Proc. Nat. Acad. Sci. USA
Vol. 69, No. 9. pp. 2637–2639, September 1972

Redistribution of Visual Projections in Altered Optic Tecta of Adult Goldfish

(retinotectal connections/neuronal specificity)

S. C. SHARMA*

Biology Department, Washington University, St. Louis, Missouri 63130

Communicated by Viktor Hamburger, July 17, 1972

ABSTRACT **The retinotectal connections formed in adult goldfish after removal of the rostro-caudal strip of dorsal tectum were mapped electrophysiologically. Initial maps recorded 1 month after surgery revealed visual scotoma corresponding to the ablated tectum. Subsequent maps recorded 4–5 months after the operation showed that the entire visual field redistributed appropriately over the remaining tectum. It is suggested that, in spite of an abnormal optic tectum, the reformed projection demonstrates a tendency to retain its completeness.**

The formation of selective synaptic connections in the visual system has been thought to be brought about by the development of specific chemoaffinities between retinal ganglion cells and the tectal elements on which they synapse (1, 2). The development of differential specificities in retinal ganglion cells occurs in the prefunctional stages (early tail-bud stages in amphibians) of neurogenesis (3–5). The prediction that each ganglion cell possesses a unique property was based on the results of eye rotation experiments (6): when an eye was rotated before tail-bud stages, normal retinotectal connections were found in an adult; however, eye rotation later than this stage resulted in an inverted visual projection that remained permanent (5, 6). Moreover, anatomical and electrophysiological experiments on optic-nerve regeneration with size disparities in the retina and the tectum of adult goldfish (7, 8) indicated that solely appropriate connections are formed, thereby suggesting a rigid form of place specificity that controls the regenerating optic axons.

The nature of retinotectal connections in an embryo, however, is not very rigid, as is quite apparent from experiments with surgically formed compound eyes in *Xenopus* (9–11). In these experiments, two nasal or temporal half-retinae at stage 32 in *Xenopus* were grafted together to form a compound double nasal or temporal retina. The optic fibers from each half-retina of the compound eye extended their fiber connections over the entire rostro-caudal tectum. In other words, a half-compound retina connected not only with the half of the tectum with which it normally would have connected, but also with the other half of the tectum.

In fact, the rigidity of retinotectal connections as postulated in the hypothesis of neuronal specificity has been found to be lacking, even in adult goldfish (12–14). After removal of caudal half-tectum from an adult goldfish, the entire visual field is distributed in an orderly fashion in the remaining rostral half-tectum (12, 13). Similar redistribution of retinal fibers leading to a topographically ordered projection after rostral tectal removal was recently reported (14).

The apparent contradiction between earlier work (7, 8) on retinotectal connectivity in adult goldfish, which suggested a rigid form of specificity controlling the regenerating optic fibers, and the recent results (12–14) in the same animal which suggest a plastic nature of these connections led to the formulation of the present experiments. A rostro-caudal strip of the dorsal tectum was removed (with and without simultaneous cutting of one optic nerve) and the resultant retinotectal projection was mapped electrophysiologically in a few animals after one month and in others 4–5 months after initial surgery.

METHODS

After anesthesia of each adult goldfish (*Carassius auratus*, 50 mm in length) in an aqueous solution of 1:1000M S222 (Sandoz; Tricaine), the animal was continuously perfused over the gills via a tube in its mouth with diluted tricaine solution. The skull was opened over the mid-brain with a dental drill and two deep longitudial rostro-caudal lesions were made on the dorsal tectum, separating the medial and lateral tectum. The cuts extended down to the ventricle; the excised tectum was then sucked out. The cranium flap was sealed in place with isobutyl–cyanoacrylate monomer (Ethicon). In some animals the contralateral optic nerve was crushed in the orbit at the time of tectal operation. After operative recovery, animals were kept for periods of 30–145 days and were then used for mapping the left visual field to the operated right tectum. After anesthesia and paralysis with tubocurine, the dorsal surface of the tectum was exposed and the eye was centered on the projection perimeter. The methods for recording action potentials and for the projection map were as described (12). At the end of each experiment, the head of the fish was fixed in Susa fluid, serially sectioned at 15 μm, and stained by Holms' silver method.

RESULTS

Retinotectal projection in normal goldfish has been described in detail by Jacobson and Gaze (16). The retinal projection map to the unoperated left tectum (Fig. 1) provided a control and allowed comparison of the maps obtained after surgical ablation to the right tectum. The normal map shows that the nasal field is represented on the rostral tectum and the temporal field on the caudal tectum. The central visual field represents the lateral edge of the tectum and the superior field projects to the medial edge of the tectum.

The visual projection to the operated tectum was mapped in ten animals at intervals ranging from 30 to 37 days after the initial operation. The contralateral optic nerve in four of these animals was also crushed in the orbit, together with removal of the tectum. In both cases, visual maps showed a clear evidence of deficit in the projection that corresponded with the area of the ablated tectum. One such case is shown in

---

* Present address: Department of Ophthalmology, New York Medical College, 1249 Fifth Ave., New York, N.Y. 10029.

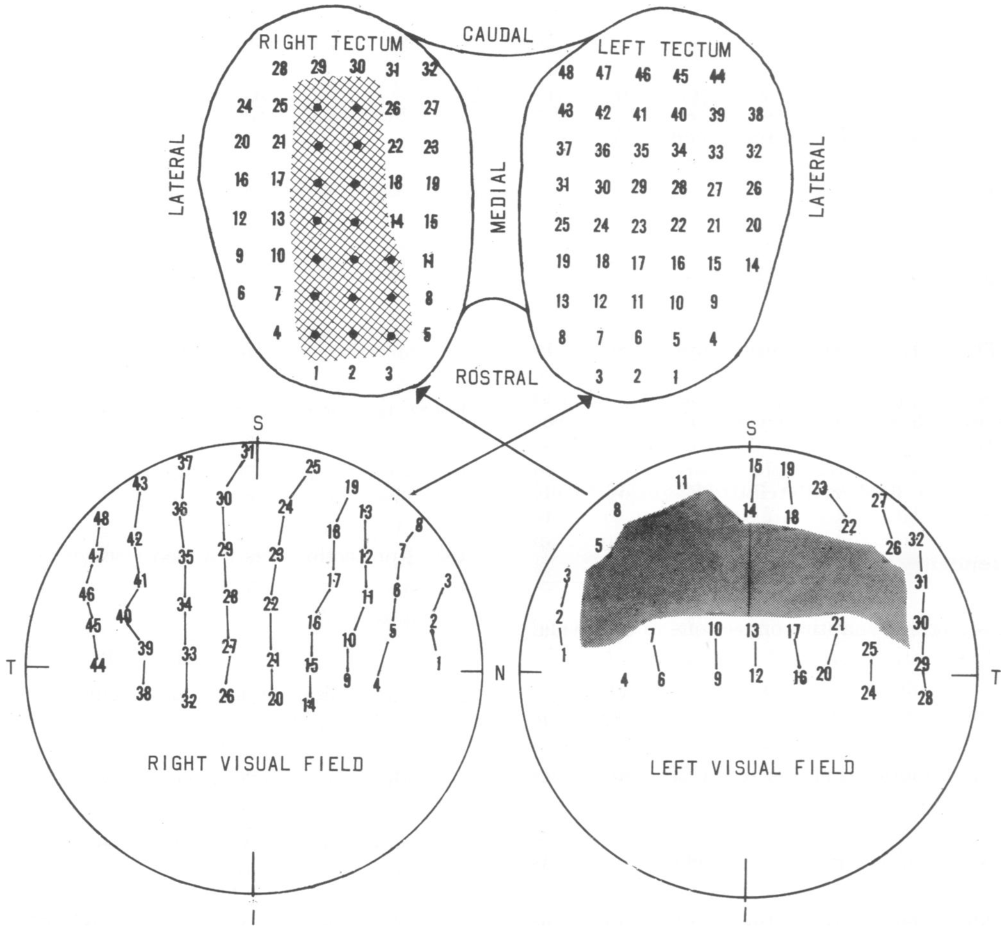

FIG. 1. The retinotectal projection 37 days after removal of the rostro-caudal strip of the right tectum. The projection from the right eye to the left (normal) tectum shows that the entire visual field is extended over the entire surface of the tectum. The *numbers* on the tectal diagram represent electrode positions, for each of which the corresponding optimal stimulus position is indicated on the chart of the visual field. The *stippled area* on the perimeteric chart of the left visual field indicates the region of visual field which, in a normal animal, would project to the *crosshatched* region of the right tectum. *Filled circles* on the right tectum represent electrode positions from which no responses could be obtained. The tectal electrode positions were spaced 200 μm apart. The perimeteric chart extends for 100° outwards from the center of the field. *S*, Superior; *T*, Temporal; *I*, Inferior; *N*, Nasal.

Fig. 1. In this case, normal projection was restored from the central and superior part of the visual field to the lateral and medical tectum, respectively. The fibers from nasal and temporal visual field retained their normal positions in the tectum. The results suggested that a normal projection to an intact tectum was restored and the fibers that should have gone to the ablated tectum failed to make tectal connections.

Furthermore, in nine other animals, the left visual projection to the operated right optic tectum was mapped at intervals ranging from 126 to 133 days after surgery. The contralateral optic nerve was crushed in three of these animals at the time of initial operation. The resulting maps were all essentially similar in their topographical organization of the visual field. One such result is shown in Fig. 2. In this case, there was compression of the visual field such that the entire field, which in the normal animal projects to the whole tectal surface, now projected over the remaining medial and lateral tectum—indicating restoration of the missing field projection. Thus, present experiments suggest that at early stages in the restoration of retinotectal projection, orderly connections appropriate to the original map are formed. However, in the later stages (maps recorded from different animals), the field projection that should have gone to the ablated tectum, together with the remaining normal projection field, is appropriately redistributed to compensate for the missing tectum.

DISCUSSION

The partial retinal ablation studies of Attardi and Sperry (7), and experiments involving disparities in the size of the retina and tectum in goldfish (8), provided strong evidence supporting the "specific chemoaffinity" hypothesis, which was presumed to be responsible for the selectivity of synaptic connections of retinal ganglion cells. Present results confirm, at least in part, the observations that retinal ganglion cells regenerate only to their correct terminal places in the tectum after partial tectal ablation. It was suggested by Jacobson (17) that retinotectal connections once determined in developing embryos (of frogs and fishes) lack modifications after surgical rearrangements. Recent reports (12–15, 18) suggest a lack of such rigid specificities in the visual system of adult goldfish.

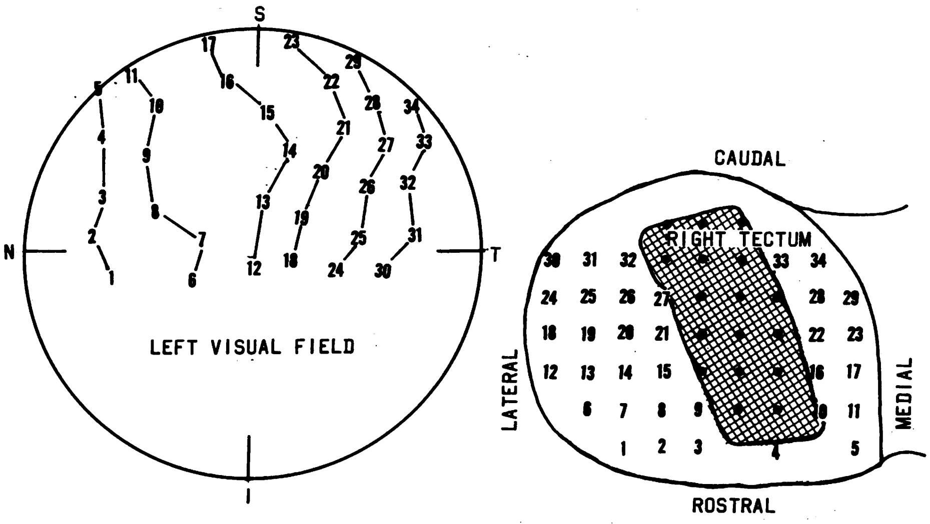

FIG. 2. Representation of the left visual field on the right optic tectum, mapped 131 days after the partial removal of the tectum (*crosshatched area*). The projection from the left eye to the operated right tectum covers the entire extent of the field, although the tectal geometry is appreciably altered. It should be noted that, although the spacing of tectal positions on the edges of the lesion (i.e., 9 & 10; 15 & 16; 21 & 22; 27 & 28; and 32 & 33) is 3–4 times that in the normal positions, which are 200 μm apart, the spacing of the field positions within rows is approximately uniform. The conventions are the same as in Fig. 1.

Rearrangement of retinal axons over the tectum retaining the normal topographic order seems to occur whenever animals are allowed to survive for a longer period after tectal ablation. It appears that the inappropriate fibers (bound for the ablated tectum) displace the appropriate fiber projection over the remaining tectum, thereby retaining the normal "correct" fiber distribution.

Recent studies of Yoon (18) are pertinent to the present work. He obtained a series of retinotectal maps from the same goldfish on three to four occasions at various intervals after surgical manipulations of the tectum and showed that a compressed visual projection to the rostral half-tectum (induced by a mechanical barrier placed in between rostral and caudal tectum) can be restored to normal by removal of the barrier, and is further reinstated by insertion of the barrier. Yoon suggested that reorganization of the retinotectal connection is a reversible phenomenon and can be induced several times over. The restoration of a normal projection from a previously compressed map was interpreted as a reinstatement of normal synaptic respecification prompted by some "unknown" biological interactions. From experiements on goldfish (12–15, 18), it would appear that readjustments of connections are primarily dependent upon the extent of the available tectum remaining, and the retinotectal connections are inconstant.

Also relevant to the present study are the recent observations of Strazincky *et al.* (19) suggesting that strict cell-to-cell specificity is lacking in the *Xenopus* visual system. These authors uncrossed the optic chiasma after metamorphosis of a *Xenopus* with one compound eye, thereby forcing the normal eye to innervate the ipsilateral tectum that was previously supplied by the compound eye, and the compound eye innervated the tectum previously supplied by the normal eye. The visual projection from the compound eye gave a typical reduplicated map across the entire ipsilateral tectum, whereas the normal eye gave a normal projection. They suggested that the tectal connections of an optic-nerve fiber may not be determined solely by the location in the retinal field of its ganglion cells, but may be determined by the nature of the total retinal and tectum system available to be matched.

Gaze and Keating (20) recently suggested that interconnecting structures (e.g., retinal ganglion cells and tectal neurons) tend to match up as systems, rather than a series of discrete, independent subunits. Their conclusions are complementary to the present observations. It is further suggested that such systems initially conform to the patterns laid out during development (i.e., rigid point-to-point specification), and are subsequently followed by adjustments of retinal fibers to compensate for the available tectal structure, thereby allowing it to remain as a complete system.

This work was supported by Grant 5R01 NB0571 of the U.S. Public Health Service to Dr. V. Hamburger.

1. Sperry, R. W. (1943) *J. Comp. Neurol.* **79,** 33–55.
2. Sperry, R. W. (1963) *Proc. Nat. Acad. Sci. USA* **50,** 703–710.
3. Stone, L. S. (1944) *Proc. Soc. Exp Biol. Med.* **57,** 13–14.
4. Szekely, G. (1954) *Acta Biol.* (*Budapest*) **5,** 157–167.
5. Jacobson, M. (1968) *Develop. Biol* **17,** 202–218.
6. Sperry, R. W. (1943) *J. Exp. Zool.* **92,** 263–279.
7. Attardi, D G. & Sperry, R. W. (1963) *Exp. Neurol.* **7,** 46–64.
8. Jacobson, M. & Gaze, R. M. (1965) *Exp. Neurol.* **13,** 418–430.
9. Gaze, R. M., Jacobson, M. & Szekely, G. (1963) *J. Physiol.* (*London*) **165,** 484–499.
10. Gaze, R M., Jacobson, M. & Szekely, G. (1965) *J. Physiol.* (*London*) **176,** 409–417.
11. Gaze, R. M., Keating, M. J., Szekely, G. & Beazley, L. (1970) *Proc. Roy. Soc. Ser. B* **175,** 107–147.
12. Gaze, R. M. & Sharma, S. C. (1970) *Exp. Brain Res.* **10,** 171–181.
13. Yoon, M. (1971) *Exp. Neurol.* **33,** 395–411.
14. Sharma, S. C. (1972) *Exp. Neurol.* **34,** 171–182.
15. Sharma, S. C. (1972) *Exp. Neurol.* **35,** 358–365.
16. Jacobson, M. & Gaze, R. M. (1964) *Quart. J. Exp. Physiol.* **49,** 199–209.
17. Jacobson, M. (1970) in *The Neurosciences Second Study Programme*, ed. Schmitt, F. O. (Rockefeller University Press), p. 116.
18. Yoon, M. (1972) *Exp. Neurol.* **35,** in press.
19. Straznicky, K., Gaze, R. M. & Keating, M. J. (1971) *J. Embryol. Exp. Morph.* **26,** 523–542.
20. Gaze, R. M. & Keating, M. J. (1972) *Nature* **237,** 375–378.