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Electrical and Mechanical Stimulation of Hair Cells in the Mudpuppy

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Summary. 1. The lateral line organ of *Necturus maculosus* was stimulated with extracellular sine wave current, and the effect on the afferent activity was compared to the effect of mechanical vibration. Both mechanical and electrical stimulation caused phase locking between stimulus and afferent spikes (Figs. 2, 3).

2. Intracellular recordings were made from the three main cell types of the neuromast: hair cells, supporting cells and afferent nerve terminals. Mechanical stimulation evoked receptor potentials of less than 1 mV in the hair cells. These potentials were synchronized with the stimulus (Fig. 6E), and mechanical stimulation caused a corresponding synchronization of action potentials in the afferent nerve terminals (Fig. 6A). The supporting cells were insensitive to vibrations (Fig. 6C).

3. Intracellular injection of sine wave electrical current into hair cells caused synchrony between stimulus and afferent spikes (Fig. 6F), whereas even ten times more intense current was insufficient to cause such synchrony if the afferent nerve terminals were injected directly (Fig. 6B). Supporting cells were insensitive to electrical current stimulation (Fig. 6D).

4. The effective intracellular current injections in the hair cells caused membrane potential changes which overlapped in magnitude with the naturally occurring receptor potentials. The conclusion is therefore that the mechanically evoked receptor potentials in hair cells have a direct effect on the transmitter release, and the potentials are not an epiphenomenon caused by the secretory activity of the hair cells.

Introduction

The acousticolateral organs in vertebrates have a common embryological origin, and the mechanoreceptive hair cells of these organs are morphologically similar. The adequate stimulus for the hair cells in all organs is shear movements of the sensory hairs, although structural differentiation reflecting functional specialization among hair cells does exist (Flock, 1971). The most sensitive lateral line organs can detect vibratory motions of subatomic magnitude, and to understand the mechanism of sensory transduction in hair cells represents an intriguing problem.

Wever and Bray (1930) were the first to discover cochlear microphonic potentials, and similar alternating potential changes synchronized with the stimulus have later been reported for most types of vibration sensitive acousticolateral organs. The site of origin of these potentials has been much debated. Von Békésy (1960) showed that the electrical energy of the cochlear micro-

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phonic potentials is considerably larger than the mechanical energy input, thus the microphonic potentials can not be due to capacitive or piezoelectric properties of the vibrating tissues. The mechanical energy triggers instead a transducer with the property of energy amplification. The microphonic potentials reverse sign when the recording electrode crosses the sensory epithelium (Jielof *et al.*, 1952; Tasaki, Davis and Eldredge, 1954; Trincker, 1957), and the potentials disappear when the hair cells are selectively destroyed by ototoxic antibiotics (Butler and Honrubia, 1963). The conclusion now generally accepted is that the summed receptor potentials from numerous hair cells contribute to the extracellularly recorded microphonic potentials.

Grundfest (1959) suggested that the synapse between the hair cells and the afferent nerve endings is chemical, and electron microscopic studies of the ultrastructure of these synapses support this idea (Flock, 1965). Hagiwara *et al.* (1962) provided indirect evidence for the existence of chemical synapses between electro-receptive hair cells and their afferent neurons. More recently Furukawa and Ishii (1967) succeeded in recording intracellularly from the primary afferent fibers of the saccular nerve in goldfish (*Carassius auratus*). They observed potential changes which showed characteristics of excitatory postsynaptic potentials in every respect, thus providing crucial evidence for the existence of a chemical synapse between hair cell and their afferent neurons.

However, the nature of the microphonic potentials is still a controversial question. Davis (1965) is in favour of a causative relationship between these potentials and the transmitter release from the presynaptic hair cell membrane. Depolarization of this membrane will then induce increased transmitter release, whereas hyperpolarization has the opposite effect. Grundfest (1965), on the other hand, has suggested that the receptor potentials may be an epiphenomenon induced by the transmitter release, and they are thus not necessarily imperative for this release.

Harris *et al.* (1969, 1970), recording from the lateral line organ of the mudpuppy (*Necturus maculosus*), were the first to observe intracellular receptor potentials in hair cells. In response to vibrational stimuli of intensities approaching the upper physiological range, they recorded intracellular sinusoidal potential changes of less than 800 μV peak-to-peak. These receptor potentials had the same frequency as the stimulus. The cells were identified by staining techniques, and whereas all cells producing receptor potentials proved to be hair cells, supporting cells were always silent. Recently, Flock *et al.* (1973) have recorded receptor potentials intracellularly from hair cells in the lateral line organ of the burbot (*Lota lota*), while Weiss *et al.* (1974) have obtained intracellular receptor potentials from hair cells in the basilar papilla of the inner ear of the alligator lizard (*Gerrhonotus multicarinatus*). With intense stimulation these potentials were less than 3 mV peak-to-peak.

Even if the recordings of intracellular receptor potentials in hair cells confirm the supposed origin of the extracellularly recorded microphonic potentials, the controversy regarding the nature of the potentials is still not settled. The receptor potentials in hair cells are very small, and at threshold stimulation the potentials may be less than 1 μV. However, this does not necessarily indicate

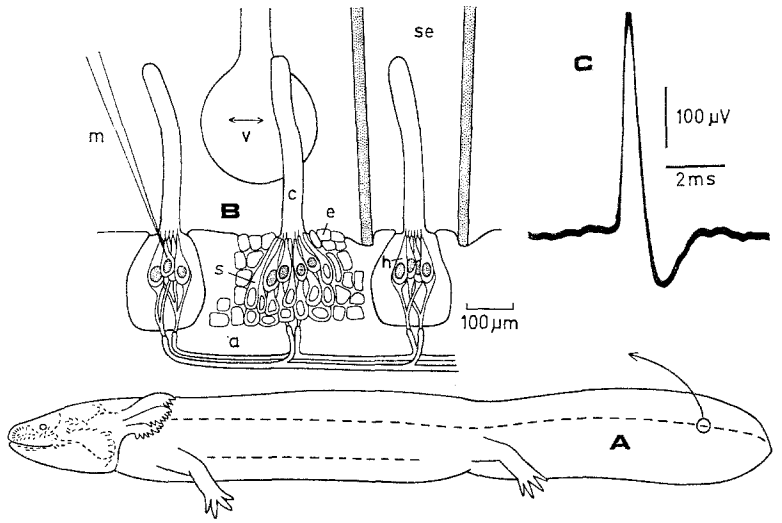

Fig. 1. (A) Sketch of mudpuppy indicating the distribution of stitches. (B) Schematical section through a stitch containing three neuromasts, each composed of hair cells (*h*) and supporting cells (*s*), and surrounded by epithelial cells (*e*). Two afferent fibers (*a*) innervates each organ. The cupulae (*c*) were vibrated through water movements induced by a vibrating sphere (*v*), Afferent spikes were picked up by a suction electrode (*se*) enclosing one organ, whereas a microelectrode (*m*) was used for intracellular recording and electrical current stimulation. Redrawn from Kingsbury (1895) and Harris *et al.* (1970). (C) Afferent spike recorded by suction electrode. Preamplifier time constant was 1 s

that the receptor potentials are epiphenomena. The electro-receptive cells in several groups of fish are closely related to hair cells, and the threshold for electrical potential detection may be less than 0.1 µV/cm (Lissmann and Machin, 1958).

No direct evidence exists so far which proves the hair cell receptor potentials to have a causative role in transmitter release, and the purpose of the present investigation was to provide such evidence. We have approached this problem by applying electrical current directly, either intra- or extracellularly. Our preparation was the lateral line organ of the mudpuppy, which was chosen because of the simplicity of the organ and the large size of the hair cells. The effect of our current stimulation was monitored by recording the activity of the afferent neurons, and then compared with the effect of mechanical stimulation.

Materials and Methods

Preparation

The mudpuppy (*Necturus maculosus*) is an aquatic salamander with free standing lateral line organs in large numbers on the head, trunk and tail (Fig. 1A, B). The anatomy of these organs in *Necturus* has been described by Frishkopf and Harris (1969) and Harris *et al.* (1970). The neuromasts on the tail are grouped in so called stitches with 2–6 neuromasts in linear rostrocaudally arrangements. A single neuromast contains 8–10 hair cells separated and surrounded by a large number of supporting cells. From

the surface of the organ a transparant cupula about 40 μm in diameter is extending 200–800 μm into the surrounding water. The cupula is secreted by the supporting cells and has a growth rate of 15–30 μm per hour (Frishkopf *et al.*, 1973). The hair cells of *Necturus* are extremely large, about 80 μm long and 15 μm in diameter at the level of the nucleus, compared to 40 and 5 μm, respectively, for a typical mammalian hair cell. The cells are narrowing towards the apical end, which is about 2 μm wide. From this end a bundle of short stereocilia and a single asymmetrically placed kinocilium project into the cupula. The direction for maximal sensitivity is parallel to the stitch, and the hair cells of individual neuromasts are divided in two populations with sensitivity axis shifted 180°. Each stitch is innervated by two large myelinated afferent nerve fibres which branch off to every neuromast within the stitch. Afferent endings terminate at the base of all the hair cells, and each fibre innervates selectively hair cells of only one particular directional population.

Our animals were obtained from a commercial dealer and kept in artificial pond water (Kishimoto, 1966). During an experiment the mudpuppy was immobilized by Flaxedil (Davis and Geck) injection of 1 mg per 100 g body weight. The tip of the tail is translucent and can be illuminated from below, and stitches in this region were selected. The condition of the animal could be followed by monitoring the capillary blood flow near the tail tip. This preparation has previously been used by several investigators (Harris *et al.*, 1969, 1970; Frishkopf and Oman, 1972; Frishkopf *et al.*, 1972; Liff and Shamres, 1972; Oman, 1972; Oman and Frishkopf, 1973; Yanagisawa *et al.*, 1974).

Recording and Stimulation

Action potentials evoked by stimulation of one particular neuromast will follow the afferent branches and enter all the other neuromasts within the same stitch. Thus, the neural activity of the whole stitch may be monitored by an extracellular microelectrode inserted into any of the neuromasts (Harris *et al.*, 1960, 1970), and this technique provides a signal to noise ratio of 4:1 for optimal conditions with the electrode tip close to the nerve terminals (Harris *et al.*, 1970; Oman and Frishkopf, 1973; Murray and Capranica, 1973). However, in the present experiments we used a different method to record spikes from the afferent nerves, and this made the recordings easier and more stable. The recording pipette was made from a glass capillary as an ordinary suction electrode filled with artificial pond water. The tip diameter was 250 μm and the edge was made smooth to secure a tight fit to the skin. The cupula and apical surface of a neuromast were enclosed by the suction electrode (Fig. 1B), and slight vacuum was sometimes applied to the electrode in order to improve the fit between the tip and the skin. The electrode was connected to a conventional a.c. amplifier (Tektronix, 122), with time constant 2 ms, and standard recording equipment. The signal to noise ratio was improved by this method, and was frequently more than 10:1.

10 Hz sine waves of either electrical current or mechanical vibration were employed as stimuli, and the response was measured as the degree of synchrony between the stimulus and recorded spikes of the afferent neurons. The afferent activity was recorded from a neuromast separated from those being stimulated. Each spike triggered a square pulse of 10 ms duration, and these pulses were averaged by a signal analyzer (HP, 5480A). The averager was triggered by the sine wave generator controlling the stimuli, and the averaged value for a certain stimulus phase thus gives the probability for an action potential to occur within $\pm 36°$ of this phase, the height of the original square pulse representing a probability of 1. The difference between the highest and lowest probability for the occurrence of an action potential during a stimulus cycle will hereafter be called the "synchronization index", and this value is used as a measure of the degree of synchrony between the stimulus and the afferent activty. It is necessary to stress that the synchronization index so defined will have a positive value even without stimulation. The index depended in such cases upon the number of sweeps being averaged, approaching 0 for an infinite number. However, to save time only 64 sweeps were usually averaged, and the typical values for the index in the absence of stimulus was then 0.15–0.25.

Our electrodes for intracellular penetrations were filled with 3 M KCl from behind by capillary action (Tasaki *et al.*, 1968), and the tip was thereafter beveled to obtain maximum sharpness (Brown and Flaming, 1974, 1975). The electrode resistances were 30–80 megohm. The electrodes were advanced by means of a manual micromanipulator, and connected to a high input impedance d.c. amplifier with an additional arrangement for current passing through the electrode (WPI, M4A). Wheras sine wave currents were used for electrical stimulation, square pulses were applied for impedance measurements of the cells using a bridge circuit.

Intracellular recordings were also performed during mechanical stimulation, and averaging techniques were then usually employed to improve the signal to noise ratio. The mechanical stimuli were provided by a sphere about 500 μm in diameter, vibrated parallel to the stitch by a coil vibrator (Harris and van Bergeijk, 1962). The distance from the sphere to the stitch was usually several diameters, and all the organs within the stitch were stimulated simultaneously. The stimulation intensity was adjusted to give a synchronization index close to 1, and further experimentation would only proceed if the stitch displayed a reasonable sensitivity.

Results

Origin of the Recorded Spike Activity

Two distinct unit activities were picked up by the suction electrode, as judged from the differences in spike height (Fig. 2A). Fig. 1C gives an example of the recorded spikes (the preamplifier time constant was 1 s in this particular case), which had amplitudes ranging from 50 to 500 μV for different recording conditions. The spike duration was about 2.5 ms, and the shape was slightly biphasic, with a large positive component followed by a much smaller negative part. Simultaneous recordings from any pair of neuromasts within the same stitch were performed by using two electrodes, and the firing patterns obtained from the two neuromasts were always the same. The origin of these potentials was confirmed by intracellular recordings from the afferent nerve terminals. The intracellular action potentials did in these cases always correspond to one of the units recorded by the suction electrode (Fig. 5), and we conclude that the extracellularly recorded spikes display the activity of the two afferent neurons innervating each stitch.

It may seem surprising that it is possible to record unit activity in this way with very coarse electrodes outside the animal. A likely explanation is that the neuromasts represent low resistance pathways for the action currents across the skin. If a tight fit between the electrode tip and the skin is obtained, it will then be possible to record the major fraction of the IR drop of the action current due to markedly reduced shunting effect of the external water.

Afferent Response to Mechanical Stimulation

Fig. 2A shows a recording of afferent spikes in the absence of mechanical stimulation. The fibres were spontaneously active, with a resting firing rate of 3–15 impulses per s. Our recording technique was not suited for detecting a possible pattern of the afferent activity in such cases, but the probability for an action potential to occur was of course independent of the future stimulus phase (Fig. 2B). However, as the intensity of the mechanical stimulus was increased, the occurrence of spikes became phase locked to the 10 Hz stimulus,

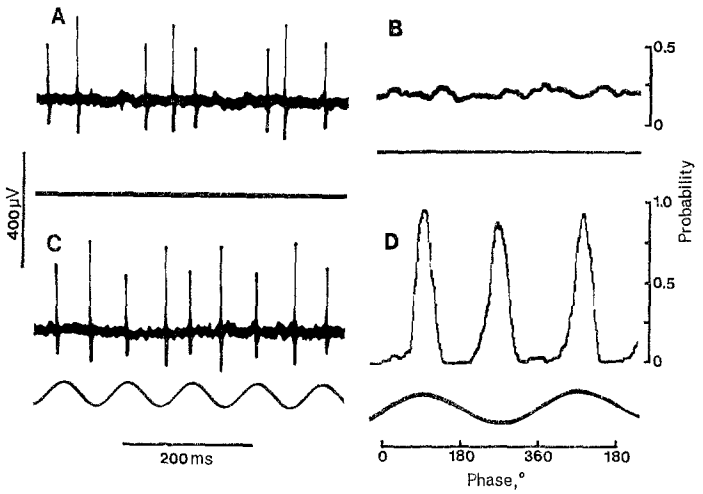

Fig. 2. (A) Recording of afferent spikes in the absence of stimulation. Note the existence of two distinct units. (B) Probability for the occurrence of spikes relative to the future stimulus phase. (C) Recording of afferent spikes during mechanical stimulation. Note that the two units are 180° out of phase. (D) Probability for the occurrence of spikes relative to the stimulus phase during mechanical stimulation. The 10 Hz stimulus is monitored on the lower trace of each recording

the two types of spikes being 180° out of phase (Fig. 2C). This fact is reflected by the corresponding probability plot (Fig. 2D), which shows probability maxima approximately 180° apart for the occurrence of spikes. These maxima approached 1 at high intensities, whereas the probabilities in between the peaks decreased towards 0 for increasing intensity.

Afferent Response to Extracellular Electrical Current Stimulation

Synchrony between stimulus and afferent spikes was also obtained during 10 Hz electrical current application through extracellular microelectrodes close to or within the neuromasts. The two afferent units were both excited at the positive current phase (Fig. 3A), and the probability maxima for the occurrence of spikes were thus separated 360° (Fig. 3B). The phase dependence of this response therefore differed from the response to mechanical stimulation.

It is important to determine whether the current excites the afferent nerve terminals directly, or if its effect is through the hair cells. An implication of the first assumption is that the effect of current stimulation should increase by decreasing distance between the electrode and the nerve terminals. Fig. 4 presents data from an experiment in which response/stimulus strength curves were obtained for several electrode positions within a single neuromast. The maximum probability difference in the absence of stimulus was in this case about 0.2, but the synchronization index increased to about 0.7 when current stimulation of 10^{-7} A was applied at the apical surface of the organ. However, advancement of the electrode tip deeper into the neuromast caused the degree of

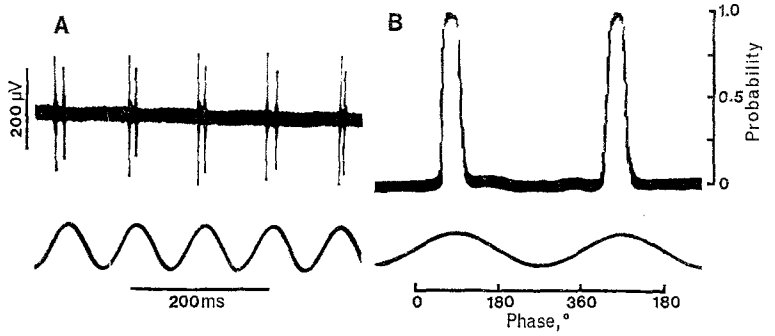

Fig. 3. (A) Recording of afferent spikes during electrical stimulation. Note that the two different units are in phase. The spikes are distorted by band pass filtering. (B) Probability for the occurrence of spikes relative to the stimulus phase during electrical stimulation. The current was applied close to the apical surface of the organ, and the intensity was 5×10^{-7} A peak-to-peak. The 10 Hz stimulus is monitored on the lower traces. Positive current corresponds to upward deflection

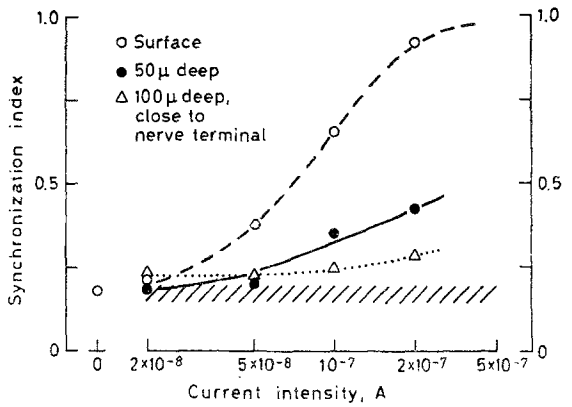

Fig. 4. Synchronization index as a function of current intensity for electrical stimulation of a neuromast. Current was applied with the electrode in three different positions. Note that the phase locking effect of the current on the afferent ativity decreased when the electrode approached the nerve terminals

synchronization to decrease. It was possible to pick up extracellular spikes from the afferent fibers with the microelectrode, but such spikes were only seen if the tip was close to the nerve terminals (Murray and Capranica, 1973). In this particular experiment current stimulation was performed during the occurrence of 700 μV extracellular spikes, which indicates that the electrode tip was very close to the nerve terminals, but an intensity of 10^{-7} A barely induced synchronization above the background level. As will be discussed later, we interpret this as strong indication for the current having its effect on the hair cells.

If this is the case, the effect of current stimulation on the afferent activity should be markedly decreased if the synaptic transmission between the hair

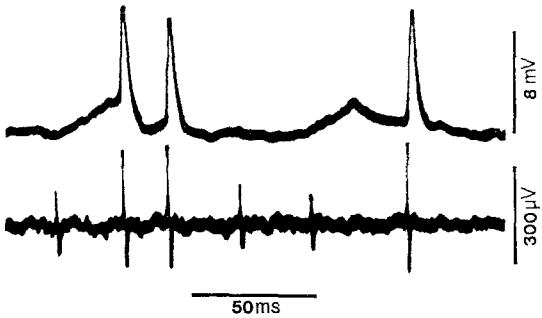

Fig. 5. Intracellular recording from an afferent nerve terminal (upper trace) compared to extracellular recording from a neighbouring neuromast within the same stitch (lower trace). Note both the existence of EPSPs in the terminal and the close correspondence between the action potentials and one of the two extracellularly recorded units. Resting potential for the terminal was −60 mV. Time constant was 100 ms due to a–c recording

cells and the afferent neurons was blocked. It has been suggested that the transmitter of this synapse is GABA (Flock and Lam, 1974), and we therefore applied intramuscular injections of picrotoxin (Sigma) or bicuculline (ICN) in concentrations of up to 100 mg/kg or 2 mg/kg body weight, respectively. In a variety of animals these drugs are known to block selectively synapses where GABA is the transmitter substance, and the necessary concentrations to obtain blocking have been less than 30 mg picrotoxin/kg and less then 0.2 mg bicuculline/kg body weight (Takeuchi and Takeuchi, 1969; Curtis *et al.*, 1970; Obata *et al.*, 1970). However, treatment of the mudpuppies with the drugs had no effect neither on the spontaneous resting firing rate, nor on the sensitivity to mechanical or electrical stimulation. Our attempt to block the afferent synapses by applying picrotoxin or bicuculline was thus unsuccessful. The drugs used were tested on other tissues, and the expected blocking effects were then observed.

Intracellular Recording and Current Stimulation

Neuromasts from 13 animals were penetrated, and intracellular recordings were made from 156 cells with resting potentials from −20 to −70 mV. The cells could be divided in three different groups, based on their response to mechanical stimulation together with the afferent response to intracellular current stimulation. However, frequently only one of these responses was tested, due to the short duration of the intracellular recordings.

a) Afferent Nerve Terminals

When the electrode tip was advanced through the deeper part of the neuromasts, afferent nerve terminals were penetrated in 5 cases. These cells had negative resting potentials of 40–70 mV, and were identified by the existence of spontaneous excitatory postsynaptic potentials (EPSPs) in addition to action potentials (Fig. 5). The action potentials closely corresponded to the extracellular spikes from one of the two units recorded from a neighbouring neuro-

mast. Frequently the action potentials seemed to be caused by a preceding EPSP reaching a certain threshold value, but the action potentials could also rise abruptly from the baseline without any preceding EPSP. We assume that the action potentials lacking a prepotential were generated in neighbouring nerve branches, since it is known that action potentials in afferent lateral line fibers in amphibians have several separated initiation points (Murray and Capranica, 1973).

When the stitch was stimulated mechanically, the nerve terminals displayed action potentials in synchrony with the stimulus (Fig. 6A). Since the activity of only one unit was recorded in these cases, the phase separation of the action potentials was 360°. On the other hand, the nerve terminals were relatively insensitive to intracellular current injection. Even current stimulation up to 10^{-8}A peak-to-peak was insufficient in causing phase locking of the afferent activity (Fig. 6B).

b) Supporting Cells

Supporting cells are the most abundant cell type within the neuromast of *Necturus* (Harris *et al.*, 1970), and approximately 80% of our penetrated cells were tentatively judged to be supporting cells. These cells did not show any spontaneous electrical activity, and even strong mechanical stimulation was inefficient in causing alternating membrane potential changes (Fig. 6C). The resting membrane potentials varied between −20 and −60 mV, the mean value being 31 mV ± 15 mV (s.d.). Our assumption that these cells were supporting cells is based on the work by Harris *et al.* (1970), who histologically identified mechanically unresponding cells within the neuromast to be of this type by using intracellular dye injection. However, the resting potentials of our penetrated cells were frequently less than −30 mV, thus indicating cell injury (Flock *et al.*, 1973). It is therefore likely that some of the unresponding cells were injured hair cells, rather than supporting cells. To eliminate this possible error, only supporting cells with a resting potential more negative than −40 mV were chosen for the current injection experiments. Intracellular current injection was applied to mechanically insensitive cells in 12 cases, and current stimulation up to 10^{-8} A failed to induce phase locking of the afferent activity (Fig. 6D). Cells which both lacked spontaneous electrical activity and sensitivity to current stimulation were therefore classified as supporting cells, even if the recording was too short lasting to allow testing of the mechanical sensitivity. This assumption is valid since hair cells turned out to have a different response to current stimulation.

c) Hair Cells

The remaining 31 penetrated cells were assumed to be hair cells, due to their unique responses to both mechanical and electrical stimulation. These cells had no spontaneous electrical activity, and the resting membrane potentials ranged between −20 and −60 mV, the mean value being 33 mV ± 13 mV (s.d.). However, an a–c component of 100–500 μV peak-to-peak became evident by averaging the potentials during mechanical stimulation of the stitch (Fig. 6E).

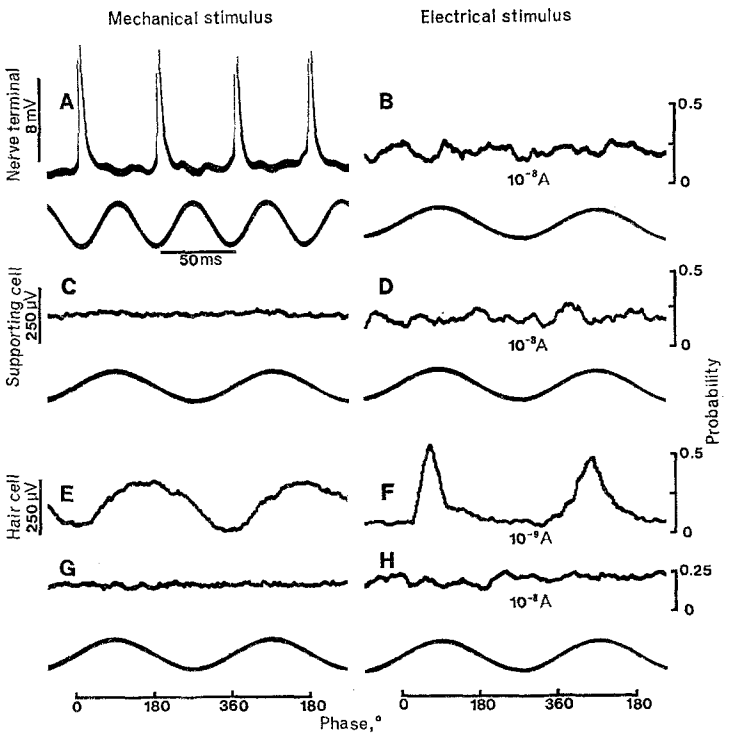

Fig. 6A—H. Intracellular potential recordings during mechanical stimulation (A, C, E) and probabilities for occurrence of afferent spikes during intracellular current injection (B, D, F). Three different cell types are presented. Nerve terminals (A, B) exhibit action potentials phase locked to the mechanical stimulus, wheras they are relatively insensitive to current injection. Supporting cells are neither sensitive to mechanical nor electrical stimulation (C, D). Hair cells show receptor potentials of the same frequency as the mechanical stimulus, and current injection into these cells causes synchrony between stimulus and afferent spikes (E, F). G and H are recordings during mechanical and electrical stimulation just after the electrode had been advanced through the hair cell. Each pair of recordings is from the same cell. Resting potentials were −50 mV for hair and supporting cells, and −70 mV for the nerve terminal. The stimuli are monitored on the lower trace of each recording. The current intensities are indicated on the figure. Positive current corresponds to upward deflection; stimulation frequency was 10 Hz for all recordings except A, where it was 20 Hz. Time constant for this recording was 100 ms

The frequency of the potential changes was the same as the frequency of the mechanical stimulus. These receptor potentials were only recorded with the electrode in an intracellular position (Fig. 6G), and were thus not an artifact due to vibration of the electrode. Harris *et al.* (1970), by applying intracellular staining techniques, found this response to belong exclusively to hair cells.

Our main purpose of the present investigation was to test if these small receptor potentials have effect on the synaptic transmitter release from the hair cells. Intracellular current stimulation might in that case cause phase locking

of the afferent activity in the absence of a mechanical stimulus by mimicking the receptor potentials. Fig. 6F shows the effect on the afferent activity of 10^{-9} A peak-to-peak intracellular current stimulation of a hair cell. The probability for the occurrence of spikes was markedly increased during the outward current phase, hence confirming the hypothesis being tested. A ten fold increase in current intensity was insufficient in causing similar phase locking after the electrode had been advanced through the cell (Fig. 6H). Intracellular current injection of less than 3×10^{-9} A peak-to-peak intensity was in 9 cases applied to cells showing receptor potentials evoked by mechanical stimulation, and a certain degree of synchronization between current stimulus and the afferent spikes was always observed. Cells which displayed similar sensitivity to intracellular current stimulation were therefore judged to be hair cells, even if recordings of mechanically induced receptor potentials were not obtained due to short lasting penetrations. 21 cells showed sensitivity to intracellular current stimulation of intensities between 0.5×10^{-9} A and 3×10^{-9} A.

It is important to know the input impedance of the hair cells, to be able to compare the membrane potential changes induced by current stimulation with the naturally occurring receptor potentials. Unfortunately, the input impedance of the cells proved to be only a small fraction of the electrode resistance, and the measurements obtained by using the bridge circuit were therefore rather unreliable. However, in the five cases where the electrode resistances were most stable, we estimated the impedances of the hair cells to range between 1 and 3 megohms. This implies that the peak-to-peak membrane potential changes during current stimulation varied from slightly less than 1 mV up to about 9 mV.

Discussion

Extracellular Current Stimulation

Several investigators have stimulated acousticolateral end organs by electrical current (Katsuki and Yoshino, 1952; Tasaki and Fernández, 1952; Lowenstein, 1954; Murray, 1956; Dodson *et al.*, 1974; Strelioff and Honrubia, 1974), and clear effects of the afferent activity were unanimously obtained. On the other hand, few attempts have been made to pin-point the action site of the current stimulation. Murray (1956) concluded that electrical currents applied to the end organ effected the nerve terminals directly, and he suggested that the microphonic potentials evoked by mechanical stimulation of the organ were sufficient to have a similar effect on the afferent fibers. The function of the hair cells were then to act as mechano/electrical transducers and generate an external field which modified the afferent activity. However, Murray's experimental data do not conflict with the assumption that current stimulation has its effect on the afferent activity by changing the rate of transmitter release from the hair cells.

Dodson *et al.* (1974) and Strelioff and Honrubia (1974) have recently studied in detail the effect of extracellular current stimulation on lateral line organs in *Xenopus laevis*. Transepithelial application of electrical current could produce most of the effects obtained by mechanical stimulation of the same organ, and the afferent response to both stimuli showed a similar decline under anoxic

conditions. They suggested that the electrical stimuli effected the hair cells rather than the afferent nerve terminals.

This interpretation fits well with the present experiments involving extracellular current application to the neuromasts. The cells within the neuromasts are elongated and orientated normal to the skin, which might cause the neuromasts to represent low resistance paths across the skin. However, the cells are very densely packed at the apical surface of the organ (Jørgensen and Flock, 1973), and a relatively large part of the current applied extracellularly in this area will enter the neuromast cells. The cells are less densely packed in the deeper parts of the neuromasts, and the extracellular space may therefore have a larger shunting effect on the current from an electrode in this deep position. Less current will therefore enter supporting cells and hair cells, while the amount of current entering the nerve terminals in this area even may increase. Since we found the current stimulation to be most efficient with the electrode in an apical position (Fig. 4), we conclude that the current intensities employed effected the hair cells and not the afferent nerve terminals directly.

Evidence for a Causative Relationship between Hair Cell Receptor Potentials and Transmitter Release

Only hair cells proved sensitive to our intracellular current stimulation. The depolarizing current phase then caused excitation of one of the afferent fibres innervating the organ, while even ten times more intense current injection directly into the afferent nerve terminals or supporting cells had no effect on the afferent activity (Fig. 6). It is therefore likely that current stimulation of the hair cells affected the afferent neurons by modifying the transmitter release from the hair cells, which suggests that the receptor potentials induced by mechanical stimulation have a similar effect. A possible existence of electrical synapses between hair cells and the afferent nerves is disproved by the fact that afferent action potentials were more easily evoked by current injection in the hair cells than in the nerve terminals directly.

We never recorded hair cell receptor potentials larger than 500 μV, whereas our effective intracellular current stimulation might cause potential changes of about 1–9 mV peak-to-peak given that the input impedances of the cells were in the range of 1–3 megohms. However, these potentials are not directly comparable. During mechanical stimulation all the hair cells within the stitch were stimulated simultaneously, whereas only one cell was stimulated by intracellular current injection. It is therefore reasonable to assume that a larger potential change is required in the last case to obtain a certain degree of synchronization between stimulus and afferent activity. Furthermore, receptor potentials of up to 3 mV peak-to-peak have been recorded from hair cells in the lizard ear (Weiss *et al.*, 1974), and the receptor potentials measured by our electrodes might be too small due to cell injury.

The conclusion is that the effective intracellular current injections in mudpuppy hair cells might have caused membrane potential changes which overlapped in magnitude with the naturally occurring receptor potentials. We

interpret this as strong support for the assumption that mechanically evoked receptor potentials in hair cells have a direct effect on the transmitter release, and these potentials are thus not an epiphenomenon caused by the secretory activity of the hair cells.

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