

# Optic Nerve Regeneration in Goldfish Under Light Deprivation<sup>1</sup>

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LEITNER, D. S., A. FRANCIS AND M. S. GAZZANIGA. *Optic nerve regeneration in goldfish under light deprivation.* BRAIN RES. BULL. 8(1) 105-107, 1982.—Regeneration following bilateral optic nerve crush was studied in groups of goldfish housed either on a standard diurnal cycle or under total light deprivation. Unoperated fish were included in each deprivation condition. Regeneration was only slightly delayed in the surgical group housed under light deprivation (68-81 days), compared to fish undergoing regeneration on the diurnal cycle (59-63 days). Visual capacity, as judged by days to meet criterion on a visual pattern discrimination task, was unrelated to deprivation condition, but significantly lessened in fish having regenerated optic nerves. These results are discussed in terms of visual acuity following optic nerve regeneration and mechanisms of axonal reconnection.

Regeneration	Optic nerve crush	Goldfish	Light deprivation	Pattern discrimination
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THE regenerative ability of the optic nerves in fish and amphibians has been well-documented. After surgical section of the optic nerve, animals with this ability regain vision upon reconnection of the optic nerve [1, 4, 5]. Anatomical and electrophysiological studies have indicated that there is specificity in the reconnection to the principal target, the optic tectum [2, 6, 8]. In addition, behavioral studies have shown that the reconnected optic nerve can subserve pattern and color vision [1,11] as well as mediate interhemispheric transfer of visual information [4]. The regenerated visual pathway of cichlid fish allows slightly less visual acuity than the intact system [11].

The apparent specificity of regeneration in the optic nerve led Sperry to postulate the existence of intrinsic chemical gradients that direct the regrowing axons to their appropriate targets [9,10]. If such a mechanism were operative, regenerating axons may not require extrinsic functional activity for reconnection. Surprisingly, this question has not been directly tested.

Jacobson and Hirsch [7] occluded the eyes of tadpoles at stage XIX with skin grafts to diminish pattern vision, and observed the development of ipsilateral and contralateral visual evoked responses in the tectum after metamorphosis. Monocular deprivation did not affect the development of ipsilateral retinotectal projections, but deprivation continued for several months led to enlarged multiunit receptive fields from both the intact and occluded eyes. Binocular deprivation, in contrast, did not produce detectable abnormalities even if continued beyond metamorphosis. Yoon studied the

reorganization of the retinotectal projection following partial tectal lesions in goldfish [12,13]. His data indicate that the compression phenomenon could occur under light deprivation.

These observations suggest that optic nerve regeneration can occur in the absence of extrinsic visual stimulation. The present report confirms optic nerve regeneration in goldfish maintained under light deprivation although the time required for restoration of vision is slightly greater than in fish maintained under standard diurnal conditions. In addition, impaired acquisition of a visual pattern discrimination task suggests a lessened visual acuity after optic nerve regeneration, which is not dependent on visual deprivation.

## METHOD

### Subjects

Eighteen goldfish (*Carassius auratus*), measuring 8-10 cm from snout to base of tail, were obtained locally. Nine fish were subjected to bilateral optic nerve crush. Of these, four were immediately light deprived by enclosing their home tank in three separate layers of opaque vinyl. A control group of four intact fish was light-deprived similarly. The only light these fish received was for approximately 30 sec each day when they were fed with Tetramin. Four other operated fish and five intact fish were housed on a diurnal cycle (12 hr light, 12 hr dark). All tanks were continuously aerated. Each group was housed in a separate tank.

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### Surgery

The fish were anesthetized by immersion in dilute tricaine (Finquel, Ayerst). Surgery was performed out of the water with the aid of a Zeiss operating microscope. A small notch was cut in the bone immediately above the eye, and the eyeball was pulled outward to expose the optic nerve, which was repeatedly crushed with a small forceps under direct visual control. The fish were tested at intervals for returning vision by their response to food baiting, presented on either side of the head. When the operated fish on the diurnal cycle had restored vision in both eyes, the two groups that had been light-deprived were removed from the vinyl enclosure, and then tested for vision. The intact light-deprived fish showed vision within eight hr after removal from the vinyl enclosure. When all fish had attained vision, discrimination training began.

### Apparatus

Fish were trained in a double-T maze using one pair of discriminanda as previously described ([4], Fig. 1).

### Procedure

The fish were taken individually from their home tanks and placed in the maze for training. They were initially habituated to the maze and then fed in it with a mixture of Tetramin and Gerber's beef baby food, delivered manually at the end of a fine blunt wire hook. When the fish were accustomed to the maze and would pursue the hook and strike at it on either side of their heads, brightness discrimination training was begun. The fish were given 14 trials alternating from one end of the maze to the other, six days a week [3,4]. The position of S+, an activated light bulb, was varied according to a Gellerman order. The criterion for learning was 12 correct responses on each of four consecutive days. The day following this criterion, pattern discrimination training began. The procedure was the same as above, but the fish were required to swim into the chamber containing one of the two stimulus cards. Fish were randomly assigned to the stimulus that would be their S+. A criterion of 44 correct responses over 4 days was used. No corrections were allowed.

### RESULTS

The days to meet the behavioral criterion of bilateral optic nerve regeneration for the two surgical groups are shown in Table 1. Fish undergoing nerve regeneration under light deprivation took significantly longer to regain vision (Mann-Whitney U,  $p < 0.01$ ).

The days to meet criterion on the pattern discrimination task are shown in Fig. 1. There was no significant difference between deprivation conditions for either intact or lesioned fish (Mann-Whitney U,  $p$ 's  $> 0.1$ ). However, the pooled scores for the fish with regeneration were significantly higher than those of the intact fish (Mann-Whitney U,  $p$ 's  $< 0.02$ ).

### DISCUSSION

The present results support the observation of Yoon [12,13] which suggested that the goldfish optic nerve regenerates and reinnervates the optic tectum after manipulation of the postoperative visual environment. However, in the present data (Table 1) regeneration is slightly delayed (from 61 to 71 days) under total light deprivation. These observations suggest that extrinsic visual stimulation is not neces-

TABLE 1  
MEDIAN DAYS TO REGAIN VISION FOLLOWING  
SURGERY (RANGE)

Allowed light	61 (59-63)
Light deprived	71 (68-81)

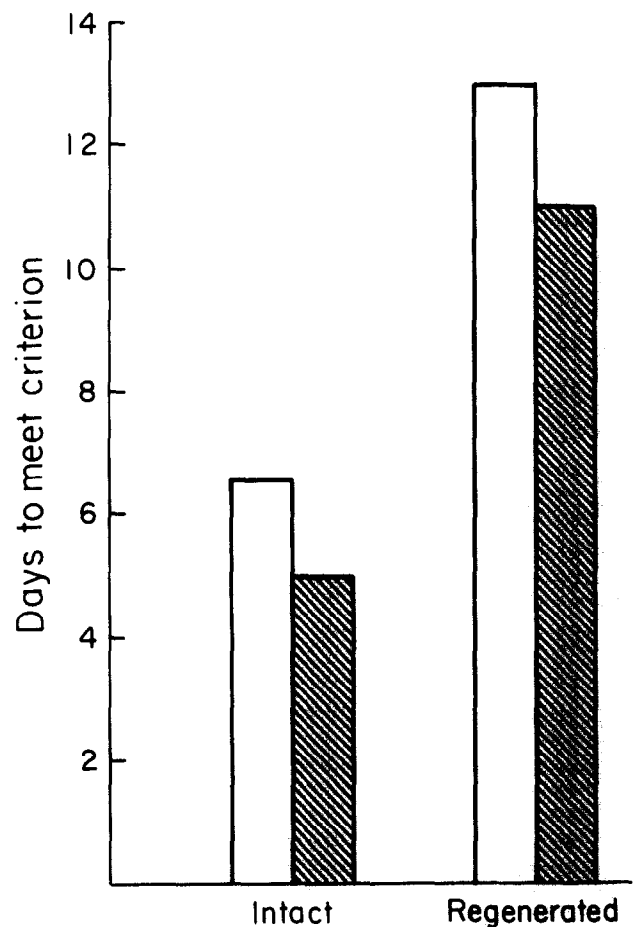


FIG. 1. Median scores (days to meet criterion) on a pattern discrimination task. White bars: fish housed on a diurnal cycle. Cross-hatched bars: fish housed under light deprivation.

sary for outgrowth and reconnection of optic nerve axons, but its presence speeds the process slightly. Regeneration that is independent of extrinsic visual stimulation is consistent with Sperry's observations [9,10] on the stability of optic reconnections in animals with rotated eyes. In these animals, functionally reversed (but topographically correct) connections persist. Thus, our data, together with that of prior workers [1, 2, 6, 7, 10], suggest that intrinsic properties of the retinotectal system are sufficient for axonal outgrowth and nerve reconnection.

Data from the pattern discrimination task indicate that goldfish with regenerated optic nerves require approximately twice the time of intact fish to meet a criterion of learning

(Fig. 1). Scores for intact or lesioned fish did not vary with the light deprivation condition, suggesting that the deficit is related only to optic nerve regeneration. Similar behavioral studies have been reported with cichlid fish. Weiler [11] found less acuity (78% of control) in cichlids after optic nerve regeneration. There was a slight, but statistically insignificant trend (in the two fish studied) toward a slower rate of learning a random vs orderly dot discrimination task. Arora and Sperry [1] showed normal rates of learning on color discrimination tasks in cichlids having optic nerve regeneration.

Since slower acquisition may reflect poorer visual acuity, the present data are consistent with an interpretation that goldfish, like cichlids, have diminished visual acuity after optic nerve regeneration. The acuity may be considerably compromised in goldfish, compared to the mild defect seen in cichlids. No study has been made of the receptive field sizes in optic tectum before and after regeneration in these two species. Jacobson and Gaze [6] did report, however, that one class of visually evoked response was absent from tectum after optic nerve regeneration.

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