J. Physiol. (1975), **246**, *pp.* 673–694
With 5 plates and 8 text-figures
Printed in Great Britain

EFFECTS OF POST-OPERATIVE VISUAL ENVIRONMENTS ON REORGANIZATION OF RETINOTECTAL PROJECTION IN GOLDFISH

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(*Received 12 August 1974*)

SUMMARY

1. Possible influence of different visual environments on the reorganization of retinotectal projection was studied with neurophysiological mapping methods following excision of the caudal half of the optic tectum in adult goldfish.

2. Post-operative light-deprivation showed no significant effects: in the absence of visual input, the visual projection from the whole retina became compressed on to the remaining rostral half-tectum in correct retinotopic order within 4 months, regardless of whether the contralateral optic nerve was left intact, or severed and then allowed to regenerate.

3. When the operated goldfish were continually exposed to visual stimuli without any dark period (post-operative dark-deprivation), two different results were observed: if the optic nerve was sectioned, in addition to excision of the caudal tectum, an orderly field compression was observed within 70 days in the re-established retinotectal projection; on the other hand, if the optic nerve was left intact, the dark-deprived fish retained the original connexions between the remaining rostral half-tectum and the temporal hemiretina without showing any sign of field compression for up to 253 days.

4. When the dark-deprived fish was then transferred into darkness, the suppressive effect disappeared: a compression of the retinotectal projection was induced within 2 or 3 weeks after the transfer.

5. Histological preparations of the fish brains showed consistent morphologic changes in the laminar structure of the remaining half-tectum. The *stratum opticum* and the *stratum fibrosum et griseum superficiale* merged together to form a new layer which contained an intricate network of thick fibre bundles.

INTRODUCTION

The visual pathways in goldfish provide a model system for studying the factors regulating the growth of highly ordered neural connexions between the retina and the mid-brain optic tectum. During neurogenesis, many hundred thousands of axons sprouting out from the ganglion cells in the retina select particular routes to their destinations and innervate the optic tectum in a consistent topographic order. If the original retinotectal projection is interrupted by severing the optic fibres in an adult goldfish, an orderly regeneration of the proximal parts of the sectioned optic fibres restores the visual projection: the scrambled regenerating optic fibres somehow unsort themselves, preferentially select particular routes to their respective destinations, and eventually form functional reconnexions with predesignated target zones in the tectum (for review see Sperry, 1963, 1965; Gaze, 1970; Jacobson, 1970; Hunt & Jacobson, 1974).

In addition to the ability of regeneration of optic fibres, the visual system of an adult goldfish shows another important property. It maintains a high degree of plasticity in its capacity to readjust to various types of size disparity experimentally induced between the retina and the optic tectum (Gaze & Sharma, 1970; Yoon, 1971, 1972*a*, *b*, 1973*a*; Sharma, 1972*a*, *b*, 1973; Holder, 1971). Using neurophysiological mapping methods, Gaze & Sharma (1970) found that, if the caudal half of the tectum was removed in an adult goldfish, there occurred an orderly compression of visual projection from the entire range of the contralateral visual field on to the remaining rostral half-tectum (which previously received projections from only the nasal half of the visual field). The compression of retinotectal projection may be induced not only along the rostrocaudal axis but also along the mediolateral axis of the optic tectum (Yoon, 1971). Furthermore, the field compression has been shown to be a reversible phenomenon (Yoon, 1972*a*): a compression of visual projections from the whole retina on to the rostral half-tectum can be induced by surgical insertion of a mechanical barrier between the rostral half and the denervated caudal half of the tectum. If the barrier is either removed or absorbed later, the field compression disappears, and a normal projection from the retina on to the whole extent of the rejoined rostral and caudal halves of the tectum is restored. In a further study (Yoon, 1972*a*), the newly decompressed visual projection was found to become compressed again following an excision of the caudal half of the optic tectum.

The compression of retinotectal projection in goldfish is a slow and gradual process. It usually takes about 2 to 3 months to complete after the tectal surgery if the operated goldfish have been kept at about 25° C and on a regular daily cycle of 12 hr in light and 12 hr in darkness (Yoon, 1971,

1972*a*). Suppose that the operated goldfish are exposed to different visual environments during their post-operative recovery periods. Would the compression of retinotectal projection depend on the specific conditions of the post-operative visual input? In the present experiments, a possible influence of visual environments upon the compression of retinotectal projection was studied under three different conditions: in the case of a light-deprived group, the experimental fish had been kept in darkness just after excision of the caudal tectum until their visual projections were mapped at various post-operative recovery periods; in a dark-deprived group, the operated fish had been continually exposed to visual stimuli during the entire post-operative recovery periods; in a transferred group, the experimental fish had been continually exposed to light for a certain post-operative period, and then transferred into either darkness or a normal visual environment (under a regular daily cycle of 12 hr in light and 12 hr in darkness) for an additional post-operative period. A preliminary account of the results was presented elsewhere (Yoon, 1973*b*).

METHODS

Common goldfish (*Carassius auratus*) used in the present experiments weighed about 8·5–14 g and were about 65–77 mm long from the nose to the base of the tail at the time of surgery. Before either surgical operation or neurophysiological recording, 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 that restrained the fish to a desired position within a holder. The gills were continually infused with aerated water through a tube in the mouth. The fish were not paralysed excepting for the terminal mapping session, and instead were hyperinfused with water at the rate of 1400 ml./min for about half an hour. In the majority of cases, this hyperinfusion eliminated most movements during a mapping session. The infusion rate was reduced to about 500 ml./min thereafter. The optic tectum was exposed by opening a single cranial bone flap that was restored at completion of surgery. The experiments involved the following surgical operations: the caudal half of the left tectum was completely excised with microscissors and a curved microforceps (Pls. 1 and 4). In some cases, the contralateral optic fibres were also sectioned by squeezing and teasing the optic nerve with finely pointed forceps at a distance about 1 mm from the posterior pole of the eyeball. All surgery and neurophysiological recordings were performed with the aid of a stereomicroscope at magnification of 6–40 times.

The operated fish had been either kept in darkness (light-deprived group) or continually exposed to visual stimuli (dark-deprived group) just after the tectal surgery until their retinotectal projections were mapped at various intervals of post-operative recovery periods. The light-deprived group was kept in light-tight aquaria inside a dark room. The dark-deprived group was housed in transparent glass aquaria which were continually illuminated with incandescent lamps. An average luminance at the bottom of these aquaria was about 0·8 log ft. lamberts and that at their walls was about 1·7 log ft. lamberts. The mean water temperature in these aquaria was about 22° C.

Standard neurophysiological methods were used for mapping retinotectal

projections (Hamdi & Whitteridge, 1954; Gaze, 1958; Lettvin, Maturana, McCulloch & Pitts, 1959; Jacobson & Gaze, 1964, 1965; Schwassmann & Kruger, 1965; Yoon, 1971, 1972*a*) with some modifications: action potentials were recorded from deep tectal layers by advancing tungsten micro-electrodes at a depth between 200 and 300 μm from the dorsal surface of the tectum. Electrolytic lesions, made through the recording micro-electrodes (by passing 5–7 μA d.c. for 5–10 sec), were later identified in histological preparations of the brain. Most of the lession were found to be located in the stratum griseum centrale (layer *d* in Pl. 3 and Pl. 5) or near its dorsal boundary with the stratum fibrosum et griseum superficiale (layer *c* in Pl. 3). In most cases the recorded action potentials were mixtures of two or three units, but occasionally single units were also isolated. The size of receptive fields for these deep tectal units ranged from about 10 to 25 degrees in diameter. When a small amount (0·3–0·5 mg) of D-tubocurarine chloride (Squibb) was topically applied on to the exposed optic tectum, visual responses of these deep tectal units usually diminished and then disappeared within 10–15 sec. These results suggest indirectly that the extracellularly recorded action potentials originate from post-synaptic visual neurones in the deep inner plexiform layers of the tectum. Some characteristics of the goldfish tectal units recorded at different levels of tectal layers and their pharmacological properties will be reported elsewhere (M. G. Yoon, in preparation). 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. This device made it possible to keep the cornea clear during a mapping experiment, which usually took about 2–5 hr. In some cases, the extra-ocular muscles were cut in order to immobilize the eyeball during a mapping session.

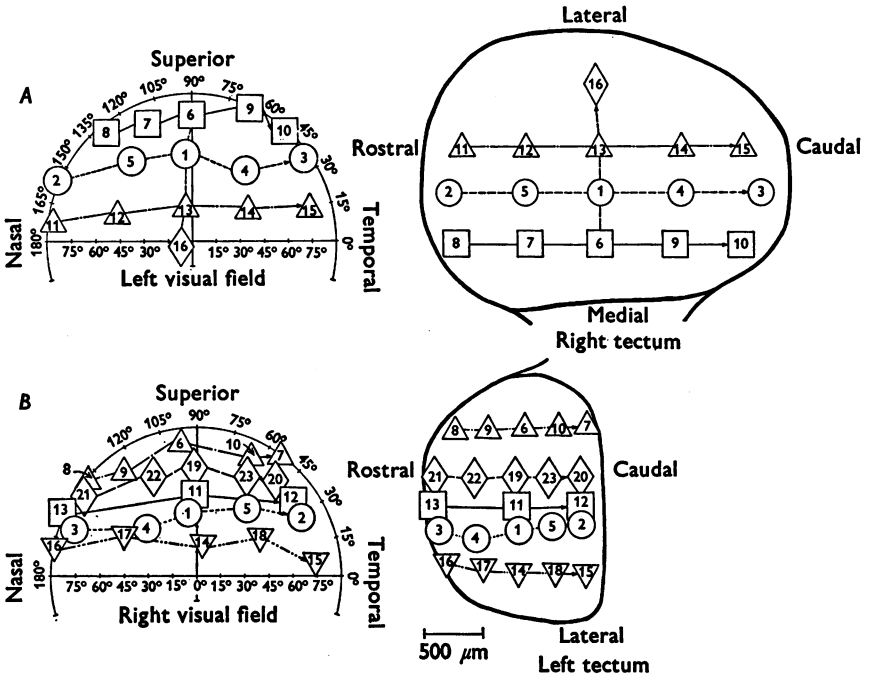
At the end of a terminal mapping session, the fish head was immersed in a mixture of 18 parts of 80% ethanol, 1 part glacial acetic acid and 1 part formalin. The brain was dissected free, embedded in paraffin and serially sectioned at 15 μm. These sections of the brain were stained with a modified Bodian protargol method (Bodian, 1937; Attardi & Sperry, 1963).

RESULTS

Post-operative light-deprivation

Experiment 1. In twelve goldfish the caudal half of the left tectum was completely excised and the remaining rostral half-tectum was also denervated by severing the contralateral optic nerve near the posterior pole of the right eyeball. These fish had been kept in darkness until their retinotectal projections were mapped at various post-operative intervals between 80 and 332 days. During the period of light-deprivation, the fish showed a remarkable reduction in their swimming activities. Four fish died during the recovery period. Retinotectal projections were mapped for the other eight fish. The remaining half-tectum in these light-deprived fish gave brisk visual responses. All eight fish showed an orderly compression in their re-established retinotectal projection. Text fig. 1 shows the result obtained from one of the light-deprived fish 80 days after the surgical operations. A normal projection from the left visual field to the

intact right tectum is shown in Fig. 1*A*. The nasal half of the visual field projects to the rostral half of the contralateral tectum and the temporal half of the visual field projects to the caudal half of the tectum. A naso-temporal series in the visual field is represented by a rostrocaudal series on the contralateral tectum whereas a dorsoventral series in the upper half of the visual field is represented by a mediolateral series on the dorsal



Text-fig. 1. Projections of the visual field on to the contralateral optic tectum in an adult goldfish. The numbers marked on the enlarged drawing of the tectum indicate the loci of recording micro-electrodes on the dorsal surface of the tectum in the order of recording visual responses from deep tectal layers. The corresponding 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 tectum.

A shows a normal visual projection from the left eye to the intact right tectum. The nasal half of the visual field projects on the to rostral half of the tectum and the temporal half-field projects onto the caudal half-tectum.

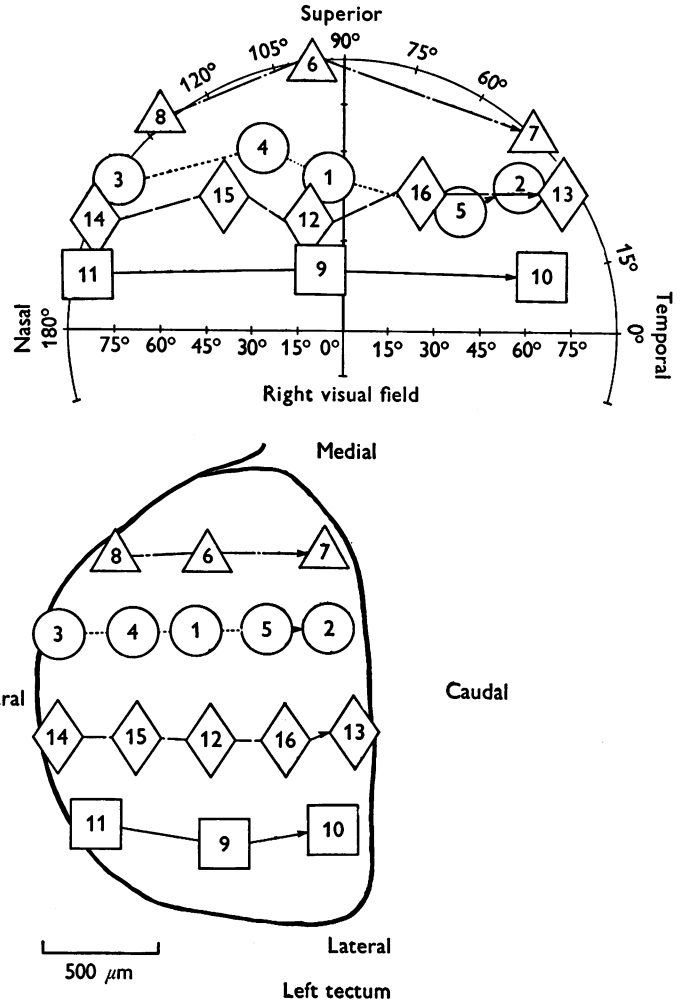
B shows a re-established visual projection from the right eye to the rostral half of the left tectum 80 days after excision of the caudal part of the tectum and section of the right optic nerve in the same fish. Note that the projection from the nasal half-field is compressed on to the rostral quadrant and that from the temporal field is compressed on to the caudal half of the remaining rostral half-tectum in correct retinotopic order. The fish has been kept in darkness during the post-operative recovery period.

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 contralateral visual field (Leghissa, 1955; Attardi & Sperry, 1963; Schwassmann & Kruger, 1965). Fig. 1*B* shows the re-established projection from the right visual field to the remaining rostral half of the left tectum in the same fish. The map indicates that the rostral half-tectum (which previously received projections from only the nasal half of the contralateral visual field) re-acquired projections from the entire range of the visual field in correct retinotopic order: the projection from the nasal half-field is compressed on to the rostral quadrant and that from the temporal field is compressed on to the foreign caudal area of the remaining rostral half-tectum in the light-deprived fish.

Experiment 2. In another group of twelve goldfish the caudal half of the left tectum was completely excised. The contralateral optic nerve, however, was left intact so that the remaining rostral half-tectum was allowed to retain its original connexions with the temporal area of the retina. These fish had also been kept in darkness until their retinotectal projections were mapped at various post-operative intervals between 65 and 162 days. Three fish died during the experimental period. In the case of four fish tested between 65 and 82 days after the tectal surgery, only the nasal half of the visual field projected to the remaining rostral half-tectum without showing any sign of compression. The other five fish tested at a later period between 117 and 162 days, however, showed an orderly compression in their retinotectal projection maps. Text-fig. 2 shows one of the latter maps obtained 117 days after the tectal surgery. The map indicated that, in the absence of visual input, the rostral half-tectum acquired visual projection from the whole retina in correct retinotopic order.

Post-operative dark-deprivation

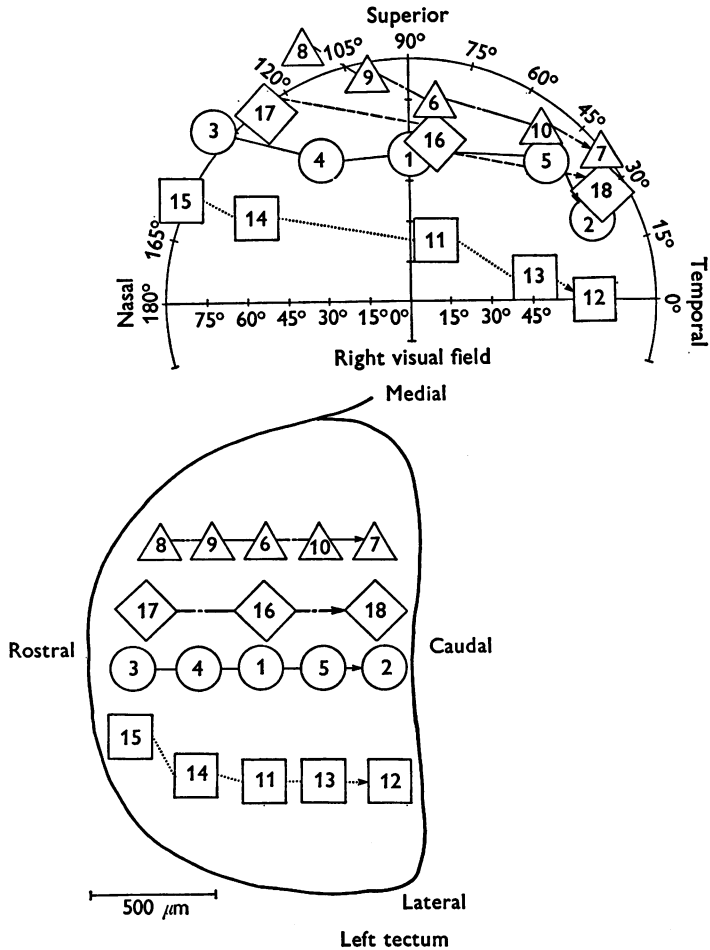
Experiment 3. In twelve goldfish, the caudal half of the left tectum was excised and the remaining rostral half-tectum was also denervated by severing the contralateral optic nerve at the same time. The operated fish were kept together in a transparent glass aquarium that was continually illuminated throughout the experimental period. These dark-deprived fish looked healthy and very active. Only one fish died during the recovery period. Retinotectal projections were mapped for the other eleven fish at post-operative intervals between 68 and 153 days. All of these dark-deprived fish showed an orderly compression in their re-established retinotectal projections. Text-fig. 3 shows one of these maps obtained 68 days after the tectal surgery and optic nerve section. The map suggested that the once-denervated rostral half-tectum was able to accommodate



Text-fig. 2. Compression of retinotectal projection during post-operative light-deprivation. The retinotectal projection was mapped 117 days after excision of the caudal half of the left tectum. The right optic nerve was left intact. The fish has been kept in darkness during the post-operative recovery period.

regenerating optic fibres from the whole retina which had been continually exposed to light throughout the experimental period.

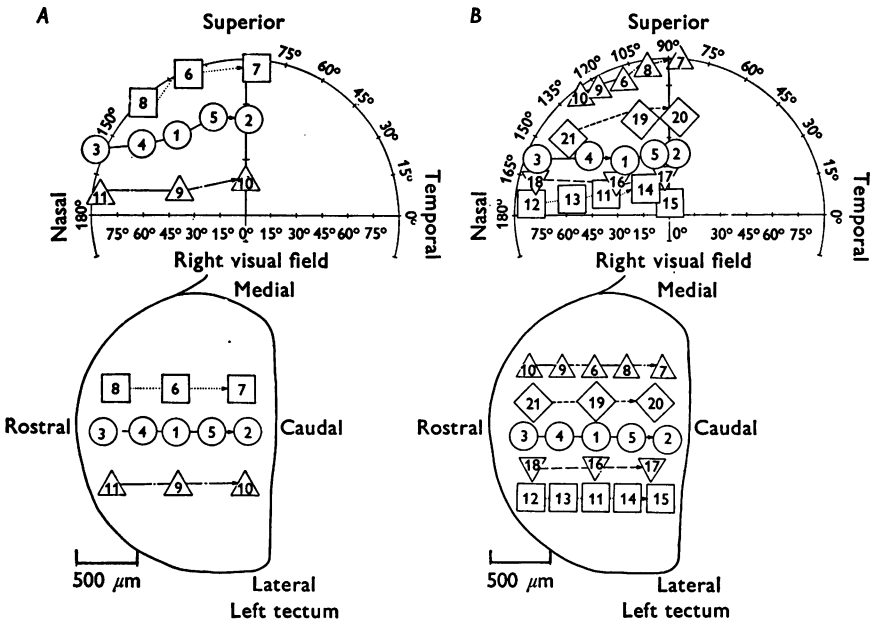
Experiment 4. In another group of twelve fish, the caudal half of the left tectum was completely ablated but the original connexions between the remaining rostral half-tectum and the temporal hemiretina were left intact. These fish had also been continually exposed to light until their



Text-fig. 3. Compression of retinotectal projection during post-operative dark-deprivation. The map was obtained 68 days after excision of the caudal half of the left tectum and section of the right optic nerve at the same time. The fish had been continually exposed to visual stimuli without any dark period during the post-operative recovery period.

retinotectal projections were mapped at post-operative intervals between 82 and 239 days. Two fish died prematurely. Retinotectal projections were mapped for the other ten dark-deprived fish. In contrast to the foregoing results, however, none of the ten tested fish showed any sign of compression in their retinotectal projection maps. Five out of the ten fish were revived after their first mapping experiments. These five fish were re-exposed to continuous light until their retinotectal projections were remapped at

later periods. Only two of the five fish survived long enough for a second mapping experiment. Neither of these two fish showed any sign of compression in their second maps. Text-fig. 4 shows the result obtained from one of these dark-deprived fish. The first map obtained 167 days after the tectal surgery is shown in Fig. 4*A*. The map indicates that only the nasal half of the right visual field projected on to the remaining rostral half of the left tectum. The second map shown in Fig. 4*B* was obtained from the same dark-deprived fish 72 days after the first mapping experiment (239 days after the tectal surgery). The latter map does not show any sign of compression either.



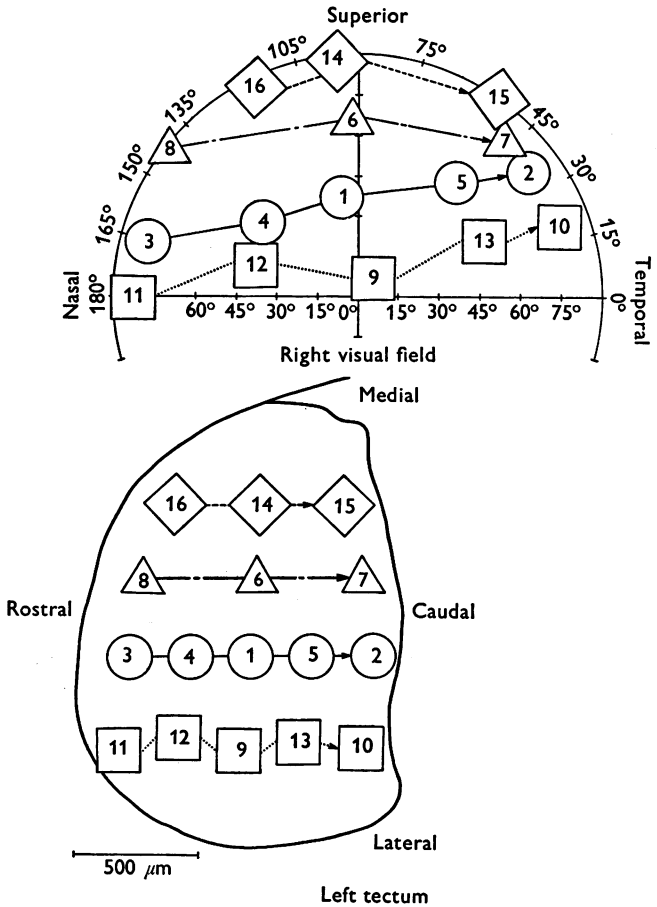
Text-fig. 4. Suppression of field compression during post-operative dark-deprivation. The caudal half of the left tectum was excised but the right optic nerve was left intact in this dark-deprived fish. *A* was mapped 167 days after the tectal surgery. *B* was obtained from the same fish after a more prolonged post-operative dark-deprivation for 239 days. Neither of these maps show any sign of field compression.

Change of the post-operative visual environment

The result of Expt. 4 suggests an unexpected possibility: if the original neural connexions between the temporal half of the retina and the remaining rostral half-tectum have never been surgically interrupted, a continual exposure of the retina to visual stimuli somehow delays or even suppresses a re-organization of retinotectal projection. Then what

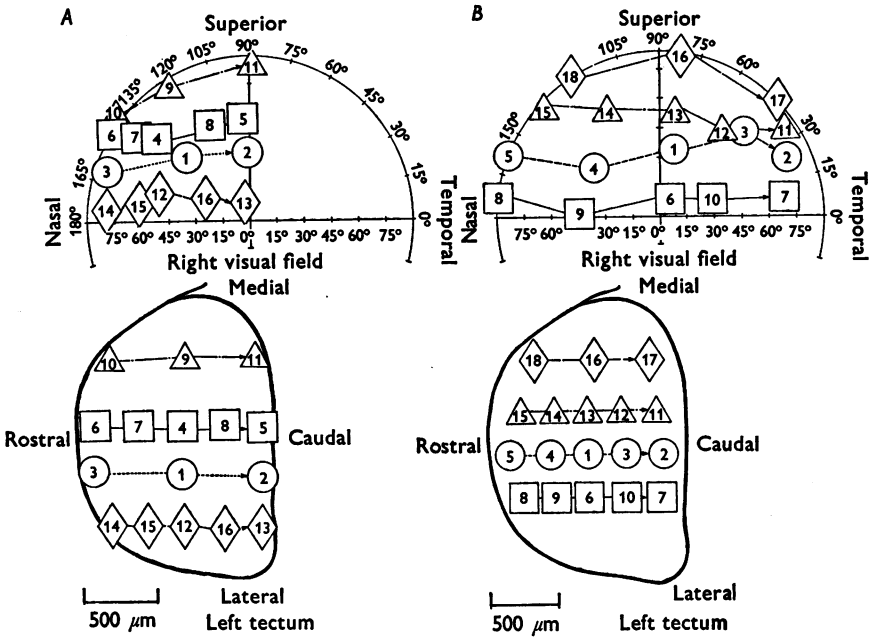
happened to those optic fibres originating from the ganglion cells in the nasal area of the intact retina? Did they undergo a degeneration during the periods of dark deprivation? Suppose that a dark-deprived fish is transferred to a normal visual environment, or into darkness for an additional post-operative recovery period. Would it be possible to induce a compression of retinotectal projection by the change of post-operative visual environment? If so, how long would it take to induce a field compression in the new visual environment?

Experiment 5. In thirty goldfish, the caudal half of the left tectum was excised without interrupting the original connexions between the tem-



Text-fig. 5. Compression of retinotectal projection under a normal visual environment. The map was obtained 84 days after excision of the caudal tectum. The right optic nerve was left intact. The experimental fish has been kept on a daily cycle of 12 hr in light and 12 hr in darkness during the post-operative recovery period.

poral hemiretina and the remaining rostral half-tectum. The tectal surgery was performed in all thirty fish within a day. Six fish out of the thirty experimental fish were kept on a regular daily cycle of 12 hr in light and 12 hr in darkness. Two of the six fish died prematurely. Retinotectal projections were mapped from the other four fish at post-operative periods between 84 and 87 days. All four fish showed an orderly compression in



Text-fig. 6. Release from a delay in field compression following a change of the post-operative visual environment. *A* was mapped 94 days after excision of the caudal tectum in the dark-deprived fish. The right optic nerve was left intact. *B* was obtained 77 days after the transfer of the same fish to a normal visual environment. The maps show that field compression was induced after the transfer.

their retinotectal projection maps, one of which is shown in Text-fig. 5. The result indicates that under the present experimental conditions a compression of retinotectal projection becomes complete 84 days (or even earlier) after the tectal surgery if the operated fish has been kept on a normal daily light-dark cycle during the post-operative recovery period.

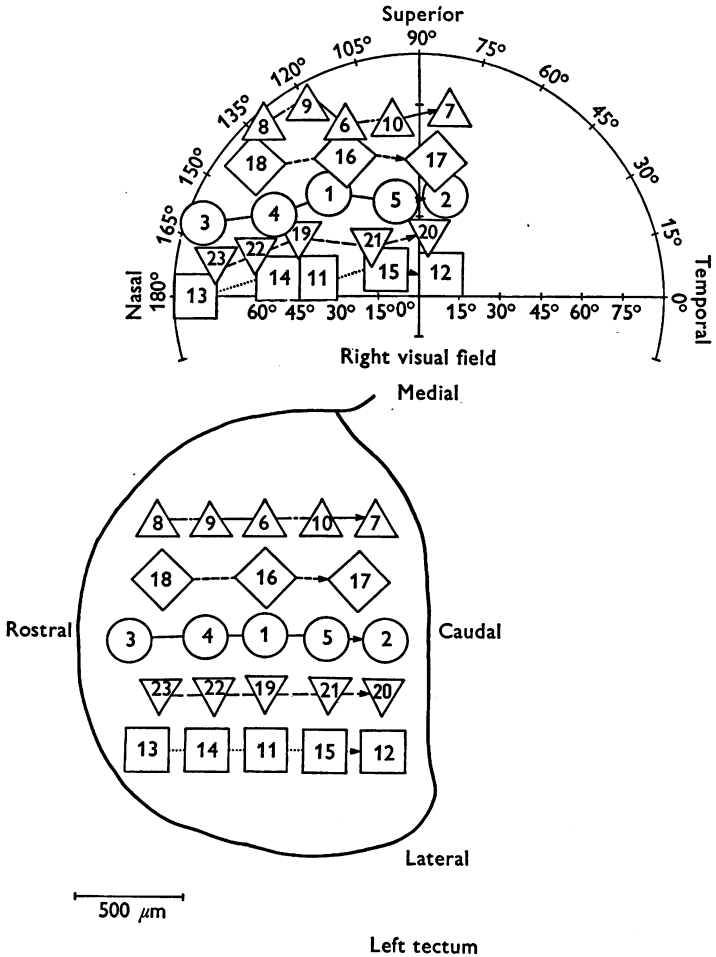
The other twenty-four out of the thirty experimental fish had been continually exposed to light just after the tectal surgery until they were tested later. Retinotectal projections were mapped from ten of these dark-deprived fish at post-operative intervals between 87 and 94 days.

None of these ten fish showed any sign of compression in their maps. Seven out of the ten fish were successfully revived after their first mapping experiments. These seven fish were transferred to a normal visual environment where they were kept on a regular daily cycle of 12 hr in light and 12 hr in darkness. Four of the transferred fish died before a second mapping experiment was attempted. Retinotectal projections were remapped from the other three fish at intervals between 72 and 84 days after their transfer. All three maps obtained from these transferred fish indicated that an orderly compression of retinotectal projection had been induced after the transfer. The result obtained from one of these transferred fish is shown in Text-fig. 6. The first map shown in Text-fig. 6*A* was obtained at the end of the dark-deprivation for a post-operative recovery period of 94 days. The map showed that only the nasal half of the visual field projected on to the remaining rostral half-tectum with no sign of compression at that time. Text-fig. 6*B* shows the second map obtained from the same experimental fish 77 days after the transfer to the normal visual environment. The latter map indicated that projections from the entire range of the visual field became compressed on to the remaining rostral half-tectum after the change of post-operative visual environment.

The other fourteen fish out of the thirty operated animals had been continually exposed to visual stimuli until they were tested at post-operative intervals between 164 and 253 days. Three fish died prematurely. In agreement with the previous result of Expt. 4, none of the eleven surviving dark-deprived fish showed any sign of field compression in their maps. Text-fig. 7 shows one of these maps obtained at the end of post-operative dark-deprivation for 253 days. The map indicates that only the nasal half of the visual field projects on to the remaining rostral half-tectum.

Four fish out of the above eleven dark-deprived animals were successfully revived after their mapping experiments. These four fish were then transferred into darkness for an additional period between 2 and 21 days. One fish kept for 2 days in darkness showed no change in its non-compressed projection. Two other fish kept for a longer period in darkness (15 and 21 days respectively), however, showed an induction of field compression during these dark periods. The last fish in this series yielded three consecutive maps as shown in Text-fig. 8. The first map in Text-fig. 8*A* was obtained at the end of the post-operative dark-deprivation for 202 days. At this time, no sign of field compression was observed. This fish was then transferred into darkness. A second map (Fig. 8*B*), obtained 9 days after the transfer into darkness, revealed an intermediate state: a field compression occurred only in the medial side of the half-tectum. The lateral side of the same half-tectum showed no sign of compression at this stage.

The fish was revived again, and then kept in a normal visual environment (12 hr in light and 12 hr in darkness) for additional 12 days. The final map in Fig. 8*C* indicated that the field compression became complete during the last 12 days in the normal visual environment.

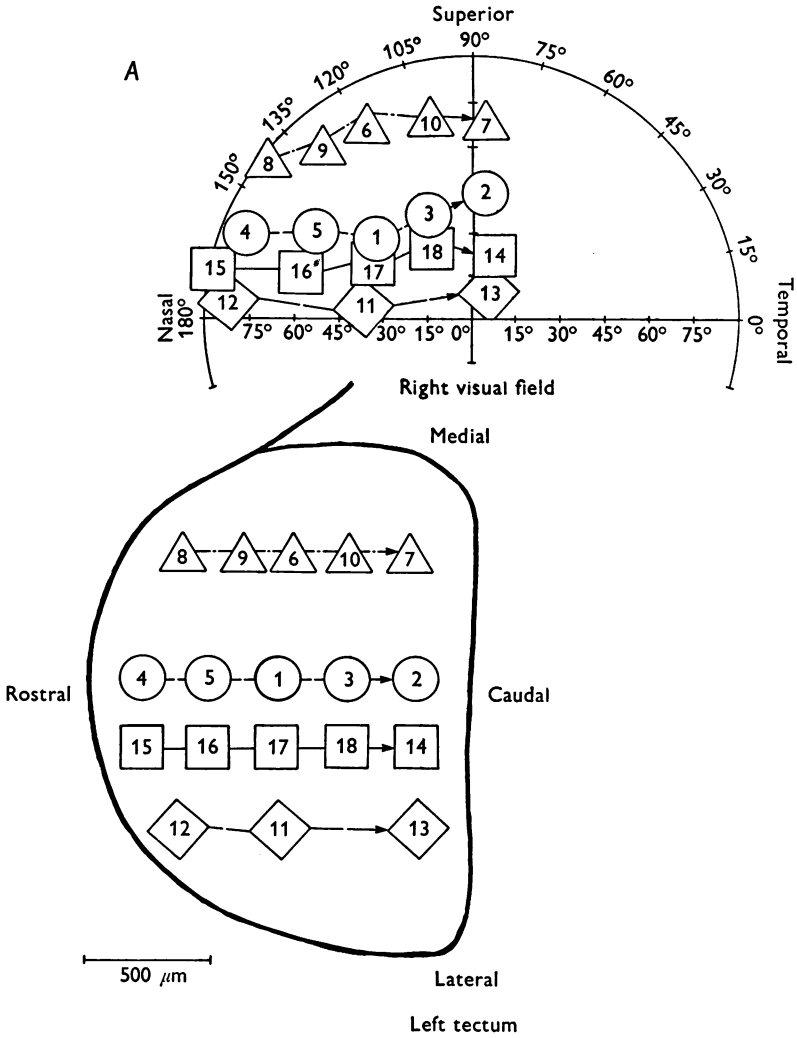


Text-fig. 7. Delay of field compression during post-operative dark-deprivation. The map was obtained 253 days after excision of the caudal half of the left tectum. The right optic nerve was left intact. This experimental fish has been continually exposed to visual stimuli during the post-operative recovery period.

Morphologic changes in the laminar structure of the remaining half-tectum

The compression of retinotectal projection suggests the possibility that the remaining half-tectum would undergo a reorganization of its neural

structure. Histological examinations of the operated tectum and its contralateral intact tectum show the following morphological differences in their laminar structures.



Text-fig. 8. Changes in retinotectal projection under different post-operative visual environments. The caudal half of the left tectum was excised but the right optic nerve was left intact. *A* was mapped at the end of a post-operative dark-deprivation period of 202 days. *B* was obtained from the same fish 9 days after its transfer into darkness. This fish was revived again, and then transferred to a normal visual environment. *C* shows the final map obtained from the same fish 12 days after the latter transfer.

Normal tectum. An intact optic tectum in goldfish has the shape of a spheroidal shell (Pls. 1 and 2). A parasagittal section of the normal tectum, stained by a Bodian protargol method, reveals a stratified structure as shown in Pls. 2 and 3. Six horizontal layers are distinguishable in the

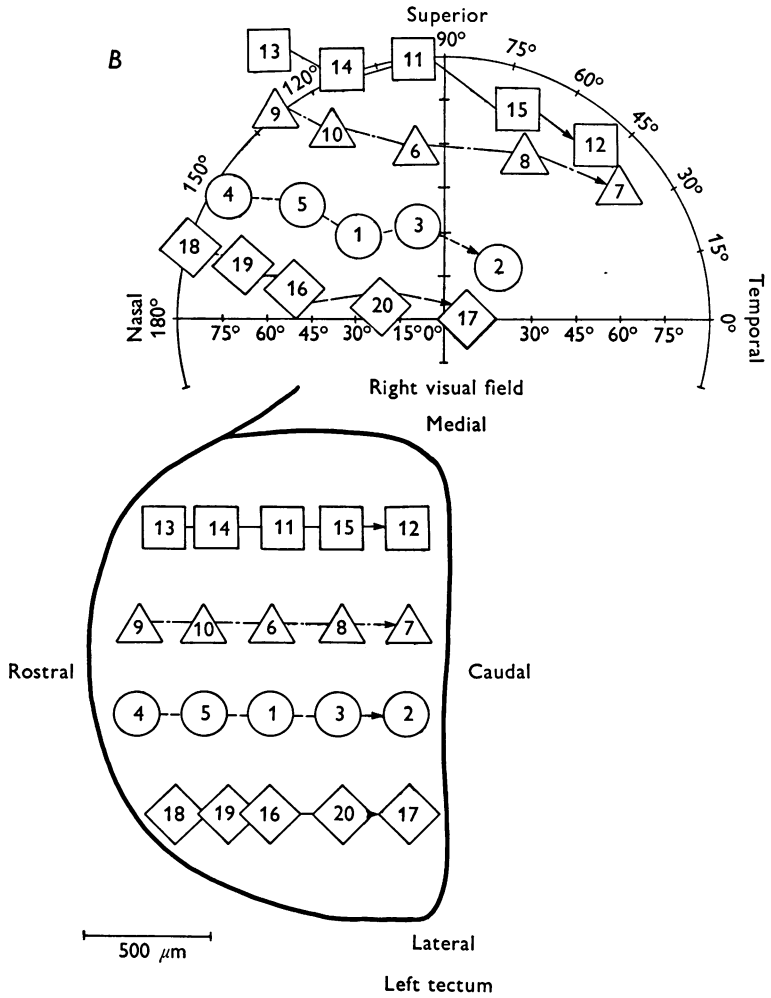


Fig. 8*B*. For legend see facing page.

present histological preparations. In the following descriptions, the terminology used by Vanegas & Ebbesson (1973) for a teleost optic tectum is adopted.

The outermost layer (labelled *a* in Pl. 3) beneath the pia matter corresponds to stratum fibrosum marginale. It is about 30–40 μm thick. This

layer does not show any prominent neural structures. The next lightly stained layer (labelled *b* in Pl. 3) corresponds to stratum opticum. This fibrous layer is about 50–70 μm in thickness. It gradually tapers off at the caudal end of the tectum. Incoming optic fibres at the rostral end of the

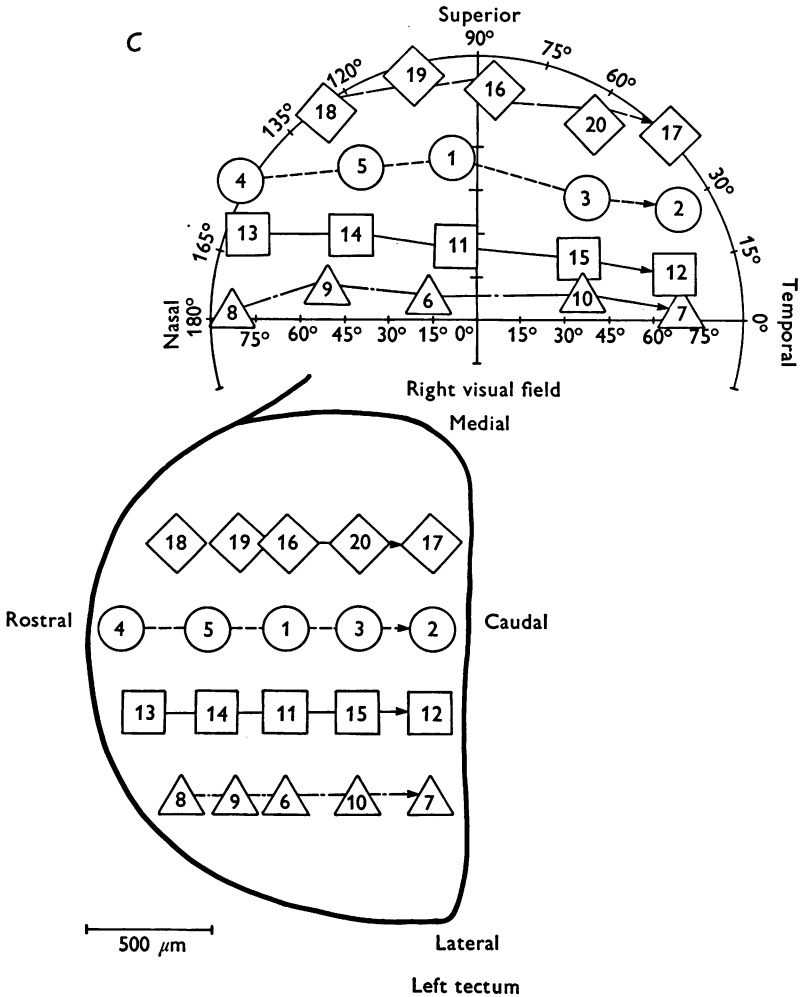


Fig. 8*C*. For legend see p. 686.

tectum spread fan-like, and then course through the layer towards the caudal end along the circumferences of the tectum. The upper zone of the central plexiform layers (labelled *c* in Pl. 3) corresponds to stratum fibrosum et griseum superficiale. Its thickness is about 120–150 μm in a normal tectum. This outer plexiform layer shows an intricate neuropil. It

also contains cell bodies of various tectal neurones. This stratum has been shown to be the main target zone of retinotectal projection (Leghissa, 1955; Attardi & Sperry, 1963; Jacobson & Gaze, 1964; Schwassmann & Kruger, 1968; Sharma, 1972c). The next plexiform layer (labelled *d* in Pl. 3) corresponds to stratum griseum centrale. This inner plexiform layer, which usually stains denser than the above outer plexiform layer, is also about 120–150 μm thick. The inner plexiform layer contains cell bodies of various neurones, embedded in a very dense and intricate neuropil. Optic fibre degeneration studies in goldfish (Sharma, 1972*c*) and in other teleosts (Ebbesson,1968; Campbell & Ebbesson, 1969; Vanegas & Ebbesson, 1973; Vanegas *et al.* 1974; Laufer & Vanegas, 1974*a*, *b*) showed that the stratum griseum centrale did not contain optic fibres: terminals of degenerating optic fibres were found in the stratum fibrosum et griseum superficiale, but they disappeared abruptly below its boundary with the stratum griseum centrale. The deeper fibrous layer (labelled *e* in Pl. 3) corresponds to stratum album centrale. It is about 30–40 μm in thickness. This stratum has been shown to contain a fraction of incoming fibres from the contralateral retina (Jacobson & Gaze, 1964; Sharma, 1972*c*) as well as efferent fibres from tectal neurones, and fibres of other origins (Leghissa, 1955). The innermost layer (labelled *f* in Pl. 3) corresponds to stratum periventriculare. This layer contains several sheets of tightly packed cell bodies. Its thickness is about 80–100 μm. Beneath this layer are ependymal cells which form the inner boundary of the optic tectum.

Different visual environments did not affect the above common features of an intact optic tectum: a fish deprived of darkness for 253 days (as shown in Pls. 2 and 3), or a fish deprived of light for more than one and a half years, showed the same features as a normal goldfish.

The half-tectum. In a majority of the operated goldfish, the remaining rostral half-tectum became well revascularized as shown in Pl. 1. Neither its shape nor its relative size, as compared with its contralateral intact tectum, showed any significant change during a post-operative recovery period of up to a year and a half (Pls. 1, 2 and 4).

Histological preparations of the half-tectum which showed a compression of retinotectal projection in a previous mapping experiment, revealed the following morphologic changes (Yoon, 1974): the stratum opticum and the stratum fibrosum et griseum superficiale merged together to form a new layer. The new layer (labelled *x* in Pl. 5) contained prominently thick fibre bundles. They formed an intricate network throughout the whole extent of the new layer. This layer contained also cell bodies of tectal neurones. The half-tectum usually showed a hypertrophy in the layer *x*. Other tectal layers (*a*, *d*, *e*, and *f*) in the half-tectum did not show any significant change. The half-tectum showed no sign of cellular regeneration

at its severed caudal margin. The density of tectal neurones appeared to be slightly less in the half-tectum than in the intact contralateral tectum.

Suppose that one delays a field compression in the half-tectum by a prolonged dark-deprivation of the operated goldfish. Would the non-compressed half-tectum show certain morphologic changes, or retain a normal laminar structure? Pls. 4 and 5 show a parasagittal section of the non-compressed half-tectum in an experimental fish which has been dark-deprived for 253 days. (Its retinotectal projection map is shown in Text-fig. 7.) The non-compressed half-tectum shows almost identical morphologic changes as does a compressed one: the stratum opticum (layer *b* in Pl. 3) and the stratum fibrosum et griseum superficiale (layer *c* in Pl. 3) in the intact contralateral tectum were replaced by a new layer (labelled *x* in Pl. 5) in the half-tectum, even if the latter showed no compression in its retinotectal projection (Text-fig. 7). Thick fibre bundles appeared to form an intricate network within the layer *x*. The underlying layer *d* (stratum griseum centrale), however, did not show any significant change. Other tectal layers (*a*, *e*, and *f* in Pl. 5) also remained unchanged in the half-tectum.

DISCUSSION

The present experiments show a differential effect of various visual environments on the reorganization of retinotectal projections in adult goldfish. In the case of post-operative light-deprivation, no significant effects were observed. A compression of retinotectal projection occurred in a light-deprived fish at about 80 days after excision of the caudal half of the tectum and section of the contralateral optic nerve. The result suggests that, in the absence of visual inputs, regenerating optic fibres from the whole retina are able to reinnervate the half-tectum in an orderly compressed form.

Compression of the retinotectal projection occurred in a light-deprived goldfish even when the original connexions between the remaining rostral half-tectum and the temporal half of the retina were left intact. In the latter case, however, the field compression took a longer post-operative recovery period (about 120 days) than when the optic nerves had been severed (about 80 days). The field compression suggests the possibility that excision of the caudal part of the tectum somehow induces an activation of regulative mechanism in the remaining rostral half-tectum (Yoon, 1971, 1972*a*, *b*, 1973*a*): the half-tectum would undergo a regulation of the halved field of its organizing factors into a whole (Spemann, 1938; Weiss, 1939) through a systematic re-specification of the synaptogenic affinity factors of its neural elements (Sperry, 1943*a*, *b*, 1944, 1945, 1948, 1950, 1951*a*, *b*, 1955), so that the half-tectum becomes able to accommodate

incoming fibres from the whole retina in correct retinotopic order. Meanwhile a majority of the optic fibres originating from the temporal area of the retina would disengage from their original synaptic linkages with the rostral half-tectum, and migrate rostrally in the half-tectum, and then reinnervate only its anterior quadrant in an orderly compressed form. The regenerating optic fibres from the nasal area of the retina (which previously innervated the excised caudal part of the tectum) eventually reinnervate the posterior part of the remaining rostral half-tectum in correct retinotopic order. Note that these dynamic processes of neural re-organization may occur even in the absence of visual inputs to the operated goldfish.

In the case of post-operative dark-deprivation, one finds two different effects: when the caudal half of the tectum is excised and the optic nerve is also sectioned at the same time, regenerating optic fibres from the whole retina re-innervate the half-tectum in correct retinotopic order within 70 days of post-operative dark-deprivation. On the other hand, if the optic nerve is left intact, the operated fish retains the original connexions between the temporal hemiretina and the remaining rostral half-tectum without showing any sign of a field compression for up to 253 days under the post-operative dark-deprivation. Furthermore, when the dark-deprived fish is transferred into darkness, or a normal visual environment (with a daily cycle of 12 hr in light and 12 hr in darkness), the suppressive effect of the previous dark-deprivation disappears. Within 2 or 3 weeks after the transfer, the visual projection from the whole retina becomes compressed on to the remaining rostral half-tectum in correct retinotopic order.

The results of the transfer experiment suggest intriguing possibilities: during the period of dark-deprivation, the continual input of visual stimuli somehow delays or even suppresses disengagement of the original synaptic linkages between the remaining rostral half-tectum and the temporal hemiretina. Under these conditions, the visual neurones in the rostral half-tectum are prevented from making new neural connexions with the foreign optic fibres (which previously innervated the excised caudal half of the tectum). These optic fibres originating from the nasal area of the retina, however, did not undergo a permanent degeneration during the dark-deprivation; they re-innervated the posterior half of the rostral half-tectum shortly after the fish was transferred into darkness.

Histological preparations of the operated goldfish brains by a modified Bodian protargol method showed consistent morphologic changes in the laminar structure of the remaining rostral half-tectum: the stratum opticum and the stratum fibrosum et griseum superficiale merged together to form a new layer, which contained an intricate network of thick fibre

bundles. In the present histological preparations, the half-tectum showed neither apparent cellular regenerations at its severed caudal margin nor an increase in the density of tectal neurones. Furthermore, the same morphologic changes were observed in the half-tectum even if a field compression was delayed by a prolonged post-operative dark-deprivation of the operated goldfish. The latter result suggests two possibilities: the aforementioned morphologic changes in the laminar structure of the half-tectum may have nothing to do with the reorganization of retinotectal projection. Or, alternatively, the morphologic changes may reflect a preliminary stage which precedes a final reorganization of neural connexions in the goldfish visual pathways.

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

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EXPLANATION OF PLATES

PLATE 1

Polaroid microphotograph of the dorsal view of the mid-brain in a live adult goldfish. The caudal half of the left optic tectum was excised and the right optic nerve was also severed, and then allowed to regenerate. The fish had been kept in darkness during the post-operative recovery period of 332 days. Note that the remaining rostral half of the left tectum became well revascularized. Brisk visual responses were recorded from the half-tectum as well as from the intact tectum in this light-deprived fish. Neither the shape of the remaining half-tectum nor its relative size as compared with the intact right tectum showed any significant change during the post-operative recovery period.

PLATE 2

Micrographs of the tectal layers in an adult goldfish. The fish survived a post-operative dark-deprivation for 253 days following excision of the caudal half of its left tectum. The optic nerves were left intact. Parasagittal sections (15 μm thick) of the optic tectum were stained by a modified Bodian protargol method. It shows the over-all view of a parasagittal section of the intact right optic tectum.

PLATE 3

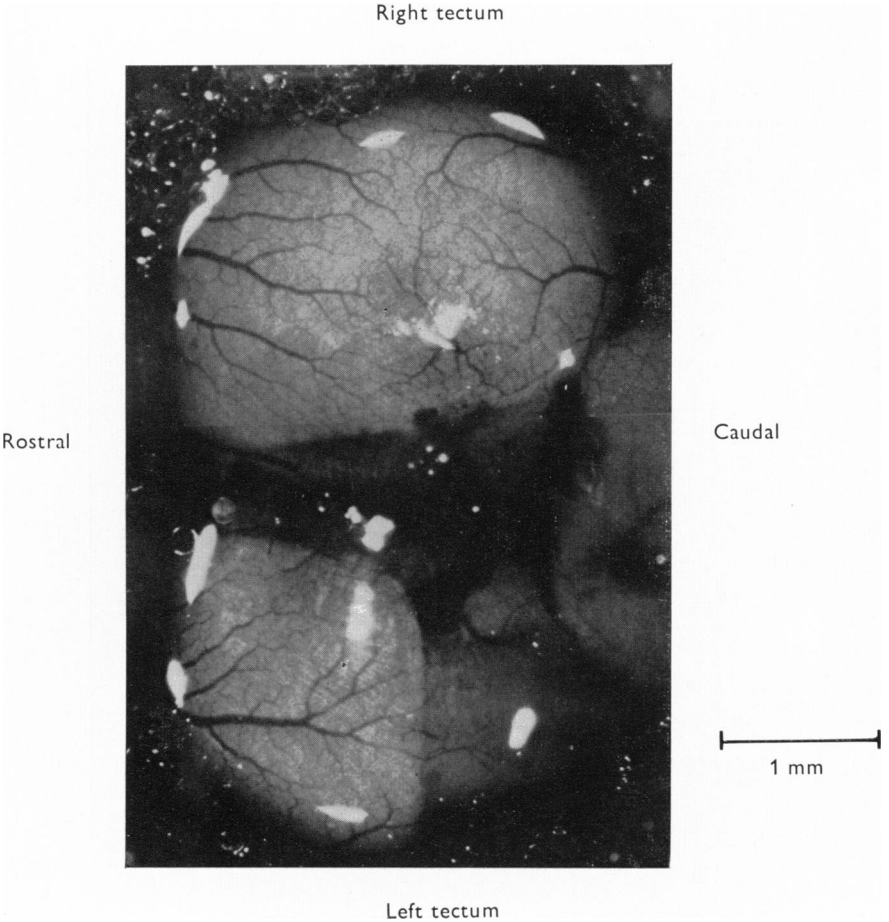
Shows a part of the same section at a higher magnification. It reveals a stratified structure with six distinguishable layers in the normal tectum. *a*, Stratum fibrosum marginale; *b*, stratum opticum; *c*, stratum fibrosum et griseum superficiale; *d*, stratum griseum centrale; *e*, stratum album centrale; and *f*, stratum periventriculare.

PLATE 4

Shows the over-all view of a parasagittal section of the remaining rostral half of the left tectum in the same fish at a level comparable to that shown in Pl. 2.

PLATE 5

Shows the caudal area of the same operated tectum at a higher magnification. In the operated half-tectum, the layer *b* (stratum opticum) and the layer *c* (stratum fibrosum et griseum superficiale) were replaced by a new layer, labelled *x*, which contained an intricate network of thick fibre bundles. *R*, rostral; *C*, caudal; *D*, dorsal; *V*, ventral.



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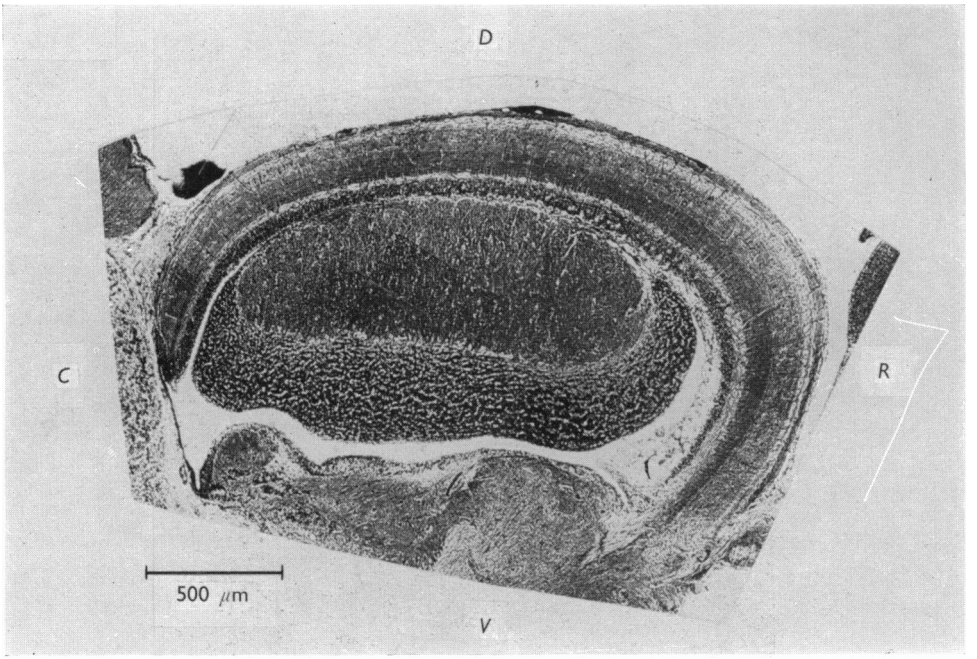
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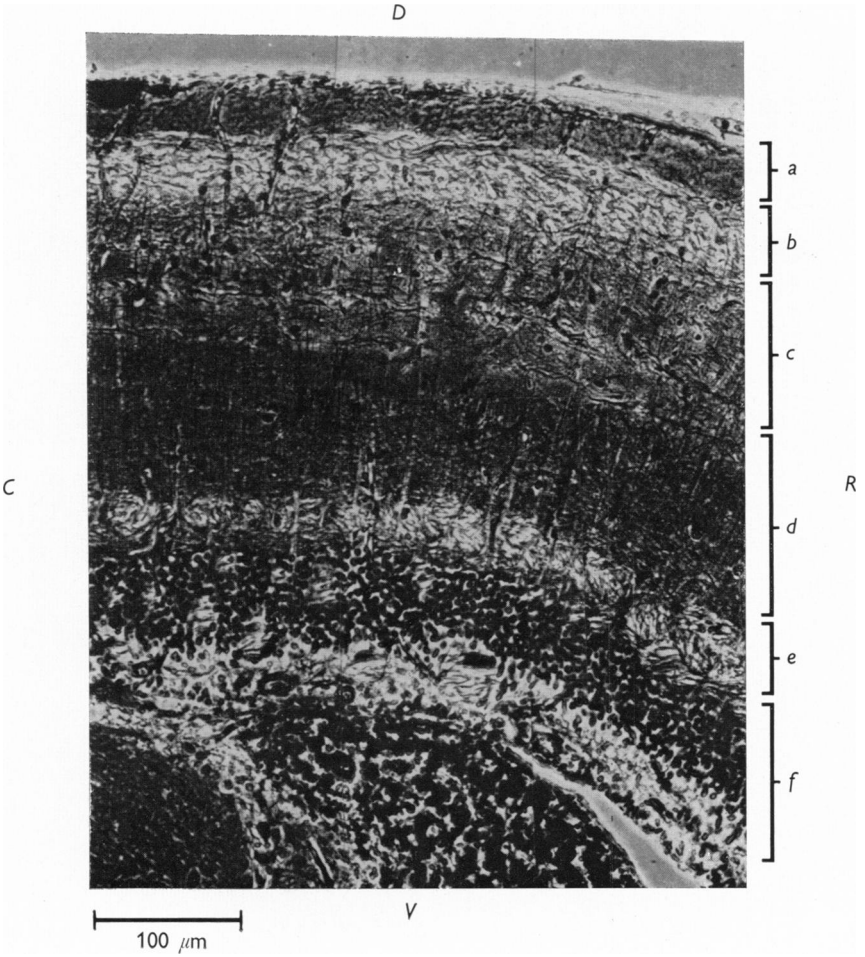

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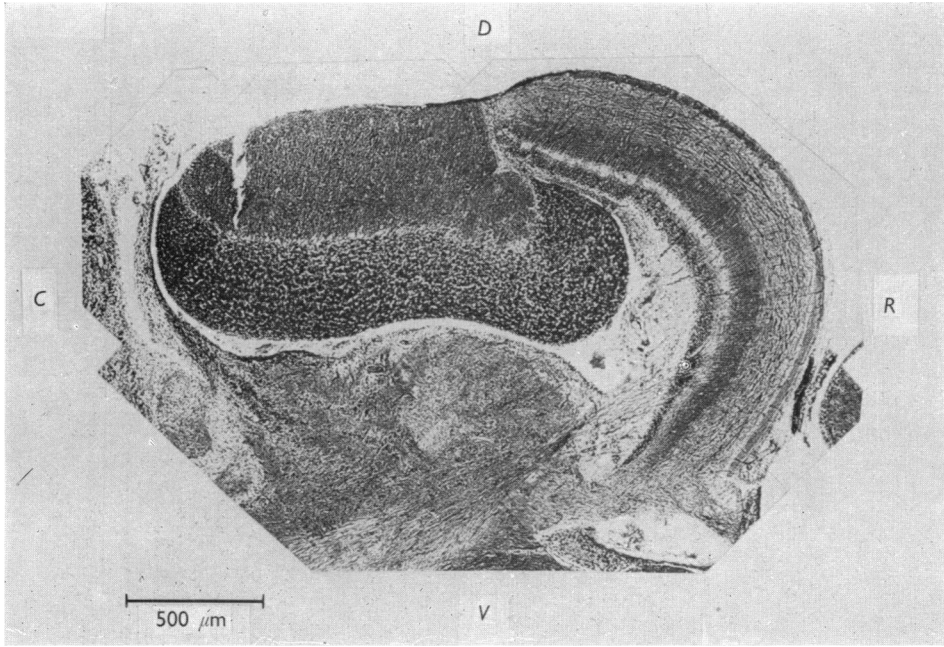

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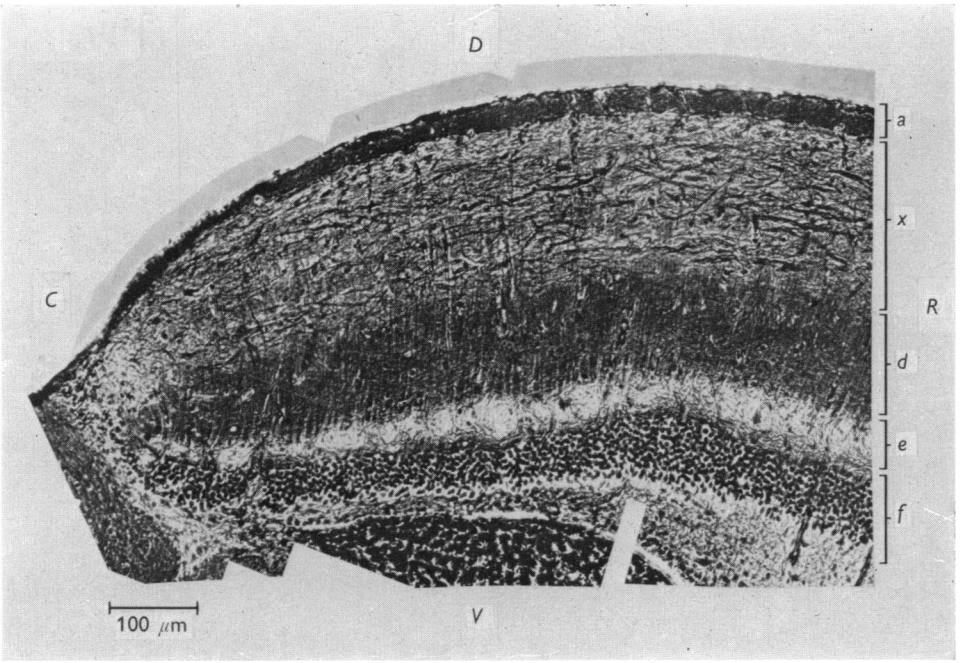

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