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Visual recovery in goldfish following unilateral optic tectum ablation: evidence of competition between optic axons for tectal targets

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Anatomical studies suggest that regenerating optic axons which invade the ipsilateral lobe of the optic tectum following ablation of the contralateral lobe compete with resident optic axons for synaptic sites on tectal neurons. Invader optic axons are initially uniformly distributed over the entire tectal lobe. With time, the invader and resident optic axons progressively segregate so that the invaders are localized in bands or islands separated by areas that are innervated mainly by the residents. When the resident optic axons are destroyed by ablating the eye opposite to the experimental eye, the invader axons remain continuously distributed and the segregation process apparently does not occur. We investigated the relationship between the segregation process and the recovery of visual function by the invader axons. Visual recovery was measured with a behavioral method in which the index of vision was the occurrence of a branchial suppression response to a moving spot of red light that was classically conditioned to an electric shock stimulus. The minimum time to reappearance of vision following ablation of the contralateral lobe of the tectum in two-eye fish was similar to the reported time of onset of the segregation process. Visual recovery occurred sooner when the opposite eye was removed. The restored vision in both groups disappeared following subsequent ablation of the remaining lobe of the tectum. These results suggest that the goldfish optic tectum normally contains no free synaptic sites for anomalous optic afferents and that the invader axons must compete for targets with the resident optic afferents. The invader axons can apparently remain unconnected or non-functional for several weeks following their arrival in the ipsilateral tectal lobe.

In normal goldfish, the majority of the ganglion cells of each retina project to the contralateral lobe of the optic tectum and their axons terminate retinotopically over the entire lobe. When one tectal lobe is ablated, the optic axons that are thereby severed grow across the midline of the brain and terminate in the ipsilateral lobe¹³. The so-called induced ipsilateral retinotectal projection has been studied electrophysiologically^{7,13} and anatomically^{8,18}. The picture emerging from these studies is that within 4 weeks postaxotomy (30 °C) the invading optic axons extend over the entire tectal lobe and are relatively uniformly dis-

tributed with the resident optic axons from the control eye. At approximately 7 weeks the two axon populations begin to segregate and each becomes discontinuously distributed over the tectal lobe. The segregation appears to be complete at 10–12 weeks when the invader axons are localized in distinct bands or islands of irregular width that are separated by areas containing mainly resident axons.

The visual function of the invader axons has received little attention. A behavioral brain lesion experiment in 3 goldfish suggests that 18 weeks following contralateral tectal lobe removal the

induced retinotectal projection can mediate visual behavior^{22,23}. The present study was carried out to investigate the possibility that visual recovery occurs after segregation ensues (7 weeks; 30 °C) and not before, which would support the thesis that the segregation phenomenon is a manifestation of competition between the invader and resident axons for synaptic sites^{7,8,18}. The competition thesis also predicts that removal of the resident axons facilitates synapse formation or recovery of function by the invader axons. Recovery of vision was assessed with an objective psychophysical technique, similar to those used by others^{11,22} that permits monocular and sequential testing in each fish^{3,4}. The index of vision was a branchial suppression response (SR) that was evoked by a moving spot of red light (the CS) that was classically conditioned to an electric shock unconditioned stimulus (US). The response is denoted as the moving-spot SR.

Goldfish (*Carassius auratus L.*), 8–12 g, obtained from Ozark Fisheries, Stoutland, MO, were kept in individual home tanks at 30 °C as previously described^{3,4}; the water temperature was similar to that used by Springer and Cohen¹⁸. The experiments were carried out during the months of August through December. Fish were conditioned individually in 3 glass tanks, 15 × 15 × 30 cm, the water being changed at the start of each session⁴. Branchial ventilation movements were detected by the thermistor method^{2,11}, the amplified thermistor signal being recorded on an ink-writing polygraph in a separate room. The CS consisted of the flickering movement of a spot of red light produced by alternately illuminating two light emitting diodes (LEDs) that were centered one above the other 1 cm apart⁴. Similar pairs of LEDs were submerged in the water opposite each eye. The lower LED was kept lit during the intertrial interval. The duration of the CS interval in a trial was 2- or 5-s during which the upper and lower LEDs were illuminated alternately every 250 ms. The US was a 0.5-s pulse from 7 to 8 mA (RMS) 60 Hz constant current that was passed between two steel electrodes on opposite sides of the fish holder.

To condition fish to respond to the CS, they were administered a sequence of two or three

sessions of conditioning trials over a period of 1–3 weeks⁴. The CS was turned on for 2- or 5-s and the US was presented at the instant that the CS was terminated. The 2-s trials were initiated automatically at random intervals and the CS was presented to the R or L eye in pseudorandom order. The fish's branchial SR to the CS was measured in the 5-s trials using the tachograph method³ or digital conversion of the analog of the branchiogram. In the digital representation method a microprocessor was used to measure the length of the branchiogram trace during the 5-s CS–US interval (B) and during the 5-s interval (A) preceding the onset of the trial. The fish's SR was expressed as the percentage change in the length in interval B relative to the length in A, $SR = [100 \times (1-B/A)]$. A deceleration of greater than 30% was accepted as a SR indicative of visual detection of the CS³.

Fish that responded with each eye subsequently received a test session consisting of 4 2-s warm-up trials followed by 4 5-s test trials. A blank trial was administered prior to each test trial. Fish that responded in each of the 4 test trials and showed no false positives in blank trials were accepted as subjects^{3,4}. The preoperative test session was administered 2–7 days prior to the day of surgery which is designated as experimental Day 0.

The fish received right (R) optic tectum ablation (UOT, n = 8) or UOT and enucleation of the R eye (UOT-X, n = 9). Anesthesia was by immersion in 0.04 percent trimethane methyl sulfonate (Sigma), buffered with Tris fish buffer (Sigma) to pH 6.5–7.5. The tectum was exposed by making a 3 sided incision in the dorsal cranium, two medial-lateral cuts and one rostral-caudal cut, forming a bone flap. The R tectum was removed by aspiration. The flap was repositioned and sealed with Permabond superglue. After the tectal surgery, the R eye was removed in the UOT-X fish. Fish were returned to their home tanks to recover.

The fish's response in R and L trials was tested weekly beginning on Day 7. The procedure was the same as in the preoperative test session. Initial recovery of L-eye vision in individuals was inferred when the fish responded (SR > 30%) in L trials in two successive sessions and the day of

the first session was designated as the day of recovery. The effects of the two surgical manipulations on the time to visual recovery were analyzed in a *t*-test contrasting the mean recovery times in the two groups.

Following visual recovery, 5 UOT and 4 UOT-X fish received L-optic tectum ablation to investigate whether the restored L-eye vision was mediated by optic axons projecting to that structure. The bilateral tectal ablates (BOT) were returned to the home tank to recover for 1 week prior to receiving the first of two weekly test sessions. All fish, including the remaining four UOT and 4 UOT-X-fish, were then sacrificed by immersion in the anesthetic and their heads were fixed in alcohol-formalin-acetic acid. Brains from several fish in each group were embedded in paraffin to obtain 15 μ m-thick transverse sections of the optic tectum to evaluate the completeness of the lesions.

L-eye responding was blocked in every fish during the first 3 weekly test sessions (Fig. 1). The UOT fish continued to respond with the R eye throughout the experiment. The UOT-X fish, which had no R eye, did not respond in R trials confirming that the moving-spot SR was evoked visually and not extraretinally³ and was monocular.

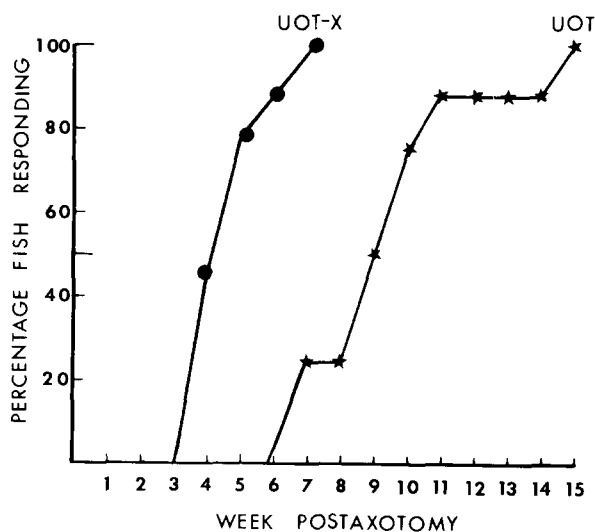


Fig. 1. The time to recovery of response with the L-eye following removal of the R-optic tectum (UOT) was decreased when the R-eye was also ablated (UOT-X).

As shown in Fig. 1, UOT-X fish regained L-eye vision within 4–7 weeks postaxotomy (WPA) ($x \pm s.d. = 34 \pm 7.38$ days) and the UOT fish regained vision within 7–15 WPA ($x = 68 \pm 17.3$ days). The difference in mean recovery time was significant ($t_{15} = 5.36$, $P < 0.01$). The UOT and UOT-X fish that subsequently received L-optic tectum ablation, producing BOT fish, ceased responding in L trials. The microscopical sections of the brain revealed that the R tectal lobe was removed in the UOT fish and that both lobes were removed in the BOT fish (Fig. 2).

The longer time to recovery of vision in UOT, as opposed to UOT-X fish, could in part be owed to the UOT fish's continued experience with the CS in R-eye trials. The time to formation of functional synaptic connections between the L-eye fibers and their targets in the L-optic tectum may depend in part on perceptual processes which act to inhibit responding to input from the

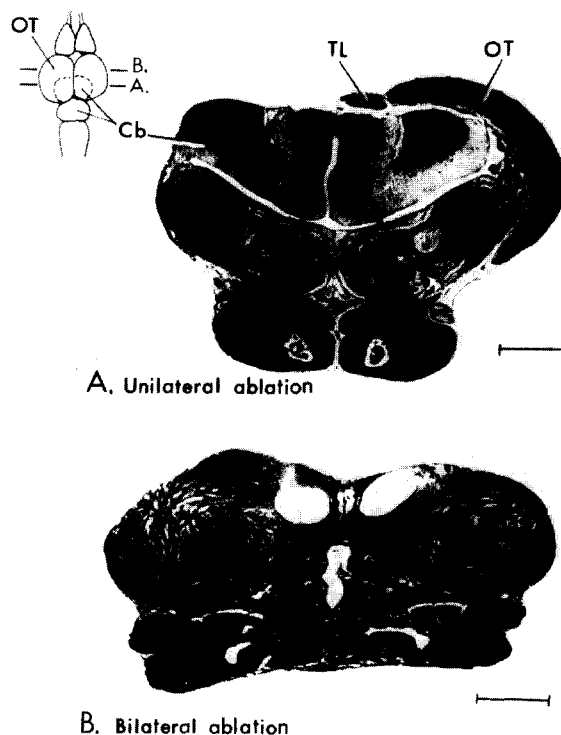


Fig. 2. Transverse sections (A,B) through the brain of representative UOT and BOT goldfish. A was caudal to B as illustrated in the diagram of the dorsal view of the brain on the left of the figure. Scale bar = 0.5 mm. Abbreviations: Cb, cerebellum; OT, optic tectum; TL, torus longitudinalis.

invader axons when the fish continues to receive input from the intact (R) eye.

To examine this possibility, 6 additional UOT fish were prepared, as described above, and administered weekly test sessions beginning 1 week following surgery. In the first 10 sessions the CS was delivered only to the L eye; during R trials the fish received the US but not the CS; this was similar to the experience of the UOT-X fish which were R-eye ablated. None of the 6 fish responded prior to 7 WPA and only one recovered at 7 WPA; this is the same result obtained in the UOT fish which received the CS in R trials. The other individuals recovered at 9, 10 or 12 WPA. The CS was reinstated in R trials beginning with the session at 10 WPA and every fish responded to it from the first trial.

The minimum time to reappearance of vision following ablation of the contralateral optic tectum (UOT fish) in our experiment is similar to the time of onset of conspicuous segregation of resident and invader optic axon terminations in the ipsilateral tectum reported by Lo and Levine⁸ and Springer and Cohen¹⁸. The similarity suggests that the segregation process is necessary to enable the invader axons to form synapses with tectal neurons. This conclusion is supported by the finding that removing the opposite eye (UOT-X fish) results in earlier recovery of vision, presumably by eliminating the resident optic axons and thus freeing postsynaptic sites to accommodate the invader axons. The distribution of invader axons in the ipsilateral optic tectum of UOT-X fish is reported to be continuous^{8,18} but there are inexplicable exceptions. Lo and Levine⁹ report a case of a UOT-X fish in which the distribution of radiolabeled invader axons was discontinuous. Autoradiographic studies in progress in our laboratory confirm that in a small proportion of UOT-X fish invader axons appear to be localized in irregular bands resembling the pattern seen in UOT fish 7–13 WPA. Further study of the segregation phenomenon and its relationship to the maintenance or recovery of function of optic afferents in the tectum is clearly needed.

In addition to the presence of resident optic afferents other factors may inhibit invader axons

from forming synapses in the ipsilateral tectum. Springer and Cohen¹⁸ reported that invader axons are distributed throughout the ipsilateral tectum within 3 WPA (30 °C). The UOT-X fish in our study regained vision between 3 and 7 WPA. The delay of recovery of function, following the arrival of regenerating axons in the tectum, is greater than we would expect based on studies in optic nerve lesion fish, in which regenerating axons regain their positions in the contralateral tectum. Following optic nerve crush, regenerating optic fibers fully reinnervate the contralateral tectum in 14–16 days (30 °C^{16,17}) and, in most fish, the moving-spot SR reappears within 14–21 days⁴. The implication for the UOT-X fish in our study is that if the ipsilateral tectum was innervated by regenerating optic axons within 3 WPA, the recovery of function of connections sufficient to mediate the moving-spot SR was delayed for several weeks.

Sperry¹⁵ induced ipsilateral retinotectal projections in amphibians. He observed recovery of visual behavior indicating that regenerating optic axons found and functionally innervated post-synaptic targets in the ipsilateral tectum. More recently, Misantone and Stelzner¹⁰ demonstrated competition between invader and resident optic axons in the optic tectum of frogs using behavioral methods. Recovery of function of invader axons following removal of one eye and the ipsilateral tectum (UOT-X) occurs earlier than in frogs that receive only the tectal ablation (UOT). Segregation of induced and normal retinotectal projections occurs in amphibians^{1,6}, but its relationship to the recovery of function of the invader axons has apparently not been investigated.

That removal of the R-optic tectum blocks responding with the L but not the R eye implies that the moving-spot SR is normally mediated by input to the contralateral optic tectum. Similar experiments by others suggest that most visual behaviors are tectally mediated^{5,19}. The possible role of retinal projections to the ipsilateral optic tectum or to non-tectal brain nuclei^{12,16,20,21} in the moving-spot SR or most other visual responses is unknown.

That the restored L-eye responding disappeared following removal of the L tectum indicates

that the recovery of function was mediated by regenerated optic axons which innervated that structure. Very little is known about the visual capacities of bilateral tectal ablates (BOT) and we are currently investigating whether long term ablates show recovery of response to various types of CS. Yager et al.²³ found that BOT goldfish kept at 25 °C show recovery of a branchial SR to a flashing spot CS within 3 weeks following the surgery but the threshold of response was greatly increased. The recovery was attributed to the establishment of connections between regenerating optic axons and non-tectal visual targets. Whether the restored flashing-spot SR was strictly visual, that is, evoked by retinal as opposed to extraretinal input³, or, if visual, a result of optic axon regeneration, as opposed to plasticity of undamaged visual system circuits, was not discussed. Regenerating optic axons that invade the valvula of the cerebellum and other non-visual areas in BOT goldfish are reported to terminate retinotopically¹⁴ but whether they form connections with postsynaptic targets that can mediate visual behavior remains to be investigated.

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