

THE INDIVIDUAL AND INTEGRATED ACTIVITY
OF THE SEMICIRCULAR CANALS OF THE
ELASMOBRANCH LABYRINTH

BY O. LÖWENSTEIN AND A. SAND

*From the Department of Zoology, University of Glasgow, and the
Laboratory of the Marine Biological Association, Plymouth*

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THE recent work of Steinhausen [1931, 1935] has furnished direct experimental confirmation of the classical theory of the mechanism of the semicircular canals, inasmuch as it was clearly shown that the cupula terminalis, the gelatinous auxiliary structure enclosing the sensory hairs of the crista ampullaris, is deflected from its normal position during an appropriate angular displacement of the labyrinth. At the same time, Steinhausen was able to demonstrate and analyse the correlations between the deformation of the cupula and the occurrence of reflex eye movements. Our own electrical study of the horizontal canal of the dogfish [Löwenstein & Sand, 1936] disclosed the unexpected fact that the sensory excitation which, on the grounds of reflex phenomena, could be presumed to occur during ipsilateral rotation, manifested itself as an increase in the volume of a persistent impulse discharge which occurs spontaneously in the stationary labyrinth, and that, further, this spontaneous discharge was reduced, or even completely inhibited, during a rotation in the direction contralateral with respect to the canal under investigation. It appears, therefore, that the sensory mechanism of the ampulla is such that rotation in the plane of the canal in either direction evokes a characteristic sensory response, and since it is certain that the increased excitation of the ampulla during ipsilateral rotation is the causal agent in the release of responses such as reflex eye movements, it may be surmised that the inhibition which occurs at the same time in the corresponding ampulla on the other side of the head may also play a part in the central nervous processes which determine the pattern of reflex motor discharges [Löwenstein, 1937].

In order to gain some insight into this problem it is clearly desirable to extend the observations to include all the semicircular canals, so that one may be in a position to specify the events which occur in the sensory connexions of all six ampullae during an angular displacement of the head in any given plane. This we have attempted to do, and the results are the subject of the present communication.

METHODS

Electrophysiological analysis of the activity of the separate parts of the vertebrate ear has progressed slowly because of the extreme inaccessibility of these structures. Our own experiments on the horizontal canal of the dogfish (*Scyllium canicula*) were the first attempt to record the sensory discharges from an individual ampulla. The ampullae of the other two semicircular canals, the anterior vertical and posterior vertical, are even less accessible, and the task of obtaining records from the nerve twigs supplying them appeared at first sight almost hopeless. The successful experiments of Ross [1936], who recorded action potentials from the isolated labyrinth of the frog, and the experience of one of us [Sand, 1938] with isolated elasmobranch sense organs encouraged us to explore the possibility of working with the isolated labyrinth, in which the exposure of individual ampullae and their nerves can be accomplished with a certainty and neatness which would be impossible in the whole animal. The attempt was successful; the isolated labyrinth remains functionally active for several hours, and it was possible to obtain satisfactory oscillographic records from all three semicircular canals.

Mature specimens of the thornback ray, *Raja clavata*, were used, having a wing span of 18–20 in. The fish is killed by pithing, the entire jaw apparatus is quickly removed, the eye is excised, and by a median longitudinal cut through the head one-half of the cranium is separated and pinned in a convenient position by the remaining strip of rostrum to a board. The jaw muscles and eye muscles attached to the cranium are removed, and the cartilaginous auditory capsule is now cleanly exposed for dissection. The posterior ampulla, which lies at the back of the labyrinth, is approached from the floor of the auditory capsule, i.e. from the posterior region of the palate. The anterior and horizontal ampullae are approached from the outer anterior surface of the capsule, i.e. from the posterior region of the orbit. When a little cartilage in the appropriate region has been sliced away, and before the cavity of the labyrinth is actually opened, the ampulla and its nerve become clearly visible, and one can then proceed carefully shaving away a groove of cartilage sufficient to allow the nerve to be exposed, ligated, cut centrally, and lifted at its free end, its attachment to the ampulla remaining, of course, intact. Thus only a small opening is made into the perilymphatic cavity, and very little perilymph escapes. The membranous labyrinth itself must not be perforated, and this is not difficult to avoid owing to the excellent visibility of the preparation. Should the sacculus or utriculus be perforated, the preparation is spoiled, and this is soon evidenced by its behaviour when installed for electrical recording

It is advisable to use a dissecting microscope for the later stages of the preparation, though the whole process can be carried out with the naked eye. About 4 mm. of nerve is available from the posterior ampulla, and 2 or 3 mm. from the anterior and horizontal ampullae.

When a nerve from one of the ampullae has been prepared, the cartilaginous capsule containing the labyrinth is severed from the remaining anterior portion of the cranium and rostrum which has served for its fixation during the dissection, and is mounted in one of the two electrode-holders illustrated in Figs. 1 and 2.

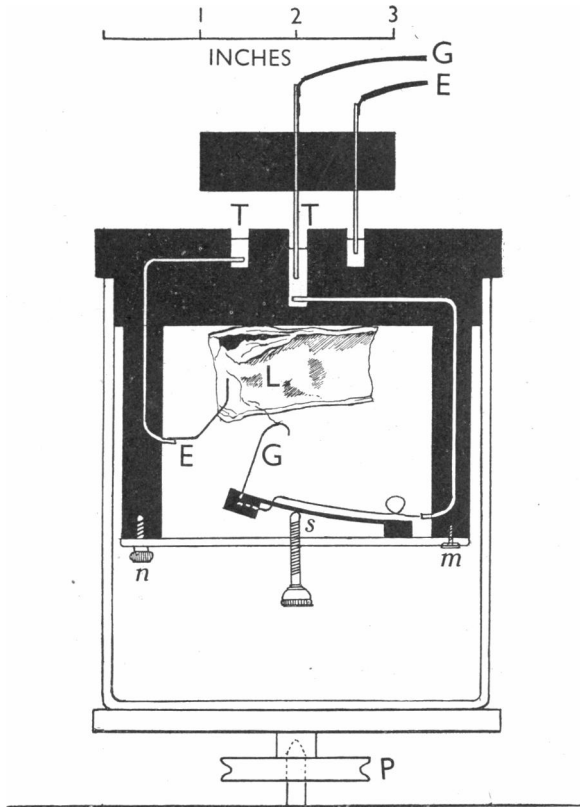


Fig. 1. Diagram of rotating holder. Explanation in text.

The rotating holder (Fig. 1) is a circular slab of ebonite turned to fit accurately, like a lid, into a cylindrical glass jar. Two pillars set opposite each other on the under side of the slab are joined by a brass strip which swings aside about the pivot *m* and can be secured by a screw *n*. In the centre of the slab is a well of circular cross-section, and concentric with it is an annular trough less deep than the well. The well and the annular trough *T, T*, are filled with mercury, and into each dips a stout copper rod. The two rods are held in a block of ebonite, which is clamped in an adjustable stand so that it may be raised and lowered. The input leads to the amplifier are soldered to these two copper rods. The ebonite slab and pillars are drilled to take the two copper wires which connect the mercury in the trough

T, T with the platinum electrodes *G, E*. The grid electrode *G* is given a mechanical vertical adjustment by being mounted on the spring *s*, attached at one end to the brass strip *nm*. The glass jar is glued in an accurately centred position to the turn-table, which is driven by means of its pulley, *P*.

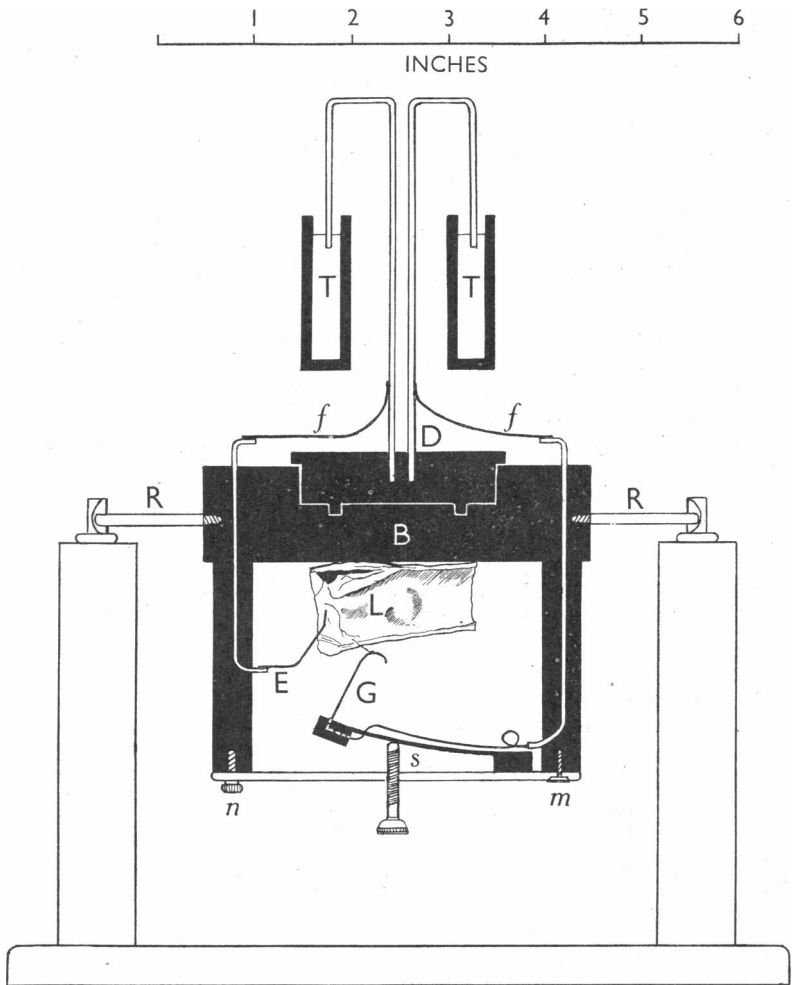


Fig. 2. Diagram of tilting holder. Explanation in text.

For mounting a preparation the ebonite holder is removed and laid upside down on the bench. The labyrinth *L* is then secured firmly in position by means of three rubber bands which cross over it and are fastened to six screw hooks set peripherally around the under surface of the slab. The brass strip *nm* is swung into position, and the nerve adjusted on the electrode *G*. The neutral electrode *E* is brought into contact with the body of the preparation. The holder is then turned the right way up and lowered into position in the jar on the

turn-table. Mercury is deposited in the troughs, the copper rods are brought down to make contact with it, and the installation is now ready for recording. The mercury has to be emptied and renewed every time a fresh preparation is mounted. It is a convenient circumstance that the upper surface of the head in a ray, above the auditory capsules, is flat and horizontal. Therefore, when a labyrinth is mounted as described, its orientation corresponds fairly accurately with its normal position in space in a ray lying flat on a horizontal surface.

The tilting holder (Fig. 2) is similar in design except that the ebonite slab *B* is tapped to take four brass rods, *R, R*, at intervals of 90° around its circumference. These serve to pivot the holder for tilting the labyrinth about its longitudinal or transverse axis. Connection with the amplifier is made through two long parallel mercury troughs, shown in section in the figure (*T, T*). Into each trough dips a copper rod having two right-angle bands. These rods are connected by flexible leads, *f, f*, with the copper wires leading down through the ebonite to connect with the platinum electrodes, *E, G*. The flexible leads allow the disk *D*, which carries the copper rods, to be lifted, turned through 90° and slipped into position again when the holder is changed from one axis to the other. The troughs *T, T*, therefore, need not be disturbed during this operation. If the troughs are made long enough (about 6 in.) the holder can be tilted 40° from the horizontal in either direction.

The recording system (amplifier, Matthews oscillograph, loud-speaker and moving-paper camera) was the same as used in previous work [Löwenstein & Sand, 1936; Sand, 1937, 1938]. The rotation of the turn-table and of the tilting holder were recorded by means of a mechanical signalling device which controlled the displacement of a wire intercepting the oscillograph beam in front of the cylindrical lens of the camera.

RESULTS

The effects of angular displacement of the labyrinth about its vertical, transverse and longitudinal axes were observed in eighteen preparations. Of these, twelve were preparations of the posterior vertical ampullae, five were anterior vertical ampullae, and one was the right horizontal ampulla. Our original observation on the left horizontal ampulla of *Scyllium* was fully confirmed. The pattern of behaviour that was then described is thus typical of all the semicircular canals of the elasmobranch labyrinth. The ampullary sense organs are constantly in a state of spontaneous activity when the labyrinth is at rest, and discharge a stream of asynchronous impulses along their nerves. These discharges are increased during angular displacement in the plane and direction appropriate for each individual semicircular canal, and they are inhibited during displacement in the direction antagonistic to that which excites them.

In Fig. 3 are shown records of the responses of the right horizontal ampulla to horizontal rotation. Record A shows the spontaneous resting discharge, and the response to a clockwise (ipsilateral) rotation through an arc of 90° , signalled by the upward displacement of the white line. The time intervals at the bottom of the record are seconds. The average rate of rotation in record A was therefore of the order of one revolution

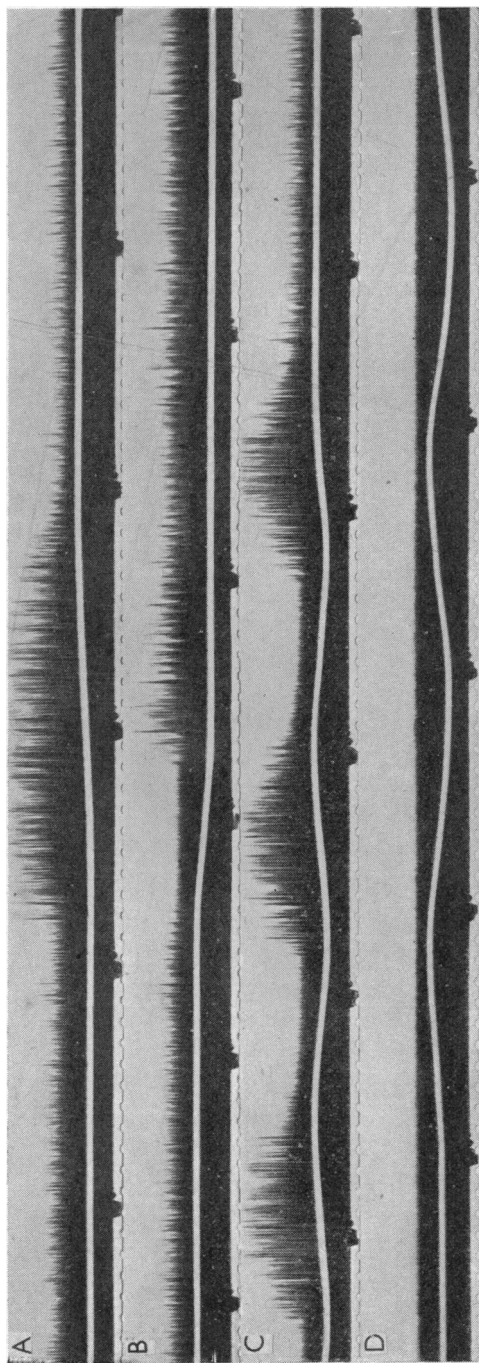


Fig. 3. Records of the responses of the right horizontal ampulla to horizontal rotation. A. Spontaneous resting discharge and response to clockwise rotation. B. Spontaneous resting discharge, response to anticlockwise rotation, and after-discharge. C. Response to consecutive clockwise and anticlockwise swinging of the turn-table through an arc of about 60° . D. Control record after crushing of nerve. Explanation of white signal-line in text. Time intervals = 1 sec. The records read from left to right.

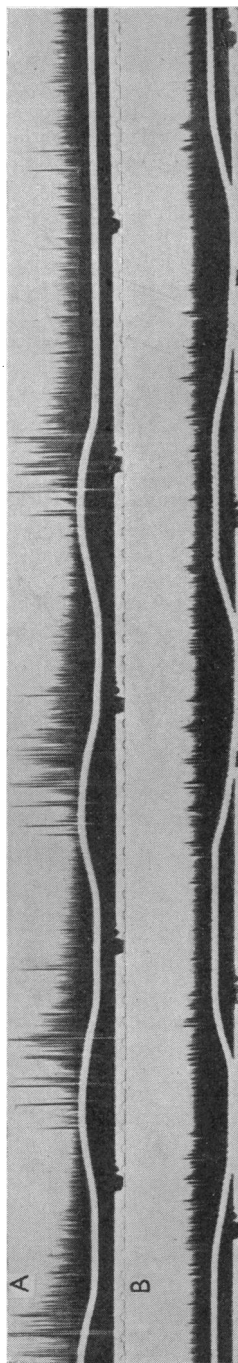


Fig. 4. A. Response of left posterior ampulla to consecutive backwards and forwards tilting about the transverse axis. B. Control record after crushing of nerve. Explanation of white signal-line in text. Time intervals = 1 sec. The records read from left to right.

in 6 sec. Record B shows the inhibition of the discharge during 90° of rotation in the opposite sense (anticlockwise). In record C the turn-table was swung to and fro through an arc of about 60° , producing an alternation of excitation and inhibition of the discharge, and, at the end of this record, where the turn-table is brought to rest, the spontaneous discharge is re-established. Control record D was taken after the ampulla had been crushed, the nerve itself remaining intact on the electrode. The amplification remained constant for all four records.

Fig. 4 shows a similar record from the left posterior ampulla during tilting backwards and forwards about the transverse axis through an arc of about 70° . Downward displacement of the white line indicates backward tilting of the labyrinth, which is excitatory in the case of the posterior ampulla, upward displacement of the line indicates forward tilting which is inhibitory. A record from the ampulla of an anterior canal would look exactly the same with the one essential difference that in that case forward tilting would be excitatory and backwards tilting inhibitory. Technically the tilting holder was less satisfactory than the turn-table, for there was a slight roughness in its movement which produced a microphonic unsteadiness of the base line, which is still shown in the control record B, taken after the ampulla had been crushed.

After-effects. In Fig. 3, record B, it will be observed that the inhibitory response to the rotation of the labyrinth is followed by an outburst of discharge which is significantly greater than the resting discharge before the beginning of the rotation. Such an after-discharge has appeared in the majority of our experiments, but was considerably more pronounced in some preparations than in others. In those cases where the after-discharge was prominent, the converse phenomenon was also observed, namely a silent period following the response to an excitatory stimulation. This was strikingly reminiscent of the silent period which occurs in single-fibre preparations of the lateral line following stimulatory perfusion of the canal [Sand, 1937].

The dynamic responses of a semicircular canal to rotation undoubtedly depend upon the integrity of the entire structure. Any damage which interferes with the continuity and free circulation of the endolymph inside the canal abolishes the dynamic responses. Preparations in which the canal was ligated or cut, or in which the sacculus was perforated ceased to respond either with excitation or inhibition to angular displacements. The spontaneous discharge in such damaged preparations, however, continued unaffected. The interpretation of these facts depends on the recognition of the spontaneous discharge as a property of

acoustico-lateral receptors in general, a property which is independent of the peculiar structural features of the labyrinth. It continues as long as the sensory elements of the crista are themselves intact and in good physiological condition. But the complete apparatus of semicircular canal, ampulla and cupula determines and restricts the mechanical conditions which can bring about a deformation of the cupula, with the resulting changes in the impulse discharges.

In Table I are summarized the responses of all the semicircular canals to rotation about the three primary axes. It will be observed that the horizontal canals are affected only by rotation about the vertical axis,

Table I. Responses of the six semicircular canals to angular displacements about the three primary axes. ● excited; ⊗ inhibited; ○ unaffected. ant. vert. = anterior vertical; post. vert. = posterior vertical.

Semicircular canal	Rotation about the					
	Longitudinal axis		Transverse axis		Vertical axis	
	Right	Left	Forwards	Backwards	Clockwise	Anticlockwise
Right ant. vert.	●	⊗	●	⊗	⊗	●
Left ant. vert.	⊗	●	●	⊗	●	⊗
Right post. vert.	●	⊗	⊗	●	●	⊗
Left post. vert.	⊗	●	⊗	●	⊗	●
Right horizontal	○	○	○	○	●	⊗
Left horizontal	○	○	○	○	⊗	●

whilst the four vertical canals respond either with excitation or inhibition to any rotation about any of the three primary axes. For example: the right anterior vertical canal is excited (i) by tilting towards the right about the longitudinal axis, (ii) by forward tilting about the transverse axis, and (iii) by anticlockwise turn-table rotation about the vertical axis.

It may be emphasized that the electrical responses in the three cases are entirely identical with the possible exception of differences in threshold which could, however, not be analysed.

The table shows further that the four vertical canals are functionally grouped in pairs, the grouping differing in the three types of rotation. Thus, during rotation about the longitudinal axis (tilting sideways) they may be described as *laterally synergic*, during rotation about the transverse axis (tilting forward and backward) they are *transversely synergic*, and during rotation about the vertical axis (turn-table rotation) they are *diagonally synergic*. For example: tilting towards the right excites the right anterior and posterior canals (lateral synergy), tilting forwards

excites the right and left anterior canals (transverse synergy), and clockwise turn-table rotation excites the left anterior and the right posterior canal (diagonal synergy).

DISCUSSION

An important difference is found to exist between the mode of function of the horizontal and of the vertical canals. In the horizontal canals excitation occurs when the ampulla follows the canal during angular displacement, the stimulus being ampullopetal inertia movement of endolymph. In the vertical canals excitation is caused by angular displacements in which the ampulla is leading, the stimulus being ampullofugal inertia movement of endolymph. From the days of Breuer and Ewald this difference between the two groups of canals has been repeatedly postulated by many authors [cf. Ross, 1936]. No obvious morphological character is known that could account for this functional difference.

In attempting to correlate the observed electrical activity of the individual semicircular canals with the compensatory effector responses occurring during angular displacement of an animal, the dynamic reflex responses of the eyes may serve as suitable test-reactions. A detailed description of these reflexes may be found in a recent review on the subject [Löwenstein, 1936]. Only a few facts need, therefore, be described here. Sideways tilting is accompanied by so-called vertical eye deviations which are brought about mainly by the action of the superior and inferior rectus muscles of the eye. Forward and backward tilting leads to rotatory eye deviations which are due to the action of the superior and inferior oblique eye muscles. These reactions are usually considered to be correlated with the integrated activity of the vertical semicircular canals. The eye reaction following rotation around a vertical axis is the so-called horizontal eye deviation followed by nystagmus. This characteristic reflex is brought about by the action of the internal and external rectus muscles, and has generally been considered to be evoked by the activity of the horizontal canals. It will be recalled that our experiments have shown that, apart from the horizontal canals all four vertical canals react to horizontal rotation. The question arises, therefore, whether this response of the vertical canals is in any way involved in the mechanism of the horizontal eye responses.

It would be quite plausible to assume that the pattern of afferent impulses correlated with diagonal synergy of the vertical canals might be received by the centre as a signal specific for horizontal rotation, as

opposed to the patterns of lateral and transverse synergy, denoting tilting about the longitudinal and transverse axes. If this be so, the horizontal canals might well be considered to be superfluous. However, it has been shown by elimination experiments in the pike, *Esox lucius* [Löwenstein, 1937], that section of the nerves supplying both horizontal ampullae totally abolishes the horizontal eye reactions, although the function of the vertical canals is completely unimpaired by this operation. It is clear, therefore, that the response to horizontal rotation does not enable the vertical canals themselves to evoke the horizontal eye reactions. The following considerations may suggest, however, in what way this response might be involved in the final adjustment of the mechanism of these reflexes.

Table II shows a number of diagrammatic representations of the labyrinth viewed from above with the six canals projected into one plane. A, B, C, and D indicate the state of excitation and inhibition arising on the outset of each one of the four tilting movements carried out in our experiments. Column 2 shows the eye reflex that would be observed in a reflex test, and column 3 the eye muscle whose contraction may be considered to be the main cause of that reflex. E shows a similar picture of what happens on the outset of clockwise turn-table rotation about a vertical axis. In order to get an idea of what might be the reflex effect of the diagonally synergic excitation and inhibition of the four vertical canals during this horizontal rotation, it may be permissible to abstract from A, B, C, and D a correlation between the excitation of a given canal and the reflex contraction of eye muscles to which this can give rise. This is shown in scheme F. All four vertical canals are represented there in the excited state, and against them are noted the muscles of the right eye whose contractions can, according to A-D be correlated with that state of excitation. At the same time the oblong rectangles indicate the three types of synergy observed in our experiments.

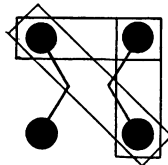
The result of this analysis is strikingly satisfactory. It shows that during sideways tilting (lateral synergy) the contraction of the superior rectus muscle (and, of course, the simultaneous relaxation of its antagonist, the inferior rectus, omitted for the sake of clarity) brings about the vertical eye deviation. At the same time the other pair of antagonists, the superior and inferior obliqui are both made to contract simultaneously. Similarly, during forward tilting (transverse synergy), the contraction of the inferior oblique (and the simultaneous relaxation of the superior oblique, not included in the scheme) bring about the rotatory deviation of the eye and at the same time the antagonistic

Table II. Correlation between the responses of the six semicircular canals to angular displacement, and the ensuing reflex contractions of the six eye muscles. ● excited; ⊗ inhibited; ○ unaffected; *a.v.* anterior vertical canals; *p.v.* posterior vertical canals; *l.h.* left horizontal canal; *r.h.* right horizontal canal.

Response of canals to	Reaction of right eye	Contracting muscle, mainly responsible for reaction
<p>A. Tilting right</p>	Vertical deviation upwards	Superior rectus
<p>B. Tilting left</p>	Vertical deviation downwards	Inferior rectus
<p>C. Tilting forwards</p>	Rolling upwards	Inferior obliquus
<p>D. Tilting backwards</p>	Rolling downwards	Superior obliquus
<p>E. Clockwise rotation</p>	Horizontal deviation forwards	Internal rectus

F. Correlation between excitation of the vertical canals and contraction of the superior and inferior recti and obliqui

Inferior rectus
Inferior obliquus



Superior rectus
Inferior obliquus

Inferior rectus
Superior obliquus

Superior rectus
Superior obliquus

superior and inferior recti are simultaneously made to contract. This simultaneous contraction of pairs of antagonistic eye muscles not directly concerned in the reflex movement at progress, has actually been observed in the rabbit by Lorente de No [1931], who ascribes to it the function of providing a fixed pivot around which the actual reflex movement of the eye can take place. In the case of horizontal rotation about a vertical axis Lorente de No [1931, p. 111, Fig. 10] shows that during the nystagmic activity of the internal and external recti, both the superior and inferior recti and the superior and inferior obliqui contract simultaneously. Exactly the same results follow from our analysis. Scheme E shows the state of excitation and inhibition of the canals during clockwise rotation. Choosing the excited vertical canals (left anterior and right posterior) and transferring the pattern of their diagonal synergy to scheme F, we see that the theoretical result would, in fact, be the simultaneous contraction of the two antagonistic pairs of eye muscles (superior and inferior recti, superior and inferior obliqui). It may well be assumed, that, if these contractions do occur in our animal, the effect would be the creation of a pivot around which occurs the horizontal eye deviation with nystagmus, which is brought about by the antagonistic working of the horizontal eye muscles, and which is solely due to the activity of the two horizontal canals. It may be emphasized that it is not known whether one is really justified in coordinating in this simple way the activity of individual semicircular canals with that of individual effector muscles. It has been claimed [Lorente de No, 1931] that in mammals the final pattern of reflex responses is largely due to the highly complex integrating activity of the centre. We have, nevertheless, thought it useful to carry out and describe at length the above analysis, in order to demonstrate that much of the complex picture of the integrated labyrinthine reflex responses to rotation can be explained more or less exclusively on the basis of the activity of the sense organ, once the exact nature of that peripheral activity has been cleared up by means of the electrophysiological method used in our experiments.

SUMMARY

1. The individual behaviour of the semicircular canals of *Raja* has been investigated in surviving preparations of the isolated labyrinth by the oscillographic method. The effects of rotation or tilting about the vertical, longitudinal and transverse axes were determined.
2. There is a spontaneous discharge of sensory impulses from each ampulla when the labyrinth is at rest.

3. During angular displacement in the appropriate direction the discharge of impulses is increased or inhibited.

4. The horizontal canals respond to rotation about the vertical primary axis, but are unaffected by rotations about the two horizontal primary axes.

5. The anterior and posterior vertical canals respond to rotation about all three primary axes.

6. During rotation about the horizontal longitudinal axis the four vertical canals are laterally synergic and during rotation about the horizontal transverse axis they are transversely synergic. During rotation about the vertical axis the four vertical canals are diagonally synergic.

7. The occurrence of an after-discharge following inhibitory rotation and of a silent period following excitatory rotation has been observed.

8. Ligating or cutting the canal or perforation of the sacculus abolishes the dynamic responses to rotation, but leaves the spontaneous discharge unaffected.

9. The integrated action of the six semicircular canals during rotation about the three primary axes are analysed in relation to the eye-muscle reflexes evoked by these rotations.

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