Sparse tectal neurones of bipolar and granular types were irregularly dispersed in the deranged structure of the inverted reimplant.

Portions of the intact rostral and the caudal zones of the same tectum are shown in the lower right side and the lower left side of the montage, respectively.

PLATE 6

Micrographs of a parasagittal section of the operated tectum shown in Text-fig. 5. The brain was fixed 336 days after reimplantation of the inverted tectal layers along the same mediolateral axis of the tectum. The optic nerves were left intact.

The whole extent of the inverted tectal reimplant is shown in the upper half of the montage. The inverted tectal tissue shows a severe derangement. The entire laminar structure disintegrated, and was replaced by irregular vortices of tangled fibre bundles. Sparse tectal neurones were irregularly scattered in the chaotic structure of the inverted tectal tissue. Portions of the intact rostral and the caudal parts of the same tectum are shown in the lower right side and in the lower left side of the montage, respectively.


A
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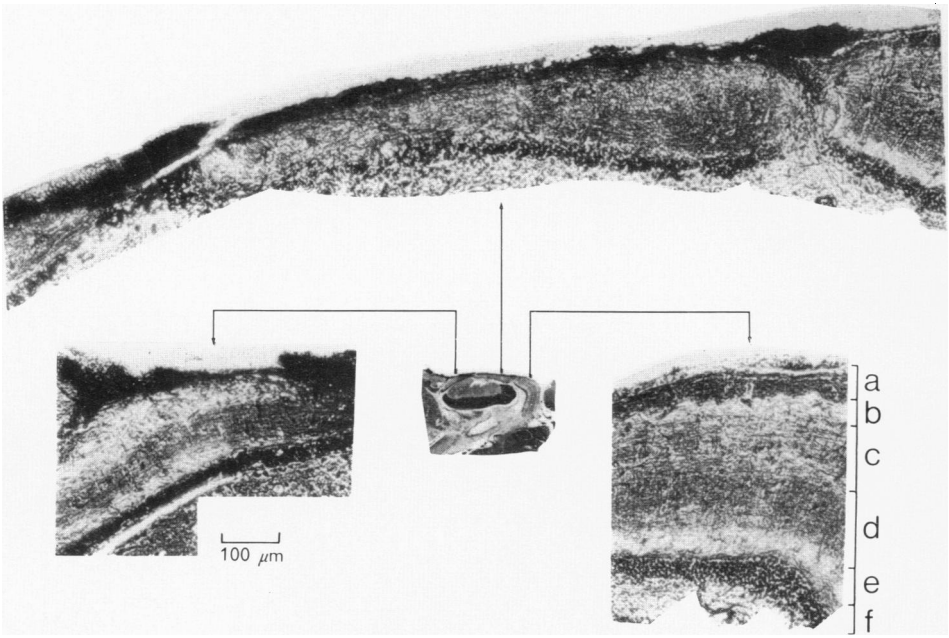
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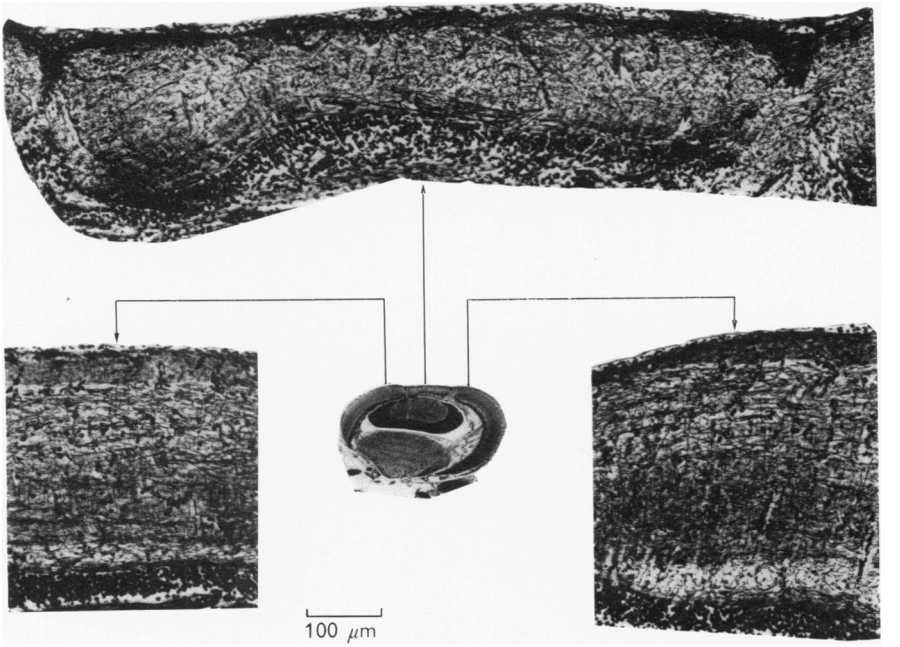
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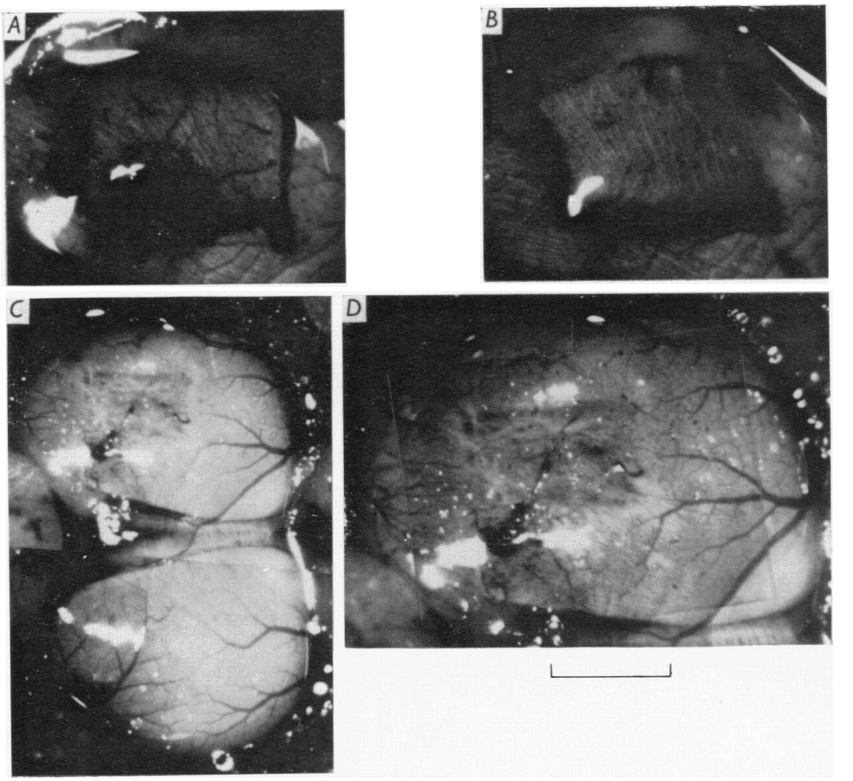
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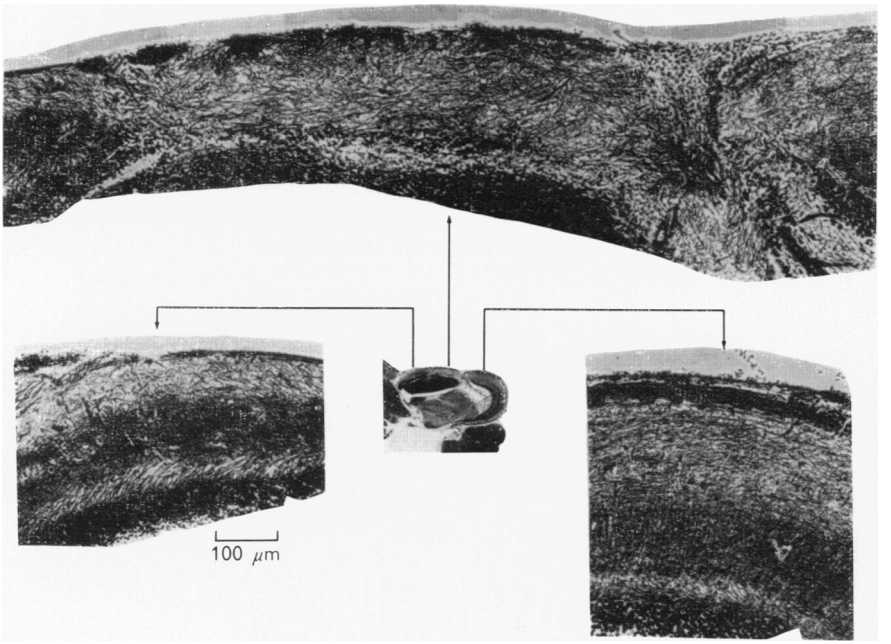
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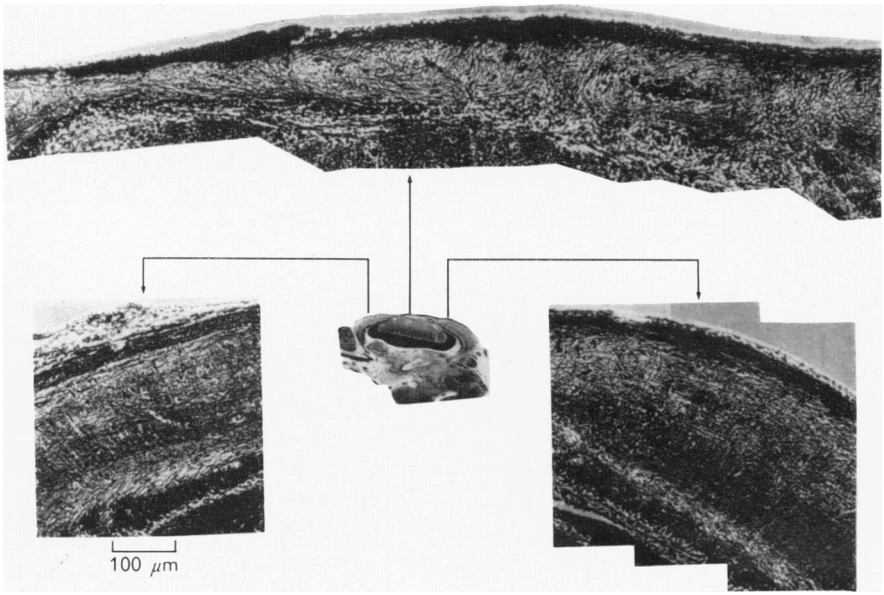
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