

Horizontal Compensatory Eye Movements in Goldfish (*Carassius auratus*)

II. A Comparison of Normal and Deafferented Animals

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Received February 8, 1974

Summary. 1. Goldfish were deprived of visual input and/or normally functional horizontal semicircular canals.

2. Their horizontal eye movements were measured from cinematographic records, and the extent to which the eyes compensated for horizontal rotations of the head was given by the "compensation factor," CF, the ratio (rotation of the eyes relative to the head)/(rotation of the head). All data were obtained from freely-swimming fish.

3. The CF's for normal, canal-lesioned, blinded, and blinded/canal-lesioned animals were: -0.95 ± 0.10 , -0.80 ± 0.10 , -0.50 ± 0.04 , and -0.41 ± 0.06 (means ± 2 S.E.M.), respectively.

4. These figures lead to the conclusion that the visual input contributes -0.39 to -0.45 to the CF, while the canal input contributes only -0.09 to -0.17 . Thus, the visual input is the major factor; the canals are quantitatively much less important. There is in addition a third source (or sources) contributing to the CF, since the blinded/canal-lesioned fish compensated partially.

5. Experiments of partially restrained animals showed that this third source is not a preprogrammed instruction, nor is it dependent on sensory feedback from the rest of the labyrinth, tactile receptors, lateral line current detectors, or proprioceptors in the trunk. Its identity remains a mystery.

Introduction

In the preceding paper (Easter, Johns and Heckenlively, 1974) we have described the eye movements made by freely swimming normal goldfish. We have specified the accuracy with which the eyes compensate the rotation by the head, and we have discussed the visual consequences of the fact that during a turn, the inner eye undercompensates. This discussion was aimed at showing how the eye movements aided the animal in its reception of visual stimuli from the outside world.

In this paper, we direct our attention inside the fish and try to learn what sources of information the animal uses when making these compensatory movements. Walls (1962) suggested that their main function was to stabilize the retinal images of the outside world as the animal moved about. In this context, the retinas themselves seem the most

likely sources of information to be employed, since movement of the retinal image could be sensed directly and minimized servomechanistically by counter-rotation of the eyes. Such a smooth pursuit system has been demonstrated in virtually all animals that have been tested, *e.g.*, primates (reviewed by Robinson, 1968) rabbits (Ter Braak, 1936; Collewijn, 1969), decapod crustaceans (Dijkgraaf, 1955, 1956; Horridge, 1966), and goldfish (Easter, 1972a). But in most of these examples, little attention has been paid to the compensatory movements made under natural conditions; that is, when the retinal image moves as a result of motion initiated by the animal itself.

Exceptions to this general assertion are Dijkgraaf (1955, 1956), Harris (1965), and Bizzi, Kalil, and Tagliasco (1971) who studied eye movements made by mobile decapod crustaceans, elasmobranchs, and primates respectively. Their findings made the retinal signals seem unimportant. To review these results briefly, Dijkgraaf's crabs and lobsters have stalked eyes, and ordinarily signal a turn of the body by flicking their eyes in that direction. Then as the head turns, the eyes slowly rotate in the opposite sense, approximately maintaining their initial orientation in space until they flick again, should the turn continue. Dijkgraaf determined that this sequence of eye movements was unaffected by blinding and/or elimination of the statocysts, provided the animal was free to control its own movements. He concluded that the eye movements were centrally programmed and closely coordinated with the turning movements of the legs, essentially independently of sensory feedback. These findings anticipated later neurophysiological work, by Wilson and others, which revealed central motor pattern generators in arthropods (reviewed by Evoy and Cohen, 1971).

Elasmobranch swimming is another locomotory pattern in which eye movements are closely coordinated with the rest of the body. This was first studied by Lyon (1899, 1900) who noted that the head of the dogfish swings from side to side during swimming, and the eyes counter-rotate with each swing. Harris (1965) examined these counter-rotations quantitatively, and found that typically the head rotates by 25 degrees, but the eyes by only -10 degrees relative to the head. Thus, the compensation is 15 degrees short of complete. Harris (1965) showed that both central programming and labyrinthine signals played a role in controlling these eye movements. The labyrinth caused the eyes to rotate by an amount equal and opposite to that of the head (-25 degrees). But the central influence, of spinal origin, and correlated with patterns of contraction of the trunk musculature, drove the eyes in the same direction as the head (15 degrees). The result was a linear sum of the two influences, with the tendency toward counter-rotation the greater, hence the partial compensation. Harris did not specifically investigate the importance of visual

signals, but the two components he identified, labyrinthine and spinal, seemed to account for all the eye movement observed.

Similarly, Bizzi, Kalil, and Tagliasco (1971) showed that monkeys compensated without the help of visual information, depending instead on proprioceptive signals from neck and labyrinth. But, in contrast to the others cited, the hypothesis that the compensatory movement was preprogrammed was specifically rejected experimentally.

Goldfish, like most teleosts, make frequent turns, anticipated typically by saccades of 10–15 degrees, following which the eyes counter-rotate (Hermann and Constantine, 1971; Easter, Johns and Heckenlively, 1974). We have deprived goldfish of vision and/or functional horizontal semicircular canals and filmed their eye movements. Their effectiveness, relative to the normal, provides the basis for an assessment of the importance of these two sense organs in controlling eye movements. In addition, we have tested other sense organs and the preprogramming hypothesis.

We have found that visual signals are the most important determinant, the horizontal canals are much less so, but still play a role, and the preprogramming hypothesis is specifically rejected. There remains yet another sensory source involved, but we could not identify it.

Methods

The methods were essentially as described before (Easter, Johns and Heckenlively, 1974). Rigid pointers were attached by suction to the corneas, and as the eyes changed orientation, the pointers so indicated. The animals were filmed from above or below, as described in the text.

Deafferentation Procedures

The labyrinthine input was interrupted by cutting the horizontal semicircular canals, which abolishes the dynamic response to rotation, but leaves the maintained discharge intact (Lowenstein and Sand, 1940). This procedure took about an hour, so the mouth was intubated, and the gills irrigated continuously with an aerated 0.01% aqueous solution of tricaine methanesulfonate. The canals were exposed dorsal to the opercular flaps, and a section, a few millimeters long, was cut from each. The wound was left open, and the animal was allowed to recover for 2–3 days before filming. The animals had no difficulty staying upright after the operation. Moreover, they made what appeared to be normal ocular compensatory movements (Lyon, 1899; Benjamins, 1918; Trevarthen, 1968) to rotations about the transverse and longitudinal axes, even when visual inputs were removed by corneal masks (see below). These observations suggest that the rest of the labyrinth was still functioning (Traill and Mark, 1970).

The animals were deprived of vision in three ways. The least traumatic method was, just before filming, to attach opaque masks over both pupils. This was done to four sighted animals. The masks were made of black electrical tubing, sealed at one end, open at the other, and filled with water. The open end attached by suction to the cornea, but, unlike the normal measuring stalks described above, these masks

covered the entire pupil. When viewed frontally, they presented a triangular cross-section, with the base 5–6 mm wide on the cornea, and the apex 11 mm lateral to the cornea.

Three fish were blinded by intracranial section of the optic nerves. The animals were anesthetized by immersion in a 0.05% aqueous solution of tricaine methanesulfonate, and the surgery, which lasted less than ten minutes, was carried out in air, with no irrigation of the gills. The cranium was opened dorsally by cutting out a small flap of bone. The telencephalon and the fatty material around the brain were removed by aspiration to expose the optic nerves and the chiasm on the floor of the cranial cavity. There was often considerable bleeding during this procedure, and in order to keep the optic nerves visible, blood was continually aspirated away. Both nerves were sectioned distal to the chiasm, after which the blood was allowed to clot, and the cranium was sealed by repositioning the bone flap and covering it with dental acrylic. Filming followed 2–3 days later.

The third method of blinding was retrobulbar crush of the optic nerves. This procedure was carried out on four anesthetized animals. A small wedge of bone was cut from the dorsal-caudal quadrant of the orbit. The eye was pulled forward and ventrally, exposing the optic nerve to view between the superior and posterior recti. It was crushed in several places with fine curved forceps, under visual control with the aid of the dissecting microscope. Filming followed 2–3 days later.

In all cases, blindness was confirmed just prior to filming by testing the animal's ability to make pursuit eye movements when restrained and surrounded by a rotating striped drum (Easter, 1972a). Failure to make pursuit movements was taken as proof of blindness.

We recognize that each of these methods has potential problems since each might result in changes over and above removal of visual signals. The attachment of the masking stalks added a mechanical load to the eyes which might impede eye movements. The intracranial operation might damage some centers concerned with ocular motility. The retrobulbar crush of the optic nerves might inadvertently damage the other nerves and muscles in the orbit. But these effects were probably not very significant, to judge from other experiments carried out on restrained fish with one eye sighted but immobile and the other eye blinded but free to move. Under these conditions, targets seen by the stationary eye result in "pursuit movements" by the blind one, and the maximum pursuit velocities obtained were comparable to normal no matter which of the three methods of blinding was used (Easter, 1972b; and unpublished). Since the time course of compensatory movements resembles smooth pursuit, and the blinding procedures did not diminish the latter, we believe that changes in compensatory eye movements after blinding can be attributed to the loss of visual information.

Restraint Procedures

We also filmed eye movements made by partially restrained fish.

They were passively rotated in either of two ways. In the first, they were gripped by hand or a sponge-lined holder, and manually rotated about a vertical axis caudal to the head. Angular velocities were 5–30°/sec, about the same range as was observed in the freely swimming animals. This method of restraint kept the animal's trunk from bending and prevented the surface of the body from contacting the water, thus removing two concomitants of normal turns: change in posture and currents alongside the body.

The second method of passive rotation was less restrictive, and is illustrated in the sketch in Fig. 1. The dorsal surface of the cranium was attached with machine screws to a flange, which in turn was attached to a vertical shaft.

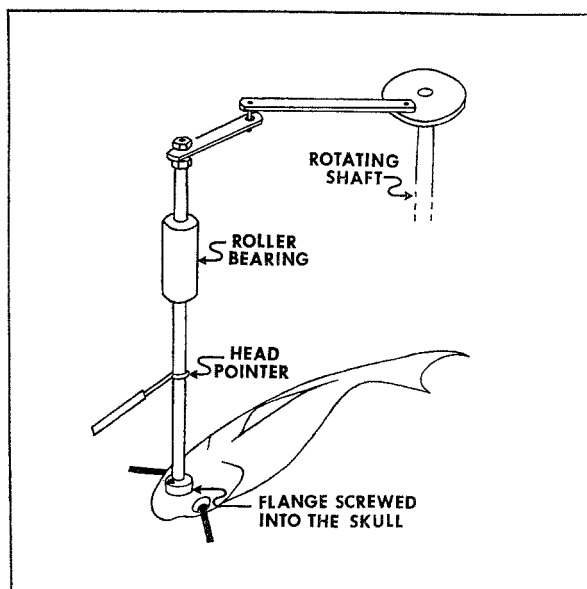


Fig. 1. This sketch shows the partial restraint apparatus. The vertical shaft in the foreground was rigidly attached to the skull and the pointer, a dark rod. The camera filmed from below and recorded the orientations of the eye stalks and pointer. Motor-driven rotation of the shaft in the background resulted, via the mechanical linkage shown, in sinusoidal horizontal oscillations of the head. The two vertical shafts could be disconnected. Then the shaft to the head could be clamped in place, restricting the animal's movement, or unclamped, to permit free rotation

The shaft rotated within a roller-bearing, and the animal was suspended in water, out of contact with the bottom or sides of the tank. The shaft was rotated approximately sinusoidally by a motor, and the fish filmed as before. The axis of rotation passed through the tectum, anterior to the labyrinths.

In other experiments, this same system was used, without the sinusoidal rotations. The shaft was sometimes clamped in place, thus preventing rotation, or unclamped, under which conditions the fish could rotate freely, restricted only by the water and the very slight friction in the bearing.

Results

Freely-swimming Fish

The data are summarized in Fig. 2, which includes (in Group I) Fig. 4 of the preceding paper (Easter, Johns and Heckenlively, 1974). Details of the column headings can be obtained in the legend. The data have been divided into four groups: I-IV, corresponding to normal, canal-deprived, vision-deprived, and canal-and-vision-deprived animals respect-

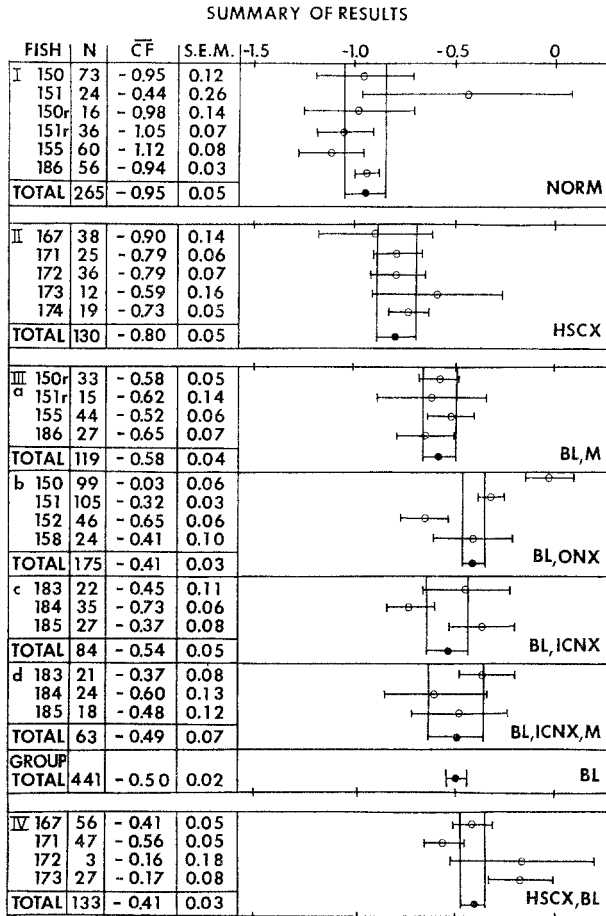


Fig. 2. The column on the left designates the fish, by number. The suffix, *r*, indicates that this animal had regenerated vision after having been blinded. The second column indicates the number of intersaccadic intervals measured. *CF* is the mean compensation factor, defined in the text. *S.E.M.* gives the standard error of this mean. The graph on the right shows the mean ± 2 *S.E.M.* for the fish in that row. The four groups, I–IV, were normals (*NORM*), canal-lesioned (*HSCX*), blinded (*BL*), and blinded/canal-lesioned (*HSCX, BL*). The four subgroups IIIa–III d were blinded by corneal masks (*BL, M*), retrobulbar optic nerve crush (*BL, ONX*), intercranial optic nerve section (*BL, ICNX*), the latter plus corneal masks (*BL, ICNX, M*). In all cases, statistics for individual animals are given by open circles; pooled data from a group or subgroup are given by filled circles. The 95% confidence limits of subgroup and group means are extended upward to facilitate comparisons. Notice that the statistics for subgroup III b and group III have omitted the samples for 150

ively. Group III is further divided into four subgroups, depending upon the method of blinding. The reader's attention is directed to the column on the right, which gives the mean ± 2 standard errors of the mean, of the compensation factor, \overline{CF} . The \overline{CF} for any rotation by the head is given by the ratio (rotation by the eyes relative to the head)/(rotation by the head); perfect compensation corresponds to a \overline{CF} of -1 . Notice that the normal fish had a $\overline{CF} = -0.95$, near perfect, and the extent to which the other groups' \overline{CF} 's differ from that value is an indication of how much the particular sensory deprivation affected the accuracy of the compensation.

Group II, the five fish with severed horizontal semicircular canals, but intact vision, behaved very differently from the normal fish. They spent much of their time motionless in the water. When they swam, they did so abnormally, usually either straight forward or backward, with the caudal fin motionless, propelling themselves by paddling with the pectoral fins. This differs from normal swimming, in which the caudal fin is very active, and turns are very frequent. In spite of this peculiar behavior, they compensated quite well. The individuals' data are all clustered together in Fig. 2, and $\overline{CF} = -0.80 \pm 0.10$ which overlaps with the estimate of the normals' \overline{CF} . Therefore the absence of horizontal canal input diminished the CF by a very small and statistically insignificant amount.

Blinding the animals seemed, on casual observation, not to modify eye movements during swimming. The fish continued to make frequent turns and they still anticipated the turns with binocular saccades. In general, they appeared to our eyes to be much more normal than the fish with severed semicircular canals. But quantitative analyses of the filmed records showed that they were actually compensating much less well than normally. They made anticipatory saccades of about the same size as normal fish, but the subsequent counter-rotation was much slower than that of the head, as Fig. 2, group III, shows. The CF was -0.50 ± 0.04 for the group as a whole, much lower than the other two groups. There were a few details which warrant comment.

Firstly, the suffix, r, (following 150 and 151 in groups I and IIIa) refers to sighted animals which had recently regenerated vision. Their behavior before blinding was measured; then they were blinded and filmed again; then they were allowed three months to recover. During this time, they were tested intermittently in the rotating striped drum to determine when they regained the ability to make optokinetic pursuit movements. After three months, they were clearly back to normal, and their freely-swimming behavior was filmed again. Fig. 2 shows that they compensated normally (Group I), and were affected by the masks much the same as other, normal fish (Group IIIa).

Secondly, one member of subgroup IIIb (150) failed to compensate ($\overline{CF} = -0.03 \pm 0.06$) This was probably due to inadvertent damage to the extraocular neuromuscular system when the optic nerves were crushed. This animal was omitted from calculation of the means for the subgroup IIIb and Group III. (Its inclusion would have lowered these \overline{CF} 's to -0.27 ± 0.07 and -0.41 ± 0.04 , respectively.)

Thirdly, subgroup IIIa, the fish with visual system intact but masked, showed slightly better compensation than the others ($\overline{CF} = -0.58 \pm 0.08$ vs. -0.50 ± 0.06 , -0.54 ± 0.10 and -0.49 ± 0.14). No explanation is offered for these small intergroup differences.

Fourthly, the non-visual effects of the corneal masks can be assessed by comparing subgroups IIIc and III d. They comprise the same three fish, blinded by intracranial optic nerve section, with normal eye stalks (IIIc) and with corneal masks (III d). Clearly, the attachment of the masks had no effect on visual input, since the animals were already blind, so any differences noted between the two subgroups could be attributed to some other effect of the masks. In fact, the \overline{CF} 's were not significantly different, which supports the conclusion that the diminished \overline{CF} of subgroup IIIa was due to absence of visual input.

Apparently, visual information played a large role in setting compensatory eye movements. This conclusion allows two interpretations. Perhaps the animals attended to the contrasts in the visual field, and moved their eyes in such a manner as to minimize the movement of the retinal image—essentially an optokinetic pursuit response as suggested in the Introduction. Then the deterioration of compensation would result directly from loss of visual points of reference. Alternatively, the animals might use the presence of visual signals only as a cue to attend to other signals, non-visual in origin, which indicate head rotation. In essence, the content of the visual image would be immaterial, only its presence would be important. These two possibilities were investigated by filming a normal fish swimming freely when surrounded by a striped drum, outside the water, which could rotate either direction about the vertical axis, or remain motionless. It was observed that the drum's rotation strongly influenced eye movements. For instance, an animal turning clockwise, surrounded by a drum rotating in the same sense, made slow eye movements in the clockwise direction, movements of a velocity which exceeded that of the head. Likewise, when fish and drum turned in opposite senses, the eyes more than held their initial positions, instead, they greatly overcompensated, following the drum. This supports the hypothesis that the visual input to compensatory eye movements acts at least partly through the optokinetic pursuit response.

Finally, when both the horizontal canals were cut and vision was blocked by corneal masks, the fish made frequent turns, sometimes

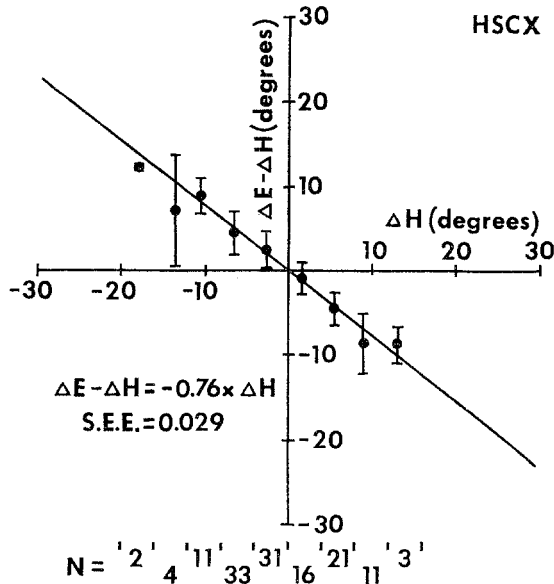


Fig. 3. Canal-lesioned fish (group II). This shows the ocular rotation relative to the head (ordinate) vs. the simultaneous rotation by the head, in laboratory coordinates (abscissa). The data were pooled in 4-degree-wide bins and the means and standard deviations computed. The filled circles give the two means within each bin; the bars indicate ± 1 standard deviation. The least squares regression line is drawn, and its equation given, along with the standard error of the estimate of the regression coefficient. At the very bottom are given the number of measurements in each bin

circling round and round many times in succession, a pattern of movement never observed in other groups. Four of the five fish which contributed to Group II were tested with masks, and the results appear in Group IV. The CF was -0.41 ± 0.06 , only 0.09 units lower than Group III, 0.017 units lower than subgroup IIIa, the most appropriate reference group. Both figures are very close to 0.15, the ΔCF noted between groups I and II, which also differed in that the latter lacked functional horizontal canals. We conclude that the horizontal canals contributed only about -0.09 to -0.17 to the CF.

An analogous comparison can be made between groups I and III and II and IV. The former pair had functional canals but differed in that III lacked visual inputs. The latter pair lacked functional canals, but IV lacked vision as well. Again, the ΔCF 's are similar to each other:

$$\begin{aligned}\overline{CF}_I - \overline{CF}_{III} &= 0.45 \\ \overline{CF}_{II} - \overline{CF}_{IV} &= 0.39\end{aligned}$$

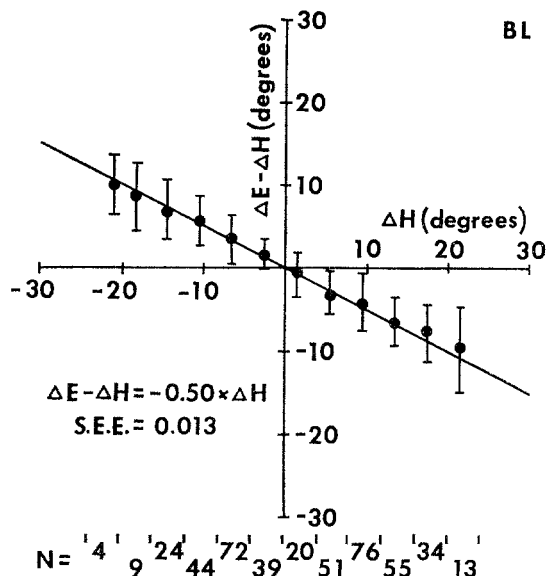


Fig. 4. Blinded fish (group III). The data were treated as described in the legend of Fig. 3

and both are very much larger than the contribution from the canals. We conclude that the visual input contributed -0.39 to -0.45 to the compensation.

In the preceding comparisons of \overline{CF} , it has been assumed implicitly that any group of animals compensated all turns by about the same fraction, on the average. But suppose the \overline{CF} depended on the magnitude of the turn, *e.g.*, small turns were compensated more accurately than big turns. Then \overline{CF} would depend on the size of turns made by the animal, and those which made mostly big turns would have a low \overline{CF} , those favoring small turns, a higher \overline{CF} . Then, intergroup differences would not indicate a change in the animal's ability to compensate, only a change in the size of turns.

Figs. 3—5 show that this was not the case. Each of these figures displays the compensation by a different class of fish, as indicated. In preparing each, the data from all animals within a group were pooled, assigned to 4 degree-wide bins of ΔH , and the mean and standard deviations computed. The least-squares regression line is drawn through each, and the linear relation in all cases is quite evident.

There are two somewhat surprising aspects to the results summarized in Fig. 2. The first is that the horizontal semicircular canals played

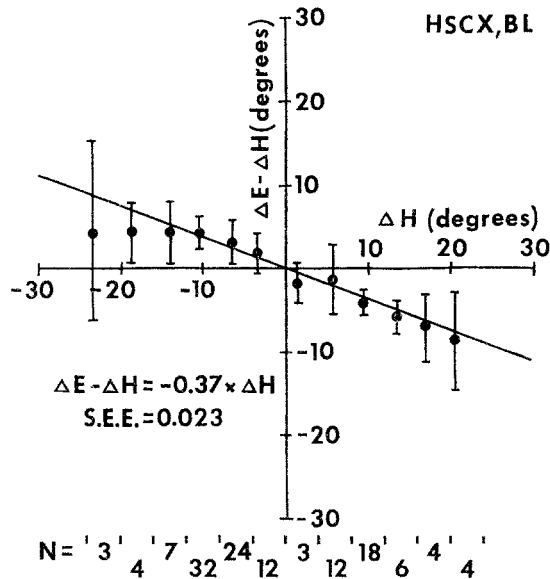


Fig. 5. Blinded/canal-lesioned fish (group IV). The data were treated as described in the legend of Fig. 3

such a minor role in compensatory eye movements. The second is that an animal deprived of horizontal semicircular canals and vision was able to compensate at all. Both of these features could be accounted for if the canal lesions had in fact been ineffective, so this possibility was checked by rotating the animals passively.

Passive Rotations

When normal, blinded, or canal-lesioned fish were rotated passively, they made compensatory eye movements, as shown in Fig. 6a—c. Note that in every case, a nystagmus occurred with repeated saccades in the direction of rotation, and intersaccadic counter-rotations. However, when the eyes were masked after cutting the canals, no such nystagmus was observed. The record in Fig. 6d shows this; the saccades were in the inappropriate direction for nystagmus. The apparently compensatory drift during the last two seconds of this record was a random event, as was shown by a determination of the CF. To make this determination, it was necessary to measure ΔH and ΔE over some period other than the intersaccadic interval, which often exceeded the period of rotation. We chose one-second intervals and found that for three fish (167, 171, 174) the CF was $\pm 0.03 \pm 0.12$ ($N = 46$).

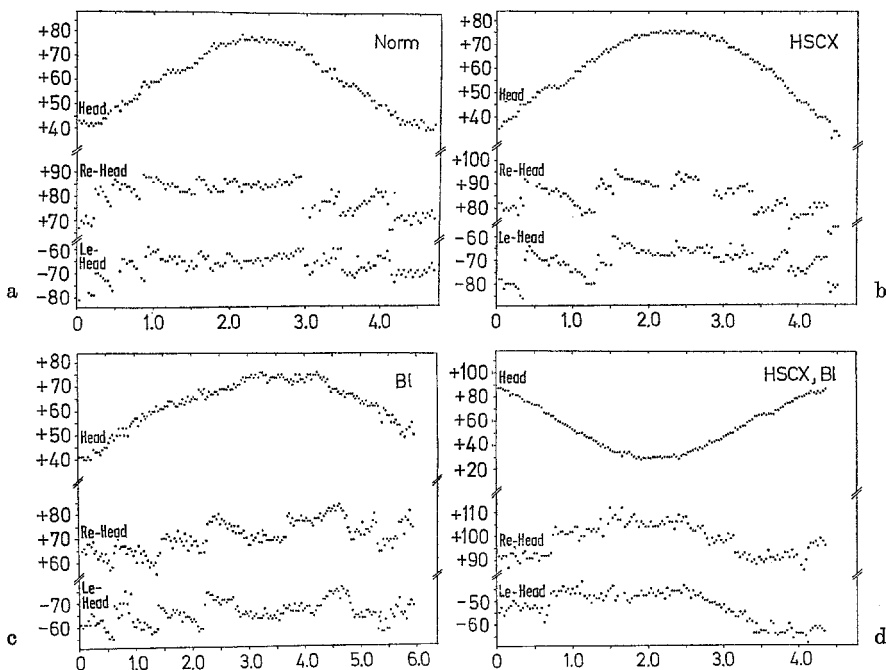


Fig. 6a—d. These are representative traces of head and eye movements made by the same passively rotated fish, using the device of Fig. 1. The upper trace is head position in laboratory coordinates; the lower two traces are the right and left eyes' positions relative to the head. a) The normal animal, with vision unobstructed and horizontal canals intact, shows a nystagmus compensating the head rotation. b) With vision unobstructed, but canals severed, nystagmus still occurs. c) With canals intact, but vision blocked by corneal masks, nystagmus still occurs. d) With canals sectioned and vision blocked by the masks, no nystagmus occurs

If the horizontal semicircular canals had in fact been spared in our surgery, the animals should have shown some compensation during passive rotation. The fact that they did not supports the assertion that the surgery was effective.

To summarize, horizontal compensatory eye movements depend in a major way on visual stimuli, to a lesser degree on inputs from the horizontal semicircular canals, and on something else as well. This hypothetical third source of information was responsible for the residual compensation by Group IV, but was not activated during passive rotation. The remainder of this report will deal with the origin of that third signal.

The Third Source: Passive, Pre-Program, or Sensory Feedback?

Before dealing with the sensory feedback vs. preprogramming hypotheses, a trivial explanation for the partial compensation must be examined. Compensatory movements made during an active turn were always from an extreme toward an intermediate position (Easter, Johns and Heckenlively, 1974). On the other hand, Fig. 6 illustrates that during passive movements, the drift was often from the intermediate toward the extreme; the saccades reset toward the intermediate position. Perhaps part of the compensation during an active turn might have been a result of the elastic forces in the two horizontal rectus muscles of each eye. When they have very different lengths, as they do just after the anticipatory saccade, they might tend to drift passively toward an intermediate position of more nearly equal muscle lengths. Then the partial compensation noted in Fig. 2, Group IV, would simply be due to the positions of the eyes. This same animal, when rotated passively, would already have its eyes at their stable positions, and would therefore show no compensation (Fig. 6d), according to this argument.

But another observation showed that there was no tendency, in the absence of sensory stimuli, to drift from extreme positions. Restrained normal animals spontaneously flick their eyes from side to side, from one extreme to another, but always hold the extreme positions for substantial periods of time, sometimes a second or more, even in absolute darkness. When the eyes return from the extremes, they do so via saccades, not slow movements. The intersaccadic drifts are ordinarily about 0.5 degrees per second or less, and always in the nasal direction, regardless of the positions of the eyes (Easter, 1971).

The case for preprogramming will be examined next. The experimental test of this hypothesis was essentially that of Bizzi, Kalil and Tagliasco (1971). Briefly, the idea is that if the animal initiates a turn, but is prevented from turning, those neuromuscular events dependent upon sensory feedback should not occur, while those events which were programmed in advance should occur, despite the absence of sensory feedback. Specifically, the preprogramming hypothesis predicts that when the animal initiates a turn by a saccade in that direction, and contracts its trunk muscles so as to turn, the saccade should be followed by a rotation in the opposite direction, even though the head has not rotated. Thus, the predicted slow counter-rotation is erroneously compensating for a rotation which normally occurs, but which the experimental conditions prevent.

This prediction was tested by using the setup illustrated in Fig. 1, but without the imposed rotation. When the animal was suspended in this way, and the shaft was free to rotate, the fish frequently made

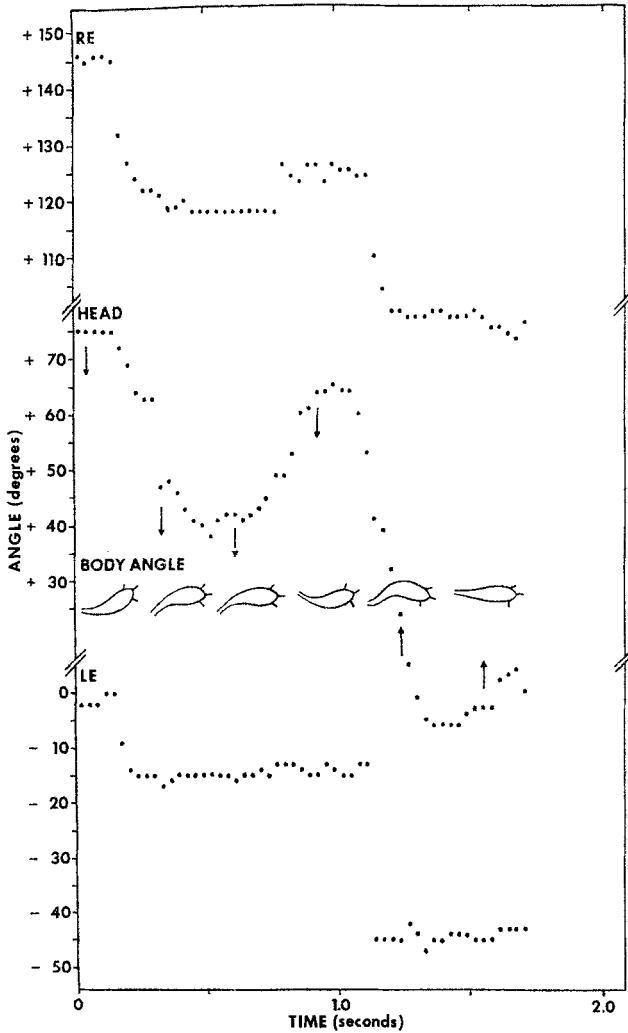


Fig. 7a

Fig. 7a and b. The three sets of dots represent the positions of the eyes and the head, in laboratory coordinates, of the same animal. The measurements were made using the device of Fig. 1, disconnected from the motor. The sketches labelled "Body Angle" show the posture at the times indicated by the arrows. a) The animal was free to rotate, and had both vision and functional canals. It turned and stabilized its eye positions between saccades. b) The head shaft was clamped, so the animal could not turn. Vision was blocked by corneal masks. The animal tried to turn, signaled the turn by binocular saccades, then kept the eyes fixed at their new positions

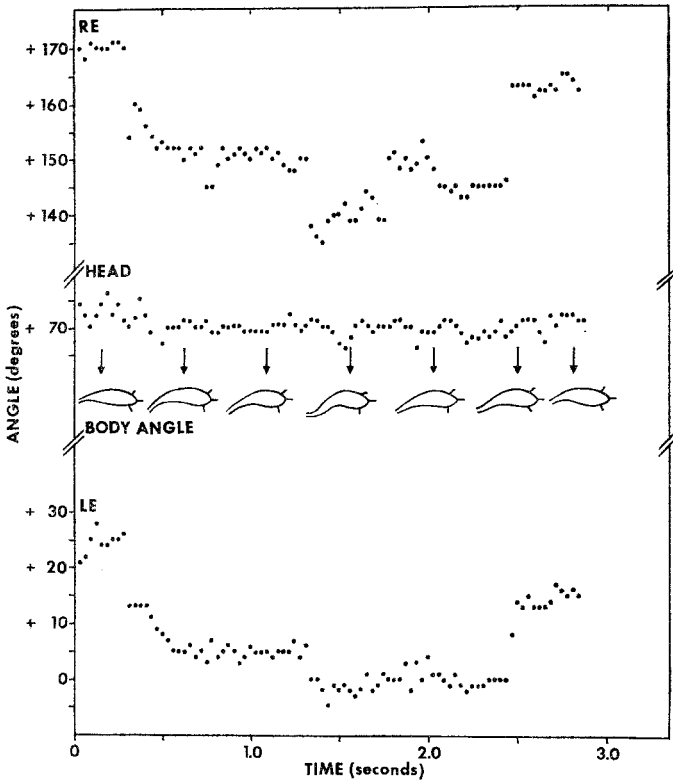


Fig. 7b

spontaneous turns, which were always signalled by: 1) binocular saccades in the direction of the turn, 2) asymmetrical paddling with the pectoral fins, and 3) a strong lateral stroke by the trunk and caudal fin in the direction of the turn. The latter stroke, for instance, made the fish concave left during a left turn. It is acknowledged that the turn so executed was not exactly like the turn made by a freely-swimming fish. In particular, there was not forward component of velocity, and the vertical axis of rotation differed in the two cases.

In spite of these differences, eye movements during these turns in place were remarkably similar to the eye movements made by the freely-swimming animals, as illustrated in Fig. 7a. This was a normal animal, with all senses intact. Note that during the nearly two seconds illustrated here, the head rotated more than 70 degrees, but the eyes counterrotated commensurately, to maintain their positions in space quite stably, between saccades, just as the normal fish did.

But when the shaft was clamped in place, and the animal vainly attempted to turn its head, the anticipatory saccades were never followed by counterrotations, even when, as in Fig. 7 b, visual signals were blocked by corneal masks. We must reject the preprogramming hypothesis, and conclude that the eyes were stabilized by sensory feedback. If so, what senses are involved?

In the remainder of this section, four likely candidates are examined experimentally. The approach was to expose the animal to conditions which exclusively stimulated one or another of these receptor systems, and then observe if predictable compensatory movements resulted.

1. *Labyrinth*. The remaining structures in each labyrinth are the other two semicircular canals, the sacculus, the utriculus, and the lagena (Lowenstein, 1971). Although none seem specifically designed to sense rotation around the vertical axis, all could, in principle, do so. The semicircular canals are particularly likely candidates, as Lowenstein and Sand (1940) have shown that in the ray, *Raja clavata*, single units from the anterior and posterior vertical canals respond to rotation about all three body axes.

If they were involved, then it would be predicted that the animal should make compensatory eye movements when rotated passively, even though the horizontal canals were cut and vision was blocked. But it has already been pointed out that these animals did not compensate ($CF = 0.03 \pm 0.12$), therefore the labyrinth must not have been responsible for the residual compensation during active turns.

2. *Tactile Receptors*. The freely-swimming animals frequently contacted the floor of the tank, but when passively rotated, they were elevated off the floor. If they sensed the relative slip between themselves and the floor, via tactile stimuli, they could use this information in compensation, and the failure to compensate during passive rotation could result from the removal of this signal.

Accordingly, the importance of the tactile stimulus was assessed by keeping the animal in contact with the floor, and rotating either the animal with respect to the floor or vice versa. Neither of these procedures evoked eye movements, therefore the tactile sense was not important in compensation.

3. *Non-labyrinthine Proprioceptors*. There are several possibilities here: joint receptors in the vertebral column, stretch receptors in the muscles, unspecified receptors in the viscera, and the lateral line organ. The joint receptors must be considered here because when the animals turn, its vertebral column bends, and the angles of the vertebral articulations could code the turn. Another concomitant of an active turn is asymmetrical stretch and tension of the muscles on either side of the trunk. These two are signalled by the stretch receptors in the muscles, and the turn

could be computed from the difference. The visceral proprioceptors have been implicated in compensatory movements by the wings and tail of birds (Biederman-Thorson and Thorson, 1973; Delius and Vollrath, 1973) and therefore must be considered. And finally, the lateral lines must also be invoked, for Roberts (1972) has shown electrophysiologically that they signal changes in posture in the dogfish, *Scyliorhinus canicula*.

The experimental test of the role of these proprioceptors was the same as the test of the preprogramming hypothesis. When the fish was bolted to the shaft and initiated a turn, it assumed the posture appropriate to a turn whether the shaft was free to rotate or was clamped in place. Therefore, the proprioceptive messages should have been essentially similar in both cases. This similarity leads to the prediction that ocular counter-rotation should follow the anticipatory saccades, even when the head did not rotate. This did not occur, as already noted, even when vision was blocked (Fig. 7 b), and so the role of the proprioceptors must be discounted.

4. *Lateral Lines*. This organ system has another function germane to this problem, the detection of currents (reviewed by Dijkgraaf, 1963). During a turn, the velocity of water relative to the animal's surface is greater on the outer than on the inner side. This difference could be used to compute the sign and magnitude of the turn, and therefore the compensatory movement.

This possibility was partially tested by passive rotation, but no attempt was made to give the animal any forward propulsion, so the currents during passive rotation probably did not simulate very well those encountered in a turn. To improve upon this, jets of water were directed (underwater) toward one side or the other of the blinded, canalectomized animals. This never elicited compensatory eye movements by either eye. It is concluded that the current-sensing function of the lateral lines did not play a role in compensation.

Thus, we are left with the conclusion that there exists some unidentified source of sensory information, in addition to the retinas and the horizontal semicircular canals, that the fish used to compute its compensatory movements.

Discussion

The conclusion that the fish used both visual and labyrinthine signals to tailor its horizontal compensatory eye movements is not surprising. But the relative ineffectiveness of the horizontal semicircular canals is, because earlier work had suggested otherwise. For instance, Skavenski and Robinson (1973) found that the velocity gain of the vestibulo-ocular reflex in primates is about -0.85 , much greater than the figure of -0.09 to -0.17 reported here. To be sure, the latter figures refer to

position rather than velocity, but in view of the fact that head movements were smooth and velocities moderate, the comparison is justified. Perhaps the difference is attributable to the different species being compared. Or it may be due to the fact that the monkeys were passively rotated, periodically, whereas the fish actively turned, sporadically. We did not do enough passive rotations, nor analyze the data carefully enough, to learn if the sinusoidal gain in goldfish is comparable to that of monkeys.

Another conflicting report is that of Lowenstein (1937), who severed the nerves from the ampullae of both horizontal canals in the pike, *Esox lucius*, and observed the effects on the eyes. He found that these animals were incapable of responding to rotations about the vertical axis, even in the light. The observation that they did not respond at all seems to contradict the results of the present report, in which it was found that visual cues alone sufficed to evoke some compensation. The disagreement might be attributed to the species difference, but Harden-Jones (1963) has found that, in water, the pike and related species are extremely attentive to movement of visual contrasts, and pursue them with both eyes and body. A more likely explanation lies in the fact that Lowenstein's experiments were carried out in air, where pike are 33-50 diopters myopic (Zenkin and Pigarev, 1969). Under these conditions, the blurred retinal image probably contained no boundaries sharp enough to evoke optokinetic pursuit.

In a later paper, Lowenstein and Sand (1940) pointed out that the vertical canals signal rotation about the vertical axis, yet these signals were not used by the animals to generate compensatory movements. This result was confirmed in the present report, and provides an interesting example of an animal's inability to make use of all the information available to it. Apparently, the horizontal rectus muscles receive a relatively direct report from the ampullae of the horizontal canals, and are not informed about rotations sensed by the other canals (cf. Szentágothai, 1950).

It was mildly surprising to find that the compensatory eye movements were not preprogrammed, even in part. The surprise stemmed from the fact that the elasmobranch eye movements seem to be partially preprogrammed (Harris, 1965), and it was supposed that since both were fish, they might share this mode of control. The difference is doubtlessly related to their contrasting swimming patterns. The very regular, periodic, lateral movements of the dogfish are probably so predictable that the open-loop preprogrammed eye movements can be made accurately enough to satisfy the visual demands of this creature. But the goldfish turns its head only sporadically, and then through an angle which can assume a wide range of values. Preprogramming would undoubtedly be more complicated under these variable conditions.

If, as the results indicate, visual input is the major determinant of compensation, how did it happen that the inside eye stabilized a region of space where there were no borders? In all this work, the high contrast stripes were about 50 cm distant, yet the animal's inside eye frequently pivoted about a point much closer than that (see Fig. 6, Easter, Johns and Heckenlively, 1974). This disparity is probably a consequence of the low velocity-gain of the optokinetic pursuit system in goldfish. The stripes subtended only 12 degrees in the vertical meridian; it was shown earlier that under these conditions, the velocity gain is only about 0.4 (see Fig. 9b, Easter, 1972a). The geometry of rotation plus translation requires that the eye undercompensate if it is to stabilize contrasts 50 cm distant. The low velocity-gain decreases this compensation still further.

Finally, the unidentified source of sensory information must be discussed. The notion that such a signal exists is not new; Lyon (1900) had found that dogfish eyes assumed different positions depending upon their animal's posture, and he concluded that "compensatory motions independent of visual impressions and equilibrium sense organs of the inner ear do exist" (Lyon, 1900, p. 81). This was presumed to be a somatic proprioceptor. Harris (1965) confirmed these observations, but claimed that the eye positions correlated not with posture, but with the pattern of axial muscular activity. This suggests that the source could be something like a tendon organ or perhaps efference copy. Whatever the source in dogfish, we could not reproduce Lyon's observations on our goldfish. Thus we have arrived at a similar conclusion through very different results.

As to why we could not identify the source, several possibilities are available. Either the list of possible senses was incomplete, and the other receptor was not tested experimentally, or the attempts to selectively stimulate one or more types of receptors were not well executed. A third possibility is that multiple stimulation of some of the receptors tested is required to evoke compensation. In any case, the absence of a pre-programmed component seems clear.

This work was supported by grant EY-00168 from the National Eye Institute of the United States Public Health Service.

We thank Drs. M. Alpern, R. Knighton, and E. Pugh for useful critical discussions, C. Gans for the extended loan of the motion picture analyzer, Mr. L. Martonyi for photographic services, Ms. J. Smith for typing, and the staff of the Statistical Research Laboratory for the use of the MIDAS Program.

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