FACTORS AFFECTING REGENERATION OF THE RETINOTECTAL PROJECTION

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ABSTRACT

Factors affecting the orderliness of the regenerated retinotectal projection were studied by inducing various size disparities between the retina and tectum of goldfish and tree frog. Behavioral, autoradiographic, and electrophysiological measurements showed that the plastic compression of the projection previously found and here confirmed for goldfish does not occur in half tectum frog. In fish, eye lesions and optic fiber transplants between tecta confirm Sperry's chemoaffinity hypothesis but show that this retinotectal affinity is not sufficient to prevent misgrowth. In addition, competitive and possibly chemoaffinity interactions between optic fibers were found that might explain the plasticity of the goldfish retinotectal system.

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I. GENERAL INTRODUCTION

Some controversy exists at present concerning the mechanism whereby the highly refined topographic projection of the retina onto the midbrain optic tectum is achieved in development and regeneration. In the early 1940's Sperry was able to establish that severed optic fibers grew back to their appropriate tectal locus despite severe disruption of their growth path and absence of timing factors and under conditions that ruled out functional adjustment (Sperry, 1943; 1944; 1945). His experiments on this relatively simple model system contradicted the prevailing opinions of several prior decades that the formation of connectivity was governed by mechanical or electrical factors and that neural function was determined primarily by learning rather than selective connectivity. Sperry (1944; 1945) proposed the existence of a highly refined cytochemical specificity among neurons with selective chemoaffinities regulating chemotactically guided growth to the synaptic zone to achieve selective reinneration of target cells.

These results have since been confirmed by numerous electrophysiological, anatomical, and behavioral studies and have been found to apply equally well to other neuronal systems like the vestibular, cutaneous and tectospinal systems (Sperry, 1951b; 1965; Gaze, 1970; Jacobson, 1970) and also to invertebrates (Hunt and Jacobson, 1973c). Attardi and Sperry (1963) were able to further obtain a direct histological demonstration of the chemoaffinity hypothesis by combining nerve regeneration with retinal lesions in goldfish. Theirs and similar subsequent experiments on both goldfish (Jacobson and Gaze, 1965; Westerman, 1965; Roth, 1972) and chick (DeLong and Coulombre,

1965; Kelly, 1970) appeared to confirm that the growing optic fibers find their correct tectal regions despite opportunities to terminate incorrectly in neighboring denervated areas.

Meanwhile, more recent studies principally by Gaze and associates have been interpreted by them as showing results inconsistent with the original hypothesis (Gaze, 1970). Compound eyes formed in frog embryos from 2 nasal or 2 temporal half retinas were found to form their synaptic connections across the entire tectum (Gaze et al., 1963). Also in goldfish it was found that a remaining anterior half tectum after ablation of the posterior tectum accepted orderly connections from the entire retina (Gaze and Sharma, 1970). In order for the whole retina to remap on the half tectum it appeared that the original synaptic connections in the intact half must break down and reform more anteriorly, while the optic fibers formerly connected to the excised tectum must form synapses with entirely new cells to which they normally never connect. To account for these and related findings, Gaze (1970) proposed a modified sliding scale or "systems matching" hypothesis whereby optic fibers form their terminals not at predestined targets but at correct relative positions along whatever fraction of tectal gradient is available. Later work by Yoon (1971; 1972a) and Sharma (1972a; 1972b) seemed to further confirm the lack of any predetermined connectivity.

Yoon in particular reported further that the process of compression of the retinal projection map onto a half tectum was reversible under conditions where the tectum was divided with a barrier of Gelfilm which later resorbed.

Firm conclusions, however, were hindered by some apparent inconsistencies in the goldfish work and by an absence of critical experiments. There was disturbing lack of agreement between the electrophysiological data on which all of the evidence for plasticity rested and the correlated anatomical data. Yoon (1972b) and Horder (1971) both reported electrical evidence for uniform spreading of a surgically formed half retina over an entire normal tectum. In contradiction to this, the original study of Attardi and Sperry (1963) using Bodian staining and Roth's (1972) recent similar work showed that these same half retinas preferentially terminated in the appropriate tectal region even after long survival periods. Yoon (1972b) and Horder (1971) had further electrophysiological data apparently showing that if noncomplementary retinal and tectal halves were removed, the remaining retina spreads over the entire inappropriate half tectum. Under these same conditions the anatomical evidence of Roth (1972) indicated that innervation was restricted to the region near the lesion leaving much of the tectum without optic fibers.

Worse yet the electrophysiological evidence seemed self-contradictory. While Yoon (1972b) claimed both nasal and temporal hemiretinas showed plasticity, Horder (1971) had evidence that only a small nasal half retina expanded in this manner and Jacobson and Gaze (1965) had data suggesting neither half did this. Although only a rough mediolateral incision was sufficient to induce complete field compression onto the rostral tectum in Yoon's hands (1971), a similarly placed even larger lesion did not produce this result in Sharma's (1972b) experiment. Medial or lateral tectal ablations had been

reported not to result in plastic remapping (Gaze and Jacobson, 1965) and the thought that this may be a consequence of interference with the medial optic tract was supported in the results of Yoon (1971). Yet, after removal of rostral tectum causing comparable tract damage, a compressed visual field representation onto caudal tectum was nevertheless found, according to Sharma (1972b). In the original Gaze and Sharma (1970) study, simple removal of caudal tectum invariably resulted in tectal positions from which two widely separated receptive fields could be recorded. Not one instance of this field reduplication was found by Yoon (1971).

In the face of the numerous discrepancies, contradictions, and confusions in the retinotectal literature it was becoming increasingly difficult to see any unifying consistent interpretation. It seemed highly probable that some additional unaccounted factors were involved in the formation of connectivity that remained to be disclosed. The following represents an effort toward resolving the uncertainties in the retinotectal story with further experiments on some of the phenomena and issues at stake. Each of the following sections has its own introduction and discussion dealing with the specifics of each experiment.

II. TESTS FOR NEUROPLASTICITY IN THE ANURAN RETINOTECTAL SYSTEM[1]

Introduction

Regeneration of the optic nerve in lower vertebrates after section and mechanical scrambling of fiber relations is followed by an orderly reestablishment of the normal topographic retinotectal projection (Cronly-Dillon, 1968; Gaze, 1959; Maturana et al., 1959; Sperry, 1943; 1944; 1948; 1955). If a substantial sector of the retina is removed, the remaining optic nerve fibers regenerate selectively to those tectal positions to which they normally connect (Attardi and Sperry, 1963). To explain this and other cases of selective reinnervation, Sperry proposed that during development the cells of the retina and tectum acquire individual position-dependent cytochemical specificities, which determine optic fiber outgrowth patterns and enable central synaptic connections to be formed in topographic order on the basis of preferential chemical affinities between matching retinal and tectal loci. It was suggested that the cytochemical labeling of individual neurons is achieved through a polarized field type differentiation of the retina and tectum separately along the three main embryonic axes (Sperry, 1945; 1951a). Most of the subsequent work on the formation of retinotectal connections in amphibians, fishes, and birds (DeLong and Coulombre, 1965) has supported this interpretation.

In apparent conflict with the foregoing, it has been found by Gaze and coworkers (Gaze et al., 1963; Straznicky et al., 1971) that

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[1]This work was published by R. L. Meyer and R. W. Sperry in Experimental Neurology 40, 525-539 (1973).

the projection from each half of the retina of a surgically formed double-nasal or double-temporal composite eye in amphibians may expand over the entire tectum. Other studies involving ablation of the caudal tectum, transtectal lesions, and Gelfilm implants in goldfish have shown that the projection of the entire retina may become compressed onto the rostral half tectum and may reexpand with absorption of the film (Gaze and Sharma, 1970; Yoon, 1971; 1972a). It has been shown further by Horder (1971) and Yoon (1972b) that a remaining hemiretina after retinal excisions may eventually expand its projection in time to cover an entire intact tectum or an entire inappropriate half tectum formed by a caudal hemitectal ablation.

In the light of these and related manifestations of plasticity in the retinotectal map it has been argued that Sperry's original interpretation is inadequate (Gaze, 1970; Gaze and Keating, 1972; Gaze and Sharma, 1970; Jacobson, 1970; Sharma, 1972a; Straznicky et al., 1971) and Gaze (1970; Gaze and Keating, 1972) has proposed a modified hypothesis in which the retinal fibers as a system compete for relative positions along the tectal gradients regardless of specific values within the gradient. However, all the foregoing experiments indicating synaptic plasticity have been carried out on embryonic anurans or on rapidly growing goldfish, where embryonic type regulative processes might still be operative and thus account for the observed readjustments. In terms of the original model, the synaptic plasticity effects may mean merely that the position-dependent cytochemical tags are still labile and the local sign properties are simply reorganized as a

developmental morphogenetic field following the imposed ablations, implants, and transplantations. Although this latter interpretation has been long favored in this laboratory (Sperry, 1965; Yoon, 1972a), critical experiments have not been advanced to distinguish between this and the alternative model proposed by Gaze.

In attempts to find a critical test between these alternatives, we have carried out half-tectum ablations and related interventions, where developmental regulation should be less likely to occur and where synaptic reconnection can be evaluated not only with electrophysiological but also behavioral methods. Postmetamorphic tree frogs, Hyla regilla, were selected because their tectal development is largely complete by metamorphosis (Larsell, 1929; Straznicky and Gaze, 1972), and, hence, the tectal cytochemical tags might be expected to be more stable than in 5-11 cm goldfish, where the tectum is apparently still growing (Kirsche and Kirsche, 1961). The Hylidae are also highly visual, have been found to show good optic nerve regeneration (Sperry, 1944; 1945) and are further well suited for behavioral perimetry testing as well as for electrophysiological recording.

Given stabilized tectal specificities, a lasting behavioral hemianopia would be predicted to follow hemitectal ablations according to the original explanation with no compression of the visual field measured electrophysiologically. If, on the other hand, nerve connections are formed according to the hypothesis of Gaze (1970), there ought to be no behavioral hemianopia after recovery and electrophysiological mapping should show a compression of the whole visual field

projection onto the remaining half tectum. The present findings support the first alternative, and it is shown how our original interpretation could account also for the various examples of plasticity that have been observed to date. A preliminary account of these findings has been given by Meyer (1972).

Materials and Methods

Animals. Adult Pacific tree frogs, Hyla regilla, 30-40 mm in body length, were used throughout. They were housed in groups of three to six in small glass terraria of 0.5 cu ft at room temperature. Unlike most larger anurans, H. regilla and related Hylidae possess a cartilagenous cranium that is easily opened and closed for surgery and electrophysiology. Unlike the clawed toad, and more than the goldfish, these Hylidae are highly visual predators. The large optic tectum and easily obtained, large evoked tectal potentials are advantageous for tectal mapping. In addition, these frogs tame quite readily and maintain aggressive feeding behavior in captivity, qualities that proved important for the behavioral tests.

Surgical Procedure. Anesthesia was administered by placing the animal in an etherized atmosphere within a glass container for several minutes prior to surgery. This was sometimes repeated during surgery if indicated. All operations were performed through a dissecting microscope with variable magnification and coaxial illumination. The optic nerve was severed with the frog on its back and its mouth held open with special retractors. The mucosa was incised, exposing the optic nerve at the point where it enters the cranium, and the nerve

was repeatedly crushed with sharpened jewelers forceps until a clear discontinuity within the intact optic sheath could be seen.

The tectal surgery was done with the frog in the prone position. After a skin flap was cut free and reflected, a flap of cartilagenous cranium over the midbrain was incised with a small knife made of razor blade and reflected back underneath the nearby skin. The dura was opened with forceps.

The tectal region to be ablated was first outlined by inserting fine electropolished tungsten needles down to the ventricle after which the area was aspirated with a small glass pipet. Care was taken to preserve the blood supply to the remaining tectum and at the midline. Gelatin film was implanted by inserting a cut rectangular piece of sterile ophthalmic Gelfilm (made by Upjohn of purified specially treated gelatin) 50-100 micrometers thick into a transverse mediolateral incision, so that it extended from above the tectal surface down into the ventricle and from the midline to the lateral tectal border, where it remained without significant absorption through the course of the experiments. The cranial and skin flaps were returned to their original positions.

Behavioral Testing. A calibrated glass hemisphere 23 cm in radius was used for the perimetry measurements. It remained freely movable, resting its concave surface down on a white topped testing table. The frog's head was centered under the half dome by sighting through a small peep hole at the dome's vertical apex onto thin cross wires stretched inside of the hemisphere about 210 mm below the apex.

The 0-180° wire was positioned to bisect the head down the midline, while the 90-270° wire bisected the two eyes. Small black circular or rectangular lures up to 4° in size were found to be highly effective as stimuli. They were presented on the end of thin glass rods against a white background formed by a cardboard cylinder extending 50° upwards around the outside of the hemisphere. To avoid extraneous visual stimuli, the examiner worked from within the posterior field of the right eye which was blinded by an optic nerve crush. The lure was advanced from behind along the outside of the perimeter into the posterior field of the left eye.

On each trial, the lure was moved in stepwise manner from the caudal pole toward the rostral pole along any given 10° parallel from the 0° horizon to 50° and oscillated over 2-3° for about a second or two at every 10° meridian. At some point in the progression, this typically produced a clear orienting response involving a sudden turning of head and body. At each response, the lure was immediately withdrawn, and the position recorded. The frog was then either allowed to spontaneously reassume its normal sitting position or was stimulated to do so by feeding a small fruit fly attached to the end of a glass rod, whereupon the perimeter was realigned. To minimize habituation the lure path was frequently varied, and an intertrial interval of a minute or more was preserved. Flies were periodically offered as reinforcement, and testing was carried out in sessions spaced over several days. Frogs that were highly reliable responders were selected in advance for the experiments, discarding about one-third of the initial supply.

Responses were collected at each tested parallel until at least five within 20° of the most posterior response were obtained. When responses beyond this area became frequent, or when they became obviously inconsistent, the testing session was terminated. Since responses beyond this 20° mark could be rather arbitrarily obtained by overtesting and were apparently more a function of habituation than variability in the measuring procedure, they are not included in the data presented. The remaining visual area outlined by these posterior response points was taken as the experimental blind region.

Electrophysiology. The method of electrophysiological mapping was similar to that employed and described by Gaze (1958). Under ether anesthesia the cranium over one tectum was opened, and the animal subsequently immobilized with d-tubocurarine and pinned onto a small platform. The dorsal surface of the tectum was then photographed at 47 X through a micrometer grid which gave 180 micrometer tectal spacings. This provided a rectangular array of potential electrode positions that could be identified on the real tectum by the tectal pigmentation pattern. The eye ipsilateral to the exposed tectum was covered with a small plastic occluder, and the animal was then placed in the center of a modified Brombach perimeter (American Optical Company). The contralateral eye axis was aligned by placing the trans-illuminated image of the optic disc along the central axis of the perimeter as viewed by a small telescope. This gave an axis of alignment some 5-10° anterior and inferior of that utilized by Gaze but was found to be more convenient and more reliable for these animals. The

perimeter's horizontal meridian was adjusted to coincide with the long axis of the frog's oval pupil. Platinum plated tungsten microelectrodes with tip diameters of 2-5 micrometers were inserted perpendicularly up to 250 micrometers into the tectum. They typically recorded multiple units evoked by black disc-shaped stimuli from 2° to 3° in diameter presented on a white background. Usually the excitatory receptive field was $5\text{-}15^{\circ}$ in diameter with a $3\text{-}5^{\circ}$ central area of maximal response. The recordings were confined to the dorsal half of the tectum, since the ventrolateral surface was not readily accessible to probing. Following mapping sessions, the animal was either allowed to recover for subsequent recording or was sacrificed for histology.

Histology. The frogs were anesthetized with ether vapor, and the cranium opened over both tecta. The head was then cut free and immediately immersed in a mixture of 18 parts 80% ethanol, 1 part formalin, and 1 part glacial acetic acid. After several hours, the brain was dissected free and placed in fresh fixative for 12-24 hrs, then embedded in paraffin, and serially sectioned at 15 micrometers. Each brain was stained with cresyl violet or a modified Bodian protargol stain. The gelatin implants were left in place throughout the histology.

Results

Behavioral Perimetry: Normal Controls. The posterior contour of the visual field of the left eye was mapped behaviorally in three normal animals. Testing was conducted between 4 and 5 days after the right optic nerve had been crushed, eliminating the right visual field

on that side. Measured counterclockwise from 0° midline meridian, the left eye field was found to extend along the 0° parallel to 170°, along the 10° parallel to 180°, and along the higher parallels to 180-190°, depending on the individual animal (Fig. 1A). For each animal, the response points obtained at each parallel grouped quite tightly; often five or more responses were obtained at the same meridian, and none were separated by more than 10°. The posterior boundary of the visual field in different individuals differed less than 10° along any given parallel, suggesting a resolution of about 10° obtainable with this method. Since we are primarily concerned here with the presence or absence of a large half-field scotoma, accuracy of this mapping procedure is entirely adequate.

Optic Nerve Regeneration: Tectum Intact. Occasional responses could be obtained as early as 30 days postoperatively, but about 60 days were required for reestablishment of reliable responses throughout the whole visual field. The left visual field of three frogs was tested with the perimeter 110-122 days after crushing the left optic nerve and 34-48 days after recrushing the right optic nerve. It was clear that in each animal the regenerated map of the left visual field was normal in conformance with earlier reports (Sperry, 1944; 1945). Open field testing also confirmed this observation. Thus, with the tectum intact, regenerating fibers were found to reestablish the normal retinotectal map in this species as in other anurans.

Caudal Half Tectum Ablation. Behavioral perimetry testing of the left field was carried out in nine frogs which had the right caudal

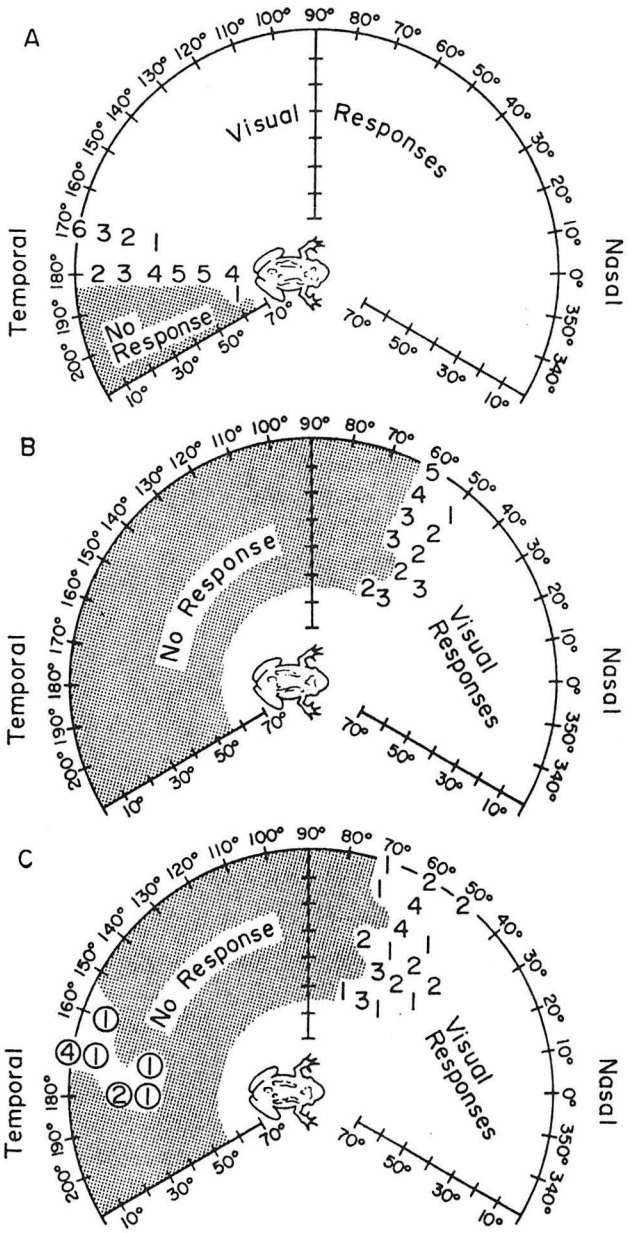


FIG. 1. Behaviorally obtained maps showing the posterior contour of left visual space. The stimulus was advanced in a temporal nasal direction along each tested 10° parallel until an orienting response was obtained. The number of responses at each position is shown, and the stippling indicates the visual field giving no responses. The right eye was blinded by nerve interruption in all cases. (A) Normal. (B) Frog with right caudal half tectum ablation sustained 7–10 days prior. (C) Same animal as in B, but tested after 124 days tectal surgery. Circled numbers represent contralateral orientation movements to the mirror image position.

half tectum ablated with simultaneous crushing of the right optic nerve 6-9 days prior to testing. As expected (Sperry, 1944), a large posterior scotoma was found in all animals (Fig. 1B). Typically the caudal posterior edge of the visual field fell approximately along the 60° meridian. Since the optic axis of the eye diverges from the midline by about 55-60° (Gaze and Jacobson, 1962), this represents approximately a half-field scotoma. There was, however, some variation between animals. In one animal, the field bulged posteriorly as far as 100°. In another, the border fell along most of the 30° meridian and decreased to 10° along the 50° parallel. This variability in the maps is to be expected from individual differences in extent of the lesions and surgical trauma at the lesion border.

Between 130 and 133 days after the initial ablation, behavioral perimetry was carried out on four of the healthiest frogs to test for expansion of the visual field into the scotoma. The left optic nerve was not crushed because expansion appears to occur more rapidly under this condition (Yoon, 1971). For testing purposes, the right nerve was crushed 14-15 days prior to the testing sessions. In three frogs, the border of the visual field was found to be not more than 10° different from that measured shortly after surgery (Fig. 1C). In the fourth frog, some expansion up to 30° was found along the caudal edge. However, the original field of this animal was the smallest of the group and, as described above, extended temporally to only the 30° meridian. The initially enlarged scotoma could have resulted from reversible damage near the lesion that recovered to give a more

typical half field size. In summary, the results in all four animals showed that the half-field scotoma had been preserved in essentially its original form up to 133 days postoperatively.

An incidental exception to the foregoing was observed; a false counter-reversal of responses aimed at the mirror image position in the visual field was obtained in all four frogs from the extreme posterior left field between the 160° and 180° meridians and between the 0° and 40° parallels (Fig. 1C). Such reversed orienting movements have been described (Sperry, 1945) and can be attributed to the sprouting and regrowth of some of the optic fibers of the ablated tectal zone to the corresponding loci in the opposite tectum. The total area of the field giving incorrect responses was less than 30° of solid angle, and hence it would appear that only a small fraction of fibers was involved. Collateral sprouting from the ablated border or even at the chiasm could account for the result. The lack of any difference between the results from unilateral and bilateral tectal lesions described below indicates that this misdirected growth (not ruled out in goldfish) cannot be interpreted as having been responsible for the absence of field expansion.

Electrophysiological Mapping: Normal Controls. For reference purposes and comparison with previously published results, the left visual field was mapped on the right tectum in a total of 15 normal H. regilla. Good multiunit responses were easily obtained having excitatory receptive fields of 5-15° and a central maximal response region of 3-5°. The source of these action potentials is thought to

be the terminal arborization of optic nerve axons (Lettvin et al., 1960) and probably reflects the pattern of functional connectivity between retina and tectum under normal conditions. All the maps were essentially the same and proved quite similar to those obtained from Rana and Bufo (Gaze, 1958; Jacobson, 1962).

A typical map is presented in Fig. 2A and is self-explanatory. The order of recordings did not seem to affect the map. Owing to the difficulty of recording from the undercurved caudal and lateral edges of the tectum, the actual extent of the tectal representation of the visual field is estimated to be larger than indicated by about 30° at the temporal extreme and 100° into the inferior field. This presents no particular difficulty for these experiments, since the experimental manipulations fall well within the field covered by these maps.

Regeneration with Tectum Intact. As expected from work on other species, H. regilla was found to regenerate the normal retinotectal projection after optic nerve interruption in the six cases tested between 60 and 151 days after complete axon interruption. Visually driven units could be recorded as early as 25-55 days in four other animals, but mapping was not attempted because of low amplitudes and large receptive fields. The recording improved with time, and at 60-80 days multiunits with normal receptive fields located in the appropriate region of visual space could be obtained over the entire tectum. Consequently, mapping in these experiments was carried out subsequent to 60 days after nerve interruption.

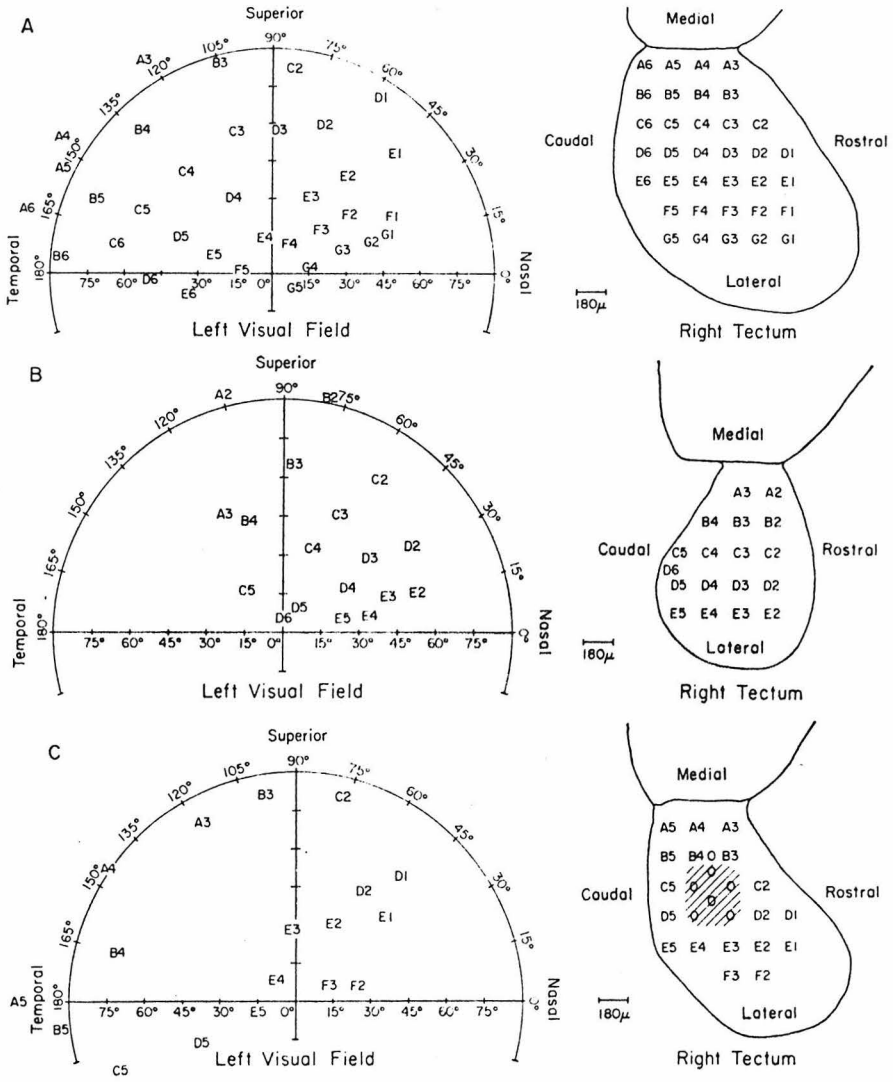


FIG. 2. Electrophysiological map of left visual space onto right tectum with numbers and letters indicating corresponding electrode placements and receptive field location. (A) Normal. (B) Right caudal half tectum ablation and left optic nerve crush 339 days prior. (C) Ablation of a rectangular piece of dorsal right tectum and left optic crush 106 days prior. Crosshatching indicates pia-covered ablation area with ○ indicating electrode positions giving no response.

Unilateral Half Tectum Ablation. Unilateral caudal tectal ablations produced the predicted electrophysiologically-measured scotoma at up to 2 weeks after surgery. This same half-field scotoma was found essentially unchanged for as long as 339 days. Excluding the seven animals used only for behavioral testing, fifteen frogs sustained right tectal ablations, of which ten of the healthiest survivors were selected for electrophysiological recording. In three of these, the left optic nerve was left intact, and retinotectal maps were obtained 114-147 days postoperatively. In the other six frogs, the left optic nerve was crushed within a day of tectal surgery and recordings obtained from 148-339 days after tectal ablation (Fig. 2B). There was no significant difference between the groups, and they will be treated together. A complete recording from the remaining animal was not obtained.

Temporal extent of the experimentally reduced visual field ranged from the 135° meridian to some 30-40° nasally. Despite probing near the lesion at close-spaced intervals, the temporal contour was found to be within 20-30° of that initially produced. The small changes sometimes found are most simply accounted for on the basis of reversible damage or differences in electrode probing. The field remaining was a well-structured fairly normal half field. There was no apparent difference between these and normals in the separation of receptive fields; whereas, a full field expansion ought to have produced inter-receptive field distances about twice that of normals.

Minor departures from the normal field organization seen in four animals seem best explained by topographical distortion of the tectum, caused by its tendency to move gradually into the space created by the lesion. Thus, in nine animals with half-tectum ablation, the electrical mapping indicated a preservation of the original patterns of retinotectal connections.

In two additional Hyla with right tectal ablation and left optic nerve crush electrical recording was done at 35 and 37 days. As in 30-day regenerating normals, the units obtained were of low amplitude with diffuse, poorly ordered receptive field suggesting the potentials were from exploratory fibers and fibers in passage rather than from finalized axon terminal arborizations. Among these were units in the mediocaudal right half tectum that had their receptive fields within the scotoma, indicating again that the lack of field expansion after nerve regeneration was not attributable to absence of the appropriate fibers.

Bilateral Ablation of Caudal Tectum. Bilateral half tectum ablations were performed in eight animals to deprive the disconnected fibers of alternative "correct" sites on an intact tectum and also to test the possibility that visual field expansion might be indirectly facilitated through chemical or other effects. Mapping of left visual space onto right tectum was performed in the first four operated animals between 60 and 106 days after bilateral optic nerve crush combined four days later with bilateral caudal tectal ablation. The resultant electrophysiological maps were the same as those obtained from animals with unilateral lesions.

Rectangular Tectal Lesions. Rectangular lesions similar to those reported to cause compression of retinal fibers in goldfish (Sharma, 1972a; Yoon, 1971) were placed in the right tectum of five frogs, and the left optic nerve was divided at the same time. The 600 X 800 micrometers tectal ablation was centered rostrocaudally with the 600 micrometers edge parallel to about 100-200 micrometers from the midline. This spared much of the tectum, including a medial strip containing the medial optic tract.

Maps obtained from three of the four survivors between 76 and 171 days postoperatively were essentially normal with a large scotoma corresponding closely in dimension and location with placement of the lesion (Fig. 2C). No evidence was found for compression of fiber projection along either the rostrocaudal or the mediolateral axis. In the fourth animal, only six receptive fields could be mapped at this date, but none fell within the scotoma area.

Gelatin Film Tectal Implants. Mediolaterally-oriented gelatin film implants in the goldfish tectum induce the same kind of fiber compression that is produced by caudal lesions (Yoon, 1972a). Pieces of Gelfilm were inserted into the right tectum of nine animals, essentially cutting off the posterior from the anterior half of the tectum. Recording was carried out in two of these at 110 and 204 days postoperatively. In a third case, the left optic nerve was crushed at 114 days, and mapping was performed at 291 days after tectal surgery. In all three of these cases selected for postsurgical recording, mapping of the rostral half tectum yielded a half visual field entirely

similar to that for the same region of a normal tectum. Histology in these three and five additional specimens with implants showed the implant to be intact in all cases, properly placed, and apparently not invaded or crossed by any nerve fibers.

Histology. The intact tectal tissue appeared normal and extended to the edge of the lesion. The tectal cytoarchitectonics seemed well preserved except at the very edge of the lesion. The tectal tissue tended to move gradually into the space created by the lesion, but the flat sheet-like tectal structure was preserved with no dorsal or ventral curling. Scar tissue appeared to be limited to the immediate vicinity of the lesion. There was no indication of tectal cell regeneration nor of any abnormal growth covering the intact tissue. In short, the remaining tectum appeared to be healthy and normally accessible to microelectrode probing. This conclusion is based on examination of all surviving experimental animals, 15 with unilateral lesions, 8 with bilateral lesions, 9 with gelatin implants, and 2 with rectangular lesions. The optic fibers deprived of tectal connections could not be distinguished from nonoptic fibers in these preparations, and their fate remains to be determined.

Discussion

Behavioral perimetry testing following ablation of the caudal hemitectum showed a stabilized temporal half-field visual scotoma that remained essentially unchanged for 130-133 days. Similarly, electrophysiological mapping after unilateral and bilateral caudal tectal ablations, midtectal rectangular ablations, and mediolateral gelatin

tectal implants all showed a corresponding stabilized scotoma with no significant compression for periods up to 339 days. The results were the same with and without optic nerve division and regeneration. These findings stand in direct contrast to those obtained in goldfish, where the initial scotoma produced by essentially the same lesions vanished within 90 days, correlated with compression of the total retinotectal projection map onto the remaining tectum.

The observed stability of the projection map in H. regilla, and probably also in Xenopus, according to preliminary evidence (Gaze, 1970), appears to rule out the alternative hypothesis of competitive innervation and favors the original interpretation based on specific cytochemical affinities between retinal and tectal neurons. The various plasticity effects reported in goldfish can be explained on the assumption that the presumably actively growing goldfish tectum (Kirsche and Kirsche, 1971) still possesses developmental lability in its neurospecificity properties. The surgically isolated tectal half field may be presumed to undergo reorganization into a new whole field bring corresponding changes in its cytochemical local sign or "position-dependent" properties that determine fiber connections. This would be predicted for early intervention in any developing morphogenetic field by definition; it is only surprising that these cytochemical dynamics should remain labile so late in growth. The adult frog tectum by contrast appears to have completed growth by cell addition at metamorphosis (Larsell, 1929; Straznicky and Gaze, 1972).

The original explanatory model (Sperry, 1945; 1951a; 1965) was expressed from the start in terms of the already established concepts of the embryonic morphogenetic field and/or gradient (Weiss, 1939), which by definition has plastic "systems" properties such that, if cut in two, either isolated half will form a whole. To impute a "rigid place specificity" (Gaze, 1970; Gaze et al., 1963; Gaze and Sharma, 1970; Straznicky et al., 1971) or "rigid cell-to-cell connection" (Gaze and Keating, 1972; Jacobson, 1970; Sharma, 1972a) reflects a misinterpretation of these basic concepts in developmental biology. The retinal unit specificities were always inferred to be plastic and reversible with the dorsoventral axis retaining plasticity later than the rostrocaudal (Sperry, 1945), and individual retinal fibers were described as typically connecting over a wide tectal area with considerable overlap among terminals of neighboring ganglion cells depending on the steepness of the retinal and tectal gradients (Sperry, 1951b; 1955).

The findings for experimentally-formed composite eyes (Gaze et al., 1963; Straznicky et al., 1971) may be explained by supposing that each hemiretina of the composite eye reorganizes itself to differentiate into a complete retinal field (Sperry, 1965; Yoon, 1972b), with each twin field a mirror image of the other. Such twinning occurs also after similar surgical manipulations of limb buds (Amprino, 1965). That the composite eye is in this and in other respects a double or twinned, rather than single, system is indicated in the tendency for such eyes to form double lenses (Gaze et al., 1970).

Expansion of the tectal projection from a hemiretina to cover the entire tectum (Horder, 1971; Yoon, 1972b) or a presumably reorganized half tectum (Yoon, 1972b) would be predicted for rapidly growing goldfish when sufficient time is allowed for the isolated half retinal field to reorganize itself into a whole field, especially where such reorganization is facilitated by destruction of the outer layers of the eyeball along with the retina (Yoon, 1972b). These were avoided in the early experiments showing selective tectal reinnervation (Attardi and Sperry, 1963; Jacobson and Gaze, 1965). Plastic mapping of tectal projection upon absorption of a dividing Gelfilm implant in goldfish (Yoon, 1972a) also is what our interpretation would predict upon restoration of cell contacts across the division line and resumption of intercellular exchange of field position information. This plasticity with respect to expansion or compression of a developing field should be distinguished from that involving reversal of polarity. The expectation that plasticity for a complete change of polarity of a gradient would be lost before that for mere expansion or compression seems to be borne out in early data (Sharma and Gaze, 1971; Yoon, 1972c). Our interpretation hardly permits the designation of an "unspecified state" (Hunt and Jacobson, 1973a) for the retinal field, especially without reference to these two very different aspects of field dynamics. By our model, most of the experiments that have seemed to deal with a critical period for the specification process (Jacobson, 1970) have dealt instead with the period in which specificities already long established become irreversibly fixed,

i.e., a given aspect of field plasticity can no longer be altered by a particular experimental manipulation. The initiation of retinal specification is assumed to begin in the early embryo and to emerge gradually out of the larger main axial gradients of the whole embryo (Sperry, 1965). A molecular model is proposed by McMahon (personal communication), describing development and behavior of these morphogenetic fields in terms of cellular contacts regulating morphogenetic substances.

The present findings, as well as those of all the other retinotectal experiments to date, seem to be most simply accounted for in terms of the original chemoaffinity hypothesis. If our interpretation is correct, most of the retinotectal experiments demonstrating neuroplasticity can be seen to deal, not so much with direct regulative factors in nerve growth and synaptic formation, as with variables in the organizational dynamics of the developing morphogenetic field of the eye and tectum.

III. EVIDENCE FOR SELECTIVE REINNERVATION OF THE OPTIC LOBES OF THE GOLDFISH AFTER VARIOUS RETINAL INSULTS

Introduction

The demonstration that optic fibers preferentially grow along specific routes to the appropriate tectal regions even if neighboring foreign tectal regions are denervated is a critical experiment in favor of chemoaffinity and against timing and mechanical guidance as the essential pattern determining mechanism in the formation of retinotectal connections. Chemoaffinity, as put forward by Sperry (1945, 1951a, 1965), assumes that the individual retinal and tectal cells acquire position dependent cytochemical specificities and that the optic fiber growth is chemotactically guided to the tectal cells having the matching cytochemical tag. There is substantial evidence indicating that the general topography of the retinotectal projection can be explained in these terms. In goldfish Attardi and Sperry (1963) and more recently Roth (1972) showed with a modified Bodian stain selective for regenerating fibers that optic fibers from surgically isolated retinal remnants tend to exhibit this kind of goal directed growth during regeneration. Using electrophysiological methods, similar observations were made by Westerman (1965) and Jacobson and Gaze (1965) also showed this following regeneration of the hemisected goldfish optic nerve. Retinal lesions in chick embryos prior to the outgrowth of optic fibers produce similar growth and termination patterns that can be seen with a variety of anatomical techniques and postoperative period (Delong and Coulombre, 1965; Kelly, 1970). Compound eye experiments

performed on Xenopus tadpoles and once thought to represent an exception to this picture can now no longer be considered as a counter example in light of recent experiments (Hunt and Jacobson, 1973b). These show, contrary to what was assumed, that the locus specificity properties governing the site of termination are not stable but appear to regulate under these conditions.

What appears directly contradictory is electrophysiological work in goldfish (Horder, 1971; Yoon, 1972b) suggesting connections from an isolated half retina can spread in an orderly topographic fashion over an entire intact tectum. However, there is reason to think that these experiments are not strictly comparable to those showing selective innervation and that they may not contradict the idea of chemoaffinity. In the goldfish work showing plasticity relatively long survival periods were used leaving the possibility that optic fibers may first grow to the appropriate tectal region and later spread over the rest of the tectum. Also the surgical procedures of these studies may have been significantly more traumatic. Yoon's diathermy technique can be expected to produce extensive destruction of the outer layers of the eye in addition to the retina. This, as well as the longer survival period, may have allowed regulative type changes in the chemospecificity properties of the remaining retina. The goldfish retinotectal system seems still to be growing by cell addition, and, as found for other growing systems, the half retina may reorganize into a whole retina. The finding that the non-growing frog half tectum (Meyer and Sperry, 1973; Straznicky, 1973) does not exhibit the

plasticity found for the goldfish half tectum (Gaze and Sharma, 1970; Sharma, 1972b; Yoon, 1971) supports this notion as does the regulative plasticity shown by developing tadpole eyes (Hunt and Jacobson, 1973b). The late occurring growth of the goldfish nervous system raises the additional possibility that some de novo regeneration of optic neurons has occurred after retinal lesions to complicate the results either by altering any regulative processes that may be occurring or by contributing fibers having growth properties of questionable normalcy. With the exception of a few controls in Roth's study, no retinal histology has been reported in these studies to evaluate this possibility.

Both small and large goldfish sustained eye lesions of various sizes either by surgical ablation of retinal tissue or by diathermy coagulation. Short and long term effects of the lesions on the retinal and other ocular tissue was evaluated histologically. After varying survival periods a heavy dose of tritiated proline was injected into the vitreous of the eye. The label is known to be taken up by ganglion cells and transported to the terminals where it accumulates (Grafstein, 1967; Neale et al., 1972). The distribution of these endings was then determined by autoradiography of serial sections.

Materials and Methods

Animals. Common Carassius auratus 4-9 cm in standard length were housed in glass aquaria of 5-15 gallons at various densities but less than one animal per gallon. The fish were kept at about 19° under a 12 hr light 12 hr dark schedule.

Surgical procedure. The fish were anesthetized in a 0.05% solution of Finquel (Ayerst), wrapped in wet gauze, and placed in a small holder. Aseptic surgery was performed under a stereomicroscope having coaxial illumination.

Optic nerve interruption was done in the orbit near the cranial entrance by repeatedly crushing with sharpened jewelers forceps until a clear discontinuity in the nerve could be seen. The approach was through a small incision in the dorsal conjunctiva with gentle downward deflection of the globe. There was little bleeding and only minor eye muscle damage.

All eye lesions were unilateral with the contralateral eye left untouched. The retinectomies were similar to those of Attardi and Sperry (1963) and Roth (1972). A cut was made along more than half of the limbus but avoiding the ventral fissure which was used as the orienting landmark. The cornea and lens were then retracted to one side. With fine tungsten needles the nasal or temporal half retina was cut from the remaining retina and peeled off as a sheet. Occasional fragments were removed with an aspirator or forceps with care taken to avoid the optic disc. The lens and cornea were then reflected back into position. Reattachment was successful in about 60% of the animals. The rest showed gross eye degeneration and were discarded.

For the diathermy lesions of nasal or temporal hemiretina the approach was as above through the vitreous. The electrode was a glass insulated tungsten needle with an exposed tip of 700-1200 micrometers and a maximum diameter of 50 micrometers. Under visual observation

the electrode was repeatedly inserted by hand at various locations in the retina and 2 megacycle current at a density just less than that causing gas formation was passed for 1-3 seconds. An obvious white disc of damaged tissue formed around each insertion point, and this was used to estimate the extent of damage. Reattachment of the cornea was as above.

For small diathermy lesions the eye was not opened. Instead it was rotated in its orbit to expose the posterior surface and an electrode similar to the above was then inserted through the sclera up to the glass insulation. An additional 300-500 micrometers of epoxy insulation beyond the glass confined the highest current densities to just beyond the sclera. Current was passed for several seconds at a level below that causing gas formation. In some control animals only a single lesion was done. For the rest multiple penetrations, 15-30 points, produced damage to 15-50% of the retina and neighboring tissue. Some local damage to eye muscles was done but the globe itself remained essentially intact.

Autoradiography. From 10-50 microcuries of L-Proline (5-3H) with a specific activity of 18 or 31.5 C/Mmole was dissolved in 1-3 microliters of sterile distilled water. Under the stereomicroscope a 30 g needle delivering the label was inserted through the sulcus of the sclera into the vitreous, and its opening advanced to a point directly behind the lens. After 12-24 hr the cranium was opened and the head immersed in a mixture of 18 parts 80% ethanol, 1 part formalin, and 1 part glacial acetic acid for several hours. The brains and in

a few cases eyes as well were then dissected free and immersed in fresh fixative for 12-48 hr. Sagittal or frontal serial sections at 15 micrometers through the entire tectum or eye were taken and coated with undiluted NTB-2 emulsion (Kodak) similar to the method of Rogers (1967). An exposure period of usually 7 days was followed by development and staining with cresyl violet.

Retinal Histology. The lesioned eye and frequently for size comparison the normal eye were examined in all the labeled animals as well as in a number of surgical controls. After the initial fixation period described above a hole in the cornea was cut and the lens removed. Further fixation was followed by 15 micrometers serial sectioning and cresyl violet staining.

Evaluation. Simple inspection by light microscopy was generally adequate for evaluation of the autoradiographs although some grain counting was done. Estimates of short linear distances were done with an ocular micrometer, while longer curvilinear contours were traced with a map measure off an enlarged projected image. Measurement of these contours from every eighth section was used to calculate retinal and tectal surface areas from 15 representative animals.

Results

Normal animals. The innervation pattern indicated by the accumulation of label in the terminals was similar to that of previous autoradiographic studies (Grafstein, 1967; Neale et al., 1972) and paralleled closely the degeneration picture seen by Nauta staining

(Roth, 1972). In all nine animals specific labeling was found only contralateral to the injected eye and nearly all of it in the optic tectum. Most of this in turn was restricted to the plexiform layer of Attardi and Sperry (1963) and to a thin lamina just superficial to it. These two layers appear to be the source of most units in the electrophysiological studies on regenerated nerves and have warranted most of the attention here. Two deep minor laminae could usually be seen but, as previously reported, were somewhat variable in appearance and quite thin.

Optic fibers are known to enter these minor laminae at the rostromedial and rostrolateral tectal margins and grow inward and caudally to terminate at the appropriate topographic tectal locus. Similarly, optic fibers enter the thin parallel layer but turn downward to arborize in the plexiform layer immediately below (Attardi and Sperry, 1963; Roth, 1972).

Regeneration of Optic Nerve. Two to three weeks after optic nerve interruption evidence for fibers over the entirety of the tectum could be seen. The label tended to be heavier at the margins and at the rostral tectum and to accumulate in clumps. These local high densities probably correspond to fasciculation of fibers.

However, by 4 weeks the label became more evenly distributed appearing essentially normal. This is based on six animals injected 5-30 days after neurotomy. An additional animal at 164 days indicated this pattern remained unchanged.

Retinal Histology. The operated eyes exhibited substantial reconstitutive growth following surgery. After initial shrinkage, especially prominent after diathermy lesions, there was a gradual recovery of shape, usually resulting by 4-5 months in an eye ± 20% of the size of the contralateral eye and asymmetrical in shape. Histological examination at various postoperative intervals indicated rapid and substantial proliferation of nonretinal tissue at the vicinity of the lesion. Especially after radio frequency lesions gross structural changes involving scleral growth and movements of the optic disc off axis were also in evidence. Except very near the lesion the unoperated retina appeared essentially normal and evidence for initial regeneration at its margins could be seen at 6-9 days. Putative regenerated retina was characterized by various degrees of cytoarchetectonic aberrations ranging from occasional misplaced cell bodies in the plexiform layers to gross distortions in lamination seen most frequently after diathermy lesions. The magnitude of apparent regenerated retina was variable and often greater at longer survival periods being up to 25% of total retina. This and differences in the size of the original lesion provided a spectrum of retinal deficits ranging from 0 to -56% of contralateral retina.

The observations were based on eyes sustaining nasal or temporal lesions 18 by ablation at 1-111 days prior, 10 by transvitreous diathermy at 6-195 days prior, and 34 by multiple penetration transscleral diathermy at 1-168 days prior. For comparison, 21 contralateral normal eyes obtained from survivors of diathermy lesions as

well as numerous normal eyes were examined. In addition, 14 eyes sustaining single point transscleral radio frequency lesions which were used for normal mapping controls were examined 1 day post-operatively for lesion placement.

Normal projection. As seen in seven animals successfully given intraocular injection of tritiated proline 1-9 days postoperatively, an isolated temporal retina produced grains confined to rostral tectum while a similar nasal retina gave labeling largely in caudal tectum but with some very light labeling from fibers of passage growing caudalward from the medial and lateral margins. This corresponds to the innervation pattern seen in previous investigations. Finer details as well as better labeling were achieved in a series of 16 animals given small, usually single point diathermy lesions at various locations in the retina followed immediately by labeling. Discounting two technical failures, these gave relatively smaller grain-sparse zones of the appropriate size and location. The transition from normal heavy labeling to nearly background levels was frequently quite sharp occurring over as little as 100 micrometers.

Projection from lesioned eyes. Labeling was found exclusively in the appropriate tectal laminae and only contralateral to the lesioned eye. Twelve of 13 animals sustaining nasal lesions and optic nerve crush 37-180 days prior to sacrifice showed normal labeling of rostral tectum followed by a gradual gradient of decreasing grains into the extreme caudal tectum. The remaining animal showed gross retinal degeneration and no tectal label. In nine of these, including

the two longest surviving, caudal labeling was very sparse not even filling up the plexiform lamina at its posterior extremes. This asymmetry was most pronounced with the larger original lesions regardless of surgical technique and sometimes less marked where substantial regeneration occurred. The gradient between rostral and caudal halves appeared steeper at short survival periods but was never nearly as sharp as that seen in controls. This made a precise determination of labeled tectal area difficult, but, in general, dense label occupied the region roughly appropriate to the size of the intact retina.

After temporal lesions and nerve interruption in 13 animals with a 29 to 236 days survival range, normal labeling was seen in caudal tectum again only contralateral and in the correct laminae. In nine of these sustaining lesions of 40% or larger of retina and injected 48-236 days postoperatively rostral densities were lighter than caudal but, in general, substantially higher than controls. However, rostral label was not significantly lighter in four animals with estimated original lesions of about 35% or less of the retina. These included two survivors at 29 and 63 days in which there was little evidence for retinal regeneration. The transition to normal labeling was again quite gradual. Retinal regrowth did seem to make a somewhat variable contribution to rostral labeling, and this was further investigated in two animals with temporal lesion 100 days prior but no optic nerve section. One animal showed little retinal regrowth and very light rostral labeling. The other had significant retinal regeneration and obviously more but still light rostral densities. In both cases rostral label was more than short surviving controls and

less than after nerve interruption. Technique of surgery had no apparent effect except as it permitted retinal reconstitution. In addition, there was some suggestion that longer survival periods permitted a more marked rostrocaudal asymmetry of label as long as retinal regeneration was not too great.

To check for abnormal growth of fibers from the intact normal eye, it was injected in a 237 day nasal lesioned, a 236 day temporal lesioned, and a 33 day enucleated animal. No substantial ipsilateral label was seen.

Discussion

Regenerating optic fibers from isolated nasal or temporal retinal remnants showed a preference for terminating in the approximately correct tectal region in general conformance with most previous work. This finding appears at variance with Yoon's and some of Horder's observations indicating these fibers spread across the entire tectum showing no apparent regional affinity. Survival period, surgical procedures, animal size seem comparable, and there is no good reason to suspect that the autoradiographic technique used here failed to demonstrate the presence of fibers. Extensive histology on these eyes showed the retina was accessible to intravitreous labeling, and this was confirmed in three cases in which autoradiography was done on three lesioned eyes. In addition, the finding here is in general agreement with those using different anatomical methods.

The apparent plasticity is probably not a simple electrophysiological artifact since Gaze and Jacobson (1965) recorded only

appropriately located putative terminals. The fact that they avoided surgical trauma to the retina itself together with the evidence here for substantial regeneration of retinal and other tissue and for changes in eye shape and location of retina suggest problems with recording from operated eyes. The projection of this retina into the visual space as defined by the eye alignment and recording techniques used is by no means simple. In addition, the abnormalcy of some of the regenerated retina opens to question the assumption of proximity of stimulated receptor to the ganglion cells that respond. Attempting to estimate the appropriate tectal area innervated by recording immediately after eye lesion is complicated by subliminal retinal damage and subsequent recovery. The histology on lesioned eyes indicates these problems are more acute after diathermy lesions, and it is interesting that in Yoon's hands this produced extensive spreading after both nasal or temporal lesions. By contrast Horder using a surgical ablation method finds this plasticity after only temporal lesions and not after nasal, dorsal, or ventral retinectomies. The evidence here also suggests there may be spreading of connections after temporal lesions but that this may be a temporary state. Horder did not describe survival periods for these animals so these may be transitory to a more confined projection. Alternatively, there may be some few misplaced terminals which are easily sampled by electrophysiological methods but do not represent the majority of normal fibers. Perhaps more likely there may indeed be an organized expansion of the projection from at least an isolated nasal retina but its

magnitude may have been overestimated. Whatever the explanation it seems reasonable to regard these preliminary electrophysiological reports showing extensive topographic spread of connections from half retinas with caution until some of these difficulties are adequately dealt with. The organization and origin of fibers seen here to apparently grow into incorrect tectal regions should be considered an open question requiring further study.

The extensive regulative changes resulting in reorganization of chemospecific properties of a half retina into those of a whole retina as indicated for Xenopus tadpoles seems not to occur here. This presumed stability of at least most of the original cytochemical tags of these retinal remnants as well as of chick half retina and of frog half tectum would be expected according to a chemoaffinity scheme to give the selectivity of innervation found. When account is taken for possible regulative changes then the spread of a half retina across a whole tectum in compound eyes of Xenopus and the compression of a whole eye onto a growing goldfish half tectum are also in accord with this hypothesis. Thus, up to this point there is no unambiguous evidence for the kind of organized mismatching of retinal and tectal elements indicating chemoaffinity is not sufficient to account for the topography of this projection.

However, chemoaffinity does not exclude the possibility of some kinds of misgrowth under abnormal conditions or of the participation of other growth directing agents and these are both suggested by the results here. At short survival times where little regeneration

has occurred and with smaller lesions at least an isolated nasal retina seems not to be able to by-pass rostral tectal sites for its normal caudal terminations. Since a temporal retina does not exhibit this extensive spread of label, and complicating processes such as regeneration or partial regulation might be expected to be the same in both cases, this implies affinity between retinal and tectal neurons is not sufficient to prevent growth and perhaps termination in inappropriate tectal regions. This argues against a simplistic chemoaffinity model where growth of a fiber is chemotactically guided independently of the presence of other fibers to exactly achieve its appropriate course and point of termination. The growth of other fibers, occupation of synaptic space, ontogenic factors, or other processes can be assumed to participate with chemoaffinity to mold the normal retinotectal innervation pattern. There has already been some suggestion of this from work in neonatal hamsters (Schneider, 1973) but unanswered questions about longevity of optic fiber growth, cell populations surviving the kind of heat lesions used, and possible regulation require interpretive caution. This multifactored scheme has been previously suggested (Sperry, 1951b), but except for long known mechanical factors clear evidence for it in the retinotectal system has been lacking.

IV. TECTAL RETINOTOPIC ORDER OF REGENERATING OPTIC NEURONS

Introduction

The less than perfect growth of optic fibers from partial retinas as demonstrated by the previous experiment raises questions about the precision of regeneration from intact retinas. While the end state as judged by electrophysiology (Gaze, 1959; Maturana et al., 1959; Jacobson and Gaze, 1965) and Nauta staining (Roth, 1972) appears to be a fairly close approximation to the normal pattern, it is not clear the initial innervation is so well organized. Gaze(1970)and Jacobson (1970) reported electrophysiological evidence for a disorganized projection during the early stages of regeneration of the frog optic nerve. However, the arguments for selectively recording from nerve terminals are in large part based on knowing the anatomy of terminals and on finding an orderly arrangement of recordable structures. This latter condition is not met, and the former is unknown for regenerating optic fibers. In addition, this finding is contradicted by recordings from goldfish where even the initial regeneration was retinotopically organized (Jacobson and Gaze, 1965).

In an attempt to determine whether or not this reestablishment of the retinotectal projection is achieved by precise initial growth, the topography of this regenerating system was studied anatomically. At various intervals following optic nerve interruption, the eye sustained a partial lesion or some of the optic fascicles of the tectum were cut. The eye was immediately labeled with tritiated proline, and the fate of transported label was then determined autoradiographically.

Materials and Methods

Transscleral single point diathermy lesions were used throughout with para-axial sections taken of all lesioned eyes and frontal sections of tecta. Controls with intact optic nerves are those from the preceding eye lesion study. The eye surgery, histology, and autoradiography were done as previously described there. The tectum was incised down to the ventricle with tungsten needles as in Chapter 6.

Results

As partly described above, a single point diathermy lesion placed between 1/2 and 3/4 of the distance from limbus to optic nerve damages not only ganglion cells in the immediate vicinity but can also be expected to disrupt axons coursing past the area in route to the optic nerve head. If these animals are labeled immediately subsequent to this lesion, there appears in the appropriate area of tectum a label free zone shaped roughly like a sector of a disc. In the mediolateral dimension the transition area from normal density label to background is generally 50-100 micrometers wide. The denervated area is about 10-20% of the total area labeled in a normal animal.

Between 41 and 149 days after optic nerve interruption, either nasal or temporal lesions were made prior to labeling with tritiated proline. At 41 days grains were found evenly distributed over the entire tectum with no evidence of any light areas. In these two animals the lesion appeared adequate. At longer survival periods an area of lighter than normal label began to appear in the appropriate tectal region. This zone had substantial labeling within it and no sharp

boundaries. This gradually improved to where at 149 days normal density label was seen to fall off within 100-200 micrometers to light labeling evenly distributed within the hole which was roughly the correct size and shape.

As will be shown in Chapters 6 and 8, in the normal animal a mediolateral midtectal cut through dorsal tectum denervates caudodorsal tectum while leaving caudolateral tectum undisturbed. In contrast, similarly treated fish with nerve section 59, 88, and 206 days prior all showed substantial dorsal label posterior to the incision. This was quite light near the midline but increased in gradient manner to join normal density labeling of lateral tectum. There was no marked differences between animals.

Discussion

There appears to be significant looseness in the regenerative reconstitution of the retinotectal projection. Even if it is assumed that optic fibers are growing along correct routes but have not yet reached the appropriate locus for termination, a caudal label light zone should have appeared after a nasal lesion or tectal incision. At the longer survival periods or with tectal incisions this should not be a complication, and yet significant inappropriate label is still in evidence. This light label remaining after retinal lesions could represent either incorrect terminations or fibers of passage that have grown through wrong routes to reach their appropriate termination zone. The orderly electrophysiological maps favor the latter, as does the presence of fairly dense label caudal to tectal incisions.

However, this regenerative growth should by no means be considered completely random since Attardi and Sperry (1963) and Roth (1972) have shown optic neurons from partial retinas exhibit some preference for the correct central route during regeneration. The labeling gradient found after tectal cut also argues for some path preference; but it does mean there is significant exploratory growth into regions in which termination is not achieved.

Estimation of retinotopic fidelity recovered at 149 days agrees with a previous electrophysiological estimate from frog (Maturana et al., 1959) that it is worse than normal by a factor of 2. Even this may not yet represent the best achievable condition.

V. UNDERWATER VISUAL FIELD MAPPING STUDIES ON GOLDFISH

Introduction

There have been a number of recent studies following the initial report of Gaze and Sharma (1970) that the retinotectal projection of goldfish exhibits substantial reorganization after various tectal insults. All of these data have relied on unit tectal recording of optic axon terminals with the eye in air. This method, however, suffers from some inaccuracies. The refractive index of the cornea of fish is approximately that of water (Walls, 1942) and hence not normally a refractive interface. In air the cornea introduces additional positive lens giving a large refractive error of 52 diopters myopic for goldfish (Wartzok and Marks, 1973). This error produces large apparent receptive fields which can be expected to be more difficult to localize. While Cronly-Dillon (1964), Schwassman and Kruger (1965), and Wartzok and Marks (1973) report on average receptive field size of from 2-4^{o} with the eye in water Jacobson and Gaze (1965) and Yoon (1971) give a value of 10-30^{o} for the eye in air. Since the cornea is somewhat flattened (Walls, 1942) one might also expect that the receptive field position would be artificially displaced away from the optic axis when the cornea is in air. In addition, methods of eye alignment and measurements of eye drift have not been rigorously described in these studies. Further, all these studies have employed visual placing of electrodes which can be expected to be less precise than mechanical methods.

There may also be a tectal sampling problem. The undercurved ventrolateral tectum corresponding to most of this inferior field is not readily accessible to superficial probing and has been neglected in all regeneration studies. In most experiments this is not a serious deficiency as long as one can record nearly all of the superior field projection. While this seems to be the common assumption (Jacobson and Gaze, 1965; Gaze and Sharma, 1970; Yoon, 1971; Horder, 1971), it may not be entirely justified. The preceding anatomical study of the retinotectal projection indicated that only about two-thirds of the superior field projected to the dorsal aspect of the tectum. The nasalmost one-third mapped to extreme rostral tectum which curved downward in a manner similar to lateral tectum. This raises the question of whether significantly less than an entire upper field was mapped but was interpreted to be relatively complete because of the artificial field expansion caused by having the eye in air. This view is encouraged by examination of published maps using this technique. Most, in fact, show a deficit in the representation of the nasal field. While these potential errors are probably not serious enough to undermine the existence of plasticity, it may be significantly different than previously supposed. For example, instead of a uniform compression involving the entire retina, the nasal field projection might remain unchanged with the compression confined to temporal field. This would seem more in accord with the idea of optic fibers having higher affinity and being more competitive for their correct tectal area. For these reasons an eye in water electro-

physiological mapping study was conducted on the goldfish retinotectal system using mechanical electrode placement and a well-defined eye alignment procedure. Normal goldfish as well as fish with prior optic nerve section and half tectal ablations were used.

Materials and Methods

Electrophysiological recording. The basic method for correcting refractive errors associated with eye in water mapping is after Schwassman and Kruger (1965) who have previously mapped goldfish. The apparatus may be viewed essentially as a small aquarium with one side formed by the flat surface of an enclosed water-filled acrylic hemisphere 60 cm in diameter. After cranial opening under Finquel (Ayerst) anesthesia common Carassius auratus 4-9 cm in standard length were immobilized by injection of 2-10 micrograms of d-tubocurarine given intramuscularly. The fish was then wrapped in wet gauze and strapped into a rigid acrylic holder. A hollow mouthpiece maintained water circulation through the gills at a rate of about 50 ml/minute. By means of a mechanical manipulator the eye was positioned at the center of the hemisphere wall and the water level raised to 10-15 mm above the eye. A form fitting raised wax barrier prevented water from entering the cranial opening. By transilluminating the animal it was possible to visualize the optic disc and the fish was turned to place this image at the vertex of the hemisphere. A modified Brombach perimeter (American Optical Company) having a diameter of 66 cm was placed next to the curved surface of the hemisphere. By means of a telescope at its center the superior-

inferior axis was defined to lie along the line connecting the ventral eye fissure and the center of the pupil. Eye position was monitored through and at the end of the recording session. Changes in disc location or torsion were nearly always less than 3^{o}. Rerecording from previous tectal positions at the conclusion of the experiment gave receptive field positions generally within $3\text{-}5^{o}$ of the previous position. Systematic electrode positioning was done with an X,Y,Z micrometer driven translation place micropositioner adjusted so that the X axis paralleled the midline axis. Placement was generally in a grid pattern at 250 micrometer intervals and a vertical depth of 50-200 micrometers. Some marking of electrode position was done by passing dc current through the electrode or by advancing the electrode deep into the tectum. Receptive field mapping was done with $1\text{-}3^{o}$ black discs against the white background of the perimeter. It was possible to map 170^{o} of visual field with refractive error of only $1\text{-}2^{o}$ at the extreme periphery.

Surgery. The techniques were similar to those described elsewhere. Finquel anesthesia was used in all cases. Optic nerve interruption was done in the orbit. Tectal removals were accomplished with tungsten needles and aspiration.

Results

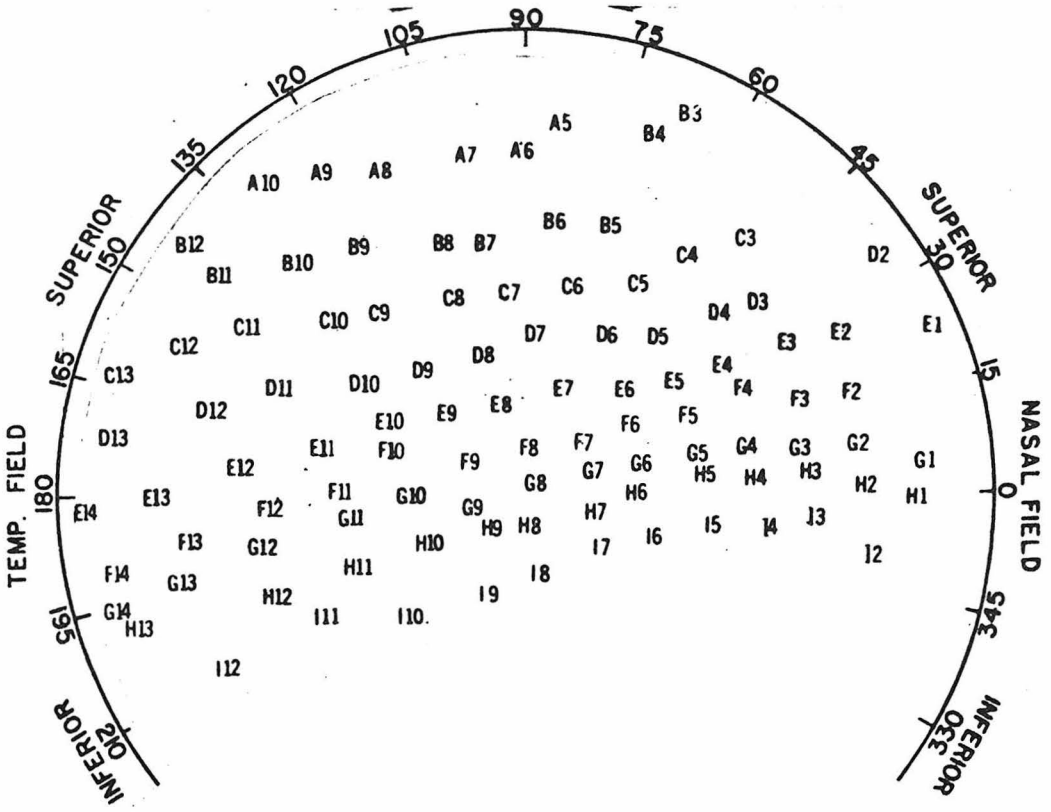
Normal retinotectal projection. The general organization of the retinotectal projection is in agreement with previous workers. This is based on successful mapping of 25 animals. As previously reported, the curved lateral tectum and corresponding inferior field is relatively inaccessible to microelectrode probing. It was also

found that the extreme rostral tectum was difficult to probe. If the long body axis of the fish was perpendicular to the electrode path, only the dorsal part of the tectum corresponding to temporal superior field could be easily recorded from by superficial probing. By tilting the fish head up at various angles more of the nasal field became accessible. At 70° up from horizontal the extremes of the temporal field began to show mapping distortions as did the far nasal regions. However, this angle gave a fairly balanced representation of the whole field and was considered optimum (Fig. 3). In 13 animals various electrode positions were marked and serial sections through the tectum taken. This showed that one-fourth to one-third of the tectum corresponding to frontal field curved downward at the rostral region so that at its far extreme it was nearly perpendicular to mid dorsal tectum. Examination of eyes sectioned along the nasotemporal axis showed equal areas of retina on either side of the optic disc, justifying the eye alignment method.

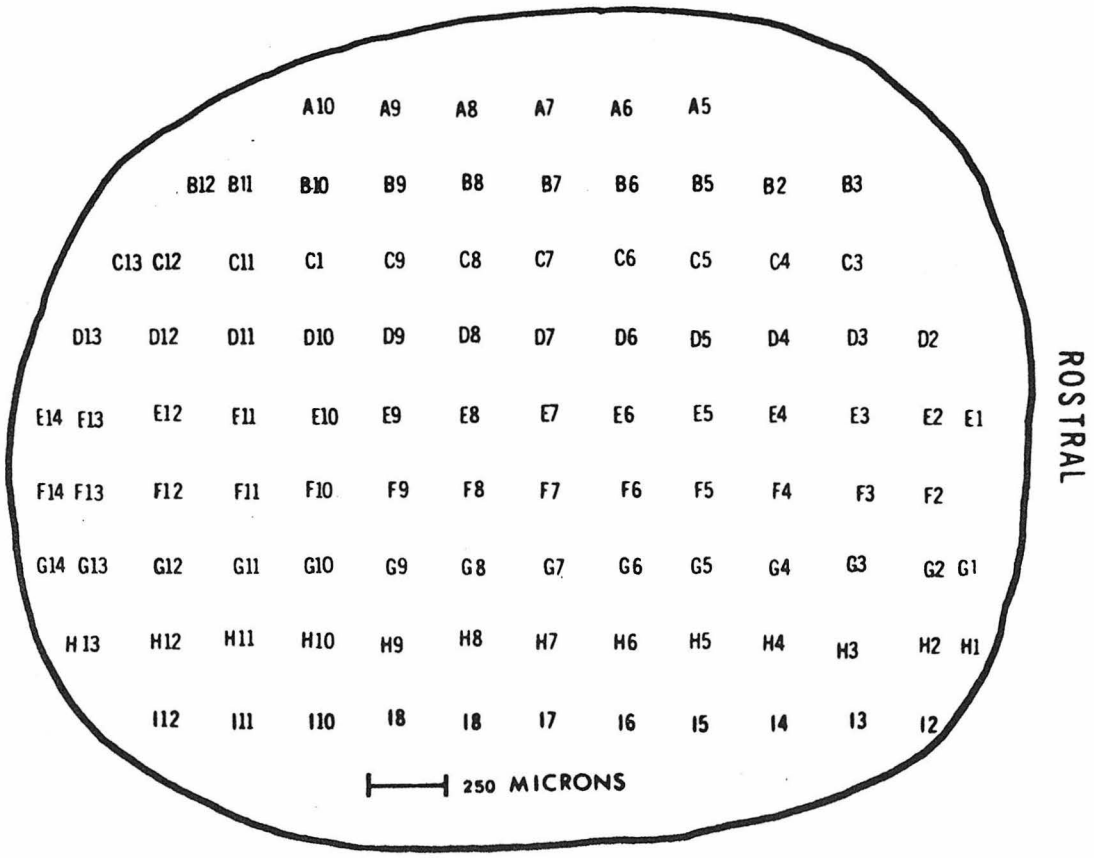
For comparison two animals were mapped using visual placement of electrodes. A microphotograph of the tectum was taken through a micrometer grid and the electrode placed with some difficulty at grid intersections identifiable on the real tectum by the blood vessel pattern. Although there was more apparent variability, the general organization of the map was comparable to that obtained with mechanical placement.

To determine the effect of having the eye in air, four fish were mapped under this condition and three of these with the eye under

Fig. 3. Electrophysiological map of left visual space onto right tectum with numbers and letters indicating corresponding electrode placement and receptive field location. Head was elevated by 70^{o}.



MEDIAL



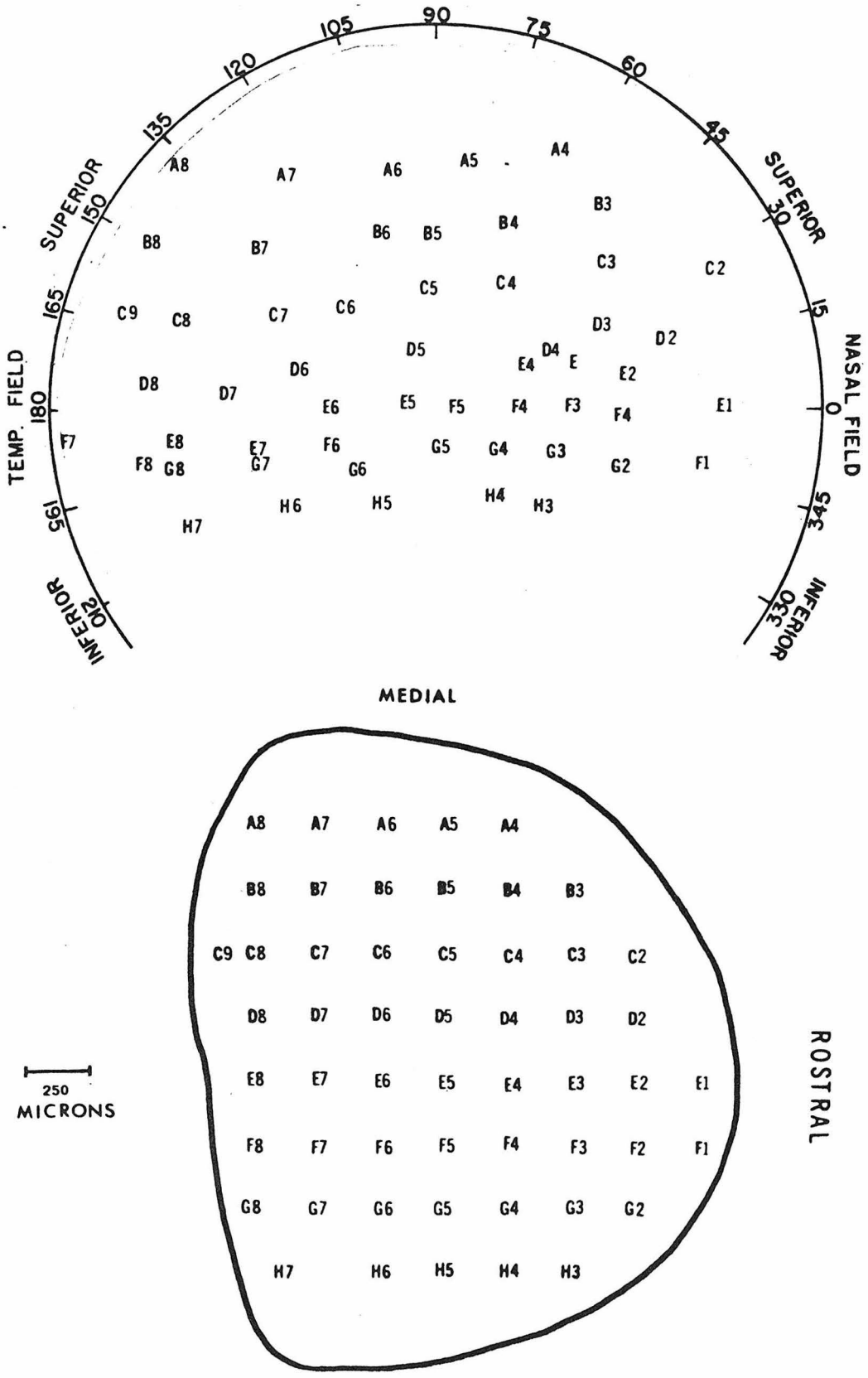
ROSTRAL

water as well. Although the general pattern of the projection was again comparable, there was significant displacement of receptive field location away from the optic axis. Receptive fields 75° from the disc with the eye in water were displaced to around 95° when in air.

Regenerated retinotectal map. As previously reported (Gaze and Sharma, 1970; Yoon, 1971; Jacobson and Gaze, 1965) the map formed by a regenerated optic nerve was approximately normal. The two projections measured at 76 and 82 days after optic nerve crush were, however, somewhat less orderly than that of normals and gave abnormally small amplitudes.

Retinotectal map onto a half tectum. Five fish were measured 63 to 214 days following caudal tectal ablation and optic nerve crush. At the short postoperative intervals the original projection to rostral tectum was approximately reestablished with relatively little evidence for compression. There was some compression at the intermediate periods and by 214 days it appeared fairly complete with only minor departures from uniformity (Fig. 4). Except for the longest survivors the projection was less orderly than in animals with only optic nerve crush but except for a few tectal points order was present along both nasotemporal and superioinferior axes. In all except the 63 and 214 day animals the responses obtained from the caudal 200-400 micrometers of the rostral remnant were generally abnormal. Units were of low amplitude and difficult to isolate. These multiunit receptive fields were unusually large, often 10-20°,

Fig. 4. Electrophysiological map of left visual space onto a right tectum 214 days after right caudal half ablation and left optic nerve interruption.


90
75
60
45
30
15
0
345
330
315
300
285
270
255
240
225
210
195
180
165
150
135
120
105
SUPERIOR
SUPERIOR
NASAL FIELD
TEMP. FIELD
INFERIOR
INFERIOR
A8 A7 A6 A5 A4
B8 B7 B6 B5 B4 B3
C9 C8 C7 C6 C5 C4 C3 C2
D8 D7 D6 D5 D4 D3 D2
E8 E7 E6 E5 E4 E3 E2 E1
F8 F7 F6 F5 F4 F3 F2 F1
G8 G7 G6 G5 G4 G3 G2
H7 H6 H5 H4 H3
MEDIAL
ROSTRAL
250
MICRONS

and frequently not well demarcated. The location of these fields were in the temporal field usually beyond 60° from the disc and as far as could be determined were topographically ordered.

Discussion

The retinotectal projection determined with the eye in water technique is in approximate conformance with that of Schwassman and Kruger (1965) but they did not give details of eye alignment to permit an exact comparison. These records also confirm the general organization of the projection as obtained with eye in air methods (Jacobson and Gaze, 1965; Yoon, 1971; Sharma, 1972) but shows some limitations of this procedure. With the large receptive fields high accuracy mapping did seem more difficult. This is suggested by the more uniform progression of receptive field locations obtained with the eye in water. However, other methodological differences may have also contributed.

The displacement of receptive field positions away from the optic axis with eye in water will give the impression that the recordable visual field is larger than it really is by some 40°. A deficit in the field, such as in far nasal field, would then be less noticeable, especially if the eye alignment procedure tends to center the recordable field. In some published maps (Jacobson and Gaze, 1965; Yoon, 1971) there is evidence of this nasal deficit though it is smaller than the one seen in the recordings here.

For many questions about retinotectal connectivity formation these sources of potential error are not of great importance. Usually what is of interest are changes in connectivity seen as changes in the

recordable projection pattern. However, some experiments may require more absolute measurements. Yoon (1972b) combined a caudal tectal ablation with a temporal hemiretinectomy and found with his recording method that this inappropriate half retina spread over the entirety of the rostral remnant. Roth (1972) repeating the same surgical procedure but using Bodian staining found innervation only in the caudal half of the tectal remnant. One possible explanation for the discrepancy is that Yoon was not sampling the far rostral regions.

The half tectal ablation with optic nerve crush supports the general findings of Gaze and Sharma (1970) that there is an initial tendency to reestablish the original connectivity pattern onto the rostral remnant. This is followed by varying degrees of compression and disorder eventually resulting in moderately well organized whole field projection onto rostral tectum. What has gone undescribed is that the responses from temporal field roughly in the area corresponding to the original lesion are significantly abnormal not unlike responses seen from regenerating neurons. The low amplitudes and large fields indicate that large precisely ordered axonal arborizations do not exist in this area at least not by 130 days. Inspection of the brain in vivo under a stereomicroscope as well as examination of several Bodian stained brains shows fascicles of optic fibers of normal orientation running up to the lesion and turning abruptly to form a small neuroma at the cut edge. With the additional observation that temporal field responses were not much in evidence at the shortest survival period, the following sequence of events suggests itself. Optic fibers

initially grow in and arborize at their previously occupied tectal locus if available and continue to grow if the appropriate site is missing. These latter fibers eventually turn back or retract into the tectum and displace rostral tectal fibers from their normal positions. This displacement is very slow and gradual and may not reach completion at up to 214 days.

VI. REGROWTH OF OPTIC FIBERS PAST SURGICALLY DISRUPTED TECTAL ZONES

Introduction

The normal retinotectal topographic projection of the goldfish appears to undergo systematic reorganization after various tectal insults including an orderly compression of the entire visual field onto a surgically reduced half tectum (Gaze and Sharma, 1970; Yoon, 1971). This compression can also be induced by blocking caudal bound fibers with a mediolateral incision or insertion of a mechanical barrier in middle tectum and is followed by reestablishment of the original projection over the whole tectum when the barrier is removed (Yoon, 1972a).

However, there are some observations which complicate this simple picture. Sharma (1972c) finds no compression after tectal incisions and even large rostral or midtectal lesions are not able to prevent reacquisition of the visual projection onto the caudal remnant. In contrast to the foregoing electrophysiological investigations, Arora's (1973) preliminary Bodian study failed to show any fiber growth past incised regions raising the specter that the electrophysiological reorganization may not represent changes in axon terminal distribution but some complex postsynaptic event. Also there has been no report of significant regenerative reconstitution of goldfish tectum following lesions. Yet, Kirsche and Kirsche (1961) frequently find this in the closely related Carassius carassius.

What seems needed is basic information on the growth responses of tectum and optic fibers to these kinds of injury. To this end the

tectum of goldfish was variously lesioned, and at different post-operative intervals tritiated proline injected into the contralateral eye. Autoradiography of serial sections shows substantial reconstitutive growth of both retinal fibers and tectum.

Materials and Methods

Animals. Common Carassius auratus 4-9 cm in standard length were housed in glass aquaria at about 19° under a 12 hr light dark schedule.

Surgery. The fish were anesthetized in 0.05% solution of Finguel (Ayerst) and aseptically operated under a stereomicroscope. A three-sided bevelled cranial flap was incised and retracted permitting the appropriate incisions and ablations as described below in context with electropolished tungsten needles. The flap was then reflected back in place without glue or stitches.

Autoradiography and histology. A 12-48 hr postinjection survival period was used, but otherwise the techniques were as previously described.

Results

Fascicles of optic fibers coursing rostrocaudally in parallel fashion can be seen on the dorsal tectum in vivo. A mediolateral midtectal incision 500 micrometers in length made a few minutes prior to retinal labeling produced in three animals normal grain distribution in the plexiform and other lamina except rostral and caudal to the incision. Posteriorly there was a rectangular hole in the label running almost to the caudal extreme. Anteriorly there was a similar region of

denser than normal grains for several hundred micrometers. The density was highest near the incision and decreased in gradient manner to be just noticeable at the rostral end. This was presumably the effect of damming axonally transported material. Three other animals were examined 11 and 20 days after similar surgery. These showed no label free area in the caudal tectum and local areas of very dense labeling near and rostral to the incision. Within about 200 micrometers anterior and 500 micrometers posterior to the incision site the dense labeling was more scattered than in the previous animal with some label in deeper tectal layers not normally innervated. More caudally the pattern was essentially normal. Anterior to the abnormal labeling pattern a narrow ribbon of heavy label in the parallel layer could be traced down into the optic tract and nerve. This path is approximately that expected of the cut fibers indicating increase of axoplasmic transport after injury. An additional animal examined at 69 days after this surgery showed a normal innervation pattern.

To more clearly see whether the optic fibers could transverse the lesion and to examine the tectal response to complete section, five animals sustained a midtectal medial lateral incision across the whole dorsal surface. The cut was down to the ventricle, and a tungsten needle with a large right-angle bend was repeatedly passed between ventricle and tectal surface to insure complete section. An animal labeled immediately after surgery showed as expected virtually no label on the caudal tectum. By contrast four other animals examined at 70-125 days postoperatively showed an essentially normal label

pattern posterior to the cut. The area of the incision was bridged by tissue and in many areas there was a very close approximation of rostral and caudal halves. In places a band of cresyl violet staining cells could be seen extending from the deep periventricular gray and ependymal layers up into the zone of surgical trauma suggesting migration of cells from these layers. In this zone label could invariably be seen and was frequently in the form of narrow ribbons between the two tectal halves. No evidence of denser than normal grains at the lateral or medial edges of the cut was seen denoting substantial fiber growth through and not around the lesion.

To obtain clearer evidence for this kind of growth and to explore the consequences of larger more traumatic lesions two animals sustained ablation of a 500 micrometer wide tectal band between rostral and caudal tectum. By 34 days this zone became reconstituted with tissue which was fairly disorganized in parts but in a few places looked like shrunken poorly laminated tectum. In this tissue were numerous round cresyl violet stained cells which resembled neurons. Evidence of label was seen throughout this region with again no suggestion of high densities at the edge. The transport rate appeared elevated as indicated by denser labeling both rostral and caudal to the incision. A similarly lesioned animal was recut through the original ablation at 120 days after the initial surgery. The original site was identified by a more translucent appearance than the surrounding tectum. The caudal tectum appeared largely denervated as a consequence with only slightly more label than seen in a normal after

this kind of cut. This label was more pronounced laterally and caudally than medially. Rostral to the recut the dense label from axoplasmic damming was seen in the normal zones. The histology of the ablated area was similar to the preceding animal but with more tectal-like structure.

To see if the fibers would prefer an existing tectal bridge rather than grow through a surgically traumatized zone, an animal was lesioned as above but sparing a 200-300 micrometer remnant near the midline. At 34 days grains were seen over the entire tectum, in the zone of trauma, and not particularly concentrated in the midline remnant.

The consequences of large tectal lesions were assessed in five animals: after a caudal tectal ablation at 37 and 59 days postoperatively, after a mediocaudal quadrant ablation at 214 days, and following a medial quarter ablation at 37 and 90 days. There was some evidence of tectal reconstitution after medial and mediocaudal lesions, and some invasions of this tissue by optic axons. This was somewhat variable, however, being practically nonexistent in some areas and comprising several hundred micrometers of tissue in others. Relative to whole tectum volume this tissue and labeling was nonetheless small, perhaps 5%. After medial lesion an abnormally thick band of grains was seen in the plexiform layer near the cut margin and in the 37 day survivor significant label was found in inappropriate tectal layers and various subtectal structures.

Somewhat different tectal reconstitution was seen following the caudal ablation and this has been also confirmed in nine other cases of up to 214 days survival but using only cresyl violet or Bodian stain. At the caudal end of the remnant there was frequently some small amount of additional tissue growth somewhat variable in appearance and extent and often connected to the subtectal valvula cerebelli. Especially after longer survival there was sometimes apparent new tectal growth at the original caudal extreme. This was up to a few hundred micrometers in length and looked much like a piece of abnormally thin tectum. Except for a pia-like connection, it was largely isolated from the rostral half and not seen to acquire optic fibers. The 37 day animal showed substantial abnormal growth into lower tectal laminae, and this could be seen well rostral to the lesion. Similarly grains were seen in subtectal areas and even in the cerebellum. This inappropriate label, however, was largely absent in the 59 day survivor.

Discussion

The innervation of dorsal tectum by rostrocaudal running fibers as suggested by previous studies (Attardi and Sperry, 1963; Yoon, 1972a; Roth, 1972) was confirmed here. The denervation of caudal tectum consequent to mediolateral tectal transection contradicts the suggestion of Sharma (1972b) that caudal tectum is supplied by the lateral optic brachia and that its innervation is not disrupted by surgical manipulations of dorsal tectum. All but perhaps a few fibers are disrupted by these procedures.

The growth response of optic fibers to axotomy as seen after short survival periods is quite vigorous. The marked increase in axoplasmic flow seen previously by other methods (Grafstein and Murray, 1969) was easily seen by autoradiographic techniques, which makes this a potentially useful method for tracing fiber paths as well as for following regenerating neurons. There is significant and rapid growth even into regions not normally innervated but most growth is in the appropriate direction. The fact that no denervated caudal areas were seen at 11 days after small tectal incisions before the heavily labeled fibers could be seen in the caudal extremes opens the possibility that collateral sprouting from intact fibers neighboring on the denervated area may have also occurred. Extensive permanent sprouting, however, is incompatible with the electrophysiological data. The normal appearance of label after long survival periods indicates some of the apparent misgrowth may be only exploratory and later resorbed. This together with the presence of label in the disrupted tectal regions even following large ablations, the absence of regional high densities at the edge of these regions, and the denervation of caudal tectum following recut all argue optic fibers can regrow onto caudal tectum and perhaps prefer to do this through the original lesion.

The tectum also exhibited significant reconstitutive growth in a manner similar to that reported by Kirsche and Kirsche (1961). They found regrowth was from ependymal cells and from matrix growth zones located at caudal, laterocaudal, and mediocaudal tectal margins.

The finding here that small lesions preserving these zones gave marked regeneration while larger lesions gave poor regeneration is in accord with their observations. The staining characteristics and cell types suggest that at least some of this growth involves neurons.

There are several consequences of these findings for studies of neural connectivity. Contrary to the suggestion from Arora's (1973) work the electrophysiological studies indicating visual projection onto caudal tectum after rostral lesions would seem to represent the distribution of optic fibers. Yoon's compression seen as long as 112 days following an incision between rostral and caudal tectum is somewhat surprising in view of the readiness of fibers shown here and in Sharma's electrophysiological experiments to rapidly grow past simple incisions (Yoon, 1971; Sharma, 1972c). It can be supposed that Yoon's original use of scissors was very traumatic and inhibited the tectal reparative processes needed to permit fiber growth. This is supported by a recent observation (Yoon, personal communication) that with the use of more refined techniques this compression does not occur.

Fortunately, tectal repair and invasion of this tissue by optic fibers was of minor significance after the large tectal ablations used in most studies on retinotectal plasticity and can probably be neglected. The substantial growth into inappropriate tectal and non-tectal regions after short survival following an ablation appeared transient largely disappearing after longer recovery periods. Thus, the steady state condition resulting in abnormal density of terminating fibers onto the appropriate laminae of the tectal remnant is really a small subset of the area to which optic fibers are capable of growing.

VII. GROWTH OF THE GOLDFISH TECTUM AND RETINA

Introduction

The retinotectal system of goldfish has been the subject of a number of recent studies showing flexibility in the pattern of optic nerve connectivity following various surgical size reductions of tectum (Gaze and Sharma, 1970; Yoon, 1971; 1972a; Sharma, 1972a; 1972b). Ths kind of plasticity is also seen after retinal manipulations on growing Xenopus tadpoles (Gaze et al., 1963; Straznicky et al., 1971) but not after tectal lesions in the adult frog (Meyer and Sperry, 1973; Straznicky, 1973) whose retinotectal system no longer grows by cell addition (Larsell, 1929; Straznicky and Gaze, 1972). Since there is some suggestion the goldfish tectum may still be acquiring new cells, it has been asked whether these plasticities are related to retinotectal growth (Meyer and Sperry, 1973).

Evidence for this growth in goldfish is based on light microscopy studies on the tectum of the closely related Carassius carassius (Kirsche and Kirsche, 1961). A zone of dividing cells was found at the caudal, lateral, and medial tectal rim from which neuroblasts appeared to migrate inward to central tectum. However, this kind of observation makes it difficult to determine the final site of the migrating neuroblast and the extent of cell death. Extrapolation from other systems is of limited use because of marked species differences. Retinal growth was not studied, leaving this an open question. The extent and pattern of cell addition in the retinotectal system of goldfish is important because it may indicate why plasticity can be

expected in this system. If the tectum grows, as suggested for Carassius carassius, by adding cells at only three sides and the retina grows, as suggested from frog (Staznicky and Gaze, 1971) by adding an annulus of cells at its ciliary margin, then there must be some way of sliding retinal connections across the tectum during growth in order for the retinal projection to remain centered on the tectum. In addition, this would argue that change in synaptic connectivity can normally occur in a well-differentiated functional neural system.

To determine whether cell addition does occur in juvenile goldfish and what form it takes, a thymidine study of retinal and tectal growth in Carassius auratus was conducted.

Materials and Methods

Common Carassius auratus were housed in glass aquaria at a density of about 1 fish per 5 gallons of water at 19°. Thymidine (methyl-^{3}H) was injected intraperitoneally in sterile aqueous solution at about 20 microcuries per gram weight of animal which ranged from 1.1 grams to 4.1 grams. The corresponding standard length range was 30 to 50 millimeters. Serial sections at 15 micrometers were taken through the entire eye and through most of the brain at either sagittal or frontal orientations. Autoradiography and cresyl violet post-staining was done as previously described.

Results

In the three animals sacrificed 24 hr after injection dense compacted labeling was found in the ciliary margin with a smaller number of scattered labeled cells throughout the retina. Some of these were consistently seen in the receptor layer within a few hundred

micrometers from the margin. Other label was in the inner limiting membrane and obviously not neural. The rest was distributed in the various retinal cell layers. The two animals fixed 11 days after labeling showed a similar picture but with the zone of dense labeling moved inward toward the fundus some 50 micrometers. The two 79 day animals also showed some scattered labeling in all retinal cell layers but slightly less than at short survival times. The retinal region having densest grains was some 300 micrometers in from the ciliary margin as compared to some 3200 micrometers of retina from the margin to the fundus. The edge closest to the fundus was often quite sharp but there were some differences between the layers. The receptor layer label was closer to the fundus and more scattered than the horizontal layer and this in turn closer and more confined than the ganglion cell layer. The area of high grain density was about 25 micrometers wide followed by about 50 micrometers of lighter grain deposition.

After 24 hr all 3 brains showed substantial thymidine uptake in the areas previously described by Kirsche and Kirsche (1961), the basal (lateral), caudal, and dorsal (medial) matrix zones. There were also a few labeled cells scattered throughout the tectum. At 11 days the heaviest label had moved slightly inward but extended in gradient manner to the growth zones. There were more labeled cells scattered apparently randomly throughout the tectum. By 79 days the heavy zone of label had moved up to 300 micrometers in from the margin out of a total tectal length of about 2500 micrometers. This zone of label was mostly in the periventricular gray and was some 25-50 micro-

meters thick across the highest density region with lighter label extending another 50-75 micrometers toward the margin. Several labeled cells in the upper tectal regions above this area of heavy label could usually be seen in every section. In addition, labeled cells were scattered in both upper and periventricular gray layers through the tectum, including the rostral regions, but the number was less than that seen in the 11 day survivors.

Discussion

The retina of the juvenile goldfish appears to be growing largely by the addition of concentric rings of cells at or near the ciliary margin. The consistent uptake of thymidine at the margin together with the slow movement of an area of heavily labeled retina away from the edge is difficult to fit with any other scheme. The fact that the outer layers are nearer to the fundus than the inner layers shows that for a given locus early ontogeny is recapitulated in this growth process with the ganglion cells being older than the horizontal cells and these older than the receptors. Although the retina may not grow at equal rates along all its edges, there is at least not marked growth asymmetry as in the tectum. In small goldfish this new growth can result after 2 1/2 months in an approximately 10% increase in retinal volume.

Tectal growth was also occurring by cell addition at its margins but some cell migration may also take place. The relative absence of label in central tectum 24 hr after injection, and its appearance at 11 days, suggests migration of these cells perhaps from the growth

matrices at the margins. However, the subsequent disappearance of many of these cells indicates that they were not long-lived neurons or glia. It also is possible that some of the label was hematogenous. A few labeled cells did persist in central tectum, however, but these may be neuroglia.

What is clear from the label pattern at various survival times is that a significant number of cells are added at the lateral, medial, and especially at the caudal margins but not apparently at the rostral end. The prominence of this label in the periventricular gray and its frequent association with cresyl violet staining cells having large round eccentric nuclei in the adjacent upper tectum indicate these cells are neural. Under the conditions here this growth can contribute some 10-15% increase in the rostral-caudal dimension in 79 days. While the exact form and magnitude of growth must await careful mapping studies and longer survival periods, it seems inescapable that growth is asymmetric.

This asymmetrical enlargement of tectum and symmetrical enlargement of retina suggests that during this phase of life the retinal projection may have to shift its connections across the tectum to maintain a centered retinotectal map. This has been previously suggested to occur in Xenopus tadpoles where an analogous mismatching of growth patterns takes place (Staznicky and Gaze, 1971; 1972). Gaze and coworkers have been able to record electrophysiologically from these tadpoles during this growth phase and have shown a migration

of the retinal projection (Gaze et al., 1972). However, functioning of the tectum and formation of working synapses by optic fibers is contraindicated in these tadpoles. Complete removal of tectum has no observable effect on their optokinetic and other visual behaviors (Mark and Feldman, 1972). Initial results from recordings from tectal neurons show no evidence of the preliminary mismatching of connections suggested by Gaze (Hunt and Jacobson, 1973c).

The import of these findings in goldfish is that the tectum is a well-differentiated system and retinotectal synapses are certainly formed. Thus, changing connectivity appears compatible with neural function and can occur in normal animals. Continuous growth of neural processes even in adult animals has been previously suggested based on sprouting following denervation of contiguous neurons (Liu and Chambers, 1958; Rose et al., 1960; Raisman, 1969), but it has never been clear that this is simply abnormally initiated growth in response to injury. In addition, this flexibility of termination locus indicates a priori that plasticity can be expected to occur after tectal size reduction. These lesion experiments can in these terms be considered a kind of negative growth.

VIII. DEFLECTION OF TECTAL OPTIC FIBERS ONTO THE CONTRALATERAL TECTUM

Introduction

Although there are now a number of reports of plasticity of connections in the goldfish retinotectal system, these experiments have invariably produced structural discontinuities in either tectum or retina. Since this disruption of tissue could permit reorganization or regulation of the properties of neurons responsible for determining connectivity, these experiments do not permit unambiguous conclusions about the essential pattern determining mechanism of this system. For this reason many observers (Sperry, 1965; Meyer and Sperry, 1973; Yoon, 1972b; Hunt and Jacobson, 1973c) have argued that these data are not incompatible with Sperry's original chemoaffinity hypothesis.

In an attempt to control for these problems and obtain further information on factors affecting fiber growth, a study was done on the innervation of optic tectum by relatively small numbers of optic fibers while avoiding the kind of tissue discontinuity causing regulative changes. By deflecting optic fascicles from one tectum onto the contralateral tectum, the growth of these fibers could be followed autoradiographically by injection of the appropriate eye with tritiated proline. This deflection was done with the contralateral tectum either normally innervated or deprived temporarily or permanently of its normal optic connections. The results suggest that growth of optic fibers is not only controlled by tectal affinities but by other factors as well.

Materials and Methods

Surgery. Common Carassius auratus were operated under Finquel anesthesia as previously described. With tungsten needles a rostrocaudally oriented rectangular tectal strip 400-500 micrometers wide and about 1200-1500 micrometers long was cut on three sides leaving the rostral end intact. This strip originated at rostral right tectum near the anterior end of the tectal commissure and either followed the optic fascicles growing along the midline or those growing toward lateral and caudal edge. The last 1/4 caudal tectum was spared. This tectal strip was lifted out and inserted into a small rostrocaudal incision in mediorostral left tectum.

Eye enucleation was done by cutting away the conjunctiva and with forceps tearing off the muscle attachments to the globe. The optic nerve and blood vessels were then cut with scissors.

Autoradiography and histology. Autoradiography was done as previously described 12-48 hr after injections of 25-50 microcuries of tritiated proline. Serial sections at 15 micrometers through the entire tectum were taken and post stained with cresyl violet. Most sections were at a frontal orientation and representative brains were reconstructed in the following manner. Since most label of interest was on dorsal tectum, a vertical rectilinear projection of the tectal label was made. An ocular micrometer grid was oriented so that one axis was along the dorsal-ventral axis of the section. The pattern of label was then plotted onto graph paper relative to the estimated midline. The sampling interval along the rostrocaudal axis was 120

micrometers. The attempt was made to classify label into one of five categories: heavier than normal labeling such as seen with axoplasmic damming, normal heavy labeling, moderate labeling which was distinctly lighter than normal but visible at low magnification (40X), light labeling seen clearly at medium magnification (100X), and very light labeling barely above background and seen at only high magnification (400X).

Electrophysiology. Eye in water recording was done as previously described, but the electrode approach was perpendicular to dorsal tectum where the majority of terminals from transplanted fibers were confined.

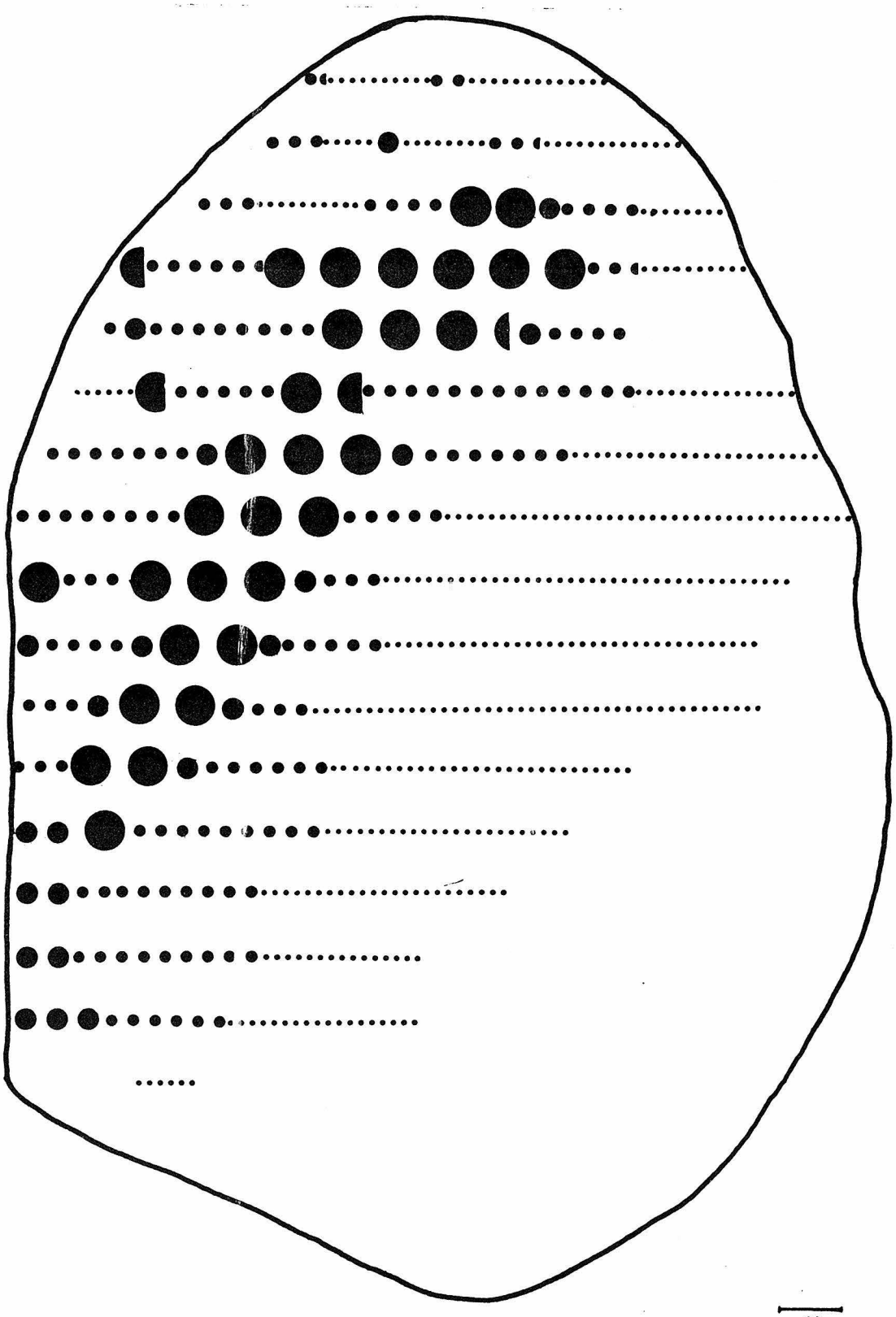
Results

It is known from Attardi and Sperry (1963), Roth (1972), and work here that the medial brachium of the optic tract supplies dorsal tectum with caudolaterally oriented fascicles of optic fibers originating from rostromedial tectum. The more lateral fascicles innervate rostrolateral tectum while those medial innervate caudomedial tectum. The surgical procedure used here can be expected to disrupt some of these fibers and create a caudally oriented elongated area of denervation. This was checked in two animals in which medial fibers from right tectum were inserted into rostromedial left tectum and the right eye injected immediately after surgery. In both cases there was normal labeling throughout the tectum except for a 100-300 micrometer wide area of denervation running caudally from the point of insertion. In one animal this hole paralleled the medial tectal margin at 150

micrometers lateral to it and enlarged at the caudal extreme. In the other this area was some 300 micrometers lateral to the margin at the rostral end and at middle tectum curved off laterally not reaching the caudal extreme. The cut off of label was quite sharp, progressing in 50-100 micrometers from normal density to very light label which was distributed throughout this region, except near the insertion point.

In five animals medial fibers were similarly implanted, and the left eye injected 106-168 days after surgery. Except for one animal where the surgery failed, the innervation pattern of left tectum was approximately complementary to the previous denervation pattern (Fig. 5). As in all subsequent cases label was in the appropriate layers but for the region immediately adjacent to the insertion point. The areas of heaviest labeling were sharply defined and most followed the course of the putative disrupted fascicles being as far as 500 micrometers from the medial margin. However, the width appeared more variable. There was frequently a small (up to 100 micrometers in width) additional area of heavy labeling along the medial and caudal margin, but its appearance was also quite variable. Light and very light label was distributed over most of dorsal tectum. Three attempts were made to insert fibers into a more lateral position. In two animals relatively few fibers crossed over. In one of these the light label was spread over dorsal tectum. In the other the pattern was similar to a third animal injected 51 days after surgery in which there was substantial label in left tectum. The insertion

Fig. 5. Disposition of label transported by left eye onto a dorsal left tectum 106 days after deflection of medial fibers from right to left tectum. Density category of label is indicated by relative dot size.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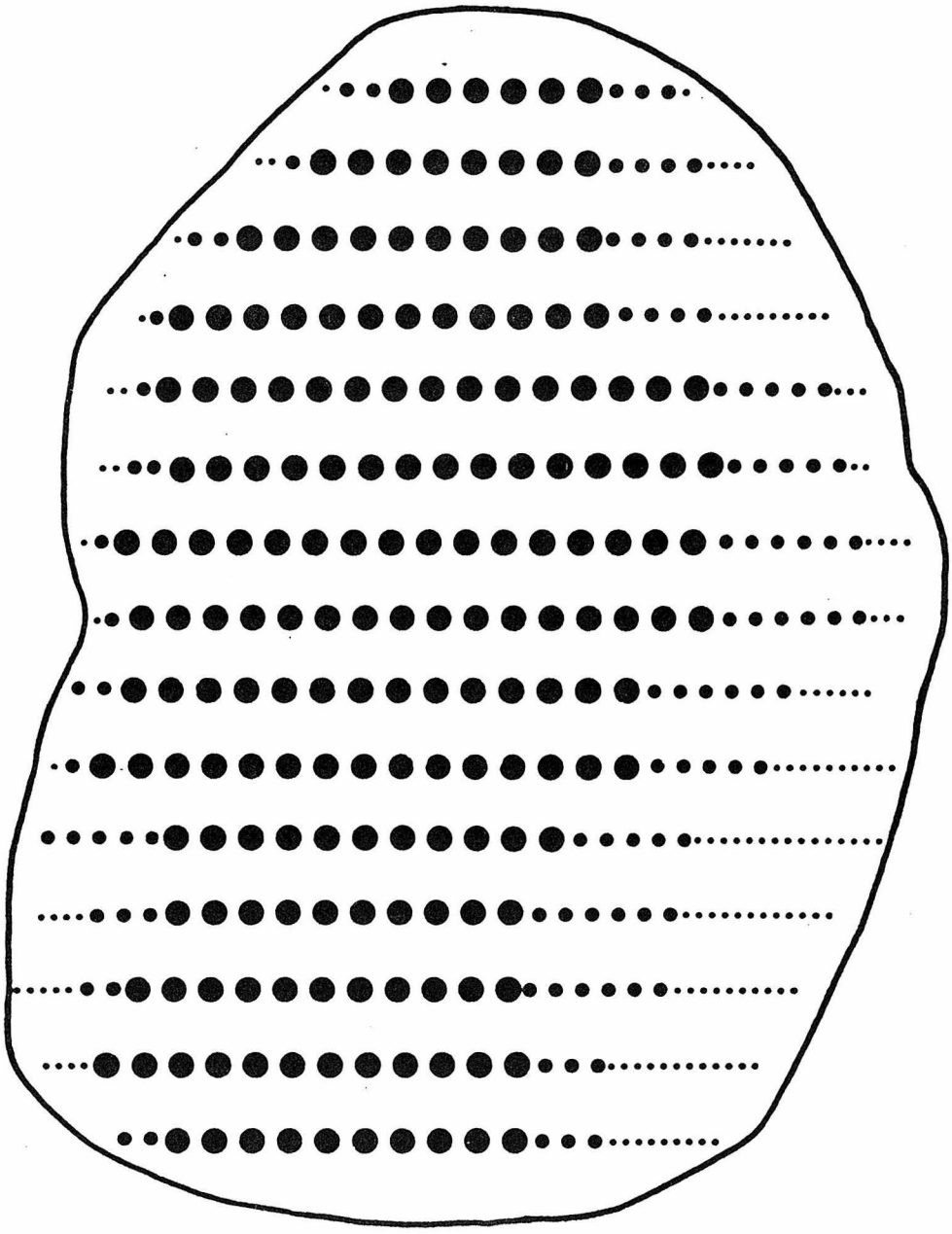
point was about 500 micrometers lateral to the medial margin. Posterior to it was a variable 150-300 micrometer wide zone of dense grains running back 2/3 of the tectal length and up to 1000 micrometers lateral to the margin. This was followed by more widespread lighter label and then heavy well-defined labeling at the caudal margin.

There were five attempts at crossing more lateral fascicles onto mediorostral left tectum but in each case relatively few fibers crossed. Light labeling was spread over much of dorsal tectum but the more medial regions were relatively label free.

In two animals the long-term effect of insertion of medial fibers from right tectum into left rostral tectum on the normal innervation of left tectum was assayed by right eye injection at 51 and 130 days. The result was essentially a negative image of the case with left eye injection. There were holes in which only light labeling was observed where previously heavy labeling was seen. This included medial and caudal margin.

In a series of animals these deflections were performed onto a left tectum which was denervated by right eye enucleation two weeks prior. In three fish medial fascicles were inserted at the usual rostromedial region of left tectum and were injected 31-238 days postoperatively. All showed extensive labeling of the entire dorsal tectum including regions rostral to the insertion (Fig. 6). The density of grains was distinctly lighter than the normal right tectum but nevertheless substantial. This medium density label extended the

Fig. 6. Disposition of label transported by left eye onto a dorsal left tectum 52 days after deflection of medial fibers from right to left tectum and 66 days after right eye enucleation. Only moderate or lighter densities were seen.



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entire rostrocaudal dimension and from 50-100 micrometers of medial margin to some 1000 micrometers laterally. The rest of the dorsal tectum, including an additional 500-700 micrometers more laterally, was filled with light and very light label. The lateral 1/3-1/4 tectum remained free of optic fibers. The 238 day animal was examined in sagittal section, and there was no suggestion that caudal label was more dense than rostral label. In two fish where more lateral fibers were deflected followed by injection at 46 and 60 days postoperatively, there was also extensive spread of label. However, the medium label was shifted 250-500 micrometers laterally from the medial edge, and the posterior few hundred micrometers were label free. In all cases this dispersed label appeared to include the parallel layer as well as the plexiform layer. This was seen more clearly in one other animal with crossed medial fibers which was injected after only 13 days. Though only relatively light label was observed, there was extensive spread even into the caudal extreme. Much of this appeared as isolated clumps confined to the parallel layer particularly in regions several hundred micrometers removed from the insertion point.

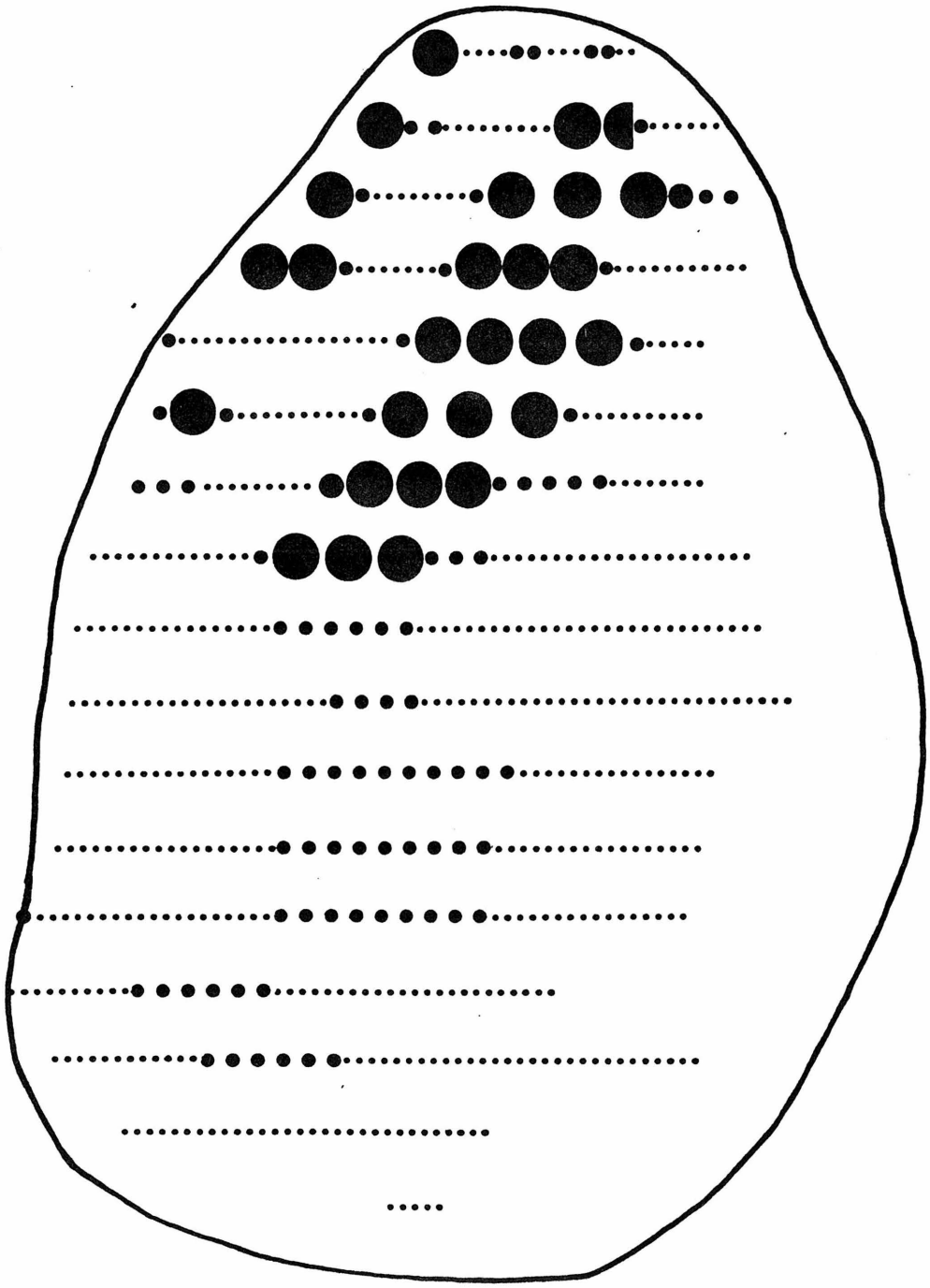
In four specimens medial fascicle deflection was combined with a mediolateral superficial incision across dorsal left tectum. Injection of right eye immediately after surgery showed, as expected, that while lateral tectum was normally innervated, dorsal tectum was virtually label free. At 75 and 95 days the left eye was injected in two fish. In both cases there was only light rostral labeling followed

in the caudal half by several generally very sharply defined islands of heavy innervation (Fig. 7). The location and shape of these zones appeared different in the two animals but in both cases heavy label extended some 700 micrometers laterally. The fourth animal had right eye labeling at 95 days postoperatively, and it showed label-sparse zones in caudal and mediolateral tectum but normal labeling elsewhere.

Electrophysiological mapping of the left field was done 85, 168, and 260 days after deflection of right tectal medial fibers onto a left tectum denervated two weeks prior. In all cases units from right tectum were obtained only from superior temporal field and were greater than 60° from the optic disc. In another fish mapped 294 days after more lateral right tectal fibers were transplanted onto a denervated tectum, units were similarly obtained from superior temporal field but were restricted to a small area near the horizontal meridian $25\text{-}55^{\circ}$ from the optic disc. In all four specimens there was little evidence of retinotopic order.

Correct topographic order was seen in a fifth animal 86 days after medial fascicles were deflected onto a left tectum enduring simultaneous section of its dorsal fascicles. The units driven by left eye were from superior temporal field as above and were found only in caudal tectum. From these electrode positions it was usually not possible to find units driven by right eye even though these could be easily found elsewhere. Recording from the left tectum of three normal animals failed to show any optic fiber type units that could be stimulated from left visual field.

Fig. 7. Disposition of label transported by left eye onto a dorsal left tectum 75 days after deflection of medial optic fibers from right to left tectum and section of dorsal optic fascicles of left tectum.



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Discussion

Some of these results clearly support the chemoaffinity hypothesis. Medial fascicles deflected onto a denervated tectum innervate the appropriate medial and caudal tectum and are rigidly restricted to the correct lamina. Similarly placed lateral fascicles innervate more lateral tectal regions leaving medial and caudal tectum relatively denervated. However, the extensive spread of label over most of dorsal tectum shows this retinotectal affinity is not sufficient to prevent very substantial misgrowth. Electrophysiological data confirm that these widely dispersed fibers originate from a very restricted retinal region and that they form terminal-like recordable structures.

If the receiving tectum is normally innervated except for the region disrupted by the surgical insertion, the transplanted fibers will preferentially innervate this denervated area. This area can be significantly displaced from the region these fibers originally innervate. This is in contrast with several reports on both frogs and goldfish (Ingle, 1973; Gaze and Jacobson, 1963; Gaze and Keating, 1970; Sharma, 1973) in which optic fibers miscrossing at the chiasm become superimposed in correct retinotopic order on the normal projection. In these cases the normal innervation was not interrupted, and the fibers probably did not grow in as organized fascicles.

However, there appear to be limits to this misgrowth. Little innervation occurred when lateral fascicles were inserted into a region occupied by more medial fibers and to a lesser extent *vice versa*.

However, even with this surgery some growth did occur and was substantial when the tectum was denervated so that this limitation of growth is not due to simple mechanical difficulties. This result is at first sight at variance with Arora's (1966) previous finding that lateral brachial fibers transplanted into an innervated contralateral tectum grow across dorsal tectum to reach the lateral areas. However, the species, the surgical procedure, the number and origin of deflected fibers are all different, and there are no controls to judge the relative growth success of different fibers. Since not even the number of successful cases was reported, this result can be neglected for purposes of this discussion. It thus appears these fibers are either effectively repulsed by the optic fibers originating from substantially different retinal regions, or they cannot successfully compete against correct fibers for terminal space in this foreign tectal region.

However, some kind of interfiber interaction is favored by the results from fiber deflection accompanied by cutting the dorsal fascicles of the receiving tectum. There is a marked tendency for the transplanted fibers to form islands of innervation apparently to the exclusion of an identical population of optic fibers from the other eye. This could represent an effective self-affinity of fibers originating from neighboring retinal regions so that when a critical number collects in an approximately appropriate tectal region, they tend to selectively colonize it in a self-organizing manner. This could discourage fibers from the other eye from innervating the same

region because they might be obliged to grow across foreign fibers to arrive at the appropriate area. The sharp boundaries seen under these conditions but not when the tectum is relatively denervated suggests inhibition of growth by other fibers. Relative exclusion is also seen here when the fascicle implant is made into a normally innervated tectum, and Schneider (1973) has observed this competitive tendency after unilateral tectal removal in neonatal hamsters. At the very least this all argues that in some way fiber growth is affected by the presence of other fibers and that retinotopic organization is governed by factors additional to chemoaffinity between retinal and tectal cells.

IX. GENERAL DISCUSSION

A number of findings here further confirm Sperry's original hypothesis. In the goldfish eye ablation and intertectal fiber deflection experiments the selected subset of optic fibers showed a clear propensity for terminating in the appropriate tectal region even despite widespread denervation of the tectum. In frog this affinity for the correct tectal locus appears to be so specific that fibers deprived of this region will not terminate on neighboring tectal regions but prefer to grow to the correct regions of a contralateral tectum to superimpose on its normal innervation. Even fibers growing onto a goldfish half tectum showed a marked initial tendency to terminate on the correct half to the relative exclusion of caudal fibers (Chapter 5 and Gaze and Sharma, 1970). The widespread exploratory growth of optic fibers seen here autoradiographically at short but not long periods after simple removal of caudal or medial tectum indicates that hyperinnervation of the correct laminae is favored over termination in hindbrain, non-tectal midbrain, and incorrect tectal laminae.

In contrast several results appear to rule out Gaze's sliding scale proposal. Whereas orderly compression of an entire retinal projection onto a frog half tectum is predicted by this model, only normal innervation to the remnant was found. He should further infer that a group of optic fibers originating from a limited area of retina would spread over an entire denervated tectum in topographic order. However, the goldfish eye lesion study and

intertectal optic fiber deflection study demonstrated regional affinity, limited spreading, and in the latter experiment no topographic ordering. The confirmation here that in half tectum goldfish severed optic axons initially regenerate a relatively uncompressed map hopefully eliminates doubts about its reality (Gaze and Sharma, 1970) and seems to require additional postulates.

One of the points at issue here is whether regulative changes in the intrinsic cytochemical properties of neurons in the context of the chemoaffinity hypothesis is what causes expansion and compression. There is now some direct evidence for processes at least analogous to regulation in tadpole compound eyes (Hunt and Jacobson, 1973b; 1973c). However, in the highly differentiated goldfish tectum its occurrence would be somewhat surprising, and there are some indications of cytochemical stability rather than plasticity. Tectal reimplants rotated 90 or 180° appear to retain locus specificity properties rather than regulate to be in accord with the surrounding tissue or to become a small complete tectum. Goldfish eyes grow at a rate comparable to tectum (Chapter 7), yet they do not appear to regulate, at least not completely, following ablations (Chapter 3). However, these same half eyes growing into a half tectum (Horder, 1971; Yoon, 1972b; Roth, 1972) innervate an area larger than can be explained with any consistent assumptions about regulation. Regulation is precluded when small numbers of fibers are deflected into a denervated tectum, and growth is nevertheless very widespread. Finally, some projection patterns

seem uninterpretable in this framework. Gaze and Sharma (1970) and Sharma (1972b) have found that with caudal or rostral half tectal removal and no optic nerve interruption the original projection tends to remain in place, and the projection to the ablated area becomes superimposed in correct relative order over most of the tectal remnant giving a kind of double topography.

Until this point chemoaffinity has been considered in fairly simple terms. The affinity of an optic fiber, say from mid-retina, for its normal termination locus, in this case mid-tectum, was implied to be higher than for any other tectal locus. It was also assumed that this affinity was greater than that of other fibers, like those from nasal retina, for this locus. There are other schemes, however, which are also consistent with normal topography and which might give compression or expansion. Consider, for example, that nasal fibers really have a higher affinity for mid-tectum than mid-retinal fibers do but have an even higher affinity for caudal tectum, their normal locus. If caudal tectum is surgically removed, nasal fibers would grow to mid-tectum and displace mid-retinal fibers. Similarly, mid-retinal fibers could displace more rostral fibers to give a uniform compressed retinotectal map. The "alternative matching" of Wasserman (1972) and contextuality of locus selection of Hunt and Jacobson (1973c) are general statements of this kind of possibility. However, a concrete scheme for goldfish consistent with the relative strength of affinities as indicated by the various lesion experiments has not been forthcoming. Further, this or any other model that relies solely on matching between retinal

and tectal elements for pattern formation appears incompatible with the experiments showing double topography and with the intertectal fiber deflection experiments here showing exclusive innervation in the presence of apparently identical fibers.

A relatively simple postulate can be put forward, at this point with some caution, that may permit a more satisfactory interpretation of the retinotectal literature. Suppose that in addition to retinotectal affinities growing optic fiber endings have similar specific affinities for other optic fibers originating from the same retinal region. If the attraction between optic fibers and tectal neurons is relatively locus nonspecific, in goldfish at least, and that between fibers is highly specified, then a modest addition to Sperry's original chemoaffinity hypothesis presents itself. Optic fibers can be thought to be guided to the general tectal region appropriate for them by tectal affinities thus giving orientation to the projection and rough topography. Between fibers from different retinal areas marked differences in the tuning of retinotectal affinity would be permitted and might even be advantageous in a growing system. For instance, temporal fibers may be relatively specific for rostral tectum while nasal fibers may have only slightly greater affinity for caudal than for rostral tectum as suggested by the growth seen after eye lesions. The more refined topography can be achieved by a kind of cell sorting between terminals whereby like fibers, those from the same retinal locus, tend to aggregate together. This effect might be expected to be more significant when terminals form a

significant fraction of the total tissue such as where extensive terminal arborizations occur. With highly specific interfiber affinities very precise relative ordering could be achieved despite disordered initial growth and with some independence from less specific tectal affinities.

The sorting process may be analogous to the cell specific aggregation shown in numerous tissue culture experiments including those with retinal cells (Moscona, 1961; Sheffield and Moscona, 1970). Differential adhesivity between fibers as between different cell types (Steinberg, 1970) and between retinal and tectal cells themselves (Barbera et al., 1973) could reasonably account for this kind of sorting. Specific contact inhibition of growth between similar fibers or a specific interfiber repulsion, higher between unlike fibers than like fibers, or other processes could also result in an effective specific interfiber affinity compatible with this model. Interfiber affinity as used here leaves open these possibilities. However, the general tendency for fibers to group into fascicles and some evidence discussed below seems to favor selective interfiber adhesivity.

Such interfiber affinities would tend to give topographic cohesivity to the retinotectal projection that could be fairly independent of tectal locus and that could account for the projection pattern following expansion or compression. These altered projections might be interpreted in Steinberg's (1970) terms as achieving the lowest free energy of adhesion for the system which here includes

both retinotectal and retinoretinal interactions. However, it is possible especially in compression that some kind of flexibility in the amount of terminal territory expressed by fibers is required. If this flexibility occurs in goldfish but not in frog, then the lack of compression in anurans could be explained. It is also possible that in frogs and chicks retinotectal affinities are relatively more specific and stronger than interfiber affinities. The specificity and strength of affinity between fibers and cells and between fibers themselves could be four independent variables different not only between species but between different systems in the same animal. Finally, it may be that once initial connections are formed in frog or chick they are not easily displaced to permit compression or expansion. Unlike goldfish these species do not exhibit extensive and asymmetrical retinotectal growth while the system is functional and may not need this high degree of plasticity. Nevertheless, refined retinotopography even in these animals may be achieved by terminal sorting and some limited compression or expansion may indeed occur.

The intertectal fiber deflection experiments of the previous section can be interpreted as showing the appropriate interfiber interactions. Fibers readily grew into a normally innervated tectum when transplanted into regions having similar fibers, but generally failed to do so when put in areas with dissimilar fibers, apparently preferring to grow back or sprout into their normal tectum. The various compressions argue against this being a simple matter of being less competitive for a given tectal position. It is also not

an inability to grow into that part of the tectum under these surgical conditions since when the receiving tectum is denervated they invade very extensively. The disorganization of the map seen in this last case might reflect insufficient density of fibers to permit terminal sorting. The tendency to form islands of innervation to the exclusion of nearly identical fibers could be a reflection of the order imposed by the initial conditions of these experiments. In normal goldfish the fascicles are very highly organized and appear constrained to grow through the parallel layer in a fairly stereotyped almost parallel manner. This would be expected to discourage invasion by other fibers which might be required to leave neighboring high affinity fibers and cross through lower affinity areas to be near similar fibers. Formation of analogous islands even when composed of apparently identical cells is seen at times in tissue culture cell sorting experiments, especially with neural tissue and when the apparent adhesivities between cell types are comparable or when one tissue phase differentiates to become immobile (Steinberg, 1970). It can be predicted that if these deflection experiments are done with relatively more disordered regenerating fascicles, islands would not form. An initial result seems to bear this out. Electrophysiology of these islands shows retinotopography and further argues for some kind of fiber self-organization independent of strict tectal matching.

This scheme also provides an interpretation of some apparent discrepancies in the goldfish tectal lesion literature. Gaze and

Sharma (1970) and Sharma (1972b) reported a kind of double topography after tectal ablation without optic nerve crush. Yet Yoon (1971, 1972a) has claimed not to have seen this under what was described to be identical conditions. However, reexamination of the indicated tectal lesions shows a consistent difference. Gaze and Sharma (1970) and Sharma (1972b) appear to have ablated only dorsal rostral or caudal tectum leaving the entire lateral half intact. Yoon seems to have included lateral tectum in his caudal ablations. To obtain compression onto dorsal tectum with lateral innervation intact a marked distortion or discontinuity in topography is required. This departure from retinotopography would likely be inhibited by the large, highly ordered, lateral projection. With no alternative the fibers from the ablated region might have to superimpose on the remaining projection and by terminal sorting among themselves achieve a second topography. While a terminal sorting mechanism requires finely tuned differential affinities between neurons of neighboring origin, this is not needed for neurons farther apart. The affinity of a given ganglion cell for other ganglion cells as a function of interretinal distance can be reasonably imagined to initially fall off sharply and then plateau for a substantial distance. Thus, the topography of most of these superimposed fibers might not be specifically disturbed by the presence of foreign fibers. One might expect also that displaced fibers closely related to some already present, those near the lesion, would be affected. The published maps, in fact, tend to show either disorganization or lack

of receptive fields from this part of visual space. When the nerve is crushed permitting more random initial conditions then a more unified, albeit distorted, topography could be achieved.

Even in Yoon's (1971) maps of animals sustaining ablation of the mediocaudal quadrant of dorsal tectum a multiple topography can be seen when the optic nerve was intact but only a single, continuous topography was found when the nerve was crushed. It is particularly interesting that Sharma's (1972b) ablation of rostral dorsal tectum clearly interrupts fibers to dorsal caudal tectum so that in this case reformation not simply preservation of dorsal topography is differentially affected by the integrity of the lateral projection. This tendency to preserve the original topography under these conditions can perhaps be viewed in a differential adhesion scheme as insufficient free energy of activation to reach a lower energy state, single topography.

Constant light has been recently claimed to inhibit compression following caudal tectal ablation without optic nerve interruption (Yoon, personal communication). Assuming no systematic size of lesion differences, this only says the expression of sorting is affected by retinal input. The consequent neural or systematic metabolic changes could even affect processes that although permitting compression are quite distinct from terminal sorting such as regulation of the size or strength of connectivity of terminal arborizations.

It might be added that the tectal reimplant experiments showing independence of the projection onto the implant (Sharma and Gaze, 1971; Yoon, 1972c) do not contradict the idea of terminal

sorting. Since there is clear evidence of a substantial cyto-architectural discontinuity between normal tectum and the implant, the two can be expected to have independent sorting.

Terminal sorting is, hopefully, a reasonable working hypothesis, but it should perhaps be reemphasized that "these qualitative specificities operate in conjunction with many other factors, and the combined action of all of them determines the final pattern" (Sperry, 1951b). Undoubtedly many more of these factors have yet to be disclosed.

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