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VISION IN GOLDFISH FOLLOWING BILATERAL TECTAL ABLATION

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Visual sensitivity of optic tectum-ablated goldfish was investigated using a classical conditioning technique. Intact fish were screened to obtain individuals which showed suppression of breathing movements in response to the visual conditioned stimulus (CS) in the presence or absence of adapting illumination. Following bilateral optic tectum ablation, responding was blocked in light-adapted but not dark-adapted fish. Response threshold testing revealed no significant postoperative changes in visual sensitivity. Small remnants of tectal tissue containing cellular elements of the periventricular gray zone and optic axon terminals were detected in some ablates but there was no evident relationship to response threshold. Optic nerve crush blocked responding in ablates and recovery occurred within 2–3 weeks postaxotomy confirming that the response was mediated by retinal as opposed to extraretinal photostimulation. The experiments support the findings by others that tectum ablation results in decreased visual sensitivity and that conditioned visual responding can be obtained. However, we find no support for the suggestion that visual sensitivity in the ablates depends on functional recovery of regenerating optic axons which innervate non-tectal visual nuclei. Instead, the results indicate that the normal retinal projections to the non-tectal nuclei can mediate visual responding and, in addition, that postoperative conditioning experience facilitates recovery of response in ablates which initially appear to be blind to the CS.

INTRODUCTION

The optic tectum receives the vast majority of optic axons in goldfish[18,21] and other teleosts[12], and the functional organization of the teleost retinotectal projection has been extensively investigated[24]. Brain lesion studies in goldfish show that the optic tectum is necessary for most visual behaviors that have been examined, such as optomotor responses, the suppression of branchial movements in response to a shadow[22], and startle movements in response to a sudden increase in illumination[7]. Non-tectal visual centers, which are located in the diencephalon and pretectum, receive comparatively small projections of optic axons[1,21]. Brain lesion experiments indicate that the non-tectal visual system is sufficient to mediate optokinetic nystagmus[22] and the branchial suppression response (BSR) to conditioned visual stimuli.

The BSR to a visual conditioned stimulus (CS), consisting of a flashing spot of white light, in light-adapted goldfish is reported to disappear following bilateral optic tectum ablation and to reappear with an increased threshold within 3 weeks postsurgery[26]. The return of visual function was attributed to the establishment of increased retinal input to non-tectal visual centers. After tectal ablation, regenerating optic axons invade various visual and non-visual brain areas[11,19,20]. Axotomized optic fibers functionally innervate alternate targets elsewhere in the *tectum*

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following caudal half-tectum ablation[17] or unilateral tectum ablation[4,25]. Additional study is needed, however, to determine whether axons that normally innervate the tectum can functionally innervate *non-tectal* visual nuclei.

Experiments in our laboratory showed that the BSR evoked by a CS consisting of a moving spot of red light is blocked in light-adapted, tectum-ablated goldfish[4]. Continued testing of the ablated fish for many weeks revealed no evidence of visual recovery suggesting that their visual sensitivity might be too low to detect the stimulus. When fish were subsequently tested in darkness, all the long-term ablates responded and, most surprisingly, so did newly ablated goldfish. Although the pilot data implied that there may be no postoperative period of total blindness, the data were insufficient to indicate whether a decrease in response threshold occurs during the first few postoperative weeks. It is possible that regenerating optic axons regain visual function by innervating various non-tectal targets and the effect might be to increase behavioral sensitivity to the visual CS.

The present study was conducted to examine whether bilateral optic tectum-ablated goldfish exhibit postoperative changes in BSR threshold when tested in darkness. The BSR was evoked by the moving red light CS that was classically conditioned to an electric shock unconditioned stimulus (US). The classical conditioned BSR is widely used in studies of fish vision[14].

MATERIALS AND METHODS

Fish

Goldfish (*Carassius auratus* L.), 8–12 g, obtained from Ozark Fisheries, Stoutland, MO, were kept in large tanks at 24–26 °C for several weeks prior to being placed in individual home tanks at 30 °C[3]. The fish were fed Tetramin staple conditioning flake food once or twice daily. The daily photoperiod was 16 : 8 h L : D with fluorescent light augmented by diffuse natural light. The tank water conductivity was $400 \pm 50\ m\Omega^{-1}\ cm^{-1}$ and the pH was 7.0–7.5.

Surgery

The fish were anesthetized by immersion in 0.04% trimethane methyl sulfonate (Sigma) buffered with Tris fish buffer (Sigma) to pH 6.5–7.5. To ablate the optic tectum, a U-shaped flap was cut in the cranium over the tectum and the torus longitudinalis (Fig. 3) was removed by aspiration. The bone was then wedged back in place and the fish was returned to the home tank to recover for ca. one week prior to subsequent behavioral testing. The optic nerve was crushed in the orbit by pinching it with a pair of forceps as described previously[5]. The day of surgery was designated as experimental Day 0.

Histology

The fish were sacrificed by immersion in the anesthetic. Their heads were fixed in alcohol-formalin-acetic acid and embedded in paraffin to obtain 15 μm thick transverse sections of the midbrain which were subsequently stained with cresyl-violet acetate. To examine the extent of the tectal lesion, brain sections from experimental fish were contrasted to corresponding sections from an intact reference brain using a Bausch and Lomb slide projector and light microscopy. In the reference brain fish and 4 additional tectal ablates the retinotectal projection was traced by autoradiography[22]. The fish received an intraocular injection of 25 μCi of [^{3}H]proline (spec.act. 20–40 μCi/mmol, New England Nuclear), 20–24 h prior to being sacrificed. Drawings of the autoradiograms were made with the aid of the slide projector. A Leitz inverted microscope was used to prepare light- and dark-field photographs of brain sections. The nomenclature of Northcutt[12] was used in describing the zones of the optic tectum.

Conditioning tank and stimuli

The fish were conditioned individually in 3 glass tanks 15 cm × 15 cm × 30 cm. Each fish was restrained in a holder[2] which rested on the bottom of the tank, centered 45 cm beneath two 20 W, cool-white fluorescent lamps which were continuously illuminated. Branchial ventilation movements were detected by the thermistor method and the amplified thermistor signal was recorded on an ink-writing polygraph in a separate room. The branchial beat rate was measured

following a digital conversion of the analog signal[5] or by a tachograph method[3].

The CS consisted of the alternate illumination of two light-emitting diodes (LEDs) that were placed one above the other 1 cm apart and ca. 2.5 cm from the eye[5]. The LED produced a spot of diffuse red light. The visual angle subtended by the LED and the angle between the two LEDs were ca. 11° and 26°, respectively. The LED image was presumably unfocused[13] and may have covered most of the retina. During the CS interval the upper and lower LEDs were illuminated alternately every 250 ms and the lower LED was kept lit during the intertrial interval. The US was a 0.5 s pulse from 7–8 mA (rms) 60 Hz constant current that was passed between two steel electrodes on opposite sides of the fish-holder.

Preliminary conditioning of the BSR

The fish were administered a sequence of 2–5 sessions of 20 right and left eye conditioning trials over a period of 1–3 weeks[5]. The CS was turned on for 2 or 5 s and the US was presented at the instant that the CS was terminated. The BSR was measured in the 5-s trials by contrasting the branchial activity during CS–US interval (B) of the trial, with the activity during the 5-s interval (A) preceding the onset of the trial. The BSR was expressed as the percentage change in the rate in interval B relative to the rate in A [100 × (1 − B/A)]. A deceleration of greater than 40% was accepted as a BSR indicative of light detection.

Preoperative test session

Fish that responded to the CS in right- and left-trials subsequently received a test session consisting of four 2-s warmup trials followed by four 5-s test trials in which the BSR was measured. Four fish were given test sessions in light. The remaining fish were tested in darkness following two hours of dark adaptation. Fish that responded in each of the 4 test trials were accepted as subjects and randomly assigned to the experimental groups. The preoperative test session was administered 2–7 days prior to the day of surgery.

Postoperative test session

Visual function following surgery was assessed weekly using a similar session of conditioning trials. The CS was presented to the right eye only. Threshold of response to the CS was measured by a staircase method in which the light-intensity in different trials varied from 1–1600 cd/m^2. The intensity was decreased when the fish responded in the preceding trial and increased when the fish failed to respond until a stable threshold was obtained. The data were reduced by estimating the LED luminance which resulted in a response in 50% of trials. Fish that did not respond at the maximum CS intensity in the first 5 trials in a session were returned to their home tanks.

RESULTS

Responding in light or darkness

Fish administered a weekly session of test trials in light for 18 weeks postoperatively (n = 4) showed no recovery of response to the CS. Of fish tested similarly but in darkness (n = 27), 43% responded from the first week and all resumed responding within 5 weeks (Fig. 1). Five responders received threshold testing. Their mean threshold was 22.2 ± 38.3 cd/m^2 one week following tectal ablation and 2.0 ± 1.3 cd/m^2 at

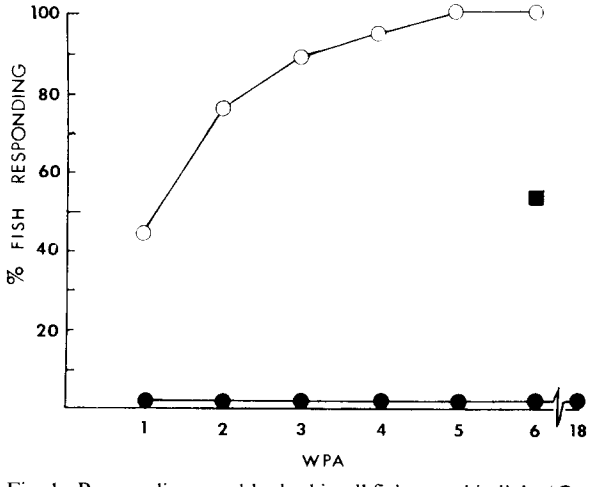

Fig. 1. Responding was blocked in all fish tested in light (●) for 18 weeks postaxotomy (WPA). All fish tested in darkness (○) recovered responding by 5 WPA. Fish tested with the CS only at 6 WPA (■) responded at the level of fish tested at 1 WPA.

6 weeks. The decrease in the mean threshold was not significant [$t(8\ df) = 1.0$; $P > 0.05$]. Four of the fish showed uniformly low thresholds at both time-points while one showed a marked decrease over the 6-week postoperative period of testing. Examination of brain sections from these fish revealed that the lesion variously spared thin strips of tissue at the extreme dorsal and/or ventral margin (Figs. 3, 5) of the tectum. Tectal remnants occurred in some fish but not in others which had showed similar low visual thresholds. The remnants were thinner than corresponding sections of the intact tectum but a layer of cells resembling the nuclear layer of the periventricular gray zone (PGZ; Fig. 5) was present. In addition, autoradiographic tracing of the retinotectal projection in other tectal ablates showed that the remnants contained optic axon terminations (Fig. 4).

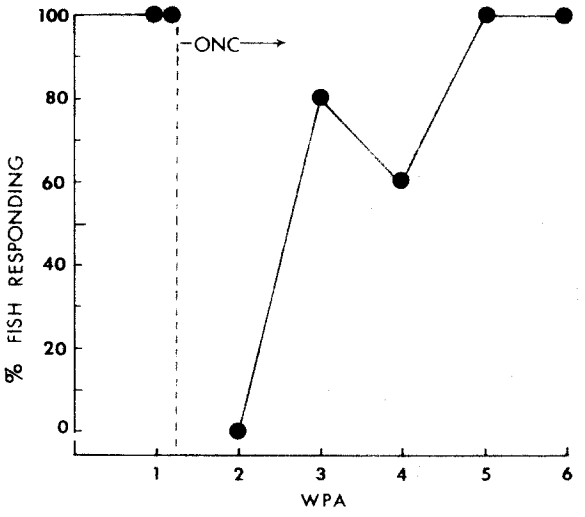

Fig. 2. Visual recovery following optic nerve crush (ONC). Bilateral optic tectum (BOT) ablation was administered on Day 0 and ONC on Day 8. Recovery occurred within 3 weeks post-ONC.

Conditioning effects on visual recovery

To examine whether the occurrence of visual responding in 100% of fish is a result of the CS–US conditioning experience, 10 fish were administered the US but not the CS during the first 5 weeks. The procedure of the weekly US-only session and a test session differed only in that the CS was withheld. When finally tested with the CS during the sixth week only half of the fish responded (Fig. 1). Moreover, the 5 fish that responded had widely varying thresholds. The mean was 169 ± 271 cd/m^2 and was not significantly different from the mean threshold of fish tested one week after tectal ablation [$t(8\ df) = 0.98$, $P > 0.05$)]. Additional fish (n = 6) which were demonstrated to respond to the CS at week one were administered US-only trials from weeks 2–5. When tested at week 6, each of the fish responded to the CS. This indicates that omitting the CS does not result in forgetting, that is, loss of response, and that the recovery of response seen in ablates receiving weekly sessions of CS–US trials could be a result of the conditioning experience.

It was possible that tectal ablates detected the CS by extraretinal photic or non-photic stimulation. To examine this, additional ablates (n = 5) which were shown to respond to the CS at Day 6 and 8 were administered optic nerve crush on the right side and enucleation on the left on Day 8. Beginning on Day 14, the fish received weekly test sessions which revealed that the crush resulted in blockade of responding followed by recovery within 2–3 weeks (Fig. 2).

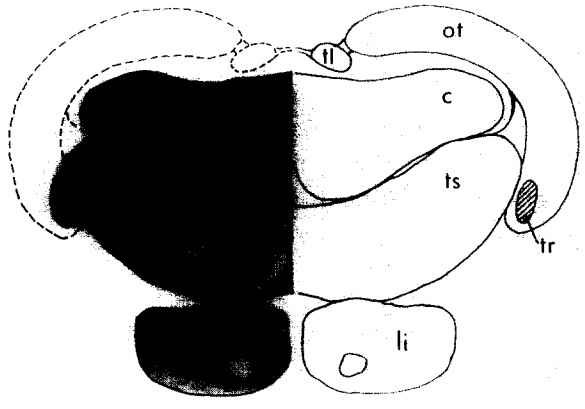

Fig. 3. Transverse section through the right half of the brain of a fish which showed recovery of vision following bilateral removal of the optic tectum and torus longitudinalis. The section is from the center of the optic tectum; c, cerebellum; li, inferior lobe of hypothalamus; ot, optic tectum; tl, torus longitudinalis; tr, tectal remnant; ts, torus semicircularis.

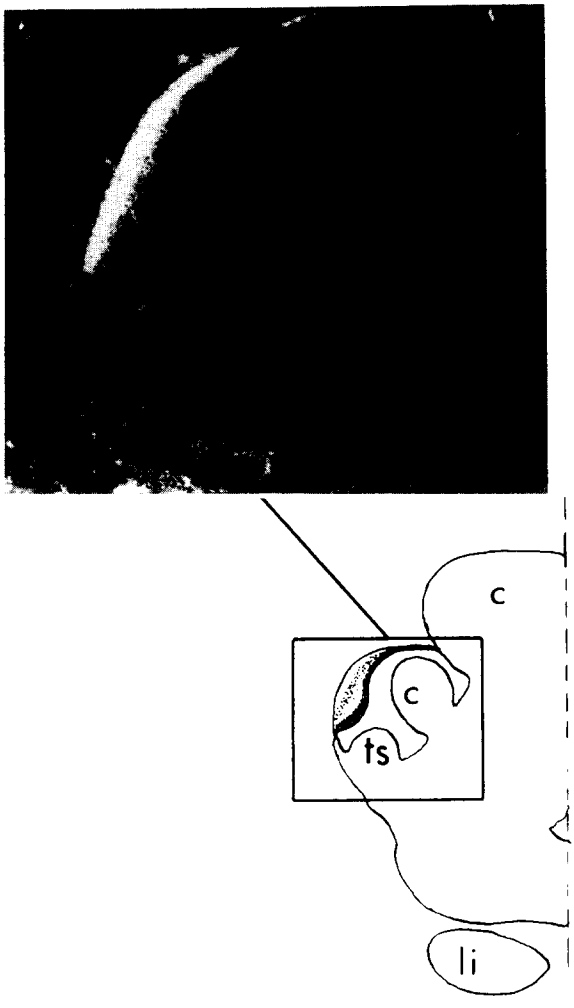

Fig. 4. Dark-field photograph of an autoradiogram of a transverse section through the right half of the brain of a goldfish that was sacrificed 6 weeks following bilateral optic tectum ablation. This section shows a densely labeled layer of optic axons and terminals in a remnant of the extreme caudal end of the optic tectum.

DISCUSSION

The visually evoked, conditioned BSR was maintained or restored within several weeks following bilateral removal of the optic tectum. Our experiments suggest that the recovery might be produced by postoperative conditioning which establishes or strengthens conditioning of non-tectal visual input and that the fish are not blind to the moving-spot CS light. Yager et al.[26] reported that the BSR to a flashing-spot CS is blocked in every fish for up to several weeks and suggested that visual recovery results from optic axon regeneration. We found no decrease in threshold of response to the moving-spot CS during the first 6 weeks following surgery. This implies that if regenerating axons add to the optic innervation of non-tectal visual nuclei, they do not materially increase behavioral sensitivity to the CS in our study. The regenerating axons would presumably have to compete with the resident optic afferents for targets[4], or wait for additional synaptic sites to be formed. The behavioral data similarly rule out regeneration of the optic tectum or of retinotectal connections as determinants of visual sensitivity in tectal ablates. Remnants of the margin of the caudal tectum might contain germinal cells, which are normally located where the PGZ meets the ventricle[16]. We could not distinguish germinal cells from neurons of PGZ, possibly owing to the limitations of visualizing cells in paraffin-embedded material, but it is conceivable that neurons were added to the outer edge of the tectal remnants during the 6-week experiment[23].

The fact that tectal remnants were present in some fish while threshold measurements remained relatively constant across most fish implies that retinotectal circuitry was not a significant determinant of the threshold. Reports on tectal ablation in goldfish by others[22,26] indicate that no tectal tissue remained following the aspiration procedure but the brain sections that were illustrated are mainly rostral to the level where we detected the tectal remnants. In pilot studies, a tectal ablate was seen to respond in light, indicating that visual sensitivity was normal or nearly so, and that the fish were found to have retained a strip of ventral lateral tectum. However, the remnant in that case was well-formed and of normal thickness and much larger than the example shown in Figs. 3 or 5.

How the non-tectal pathways might participate in the original conditioning or in retention of the CS–US association is unclear. If the non-tectal pathway in the light-adapted intact fish is insensitive to the CS illumination, as it seems to be in the tectal ablate, the conditioning might be mediated primarily by the optic tectum. Tectal efferents

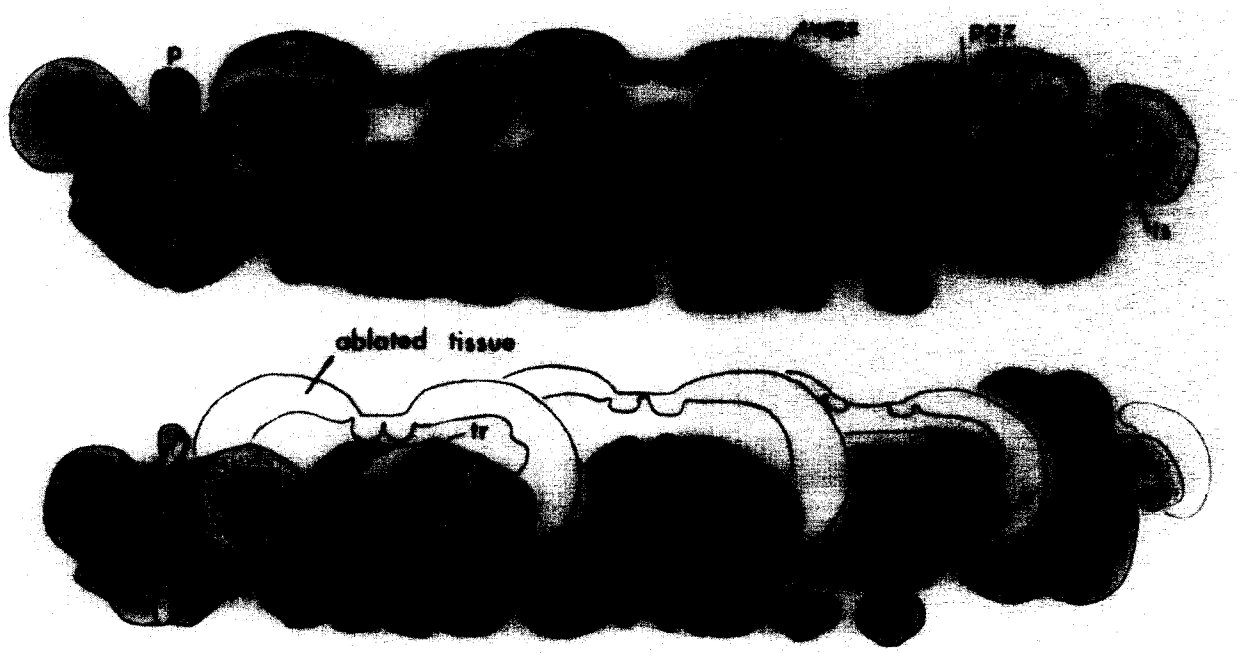

Fig. 5. Drawings of autoradiograms of transverse sections of an intact reference brain (top) and of the brain of an experimental fish that was sacrificed 6 weeks after bilateral optic tectum ablation. Arranged from left to right are sections from the rostral to the caudal end of the tectum. The stippled areas show the location of the most dense concentrations of labeled optic axons and terminals of the retinotectal projection. The principal terminal field is in SWGZ. In the experimental brain, heavily labeled optic axons formed a disorganized mass in the rostral and central midbrain floor and could be traced into many brain areas (not shown) including the remnants of the margins of the optic tectum.

project to the pretectum and thalamus and the majority of non-tectal nuclei that receive retinal input also receive tectal input (review, Northcutt[12]). Thus, conditioning of the CS may normally entail not only the tectum but non-tectal nuclei as well. It is possible that the learning also occurs in lower brain areas such as the cerebellum[9,10]. The teleost telencephalon has been shown to be unnecessary for classical conditioning (review, Overmier and Hollis[15]).

The report of Yager et al.[26] that the non-tectal visual system is comparatively insensitive to a flashing white-light CS is supported by our finding that the BSR to the moving red-light CS in ablates is blocked in the presence of an adapting light. Measurements made in studies that are in progress show that less than 0.2 cd/m^2 of diffuse, white overhead illumination is sufficient to block response to the LED when it is placed near the optic axis 30–35 cm from the eye. Some fish respond after as little as 20–30 min in darkness but response threshold decreases with increased time in darkness for ca 2 h. In contrast to the results for the BSR to a visual CS, tectal ablates show normal visual sensitivity in response to a moving field of black and white stripes which evokes optokinetic nystagmus[22]. Also, the dorsal light reaction is reported to persist following a blockade of retinotectal input by optic tract lesions[7], which suggests that non-tectal circuits are sufficient, but this is inconsistent with the finding that the reaction does not occur in tectum-ablated goldfish[22].

The possibility that the BSR was evoked extraretinally, for example by photic stimulation of the pineal complex[3], was ruled out by the finding that optic nerve crush resulted in loss of response followed by recovery within 2–3 weeks. The recovery times were similar to or slightly shorter than those that occur following optic nerve crush in fish that have an intact optic tectum and are tested in the presence of an adapting light[5].

Functional reinnervation of the non-tectal nuclei should entail shorter axonal outgrowth than is required to reach most of the tectum. For tectally mediated vision, the distance between the optic fiber lesion and the tectum can be a major determinant of the time to reappearance of vision[7]. Also, the axons that restored vision may have included ones of retinal ganglion cells which had been axotomized during the tectal lesion. Removal of the tectum could thus act as a conditioning lesion on the ganglion cell and result in decreased time to axonal outgrowth and, thereby, the time of reappearance of vision following the nerve crush[6,7].

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