J. exp. Biol. (1980), 86, 63–77
With 7 figures
Printed in Great Britain

EXTRACELLULAR RECEPTOR POTENTIALS FROM THE LATERAL-LINE ORGAN OF *XENOPUS LAEVIS*

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(*Received 26 September 1979*)

SUMMARY

1. The response of the epidermal lateral-line organ of *Xenopus laevis* to stimulation was studied by recording extracellular receptor potentials from the hair cells in single neuromasts in isolated preparations. One neuromast was stimulated by local, sinusoidal water movements induced by a glass sphere positioned at a short distance from the neuromast.

2. The amplitudes of the extracellular receptor potentials were proportional to the stimulus amplitude over a range of 20 dB. The phase of the extracellular receptor potentials with respect to water displacement was independent of the stimulus amplitude.

3. With large stimulus amplitude, and stimulus frequencies between 0·5 Hz and 2 Hz, the extracellular receptor potentials, and responses of single afferent nerve fibres, showed a phase lead of $1{\cdot}2\,\pi$ radians with respect to water displacement, i.e. they were almost in phase with water acceleration.

4. It is concluded that under conditions of stimulation with small-amplitude water movements, the hair cells respond to sensory hair displacement, whereas under conditions of stimulation with large-amplitude water movements they respond to sensory hair velocity.

INTRODUCTION

The mechanoreceptive hair cells in the acoustico-lateral organs have the same embryological origin in all vertebrates, and are morphologically and functionally similar. The epidermal lateral-line organ of the clawed frog, *Xenopus laevis*, is a convenient model for studying the mechano-electric transduction process in sensory hair cells. It is readily accessible because of its location in the surface of the skin, and single fibre recording is relatively easy. Its structure and innervation have been studied in detail (Murray, 1955; Görner, 1963; Harris & Milne, 1966; Pabst, 1977). Briefly, the sensory hair cells are grouped together in neuromasts, each of which contains 20–60 hair cells whose sensory hairs are embedded in a gelatinous cupula extending to about 100 μm above the skin. A number of neuromasts (2–14) are arranged in a row to form a 'stitch' and every stitch is innervated by two myelinated afferent nerve fibres. Every neuromast contains two populations of hair cells, which have maximum sensitivity in the opposite direction to each other (Flock & Wersäll,

1962; Dijkgraaf, 1963; Flock, 1965*a*, *b*). All hair cells of one stitch having the same orientation are innervated by the same branching afferent nerve fibre. Each neuromast has its own impulse generation site (Pabst, 1977), and an impulse generated in one neuromast resets all other impulse generators on the same nerve fibre (Harris & Milne, 1966; Harris & Flock, 1967; Murray & Capranica, 1973).

It is generally assumed that lateral-line organs are transducers of cupular displacement (for references, see Flock, 1971; Schwartz, 1974) and that the adequate stimulus for the hair cells is sensory hair displacement (Flock, 1965*b*, 1971; Schwartz, 1974; Hudspeth & Corey, 1977; Hudspeth & Jacobs, 1979). It has been suggested that the hair cells also respond to sensory hair velocity (Oman & Frishkopf, 1973; Gallé & Clemens, 1976; Strelioff & Honrubia, 1978). In the lateral-line organ of *Xenopus* the direction of maximum sensitivity of a neuromast is parallel to the major axis of the flattened, flag-like cupula. Therefore, the displacement of the cupula will be the result of viscous drag along its flat sides and is consequently proportional to water velocity (Görner, 1963; Harris & Milne, 1966). It has been shown that the afferent nerve response of a stitch to a constant, unidirectional water flow is proportional to the water velocity (Strelioff & Honrubia, 1978). Recently, linear system frequency response analysis has demonstrated that the lateral-line organ of *Xenopus*, when stimulated by small amplitude, sinusoidal water movements actually responds as a water velocity detector (Kroese, van der Zalm & van den Bercken, 1978).

The input–output relation of the hair cells is linear only for small deflections of the sensory hairs (Flock, 1965*b*; Furukawa, Ishii & Matsuura, 1972; Hudspeth & Corey, 1977). Since a neuromast contains two oppositely orientated hair-cell populations it is to be expected that the depolarizations and hyperpolarizations evoked by small amplitude, periodical water movements will cancel each other, i.e. no extracellular responses will occur. At larger stimulus amplitudes, however, the depolarizations will exceed the hyperpolarizations and then one can record extracellular receptor potentials, which represent the sum of two opposite responses of a number of hair cells. Consequently, the extracellular receptor potentials will have twice the frequency of the stimulus and they will be superimposed on a negative d.c. potential (Flock & Wersäll, 1962; Flock, 1965*b*).

Since the discovery of these 'microphonic potentials' in the canal lateral-line organ of *Acerina cernua* by De Vries (1948), extracellular receptor potentials have been measured in a number of canal lateral-line organs (for references see Flock, 1971; Boston, 1976). In the present study these potentials are recorded from single neuromasts of the epidermal lateral-line organ of *Xenopus*, together with single fibre afferent nerve activity from the same neuromast. This makes it possible to distinguish between the transduction process in the hair cells and the transmission steps following transduction.

METHODS

Preparation

The experiments were performed on small specimens (6–9 cm) of the clawed frog, *Xenopus laevis*. An animal was gently trapped in a glass beaker and anaesthetized by cooling to about 0 °C with ice-cubes. It was decapitated and pithed, and a piece

of skin containing several stitches was removed. The skin was fixed between two perspex rings, care being taken to avoid touching and stretching, and was placed with the outer surface upwards in an experimental chamber (Fig. 1 A). The chamber was filled with Ringer solution containing (in mM): NaCl 115, KCl 2·5, $CaCl_2$ 2·0, HEPES 3·0; pH was adjusted to 7·4. The outer surface of the skin was covered with tapwater. The experiments were performed at a room temperature of 21–23 °C.

Stimulation

Local, sinusoidal water displacements were induced by a small glass sphere (300 μm in diameter) located in the water just above the skin, next to a cupula, and driven by a modified loudspeaker which was connected to a gated sine-wave generator. The displacement of the sphere was recorded by measuring the capacity variations between the coil and the frame of the loudspeaker. The driving device of the loudspeaker used these capacity variations for a displacement feedback. Amplitude and phase of the displacement of the sphere were independent of the frequency over the whole range of frequencies used (0·5–100 Hz). The sine-wave generator was gated for periods of 0·1–10 s at intervals of 3–10 s, depending on the frequency of stimulation. In this way 5–30 stimuli, each consisting of 5–20 sinusoidal sphere movements, were applied and the responses averaged.

The movement of the sphere was parallel to the skin and in the direction in which the neuromast had its maximum sensitivity, i.e. parallel to its longitudinal axis (Fig. 1 B). The distance between the sphere and the cupula was measured with an eyepiece-micrometer and was between 100 and 500 μm. The amplitude of displacement of the sphere could be varied from 2 to 67 μm.

In the experiments in which afferent nerve activity was recorded, continuous, sinusoidal stimulation with a maximum duration of 60 s was followed by a period of 4 min without any stimulation.

Extracellular receptor potentials

Extracellular receptor potentials were recorded by platinum–iridium microelectrodes with platinum blacking and with a fine tip of 1 μm, insulated except for 5 μm (Frederick Haer, type B30-10-3). The electrode resistance was between 5 and 50 MΩ, measured at 2500 Hz. In order to be able to position the recording electrode accurately, the cupulae were made visible at the onset of an experiment with indian ink (Rotring). By means of an injection needle about 0·05 ml of undiluted ink was ejected above a stitch, just below the surface of the water. The ink settled on the surface of the skin and after 2 min it was sucked away very gently. The cupulae were then clearly visible in a light beam directed parallel to the skin. In some preparations a cupula was present on all neuromasts, but in others several neuromasts lacked a cupula, probably as a result of the dissection. In the course of an experiment the ink disappeared slowly from the cupulae without there being any change in electrical response of the neuromasts. No difference was observed between extracellular receptor potentials from neuromasts to which indian ink had been applied and neuromasts in which no cupula had been made visible.

The cupulae were flag-like in shape and had a length of about 100–150 μm, as described previously (Görner, 1963). They kept growing for several hours at a rate of

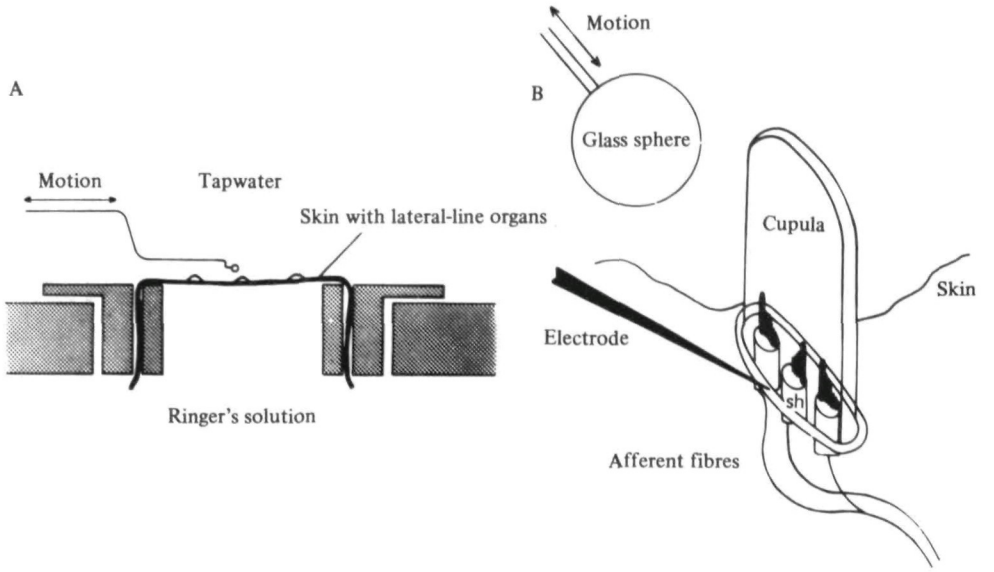

Fig. 1. Schematic diagram of the lateral-line preparation of *Xenopus laevis*. (A) Skin with lateral-line organs in experimental chamber. (B) Single neuromast, showing the position of the glass sphere and the recording electrode; sh, sensory hair cells.

approximately 15 μm/h. A similar growth rate has been reported for the cupulae of the epidermal lateral-line organ of *Necturus* (Frishkopf & Oman, 1972).

The recording electrode was positioned at an angle of 45° to the surface of the skin and perpendicular to the longitudinal axis of the neuromast; its tip was just next to the base of the cupula, near the apical surface of the hair cells (see Fig. 1 B). In this way possible hindrance of the motion of the cupula by the electrode as well as mechanical artifacts evoked by movements of the cupula were prevented. The electrode signal was fed into a d.c.-coupled pre-amplifier with offset correction and further amplified by an a.c.-coupled amplifier with a gain of 10000 and variable passband. An Ag/AgCl electrode with platinum blacking (Frederick Haer, type 18-20-2) was used to ground the tapwater covering the skin. The extracellular receptor potentials, together with a signal proportional to the instantaneous position of the glass sphere, were recorded on magnetic tape for later analysis. During the experiments the presence of extracellular receptor potentials was ascertained with the aid of a storage oscilloscope. Preparations remained in good condition for a period of 3–4 h.

Afferent nerve activity

To record afferent nerve activity under the same conditions of stimulation as used for the recording of extracellular receptor potentials, the preparation was modified in the following way. Before the preparation was placed in the experimental chamber the nerve innervating the lateral-line organ was dissected free from the skin and all stitches except one were disconnected. An incision about 0·5 mm long was made in the skin, from the inside out, with a razor blade. The nerve was carefully pushed through the incision to the outer side of the skin and the skin was sealed with silicone

grease. Under these experimental conditions the outer surface of the skin was covered by Ringer solution. The preparation was placed in the experimental chamber and afferent nerve activity was recorded with the aid of a silver wire electrode, as described previously (Kroese *et al.* 1978). The impulses from the two afferent fibres of the stitch were separated by their different amplitudes, and single fibre activity was measured. The nerve signal was amplified and filtered (0·02–3 kHz).

Data processing

For analysis of the extracellular receptor potentials a number of responses (5–30) were digitized by means of a transient recorder (Biomation Model 802) and averaged on a minicomputer (Hewlett-Packard 21M20). The averaged response together with the actual displacement of the sphere were plotted (see Fig. 2). Signals with an amplitude of the order of 5 μV could be analysed quantitatively. Amplitude (μV) and phase with respect to sphere displacement (radians) of the potentials were determined by means of Fourier-series analysis of the averaged response, using the harmonic component that corresponded to twice the stimulus frequency. To exclude any transient effects the first two periods of the averaged response were discarded. Due to the high pass filter (0·2 or 2 Hz) in the recording system, the d.c. component of the extracellular receptor potentials was lost.

Period histograms of afferent nerve activity were computed as described previously (Kroese *et al.* 1978). The phase of the response with respect to sphere displacement was measured as the difference between the maximum of sphere displacement and the maximum of the first harmonic component of the response, and was expressed in radians.

RESULTS

Recording of extracellular receptor potentials

Stimulation with local, sinusoidal water movements produced extracellular receptor potentials with amplitudes of up to 200 μV. A typical response to stimulation with different stimulus frequencies is shown in Fig. 2. As noted in the Introduction, the recorded potentials had twice the frequency of the stimulus and were superimposed upon a negative d.c. component. The d.c. component is not shown in Fig. 2 because the recording was a.c.-coupled. The recorded potentials were fairly symmetrical and showed only small distortions of sine wave shape.

When a neuromast was stimulated in the direction of the longitudinal axis of the stitch no responses could be recorded. The addition of dihydrostreptomycin (0·3 mM) or of KCN (3 mM) to the tapwater covering the skin caused the response to disappear within 5 min. The characteristic features of the response disappeared when the recording electrode was only slightly displaced. These results confirm that the recorded potentials were generated by the hair cells.

Effects of stimulus amplitude

The amplitude of the extracellular receptor potentials as a function of stimulus amplitude was measured for 16 neuromasts in 16 preparations at stimulus frequencies between 5 and 60 Hz. An example of the response to a 20 Hz stimulation with

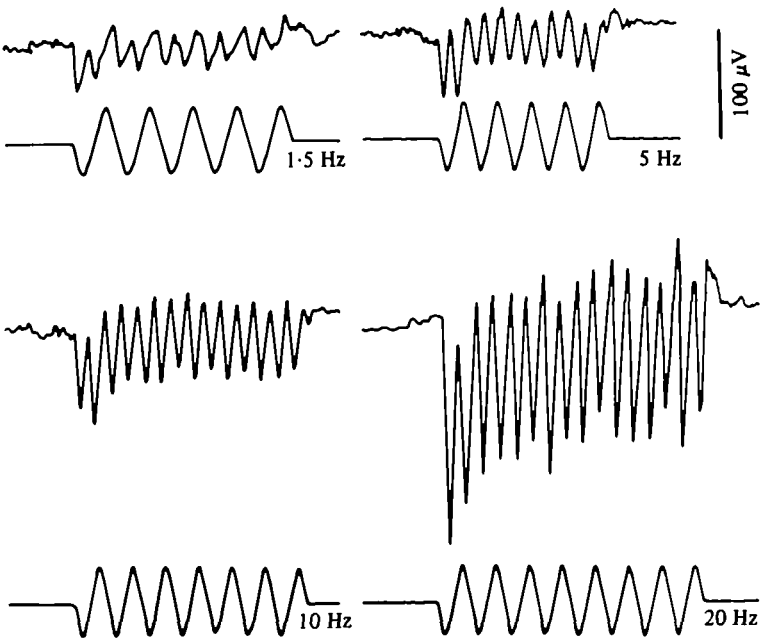

Fig. 2. Extracellular receptor potentials of one neuromast, evoked by local sinusoidal water displacements. Upper traces show the extracellular receptor potentials evoked by stimulation with constant amplitude and with a frequency of 1·5, 5, 10 and 20 Hz, respectively. Each trace represents 30 averaged responses. Lower traces show displacement of the glass sphere.

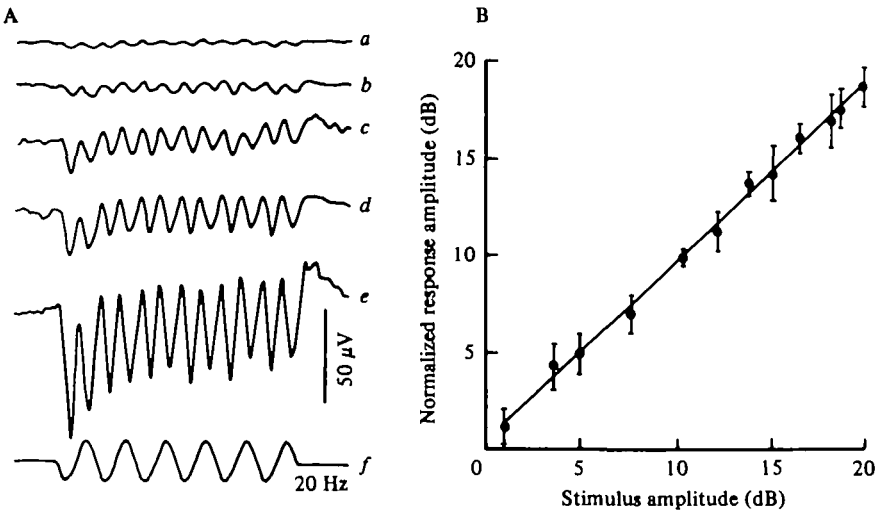

Fig. 3. Amplitude of the extracellular receptor potentials as a function of stimulus amplitude. (A) Extracellular receptor potentials evoked by stimulation with a frequency of 20 Hz and with amplitudes of 1 (*a*), 2·5 (*b*), 3 (*c*), 5 (*d*) and 8 (*e*) arbitrary units, respectively. Each trace represents 30 averaged responses. Lower trace (*f*) depicts the displacement of the glass sphere. Note that the phase of the extracellular receptor potentials with respect to sphere displacement is independent of stimulus amplitude. (B) Relationship between stimulus amplitude and amplitude of extracellular receptor potentials in 14 preparations. Stimulus amplitude is expressed in arbitrary units. Each point represents the mean value ± S.D. of nine measurements. Drawn line is best fitting straight line; the slope is 0·94 ± 0·05 (S.D.).

various amplitudes is shown in Fig. 3A. The amplitude of the potentials was plotted against stimulus amplitude on a log/log scale. Each plot was tested for linearity and the best fitting straight line was calculated. Since the amplitude of the recorded potential varied between recordings, the plots intercepted the receptor potential axis (y-axis) at different points. Statistical analysis showed 14 out of 16 input–output curves to be parallel ($P < 0{\cdot}025$). The average slope of the input–output curves was $0{\cdot}95 \pm 0{\cdot}15$ (mean ± S.D.; $n = 14$). Results for the 14 neuromasts were normalized by shifting the curves along the y-axis, as shown in Fig. 3B. This figure clearly shows that the amplitude of the extracellular receptor potentials is linearly related to the stimulus amplitude over the first 20 dB; at higher amplitudes the relationship was not linear.

Although we did not attempt to study extensively the threshold levels for the extracellular receptor potentials, the amplitude of the smallest water displacements (calculated as described below) at which potentials were measured was of the order of 1 μm (frequency 20 Hz). This is larger by a factor of 10 than the amplitude of the water displacements that have been shown to produce sinusoidal modulation of the spontaneous afferent nerve activity of the lateral-line organ of *Xenopus* (Kroese *et al.* 1978). Apparently the extracellular receptor potentials of sensory hair cells with opposite orientation cancel each other at small stimulus amplitudes.

Because of the short distance between sphere and cupula, stimulation is in the near-field (Harris & van Bergeijk, 1962; van Bergeijk, 1967; Schwartz, 1974) and the magnitude of the water displacements near the cupula can be approximated by $A = U(R/D)^3$ (van Bergeijk, 1967), where A = amplitude of water displacement near the cupula (μm), U = amplitude of displacement of the glass sphere (μm), R = radius of the glass sphere (μm), D = distance between centre of the sphere and core of the cupula (μm).

In additional experiments, the response to stimulation with constant sphere amplitude and frequencies between 5 and 40 Hz was measured for six neuromasts as a function of the distance between sphere and cupula. The amplitude of the potentials was plotted against the calculated water displacement (1·2–14 μm) on a log/log scale. For each plot the best fitting straight line was calculated, and statistical analysis showed all lines to be parallel ($P < 0{\cdot}025$). The average slope of the lines was $0{\cdot}95 \pm 0{\cdot}09$, which is in perfect agreement with the results described above.

The phase of the extracellular receptor potentials was independent of the amplitude of sphere movement and of the distance between sphere and cupula; the phase depended on stimulus frequency only.

Frequency response

The amplitude and phase of the extracellular receptor potentials as a function of stimulus frequency were measured for nine neuromasts in nine preparations. The (calculated) amplitude of water displacement was chosen in the same range as used for the input–output curves (1·2–14 μm) and was kept constant during each experiment. The curves describing the relationship between receptor potential amplitude and stimulus frequency were identical in shape for the different neuromasts, but were shifted along the amplitude-axis, due to the variation in amplitude between recordings.

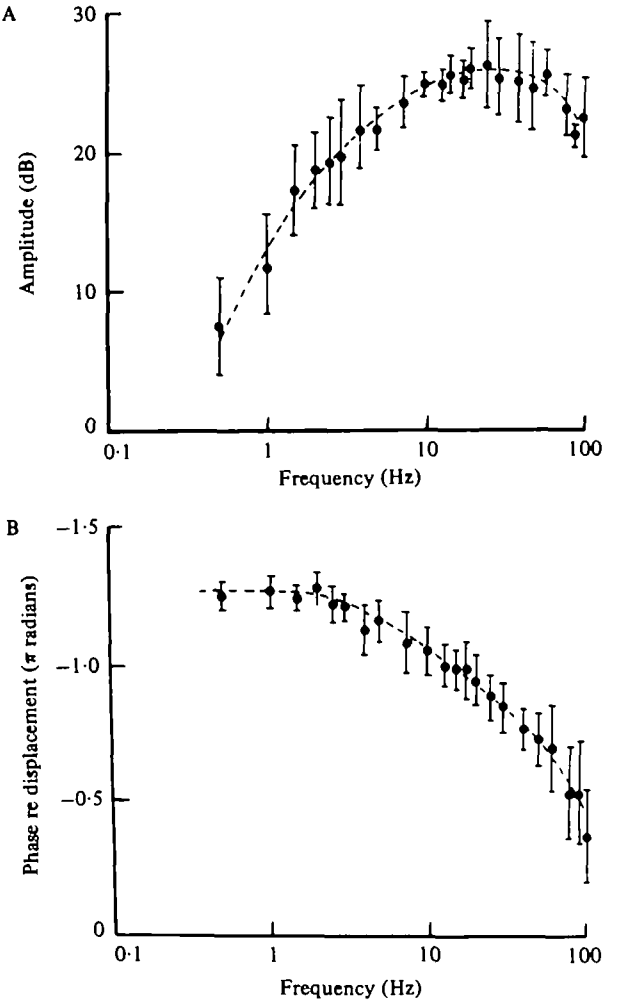

Fig. 4. Frequency response of the lateral-line organ, measured by recording extracellular receptor potentials from single neuromasts. Amplitude (A) and phase with respect to displacement (B) of the extracellular receptor potentials is given in relation to stimulus frequency. The results were obtained from 232 measurements on 9 neuromasts: the amplitude responses of the individual neuromasts were normalized at 10 Hz. Each point represents mean value ±S.D.

Curves were therefore normalized, at 10 Hz, as in Fig. 4A. The amplitude of the extracellular receptor potentials increased with increasing stimulus frequency until a maximum was reached at 15–20 Hz. For frequencies above 60 Hz the amplitude started to decrease. In several neuromasts the frequency response was measured at different stimulus amplitudes but the curves had always the same shape. Receptor potential amplitude was at a maximum at the same frequency as is the amplitude response obtained by recording afferent nerve activity (Kroese *et al.* 1978). This indicates that the frequency range where this sense organ is most sensitive is determined by the physical characteristics of the cupula.

Since the extracellular receptor potentials represent the summated recepto

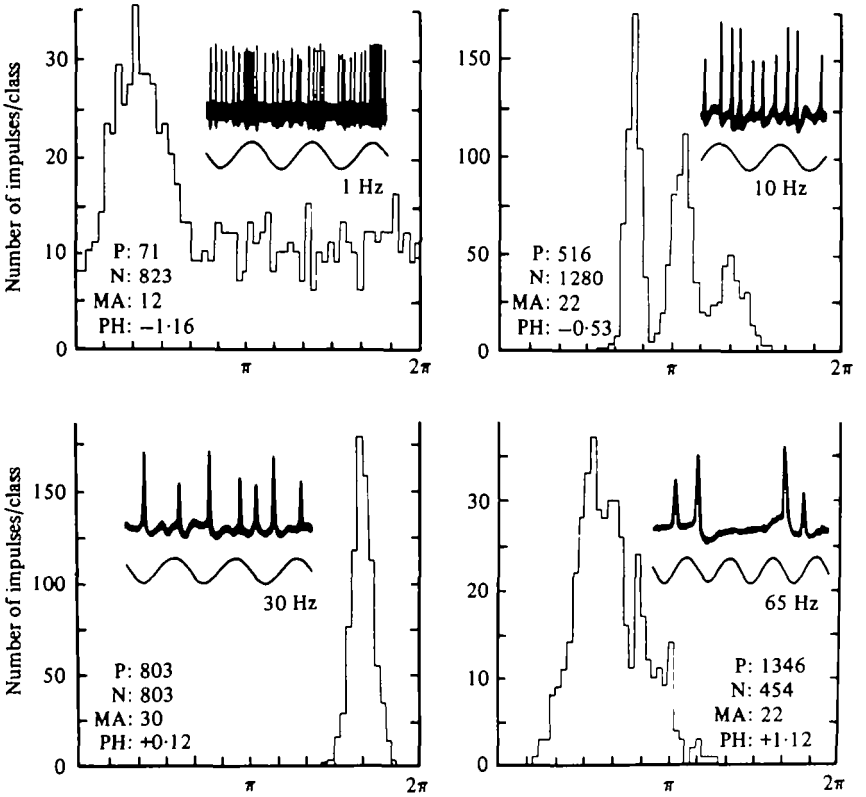

Fig. 5. Period histograms of single fibre afferent nerve activity, obtained by stimulation of one neuromast with constant amplitude and with frequencies of 1, 10, 30 and 65 Hz, respectively. Each stimulus period is divided into 60 classes. P, Number of averaged periods; N, number of impulses in the histogram; MA, mean activity (impulses/s). The phase (PH) of the response was measured as the difference between the maximum of the sphere displacement and the maximum of the first harmonic component of the response. The measured phase value was shifted π radians forwards (see text). Inset shows afferent nerve activity over several stimulus periods; the large impulses were used. Inset at 1 Hz shows only one type of impulse.

potentials of two populations of hair cells, quantitative information about the response of single hair cells can be obtained only from the phase response as a function of stimulus frequency.

The phase responses of the different neuromasts were identical (Fig. 4B). For frequencies up to 2 Hz the phase of the potentials had a constant value of $-1{\cdot}25\,\pi$ radians with respect to displacement. With higher stimulus frequencies the phase lead decreased gradually to a value of $-0{\cdot}4\,\pi$ radians at 100 Hz. A frequency-dependent phase shift is present in the microphonic potentials of canal lateral-line organs induced by direct displacement of the cupula (Kuiper, 1956; Flock, 1965*b*).

The measured value of the phase of the extracellular receptor potentials for low frequencies was $-0{\cdot}25\,\pi$ radians. Up to 5 Hz the phase response obtained by recording activity of single afferent nerve fibres and stimulation with small-amplitude sinusoidal water displacements has a constant value of $-0{\cdot}7\,\pi$ radians (Kroese *et al.* 1978). Since this would mean that afferent nerve activity would precede the receptor

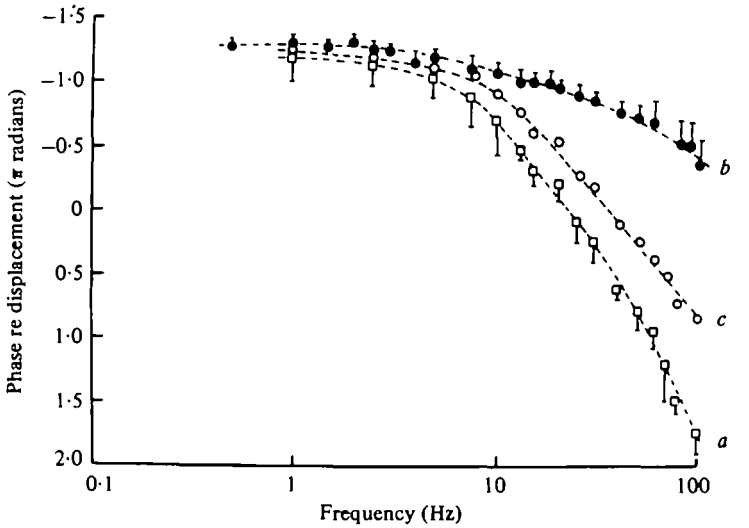

Fig. 6. Phase responses as a function of stimulus frequency of afferent nerve activity and extracellular receptor potentials recorded from single neuromasts. Curve *a* depicts the phase of the afferent nerve response as a function of stimulus frequency. The results were obtained from 138 measurements on 6 neuromasts. Each point represents mean value ± S.D. Curve *b* depicts the phase of the extracellular receptor potentials as a function of stimulus frequency. This curve is identical to the phase curve in Fig. 4B. Curve *c* was constructed by subtracting the frequency-dependent phase-shift present in the extracellular receptor potentials (see curve *b*) from the phase of the afferent nerve response (curve *a*). This curve depicts the phase characteristics of the transmission steps following mechano-electric transduction.

potentials, the phase values for the potentials were shifted forwards by π radians. The same was done with the phase response of the afferent nerve activity accompanying the extracellular receptor potentials (see next paragraph). It should be borne in mind that the phase response of the extracellular receptor potentials represents the phase response of the receptor potentials in one of the two populations of sensory hair cells in the neuromast.

Afferent nerve response

Single-fibre afferent nerve activity was recorded under conditions of stimulation identical to those used for recording extracellular receptor potentials. To ensure that only one neuromast of a stitch was stimulated, we selected neuromasts whose neighbouring neuromasts contained no cupula. At all frequencies of stimulation the period histograms clearly showed phase-locking of the impulses to the stimulus. Fig. 5 shows the afferent nerve response to stimulation of one neuromast at different stimulus frequencies. Below 5–10 Hz some impulses were not locked to the stimulus, and are presumably spontaneous in origin. Phase-locking of all impulses to the stimulus during sinusoidal stimulation (5–20 Hz) of one neuromast of a stitch has been reported before (Murray & Capranica, 1973) and is probably a result of the resetting of impulse generators not controlled by the stimulus (Harris & Milne, 1966; Pabst, 1977).

The phase of the afferent nerve response as a function of stimulus frequency was measured for six neuromasts in six preparations. For frequencies up to 2 Hz the afferent nerve response (Fig. 6, curve *a*) was in phase with the extracellular receptor potentials (Fig. 6, curve *b*). With higher stimulus frequencies the phase of the afferent

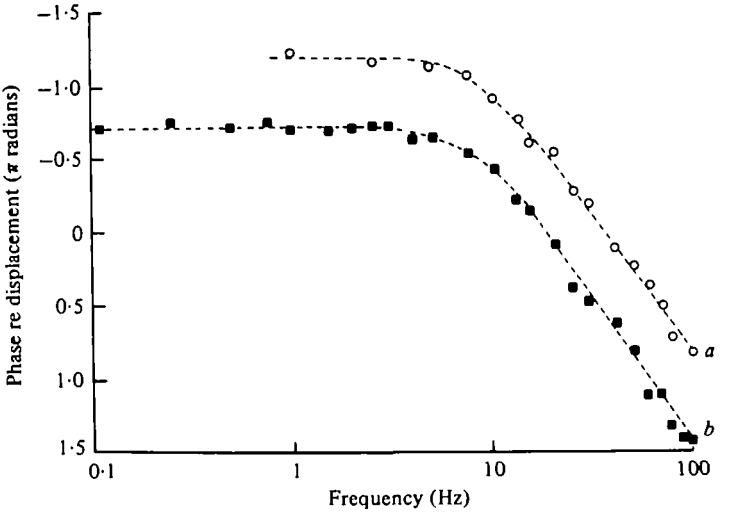

Fig. 7. Phase response as a function of stimulus frequency obtained by recording afferent nerve activity from the lateral-line organ under conditions of stimulation with small and large stimulus amplitudes. Curve *a* depicts the phase characteristics of the transmission steps in the lateral-line organ following mechano-electric transduction. This curve was obtained under conditions of stimulation with large amplitude water displacements and is identical to curve *c* in Fig. 6. Curve *b* depicts the phase of the afferent nerve response as a function of stimulus frequency obtained during stimulation with small amplitude water displacements (Kroese *et al.* 1978). Under these conditions of stimulation the lateral-line organ can be regarded as a linear system. Note that the curves are parallel at a distance of about 0·5 π radians over the whole frequency range.

nerve response changed rapidly, from a phase lead of 1·25 π radians below 2 Hz to a phase lag of 1·75 π radians at 100 Hz.

The frequency-dependent phase shift of the extracellular receptor potentials is associated with the mechano-electric transduction process and consequently contributes to the phase of the afferent nerve response. By taking the difference between the phase of the extracellular receptor potentials below 2 Hz ($-1{\cdot}25\ \pi$ radians) and the phase of these potentials at higher stimulus frequencies, and subtracting this from the phase of the afferent nerve response, a curve was obtained (Fig. 6, curve *c*) that shows the phase characteristics of the transmission steps following transduction, i.e. synaptic transmission, impulse generation and impulse propagation. The latter curve is depicted again in Fig. 7 (curve *a*), together with the phase curve of the afferent nerve response to small-amplitude stimulation (curve *b*) obtained in previous experiments (Kroese *et al.* 1978). Both curves are parallel at a distance of about 0·5 π radians over the whole frequency range. This difference can be attributed to differences in the amplitude of stimulation. The calculated amplitude of the water movements near the cupula which evoked modulation of the spontaneous afferent nerve activity varied between 0·1 and 2·4 μm (Kroese *et al.* 1978). Under these conditions of stimulation all neuromasts of a stitch are stimulated by water displacements of small amplitude and the afferent nerve response up to 5 Hz is almost in phase with water velocity. The amplitude of the water movements used to evoke extracellular receptor potentials (which are accompanied by trains of impulses in the afferent nerve fibre) varied between 1 and 14 μm, i.e. it was 5–10 times larger. In this case the afferent nerve response up to 2 Hz is almost in phase with water acceleration.

DISCUSSION

The experiments show that extracellular receptor potentials from the hair cells in the lateral-line organ of *Xenopus* can be recorded under well-defined conditions of stimulation. The input–output function obtained by varying the amplitude of the sphere displacement was identical to that obtained by varying the distance between sphere and cupula. This demonstrates that the lateral-line organ of *Xenopus* responds to water movements in the near-field (van Bergeijk, 1967; Schwartz, 1974), which is in accordance with recent observations by Bauknight, Strelioff & Honrubia (1976). The input–output function obtained in the present study gives a straight line on a log/log plot with a slope of 0·95 over a range of 20 dB. Input–output functions with the character of a power law function and with slopes varying between 0·5 and 2 have been reported for extracellular receptor potentials from canal lateral-line organs of several species (Harris & van Bergeijk, 1962; Flock, 1965*a*; van Bergeijk, 1967; Flock & Russell, 1973; Boston, 1976). The reason for this variation in slope is not known.

For frequencies up to 2 Hz the extracellular receptor potentials and the accompanying afferent nerve activity had a phase lead of 1·25 π radians with respect to displacement, i.e. they were both almost in phase with water acceleration. In a previous study we demonstrated, by recording afferent nerve activity, that the lateral-line organ of *Xenopus* responds to the velocity of small-amplitude water movements; for stimulus frequencies up to 5 Hz the phase of the afferent nerve response had a constant value of $-0{\cdot}7\ \pi$ radians with respect to displacement and between 0·1 and 20 Hz the gain increased by 7·5 dB/octave (Kroese *et al.* 1978). In the same study it was shown, however, that an increase in stimulus amplitude gradually caused a further forward phase shift up to a value of $-1{\cdot}2\ \pi$ radians with respect to displacement, and it was concluded that the lateral-line organ of *Xenopus* is also sensitive to water acceleration. The present experiments confirm that under conditions of large-amplitude stimulation the hair cells respond to water acceleration. Apparently, with increasing stimulus amplitudes the lateral-line organ of *Xenopus* changes from a water velocity detector to a water acceleration detector.

The afferent nerve response to large-amplitude stimulation and frequencies below 2 Hz was in phase with the extracellular receptor potentials. It is reasonable to assume that for small-amplitude stimulation at low frequencies the phase difference between the afferent nerve response and the receptor potentials is also negligible. Hence, it can be concluded that the difference of 0·5 π radians between the phase curves of Fig. 7 is present already in the receptor potentials and thus originates in the mechano-electric transduction process in the hair cells. The possibility that the extracellular receptor potentials are in phase with water displacement and not with water acceleration can be excluded, because of the forward phase shift of the afferent nerve response with increasing stimulus amplitude (Kroese *et al.* 1978). Thus it is concluded that the transduction process in the hair cells responds either to water velocity or to water acceleration, depending on the stimulus amplitude.

The phase curve of the afferent nerve response to small-amplitude stimulation (Fig. 7; curve *b*) depicts the phase shifts that have been caused by all the transmission steps between water movement and recording of afferent nerve activity. The phase

curve of the afferent nerve response to large-amplitude stimulation after subtraction of the frequency-dependent phase shift of the extracellular receptor potentials (Fig. 7; curve *a*) includes the phase shifts caused by the transmission steps following transduction. The fact that both curves are parallel over the whole frequency range studied strongly suggests that the phase of the receptor potentials evoked by small-amplitude water movements is independent of stimulus frequency. This implies that under conditions of stimulation with small as well as large stimulus amplitudes the phase characteristics of the transmission steps following transduction are identical. This is true only for stimulation with amplitudes not larger than those used in the present study. During stimulation of all neuromasts of a stitch by even larger water movements the afferent nerve response is shifted further forwards by several tenths of π radians, probably as a result of the refractoriness of the impulse generating mechanism.

Recently, Strelioff & Honrubia (1978) have shown that a constant flow of water past the lateral-line organ of *Xenopus laevis* caused a sustained neural response which was proportional to the water velocity. Their results and our previous experiments (Kroese *et al.* 1978) clearly indicate that the hair cells respond to cupular displacement, which in turn is a function of water velocity. Because of the great sensitivity of the lateral-line organ to transient water movements, Strelioff & Honrubia (1978) have suggested that sensory hair velocity is also a feature of the adequate stimulus for the hair cells. The present study demonstrates that the lateral-line organ of *Xenopus* actually responds to water acceleration during large-amplitude steady-state periodical stimulation, and that the sensitivity to acceleration is associated with the mechano-electric transduction process in the hair cells.

Two different mechanisms responsible for the sensitivity of the lateral-line organ to water acceleration during large-amplitude stimulation can be distinguished. (1) The hair cells respond to sensory hair displacement, but cupular displacement is proportional to water acceleration. (2) Cupular displacement is proportional to water velocity and the hair cells respond to sensory hair velocity.

As long as the relationship between water movement and cupular movement is not determined, the possibility that the sensitivity to acceleration is associated with the coupling of water and cupula cannot be ruled out. The study of this relationship is, however, very difficult because of the fragile nature of the cupula and because it is probably the movement of only the base of the cupula which is the effective stimulus for the hair cells (cf. Flock & Goldstein, 1978).

In an elegant study, Hudspeth & Corey (1977) stimulated single hair cells in the sacculus of the bullfrog, *Rana catesbeiana*, by direct mechanical deflection of the sensory hair bundle, and recorded intracellular receptor potentials. Up to 150 Hz the response of the hair cells depended on the displacement and not on the velocity of the sensory hair bundle. If the same is true for the lateral-line organ of *Xenopus*, this would mean that the sensitivity to water acceleration is brought about by the coupling of water and cupula. In the experiments of Hudspeth and Corey, however, the apical surface of the hair cells was in contact with a high-Ca^{2+}/low-K^+ medium that may have affected the receptor current that is presumably carried by K^+ ions (Davis, 1965; Russell & Sellick, 1976; Valli, Zucca & Casella, 1977, 1979).

The displacement of the cupula of the lateral-line organ of *Xenopus* is considered

to be proportional to water velocities of up to 14 mm/s (Strelioff & Honrubia, 1978). The maximum calculated water velocity in the present experiments was several times smaller than this value. It seems unlikely that for low stimulus frequencies the displacement of the cupula is proportional to water acceleration, which leads to the conclusion that the sensitivity to water acceleration resides primarily in the hair cells. However, the possibility that under certain stimulus conditions the coupling between the sensory hair cells and the cupula behaves like a fluid cannot be excluded. In this case the transduction mechanism may still depend upon the displacement of the sensory hairs.

The possibility that hair cells are sensitive to sensory hair velocity has been given some consideration before. Oman & Frishkopf (1973) stimulated the cupula of the epidermal lateral-line organ of *Necturus* directly and showed that the afferent nerve response was proportional to the velocity rather than to the magnitude of cupular displacement. Their preparations, however, showed low spontaneous activity and did not respond to static displacement of the cupula. Gallé & Clemens (1976) analysed single-unit activity from primary afferent fibres of the sacculus of the frog, *Rana esculenta*, under conditions of static and dynamic stimulation, and they assumed that the response of the hair cells to dynamic stimuli was related to sensory hair velocity. There is also evidence that in the mammalian cochlea the velocity of basilar membrane movement is a feature of the adequate stimulus for some of the hair cells, probably the inner hair cells (Dallos *et al.* 1972; Zwislocki & Sokolich, 1973; Konishi & Nielsen, 1978). This, however, does not necessarily mean that the inner hair cells respond to sensory hair velocity, but the response of the hair cells to basilar membrane velocity may be due to the coupling of the sensory hairs and the tectorial membrane (Kimura, 1965; Lim, 1971; Dallos *et al.* 1972).

In summary, it is concluded that under conditions of stimulation with large-amplitude water movements the sensory hair cells in the lateral-line organ of *Xenopus* respond to water acceleration. It is assumed that the displacement of the cupula is proportional to water velocity and that the hair cells respond not only to sensory hair displacement but also to sensory hair velocity.

The authors are grateful to Miss S. M. McNab for helping to improve the style of this article.

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