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CENTRAL CONTROL OF POSTURAL ORIENTATION IN FLATFISH

II. OPTIC-VESTIBULAR EFFERENT MODIFICATION OF GRAVISTATIC INPUT

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INTRODUCTION

A remarkable advance in understanding mechanisms of neural integration was the discovery that the central nervous system could alter the sensitivity of peripheral receptor organs, the very first stage of input to the central nervous system (von Holst, 1954; Livingston, 1959). This efferent control has been demonstrated physiologically in the visual system of crustaceans (Arechiga & Wiersma, 1969), the static system of gastropods (Wolff, 1970), the olfactory system of fish (Hara, 1967) and several sensory systems in mammals (see Livingston, 1959). Anatomical evidence also indicates an efferent innervation in the visual system of frogs (Branston & Fleming, 1968) and birds (Holden, 1968).

The sensory hair-cell mechanoreceptors of the vertebrate acousticolateralis system receive both afferent and efferent synaptic terminals (Wersäll & Flock, 1965; also see Lowenstein, 1971). Anatomical and physiological evidence for efferents has been reported for the auditory system (Petroff, 1955; Galambos, 1956), the vestibular system (Dohlman, Farkashidy & Salonna, 1958; Gacek, 1960; Sala, 1965) and the lateral line system (Hashimoto, Katsuki & Yanagisawa, 1970; Roberts & Russell, 1970). Some fishes and amphibians, in addition to mammals, have been used to demonstrate some of the vestibular efferent pathways, from the contralateral labyrinths (Schmidt, 1963; Gleisner & Henriksson, 1963; Caston, 1972), from the cerebellum (Llinás & Precht, 1969; Hillman, 1969), and from midbrain centres as well (Markham, Precht & Shimazu, 1966; Piddington, 1971).

The function of such an efferent system was at first thought to be inhibitory, but has proved to be more complex (see Gernandt, 1967). The alteration of unit activity in the afferent semicircular canal as a result of optokinetic stimuli (Klinke & Schmidt, 1970) has raised the possibility of a feedback system operating on the principle of 're-afference' (von Holst & Mittelstaedt, 1950). It would be naïve at this time to suppose this was the only possible function.

The unique asymmetrical side-lying posture of adult flatfish, following metamorphosis from a symmetrical larva, provides an interesting case of alteration in optic-vestibular relations. Once the postural change was demonstrated to be dependent on central rather than peripheral changes (Platt, 1973), optic influence on otolith-organ

activity became a possibility. This influence need not extent to the peripheral vestibular organs, but the evidence cited above showed that such control is likely.

The present paper demonstrates that optic stimuli can influence postural responses in adult flatfish, and that an efferent pathway exists from midbrain visual centres to the otolith organs to modify peripheral gravistatic neural function.

MATERIALS AND METHODS

Two species of adult flatfish were used – the bothid *Citharichthys stigmaeus* Jordan & Gilbert (speckled sanddab) and the pleuronectid *Hypsopsetta guttulata* (Girard) (diamond turbot), obtained locally by hand nets, identified using the key by Roedel (1953) and comparison with specimens in the collection of the Scripps Institution of Oceanography.

Four *Citharichthys* 8 cm long were used in the behaviour experiments. Each was tested as previously described (Platt, 1973) on a revolving platform inside a cylindrical Plexiglass tank. Two visual stimuli were used around the clear section of the tank. One was a 6 V collimated-beam microscope lamp pointing through a hole in an otherwise black cylinder; the cylinder fitted snugly around the tank and so could be rotated through 360°. The other stimulus was a half-translucent, half-black cylinder of paper and vinyl which also could be rotated around the tank, with the circular fluorescent lighting system described earlier as a light source. The behavioural response measured was the ocular compensation angle to lateral tilt, α (Schöne, 1964), and its modification by the direction of light.

Neural activity was recorded as previously described with suction electrodes on the exposed eighth nerve of *Hypsopsetta* fixed to a tilting table, subjected to artificial respiration under light (1/30000) anaesthetic (tricaine methanesulphonate, Crescent Research Chem., Scottsdale, Ariz.) and usually paralysed with 0·1 ml Flaxedil (Davis and Geck, Danbury, Conn.; 20 mg/ml). The stimulating electrode used for some experiments was made of a steel-wire pair (each 0·010 in. diam.), each wire insulated and the pair cemented together with epoxy, cut off squarely at the tip to provide a bipolar stimulus. This electrode could be placed on different points of the exposed brain surface by a simple manipulator fixed to the tilting table. A Grass S-8 stimulator with its stimulus-isolation unit was used to deliver biphasic pulses either singly or in trains at varying frequencies and pulse widths, at amplitudes from 0 to 15 V. The visual stimulus was a simple 3 V flashlight, aimed by hand, with a photocell fixed on the front. Oscilloscope output was led for later analysis to an AM tape recorder with one channel for neural activity and one for monitoring stimuli.

A total of 19 *Hypsopsetta* 22–25 cm long were used for these experiments, yielding 40 analysable single units from the intact nerve. Seven of these fish also were used for studying evoked responses in the central stump of the cut nerve. All the preparations survived for several hours.

RESULTS

1. *Visual stimuli alter ocular compensation to lateral tilt*

Visual stimuli are often integrated with gravitational stimuli to provide postural compensation to deviations from the vertical. Examples include the dorsal light reflex of free-swimming teleost fishes (von Holst, 1935) and decapod crustaceans (Schöne,

1952). Visual stimuli can also affect postural responses less dramatically in many terrestrial animals (Mann, 1952; Butz-Kuenzer, 1957; Howard & Templeton, 1966). There were two major reasons for testing for such integration in flatfish. First, a similar effect in flatfish would allow determination of the relative importance of vision. The bottom-living habit might minimize visual equilibrium function, but the visual role in feeding suggests a high behavioural priority for vision. Secondly, as discussed in a preceding paper (Platt, 1973), the postural change during metamorphosis from larva to adult is apparently accompanied by central changes in vestibular function. If visual-vestibular integration could be demonstrated, then visual influence possibly could affect postural orientation during metamorphosis.

Flatfish could not be induced to swim freely in a water current to test for the dorsal light reflex; instead, ocular compensation to lateral tilt (Schöne, 1964) was used to test for visual influences. One series of experiments utilized a collimated beam of 2 cm diameter as a directional light; the other series utilized a half-light, half-black cylinder as a horizon (see Methods). Both the beam and horizon could be rotated 360° around the test cylinder. A fish was presented at a given tilt with the light from 90° to its right, then tilted 180° so the light was from 90° to its left; the light was then re-positioned before the next tilt was made, to eliminate any purely optomotor response to rotation of the light. Four *Citharichthys* were run, each tested in 90° tilt increments over 360°. To cancel out any decline in response with time, the first fish began and ended the full circle at 0° (horizontal), the second began and ended at 90° clockwise tilt, the third at 180°, and the fourth at 270°. Since the first and last positions for each run were identical, there were a total of five sets of data on light and tilt to be compared. The same four individual fish were used for both the beam and horizon experiments, allowing direct comparison of the results. For both cases, the measure of light influence on perceived tilt is Δa, in degrees, calculated as the difference between the ocular compensation angle a to a given tilt with light from one side and from the other. This angle is termed positive if the shift in a is in the same directional sense as the shift in the light. Although light might affect an ocular movement by a mechanism other than integration into postural response, the movement of the eye on the darkened side and the tonic nature of the positional change of the eyes suggest a summation with postural mechanisms.

Fig. 1 shows the results for both the beam (*a*) and the horizon (*b*) experiments, with single-sided 95% confidence limits on the mean values. In both cases the visual effect is clear, though not large in comparison to the total ocular compensation to tilt. Maximum light influence, with the horizon, produces a change in a of roughly 10°, while maximum excursion of a to tilt alone is more than 40°. The horizon elicits nearly equal effects at all tilts; the beam has greatest effect at tilts of 0° and 180°, when the gravitational response is smallest. The horizon is not as bright a stimulus as the beam, but every value of the light effect is greater for the horizon than the beam. Either the horizon is a stronger behavioural stimulus, or the beam may be too bright for a normal response.

These data show that a postural response in flatfish can be altered by visual cues. Light direction causes an ocular compensation toward it, which is not proportional to tilt but which sums with the response to tilt.

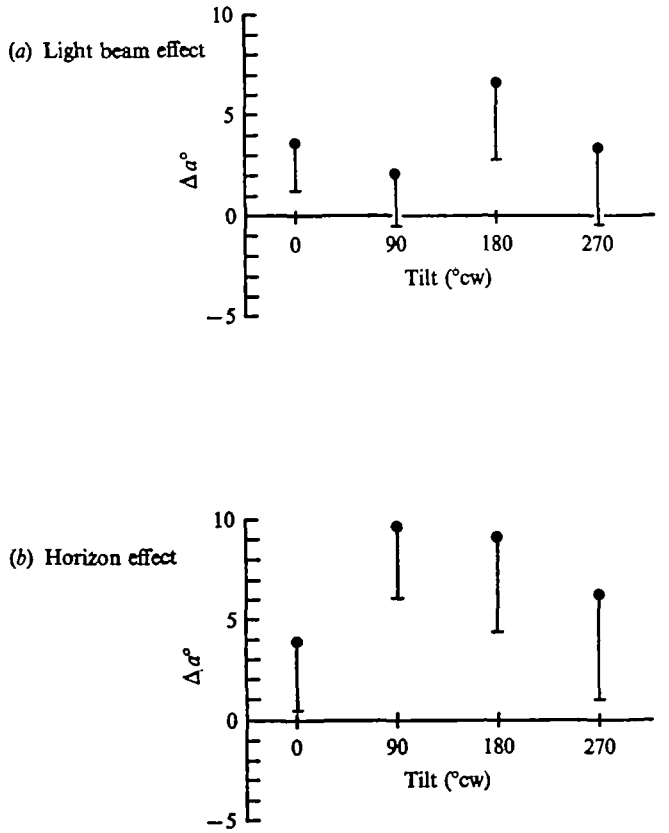


Fig. 1. Effect of directional light on ocular compensation. Change in ocular compensation, Δa, obtained by subtracting a with the light to the right from a with the light to the left of the fish, for each tilt angle. Since an a to the right (clockwise) is negative by convention, a positive Δa indicates a bias toward the light. Means and 95 % confidence intervals, from paired differences; $N = 5$ (*Citharichthys*). (*a*) Beam of collimated light. (*b*) Horizon of black-and-white cylinder. Note apparent independence of effect from angle of tilt in both cases.

2. *Tectal stimulation evokes potentials in the vestibular nerve*

Efferent fibres to the vestibular epithelium have been histologically demonstrated in several vertebrates (Dohlman, 1960; Gacek, 1966). Physiological demonstrations on the cut central stump of the nerve have shown efferent activity evoked by stimulating a contralateral otolith or semicircular canal (Gleisner & Henriksson, 1963; Klinke & Schmidt, 1968; Caston, 1972), or cerebellum (Llinás & Precht, 1969). Because of the visual–vestibular interaction it seemed that stimulation of the optic tectum might result in stimulation of efferents to modify vestibular activity.

Brief electrical biphasic square pulses were delivered by a bipolar wire pair to various locations on the brain surface, while the distal end of the stump of the cut vestibular nerve was taken into a suction electrode. Unfortunately, the anatomy of the flatfish head made stimulation or recording from the underside impossible without such extensive surgery that major damage to the brain or vessels occurred. The following results are from observations on seven *Hypsopsetta*. Though the traces

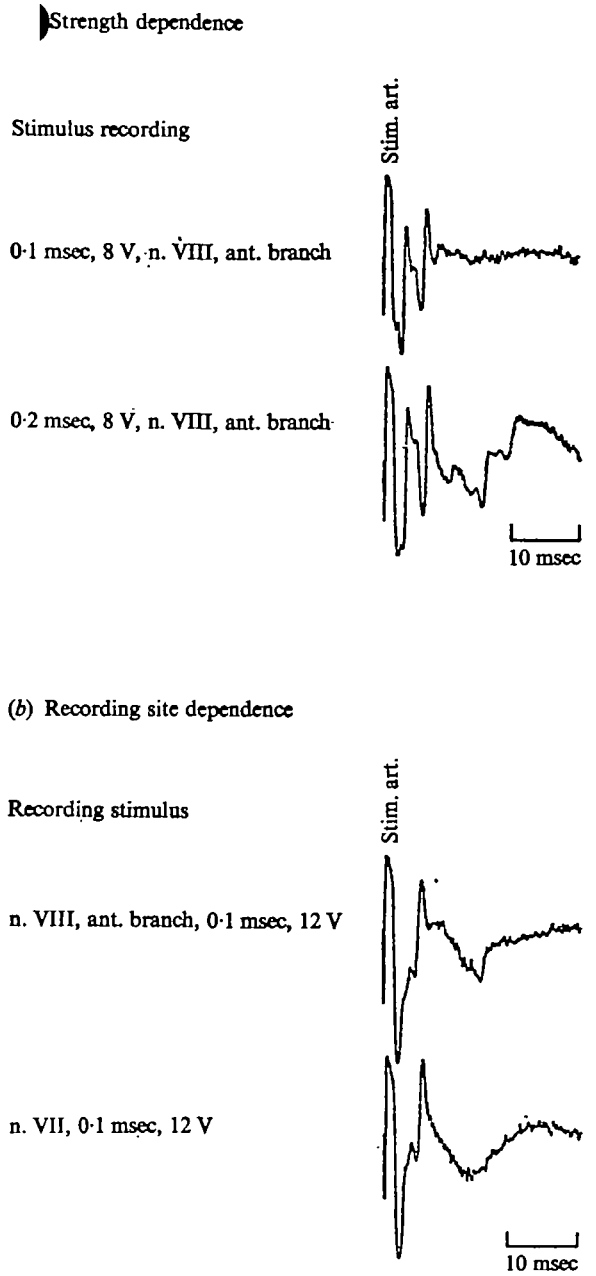


Fig. 2

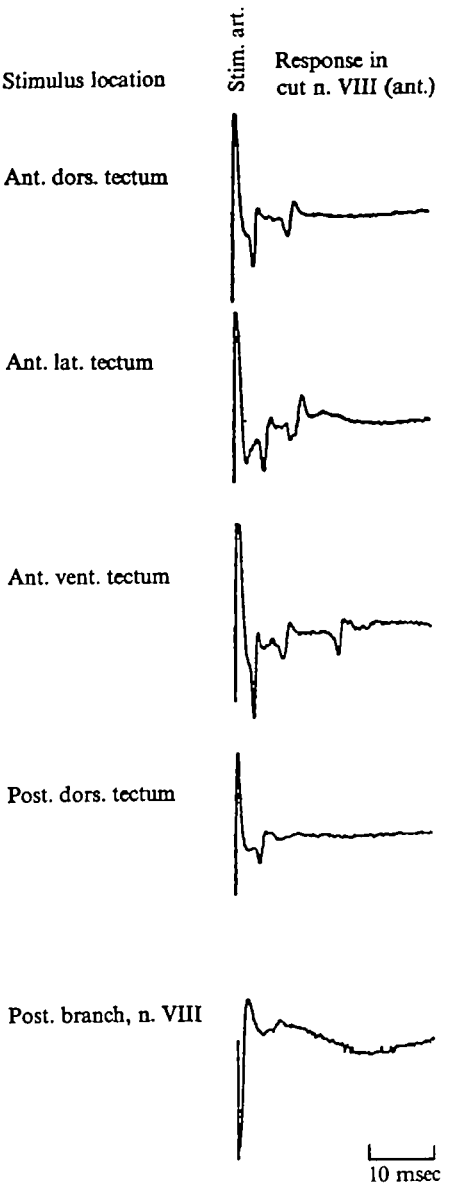


Fig. 3

Fig. 2. Dependence of evoked efferent potentials on strength of stimulus and location of recording. Bipolar biphasic stimulus pulse to anterior lateral tectum. Suction electrode records from central stump of cut ipsilateral branch of vestibular nerve of *Hypsopsetta*. Each trace averaged from eight consecutive sweeps; individual sweeps are similar. Time bar, 10 msec. (*a*) Increasing stimulus pulse duration from 0·1 to 0·2 msec evokes later potentials (near 20 msec), and increases size of early ones at 5 and 7 msec. (*b*) Changing recording site from cut stump of eighth nerve to cut stump of seventh nerve results in loss of evoked potential with 15 msec latency, but little change in response at 5 msec.

Fig. 3. Dependence of evoked efferent potentials on location of stimulus. Stimulus 0·2 msec, 8 V, biphasic, bipolar, at 1 Hz. Suction electrode records from central stump of cut anterior branch of ipsilateral vestibular nerve of *Hypsopsetta*. Each trace averaged from eight consecutive sweeps; individual sweeps are similar. Time bar, 10 msec.

shown are averaged from eight successive sweeps, each individual sweep shows the evoked potentials of the same general shape; the responses are not spike-like.

The evoked potentials depend on both the strength of the stimulus and the location of recording, as expected. Fig. 2(*a*) shows that an increase in stimulus duration from 0·1 to 0·2 msec can elicit new later responses while retaining the early ones. The increased amplitude of the early responses shows that, above threshold, there is some graded quality to the responses. The same variations also can be observed if the stimulus duration is held constant and the voltage varied; typical threshold values for the first response are 6–8 V at 0·1 msec, delivered at 1 Hz.

Different activity patterns result when recording from different nerve branches. Fig. 2(*b*) shows first an early and a later response from the vestibular nerve to stimulation of the anterior lateral tectum; below it is a record from the seventh nerve, which is primarily motor, with the same stimulation in the same fish. This control recording suggests that the early response near 5 msec may be from the same source in both cases, and thus not from a specifically vestibular efferent system. The later responses, however, consistently seen in vestibular branches, were never seen in the few recordings made from the seventh nerve. Only a few comparisons were made between anterior and posterior branches of the vestibular nerve, because of surgical difficulties in cutting the posterior branch. Although the absolute latencies of the responses vary, records from the posterior branch also show at least two sets of responses.

It is surprising to find that the responses are different when stimulating different regions of the tectum, suggesting a specificity to even this gross stimulus. Fig. 3 shows sample records from five stimulation areas, four tectal and one vestibular. The latencies of the early responses are shifted slightly for the different stimuli, with some amplitude changes, but stimulation of certain regions clearly can elicit additional responses. As a control for specificity, stimulation of the ipsilateral posterior forebrain, lateral cerebellum, or posterior branch of the ipsilateral vestibular nerve never produced any evoked potentials in the anterior branch of the vestibular nerve. Contralateral sites were not accessible for stimulation.

Data from five fish on location of stimulus and recording, and on strength of stimulus, are summarized in Fig. 4. There is a late response from the anterior tectal stimulation at 16–20 msec. While there is overlap in the earlier responses, they seem to fall into two groups – one from 8 to 10 msec and one from 3 to 6 msec. The earliest group often shows division into a 3 msec and a 5 msec response; as mentioned, the presence of this group in recordings from the seventh nerve suggest it may be not specifically vestibular.

These groups can also be separated by the differing effects of repetitive stimulation. Fig. 5 shows that the earliest response gradually decreases in size and increases in latency, but is not abolished, with stimulus frequencies increasing from 1 to 100 Hz. The response at 10 msec decreases greatly in size and slightly in latency when the stimulus frequency increases from 1 to 5 Hz. The late response at 20 msec is abolished completely by increasing stimulus frequency from 1 to 5 Hz. These responses show no differences in shape or latency for stimulus frequencies below 1 Hz, compared to the shape and latency at 1 Hz.

These tectal stimulations by single shocks show that evoked potentials in the vestibular nerve fall into at least three groups of latencies. The two longer latency

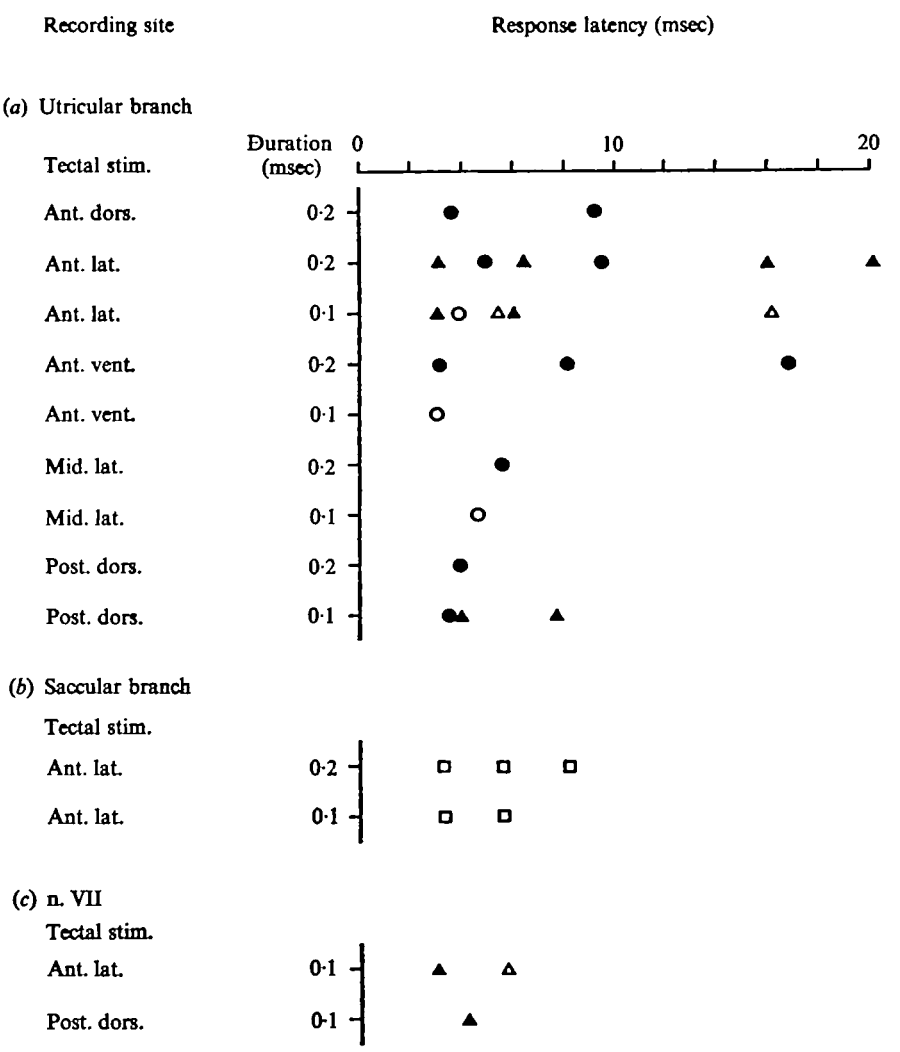


Fig. 4. Summary of dependence of evoked efferent activity on location of stimulus and recording, and on strength of stimulus. Tectal stimulus 8 V, biphasic, bipolar, at 1 Hz; duration listed vertically for three ipsilateral recording sites (central stumps of cut nerves). Latency of responses displayed horizontally. Different symbols represent different experiments (all *Hypsopsetta*); recurrence of a single symbol in one line indicates multiple responses. (*a*) Anterior (utricular) branch recording. (*b*) Posterior (saccular) branch recording. (*c*) n. VII recording. Note multiple responses in (*a*) and (*b*) to anterior tectal stimulus.

groups, near 10 and 20 msec, suggest by their delay and extinction at stimulus frequencies above 5 Hz that they are not antidromic volleys but involve at least one synapse. Their dependence on stimulus location in certain regions of the tectum alone suggests the presence of a tecto-vestibular efferent pathway.

3. *Tectal stimulation can alter tilt-responding vestibular units*

Since single tectal shocks elicit apparent efferent activity, similar central stimulation might be expected to produce changes in the primary afferent vestibular activity.

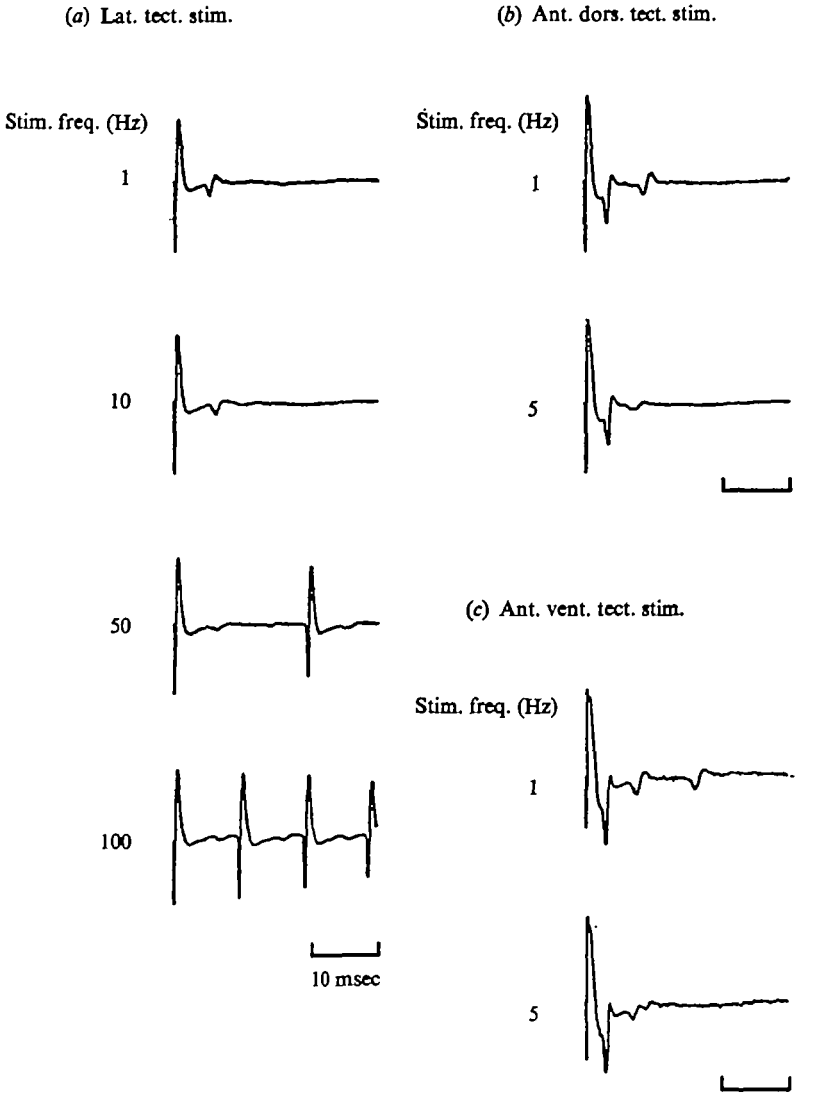
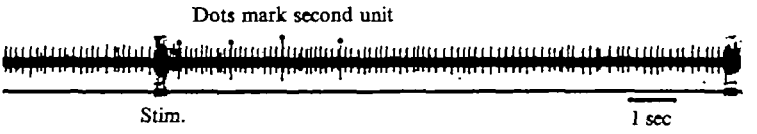


Fig. 5. Failure of later evoked efferent potentials with repetitive stimulation. Stimulus 0·2 msec, 8 V, biphasic, bipolar. Suction electrode records from ipsilateral anterior branch of vestibular nerve of *Hypsopsetta*. Each trace averaged from eight consecutive sweeps; individual sweeps are similar. Time bar, 10 msec. (*a*) Stimulate lateral tectum at 1, 10, 50, 100 Hz. (*b*) Stimulate anterior dorsal tectum at 1 and 5 Hz. (*c*) Stimulate anterior ventral tectum at 1 and 5 Hz.

Though stimulation of vestibular nuclei (Sala, 1965) and the cerebellum (Llinás & Precht, 1969) can cause such changes, the peripheral effects of stimulation of midbrain centres has not been reported, except in the special vestibular case of hearing (Piddington, 1971). Vestibular afferent single units in flatfish were tested for such central influence on their often regular rate of spontaneous discharge.

Recording was carried out using tapered fire-polished suction electrodes of 10–20 μm tip diameter from single units in the intact vestibular branches. The stimuli again were bipolar biphasic brief pulses delivered to the right anterior tectum (ipsi-

(*a*) Unit responses to tectal shocks



(*b*) Effect on unit rate

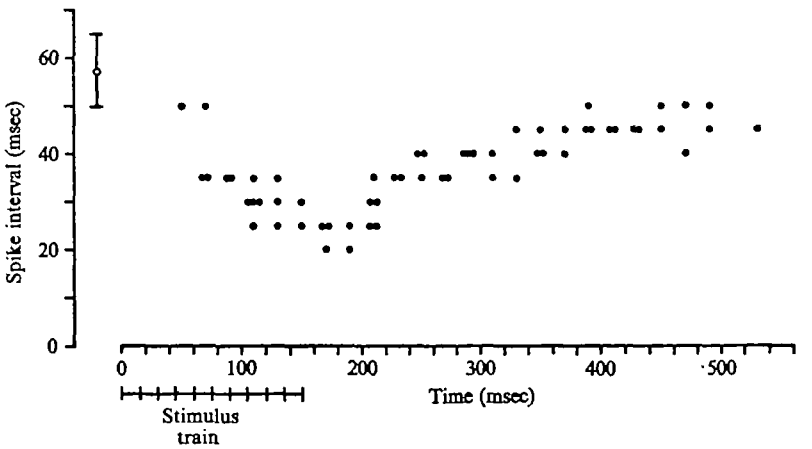


Fig. 6. Effect of tectal shocks on peripheral afferent gravistatic unit. Stimulus 0·2 msec, 8 V, biphasic, bipolar; train of 11 pulses, at 15 msec intervals, delivered at 10 sec intervals to anterior lateral tectum. Suction electrode record from intact ipsilateral vestibular nerve of *Hypsopsetta*.

(*a*) Sample record during stimulation. Gravistatic unit with spontaneous regular activity shows rate increase immediately after stimulation. Second units (dots) discharges sporadically only after stimulation. Time bar, 1 sec.

(*b*) Interspike interval plot, pooled from responses of unit shown above to five successive stimulus bursts. Open circle is mean interval (±S.D.) for 10 sec before stimulation. Increasing frequency clearly begins during stimulation, and lasts long after end of stimulation.

lateral to recording); the left tectum was not accessible. Recorded units were determined to be vestibular primary afferents by their location in the nerve branch distal to emergence from the brain, their spontaneous and often regular activity, and their response to lateral tilts. Forty units from 19 *Hypsopsetta* were tested.

Single shocks to the tectum, which had produced evoked potentials in the cut nerve, never produced any response in the single units selected on these criteria to be tilt afferents. This lack of effect is further evidence that the units being recorded are not efferents from the tectum, and moreover that the evoked responses seen earlier are not from antidromic stimulation of vestibular afferents.

If a short train of shocks was used instead, some units did show an effect. The shocks, of 0·1 msec, were delivered 10 msec apart in a burst; the minimum burst to produce an effect was usually 100 msec (10 shocks) or more, at 8 V. This response was not a vestibular response to movement; the fish were paralysed with Flaxedil, and no movement was ever noticeable anywhere in the head or body of the fish in response to the stimulus. An effect from vaso-dilation or constriction is doubtful since the

Table 1. *Effect of tectal shocks on peripheral gravistatic units* (40 *units from* 19 Hypsopsetta)

I.	No tectal effect: 30		
	A. Tonic	21	
	Dorsal-up	17	(a: 7; p: 10)
	Dorsal-down	3	(a: 1; p: 2)
	Bidirectional	1	(a: 0; p: 1)
	B. Phasic	9	
	Dorsal-up	2	(a: 2; p: 0)
	Dorsal-down	7	(a: 5; p: 2)
	Bidirectional	0	
II.	Increase to tectal stimulation: 8		
	A. Tonic	6	
	Dorsal-up	3	(a: 2; p: 1)
	Dorsal-down	2	(a: 0; p: 1; b: 1)
	Bidirectional	1	(a: 0; p: 0; b: 1)
	B. Phasic	2	
	Dorsal-up	0	
	Dorsal-down	2	(a: 1; p: 1)
	Bidirectional	0	
III.	Decrease to tectal stimulation: 2		
	A. Tonic	0	
	B. Phasic	2	
	Dorsal-up	1	(a: 1; p: 0)
	Dorsal-down	1	(a: 0; p: 1)
	Bidirectional	0	

Tectal stimulus a train of pulses, each pulse 0·2 msec, 8–10 V, biphasic, bipolar; pulse train of 50–200 msec (5–20 pulses) to surface of ipsilateral tectum. Tonic units maintained a new discharge rate for more than 1 min after change in tilt. Dorsal-up, dorsal-down and bidirectional refer to sense of tilt required to cause increase in rate of unit. Nerve branch indicated by (a) anterior, (p) posterior and (b) base. Number of units in each category includes all those of lower categories.

latency is short, and the stimulus occasionally decreases rather than increases unit rates.

Fig. 6 shows a series of consecutive responses by one regular unit which showed an increase in activity for roughly 500 msec following a 150 msec tectal burst. Analysis of these responses indicates that the rate increase did not begin until more than 50 msec (more than one interspike interval) after the start of the stimulus. This latency suggests a necessity for temporal summation in either the number of stimulating shocks or the duration of the total burst. One interesting feature of this record and a few others is the sporadic slower firing of a second unit for 1 or 2 sec after the stimulus; this activity is of the type which might be expected from an efferent unit.

Of the 40 units tested, 10 responded to the tectal stimulus. A summary of the data is shown in Table 1. Units tested include those from both anterior and posterior branches of the nerve, tonic and phasic, increasing or decreasing rate with tilts dorsal-fin upward; the tectal-responding units are not confined to any one of these categories. (Two of the responding units were located so near the base of the nerve at the entry to the medulla that they were designated 'b' rather than anterior or posterior.) Of these few units, more responded by increase (8) than decrease (2) to the tectal stimulation; of the six responding tonic units, none showed decrease. The increase or

Table 2. *Effect of directional light on peripheral gravistatic units* (22 *units from* 11 Hypsopsetta)

I.	No light effect: 16		
	A. Tonic	9	
	Dorsal-up	8	(a: 3; p: 5)
	Dorsal-down	1	(a: 0; p: 1)
	B. Phasic	7	
	Dorsal-up	0	
	Dorsal-down	7	(a: 4; p: 3)
II.	Increase to ipsilateral light: 3		
	A. Tonic	2	
	Dorsal-up	1	(a: 0; p: 1)
	Dorsal-down	1	(a: 0; p: 0; b: 1)
	B. Phasic	1	
	Dorsal-up	1	(a: 1; p: 0)
	Dorsal-down	0	
III.	Increase to contralateral light: 1		
	A. Tonic	0	
	B. Phasic	1	
	Dorsal-up	0	
	Dorsal-down	1	(a: 1; p: 0)
IV.	Increase to light to either eye: 2		
	A. Tonic	1	
	Dorsal-up	1	(a: 0; p: 1)
	Dorsal-down	0	
	B. Phasic	1	
	Dorsal-up	1	(a: 0; p: 0; b: 1)
	Dorsal-down	0	

Stimulus a flashlight beam directed to one eye. Notation as in Table 1, except no bidirectional units tested.

decrease of a unit produced by ipsilateral tectal stimulation does not appear related to the directional sensitivity to tilt of that unit.

Thus a short burst of electrical pulses to the tectum can alter the firing rate of vestibular units which respond tonically to tilt. The usual effect seems to be excitatory, independent of the directional sensitivity to tilt of the vestibular unit. Because the electrical stimulus is liable to have unforeseen effects, these results only suggest that an efferent system may control gravistatic receptor activity.

4. *Visual stimuli can alter activity of tilt-responding vestibular units*

Visual stimuli have been shown to alter afferent activity from semicircular canal organs (Dichgans, Wist & Schmidt, 1970; Klinke & Schmidt, 1970; Schmidt, Wist & Dichgans, 1972). The tectal stimulation experiments just described suggest that more natural visual stimuli might similarly affect the otolith organs.

The same criteria described above were used to determine whether suction electrode records of single units in the intact vestibular nerve were from primary gravistatic afferents. The visual stimulus used was simply a flashlight, held by hand at a distance of 10–15 cm from the head, shining directly into one eye or the other. (A horizon stimulus, of a shadowed edge cast on a translucent arch over the fish's head,

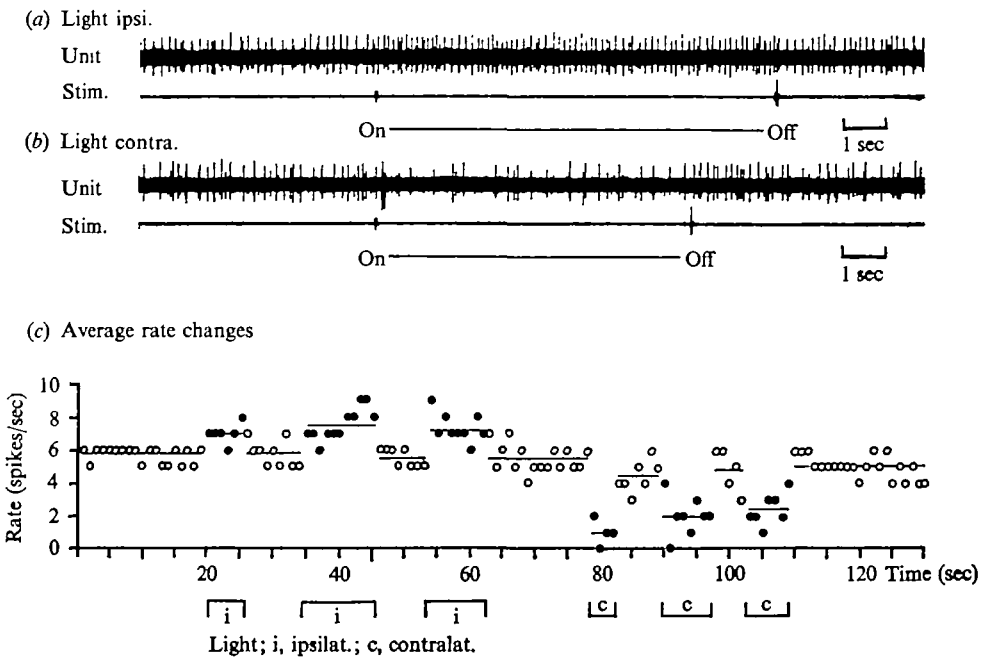


Fig. 7. Effect of directional light on peripheral afferent gravistatic unit. Stimulus a flashlight shining in ipsilateral or contralateral eye. Suction electrode record from intact vestibular nerve of *Hypsopsetta*. (*a*) Sample record shows increased rate with ipsilateral light; photocell response indicates light on and off. Time bar, 1 sec. (*b*) Same, but decreasing rate with contralateral light. (*c*) Average rate changes for this unit shown by solid points during light stimulation, open circles when not stimulated, for a series of consecutive stimuli. Rate averaged over 1 sec epochs; mean rate during a given period shown by line of period length. Note opposite effects of light.

never caused any response.) A few responses did occur to the light stimulation, and the same factors mentioned earlier for tectal stimulation argue against these responses as movement or autonomic artifacts. Twenty-two units from 11 *Hypsopsetta* were tested.

Of the 22 tilt-responding units, 6 (from three fish) responded to light; the data are summarized in Table 2. The distribution of light-affected units is not clearly confined to any category of tilt responses. However, the light has clearly demonstrable directional effects. Only two of the six units showed a rate increase to light presented from either side; none showed bidirectional decrease. The other four showed a noticeable rate increase only when light was presented from one side. The most remarkable two units showed not only this increase to light from one side, but a decrease when the light was moved to the other side. This differential response provides further evidence that the effect is not simply an artifact, nor a general effect of stimulation, but a specific response. The change in rate resulting from light stimulation is usually similar to the change resulting from tilts of more than 30°, indicating a substantial effect. The light effect is not proportional to tilt, but seems to add a constant amount of increase in spike frequency. The light effect also is tonic, both increase and decrease sometimes lasting for stimulation periods of more than 1 min.

Direction-dependent responses from one unit are shown in Fig. 7, with the light stimulus monitored by a photocell. The increase in rate during ipsilateral (right) eye illumination (*a*), was from roughly 5 spikes/sec at rest to roughly 8 spikes/sec during the stimulus; the decrease during contralateral illumination (*b*) was almost to zero. A plot of average spike rate versus time for this unit (*c*) summarizes the data from a series of stimuli. Light from above the fish shining into only one eye produced less effect than light shining directly into one eye along the optic axis; either a directional sensitivity within the eye or an intensity difference could be responsible. The examples shown here are the best of a series, and illustrate that the latency for the increase or decrease is less than 100 msec, just as with the electrical tectal stimulus. Following the inhibition (7*b*), there seems to be a brief rebound excitation before return to the normal rate; the converse effect is not evident following excitation (7*a*).

These data indicate that some gravistatic vestibular units in the peripheral nerve can have their activity tonically modified by a directional light stimulus. The alteration may be either an increase in rate independent of the light direction, or a direction-dependent increase or decrease. While the proportion of tested units which were affected was small, the responses to this natural stimulus show that optic-vestibular integration can involve central control over vestibular peripheral units.

DISCUSSION

1. *Visual stimuli influence a postural response*

In free-swimming fish which show the dorsal light reflex, the optic component of postural equilibrium is proportional to the sine of the angle at which light enters the eye, just as the vestibular component is proportional to the sine of the angle of the body relative to gravity. The ratio of the optic to vestibular component has been calculated for several fishes, and ranges widely, from 0·2 to 5·7 (von Holst, 1950; Braemer, 1957; Traill & Mark, 1970). Amphibians passively tilted, tested with optic cues, showed that approximately 20% of the postural compensation was elicited by the eyes, and 80% by the labyrinths (Butz-Kuenzer, 1957). The results presented here on flatfish are more comparable to the amphibian work because of the passive tilt used, and because the angle at which light entered the eye was not held constant. The maximum values obtained indicate that the contribution of light in flatfish is 20% or less, and of the otoliths 80% or more. These results are within the wide range found for other fishes, and agree well with the amphibian work.

The von Holst–Braemer model treated the light effect as independent of the gravitational effect, although they add together in the total response. Although the Butz–Kuenzer percentages imply a proportional dependence of light to tilt effects, the visual stimulus in fact was changed by tilting, unlike the constant light-stimulus angle used by von Holst to show independence. The flatfish data on the behavioural influence of light are in agreement with both points of the von Holst–Braemer model. First, the amount of change caused by light direction is not dependent on tilt angle. (Whether the amount of change is proportional to the sine of the angle at which light enters the eye cannot be determined from these data, which measure only the net difference between effects of light angles 180° apart.) Secondly, the light effect is additive with the vestibular effect; directional light can either increase or decrease the

ocular compensation to tilt, depending on whether the light and gravitational vector add or subtract.

The mechanism for orienting responses toward directional light is probably not simply a telotactic comparison between light intensity to the two eyes. If that were the case, then the bright collimated beam, directed into only one eye with the other eye dark, should produce a greater response than the much less localized horizon test; yet the horizon in fact produces the greater response. If the edges of the horizon rather than just the lighted half were an important stimulus, then the horizon test should have more effect when the edge could be seen. At the three test positions other than 0° there is always an ocular compensation in which one eye aims at an angle upward from the platform; since the horizon edge is perpendicular to the platform, this eye shift would bring the edge more into view. At 0°, though, each eye aims nearly horizontally, and the horizon edge directly above would be in very peripheral view. The prediction that the horizon should have greater effect at tilts of 90°, 180° and 270° than at 0° is confirmed by these data. However, in the turbid coastal waters where flatfish live, a sharp horizon is unlikely to exist. The half-light, half-black cylinder instead may be an effective stimulus because the light is relatively diffuse over a wide visual angle, while retaining a directional component, just as skylight would be in turbid water.

2. *Tectal stimulation elicits vestibular nerve responses*

Direct electrical stimulation of the tectum by single and multiple shocks produced two effects in the vestibular nerve, evoked efferent volleys and alteration of afferent activity, respectively. These results are useful for comparison with work by other authors in clarifying the nature of efferent central control of the vestibular system.

Several arguments ruled out the possibility that these effects represent artifacts of current spread, muscular control or antidromic activitation. If current spread were responsible, the posterior stimulation sites would be expected to cause greater effects than sites farther from the recording electrode. Precisely the opposite was found. The greatest number of evoked responses to single shocks, as well as the strongest effects of bursts, resulted from stimulating the anterior lateral tectum; these effects were decreased by moving the stimulation site posteriorly, and also appeared only when stimulating tectal regions. Muscular control was eliminated by use of Flaxedil, and its absence was verified by visual observation. Antidromic activation of vestibular afferents was unlikely for two reasons. First, the source of stimulation was far from either the cerebellum or the oculomotor nuclei, the known terminations of primary vestibular afferents (Carpenter, 1967). Secondly, as mentioned in the Results, single shocks never produced a related spike in any of the primary afferent units recorded in the intact nerve.

The latencies of the effects of tectal stimulation were quite different for the two recording cases of the cut nerve and the intact afferent records. The early group at 3–6 msec is in the same latency range as a response also seen in the seventh nerve, which suggested it might not be specifically vestibular. However, it coincides with the 5 msec latency observed by Piddington (1971) for suppression of afferent auditory responses at the medulla following midbrain stimulation in goldfish, so it cannot yet

be eliminated as a vestibular effect. The middle group at 8–10 msec has not been reported by others. It probably involves a different synaptic pathway from the early response, rather than slower fibres of the same pathway, since it could not be driven at nearly as high a frequency. The decrease in latency at higher stimulus rates may have resulted from the repetition maintaining the responsible cells nearer their threshold than at low frequencies. The late group at 16–20 msec is similar in latency to the effects resulting from stimulating the contralateral Deiters' nucleus in cats (Sala, 1963, 1965), a known source of efferents to the vestibular system.

It seems unusual that the frequencies which produced a response from the afferents in the intact nerve, the 100 Hz stimulus of the burst, were those at which the long-latency evoked responses in the cut nerve were abolished. The latencies for effects on the afferent rates in the intact nerve, in the range of 50–100 msec, were also much longer than latencies for evoked responses in the cut nerve. These latencies are similar to those found by other authors for effects on both efferent and afferent activity from contralateral physiological stimulation of the labyrinths (Gleisner & Henriksson, 1963; Klinke & Schmidt, 1968; Caston, 1972). No latency of this length ever was seen for the evoked potentials in the cut nerve of flatfish, even with the burst stimulation to the tectum. The duration of the effect of a burst on afferents, over 500 msec in some cases, is similar to the duration of the auditory suppression following a similar stimulus reported by Piddington (1971).

These combined stimulation data suggest a possible efferent influence from the midbrain involving at least three pathways. First, a fast direct connexion might descend from the midbrain directly out via the eighth nerve to the primary afferents or the receptor cells, with a latency of roughly 5 msec, comparable to the direct cerebello-vestibular Purkinje axon efferents (Llinás & Precht, 1969; Hillman, 1969). Secondly, a connexion might descend from the midbrain to the receptors with at least one synapse, and a latency of 10 msec. It is impossible from these data to locate the synapse, but it could be in the reticular formation, a known relay in other ascending and descending vestibular pathways (Ruch *et al.* 1965, p. 216). Thirdly, a connexion might descend from the midbrain to synapse in the vestibular nuclei, similar to that described by Markham *et al.* (1966). The latency of 20 msec is close to that observed by Sala (1965) for peripheral responses to stimulation of the vestibular nuclei. Finally, the long latency and duration of the effect on afferent activity in the intact nerve could result from a need for spatial or temporal summation of the efferent activity in the sense organs, perhaps effective in changing the polarization level of the hair cells (see Hama, 1969).

3. *Visual stimulation alters tonic vestibular activity*

Correlation between optokinetic nystagmus and modulation of spontaneous vestibular activity in both goldfish and rabbits have suggested a role of 're-afference' for an efferent vestibular pathway (Dichgans *et al.* 1970; Schmidt *et al.* 1970; Klinke & Schmidt, 1970). In this model the optokinetic stimulus causes an intent to move, and simultaneously initiates an efferent activity which cancels out the change in vestibular afferent activity resulting from the movement. Thus an intended movement is not interfered with by vestibular reflexes which would otherwise cause compensations for the same movement if unintended. The same workers have recently shown

that tonic eye position, as well as nystagmus, may have a tonic effect on some peripheral units (Schmidt, Wist & Dichgans, 1972).

While the flatfish units never showed such changes in afferent rates to passive changes in eye position, similar tonic effects were dependent on the direction of a light stimulus. Unlike the units reported by Schmidt *et al.* (1972), a few units affected in flatfish were from the otolith organs, as indicated by their tonic responses to tilt. As with the behavioural effects of light, these physiological effects appear to be independent of tilt, and add algebraically with the tilt response.

The latencies for the effects of light on vestibular units in flatfish are in the same 50–100 msec range mentioned for effects of a direct electrical burst to the tectum discussed above. The units which Schmidt *et al.* (1972) reported as tonically affected by eye position also showed a latency of 100–200 msec for the effect, in contrast to the pre-saccadic effect on the other phasic units. These results are consistent with the necessity for spatial or temporal summation discussed for the direct stimulation experiments.

4. *The descending efferent control is not simply inhibitory*

These comparisons suggest that not only is there a descending efferent tecto-vestibular pathway, but also that optic cues can be a strong input to the central control mechanism. There are three main alternatives for the type of central control exercised over the peripheral vestibular units:

(1) General inhibition. If tectal stimulation (electrical or visual) decreased the rate of activity of vestibular afferents, it would suggest that visual stimuli might override gravitational stimuli in determining posture.

(2) General excitation. If tectal stimulation increased vestibular afferent rates, it would suggest that visual stimuli might enhance sensitivity to postural change; vestibular activity could be raised closer to threshold for compensatory responses in either direction.

(3) Direction-specific excitation. If tectal stimulation on one side increased the rate of units which normally increased rate on tilts to that side, and the converse decrease also occurred, it would suggest that directional visual stimuli might enhance vestibular tilt responses by a specific summating mechanism.

All these effects have been found for the similar interaction of optokinetic stimuli, rotation and semicircular canal activity, and are designated types *i*, *a* and *d*, respectively (Schmidt *et al.* 1972). The results on flatfish indicate that similar diversity exists in the responses of otolith-organ units to both electrical and visual stimulation.

A few units responded to the three test stimuli of tilt, tectal stimulation, and light. The summation of tilt and light in the behavioural experiments suggested a prediction for the responses of these single units to the three test stimuli. A gravistatic afferent modulated by light direction should show three activity patterns. First, it must be directionally responsive to tilt, increasing on tilt to one side and decreasing to the other. Secondly, the directional light effect must be such that light to the right eye causes the same type of response normally seen with a tilt to the left. If left tilt increases the resting rate, light from the right should make the fish 'feel' tilted left, and increase the rate of the unit. Finally, since fish have a completely crossed optic

chiasm, this effect of light to one eye should be reproduced by stimulation of the opposite tectum. In short, the simplest summation mechanism would enable contralateral light stimulation and ipsilateral tectal stimulation to increase the rate of a unit which increases rate when tilted in an ipsilateral sense. Of the six responding units, however, none show agreement of all three factors. One shows agreement for tilt and tectal effects but not light; one agrees for tilt and light effects but not tectal; two agree for tectal and light effects but not tilt; and two show general excitation to both tectal stimulation or light from either side.

In the case of flatfish, then, the central control mechanisms of otolith-organ activity are not simple; visual and gravistatic input can be integrated at the peripheral level, and modify orientation responses as in other vertebrates. The effects of both electrical and visual central stimulation are diverse but discrete. Central control is not exerted by only one of the three alternatives listed; it appears they all may be used simultaneously. Given such control mechanisms affecting the primary vestibular neurones and possibly the receptors themselves, a large visual role in the metamorphic postural change of flatfish becomes more rather than less plausible. The period of eye migration without a peripheral change in otolith-organ function may well be a period of central plasticity during which visual feedback influences postural adjustment.

SUMMARY

1. Visual influences on postural responses mediated by the otolith organs were studied in behavioural and physiological experiments on two species of flatfish, the bothid *Citharichthys stigmaeus* and the pleuronectid *Hypsopsetta guttulata*.

2. Ocular compensation to lateral tilt is altered toward a directional light stimulus; the light effect is independent of the tilt effect, and sums with it. Maximum light effect is roughly one-fourth the magnitude of the maximum tilt response.

3. Single shocks to the optic tectum evoke up to three responses in the cut stump of the ipsilateral vestibular nerve, falling into three latency groups at roughly 5, 10 and 20 msec; all three follow stimulation of the anterior lateral tectum. The two later groups suggest by their delay and extinction at low stimulus rates that their path involves at least one synapse.

4. Some units determined as primary otolith-organ afferents by their response characteristics to lateral tilts show a change in rate following a burst of shocks to the tectum, but no response to single shocks. The usual effect is excitatory, with a latency of 50–100 msec, and lasting 200–500 msec after the stimulus.

5. Some of these units also show tonic alteration in rate while a light is shining into one eye. The alteration is either an increase with light to either eye, or a direction-dependent increase or decrease, with a latency of 50–100 msec, and lasting tonically during stimulation times of up to 1 min. The effect is independent of tilt.

6. While the proportion of affected units is small, the elimination of mechanical, muscular, autonomic or antidromic effects and the specificity of many of the responses demonstrate that optic-vestibular integration can involve an efferent control system from the tectum to the otolith organs.

7. The presence of such visual interactions with vestibular input in adult flatfish leads to the hypothesis that the central control of the 90° postural change occurring

during flatfish metamorphosis may involve crucial optic influences during the period of eye migration.

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REFERENCES

ARECHIGA, H. & WIERSMA, C. A. G. (1969). The effect of motor activity on the reactivity of single visual units in the crayfish. *J. Neurobiol.* **1**, 53–70.

BRAEMER, W. (1957). Verhaltensphysiologische Untersuchungen am optischen Apparat bei Fischen. *Z. vergl. Physiol.* **39**, 374–98.

BRANSTON, N. M. & FLEMING, D. C. (1968). Efferent fibers in the frog optic nerve. *Expl Neurol.* **20**, 611–23.

BUTZ-KUENZER, E. (1957). Optische und labyrinthäre Auslösung der Lagereaktion bei Amphibien. *Z. Tierpsychol.* **14**, 429–47.

CARPENTER, M. B. (1967). Central connections of the vestibular system. *Archs Otolar.* **85**, 517–20.

CASTON, J. (1972). L'activité vestibulaire efférent chez la Grenouille. *Pflügers Arch. ges. Physiol.* **331**, 365–70.

DICHGANS, J., WIST, E. R. & SCHMIDT, C. L. (1970). Modulation neuronaler Spontanaktivität im N. vestibularis durch optomotorische Impulse beim Kaninchen. *Pflügers Arch. ges. Physiol.* **319**, R 154–5.

DOHLMAN, G. F. (1960). Histochemical studies of vestibular mechanisms. In *Neural Mechanisms of the Auditory and Vestibular Systems* (ed. G. L. Rasmussen and W. F. Windle), pp. 258–75. Springfield, Ill.: C. C. Thomas.

DOHLMAN, G. G., FARKASHIDY, J. & SALONNA, F. (1958). Centrifugal nerve-fibers to the sensory epithelium of the vestibular labyrinth. *J. Lar. Otol.* **72**, 984–91.

GACEK, R. R. (1960). Efferent component of the vestibular nerve. In *Neural Mechanisms of the Auditory and Vestibular Systems* (ed. G. L. Rasmussen and W. F. Windle), pp. 276–84. Springfield, Ill.: C. C. Thomas.

GACEK, R. R. (1966). The vestibular efferent pathway. In *The Vestibular System and Its Diseases* (ed. R. J. Wolfson), pp. 99–116. Philadelphia: University of Pennsylvania Press.

GALAMBOS, R. (1956). Suppression of auditory nerve activity by stimulation of efferent fibers to the cochlea, *J. Neurophysiol.* **19**, 424–37.

GERNANDT, B. E. (1967). Central regulation of the vestibular system. *Archs Otolar.* **85**, 521–8.

GLEISNER, L. & HENRIKSSON, N. G. (1963). Efferent and afferent activity pattern in the vestibular nerve of the frog. *Acta oto-lar.*, Suppl., **192**, 90–103.

HAMA, K. (1969). A study on the fine structure of the saccular macula of the gold fish. *Z. Zellforsch. mikrosk. Anat.* **94**, 155–71.

HARA, T. J. (1967). Electrophysiological studies of the olfactory system of the goldfish, *Carassius auratus* L. II. Response patterns of olfactory bulb neurones to chemical stimulation and their centrifugal control. *Comp. Biochem. Physiol.* **22**, 199–208.

HASHIMOTO, T., KATSUKI, Y. & YANAGISAWA, K. (1970). Efferent system of lateral line organ of fish. *Comp. Biochem. Physiol.* **33**, 405–21.

HILLMAN, D. E. (1969). Light and electron microscopical study of the relationships between the cerebellum and the vestibular organ of the frog. *Exp. Brain Res.* **9**, 1–15.

HOLDEN, A. L. (1968). The centrifugal system running to the pigeon retina. *J. Physiol., Lond.* **197**, 199–219.

VON HOLST, E. (1935). Über den Lichtrückenreflex bei Fischen. *Pubbl. Statz. zool. Napoli* **15**, 143–58.

VON HOLST, E. (1950). Die Arbeitsweise des Statolithenapparates bei Fischen. *Z. vergl. Physiol.* **32**, 60–160.

VON HOLST, E. (1954). Relations between the central nervous system and the peripheral organs. *Br. J. Anim. Behav.* **2** 89–94.

VON HOLST, E. & MITTELSTAEDT, H. (1950). Das Reafferenzprinzip. *Naturwissenschaften* **37**, 464–76.

HOWARD, I. P. & TEMPLETON, W. B. (1966). *Human Spatial Orientation.* New York: John Wiley.

KLINKE, R. & SCHMIDT, C. L. (1968). Efferente Impulse im Nervus Vestibularis bei Reizung des kontralateralen Otolithenorgans. *Pflügers Arch. ges. Physiol.* **304**, 183–8.

KLINKE, R. & SCHMIDT, C. L. (1970). Efferent influence on the vestibular organ during active movements of the body. *Pflügers Arch. ges. Physiol.* **318**, 325–32.

LIVINGSTON, R. B. (1959). Central control of receptors and sensory transmission systems. In *Neurophysiology*, vol. 1, Sect. 1 of *Handbook of Physiology* (ed. J. Field, H. W. Magoun and V. E. Hall), pp. 741–59. Washington: American Physiological Society.

LLINÁS, R. & PRECHT, W. (1969). The inhibitory vestigular efferent system and its relation to the cerebellum in the frog. *Exp. Brain Res.* **9**, 16–29.

LOWENSTEIN, O. (1971). The labyrinth. In *Fish Physiology*, vol. v (ed. W. S. Hoar and D. J. Randall), pp. 207–40. New York: Academic Press.

MANN, C. W. (1952). Visual factors in the perception of verticality. *J. exp. Psychol.* **42**, 460–4.

MARKHAM, C. H., PRECHT, W. & SHIMAZU, H. (1966). Effect of stimulation of interstitial nucleus of Cajal on vestibular unit activity in the cat. *J. Neurophysiol.* **29**, 493–507.

PETROFF, A. E. (1955). An experimental investigation of the origin of efferent fiber projections to the vestibular neuroepithelium. *Anat. Rec.* **121**, 352–3.

PIDDINGTON, R. W. (1971). Central control of auditory input in the goldfish. I. Effect of shocks to the midbrain. *J. exp. Biol.* **55**, 569–84.

PLATT, C. J. (1973). Central control of postural orientation in flatfish. I. Postural change dependence on central neural changes. *J. Exp. Biol.* **59**, 491–521.

ROBERTS, B. L. & RUSSELL, I. J. (1970). Efferent activity in the lateral line nerve of dogfish. *J. Physiol., Lond.* **208**, 37*P*.

ROEDEL, P. M. (1953). Common ocean fishes of the California coast. *Fish Bull. Calif.* **91**.

RUCH, T. C., PATTON, H. D., WOODBURY, J. W. & TOWE, A. L. (1965). *Neurophysiology*, 2nd ed. Philadelphia: W. B. Saunders.

SALA, O. (1963). Modifications of the vestibular nerve activity by stimulation of the efferent vestibular system. *Experientia* **19**, 39.

SALA, O. (1965). The efferent vestibular system. *Acta. Otolar.*, suppl. **197**, 1–34.

SCHMIDT, C. L., WIST, E. R. & DICHGANS, J. (1970). Alternierender Spontannystagums, optokinetischer und vestibularärer Nystagums und ihre Beziehungen zu rhythmischen Modulation der Spontanaktivität im N. vestibularis beim Goldfisch. *Pflügers Arch. ges Physiol.* **319**, R 155–6.

SCHMIDT, C. L. WIST, E. R. & DICHGANS, J. (1972). Efferent frequency modulation in the vestibular nerve of goldfish correlated with saccadic eye movements. *Exp. Brain Res.* **15**, 1–14.

SCHMIDT, R. S. (1963). Frog labyrinthine efferent impulses. *Acta Otolar.* **56**, 51–64.

SCHÖNE, H. (1952). Zur optischen Lageorientierung ('Lichtrückenorientierung') von Dekapoden. *Naturwissenschaften* **39**, 552–3.

SCHÖNE, H. (1964). Über die Arbeitsweise der Statolithenapparate bei Plattfischen. *Biol. Jahresh.* **4**, 135–56.

TRAILL, A. B. & MARK, R. F. (1970). Optic and static contributions to ocular counter-rotation in carp. *J. exp. Biol.* **52**, 109–24.

WERSÄLL, J. & FLOCK, Å. (1965). Functional anatomy of the vestibular and lateral line organs. *Contrib. Sensory Physiol.* **1**, 39–61.

WOLFF, H. G. (1970). Efferente Aktivität in den Statonerven einiger Landpulmonaten (Gastropoda). *Z. vergl. Physiol.* **70**, 401–9.