

Short Communication

Spatial discrimination in goldfish following bilateral tectal ablation

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Goldfish were classically conditioned to discriminate between right and left or nasal and temporal presentations of a spot-of-light conditioned stimulus (CS). After the conditioning, the fish were administered bilateral optic tectum ablation followed by weekly sessions of conditioning trials to test for retention or relearning of the discrimination response. As their behavioral photosensitivity is greatly decreased, the ablates were dark-adapted prior to each session and trials were administered in darkness. Right \times left discrimination was retained postoperatively but the nasal \times temporal discrimination was blocked. Sham-operated controls discriminated between the nasal and temporal CS when dark-adapted and tested in darkness. Subsequent transection of the optic nerves obliterated response to the CS, indicating that tectum ablates detect and respond to the CS retinally and not extraretinally. We conclude that memory of visual spatial learning is mediated by non-tectal brain structures and that the ablate can discriminate between right- and left-eye input but sees the CS too diffusely to distinguish its location within the monocular field.

Goldfish retinal ganglion cells project to several small nuclei in the diencephalon and the pretectum but mainly to the optic tectum that forms the entire roof of the mesencephalon^{4,10}. Bilateral ablation of the tectum produces decreased behavioral sensitivity to spot-of-light stimuli that are classically conditioned to electric shock^{7,11}, as well as blockade of whole body orientation responses to dorsal illumination, food objects and optomotor stimuli⁸. These results show that non-tectal brain areas can mediate memory of visual learning, but that the tectum may be necessary for perception of visual images and their location in space. The retinotopic distribution and laminar organization of the retinal connections and the

extensive interconnections with other brain areas also suggest that the optic tectum is responsible for processing visual spatial information and integrating it with input from other sensory modalities. Non-tectal visual pathways may be organized for processing some kinds of spatial information. Studies in a cichlid, *Haplichromis*, show that individual visual nuclei in the diencephalon and pretectum vary in receiving axons from different sectors of the retina⁶. A striking behavioral finding has been that tectum ablation blocks the swimming response to an optomotor stimulus but not optokinetic nystagmus⁸. The implication is that the tectal ablate can detect moving images.

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The present experiments examined whether the visual centers that remain following removal of the optic tectum permit goldfish to discriminate between the right (R) and left (L) visual fields and between the nasal (N) and temporal (T) zones of the right visual field. Discrimination was measured using the branchial suppression response (BSR) to a classically conditioned visual stimulus as an index of vision. The conditioned stimulus (CS) was a stationary spot of red light. The positive CS was reinforced by the delivery of an electric shock unconditioned stimulus (US). Some of these experiments have been briefly reported³.

Fish and apparatus. Goldfish (*Carassius auratus* L.), 8–12 g, obtained from Ozark Fisheries, Stoutland, MO, were maintained in individual home tanks at 30 °C. The fish were conditioned individually in glass tanks which were illuminated from above by cool-white fluorescent lamps. The methods of maintaining the fish and of conditioning and recording the BSR are described elsewhere^{1,2,7}.

The CS consisted of red diode illumination (1600 cd/m²) that was presented for 5 s. Three diodes were placed aside the fish holder, level with the horizontal axis of the window which framed the fish's field of view (Fig. 1). Each diode was at a viewing distance of 34 cm⁵. One on the left and one on the right, each located 60° from the longi-

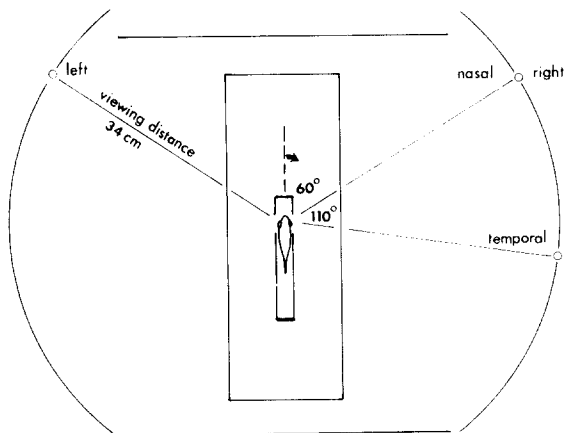


Fig. 1. Plan of the arrangement of the diodes that produced the right and left and the nasal and temporal CSs. The diodes were placed against a screen of opaque white plastic which blocked the fish's view to 18 cm above the horizontal axis of the window of the fish holder.

tudinal axis of the fish holder, provided the right and left CSs. The third was located more temporally in the right visual field, at 110° from longitudinal axis of the holder. The two diodes on the right provided the nasal and temporal CSs. The electric US was delivered between two electrodes placed on opposite sides of the fish's body.

Discrimination training and testing. Intact fish were administered semi-weekly sessions of discrimination training consisting of 3 S⁺ and 15 S⁻ trials that were presented automatically by a microprocessor in pseudorandom order. At the end of each session the fish received 3 S⁺ and 3 S⁻ test trials. The test trials were initiated by the investigator at a time when the fish's branchiogram was stable to record the fish's BSR. Digital conversion of the analog branchiogram signal² was used to measure the percent suppression of branchial activity in the 5-s CS interval (B) of the trial in relation to the activity in the 5-s interval preceding the CS [BSR = 100 (1 minus B/A)]. Intact fish screened for stable discrimination behavior were randomly assigned to experimental groups which were matched for similar mean BSRs (see Baseline data, Figs. 2,3).

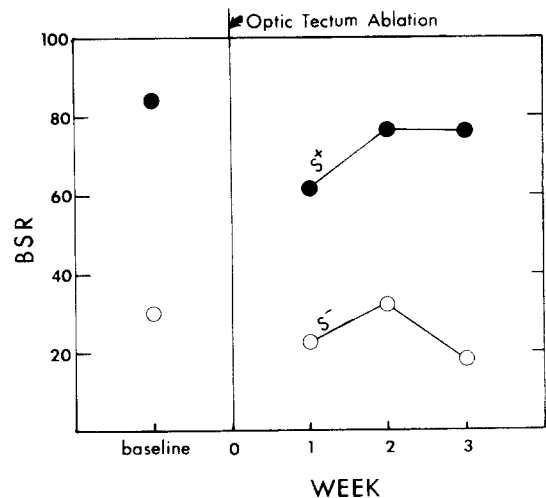


Fig. 2. Discrimination of right from left CS presentations before and after bilateral optic tectum ablation. Either the right or the left nasal CS was reinforced (S⁺), in different fish. The opposite CS was never reinforced (S⁻). The BSR is the percentage change in branchial activity during the 5-s CS-US interval in relation to the activity in the 5-s interval preceding the CS. The lesion did not significantly impair the discrimination behavior.

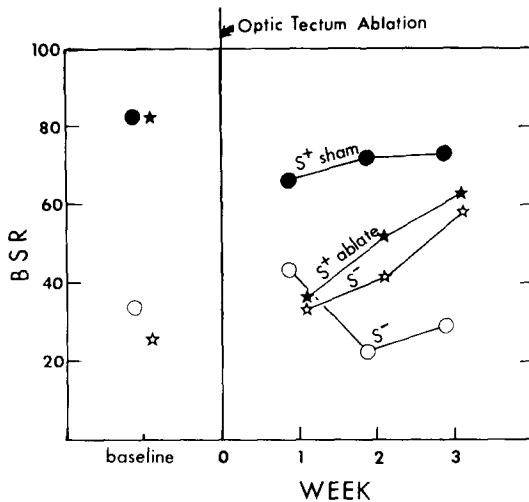


Fig. 3. Discrimination of nasal from temporal CS presentations before and after bilateral optic tectum ablation (stars) or the sham operation (circles). Either the nasal or the temporal CS was reinforced (S^+), in different fish. The opposite CS was never reinforced (S^-). The shams showed similar discrimination behavior pre- and postoperatively. The ablates continued to respond but did not discriminate postoperatively.

Optic tectum ablation and postoperative testing.

Nine fish that had been trained in the $R \times L$ task received bilateral optic tectum ablation¹. Seven of the fish trained in the $N \times T$ task received tectum ablation and an additional 5 were administered a sham operation. During surgery, the fish was anaesthetized with buffered trimethane methyl sulfonate (Sigma). The tectum was aspirated through a small opening that was cut in the roof of the cranium. The sham operation consisted of the craniotomy in which the cranium was opened and closed without otherwise disturbing the brain. The day of surgery was designated as Day 0.

After surgery, the fish were returned to the home tank to recover for one week prior to receiving a sequence of weekly sessions of discrimination trials. The trial procedure was the same as in the preoperative sessions with the essential exception that the fish was dark-adapted for 2 h prior to the session and the trials were administered in darkness. Less than 0.2 cd/m^2 of diffuse, cool-white fluorescent illumination, from overhead, blocked response to the 1600 cd/m^2 in tectal ablates.

Retention of $R \times L$ discrimination. All the $R \times L$ fish responded from the initial postoperative session (Fig. 2). A two-way ANOVA revealed a significant Discrimination effect, that is, a difference in response to S^+ and S^- ($F_{1,8} = 169.70$, $P < 0.001$), and also showed that there was no significant variation in the BSR over the sequence of postoperative sessions ($F_{2,16} = 1.49$, $P = < 0.25$). Thus, the optic tectum is not necessary for memory or performance of conditioned discrimination of right- versus left-eye stimulation from the CS light.

Loss of $N \times T$ discrimination. The $N \times T$ tectal ablates responded to S^+ and S^- (criterion, $\text{BSR} > 40\%$), but they did not discriminate between them (Fig. 3). Since fish were free to move 1–2 cm forward and backward in the holder, and the darkness would prevent the use of visual landmarks to discriminate N from the T , intact or sham operated fish might also show decreased discrimination in darkness. For example, fish might discriminate the N stimulus in part by its being rostral to the unlit diode holder at location T . The diodes would be continuously visible against the uniform, white background when the tank was lit (Fig. 1). If so, the change to administering trials in darkness should disrupt the $N \times T$ discrimination in intact fish. We found, however, that sham-operated fish performed the $N \times T$ discrimination in total darkness (Fig. 3). Thus the impaired $N \times T$ discrimination of the ablates was due to removal of the tectum and not to the change to conditioning in darkness.

The data for the $N \times T$ shams and ablates were contrasted in a multiple ANOVA. There was a significant Discrimination effect ($F_{1,20} = 66.22$, $P < 0.001$) and a Group \times Discrimination interaction ($F_{1,20} = 37.36$, $P < 0.001$), which reflects the lack of discrimination by the tectal ablates. The mean BSR of the ablates seemed to increase with postoperative sessions. There was no significant Session effect ($F_{2,10} = 1.83$, $P = < 0.18$) but the Session \times Discrimination interaction was marginally significant ($F_{2,10} = 4.40$, $P < 0.02$).

The equivalence of the N , T , R and L stimuli as CSs. We investigated whether uncontrolled differences in the illumination between LEDs could account for the discrimination behavior. Since

ablates responded similarly to N and T stimuli, though only one was reinforced, we could infer that there were no significant differences between the two stimuli. By the third postoperative session, responses to N and T were strong (BSR = 80–90%) in 3 ablates, intermediate in two (50–60%), weak in one (40–50%), and one fish showed no response indicative of light detection (BSR < 40%).

To assess whether the R and L stimuli differed as CSs, the R × L ablates were administered a session of 10 R⁺ and 10 L⁺ trials during the fourth postoperative week. The 20 trials were delivered in pseudorandom order by the microprocessor. At the end of the session, the investigator administered 3 R⁺ and 3 L⁺ test trials to measure the BSR. The mean BSR was 77% for R⁺ and 71% for L⁺, and the difference was not significant ($t = -1.68$, $r = 0.88$, $P > 0.05$). Thus the R and L stimuli were similar.

As a further test for uncontrolled differences between the diodes as stimuli we measured the 50% response threshold for the R and L diodes, using the 3 strongest responders (BSR = 80–90%), and for the N and T diodes, using the entire N × T group ($n = 9$). Threshold was measured by a staircase method in which the light intensity in different trials varied from ca. 1 to 1600 cd/m² by varying the electrical current⁷. The data for individuals were reduced by estimating the luminance which resulted in a response in 50% of trials. The mean threshold was 2.5 cd/m² for the N and also for the T diodes, and 3.2 and 3.4 for the R and L diodes, respectively. Similar thresholds were seen in previous studies in tectal ablates⁷. These results indicate that there was no behaviorally significant difference in illumination between LEDs.

Control for extraretinal cues and verification of the tectal lesion. Three ablates that responded strongly to the R and L stimuli (R⁺, L⁺) and three that responded to the N and T stimuli received optic nerve transection immediately following the behavioral test session of the fourth postoperative week. The fish was anesthetized during the optic nerve surgery¹, and returned to the home tank to recover for one week prior to administration of trials to measure the response to

the CSs. Neither group responded (BSR > 40%), indicating that the BSR was not evoked by extraretinal photic or non-photoc cues that might be associated with the CS¹.

After the session the fish were killed by immersion in anesthesia and the head was fixed in alcohol-formalin-acetic acid for one week. The fixed head was partially dissected to expose the brain and then embedded in paraffin to obtain 10- μ m thick transverse sections of the diencephalon and mesencephalon. The sections were mounted on glass slides and stained with Cresyl violet acetate. Representative sections from the rostral, central and caudal third of the optic tectum region were examined microscopically. No intact tectum could be seen (Fig. 4). Two brains had remnants of the extreme ventral edge of the tectum on one side. Similar remnants were detected in previous experiments⁷ and whether they are functional is unclear.

Interpretation. Since tectal ablates showed good retention of the R × L discrimination, the impairment of the N × T discrimination can be attributed to impaired visual input or perception rather than amnesia. The fish may see the CS only as a diffuse field of light or scattered patches of light and not as a spot of light. This would permit discriminating whether the CS is in the right or left visual field but not where it is in the monocular field. In support of this conclusion, in a pilot study with defective fish holders, we found that ablates initially did not discriminate N from T but appeared to relearn the discrimination after several postoperative sessions of conditioning trials³. Transection of the right optic nerve, to test for monocularly and for extraretinal cues, revealed a light leak in the front of the holder that permitted the fish to see the N but not the T stimulus with the opposite eye. When the leak was blocked, the discrimination disappeared, indicating that following their surgery the ablates had learned the N × T task as a L × R task.

The foregoing results suggest that the organization of retinal input to non-tectal visual centers is sufficient for discriminating right- from left-eye stimulation but not for localizing a small spot of light in the monocular field. The major non-tectal targets of retinal input include the preoptic nu-

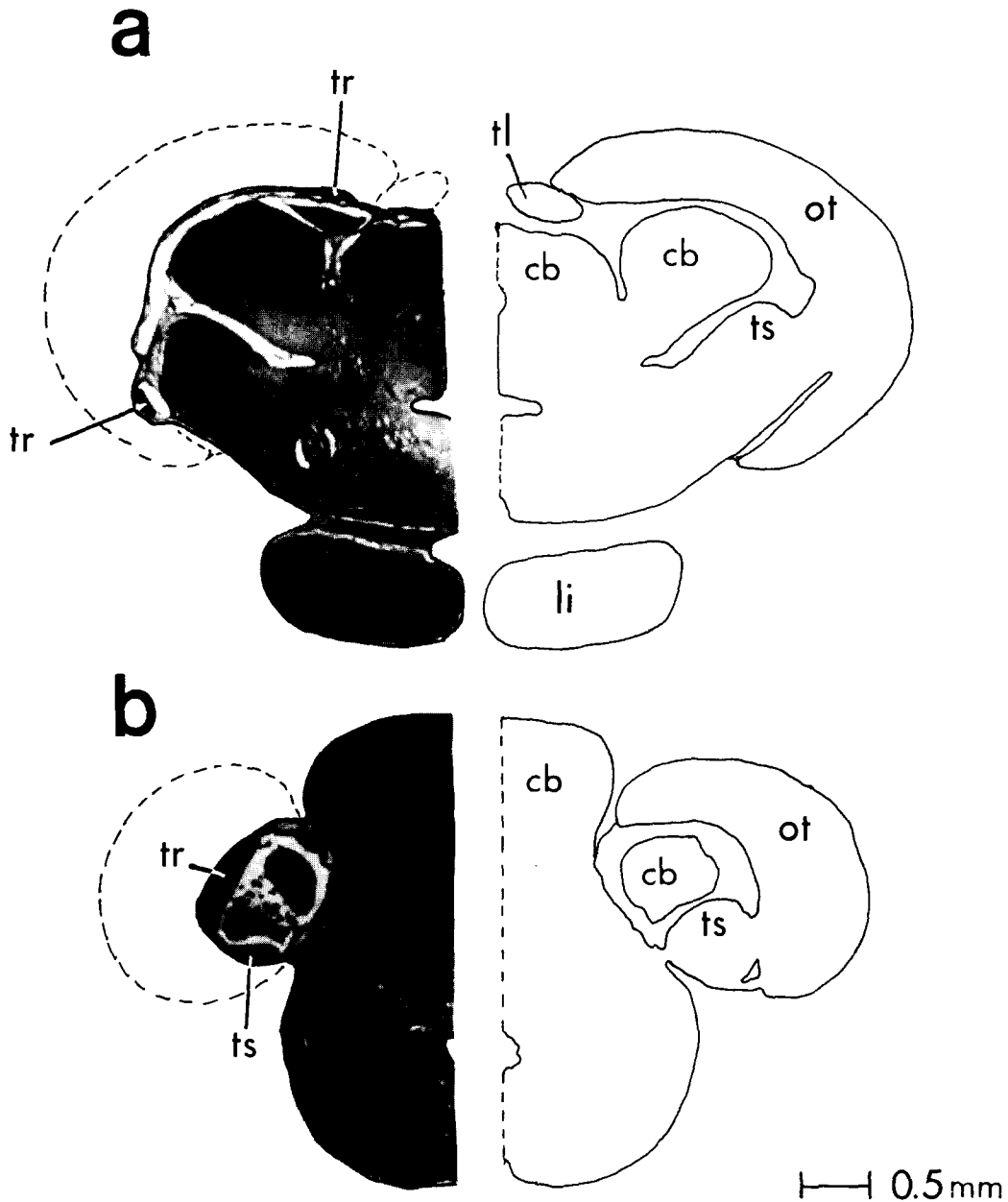


Fig. 4. Photomicrographs of transverse sections through the right half of the brain of a representative bilateral tectum-ablated goldfish showing the extent of the tectum lesion. The outline drawing is of a corresponding section of a reference brain. Section (a) is from the center of the tectum region. The ventral and dorsal remnants of the tectum merge caudally as shown in section (b); cb, cerebellum; li, inferior lobe of hypothalamus; ot, optic tectum; tr, tectal remnant; ts, torus semicircularis.

cleus, and the dorsomedial, ventrolateral and intermediate thalamic nuclei, which are innervated bilaterally, and the lateral geniculate nucleus and nucleus rotundus, which are innervated only contralaterally⁹. Anatomical mapping of non-tectal retinofugal projections in the cichlid, *Haplo-*

chromis, suggests that fish pretectal nuclei receive input from all regions of the retina and that the innervation is organized retinotopically⁶. In our study, the tectum lesion may have damaged pretectal nuclei. In goldfish examined 6–8 weeks after bilateral tectum ablation, the pretectum

region tissue is disorganized and contained many regenerating optic axons⁷. Therefore, we cannot rule out the possibility that pretectal nuclei can mediate spatial discrimination.

The finding that the non-tectal visual pathways are insensitive to the CS illumination in light-adapted ablates¹ suggests that the original learning of the R × L discrimination in light-adapted intact fish is mediated primarily by the optic tectum. As most of the non-tectal nuclei that receive retinal input also receive projections from the optic tectum⁴, non-tectal nuclei could be involved in processing the CS-US association⁷. Whatever the mechanism, after the tectum is removed, goldfish show retention of a classically conditioned visual discrimination.

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