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# THE LOCALIZATION AND ANALYSIS OF THE RESPONSES TO VIBRATION FROM THE ISOLATED ELASMOBRANCH LABYRINTH. A CONTRIBUTION TO THE PROBLEM OF THE EVOLUTION OF HEARING IN VERTEBRATES

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In a recent paper in this *Journal* (Lowenstein & Roberts, 1950) we described experiments in which we recorded oscillographically the gravity responses from the otolith organs of the isolated labyrinth of the ray (*Raja clavata*). It was found that all the three otolith organs, viz. the utriculus, sacculus and lagena, show clear responses to gravitational stimuli. This result was somewhat surprising in the light of the fact that in a number of bony fish, especially among the Ostariophysii, the pars inferior of the labyrinth, comprising the sacculus and lagena, has no detectable equilibrium function (Lowenstein, 1932). In these animals the pars inferior has been shown to be the seat of sound perception and pitch discrimination over a wide part of the sound spectrum (von Frisch & Stetter, 1932; von Frisch 1936; Wohlfahrt, 1939, 1950; Dijkgraaf & Verheijen, 1950). In the absence of reliable experimental evidence for sound perception and its localization in the elasmobranchs it was of considerable interest to ascertain whether the participation of all three otolith organs in the perception of gravitational stimuli precludes these vestibular structures from functioning as vibration receptors.

Using preparations of the isolated elasmobranch labyrinth, such as yielded the successful electrophysiological localization and analysis of gravity perception, we have now studied the vestibular responses to vibrational stimulation in experiments to be described in the present paper.

## METHODS

A description of the operative procedure in the isolation of the labyrinth preparation, the exposure of the various sense endings and their nerve branches, and the setting up of the preparation for the testing and electrical recording of its responses has been given in our previous paper (Lowenstein & Roberts, 1950).

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It may be stated at the outset that the isolation of the labyrinth preparation from its natural anatomical setting, and the fact that it has to be mounted on a solid base in air is bound to interfere, perhaps to the extent of total abolition, with any sound-conducting mechanism that may be in operation in the intact animal. In consequence, the threshold for vibrational stimulation will be greatly affected over wide ranges of the frequency spectrum. It is thus not surprising that airborne sound proved entirely ineffective.

All the results described below were obtained by the application of vibrational stimuli conducted to the preparation through the solid base on which it was mounted. As it was often necessary to supplement the vibrational stimuli by tilting tests for gravitational responses, a great number of the results were obtained with the preparations mounted on the tilting device described in the previous paper. In this case the vibrational stimulus was derived from the diaphragm of a telephone earpiece applied directly to the surface of the table to which the tilting device was clamped. The earphone was weighed down by a heavy block of lead fixed to its casing. It was energized from a beat-frequency oscillator, capable of a 5 W. undistorted sine-wave output from 10 cyc./sec. to 50,000 cyc./sec. In later experiments the usual preparation holder was fastened to a board which was suspended by wire from the roof of the screening cage. The telephone earpiece acting as the source of vibration was screwed diaphragm-downwards to the board by means of a screw soldered to the diaphragm. A second earphone was similarly fixed to the board in the immediate vicinity of the preparation holder. This could be connected to the amplifier system and served as a device for the approximate monitoring of the amplitude and harmonic distortion of the vibrational stimulus reaching the preparation. The complex nature of the conducting system, in both cases, did not allow a satisfactory assessment of stimulation intensities, and it became obvious that harmonics of great variety in frequency and strength were introduced. Consequently no attempt was made at a quantitative study of the stimulus-response relationships expressible in the form of audiograms.

## RESULTS

### *Localization*

In a later section of this paper we shall define what we consider to constitute a vibration response and give examples of characteristic types of such responses obtained from the various vibration-sensitive areas of the labyrinth. It may be useful, however, first to give an account of the topographical distribution of vibration sensitivity. Fig. 1 shows a ventro-lateral view of the labyrinth and the distribution of the main branches of the eighth nerve. For electrical pick-up nerve twigs were separated from these branches in the immediate vicinity of their entry into the maculae. Thus the origin of the responses could be fairly accurately assessed.

As the pars inferior was, on comparative physiological grounds, the most likely seat of vibration reception, we began our search by testing sacculus and lagena preparations. It was found that all the preparations definitely identified as derived from the macula lagenae showed a remarkable freedom from vibration sensitivity. Even the purely microphonic phenomena that can be recorded from many parts of the thin-walled vestibulum were generally completely absent from the lagena preparations, whose clear-cut gravitational responses to tilting have been previously described in detail.

The nerve fan leading from the sacculus can be seen in Fig. 1 to consist of a number of main branches, the final ramifications of which extend along the whole length of the ventro-lateral aspect of the sacculus macula. The gravity responses obtained from this macula were not uniformly spread over the whole of it, but were confined roughly to the posterior third of the nerve fan. When nerve twigs are taken from the extreme posterior end their responses are purely gravitational and are marked by an absence of microphonics, thus resembling the situation found in the lagena. The more anterior the origin of the nerve twig under observation the more subject the tilting response becomes to interference by microphonic disturbances, until in the anterior two-thirds of the macula the action potential picture assumes all the aspects of a true vibration response uninfluenced by the spatial orientation of the preparation. The transition from one type of response to the other has no strikingly evident parallel in the structural features of the macula and its covering otolith, apart from the fact that the inner edge of the otolith appears to be more firmly lodged in the V-shaped trough formed by the sacculus wall at the posterior end than at its anterior end where the otolith has a greater freedom of movement. It is nevertheless, remarkable, that within the same macula covered by its massive, undivided otolith are found side by side the receptive mechanisms for the detection of changes in the direction of gravitational pull and those for the detection of the phasic displacements brought about by vibration.

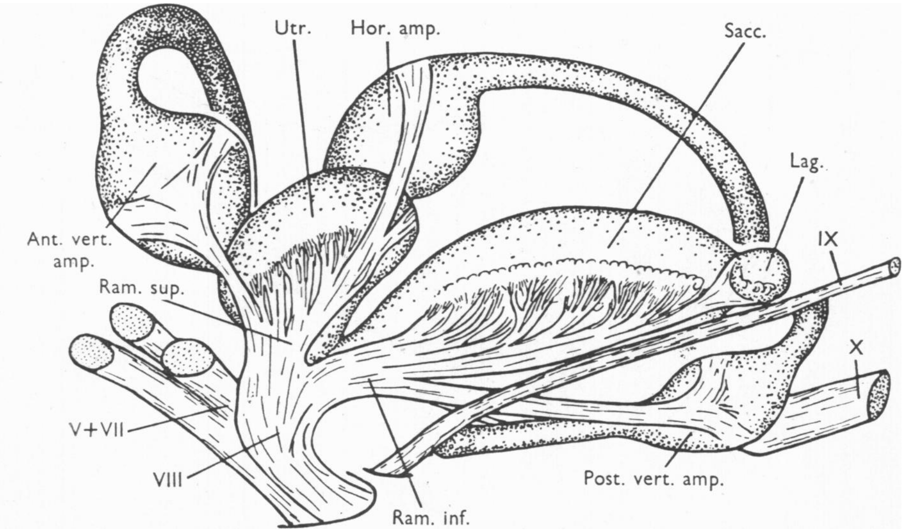

Fig. 1. Ventro-lateral view of the left membranous labyrinth of the ray. Ant. vert. amp. = anterior vertical ampulla; Hor. amp. = horizontal ampulla; Lag. = lagena; Post. vert. amp. = posterior vertical ampulla; Ram. inf. = ramus inferior; Ram. sup. = ramus superior; Sacc. = sacculus; Utr. = utriculus; V–X = 5th–10th cranial nerves.

In the sacculus the macula is wholly covered by its otolith. This is not so in the case of the macula of the utriculus. Here, only the central area, supplied by the main utricular nerve fan, is covered by the utriculus otolith and there is a posterior tongue-shaped extension of the macula towards the roof of the recessus utriculi which does not possess any otolithic covering structure. This unloaded part of the macula utriculi is known as the lacinia and is supplied by a separate nerve branch which can be seen to run posterior to the ramus ampullaris horizontalis which lies between it and the main utricular nerve fan (Fig. 2).

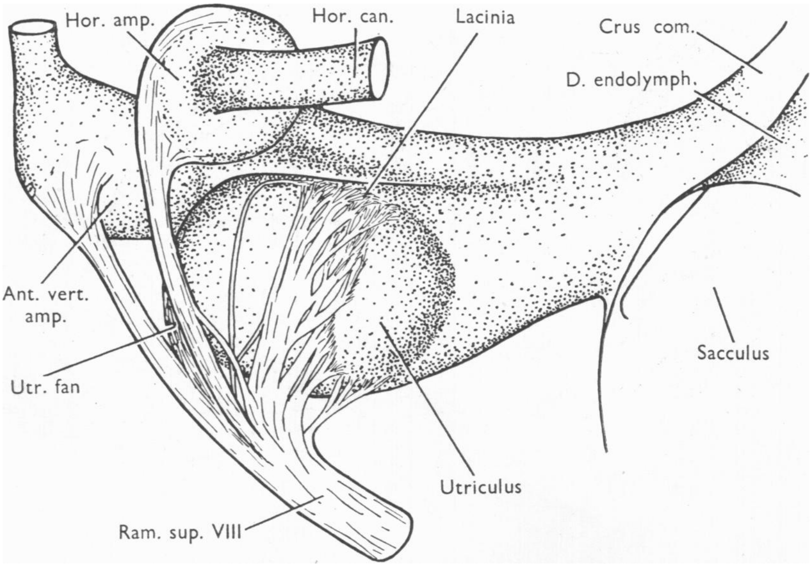

Fig. 2. Postero-lateral view of the left utriculus of the ray, showing the lacinia utriculi and its innervation. Ant. vert. amp. = anterior vertical ampulla; Crus com. = crus commune; D. endolymph. = ductus endolymphaticus; Hor. amp. = horizontal ampulla; Hor. can. = horizontal canal; Ram. sup. VIII = ramus superior of eighth nerve; Utr. fan = main utricular nerve fan.

The structural division of the utriculus macula into a loaded and an unloaded portion was found to be of functional significance. On recording from the main utricular fan, gravity responses only are obtained, and such preparations are usually reasonably unmicrophonic. Twigs separated from a nerve branch innervating the lacinia, however, do not yield responses to positional changes. Their sense endings are highly sensitive to vibrational stimulation and yield characteristic impulse responses over the same range of frequencies as the sacculus organ.

Vibration sensitivity in the elasmobranch labyrinth is thus not confined to the pars inferior. Its occurrence in the utriculus is of special interest in relation to the localization of sound perception in clupeids and other non-ostariophysean bony fish, where the participation of the pars superior in sound perception has been postulated on theoretical grounds (de Burlet, 1935; Wohlfahrt, 1936), and recently demonstrated in conditioning and elimination experiments (Dijkgraaf, 1949). The complete absence of vibration responses from the elasmobranch lagena on the other hand is surprising, when one considers that the lagena appears to play an important part in the sound perception and pitch discrimination of the ostariophysean fish.

Apart from the receptors in the sacculus macula and the lacinia utriculi we found yet another highly vibration-sensitive vestibular organ. It is the macula neglecta of hitherto unknown function. Its sensory epithelium is lodged in the medio-dorsal aspect of the sacculus cavity immediately posterior to the opening of the tubular connexion between the sacculus and the full-circle ring of the posterior semicircular canal. This remarkable organ, also sometimes called crista quarta, has no otolithic cover, but its sensory hairs are enveloped in a gelatinous secretion which in some elasmobranchs becomes fairly elaborate in shape (Vilstrup, personal communication). A long slender nerve connects the sensory epithelium of the macula neglecta with the ramus posterior of the eighth nerve. It consists of roughly a hundred nerve fibres of large diameter and is enclosed in a rather tough perineural sheath. As will be seen later, the

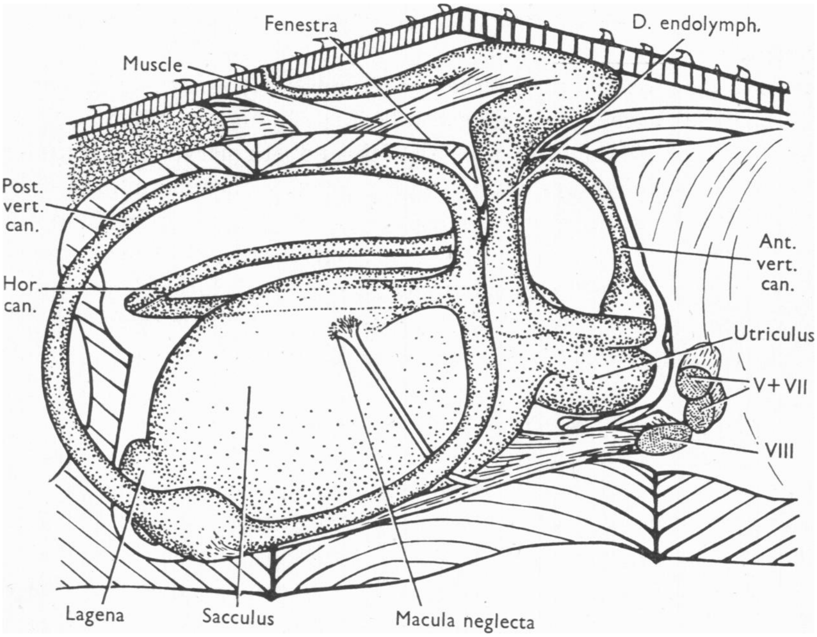

Fig. 3. Inside view of the left membranous labyrinth of the ray, showing the position and innervation of the macula neglecta and the spatial relationship between the sacculus and the fenestra of Scarpa, and the ductus endolymphaticus. Ant. vert. can. = anterior vertical canal; D. endolymph. = ductus endolymphaticus; Hor. can. = horizontal canal; Post. vert. can. = posterior vertical canal; V + VII, VIII = 5th + 7th and 8th cranial nerves.

vibration responses from the macula neglecta differ from those obtained from the utriculus and the sacculus maculae, both in their extremely low threshold and in certain peculiarities in the impulse picture. Topographically the macula neglecta offers a point of greatest interest. Its position in the labyrinth places it in the immediate vicinity of a membrane which covers an oval perforation in the cartilaginous skull, first described by Scarpa (1789) as the 'fenestra ovalis' (Fig. 3). This, in conjunction with the external opening of the ductus endolymphaticus situated nearby, may well form part of a sound-conducting mechanism aiming at the sacculus in general and the macula neglecta in particular.

Access to the proximal part of the nerve is relatively easily gained by making a small opening in the cartilaginous wall of the braincase at the level of, but posterior to, the foramina of the 5th, 7th and 8th nerves. A considerable amount of perilymph can be drained off through this opening to free a sufficient stretch of nerve from the short-circuiting effect of the surrounding fluid. Owing to the fact that the nerve is enclosed in a tough sheath of perineurium, and as its delicate insertion in the membranous sacculus wall forbids the application of the slightest pull, it is practically impossible to subdivide this nerve. It has to be picked up as a whole and single fibre recordings are thus unobtainable.

## *The nature of the vibration response*

### *Sacculus*

If in the sacculus a twig is picked up for recording from well within the anterior two-thirds of the macula it is safe to forecast that it will show no significant response to tilting. A resting discharge is often present which is uninfluenced by positional changes. Fig. 4, record 1, shows the effect of a vibrational stimulus with the beat-frequency oscillator giving a signal of 85 cyc./sec. It will be seen that there is a striking increase in the discharge activity, which appears to be due partly to the speeding up of the firing rate of the two fibres represented in the resting discharge and partly to the recruitment of new entrants. Fig. 4, record 2, shows the response of the same preparation to intermittent stimulation at the same frequency with stimuli lasting $\frac{1}{6}$ sec. repeated at $\frac{1}{3}$ sec. intervals. Here, too, the initial burst and the levelling out can be clearly seen. In both cases the response is asynchronous, i.e. its frequency is entirely unrelated to the stimulus frequency. It is interesting to note that an off-effect appears in the form of a transitory depression of the resting discharge frequency after the termination of stimulation.

Fig. 4, record 3, shows how tapping of the table can bring about discrete bursts of asynchronous potentials. The bursts occur at a frequency of 48 bursts per sec., a rhythm which could well be in synchrony with the leading rhythm of the vibrating table. The discharge frequency during the bursts is in this case six to seven times higher than in the resting discharge.

To investigate the genesis of rhythmicity in the response a preparation consisting of two to three functional units was stimulated with a signal frequency between 20 and 30 cyc./sec. The intensity was increased during the stimulation in three discrete steps of 6 db. each (Fig 4, records 4*a* and 4*b*).

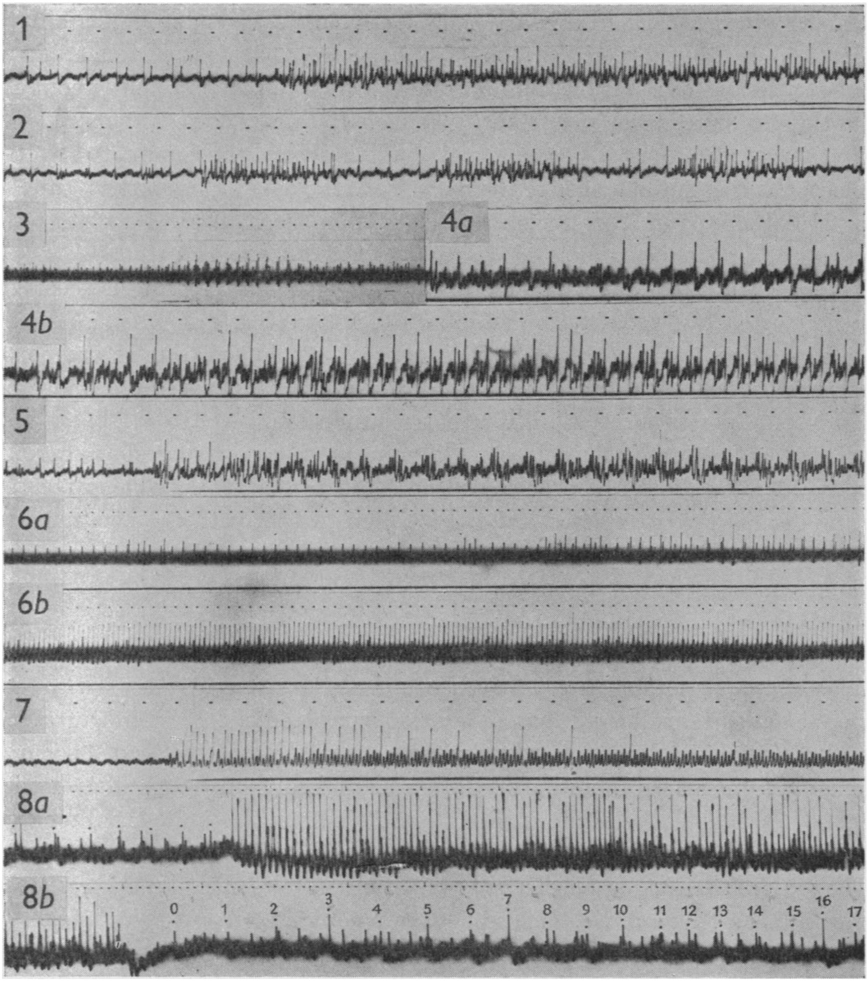

Fig. 4. Records of vibration responses from the sacculus of the ray. Detailed explanation in the text. In records 1–7 inclusive, time marker at top of record: 24/sec.; in nos. 8*a* and 8*b*: 50/sec. Nos. 4*a* and 4*b* are continuous; nos. 6*a* and 6*b* are parts of a single record with an interval of 7·5 sec. between *a* and *b*. Nos. 8*a* and 8*b* show one of the records of 'post-stimulatory inhibition' plotted in Fig. 5. There is an interval of 4·5 sec. between *a* and *b*. The marks in records 8*a* and 8*b* indicate the method of timing the impulses, which are marked with dots in the resting discharge at the beginning and with numbered dots after the cessation of the synchronized vibration response.

It will be seen that the effect of rising stimulus intensity is the appearance of high summated spikes at 28 impulses/sec. (record 4*a*). At the highest intensity this rate is doubled (record 4*b*). At this low-frequency stimulation the response is thus rhythmical, and in this case consists of summated spike potentials.

Fig. 4, record 5, shows the response from the same preparation to a stimulus of about 20 cyc./sec. with a running change of intensity brought about by a continuous sweep over the intensity range. Here again the response shows a rhythm synchronous with the signal, but this time the summated spikes appearing in the initial stages of the response are, at higher intensities, supplemented by groups of discrete new entrants. This leads to the appearance of rhythmically recurring groups of spikes at a rate of 22 groups per sec.

The recruitment of new entrants during stimulation with rising intensity is also illustrated by Fig. 4, records 6*a* and 6*b*. The resting discharge is derived from a single unit firing at a rate of 24 impulses/sec. The first sign of a response to a stimulus of approx. 55 cyc./sec. is the interpolation of three unevenly spaced spike potentials of slightly greater amplitude (record 6*a*). These new entrants then establish themselves and become more evenly spaced at approximately 25 impulses/sec. (end of record 6*a*). Meanwhile, the frequency of the resting discharge is unaffected and can be seen to beat with the new entrant, and this leads now and then to complete superposition. On further increase in stimulus intensity the frequency of the new entrant increases gradually and finally reaches approximately 50 impulses/sec. (record 6*b*). At the highest intensity reached, a second new entrant made its appearance speeding up quickly from 15 impulses/sec. and becoming almost synchronous with the first. We are thus dealing with a preparation composed of three units, of which one is unaffected by vibration, whereas two respond by an increase of discharge frequency to increased intensity of vibration. It is difficult to decide whether there is any significance in the fact that in both responding units the discharge frequency finally attained was near to the signal frequency.

The most extreme example of synchronization between stimulus and response is shown in Fig. 4, record 7. This response was obtained from a preparation of the sacculus macula which did not show a resting discharge. It showed, however, good vestibular microphonics of moderate amplitude over a range between 60 and 100 cyc./sec. with a clear peak of sensitivity to a stimulus signal of 96 cyc./sec. approx. At this frequency the vestibular microphonics showed frequency doubling (i.e. 192 cyc./sec.). At first a spike potential coincides with every second crest of the microphonic wave, then the response adapts and more and more spikes are missed out. The general phase relationship between spike and wave is maintained. In view of the considerable amplitude of the potentials, it is very likely that they are derived from a number of simultaneously firing neuromasts.

In the case of Fig. 4, record 2, attention was drawn to the appearance of an off-effect at the end of vibratory stimulation. This takes the form of a temporary depression in the basic discharge rate. Fig. 4, records 8*a* and 8*b*, show a response to a 60 cyc./sec. stimulus with perfect synchronization of the high-amplitude spike potentials. The basic discharge is derived from two to three fibres, and it is possible to assess the basic discharge rate of the highest of the spikes (record 8*a*). After the cessation of the stimulus this unit can again be identified and its rate measured (record 8*b*). Five such records were taken with increasing duration of stimulation and the post-stimulatory discharge rates plotted against time (Fig. 5). It is clear that the post-stimulatory depression in the discharge rate is a function of the length (and very likely also the strength) of the stimulus, and that there exists likewise a correlation between the length of the stimulus and the time taken for the recovery of the basic discharge rate characteristic of this unit. Apart from giving useful quantitative information with regard to the stimulus-response relationship this series of experiments furnishes additional proof that a unit involved in the basic activity of the preparation was, in this case, definitely affected by the stimulus and participated in the response. In view of the fact that it is impossible to identify the discharge from any individual unit during such a synchronized response, the existence of the post-stimulatory effect is welcome.

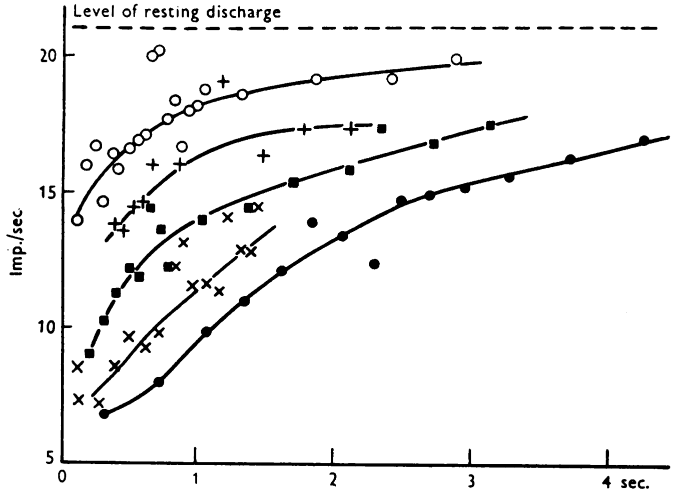

Fig. 5. Graphs showing the course of recovery of the discharge frequency from a single saccular sense ending to its basic resting level, after its 'inhibition' by vibrational stimulation of different durations. Abscissa: time in seconds after the cessation of the stimulus. Ordinate: discharge frequency in impulses/sec. Stimulus duration: ○, 0·9 sec.; +, 1·5 sec.; ■, 2 sec.; ×, 3 sec.; ●, 6·5 sec. Part of the record plotted in curve ● is shown in Fig. 4, nos. 8*a* and 8*b*.

With the sweep frequency synchronized with the signal from the beat-frequency-oscillator we photographed successive sweeps showing the vestibular microphonic wave and single unit action potential responses. Fig. 6, record 1, is a response to a 110 cyc./sec. stimulus. Eight traces are superimposed and the relatively undistorted microphonic wave carries high-amplitude spike potentials. It is clear that the action-potential discharge does not coincide with the peak of the microphonic wave. It can also be seen that the response adapts fairly rapidly, as only five or six of the traces carry a spike response. This adaptation can be very rapid indeed, and furnishes confirmation that we are dealing here with synchronized nerve impulses and not with especially high harmonic peaks of the microphonic wave (see also Fig. 4, record 7). Fig. 6, record 2, shows a response to a 100 cyc./sec. stimulus. Secondary harmonic humps of the microphonic wave are also seen to carry spike responses, which are in part summation products of two imperfectly synchronized components.

### *Macula neglecta*

When a successful preparation of the macula neglecta nerve is connected to the recording apparatus it becomes at once evident that the sense ending, which does not respond to rotation and tilting, is highly sensitive to vibration. The slightest mechanical disturbance in the room gives rise to bursts of high-amplitude potentials, one of the most remarkable examples of sensitivity being a just noticeable response to the dropping of a pin from shoulder-height to the stone floor of the laboratory. The responses as monitored in a loudspeaker are the more striking as they frequently occur in the absence of a resting discharge. Responses to purely air-borne sound stimuli were not obtained. Although the preparation will readily respond to speech, this response is at once abolished when the speaker stands or sits on a vibration-absorbing surface. The loudspeaker slung from an iron girder was thus as ineffective as a stimulator as in the case of the sacculus macula.

Fig. 6, records 3*a* and 3*b*, show a response to a 72 cyc./sec. stimulus recorded with reduced amplification. At the beginning of record 3*a* the preparation is already responding to a prolonged stimulus, but the impulse picture hardly emerges from the base line. At a sudden increase in stimulus intensity by 6 db. the amplitude of the response increases, very likely by recruitment and summation. Gradually adaptation sets in resulting in a reduction in the frequency and in the average amplitude of the discharge (beginning of record 3*b*). A renewed increase in stimulus intensity by 6 db. leads to a repetition of these events (record 3*b*). This stepwise increase in the response can only be explained by a build-up in the number of participating sense endings showing a wide range of thresholds. Fig. 6, records 4*a*, 4*b* and 4*c*, show a high-speed record of such a build-up in the response to a gradual as opposed to a stepwise increase in stimulus intensity. They show interesting summation pictures and very good synchronization.

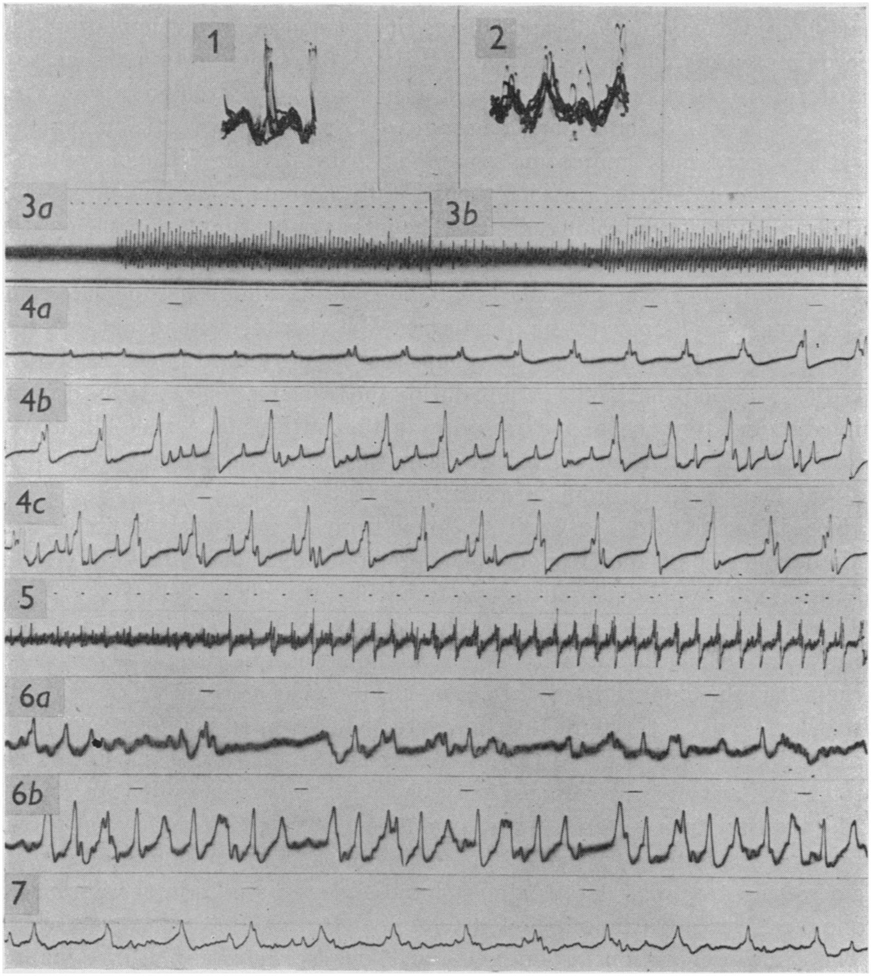

Fig. 6. Records of vibration~~al~~ responses from the sacculus (records 1~~-4c inclusive~~ and 2), the macula neglecta (records ~~4a, 4b and 4c~~ 3-4c inclusive), and the lacinia utriculi (records 5, 6*a*, 6*b* and 7) of the ray. Detailed explanation in the text. Records 1 and 2 show the constant phase relationship between microphonic waves and summated spike potentials by superposition of a number of sweeps. Record 1, stimulus frequency 110 cyc./sec., duration of single sweep 18 msec.; record 2, stimulus frequency 100 cyc./sec., sweep duration 20 msec. Nos. 4*a*, 4*b* and 4*c* are a continuous record of the response to gradual increase in stimulus intensity. Time marker at top of records 3*a*–7 inclusive: 24/sec.

### *Lacinia*

The vibration responses from the lacinia utriculi are illustrated by Fig. 6, records 5, 6*a*, 6*b* and 7. Record 5 represents a response from a preparation that showed a complete absence of gravity responses. On stimulation with approximately 17 cyc./sec. the lively two to three-fibre resting discharge is transformed into a response pattern of groups of bunched potentials at a rate of 34 groups/sec. Each group consists of two or three potentials very close together but rarely summating completely. In the intervals between the groups, spike potentials are sometimes totally absent, a fact which supports the assumption of a bunching effect of the vibrational stimulus on the existing resting discharge rather than the mere recruitment of newcomers. As will be pointed out in the discussion, this is only possible at stimulus frequencies below the frequency of the resting discharge. Whether the frequency-doubling (stimulus 17 cyc./sec., response 34 groups/sec.) is introduced in the receptor or was due to a strong harmonic arising during the transmission cannot be decided. Immediately after the above record was taken the nerve was killed with alcohol and this led as usual to the disappearance of the spike response. No vestibular microphonics remained after this treatment.

Records 6*a* and 6*b* of Fig. 6 were obtained from a highly sensitive preparation again devoid of positional response. The preparation showed a few-fibre resting discharge and responded well to low frequency stimulation (14–70 cyc./sec.). At 14 cyc./sec. its response was asynchronous (record 6*a*) and showed marked recruitment when the intensity of stimulation was raised. But even at the highest stimulus intensity (record 6*b*) no clear synchronization of the response with the 14 cyc./sec. stimulus can be detected. There is, however, a striking increase in the amplitude of the spike response due to summation, and the greater regularity of the response may be due to a synchrony with a rhythm in the transmitting system. As was pointed out in the introduction to this paper, the difficulties of accurate monitoring of the vibrational stimulus make the assessment of such details almost impossible, especially in the absence of pronounced vestibular microphonics.

The same preparation responded to a 56 cyc./sec. stimulus in the manner shown in Fig. 6, record 7. Here we found at a much lower stimulus intensity absolute synchronization of a clearly marked more or less completely summated spike potential firing at the stimulus frequency. Successful synchronization appeared thus to be easier at higher stimulus frequencies and almost impossible at the lower end of the frequency spectrum.

## DISCUSSION AND CONCLUSIONS

Vestibular vibration responses can manifest themselves in two different ways, viz. the vestibular microphonics, as first described by Wever & Bray (1930), and a response by impulse discharge into the branches of the eighth nerve. It is obvious that the more closely the point of electrical pick-up from the nerve lies to the vestibular wall the more difficult it becomes to record the two phenomena separately or to eliminate the one without affecting the other (Zotterman, 1943).

The functional interpretation of vestibular microphonics still rests on a very insecure basis (Adrian, 1932; Ashcroft & Hallpike, 1934; Rawdon-Smith, 1938), and although the phenomenon is being widely used in the analysis of quantitative stimulus-response relationships, we have preferred to concentrate our attention solely upon responses in the form of impulse discharges. Consequently, none of the conclusions put forward in this paper, with regard to either the localization of vibration sensitivity or the nature of the response, are based upon the observation of vestibular microphonics.

A resting discharge can be observed in all vibration-sensitive areas of the labyrinth. This is, however, not a prerequisite for the appearance of an impulse response to vibration, and it appears that a considerable number of sensory units are quiescent at any given moment and can be recruited to take part in the response. We have thus a dual mechanism of transmission of stimulus intensities: first, the usual increase in the discharge frequency from already firing units; and secondly, the addition of newly recruited units to the response picture. It was of interest in this connexion to see whether vibration can also lead to a slowing (inhibition) of the resting discharge activity, which is a prominent feature in the reactions to angular accelerations and positional stimuli (Lowenstein & Sand, 1940; Lowenstein & Roberts, 1950). Only in a resting discharge of significantly higher frequency than the stimulus signal can an inhibitory phase of the stimulus become effective. In that case we might find a discharge picture resembling the example of bunching shown in Fig. 6, record 5. As single units of the elasmobranch labyrinth rarely fire spontaneously at a higher rate than 30 impulses/sec. this synchronization, brought about both by acceleration and deceleration of a resting rhythm, is bound to be confined to low stimulus frequencies. A continuous suppression of a resting discharge by sustained stimulation with a certain frequency as described by Galambos & Davis (1944) for the mammalian ear was not observed in our experiments, despite the many similarities in the general response mechanism which will be discussed below.

A striking feature of our findings is the greatly restricted range of frequency response. The highest frequency which produced a clear spike response was in the neighbourhood of 120 cyc./sec. Above this frequency there is a sharp cut-off in spike response, although vestibular microphonics were observed up to 750 cyc./sec. It is very likely that this cut-off is largely due to the peculiar mode of stimulus transmission, and no physiological significance can be attached to it. The same holds at the lower end of the frequency scale, at about

10 cyc./sec., where there is a considerable drop in energy transmitted to the receptor organs.

Apart from the totally different ranges of frequencies covered by the responses recorded from the elasmobranch labyrinth and the mammalian ear, there exists striking agreement in the nature of the responses reported by Derbyshire & Davis (1935), Galambos & Davis (1943), and our own findings. Both in the mammalian cochlea and in the vibration-sensitive areas of the elasmobranch labyrinth a proportion of the sensory units show a resting discharge, the frequency of which is generally increased by the vibrational stimulus. This can lead to an asynchronous response in which the final frequency of discharge is entirely controlled by the stimulus intensity. However, in mammals below 4000 cyc./sec., and in the elasmobranch vestibulum almost over the whole explored range of vibration sensitivity, synchronization between signal frequency and spike discharge rate occurs. As we could observe a build-up of this synchronization with rising intensity, and as we also observed intermediate stages of summation leading to potentials of high amplitude, we have come to the conclusion that the synchronized spike responses generally consist of summated spikes which owe their existence to recruitment of newcomers during a certain phase of the vibrational stimulus. That this does not usually coincide with the peak of the microphonic wave is shown in the remarkable agreement both in Fig. 6, records 1 and 2, of the present paper, and in figs. 12*a* and 12*b* of Galambos & Davis (1943, p. 51). Whether or not the phenomenon of synchronization can have any relationship with pitch discrimination will be discussed in a different context.

Two more phenomena are worthy of scrutiny, viz. adaptation and the 'silent period'. They, too, are common to mammalian and elasmobranch sense endings. The adaptation observed in our preparations was sometimes extraordinarily rapid and drastic, and there is every indication that it is not shown by all sense endings to an equal degree. Neuromasts which fire spontaneously show very little adaptation, whereas newly recruited units may cease firing during the first 2 sec. of continuous stimulation. This consequently often leads to the rapid disintegration and even abolition of a synchronized response and is sometimes heralded by the dropping out of alternate spike responses and the appearance of greater gaps in the discharge picture, the phase relation of spike to stimulus remaining unchanged (Fig. 4, record 7).

The significance of the depression in the discharge rate after termination of a prolonged stimulus and its relationship to the duration of the stimulus is illustrated by Fig. 5. This phenomenon, described by Galambos & Davis (1943) as the 'silent period', occurs in the mammalian ear after high intensity stimulation and is, there, often followed by an after-discharge which we have not been able to observe in our material.

The fundamental agreement between results gained from the mammalian cochlea and the maculae of the elasmobranch labyrinth is striking. However, great caution must be employed in assessing the significance of our present results in relation to the 'Theory of Hearing'. To put it as bluntly as possible: We have been able to demonstrate the existence of the areas of selective vibration sensitivity in the elasmobranch vestibulum. The behaviour of these sense endings could be studied only within a range of stimulus frequencies drastically restricted to the lower end of the sound spectrum, a limitation inherent in the experimental technique. We are, moreover, almost completely lacking in reliable information about the very existence of sound perception in elasmobranchs, to say nothing about threshold, frequency range and localization. The results of Parker's (1910) crude experiments on the dogfish with low-frequency vibration stimulation, and a report of some recent observations by Dijkgraaf (1949) on the same animal, constitute all we know, as training, combined with operative elimination of the vestibular organ as a whole or in parts, offers considerable difficulties in the elasmobranchs.

It is not our intention to discuss at length the question of a definition of hearing. This has been repeatedly attempted by a number of authors without the emergence of a generally accepted formula of definition. von Frisch (1936) defines hearing for the vertebrates on the basis of comparative anatomical homology. This he considers to be established if sound reception can be shown to be localized within the inner ear. Pumphrey (1940) rejects the distinction between auditory and tactile sound perception according to its localization in one or other type of mechano-receptor as recommended by von Buddenbrock (1937), and suggests a difference in intensity levels of the mechanical stimulus as a practical criterion for their distinction. In a later paper, Pumphrey (1950) makes the interesting suggestion that hearing may be considered to involve perception of mechanical stimuli derived from distant sources and to be characterized by provision for more or less accurate spatial localization of the centre of mechanical disturbance. Finally, Autrum (1941), in a well-reasoned discussion of the problem, points out that a strict discrimination between hearing and tactile vibration perception is quite impossible in many cases, and without special heuristic value. To define our attitude in this matter we would say that, given reliable evidence for a utilization by the animal of the sensory input from the vibration sensitive areas of the labyrinth, we should be well content to use the term hearing. As this evidence is at present still absent in the case of the *elasmobranchs* we want to confine ourselves strictly to the description of the peripheral sensory responses to vibration. This need not, however, preclude us from speculating on the *potential* significance of vibration sensitivity in well-defined sensory areas of the labyrinth.

The facts concerning bony fish are unequivocal. It has been demonstrated beyond doubt that hearing in some cyprinoids covers a frequency range up to 13,000 cyc./sec. (von Frisch, 1936). Pitch discrimination is well marked and has its upper limit at 1260 cyc./sec. At 800 cyc./sec. minnows can discriminate a quarter tone in two simultaneously presented sound signals (Wohlfahrt, 1939, 1950; Dijkgraaf & Verheijen, 1950). Some power of transposition, a sense of absolute pitch and some other features of pitch discrimination characteristic of the human ear have been demonstrated in fishes (Wohlfahrt, 1950). The absence of any obvious pitch analyser structure like the basilar membrane of the cochlea makes these facts the more remarkable.

The upper limit for pitch discrimination at approximately 1200 cyc./sec. could be interpreted to indicate that synchronization may, after all, play an important part in pitch transmission, for this is little more than twice the maximum discharge frequency to be expected in single axons of a cold-blooded animal. The upper limit of pitch discrimination in the cyprinoids lies within the range of the physiologically possible if we assume, as we have done, that synchronization is supported by a recruiting mechanism. Such a mechanism is also postulated by Wever (1933) in his volley-hypothesis of synchronization in mammals. Impulse discharge to a frequency above 2000 cyc./sec. can in this case be assumed to be mediated by fibres with a refractory period of not much less than 1 msec. This is an important consideration, as it would furnish a means of explaining the occurrence in fishes of pitch discrimination in the absence of a place-representation mechanism based upon resonator structures as found in the basilar membrane. The fact that the phenomenon of synchronization of impulse responses is apparently general in the vertebrates may, of course, only mean that it is an unavoidable by-product of vibration perception at the lower end of the frequency scale. On the other hand, it is feasible to consider the evolution of the cochlea with its basilar membrane as a means for the extension upwards both of the perceptive auditory range and of pitch discrimination with it. With reference to a preliminary publication of our results (Lowenstein & Roberts, 1948), Pumphrey (1950) suggested that the central integration of the vibration responses from the otolith-bearing macula sacculi and the unloaded macula neglecta might form a basis for pitch discrimination. Our findings, so far, neither support nor contradict such a hypothesis.

Given the vibration sensitivity probably inherent in most mechano-receptor structures but especially in all end organs of the acustico-lateralis system (Pumphrey, 1950), the question arises whether they actually function as vibration receptors. This is entirely a matter of their exposure to, or insulation from, vibrational stimuli (de Burlet, 1935). de Burlet framed the term perilymphatic labyrinthine endings for sensory epithelia within the labyrinth which are anatomically connected and spatially related with special sound-conducting structures. The Weberian ossicles intervening between the air bladder and the vestibulum in cyprinoid fish link up with the sacculus; the ligamentous link between the air bladder and the clupeid labyrinth aims at its utriculus. The lagena lies opposite a significantly thinned-out part of the bony skull (lagena window) in the minnow. What is the situation in the elasmobranchs? This question is of the greatest importance in the assessment of the chances that the areas of vibration-sensitivity described in the experimental part of this paper are in fact functioning as vibration receptors.

In 1789 Scarpa described a membrane-covered perforation in the elasmobranch skull as 'fenestra ovalis'. It is shown in Fig. 3, from which its spatial relationships can be seen. As has been pointed out above (p. 476) this structure, in conjunction with the adjoining ductus endolymphaticus, could represent a sound-conducting mechanism aimed at the sacculus macula and the macula neglecta. Its relation to the lacinia utriculi is less striking. According to de Burlet's contention, the sacculus and macula neglecta would thus very likely be functional vibration receptors, whereas the lacinia could only be described as a prospective receptor area whose potentialities may have become realized in cases like the clupeids. It is known that even the cristae of the semicircular canals have such potentialities, and there is some suspicion that after the so-called fenestration operation in man the cristae, owing to their artificial exposure, become affected by sound stimuli (see Pumphrey, 1939). Whether the sense endings in the lateral line which are known to be vibration sensitive (Sand, 1937) are to be considered as potential or actual vibration receptors is still an open question (von Frisch, 1936; Dijkgraaf, 1934, 1949; Pumphrey, 1950).

Phylogenetically the elasmobranch vestibulum may thus be considered to represent an extremely generalized state comprising the morphological and functional raw material from which, during the evolution of the vertebrates, the one or the other potentiality has found its full functional realization. It is greatly to be desired that ways be found to subject the actual auditory capabilities of elasmobranchs to a qualitative and quantitative analysis.

## SUMMARY

1. Vibration responses in the form of impulse discharges were recorded from nerve twigs leading from part of the macula sacculi, the macula neglecta and the lacinia of the macula utriculi of the isolated elasmobranch labyrinth.

2. The otolith-bearing part of the macula utriculi, the posterior portion of the macula sacculi and the adjoining macula lagenae do not respond to vibrational stimuli. They contain gravity receptors only.

3. An appreciable number of the sense endings show a resting activity in the absence of vibrational stimulation. There exists, however, convincing evidence that, at any given time, many sensory units are quiescent. These can be recruited to take part in the vibrational responses, and they show a considerable range of thresholds.

4. Under the described experimental conditions vibration responses were recorded to stimulus frequencies extending rarely higher than 120 cyc./sec. Vestibular microphonics were observed up to a signal frequency of 750 cyc./sec., but only nerve impulse discharges are accepted as evidence for vibration sensitivity.

5. At low intensity stimulation the response consists of an increase in the discharge frequency of the 'spontaneously' firing units. Higher intensities lead to the recruitment of previously quiescent sense endings, and to a marked synchronization of the response frequency with that of the stimulus. This synchronization closely resembles the responses described for the mammalian cochlea, where it occurs at the lower end of the audible spectrum.

6. Adaptation to sustained vibrational stimulation and a 'silent period' after cessation of prolonged stimuli have been observed, and the latter has been quantitatively analysed.

7. The significance of these findings is discussed in relation to the problems of the evolution of hearing and pitch discrimination in vertebrates, and the present knowledge of the mode of function of the vertebrate ear.

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