Expansion of the Half Retinal Projection to the Tectum in Goldfish: An Electrophysiological and Anatomical Study

JOHN T. SCHMIDT,[1] CAROL M. CICERONE [2] AND STEPHEN S. EASTER [3]
Biophysics Research Division, Department of Psychology, Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109, U.S.A.

ABSTRACT The topographical retino-tectal projection of goldfish was electrophysiologically mapped at various intervals after surgical removal of the nasal half of the retina and pigment epithelium. The remaining projection was initially restricted to the appropriate rostral half of the tectum, even if the nerve was crushed and allowed to regenerate. But later, after 137 days or more, it showed a progressive expansion onto the foreign caudal half of the tectum. The magnification factor, the number of micrometers of tectum per degree in the visual field, doubled in the rostro-caudal but not in the medio-lateral direction. Analysis of the sequence of the expansion showed that a few fibers originally projecting nearest the denervated area were the first to spread over it. Then, progressively more fibers moved caudally until a nearly uniform representation of the half retina was established on the tectum. Radioautography also demonstrated that retinal fiber terminals had invaded the caudal tectum. The retinae of these fish were also examined histologically. The density of ganglion cells had not increased, but they consistently showed the axonal reaction. This was not found to be associated with any initial surgical trauma, but rather with the movement of their fiber terminals within the tectum. Frozen sections, through half retinal and normal eyes, were cut and photographed for comparison of ocular geometry. Operated eyes were normal except for a slight but consistent loss of ocular volume. Analysis of the optical geometry showed that recording with fish in air produced two effects: Myopia (10° blur circle, or less) and enlargement of the visual field by 15% to 20%.

The topographical retino-tectal projection of goldfish, which was once thought to be unmodifiable (Attardi and Sperry, '63; Jacobson and Gaze, '65; Sperry, '63), has more recently been found to be a very dynamic system (Gaze and Sharma, '70; Yoon, '71, '72a,b,c; Schmidt et al., '74). This shift in thinking began with the work of Gaze and Sharma ('70) who first demonstrated that, following removal of the caudal half tectum, the entire retinal projection could compress onto the remaining rostral half tectum.

Although the results on half tectal compression have been verified by many others (Yoon, '72a, '71; Schmidt et al., '74; Meyer, '77), there is still disagreement about reorganizations following retinal ablations. Anatomical studies have shown little or no evidence for expansion of the half retinal projection. Attardi and

Sperry ('63) waited only 30 days after optic nerve crush and partial retinal removal, and found that the regenerating fibers reestablished the original projection of the remaining retina but did not invade new areas of the tectum. More recently, Meyer ('75) using radioautography, found little evidence for expansion of the half retinal projection even after many months. Two short electrophysiological reports have also dealt with the projection of the half retina at long postoperative intervals, but include conflicting results. Horder ('71) found expansion of the nasal but not of the temporal half retinal projection. Yoon ('72b)

reported that either half could expand. Both these reports were abstracts, drawn from small numbers of fish, and from which, therefore, no firm conclusion can be drawn.

The present investigation was undertaken first as an attempt to resolve the disagreement about the reorganization following retinal ablations; here we report the electrophysiological results of 42 fish. In addition, we have used radioautography on the same fish to clarify apparent discrepancies between the anatomical and electrophysiological reports. Finally, we have investigated the time course of the changes. The results of Attardi and Sperry ('63), coupled with those of Yoon ('72b), suggested that any reorganization might take place after a long postoperative delay. Through multiple recordings from individual fish as well as recordings from many fish at varying postoperative intervals, the progressive stages and time course of the reorganization were detailed.

RESULTS

Surgery

Common goldfish (*Carassius auratus,* Ozark Fisheries, Stoutland, Missouri) 12-18 cm in length were operated and maintained at room temperature (19-23°C). In the totally crossed retino-tectal projection, surgery was restricted to the right eye-left tectum pathway. Fish were anesthetized in a 0.1% solution of tricaine methanesulfonate and operated in air. The eyeball was cut along the nasal side at the equator, and the front half of the eye with the lens attached was then pulled outward. The nasal retina and pigment epithelium were separated from the underlying choroid and sclera, cut and removed using fine rat-toothed forceps; remnants were cleared by gentle aspiration. Care was taken to avoid damage to the optic disc, both to assure continued blood supply to the remaining retina and to leave its optic fibers intact. After surgery the sclera was sufficiently rigid to hold the incision closed. The ocular media, initially bloody, cleared within a few weeks, at which time (in about 75% of the cases) the eye was virtually indistinguishable from normal. Individual fish are designated by the prefix HR (half retina) followed by a number.

In four fish the optic nerve was also crushed in the orbit (Attardi and Sperry, '63). Using fine watch-makers forceps, the nerve was repeatedly crushed under visual control until

there was no continuity in the white column inside the sheath.

In a separate group of seven fish, the optic tract was severed between the chiasm and tectum, and the retinae were later examined histologically for the axonal reaction of the ganglion cells (Murray and Grafstein, '69). Access was via an opening made in the dorsal cranium which was resealed afterward with dental acrylic. A section of optic tract was aspirated to assure complete interruption.

Electrophysiological recordings

The projection of the visual field onto the tectum was determined using a technique similar to that of Schwassmann and Kruger ('65). The fish was positioned in air with its eye at the center of a transparent plastic hemisphere 86 cm in diameter, and an aerated anesthetic solution passed through its gills. The animal was oriented so that a line perpendicular to the pupil intersected the center of the dome.

The position of the eye was monitored by locating the projection of the optic disc onto the hemisphere. A strong light placed atop the fish's head caused the disc (which lacks the pigmented layer) to appear luminous; the hemispheric projection of the disc was located at the point where its image appeared centered in the pupil. The disc projection is known to lie approximately 6° nasal and 14° dorsal to the center of the visual field, which is probably the line normal to, and through the center of, the pupil (Easter et al., '77). In some HR eyes no disc projection could be located, probably because the disc lay at the edge of the ablation, which also appeared luminous. In such cases, the center of the visual field was estimated by a corneal reflection technique. A small light was positioned on the hemisphere, and its reflection off the corneal surface, viewed from behind the source, was centered in the fish's pupil. The two methods, disc visualization and corneal reflection, gave consistent results when used on the same fish. Eye movements seldom exceeded 10° over the course of several hours, but all maps were corrected for such movements.

The tectum was exposed and a metal filled micropipette was inserted to record neural activity, believed to arise from the terminal arborizations of the retinal fibers (Maturana et al., '60). Typically, multiple units and occasionally single units were recorded and moni-

tored on an oscilloscope and an audio monitor. It was possible to distinguish the presumed presynaptic activity from occasional tectal cells which had very large and completely inhibitory receptive fields or habituated rapidly (O'Benar, '71).

The luminous tip of a fiber-optic bundle (12' arc, 16-100 ft.-lamberts brightness, white tungsten light) was moved by hand on the hemisphere to determine each receptive field, operationally defined as that region of space in which small movements of the light evoked audible changes in neural activity. Background illumination was about 1 ft-lambert. The electrophysiological results are presented as maps of the points of correspondence between the visual field and tectum. The circles indicate the approximate sizes of the receptive fields which were roughly circular. The organization of each map was quantified through the computation of rostro-caudal and medio-lateral magnification factors, RCMF and MLMF, respectively.

The magnification factor (MF) along a tectal axis is the number of micrometers (μm) on the tectal surface per degree in the visual field (Daniel and Whitteridge, '61). Tectal distances were taken from a grid in the eyepiece of a microscope positioned to view the tectum dorsally. Degrees in the visual field were measured between the centers of the receptive fields. MF's were determined as the slope of the least squares regression of tectal distances versus degrees in the visual field, with the regression line constrained to pass through the origin. In some cases, "local MF's" were computed from single pairs of tectal penetrations.

Optics of the goldfish eye in air

Recording with the fish's eye in air rather than water is known to cause significant changes in the optics, since the cornea becomes an active optical element (Charman and Tucker, '73). To quantify these changes in the normal eye and to check for differences in the operated eyes, frozen sections were cut from freshly removed eyes of three HR fish at 23 to 50 days postoperatively. The details of the procedure are given elsewhere (Easter et al., '77). The HR eye was removed under anesthesia, quickly frozen by immersion in a dry ice-acetone mixture and sectioned at 100 μm along a plane perpendicular to the pupil. After the first eye was removed, the fish was main-
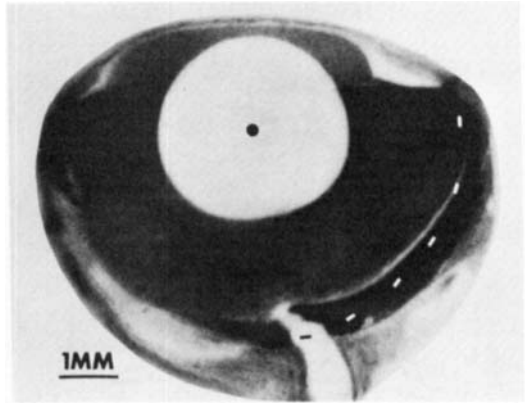

Fig. 1 Horizontal cross section of frozen half retinal eye. The lens, cornea and pupil were conformal with those of the contralateral normal eye which was identically sectioned. The retina (white tissue) had however moved slightly closer to the center of the lens (filled circle). The dashes indicate the position of the retina-pigment epithelial boundary within the contralateral normal eye. Calibration bar: 1 mm.

tained alive for approximately 30 minutes until the second, normal eye was also removed and sectioned. Sections were discarded but the frozen block and a calibration scale were photographed at × 1.11-1.29. Later, negatives were projected and traced. The one with the section containing the largest diameter of both the lens and globe was chosen for analysis, since it should contain the optic axis, the line connecting the center of the lens and the center of curvature of the cornea.

Analysis of the tracings pointed to two major conclusions concerning the optics of the goldfish eye in air. Both result from the air-cornea interface, and are treated in more detail in the APPENDIX. First, goldfish of this size are about 37 diopters myopic in air, causing a distant point of light (such as the stimulus used here) to form a retinal blur circle of about 10° diameter. Second, light rays entering the eye are bent toward the normal, since the corneal surface is not concentric with the lens. The effect is to increase the size of the fish's visual field by about 18% to 20% horizontally and 14% to 15% vertically. All the MF's given in this paper were measured in air, and have not been corrected to give their values in water.

The HR eyes showed good retention of their ocular geometry. A photograph of a cross-section of one eye is shown in figure 1. In all cases, the lens, cornea and pupil remained

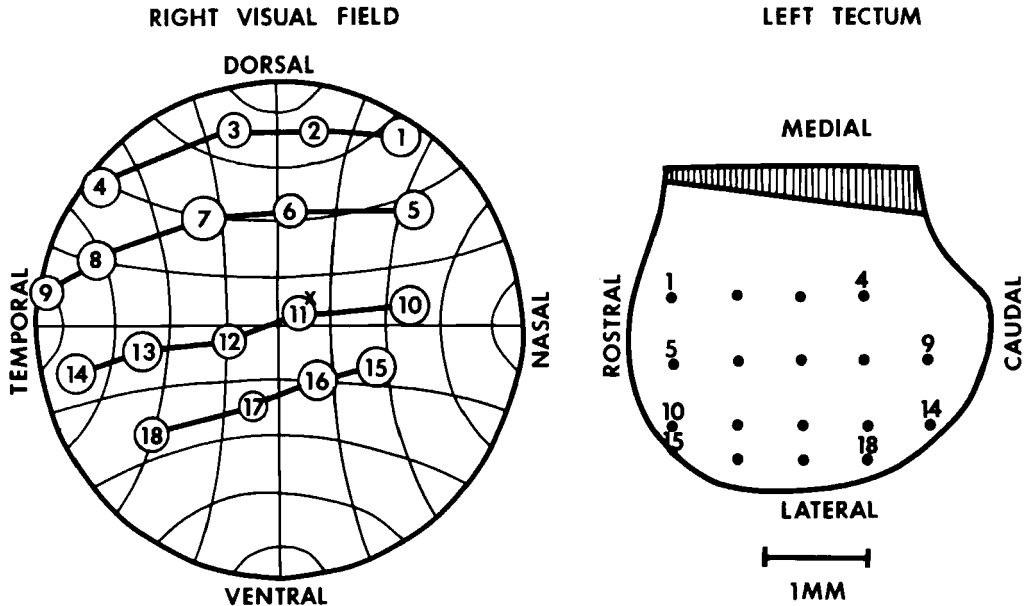**RIGHT VISUAL FIELD**   **LEFT TECTUM**

Fig. 2 Normal retino-tectal projection of goldfish. Numbered electrode positions correspond to receptive field positions in the visual field. Circles indicate the approximate sizes of receptive fields. The hemisphere representing the visual field is marked at 20° intervals. "X" marks the projection of the optic disc onto the hemisphere.

undistorted, but the ocular volume was consistently somewhat less than normal. This resulted in the retina occupying a position slightly closer than normal to the lens in the central area. Although this made these eyes less myopic in air, it also caused a slight decrease in the MF, since a fixed amount of retina subtended a larger visual angle. This has been corrected in figures 4 and 5, to allow comparison of MF's between normal and operated eyes.

Histology

Twenty-three eyes were fixed in Bouins for two to seven days, dehydrated in graded alcohols, embedded in paraffin, sectioned radially at 10 μm and stained with hematoxylin and eosin or cresyl violet. Five fish were injected intraocularly with 40 μC of L-(2,3)-³H-proline (New England Nuclear, specific activity 25-35 Ci per mmole), and killed after two days survival. The brains were fixed in Bouin's solution, embedded in paraffin and radioautographed using the method of Kopriwa and Leblond ('62). We used Kodak NTB-2 nuclear track emulsion, and developed after three to five weeks storage in light tight boxes at 5°C. Slides were stained through the emulsion with hematoxylin and eosin.

RESULTS

Normal fish

Eight normal fish of the same size range as the HR fish were mapped as standards. The organization of a typical normal map is shown in figure 2. The mean RCMF and MLMF of the normal fish are listed in table 1. The variation in mean RCMF within the size range used was not significantly related to the variation in the length of either the tectum or the body (regression, $R^2 < 0.30$). MF's from different fish within this range should therefore be comparable.

Preliminary screening of HR fish

The extent of the lesion was checked one to two weeks postoperatively, by the same transscleral illumination procedure used to locate the optic disc (METHODS). The fish was anesthetized and alaced at the center of the hemisphere where its head was strongly illuminated. The experimenter sighted through the pupil until the dark/light boundary, the edge of the ablation, was centered in the fish's pupil. Marks were made on the hemisphere corresponding to this boundary until the entire ablation was projected. The light region corresponds to that part of the fundus with no

pigment epithelium, and therefore represents a conservative estimate of the area of the ablation. Those fish in which ocular geometry seemed nearly normal, and the ablated region was estimated to include 30% or more of the retina were kept for further use; others were discarded.

Of the 180 operated fish which met these criteria at the initial examination, 126 were rechecked by trans-scleral illumination several months later (30 had died; 24 were used for frozen sections, histology or other short term experiments reported elsewhere). Compared with the earlier estimates of the lesion, it was found that 63 of them showed extensive regeneration of pigment epithelium in the ablated half. Seven of these were mapped electrophysiologically at 166 to 229 days and all showed restoration of the normal temporal field projection to the caudal tectum by fibers from a newly regenerated nasal retina. The MF's were normal (table 1: HR-regenerated). The projection from the temporal field apparently did not come from retina spared during the initial operation, since it is highly unlikely that retina would remain after removal of the underlying pigment epithelium, the absence of which was verified postoperatively by trans-scleral illumination. Histology, to be presented later, supported this conclusion. We inferred that all other fish with extensively regenerated pigment epithelium had also regenerated functional retina, and no more of them were mapped.

Expansion of the HR projection

Forty-two of the 63 fish which showed little or no regeneration of pigment epithelium were mapped from 36 to 328 days postoperatively (the other 21 were used for other experiments). Maps from the 42 fish fell into two quite different groups.

In four fish mapped 36 to 94 days after the ablation, there was no reorganization. One of these is shown in figure 3A. The caudal tectum received no visual projection, and the rostral half received a normally organized projection from the nasal half of the visual field. Both the RCMF's and MLMF's of the unexpanded maps were in the normal range (table 1). In two fish, the optic nerve had been crushed at the time of the ablation. The appropriate projection was reestablished upon regeneration at 36 days in both cases (earlier attempts to map at 32 days could not record distinct retinal units). These maps were in

TABLE 1

Average magnification factors

Group	RCMF (S.E.M.)	MLMF (S.E.M.)
	(μm/°)	(μm/°)
1 Normal fish (N = 8)	18.6 (0.8)	21.1 (1.0)
2 HR-regenerated (N = 7)	19.5 (0.8)	21.9 (2.3)
3 HR-unexpanded (N = 4)	18.3 (1.3)	21.2 (0.9)
4 HR-expanded (N = 43)	38.5 (2.1)	19.9 (0.7)

agreement with the histological observations of Attardi and Sperry ('63).

In the other 38 fish which were mapped at 137 to 328 days (and in 3 of the 4 just mentioned, which were re-mapped at 203-234 days), the projection from the remaining nasal field was found to have expanded onto the denervated caudal half of the tectum in correct retinotopic order. Typical maps are shown in figures 3B and 3C. The vertical meridian, which normally projects to the mid-tectal surface, projected to the caudal tectum. At all tectal sites, the activity showed the characteristics of retinal ganglion cell terminals recorded from normal tecta. The receptive fields were of normal size (10-13%), and the units fired briskly to a small light flashing within the receptive fields, even after repeated stimulation.

The expansion of the projection was reflected in a two-fold increase in the average RCMF, but the average MLMF was unchanged from normal (table 1: HR-expanded). This parallels the earlier finding that half tectal compression is reflected in the MF only along the shortened tectal axis (Yoon, '71; Schmidt et al., '74).

The time course of the expansion was examined, and two features stand out. The first is that it began after a considerable delay. None of the four projections recorded at or before 94 days had expanded, whereas all those recorded at 137 days or later had done so. The second feature is that once underway, the expansion required months to reach its final form. Early in the expansion, the caudal half tectum was invaded by fibers which originated near the boundary of the retinal ablation. This is illustrated in the map of figure 3B, in which equally spaced penetrations in the tectum did not result in equally spaced receptive fields, as in the normal (figs. 2, 3A). Instead the receptive fields were nearly normally spaced nasally but closely spaced near the edge of the ablation. Thus, the thin strip of the visual field temporal to the dashed line projected in greatly
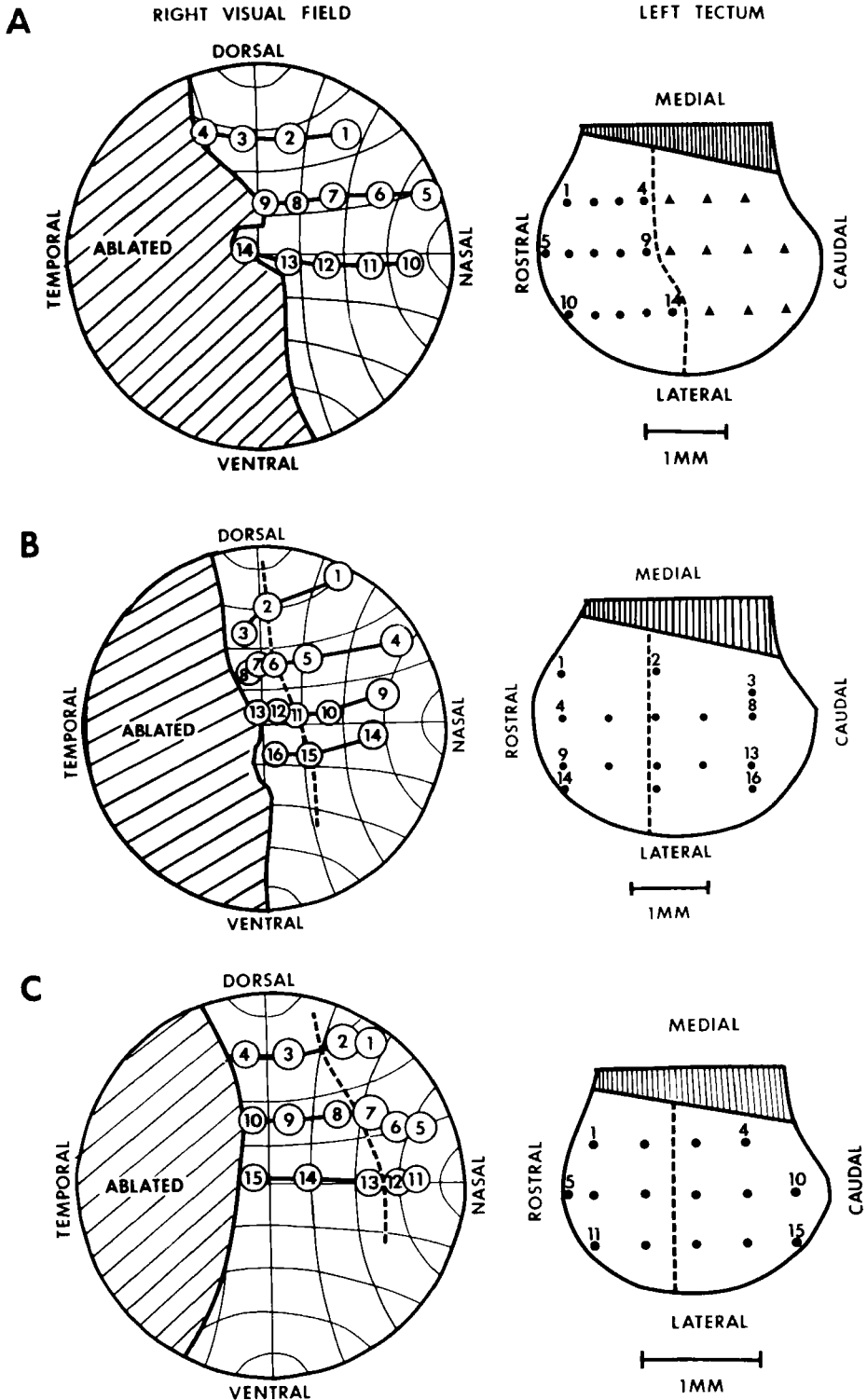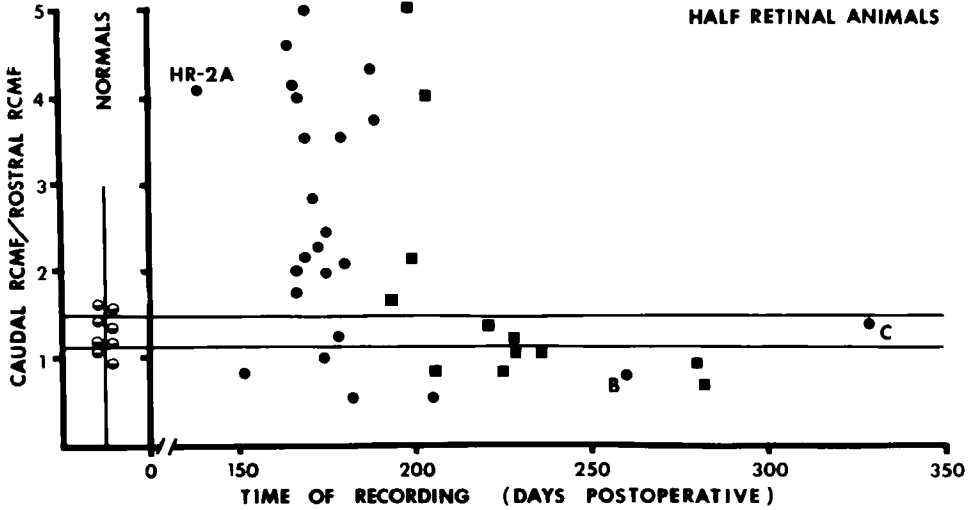
Figure 3

Fig. 4 Plot of the ratio (caudal RCMF/rostral RCMF) versus time of recording for HR fish. The bars denote ± 2 S.E.M. for the normals. Filled circles: initial group mapped in winter. Squares: second group mapped in summer.

expanded fashion over the caudal tectum. At later times the areas projecting caudally and rostrally became more nearly equal (fig. 3C).

These differences are shown quantitatively in figure 4, which plots the ratio of caudal RCMF to rostral RCMF as a function of the time of recording. Each point represents an individual map. (Data from eyes with more than 60% or less than 40% of the retina remaining were excluded.) The normal animals are shown (half-filled circles) for purposes of comparison. The filled circles represent the initial group of fish mapped during the winter months, squares a second group mapped during summer months. (The groups were operated in opposite seasons.) Both groups showed a sequential change in the ratio of the caudal to the rostral RCMF. The early ratio of 3 to 5 was followed by an abrupt drop at 180 to 200 days to a value of less than unity, and finally a

Fig. 3 Half retinal projections of three fish.

A. Unexpanded projection of HR-25, mapped 36 days after the retinal ablation and crush of the optic nerve. Triangles mark penetrations which did not record light driven units; the dashed line separates innervated and denervated areas of the tectum.

B. Early pattern of expansion in HR-28, mapped 171 days after retinal ablation.

C. Late pattern of expansion in HR-62, mapped 282 days after retinal ablation. Dashed lines on the tectum and hemisphere show the portions of visual field projecting rostrally and caudally on the tectum in each case. The striped area marked "ablated" corresponds to the ablated nasal retina. The optic axis, determined by the corneal reflection was within 4° of the centre of the hemisphere in each case.

gradual rise toward the normal value, reached at about 300 days. The group mapped during the summer months reached the drop about 20 to 30 days later, but went through the same sequence. This sequence, which has been inferred from single maps of many fish at varying postoperative intervals, was also observed in one fish (HR-2) which was recorded three times as indicated in figure 4. Following the abrupt drop at 180 to 200 days, it appeared that there might be a significant overshoot in the ratio before it reached the normal range. To test this, the ratios from all 11 maps made after 205 days were compared to those from the eight normal fish using the standard t-test. The means for the two groups, 0.99 and 1.30 respectively, were significantly different (t = 2.45, D.F. = 17, p < 0.05), supporting the notion that there was a transient overshoot in the reorganization.

A more detailed analysis of the variation in the local RCMF is given in figure 5, for the normal fish (above) and for three groups of half retinal fish (below). For each map, two lines of points near the horizontal meridian were used, and the RCMF computed for each pair of points was referred to the midpoint between them. Distances on the tectum were scaled to a standard tectum 3.15 mm in RC extent. Data were grouped into discrete 300 μm bins and averaged to give the points plotted.

In the upper graph, the normal data have an average local MF of 18.6, which decreases ros-
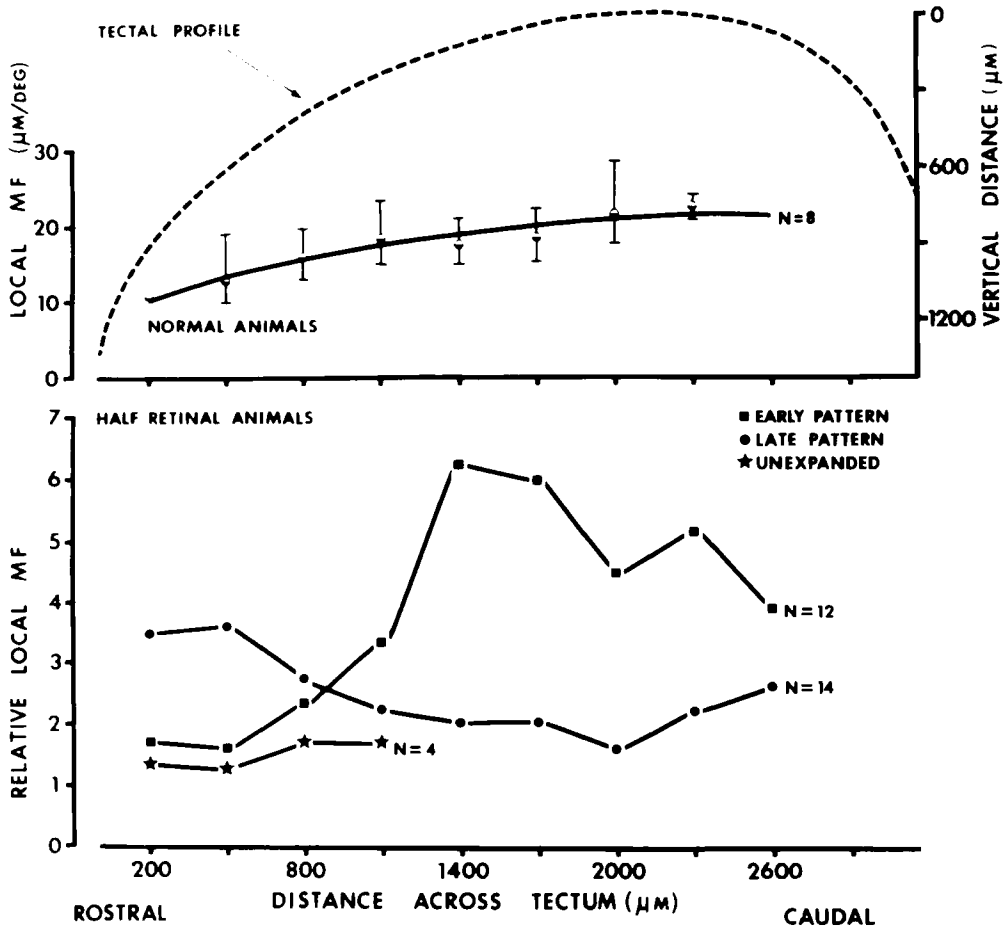
Fig. 5   Plots of local RCMF versus rostro-caudal position on the tectum. In the upper plot, the dashed line (referred to right ordinate) shows the profile of the dorsal tectal surface, obtained from a photograph of a live fish. The symbols give the mean local RCMF (2 S.E.M.) obtained from normal fish. The lower plot shows the relative local RCMF of three groups of experimental fish.

trally due to the effect of the tectal curvature, shown by the dashed line. The data for the half retinal fish, plotted below, were normalized relative to the same tectal sites in the normal animals to eliminate the effects of tectal curvature. The squares are the means for the group of animals with the ratio, (caudal RCMF/rostral RCMF), more than twice the normal value. The maps from these fish are typified by figure 3B, where a thin strip of visual field projected in expanded fashion over the caudal tectum. Maps such as this one were found in eight of the 11 cases recorded before 171 days, indicating that it is probably the early stage of expansion. The circles are the means for the group of animals with the ratio either within or below the normal range. A

typical map is figure 3C, where the receptive fields were more closely spaced in the nasal visual field, away from the ablation. This pattern was recorded in all but one of the twelve cases recorded after 200 days, suggesting that it is a later stage of expansion. Our interpretation of the progression of the expansion ("early" to "late") was further supported by the three maps obtained in the same fish, HR-2, at different times, labelled A, B and C in figure 4.

The unexpanded projections, as expected, had relative MF's over the rostral half that were close to unity. The early pattern also showed a normal relative MF at the far rostral end, but this rose sharply at mid-tectum and remained high over the caudal tectum. In the

late pattern, the peak in the local relative MF had moved to the rostral tectum, but had also declined considerably in amplitude. The MF on the caudal tectum was lower than rostrally, but still nearly twice the normal value. These shifts in the local MF will be related to density of retinal fiber terminations in the DISCUSSION.

Radioautography of the tectum

The retino-tectal projection was traced radioautographically in normal fish, HR fish immediately post-operatively, and in several fish that were mapped to study the sequence of expansion.

The normal retinal projection consists of at least three separate bands of termination, as shown in figure 6A, a parasagittal section through a normal tectum. A thin superficial band lies just above the broad main band occupying the external plexiform layer. Much deeper is a very sparse band within the internal plexiform layer, sometimes called the deep afferent layer (Neale et al., '72; O'Benar, '71).

In two fish, the nasal half retina was removed five days prior to the injection of the ^{3}H proline. Both gave the same result, shown in figure 6B; the remaining projection was confined to the appropriate rostral half tectum.

Three expanded half retinal projections were successfully radioautographed. Two of these were in the early stages of expansion; that is, the electrophysiology showed that only a small part of the half retina had projected caudally. In one (lightly labelled), there were silver grains above background only in the rostral tectum, but in the other, more heavily labelled, there were, in addition to the heavy grain density rostrally, light patches of silver grains caudally. The third fish, mapped at 282 days (fig. 3C), exhibited a more uniform projection, and the radio-autograph (fig. 6C) showed a continuous projection from rostral to caudal.

Histology of the HR eyes

The four micrographs in figure 7 illustrate the relevant findings. All are horizontal sections through or near the optic disc, with the ablated area to the left, normal retina to the right. The eye in figure 7A was operated seven days earlier. There is a clearly defined end to the remaining retina and no residual retina or pigment epithelium visible in the ablated half. Figures 7B and C are sections through the eyes of two fish with expanded half retinal projections. In some cases (fig. 7B) there was no regeneration of any retinal tissue in the ablated region. More often, as in figure 7C, the retina had partially regenerated. It was clearly abnormal, in that nuclear stratification was disorderly and the outer segments of the photoreceptors were shorter than normal. The pigment epithelium was very sparse or totally absent, confirming the observations made by trans-scleral illumination. Most significantly, there was no ganglion cell fiber layer evident at the vitreal edge. In contrast to the regenerated half, the remaining temporal retina looked normal. Figure 7D is from an HR eye which the electrophysiology had showed to have regenerated functional retina in the ablated area. In such cases, there was clear nuclear stratification, outer segments were of normal length, and a definite pigment epithelial layer was present.

Within the remaining half retina, the density of ganglion cells was examined, for it seemed possible that the half retina might have responded to the presence of available denervated tectum by increasing the number of ganglion cells. These were distinguished by their pale, round, relatively large nuclei, surrounded by an irregularly shaped ring of basophilic cytoplasm (Johns and Easter, '75). Counts were made of all ganglion cell nuclei within single sections through the centers of 17 operated eyes and 3 normal ones, corrected using Abercrombie's factor (Abercrombie, '46; Konigsmark, '70), and converted to retinal surface densities. The HR eyes had an average density of 1,916 ($\pm$ 98 S.E.M.) ganglion cells/mm^2, versus 2,333 ($\pm$ 108 S.E.M.) for the normals. This represented a slight decrease, suggesting either some cell death or physical expansion of the retina, but certainly not a proliferation of ganglion cells.

A second point of interest was the morphology of the retinal ganglion cells, for if they were actively moving their terminal arborizations over substantial distances within the tectum, they might be expected to exhibit the axonal reaction (Murray and Grafstein, '69). This was consistently found to be the case, as seen in the set of photomicrographs in figure 8. Figure 8A shows a normal case, 8B and C are from eyes with expanded HR projections, and 8D, ganglion cells whose axons had been transsected 60 days previously. The latter

TABLE 2

Measurements of the axonal reaction in retinal ganglion cells

Group	Days postoperative	Number of cells examined	Nuclear diameter		% with nucleoli	(S.E.M.)	% eccentric	(S.E.M.)
			(μm)	(S.E.M.)				
1 Normals (N = 3)	—	834	5.07	(0.03)	12	(2)	11	(1)
2 HR-expanded (N = 14)	171–284	983	7.23	(0.17)	48	(6)	51	(5)
3 HR-unexpanded (N = 2)	7	416	5.47	(0.04)	18	(2)	21	(3)
4 Optic tract	31	153	8.44	(0.14)	65		59	
section: recent	47	163	8.02	(0.07)	67		67	
(N = 3)	60	224	7.88	(0.13)	46		40	
Average			8.11	(0.14)	59	(6)	55	(7)
5 Optic tract	143	113	5.40	(0.11)	53		22	
section: distant past	395	149	5.35	(0.07)	10		13	
(N = 4)	395	153	5.27	(0.06)	13		18	
	455	142	5.37	(0.08)	18		9	
Average			5.35	(0.02)	24	(9)	16	(2)

three all show the full axonal reaction; enlarged, darkly staining (basophilic) peri-karyal cytoplasm; enlarged, pale, eccentrically located nuclei; and prominent nucleoli. This subject was investigated quantitatively on 14 half retinae and 3 normal retinae. The diameter of the nucleus of each ganglion cell was measured at × 900 with an ocular micrometer, and each was scored for the presence of a nucleolus and eccentric location within the cell body. Either every ganglion cell within a single section was measured or a smaller area was chosen at random. The two methods gave similar results, which appear in table 2, groups 1 and 2. The mean nuclear diameter of the HR ganglion cells was 43% larger than the mean of the normals, which corresponds to almost a 3-fold increase in nuclear volume. In addition, the percentages of nuclei containing nucleoli or occupying eccentric positions were both over four times the normal value.

This difference might be the residual effect of injuring the axons of the ganglion cell during the initial operation. Murray and Grafstein ('69) found that the axonal reaction in goldfish retinae was most pronounced at 28 days and declined considerably by 60 days, but they did not investigate the disappearance of the effects beyond that point. To check for possible long term effects, measurements were made in the eyes of seven fish whose optic tracts had been cut 31 to 455 days previously. Initially at 31, 47 and 60 days, the ganglion cells showed a strong reaction with larger nuclei, more nucleoli and more eccentric nuclei

than in the half retinae (table 2, group 4). However, at 143 days and thereafter, there was a marked reduction in the nuclear diameter and the percentage of eccentric nuclei to near the normal values (table 2, group 5). The percentage of cells with nucleoli dropped more slowly, but approached the normal range. The cytoplasmic volume of these cells appeared to be greatly reduced and much less basophilic. Given this reduction by 143 days, it appears highly unlikely that the axonal reaction seen in the half retinae at times up to twice as long could be due to axonal injury during the initial operation.

The question of surgical trauma to the remaining half retina was also investigated by injecting two HR eyes with ^{3}H-proline five days after surgery. The radioautographs showed high grain density in the rostral tectum (fig. 6B), indicating that the projection was largely intact after surgery. The retinae were also examined histologically, without radioautography. Had the axons been damaged, the retinal ganglion cells should have shown the axonal reaction (Murray and Grafstein, '69). Most of the ganglion cells appeared normal. Only about 25-30% in each case showed any features of the reaction, and these cells were largely confined to the area near the edge of the ablation. The quantitative data are given in table 2, group 3. This lack of widespread reaction initially, as well as the decline of the effects on axotomized retinal ganglion cells after 143 days, indicates that the effects seen in the half retinae up to 284 days post-

operatively could not have been due to the surgery. The axonal reaction is therefore most likely associated with the movement and perhaps enlargement of the terminal arborizations during the expansion of the projection.

DISCUSSION

Comparisons with earlier work

These results confirm, with different techniques, the conclusion of Attardi and Sperry ('63) that optic fibers from a half retina grow back to their original sites of termination soon after nerve crush. They also confirm the report (Yoon, '72b) that the projection expands to fill the entire tectum after a delay of many months.

The results conflict with those of Horder ('71) who used similar techniques. His report, however, was based on few animals, whereas ours was drawn from many more. Our radioautographic demonstration that fibers from the half retina spread to the caudal tectum differs from Meyer's finding (Meyer, '75). In this case, we acknowledge that all of our radioautography did not show the spread. During the early stage of expansion, when the electrophysiology indicated that a relatively small part of the half retina projected to the caudal half tectum, little label was found there. However, during the late stage, when the local RCMF was more uniform over all of the tectal surface, the label was quite clear although still thinner caudally than rostrally. In view of the fact that the electrophysiology and the radioautography sometimes gave conflicting results, we must try to assess which is the more reliable. The radioautographic grain density depends on the amount of labelled protein transported to the tectum. This in turn depends on factors such as differential uptake at the cell body, axon diameter, and degree of arborization. Arborizations which had recently moved into the foreign caudal tectum might reasonably be assumed to be of smaller diameter and less extensively arborized than those rostrally which had moved very little or not at all. Since these ultrastructural details remain unknown, it seems reasonable to assume that, in this case, the electrophysiological technique is the more sensitive of the two.

Relation of the local MF to fiber density

The relative local MF can be inversely related to the relative density of retinal fiber arbors across the tectum. (Note that we refer to a density, not of individual terminals, but of terminal arbors, the group of processes originating from one fiber.) Only two assumptions are needed: (1) The surface density of ganglion cells is uniform over the retina, and (2) Each ganglion cell has one and only one arborization in the tectum. Histological observations support the first of these (Johns and Easter, '75; Schellart, '73). The second is supported by the fact that each point in the visual field is represented at a single tectal locus (Schwassmann and Kruger, '65). Given these two assumptions, the MF in any direction is inversely proportional to the linear density of fibers terminating along that direction. Accordingly, peaks in the local relative MF correspond to zones of low fiber density and the rostral position of this peak in the late pattern is evidence for a slightly greater density of arborizations on the foreign caudal half of the tectum than on the rostral half during the late stage of expansion.

A model of inter-fiber competition

We believe the expansion can be explained by a competitive model of innervation based on the following three assumptions: (1) Each retinal fiber terminal has an intrinsic tendency to expand its arbor, (2) The tendency weakens as the arbor grows larger, and (3) It is opposed by other nearby arbors. Assumptions 1 and 3 are similar to those postulated by Aguilar et al. ('73); that is, a factor promoting continual growth, opposed by the release from neighboring fibers of an inhibitory factor. In addition, assumption 2 is necessary so that the arborizations spread evenly to fill the available surface, eventually locking each other in place. If half the fibers were removed, as in this case, those arborizations bordering on the denervated area would find their expansionist tendencies unopposed, and would enlarge to occupy the vacant area. As a result of their expansion, they would be less effective competitors with neighboring arborizations on the side away from the denervated area, and would be pushed out of some or all of the denervated area they had previously occupied. The arborizations which had expanded into that area would in turn lose some of their processes on the side away from it, and so on, until finally a new uniform array of arborizations had been achieved, half as numerous as before, but each much larger.

This sort of competitive model could also ex-

plain the reorganization following ablation of a caudal half tectum (Gaze and Sharma, '70; Yoon, '71; Schmidt et al., '74). Fibers unseated by the lesion would re-arborize at the edge of the ablation, causing a high density of fiber terminals there and resulting in displacement rostrally of fibers forming the projection from the nasal field until a new uniform density across the half tectum was achieved. This model is also compatible with the continual adjustment that is thought to occur during the growth of the retina and tectum in larval *Xenopus* (Gaze et al., '72). Surgically induced reorganizations (such as this one) may only be an exaggerated instance of the same phenomenon in goldfish (Meyer and Sperry, '73). Both the retina and tectum of the goldfish are known to grow throughout its adult life (Johns and Easter, '75), and the patterns of cell addition are geometrically different (Johns, '76), suggesting shifting connections as in larval *Xenopus*.

Competitive innervation has similarly been demonstrated in the sensory innervation of the skin (Weddell et al., '41; Aguilar et al., '73), and also in many areas of the mammalian central nervous system, including the hippocampus (Lynch et al., '72), superior colliculus (Schneider, '73; Chow et al., '73) and visual cortex (Wiesel and Hubel, '74; Shatz et al., '75). The results from monkey visual cortex are strikingly similar to half retinal expansion. After enucleating one eye at birth, Wiesel and Hubel ('74) found that the ocular dominance columns of the intact eye expanded to nearly twice the normal thickness at the expense of those from the missing eye. This similarity suggests that competition may be a general property of topographical projections.

In addition to competition, other factors, of course, must be at work to produce ordered topographic projections during both development and regeneration. Two such postulated mechanisms are the sorting out of retinal fibers (Hope et al., '76), and the matching of specific cytochemical markers between pre- and post-synaptic cells (Sperry, '63). The latter concept was strengthened recently by the demonstration of selection regeneration of retinal fibers to translocated patches of tectum (frog: Jacobson and Levine, '75; goldfish: Hope et al., '76). The initial selective regeneration found in this study (fig. 3A) is also consistent with it, although the later expansion (figs. 3B,C) is not. The following paper (Schmidt, '77) examines this problem, and demonstrates changes in these operationally defined tectal markers, both during the expansion and following long periods of denervation.

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APPENDIX

Analysis of the effects of recording with the goldfish's eye in air

Structure

The observations below apply to animals in the size range reported in the body of this report. In cross section, the lens is nearly circular (Charman and Tucker, '73; Easter et al., '77), 1.54 mm in radius. The cornea roughly conforms to the arc of a larger, non-concentric circle, but it is slightly flattened at the edges and has slightly different radii of curvature in the horizontal and vertical planes (4.34 and 3.61 mm, respectively, determined from photographs of live fish). The optic axis is defined as the line through the center of the lens and the center of curvature of the cornea. The retinal surface is roughly concentric with the lens and approximately hemispherical (182° subtense), but approaches the lens more closely off axis (4%, 8% and 14% at 30°, 60° and 90° respectively).

Myopia in air

In water, the cornea is almost optically inactive (0.33 diopters) because its refractive index and that of the aqueous humor are very close to that of fresh water (Charman and Tucker, '73). When moved into air, the cornea increases to about 63 diopters (average of horizontal and vertical values), calculated by the simple lens formula. In combination with the lens, the cornea rendered the entire system about 37 diopters myopic. (These larger fish were rendered less myopic in air than those of Charman and Tucker, '73, due to their flatter corneas.) The image was brought to focus about 0.7-0.8 mm in front of the receptor outer segments on the optic axis (F'$_{Air}$ in Appendix fig. 1), producing a blur circle from a distant point of light of about 10° on the retina. Off axis, the size of the blur circle is reduced since the retina approaches the lens more closely, and also because of the decreased

APPENDIX TABLE 1

Relation between retinal and visual field angles with the eye in air

Visual field angle	Retinal angle		Percent error	
	Horizontal	Vertical	Horizontal	Vertical
0°	0°	0°	0%	0%
10°	8.3°	8.7°	20.0	14.0
20°	16.7°	17.4°	19.8	14.9
30°	25.1°	26.2°	19.5	14.5
40°	33.5°	35.0°	19.4	14.3
50°	41.9°	43.7°	19.5	14.4
60°	50.2°	52.5°	19.5	14.3
70°	58.9°	61.2°	19.4	14.4
80°	67.4°	70.1°	18.9	14.1
90°	76.6°	79.5°	18.4	13.2
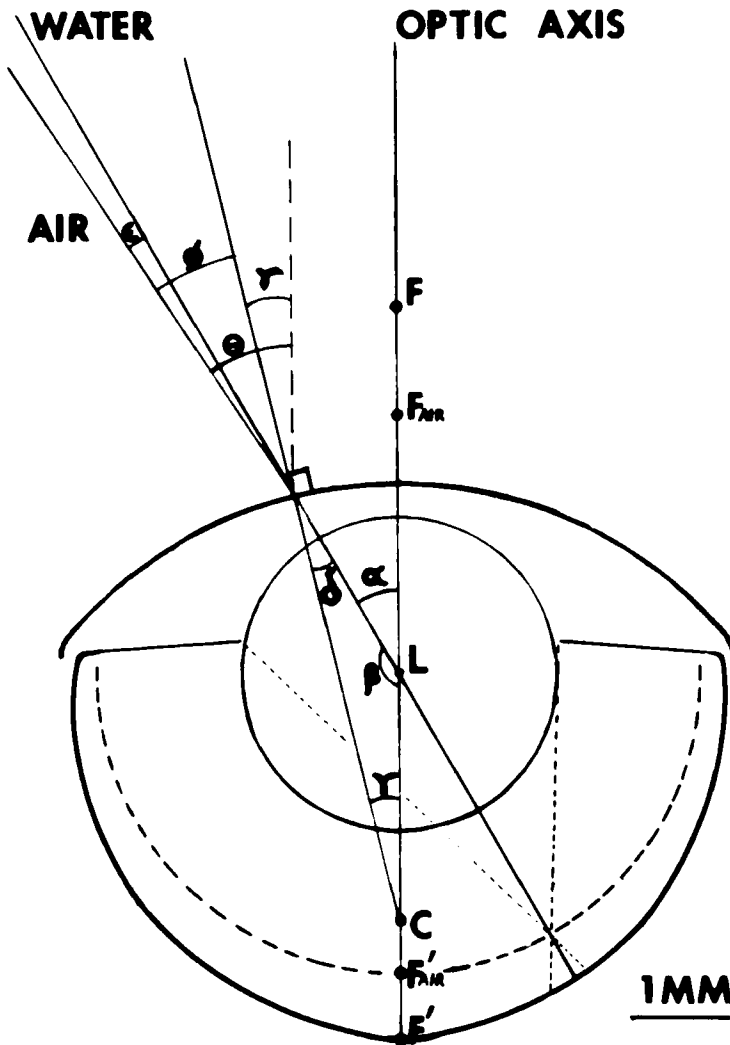
Appendix figure 1   Schematic eye of goldfish, constructed from photographs of frozen sections and showing the bending of rays in air at the air-cornea interface. F, F', F_{AIR}, F'_{AIR}: First and second focal points of the eye in water and in air respectively. The angles shown are defined in the text.

effective aperture of the pupil at increasing angles.

Enlargement of the visual field

A second effect of the air-cornea interface is that it increases the amount of visual space imaged onto the retina (Charman and Tucker, '73). This occurs because the curvature of the cornea is not concentric with that of the lens (Appendix fig. 1). As a result, a ray travelling a straight path through the center of the lens (L) does not pass normal to the corneal surface (except on axis). In water the ray would not be bent appreciably since the indices of refraction of water and the aqueous humor are nearly the same. However, in air a ray at a greater angle to the optic axis would be bent toward it. Calculation of the exact amount of bending at each visual field angle requires the following terms, also identified in Appendix figure 1:

α: Retinal angle from the optic axis; also the angle of the ray in water to the optic axis.

θ: The angle of the ray in air (to the optic axis) which corresponds to the same retinal angle α to the optic axis.

ϵ: The amount of bending at the cornea; the difference between θ and α.

ϕ, δ: The angles of the ray to the normal at the cornea, in air and inside the eye, respectively.

γ: Angle between the normal to the cornea and the optic axis.

d_{LC}: The distance between the center of the lens (L) and the center of curvature of the cornea (C).

R_c: The radius of curvature of the cornea.

n_{aq}: The refractive index of the aqueous humor.

Using Snell's law and trigonometry, it can be shown (Schmidt, '76) that the amount of bending, ϵ is equal to $\epsilon = \theta - \alpha = $ arcsin $[n_{aq} * d_{LC} * (\text{Sin } \alpha)/R_c] - $ arcsin $[d_{LC} * (\text{Sin } \alpha/R_c)]$.

Appendix table 1 gives the relation between visual field angles and retinal angles calculated from this equation for both the naso-temporal and dorso-ventral planes. The disparity between these angles (expressed as a percent of retinal angle) was nearly constant, within each plane, over the area mapped onto the hemisphere. The slight decrease in bending at large angles is a result of the assumption that the corneal profile conforms to the arc of a circle. The slight flattening at the edges (noted above) would tend to offset this decrease. We have also introduced a slight error by using the ray through the center of the lens to define the center of the blur circle. This error is exaggerated in Appendix figure 1, where the edges of the blur circle were determined by tracing lines from the edges of the pupil, instead of from the principle plane of the lens. This error never exceeded 0.5° and was therefore neglected. The net effect of the corneal bending was therefore an almost uniform demagnification of about 19% and 14% in the horizontal and vertical planes respectively.

One final point is that the two effects—myopia and enlargement of the visual field—depend in opposite ways on the curvature of the cornea, and therefore cannot both be eliminated by the use of a contact lens.[1]

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[1] As this paper was going to press, a study (Meyer, '77) appeared which had compared the visuotectal maps recorded with the goldfish's eye in water and in air. Both the peripheral shift of receptive fields and the difference in the amount of this shift along the horizontal and vertical directions were noted, as predicted here. His estimate of 20-25° maximum, although not quantitatively measured, is in reasonable agreement with these calculations. In addition a very slight enlargement of receptive field size was noted, as would be expected from the 10° blur circle.

PLATE 1

6 Dark field radioautographs of retinal projections to the tectum. The plane of section is parasagittal; rostral is to the right, dorsal at the top.

A Normal projection.

B Half retinal projection of HR-65, injected five days after surgery.

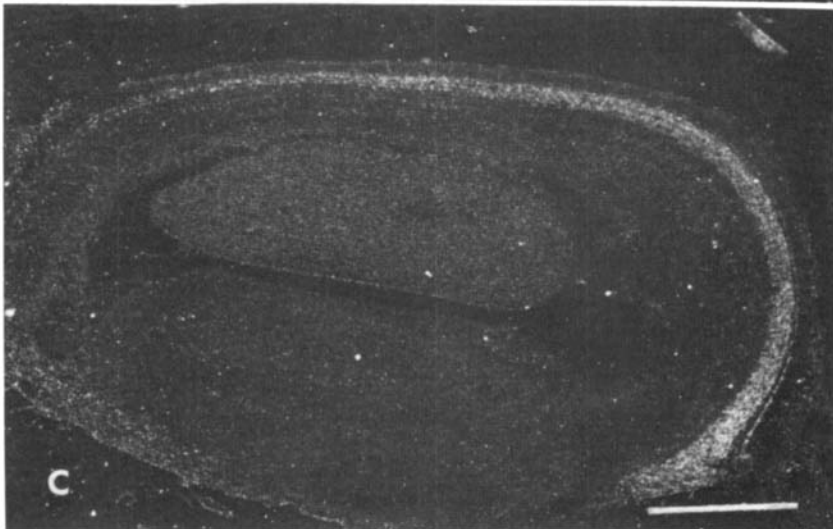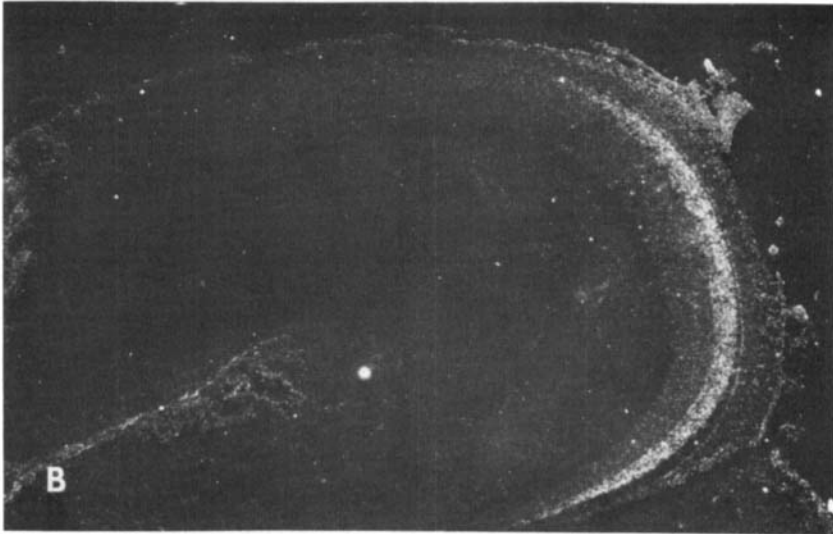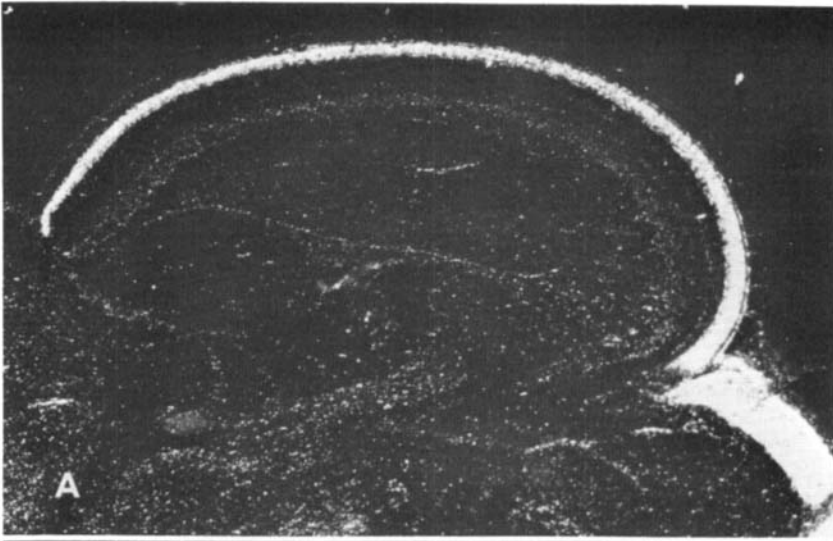C Expanded half retinal projection of HR-62, injected at 282 days. Calibration bar: 500 μm.

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7   Horizontal sections through or near the optic disc of HR eyes. The arrows mark the estimated boundaries of the original ablations. In all cases, the normal half of the retina is on the right.

A   HR-64, at seven days postoperative, showing typical extent of removal.

B   HR-3, at 189 days. Little or no retinal regeneration.

C   HR-2, at 328 days. Partial regeneration of immature retina which did not project to the tectum.

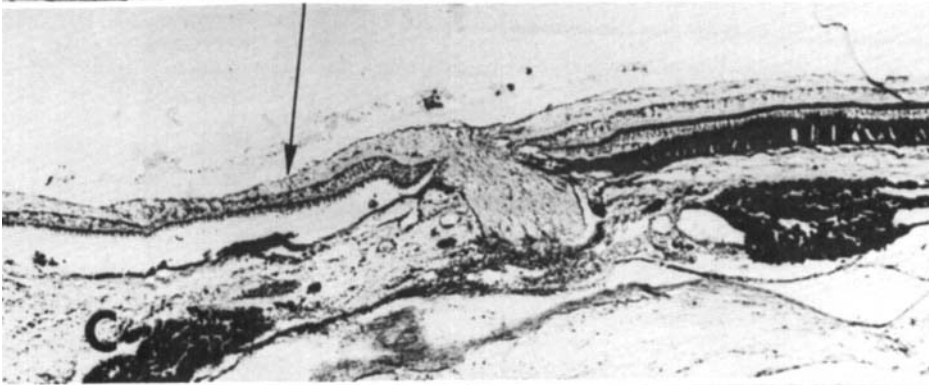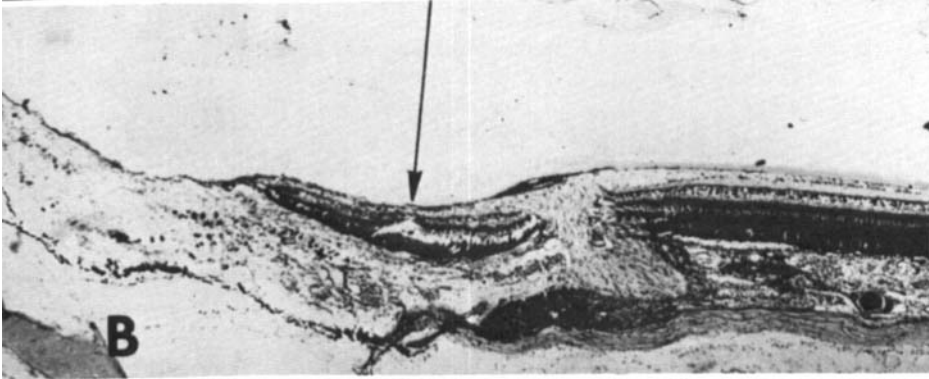D   HR-5, at 171 days. Regenerated retina found to project on the tectum. Calibration bar: 500 μm.
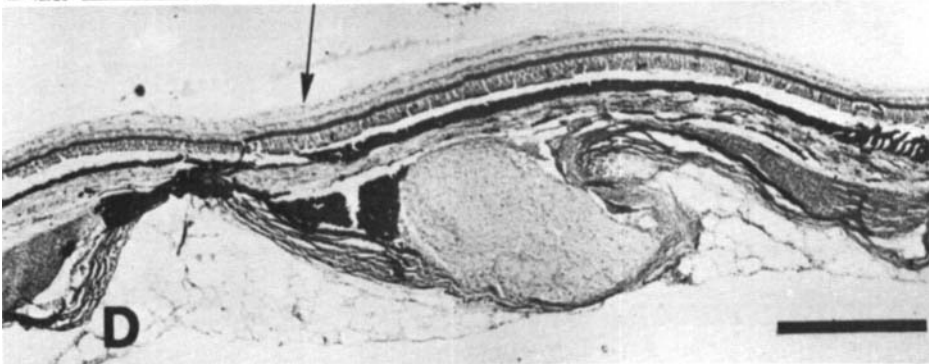
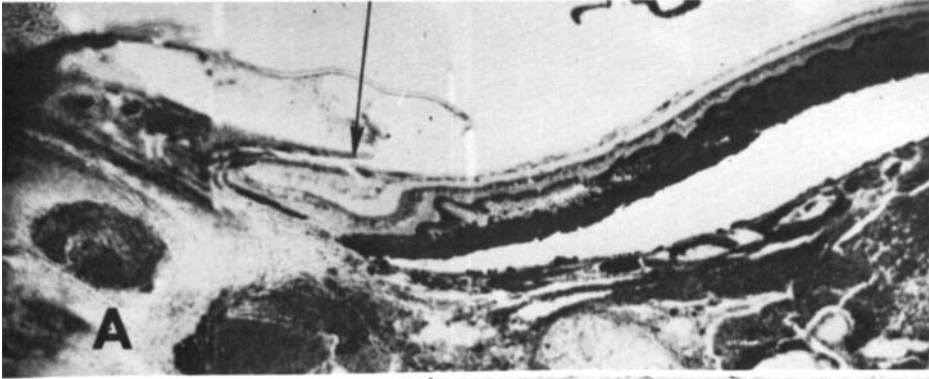8   Micrographs of retinal ganglion cells of four fish. Cresyl violet, 10-μm sections. The vitreal surface of the retina is at the top.

A   Normal goldfish.

B   HR-62, at 284 days postoperative.

C   HR-48, at 224 days postoperative.

D   Ganglion cells whose axons had been cut at the optic tract 60 days earlier. Calibration bar: 10 μm.