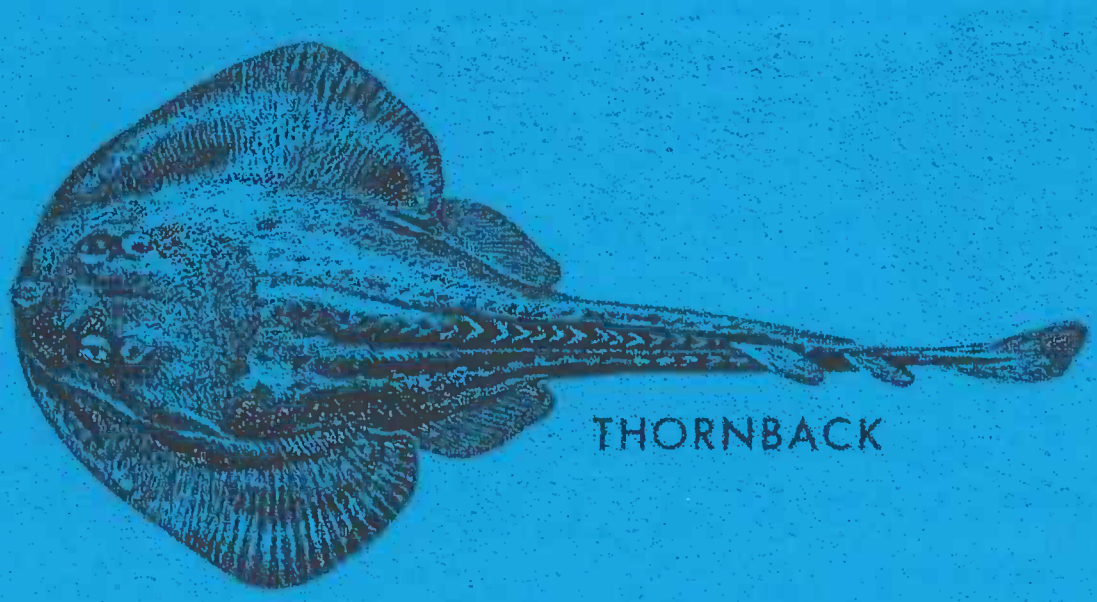
Electroreception

of the

Elasmobranch Thornback Ray,

Platyrhinoidis triseriata.



Fulco Zegwaard
March 1994.

Preface:

This report is part of my graduation in marine biology at the Rijks University Groningen, The Netherlands. It was sponsored by Dr. J Videler (The Netherlands) and Dr. A.J. Kalmijn (U.S.A.). It reflects the results of seven months of hard work in the sensory biophysics lab of Dr. A.J. Kalmijn at Scripps Institution of Oceanography in La Jolla, California.

Working and living in California is, I think, everybody's dream. My dream came true. Even though it was sometimes hard to focus on work and not to be distracted by other things, it became a good learning experience in many respects. Ad stayed very patient in trying to teach me some of the basics of biophysics, but besides the scientific part I also got a lot to learn about people (and about me). Living in International house on the UCSD campus also contributed to that last part. Therefore I want to express my thanks to everybody who made my stay in the U.S. possible and fun:

Dr. Gieskes, for bringing me into contact with Dr. A.J. Kalmijn.
Dr. J. Videler for supporting me to go to San Diego.
Ad Kalmijn for giving me the opportunity to work in his lab, and for teaching me the things he did.
Vera Kalmijn for taking care of me when I first arrived and getting me through my 'culture shock' by still being very Dutch, but in an American surrounding.
Jozien for making life in that small office bearable.
To all the friends I made, who made life in California so much fun, I'll miss you all.
And to Beth , to whom I am dedicating this paper and who has been the inspiration to many of my thoughts.

I will remember this period of my life forever and I hope to return here someday, to visit my friends or just travel around.

Fulco Zegwaard
March 1994

Abstract:

Sharks, skates and rays all have Ampullae of Lorenzini. Nowadays these ampullae are generally accepted as being electroreceptors, with which the animals can sense the (bio)electric fields in their environment.
Much biophysical research has been carried out on these electroreceptors, but most of the experiments were conducted under biologically invalid '*in vitro* ' situations and the properties of the ampullae were therefore greatly influenced. It is important to re-evaluate the data obtained to date under biologically more adequate and '*in vivo* ' conditions.
In this report the results of '*in vivo* ' experiments on live, anaesthetized thornback rays are described. One of the goals was to perform the experiments on fully submerged animals. The only difficulty we had to overcome was that by submerging the animal, the canal would no longer be insulated and the nerve signals would no longer be measurable. At the present time no solution has been found for this problem. The decision was therefore made to temperarely record the nerve spikes (as a measurement of the activity of the ampulla) without submerging the animal yet. The activity of the nerves to different pore-positive, hyperpolarizing stimuli was recorded.
With the current set-up we were able to conduct experiments on the animal up to several days, this in contrast to other workers who were only able to make stable recordings up to several hours. We found some differences with the results of previous workers, which could be a result of the differences in the experimental conditions. In our experiments we found a clearly visible reaction of the ampulla to stimuli with a frequency of up to 12 Hz. At higher frequencies the response was less evident. The oscillations we recorded did not respond to any of the stimuli in any way. This is a sharp contrast to what other researchers found and contradicts their explanation of the high sensitivity. They are however physiologically real and are probably more than just artifacts. The way to get answers to these questions is to continue with trying to conduct the experiments under biologically more valid conditions (i.e., by fully submerging the animal) and under more controlled circumstances (i.e., computer controlled amplitude, frequency and time base settings for the stimuli). A program to solve this last problem is already being developed. Another way to get the answers would be with intra-cellular recordings (sofar unsuccesfull).

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1 Introduction:

Ever since the beginning of history, there has always been an interest in the electrical properties of marine elasmobranchs. In the ancient Greek era of Aristotle, the main reasons of interest in these animals, for example in *Torpedo torpedo*, were the painfull, but also regarded as invigorating and supposedly healing effects of the electric shocks some skates and rays can produce (Wu 1984). After the Renaissance however, interest shifted to a more descriptive and investigative point of view. Francesco Redi and Stephano Lorenzini were the first to describe the electric organ responsible for producing the shocks (1671). The mechanism of how the shocks actually came to be however, was still not understood, since electricity itself was not 'discovered' until almost a hundred years later (1745, invention of the Leyden jar).
Lorenzini was also the first to describe a system of ampullary canals (1678) which are found in all cartilaginous fish (sharks, skates, rays and chimaeras) and now bear his name: the Ampullae of Lorenzini. At the time of discovery Lorenzini thought the ampullae to be glands and that the long canals served to distribute their mucus secretion over the surface of the fish (Waltman 1966). But already he commented that:

> '...the walls of the canals which being much thicker than what is appropriate for a simple duct, makes us suspect that they are intended for another more hidden function, since nature never acts casually, nor multiplies entities without necessity...'

Since then many different functions have been ascribed to the ampullae of Lorenzini, from mechanoreceptors (Parker 1909) to temperature receptors (Sand 1938), but they are now generally accepted as being electroreceptors for the detection of DC (direct current) fields and low frequency AC (alternating current) fields.
All electroreceptors i.e., the electroreceptors in elasmobranchs, the catfishes and the chondrosteans (primitive bonefishes), are often thought to be related to the acousticolateralis system, which also includes the inner ear and the lateral line. There is however no formal relationship. The electroreceptors are obviously of major importance to the fish, since the number of their sensory nerves ranks with those of the eye, ear, nose and lateral line. The electric fields that are present in natural waters, provide a wealth of sensory information the fish can detect.

They can use this information to detect prey, to receive important orientational cues and even to receive complete compass data. This discovery of such a particularly high electrical sensitivity in fish, dates back to 1917 when Parker and van Heusen reported remarkable sensitivity of freshwater catfish to galvanic fields. They did not recognize the biological significance of their findings, obviously still unaware of the electric fields in the animals natural habitat. Long after the work of Parker and van Heusen, in the year 1951, Lissmann gave a new impulse to the study of electrosensitivity. In behavioral experiments he showed that the fishes not only respond to the electric-organ fields of each other, but also can detect nearby objects as impedance inhomogeneties of their own electrical discharges (Lissmann 1951, 1958). In the years following Lissmann's discovery, Murray found that the ampullae of Lorenzini are not only very sensitive to thermal and mechanical stimuli (Murray 1960, 1962), but also respond to weak electric fields. Dijkgraaf and Kalmijn (1962, 1963) offered direct evidence for the shark's electrical sensitivity by applying purely electric fields of dipole and uniform configuration and by conducting denervation experiments. Kalmijn showed biological relevance of electroreception when performing prey detection experiments on sharks and orientation experiments on rays (Kalmijn 1966, 1971, 1982).

Much biophysical research has been carried out on the ampullae of Lorenzini in order to unravel the process of electroreception. Nevertheless is, in our opinion, most of the neurophysiological data on the highly sensitive process of sensory transduction based on experiments conducted under biologically invalid conditions. They are largely confined to experiments on excised ampullae and stimuli outside the dynamic range of the electric sense. Our project is mainly based on criticism on these experimental conditions and set-ups. Therefore to identify the true process of electroreception, the observations made to date must be reexamined for their biological validity by conducting neurophysiological experiments on the ampullae of Lorenzini under biologically adequate conditions.

In this report the results are presented of '*in vivo* ' experiments carried out on life, anaesthetized thornback rays, while trying to comply to the previously mentioned conditions. Also a short comparison is made with the ' *in vitro* ' work done by other investigators. The main question the present study focuses on, is as to where the elasmobranch's electrical sensitivity is located in the processes leading from the physical stimulus to the behavioral response.

More specifically an attempt is made to answer the question of how the afferent nerve fibers of individual ampullae respond to the receptorpotentials generated by weak electric fields. We also tried to find an answer to the role of oscillations (in our point of view mere artifacts) in the receptor potentials with regard to the sensitivity of the system. The way we tried to find the answers to those questions was by recording the action potentials of the 5-7 nerve fibers innervating each ampulla, with an electrode in the canal near the capsule in life anaesthetized rays. By insulating the canal for some length to increase the capacitive reactance of the wall, the nerve signals passively invading the canal attain heights well above the system noise. To reduce the number of active fibers to only one or a few recognizable types, the ampullae are pore-positively (hyperpolarized) biased for the duration of the recordings. The reactions of the ampulla to different frequencies and amplitudes of the stimuli were then stored on a DAT-recorder and analyzed after being printed out on an pen-recorder.
The following part of this introduction will consist of a short morphological description of the ampullary system followed by a description of its biophysical properties and its biological relevance in respect to behavior.

1.1 Anatomy:

The ampullae of Lorenzini form a system of sense organs in all cartilaginous fishes. Due to the large variety of habitats, predation habits, body shapes etc., there are many structural variations in the ampullary systems. For example the lengths of the canals vary from species to species and even within any one fish, although there is a stereo type set with variations: canals radiate in all directions from clusters. They cover a large area and have a tendency to have some overlap. Also the amount of ampullary swellings and the pore densities may vary. The pattern of pore distribution is approximately species specific (figure 1.)

The anatomical description of the ampullae will be of a more general nature in this paper, for more detailed work see Dotterweich (1932), Waltman (1966) and Raschi (1978).

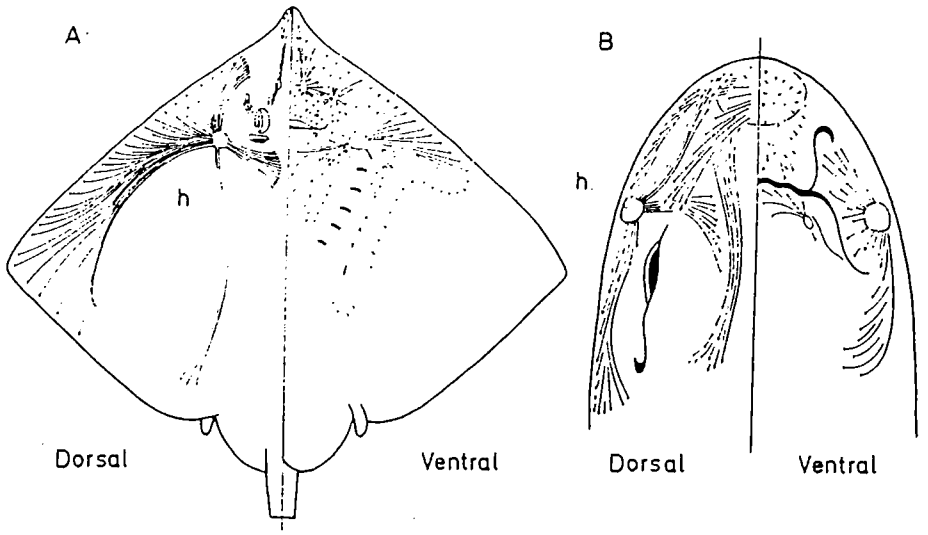

Figure 1: Distribution of the ampullary canals in (a) Rays and (b) Sharks (after Murray 1974)

1.1.1 The Ampulla:

The ampullary system consists of two main regions, divided by a transitional zone. The first region is the cylindrical jelly-filled canal which is at the distal side electrically connected with the seawater by a pore through the skin.

On the proximal side it ends blindly in the second region, a cluster of small swellings or alveoli, lined by the sensory epithelium. This is the ampulla proper. These two clearly distinguishable elements are divided by a narrow marginal zone(figure 2).

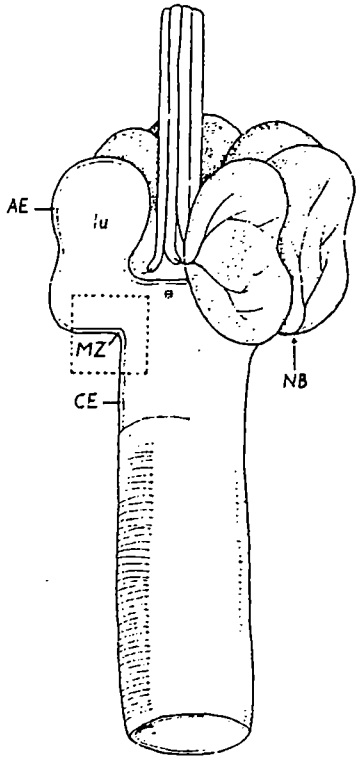

Figure 2: Ampulla of Lorenzini (after Waltman 1966). MZ is the Marginal Zone, CE the Canal Epithelium, AE the Alveolar Epithelium, Lu the Lumen and NB the Nerve Bundle

A. The canal:

The epithelium lining the canal on the inside is 1-2 μm thick and consists of two layers of pavement epithelium. The innermost pavement cells are connected together by tight junctions or zones. The outside of the canal is made up of circular and longitudinal collagenous fibers, providing mechanical strength. The lumen of the canal is filled with a gelatinous jelly, which consists of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- ions, Urea and mucopolysaccharide components. The jelly has a resistivity of 31Ω.cm (slightly more than seawater) and is anchored to hemidesmosomes in the epithelium with protein fibers

B The ampulla proper:

The alveolar epithelium is single layered and about 15 µm thick. It contains two kinds of cells: the innervated pear-shaped receptor cells and the uninnervated pyramid-shaped supporting cells. The pear-shaped receptor cells have a small apical membrane and a large basal membrane. At the apical membrane a kinocilium (0.3 µm diameter, 5 µm long, 8+1 axonema configuration) protrudes into the lumen, the function of which is still unknown. The receptor cells are isolated from each other by a surrounding of supporting cells. The flattened tops of the supporting cells carry a crown of microvilli around each kinocilium. All the cells are connected closely together just below their distal face by tight junctions, as in the canal epithelium. The tight junctions form continuous bands around the necks of the receptor cells (figure 3).

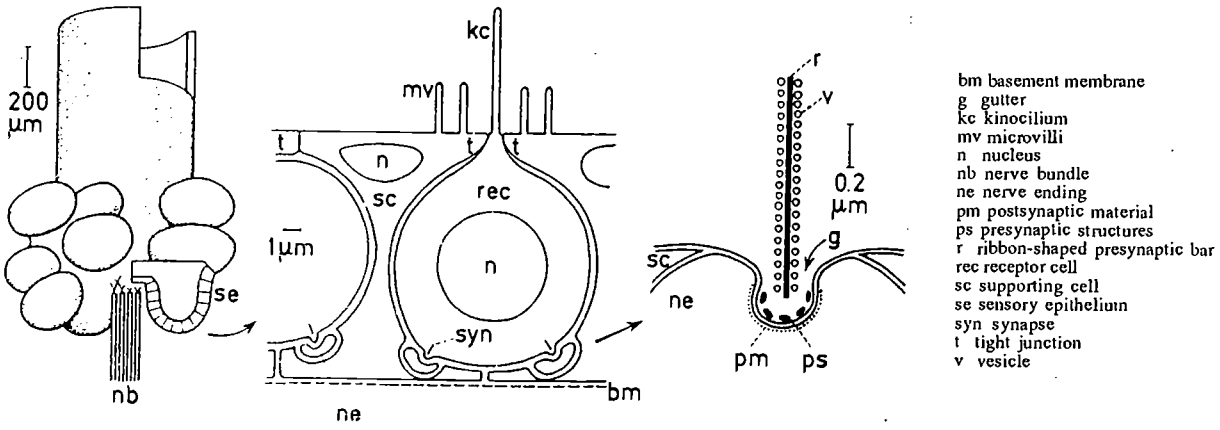

Figure 3: Receptorcell and detail of synaps (after Murray 1974)

This occlusion of the spaces between the cells lining both canal and ampulla, provides the morphological explanation for the very high electrical resistance between inside and outside of the ampullary organ.

This high resistance (6 MΩ. cm^2: Waltman 1966), combined with the 0.4 µF/cm^2 capacity value of the canal wall (typical for biological membranes) and the low resistivity of the jelly (31 Ω.cm: Waltman 1966) make the canal an almost ideal submarine cable with virtually no attenuation of DC-voltages. This forms the basis for the electroreceptor function, as will be discussed later.

Synapses occur at the basal membranes of the receptor cells (see also figure 3). Each receptor cell has between 2 and 7 separate afferent synapses with the terminal axon branches. All synapses converge to a small bundle (5-12) of afferent nerve fibers from the hyomandibular branch of the facial nerve (which is part of the cranial nerve vii), that innervates each ampulla wih its thousands of receptorcells. Efferent synapses are absent.

C: The marginal zone:

The nerves enter the ampulla through the transitional region, the marginal zone. The marginal zone forms the rim of each alveolus and so forms the transition from canal epithelium to the alveolar epithelium. Tight junctions and desmosomes characterize the lumenal ends of the lateral cell contacts, which below the tight junctions interdigitate extensively (this also accounts for the high capacitance).

1.1.2 Distribution of the Ampullae:

The ampullae are clustered into groups, which are enclosed in tight connective tissue capsules (see also figure 1). The design of the peripheral system, with the ampullae arranged in clusters and the skin pores covering the heads of sharks and additionally spreading over the wing-like pectoral fins in skates and rays, would excellently suit the detection and spatial analysis of the imposed electric fields.
The distribution of the pores is an important characteristic for a spatial analysis, since the average resistivity of the skin and body tissues is too high compared to the seawater, for the electric fields to invade the marine elasmobranchs unimpededly. The lengths of the canals just serve to bring the ampullae of far-separated pores close together. The resulting arrangement of the ampullae in clusters would allow the animals to measure the fields differentially by comparing the signals received from individual ampullae (single ended input devices) and suppressing their common-mode contents. This rejection of the common-mode signals would make the animals less susceptible to interference from their own bio-electric fields (Kalmijn 1974,1984,1987).

1.2 The Electrical Sensitivity of the Ampullae of Lorenzini:

When first discovered in 1678, Lorenzini thought the ampullae to be glands, with the canals to distribute the jelly over the fish's surface. In 1909 Parker discovered that applying pressure to the ampullary mass caused a respiratory reaction. With the ampullary nerves cut, such responses no longer occurred. Sand was in 1938 unable to confirm this mechanical sensitivity, but found them to be extremely sensitive to temperature changes instead. He found an acceleration of action potentials to cooling and a corresponding deceleration to warming. The ampullae responded to stimuli as weak as 0.1-0.2 °C. Then Murray revived in 1957 the possible mechanoreceptive function of the ampullae by finding them rather sensitive to pressure stimuli if properly applied. The stimuli had to produce pressure differences between the inside and outside of the ampullary organs. However, similar stimuli may be detected by the lateral line and other skin receptors as well. Therefore the real function of the ampullae was still uncertain at that time. Murray continued to systematically study the electrophysiological properties of the ampullae of Lorenzini. He subsequently discovered that the ampullae are not only sensitive to mechanical and thermal stimuli, but also respond to weak electric fields and salinity changes (Murray 1960, 1962).

Later Kalmijn (Dijkgraaf and Kalmijn 1962) showed the sharks indeed to be extremely sensitive to electric fields in the surrounding water. In the experiment sharks (Scyliorhinus canicula) and rays (Raja clavata) responded to uniform square wave fields of 5 Hz, at voltage gradients as low as 0.1 $\mu V.cm^{-1}$ with eye blink responses. That the responses were truly due to the ampullae of Lorenzini was demonstrated in both denervation and recording experiments (Dijkgraaf and Kalmijn 1963, 1966). In later experiments (Kalmijn 1966) Kalmijn found the sensitivity to uniform 5 Hz square wave fields to be even larger then expected. Electrocardiograms of Raja clavata showed distinct reactions of the heartbeat when the voltage gradient was lowered to only 0.01 $\mu V.cm^{-1}$. This value represented the at that time highest electrical sensitivity known in aquatic animals. It was clear that a thorough re-evaluation of the naturally occurring electric fields and their potential usefulness to sharks and rays was necessary.

1.3 Biological Significance of Electroreception:

Electrosensitive animals could use the electric fields present in their environment. They could be able to detect animate and inanimate sources, use it for social communication, for orientation and navigation, and detection of background electric fields (wave action, currents, etc).

1.3.1 Origin of Bioelectric Fields:

The existence of bioelectric fields has already been known for a long time. In 1902 Mathews observed steady electrical potentials (at mm distances) along cut, regenerating stems of the marine tubularian hydroid *Parypha*. Lund (1922) had similar results in *Obelia* and Burr and Hovland (1937) extended the investigations to salamander larvae. In his search for clues to explain the marine sharks and skates unusual electrical sensitivity, Kalmijn (1966) found low-frequency bioelectric fields at considerable distances (up to 10 cm) from the flounder *Pleuronectes platessa*. In marine fishes, the electric fields appeared mainly to emanate from the mouth and opercular slits. Other parts of the body, especially when slightly bruised, could be noticeably electrogenic as well. The externally measured fields result from spatial variations in the potential difference across the living ectoendoderm, the boundary between the animal and the seawater medium. To give an example of a measured bioelectric field, Kalmijn (1969) recorded at a distance of 10 cm from the head of a small, undamaged flounder (a natural prey for the sharks), DC fields of 0.2 μV/cm. Ventilatory and fin movements caused the field to fluctuate rhythmically at rates from 1 to a few cycles per second. This is already well within the range marine sharks, rays and skates can detect, since they give biologically meaningful behavioral responses to DC and low-frequency electric fields at voltage gradients as low as 5 nV/cm (Kalmijn 1981, 1982). Proof of the elasmobranchs electrosensitivity and its role in predation came in 1971 when Kalmijn conducted experiments on bottom dwelling sharks. Feeding responses were elicited when small flounders were presented under a layer of sand or agar, but were absent when presented under agar covered with a plastic film, electrically screening the prey.

Feeding responses were also observed when prey fields were simulated with two electrodes under agar or sand. (figure 4)

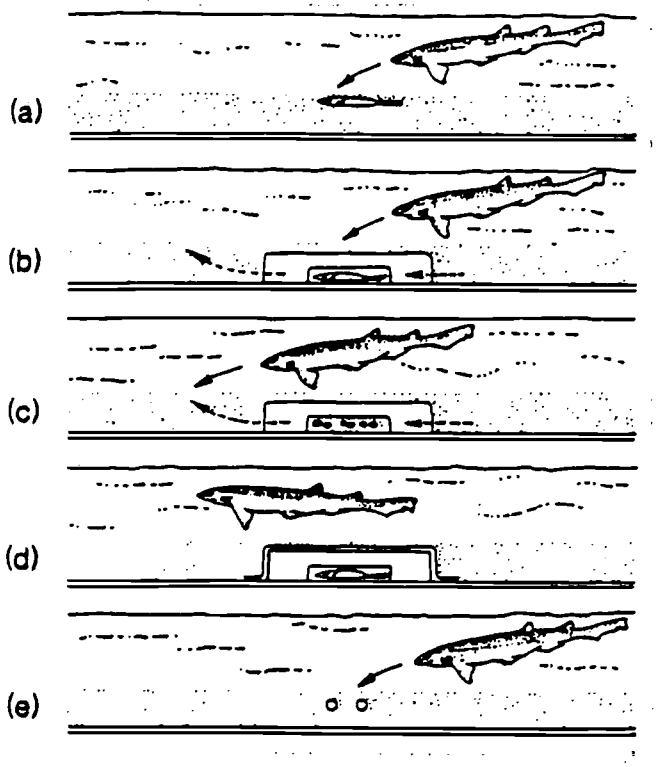

Figure 4: Responses of dogfish Scyliorhinus canicula to (a) a flounder buried under the sand. (b) a flounder in an electrically transparant agar chamber. (c) pieces of cut fish in an agar chamber. (d) a flounder in an agar chamber covered by an insulating plastic film. (e) electrodes simulating the bioelectric field of a flounder. The sharks aim their well-aimed attacks in response to the electric fields of both real and simulated prey. (after Kalmijn 1971).

1.3.2 Environmental Electric Fields:

A: Fields Due to Water Flowing Through the Earth's Magnetic Field

Besides for prey detection, the electric sense may also be used for orientation. In the oceans, the electromagnetic fields induced by the flow of water through the earth's magnetic field prevail. They were already predicted by Faraday in 1832.

The global system of wind-driven ocean currents and tidal flows induce, by interaction with the vertical component of the earth magnetic field, horizontal electric fields perpendicular to the flow of the water. This results in a world-wide pattern of motional-electric fields, ranging from 5 nV/cm to over 500 nV/cm (von Arx 1962). The induced electric currents give rise to voltage gradients, which in the stream provide the animals with directional cues during their movements in familiar territory (Kalmijn 1974). This so-called passive electro-orientation not only provides the animals with information about their drift in the water and gives them local orientational cues, but also informs them about the upstream and downstream directions and the speed of the seawater current in which they find themselves (figure 5).

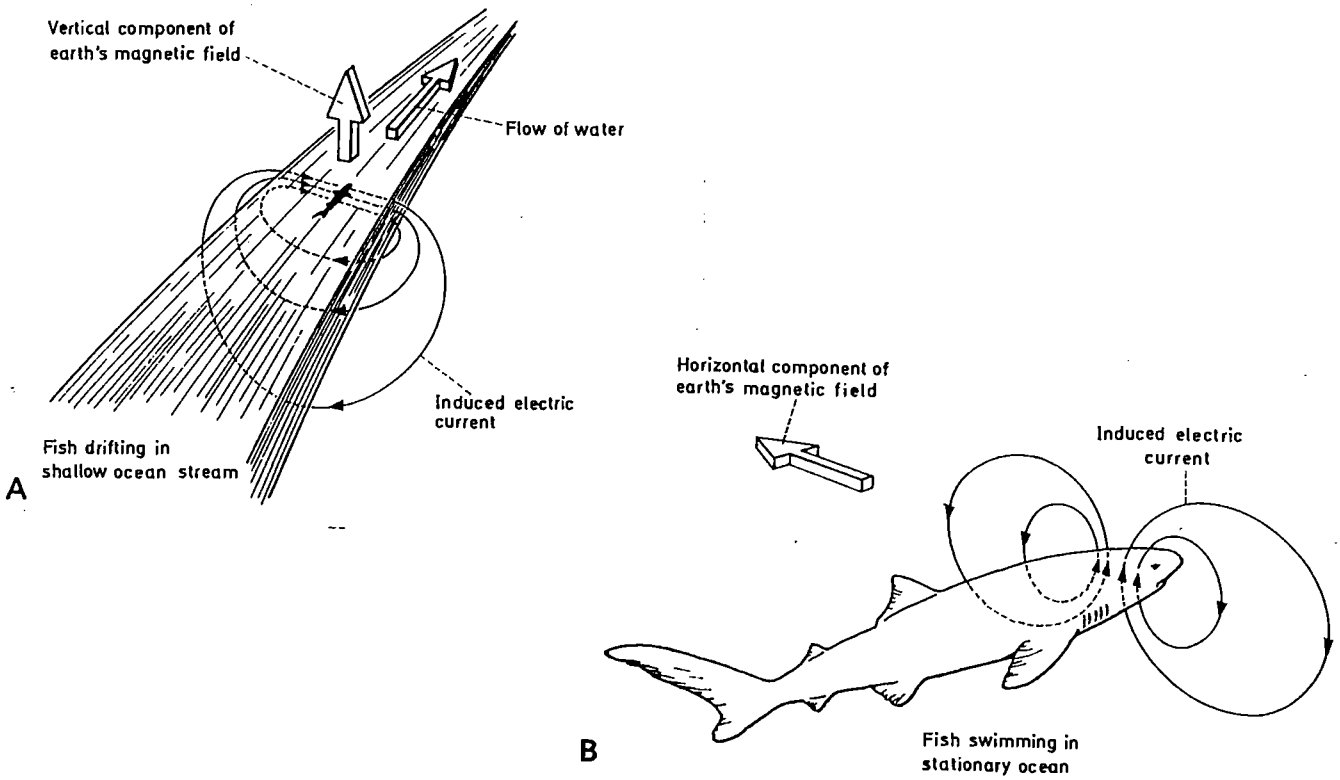

Figure 5: (a) Open-ocean currents flowing through the earths magnetic field yield electrical voltage gradients that may inform drifting animals of upstream and downstream directions. (b) Fish swimming through the earths magnetic field induce electrical voltage gradients that may constitute a physical basis for compass orientation. (after Kalmijn 1974).

B: Fields Induced by the Recipient Animal's Forward Motion:

When a fish moves relative to the water it also induces an electric field by actively interacting with the earth's magnetic field. Crossing the horizontal magnetic component, the animal induces a ventrodorsal field (the dorsal ampullae becoming negative with respect to the ventral ampullae) when heading east, and a dorsoventral field (the dorsal ampullae becoming positive with respect to the ventral ampullae) when heading west and no potential differences are induced when the animal is either swimming to the north or to the south.(figure 5) At an ambient magnetic field of 0.5 gauss, a swimming speed of 1 cm/s suffices to produce the threshold stimulus of 5 nV/cm. If the shark is able to evaluate these potential differences, it may use them as a physical basis for a true compass sense. Interaction between the animal and the vertical component of the earth's magnetic field, induces similar potential differences in the horizontally directed ampullary canals. With this additional information, the shark might sense the inclination of the earth's magnetic field, and thus the latitude of its position on the globe.
In behavioral experiments on the stingray *Urolophus halleri* Kalmijn proved (1984) beyond doubt that the elasmobranchs are capable of magnetic orientation. Further experiments to verify the electromagnetic principle of the elasmobranchs' magnetic compass sense are under way.

C: Other Fields of (Geo)- Physical Origin the Elasmobranchs Might Detect:

Currents induced in the earth's crust and the oceans by magnetic variation, known as telluric or earth currents, can also reach heights that are measurable for elasmobranchs. In the oceans these currents tend to concentrate in the coastal waters and along the continental shelf. They might interfere with, or perhaps contribute to electro-orientation. They also might just constitute, together with fields due to atmospheric electromagnetic phenomena (lightning discharges) and man-made 60 or 50 Hz electric pollution, background "noise". This may be useful either as a smoothing factor in nervous transmission or as a constant input maintaining central excitatory state (Kalmijn 1974).

D: Fields of Electrochemical Origin:

In the habitat of aquatic animals, complex electrochemical fields result from local differences in chemical composition, salinity, oxygen content, pH, temperature, etc. The fish may be able to electrically sense chemical non-uniformities in their milieu. If these non-uniformities are more or less steady, the fish might use them as orientational features in their habitat. On the other hand, if differences are, for instance, induced by the presence of plankton, a fish like *Polyodon* might electrically locate its feeding grounds (Kalmijn 1974). These are however, highly speculative theories.

1.4 Biophysical Properties of the Ampullary Receptors:

1.4.1 Frequency Range of the Electric Sense:

Waltman (1966) revealed that for DC and low-frequency signals, the Lorenzinian canal acts as an almost ideal submarine cable. When a potential difference is applied between the pore and the ampullae, no appreciable voltage drop develops along the jelly-filled canal. This results from an extremely high mural resistance (6 $M\Omega.cm^2$) and a relatively low core resistivity (31 $\Omega.cm$) of the canal and its 'blind' ampullary end.
At higher frequencies, however, the signal is considerably attenuated, because of a rather high mural capacity (0.4 $\mu F.cm^{-2}$). According to Waltman's calculations there is already a significant cut-off at 3Hz. This is however a questionable value, since he did not take the dynamic behavior (feedback) of the ampulla into account. When looking at the behavioral frequency range of sharks and rays, it can be seen that they are most responsive to DC fields; sine-wave fields of 1, 2, and 4 Hz were slightly less effective. At 8 Hz, the strengths of the stimulus had to be increased with a factor two to elicit a clear feeding behavior and at 16 Hz the sharks no longer reacted at all. The rays still responded at 16 and even 32 Hz, but only after raising the strengths of the currents respectively 8 and 32 times.

That sharks and rays are most sensitive to frequencies up to 8 Hz is in agreement with Murray's (1962,1965) and Waltman's (1966) data and seems to be an adaptation to the DC and low-frequency bioelectric fields (c.f. 1.3.1.). From an electrophysiological point of view however, the ampullae of Lorenzini do not behave as true DC receptors, because they adapt almost completely within seconds. This means that sharks and rays must move with respect to DC fields in order to bring the stimuli within the frequency range of the electroreceptors (Kalmijn 1974).

1.4.2 Electroreceptor Mechanisms:

A: General Description:

The behavioral threshold for marine sharks, skates and rays, is at voltage gradients as low as 5 nV/cm. This corresponds with a potential difference received by the receptor epithelia in the longest canals of the animals tested, of at most 25 nV (Kalmijn 1974, 1982). Observed neurophysiological responses, usually in experiments with isolated ampullae, required at least 10 to 100 times stronger stimuli.
Murray observed in 1962 that the afferent nerve fibers have a tonic resting discharge of 30-40 impulses per second. Pore-positive stimuli decrease the firing rate of the nerve, and pore-negative stimuli increase the firing rate, which is a polarity reversal when compared to teleost tonic receptors. This reversal points at the possible excitability of the lumenal faces of the receptor cells. A lumen-negative stimulus depolarizes and excites the lumenal faces; their regenerative response depolarizes the basal faces, which then secrete transmitter. A lumen-positive stimulus leads to relative hyperpolarization of the basal faces and a reduced release of transmitter. If stimuli are very strong and even outside the range of electroreception, their direct actions on the basal faces dominate the effects of changes in lumenal membrane activity and the polarity of sensitivity becomes the same as in the teleosts: a lumen-negative stimulus decreases the tonic nerve discharge and a lumen-positive stimulus increases it (Bennett and Clusin 1979). In fact, this can be seen as a mere artifact, since in the natural surrounding stimuli of that large a nature are not found.

At prolonged stimuli of moderate amplitude, the impulse frequency shows a complete accommodation, returning three-quarters the way back to its initial value in less then 5 seconds. The time-course of accommodation is similar for pore positive and negative stimuli (Murray 1962). Accommodation to a prolonged stimulus makes it possible to still be very sensitive to small voltage changes superimposed on this prolonged stimulus.

B: Generation of a Receptor Response:

A characterization of the response of the lumenal membranes is made by voltage-clamp experiments (Clusin and Bennett 1979) and perfusion experiments (Clusin and Bennett 1977). Bennett and co-workers found the following sequence of events during the generation of receptor potential, when a lumen-negative voltage was applied.

-- When the apical face of the ampulla is depolarized by applying a lumen-negative stimulus, the voltage sensitive inward Ca^{2+}-channels are opened. Ca^{2+} flows inward (I_{early}, the regenerative inward current) and depolarizes the lumenal membrane actively and the basal membrane passively.

-- The depolarized basal membrane causes basal Ca^{2+}-channels to open and as a result Ca^{2+} flows inward and transmitter (L-Glutamate, Akoev et al.,1991) is released.

-- With sufficient Ca^{2+} influx at the apical membrane, a delayed outward current is activated (I_{late}) by opening of the Ca^{2+}-activated K^+-channels. As a consequence the apical membrane repolarizes.

-- Voltage sensitive or Ca^{2+}-activated K^+-channels in the basal faces are also activated, the outward current then repolarizes the basal membrane and transmitter release ends.

-- When the voltage drops below a certain level as a result of the repolarization, the Ca^{2+}-channels are closed (I_{early} stops) and the Ca^{2+} activation of the K^+-channels rapidly declines . The basal membrane K^+ conductance turns of with some delay, and the cell thus returns to its condition at the beginning of the cycle.

This oscillatory model is however speculative (the delays in the different currents were never proven or calculated), and is proposed by Bennett and co-workers to explain the high sensitivity of electroreceptors. They ascribe this high sensitivity to voltage amplification due to the regenerative oscillatory activity of the receptor cells.

The gain of excitable cells poised near threshold approaches infinity, and they argue that it is reasonable that oscillating cells, which are repetitively passing through threshold, would also be very sensitive to stimulation (Bennett and Clusin, 1979).
Bennett and co-workers also discovered that high K^+, Co^{2+}, EGTA or tetraethyl ammonium (TEA) treatment of the basal membrane reversibly blocks the oscillations. The TEA sensitivity of the oscillations indicates that they involve a potassium conductance in the basal membranes of the receptor cells, but the true process and reason for the oscillations is still not fully explained by Bennett.

Broun and Gorvardovskii (1984) propose a different theory for the electroreceptors great sensitivity without the use of the oscillations. They claim that the regenerative responses of the receptor epithelium (the basic argument of Bennett's hypothesis) are side effects and are unrelated to the normal functioning of the electroreceptors.
Their hypothesis is that excitation of electroreceptors of the ampullae of Lorenzini in response to the action of an outward current and inhibition in response to the action of an inward current, are explained by the presence of a descending region (= negative resistance) on the stationary CVC (current-voltage characteristic curve) of the apical membrane of the receptor cell, and not by modulation of the spontaneous regenerative or oscillatory activity of this membrane. Their main argument against the theory of Bennett and co-workers, is that the spikes generated by the epithelium are unrelated to normal electroreception functioning and only arise in response to the use of stimuli outside the working range of the electroreceptor cell. In their experiments they never found any oscillations when the animal was properly loaded and biologically correct stimuli were applied. They also theorized that the apical membrane is the excitable part and the basal membrane only acts as a positive resistance and is not excitable. The main amplification of the signal, they argued, would come from amplification at the synaps.

Kalmijn proposes a third possible explanation for the extraordinary sensitivity of the electroreceptors. He agrees with the Russians (Broun and Gorvardovskii et al.) that in the live animal and with stimuli inside the working range of the electroreceptors no oscillations are found.

The delayed outward current (the main factor causing the oscillations) Bennett talks about is not calculated and also would describe the situation at stimuli of a factor 10^6 higher then is common in the natural situation. The Russian investigators get already one step closer to discribing the true process of electroreception. Kalmijn's theory differs however from their theory in several respects. The main point of disagreement is that the Russians ascribe the high sensitivity to the synapses, but then what would be the use of the receptorcells ? Kalmijn argues that there must be an amplification in the cell, probably acting in combination with an amplification at the synapses. The amplification in the cell is caused by a positive feedback at the basale membrane. This model with an infinite input impedance has proven stable in an electrical model and did not show any oscillations.

Most of the previously mentioned studies have been conducted on excised ampullae or with dead animals and to stimuli outside the dynamic range of the electric sense, applied under biologically invalid conditions. Therefore, the regenerative epithelial potentials and the stimulus-induced damped oscillations, though physiologically real, are biologically mere artifacts unless they actually occur in the life animal in response to natural stimuli. In the present study we will reexamen the observations made to date under biologically more valid conditions and in the process find more evidence to explain the extraordinary electrosensitivity in elasmobranchs.

2 Materials and Methods:

2.1 Experimental Animals:

The animals used in the experiments were thornback rays, *Platyrhinoidis triseriata*. The animals were collected around sunset in shallow waters of the Pacific Ocean, just off the coast of San Diego, California. They were caught off the pier of the Scripps Institution of Oceanography, La Jolla, California, U.S.A. Using this species has got several advantages. First : the animals are fairly abundant in this area and easy to obtain. Second: they are relatively easy to keep and to handle (they do not posses a sting, but have instead harmless thorns covering their backs). Third: Since the animals are flat it is easy to lay them down in a stable position in a stand carrying the electrodes during the experiments. When caught, the animals were first adjusted overnight to a room temperature of 10 °C. The next morning they were placed in a storage swimming pool (10°C, light: dark = 12:12hrs) where they were kept until they were used for the experiments.

2.2 Anaesthetization:

About half an hour before an animal was to be used for an experiment, it was lightly anaesthetized in a small tank of seawater (10 liters) with 50 ppm (0.500 gr.) of MS222 (Methanesulfonate Salt: 3-Aminobenzoic Acid Ethyl Ester, from Sigma Chemical Co., stored at -4°C). Since MS222 has an effect on the afferent nerve's transduction processes, the animal was subsequently tranquilized, after about half an hour, with 2µg / gr. body weight Pavulon. The effects of the MS222 on the nerve would by then almost have worn off. The solution (1ml standard pavulon solution [2mg/ml] dissolved in 19 ml Ringer [zie bijlage] was injected intramuscularly at the base of the tail. The animal was then placed in the experimental tank (0.15x0.40x0.85 m). If during the experiment the animal would become active again, another 1/2 dose or a 1/4 dose (depending on the condition of the animal) would be given.

2.3 Preparation:

The tranquilized animal was kept alive for 5-7 days, by insertion of two soft-rubber hoses into the spiracles providing a constant flow of seawater over the gills. For the experiments the cranial part of a canal having its pore at the base of the tail and its ampullae at the level of the spiracle in the hyoid capsule, was exposed by cutting a slit (3-4 cm) in the overlying skin. The hyoid capsule was not exposed. The membranous ligaments by which the canal is attached to the skin, neighboring canals, and the underlying tissue, were removed. The canal was then placed in a piece of silicone rubber with a small slit in it, to increase the capacitive value of the canal wall. Electrical contact with the canal jelly was made through a small hole cut in the wall of the ampullary canal. After three days the other side of the animal was used, because the condition of the canals on the exposed side would deteriorate slowly with time. When an animal died, about 60 liters of the total of 180 liters (45 gallons) seawater in the closed circulating system was replaced with fresh, filtered seawater.

2.4 Recordings:

The canal potentials, consisting of DC-receptor responses and the passively invading nerve spikes (evoked and spontaneous), were recorded differentially by use of a low-noise preamplifier (**Princeton Applied Research, Model 113**: low-frequency roll-off DC, high-frequency roll-off 1 kHz, gain 1000), with one electrode in the seawater at the skin pore of the ampulla and a second electrode at a hole in the canal close to the capsule. A third electrode was used as the ground electrode. The electrodes consisted of AgCl plated silver wires inserted into 4% agar-seawater filled glass capillaries till near their tips. The 4% agar-seawater solution was used to overcome diffusion artifacts and artifacts created by air-bubbles in the electrodes. From the first preamplifier the DC-receptor signals were sent to a second preamplifier (**Princeton Applied Research, Model 113**: low-frequency roll-off DC, high-frequency roll-off 300 Hz, gain 20-50) and subsequently stored on a DAT- recorder (**Biologic, Digital Tape Recorder 1801**: channel 2, gain 1).

The nerve spike signal from the first preamplifier was sent directly to a filter (**A.P. Circuit Corp., Model Ap255-5**: Band pass, high pass 10 Hz, low pass 800 Hz) and then to the DAT- recorder (channel 1, gain 10). This all led to an amplification of the signal of 10.000 times. The signals were led from the DAT and made visible on an oscilloscope (**Tektronix, Model 7313**) and audible on a stereo system. The DAT-recordings were used for further analysis. They were first copied to a tape recorder (**Honeywell, Model 5600 C**) and on this machine slowed down to 1/16 of the normal speed in order to be able to distinguish individual spikes. The slowed down version was then printed on an ink-recorder (**Gould, Model Brush 2200**: DC 0.5 volts full scale, chart speed 25 mm/sec)

2.5 The Stimulus:

The ampullae were excited by uniform electric fields in the water. The current was applied through platinum electrodes and salt-bridge manifolds to avoid the adverse effects of polarization at the electrodes. The field was produced by several instruments. First a pore-positive voltage step was created by an isolation unit (**W.P.I., Model 305-2**: output range: 0-50 μA) which got its sinc-signal from a function generator (**HP function generator, Model 3314A**). The resulting silencing of the activity of the nerve fibers did not last long, since the ampullae adapt to a voltage step with a time constant of several seconds. Subsequent to the step a ramp signal had to be applied just steep enough to offset the process of accommodation. This ramp signal was made by a function generator (**HP function generator, Model 3314A**). The steepness of the ramp could be set to the specific accommodation of the ampulla (voltage gradient of 0-10 V/10 sec.). The resulting ramp signal was then led to a second isolation unit (**Linear Stimulus Isolator, Model A395:** output range 100μA). The test stimuli, consisting of either rectangular pulses or short trains of sine waves, were superimposed on the ramp signal. They were generated by a second function generator (**Wavetek function generator, Model 112B**) which got its sinc-signal from the first function generator and was gated in a way that only during the ramp sinewaves were produced. The test stimuli were led to a third isolation unit (**Axon instruments Isolator-10 :** output range 1mA).

The outputs of all three isolation units were then connected in parallel and sent to the electrodes. The maximum voltage gradient resulting in the water varied between 0-2.3 V/cm^2.

2.6 The Experiments:

The following experiments were carried out:

- Recordings of spontaneous activity of the ampullae.
- Recordings of the reaction of an ampulla to a (symmetrical) square wave field (half the signal is inhibitory, half the signal is excitatory).
- Recordings of the reaction of an ampulla to a pore-positive voltage step.
- Recordings of the reaction of an ampulla to a pore-positive step+ramp signal.
- Recordings of the reaction of an ampulla to stimulus signals of various frequencies and amplitudes, superimposed on the ramp.
- Recordings of the epithelial potentials: -Flattening and/or reversing the signal.
  -Reaction of epithelial potentials to applied stimuli.
- We also attempted to conduct these experiments on fully submerged canals.

The main goal was to mimic the natural situation as well as possible to obtain biologically valid results, and to find an answer to the specific question of how vigorously the afferent nerve fibers of individual ampullae respond to weak electric fields.

Also a comparison was made between these *'in vivo '* results and some *'in vitro '* results of other workers.

3 Results:

In this chapter the results are presented of the experiments conducted in the period from August 1993 till February 1994. The first goal we set was to make the nerve signals that passively invade the canals ("spikes") visible, by trying to raise them above the system noise. The second and ultimate goal was to measure these signals in fully submerged animals.

To attain the first goal the canal was insulated for some length to increase the capacitive reactance of the wall and to insulate it from the seawater, otherwise the spikes would be too small. We did this in several ways. In the first set of experiments we increased this capacitive reactance of the wall by slipping a piece of lengthwise slit plastic tubing around the canal. Electrical contact with the electrode was made through a small hole in the tube at the same height as the hole in the canal wall. To insulate the canal and the hole from the surrounding seawater and prevent short circuiting of the signal, the canal and the surrounding exposed skin area were rinsed with sucrose. Unfortunately, the signals were not raised sufficiantly above the noise, presumably by short-circuiting due to seawater leaking into the plastic tube. It was essential to overcome this leakage problem if we wanted to attain our second goal, which was to fully submerge the animals. We attempted to make the tube 'water-proof' by covering the inside with silicone-grease and then snugly fitting it around the canal, but the results were the approximately the same. The only way we could detect measurable nerve signals was when we blew air underneath the canal at the ends of the tubing, thereby decreasing the opportunity for the seawater to seep in the tube. It was then that the decision was made for the moment not to concentrate on conducting the experiments on fully submerged animals, but instead to get measurable nerve signals under biologically slightly less ideal conditions. We no longer slipped a tube around the canal, but instead used a silicone rubber block with a groove in the middle in which we draped the canal. The block was too high for the seawater to be able to seep in, but low enough for the canal not to get stretched and in the process get damaged. We used the same process as before to insulate the canal.

First the canal and the surrounding tissues were rinsed with sucrose (and later urea) and then air was blown under the canal at both ends of the block. The nerve signals now attained heights well above the system noise. The next step was to record the nerve reaction to signals with different frequencies and amplitudes. The recordings at one ampulla lasted up to several hours.

3.1 Spontaneous Activity:

The spontaneous activity of the 5-7 afferent nerve fibers innervating each ampulla produces recordings too complex for sorting out the action potentials of individual units. Figure 6 shows the spontaneous activity of an ampulla over a (selected) period of 10/16 sec. The top trace depicts the nerve signals, the bottom trace are the DC epithelial potentials measured simultaneously. It can be clearly seen that on the top trace the spikes are too numerous and there is too much overlap between spikes to distinguish the activity of individual fibers. The bottom trace looks fairly stable, although there are some oscillations visible with a frequency of about 20 Hz.(see also figure 14).

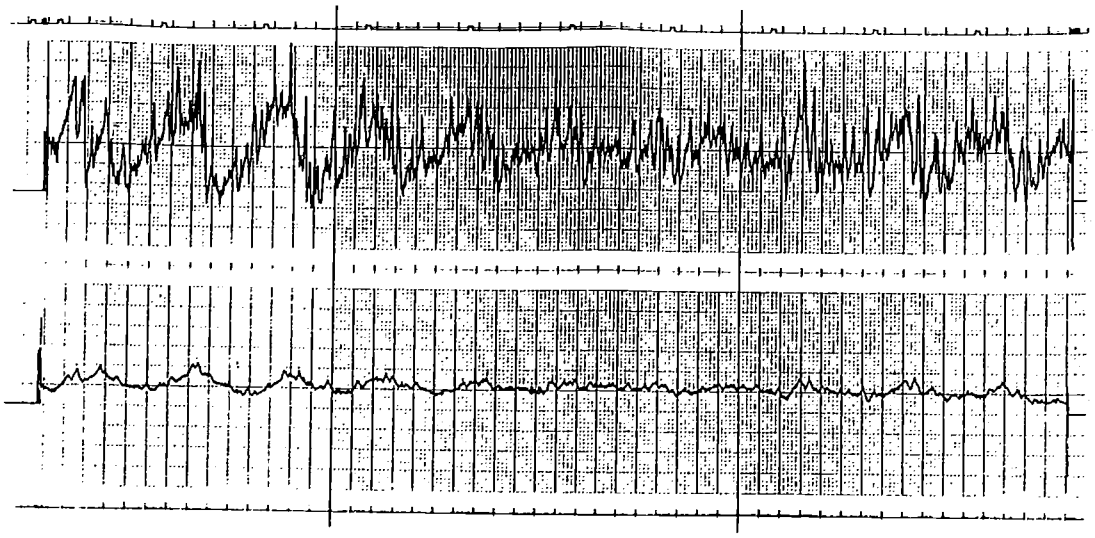

Figure 6. Spontaneous activity. Top trace = nerve spikes, Bottom trace = DC-signal.

3.2 Pore-positive Voltage Steps:

To diminish the activity of individual nerve fibers a pore-positive voltage step was applied, silencing all but one or a few of them. Figure 7 shows the reaction of an ampulla to a voltage step. The spikes are now much more clearly distinguishable, and apparently come from one or two fibers. Clearly visible in the top trace is the accommodation of the ampulla to the stimulus with a time constant of several seconds. Initially, directly after the transient, the spikes are completely suppressed, but then the number of spikes slowly starts to increase, eventually up to the point where it reaches the level of activity from before the stimulus was applied. In figure 7 the complete accomodation is not shown. After one second another signal is given.

On the bottom trace something else is visible. It can be seen that the epithelium has an active response to the voltage step. Instead of following the step passively, it responds by going against it and keep the epithelial potential flat, or, when sufficiently insulated, it can even be seen that the epithelial potential reverses. Instead of a voltage loss, the ampulla prevents loss and compensates and sometimes even overcompensates it (active response).

In both traces there are again oscillations visible.

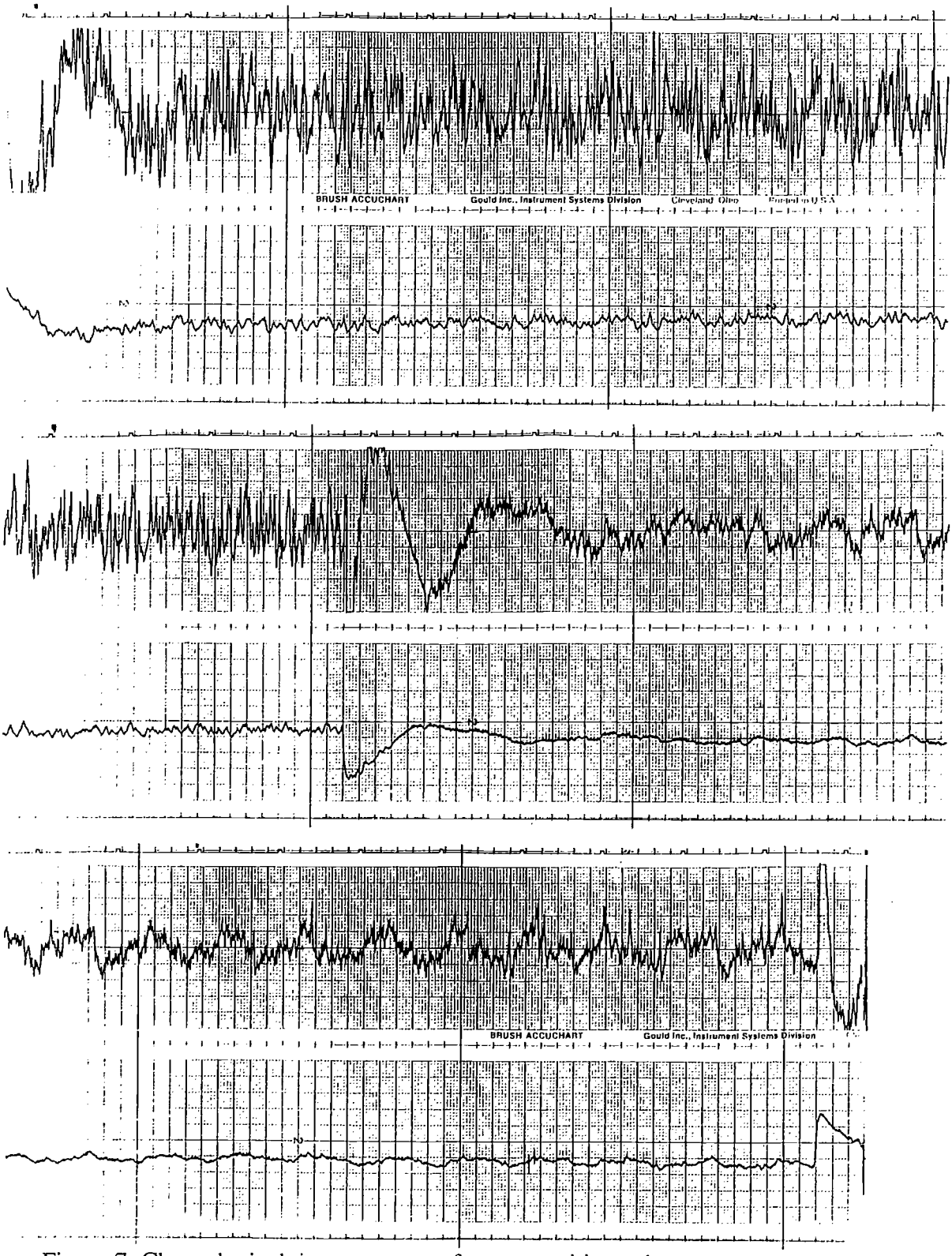

Figure 7. Chronological time sequence of a pore positive voltage step.

3.3 Applying a Ramp Signal:

Because of the accommodation of the ampulla to a voltage step with a time constant of several seconds, subsequent to the step a ramp signal was applied, just steep enough to offset the process of adaptation. For every ampulla the slope of the ramp was adjusted to maintain constant levels of nerve activity. The goal was to suppress every fiber but one and the one fiber left had to have a constant, slow firing rate of about 10-15 spikes/sec. The voltage step and the ramp have approximately an equal time period, we tried several lengths and decided that a 4 second voltage step followed by a 4 second ramp gave the best results. The steepness of the ramp could vary between 0-10 V over those 4 seconds and was usually set at values between 6-10 V (less in the water because off the max. output of 100μA of the isolation unit: ± 3mV). It was fairly difficult to set the ramp at the correct steepness. The reason was that it was not possible to see directly on the oscilloscope what the effect of the ramp would be on the activity of the spikes. Most of the settings were done by audibility. We would try to make the ramp in such a way that we would barely hear spike activity. When we then would print out the results it would usually be the case that all but one or two fibers were suppressed, but often there would still be too many fibers active or fibers would start coming back in the end. Figure 8 shows a short time frame of a 4 second ramp. In both the top trace and the bottom trace again oscillations were visible, they seemed to be unaffected by the step-ramp signal. The epithelial potential seemed to be following the ramp.

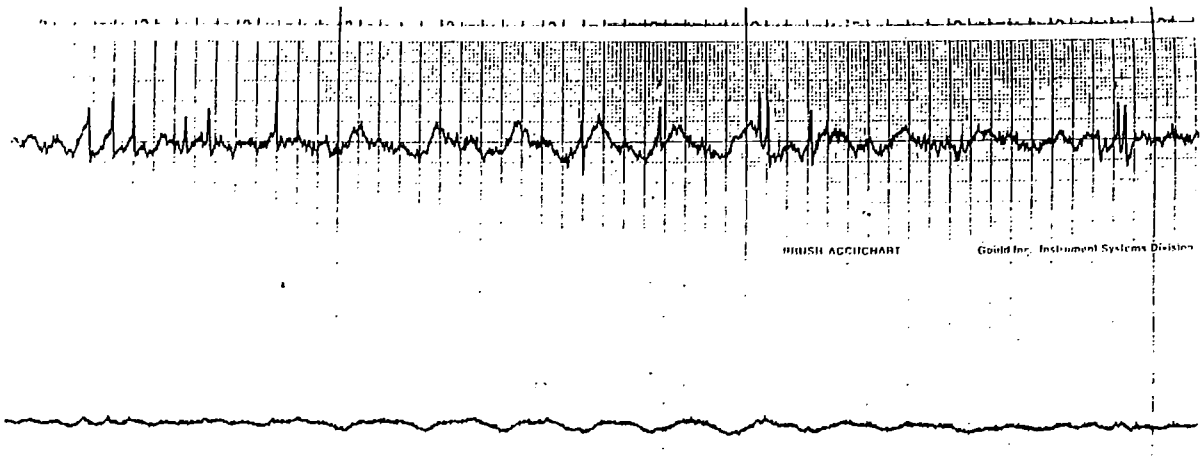

Figure 8. Short time frame (11/16 sec) of a ramp of 4 seconds.

3.4 Applying the Test Stimulus:

The test stimuli we gave were superimposed upon the ramp signal. They consisted of either rectangular pulses or short trains of sine waves. They ranged in frequency from 2-32 Hz (2, 4, 8, 12, 16, 24, 32 Hz). The amplitude of the test stimuli was more difficult to determine. We used three levels of amplitudes and made recordings of :1. Clearly audible responses, 2. Just audible responses, and 3. Doubtful audible responses. Figures 9 A-C show some sample recordings.

The reaction of the ampulla to the stimulus is clearly visible. Most of the time however, it seems that more than one fiber is being activated by the test stimulus. There were too many spikes and there was overlap between spikes. The amplitudes were for the most part too high. Even when the reaction of the ampulla was only just audible it looks like more then one fiber was responding. When the reaction of the ampulla was doubtful audible however, it seemed that only one fiber was responding, but in a very irregular manner.

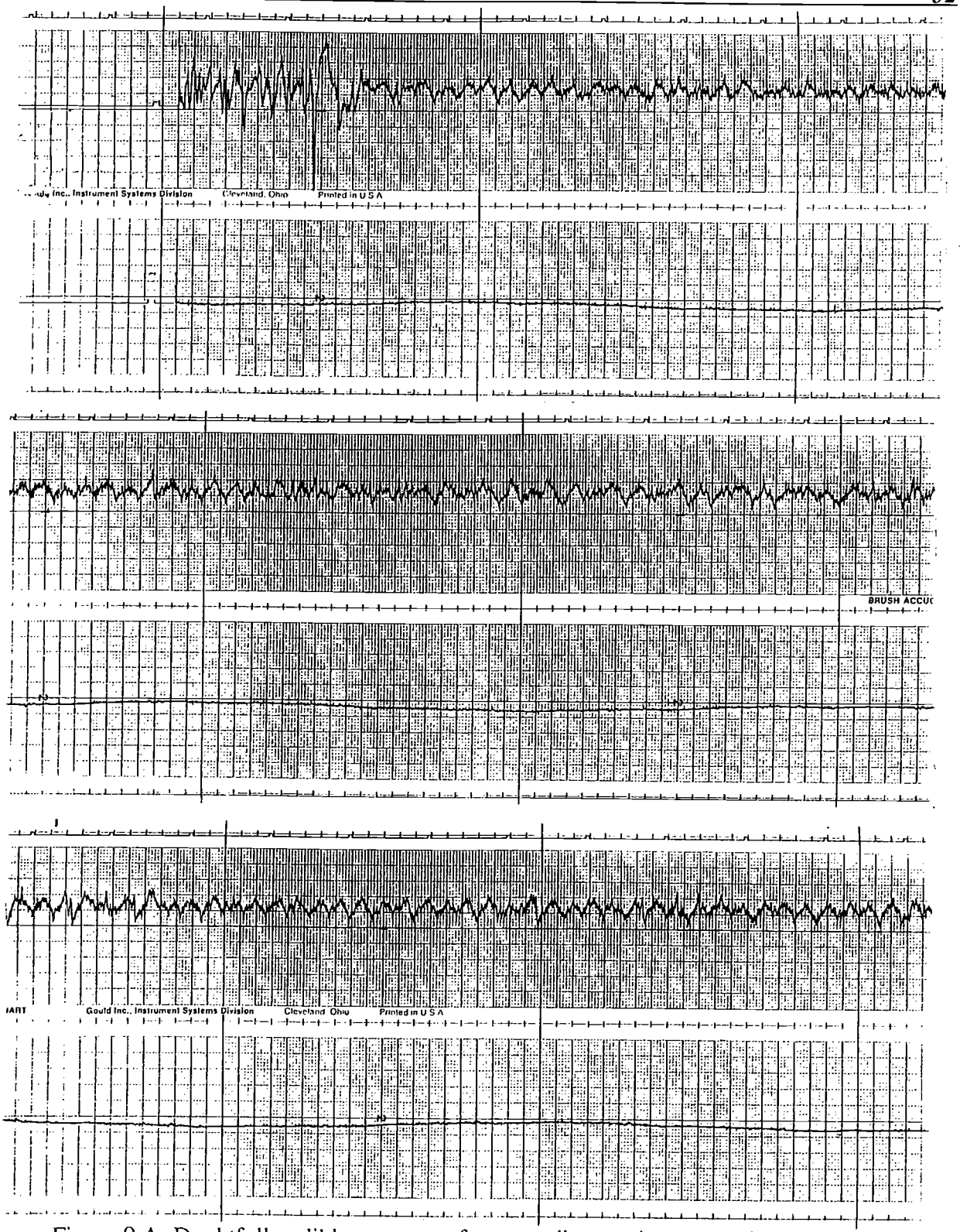

Figure 9 A: Doubtfull audible response of an ampulla to a sine wave of 2Hz.

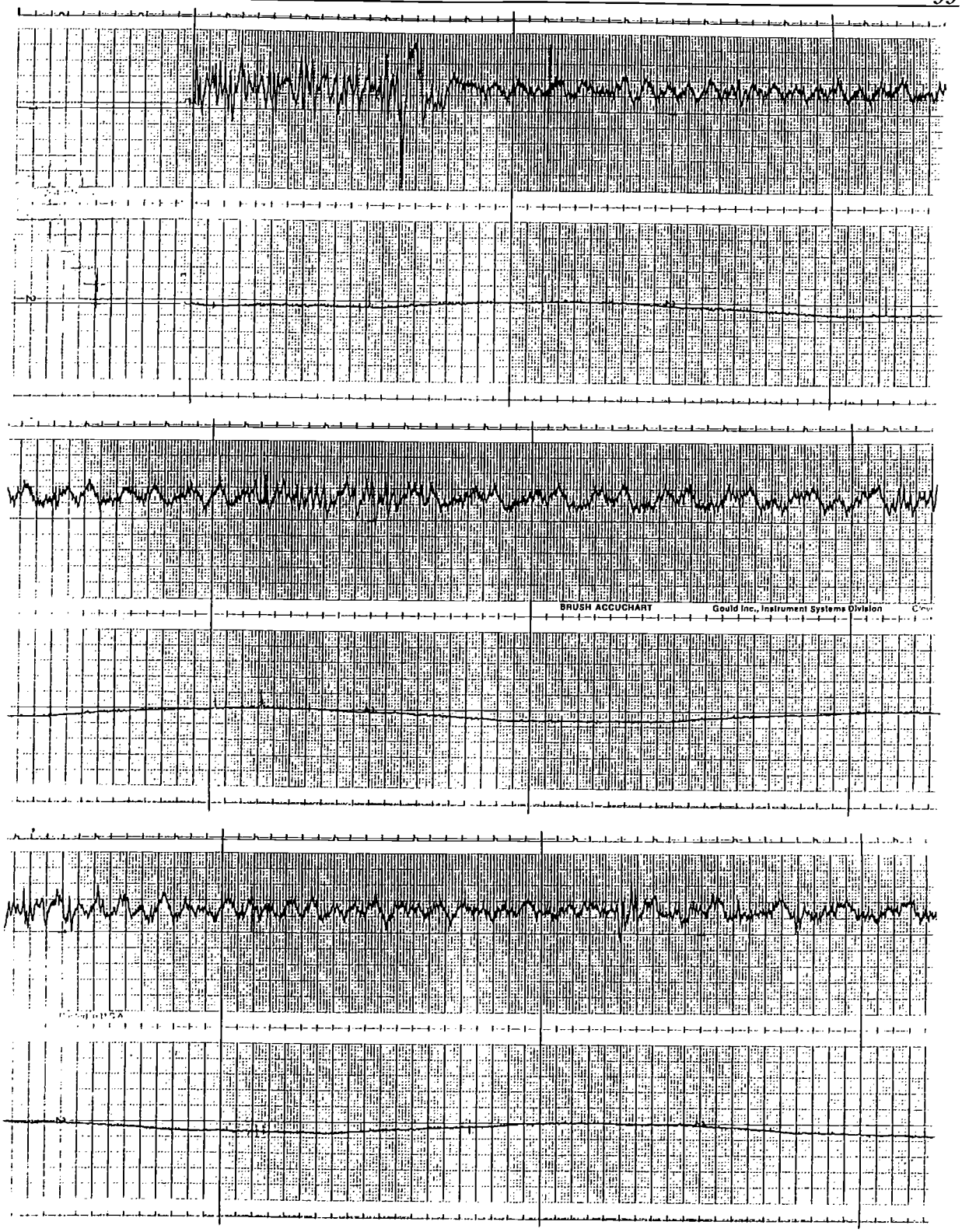

Figure 9 B: Just audible reponse of an ampulla to a sinewave of 2Hz.

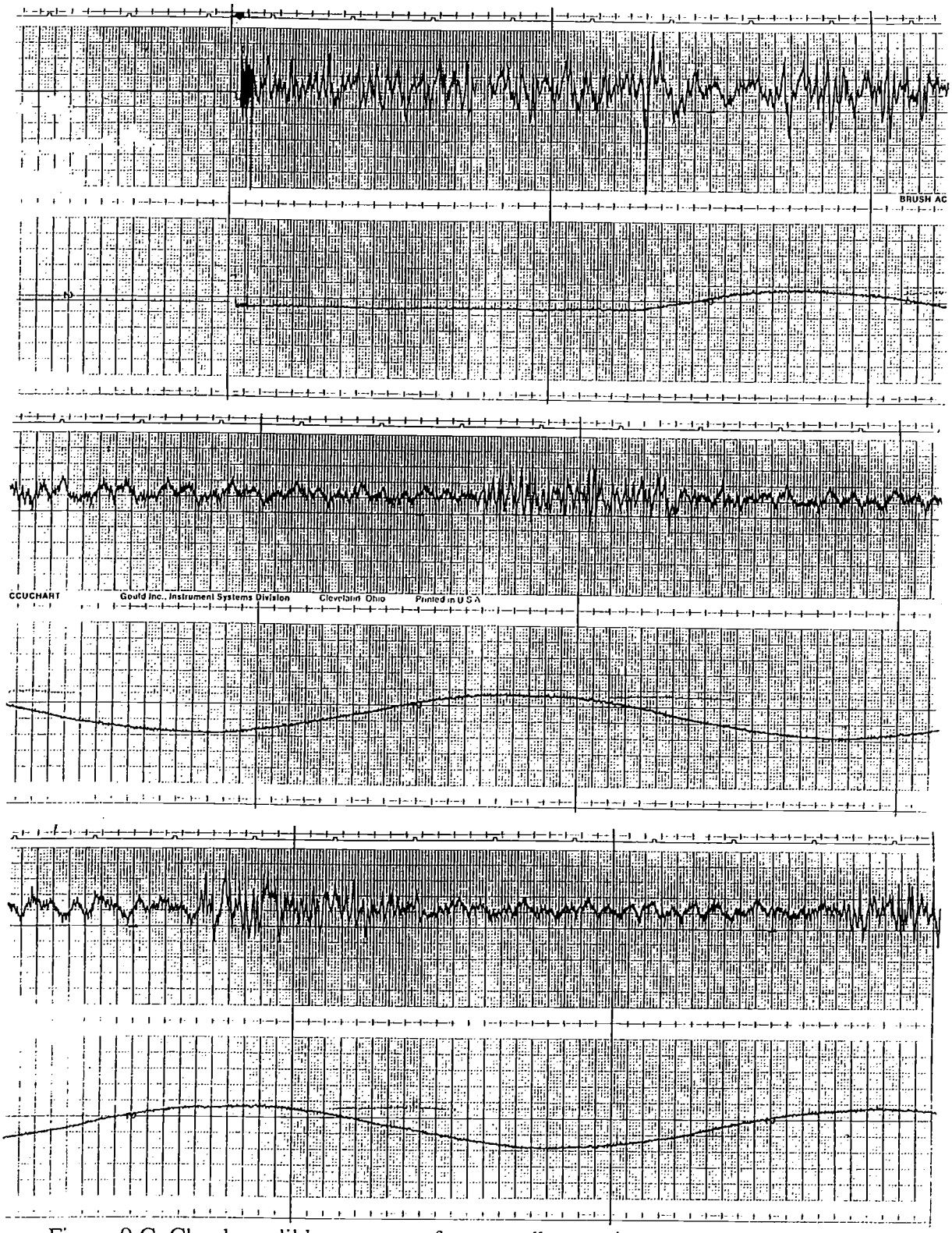

Figure 9 C: Clearly audible response of an ampulla to a sine wave of 2 Hz.

With regard to the frequency response there are some interesting things to be noted. The best responses came in the frequency range from 2-8 Hz (Figures 9 and 10). At higher frequencies ranging from 12-16 Hz (figure 11) the response did not follow the stimulus directly, but a small delay in the reaction is visible. At the highest frequencies (Figure 12) the reaction was not as clear any more, the delays were even larger and the reaction sometimes skipped a sine. Another thing that was noticeable, was that for higher frequencies (12-32 Hz) the amplitude had to be turned up by a factor two or more, to get the same audible responses (i.e., clearly, just and doubtful audible responses).
Oscillations were again visible but did not seem to react to the test stimuli.
The bottom trace no longer shows the epithelial potentials but now shows directly the stimulus signal generated in the function generator.

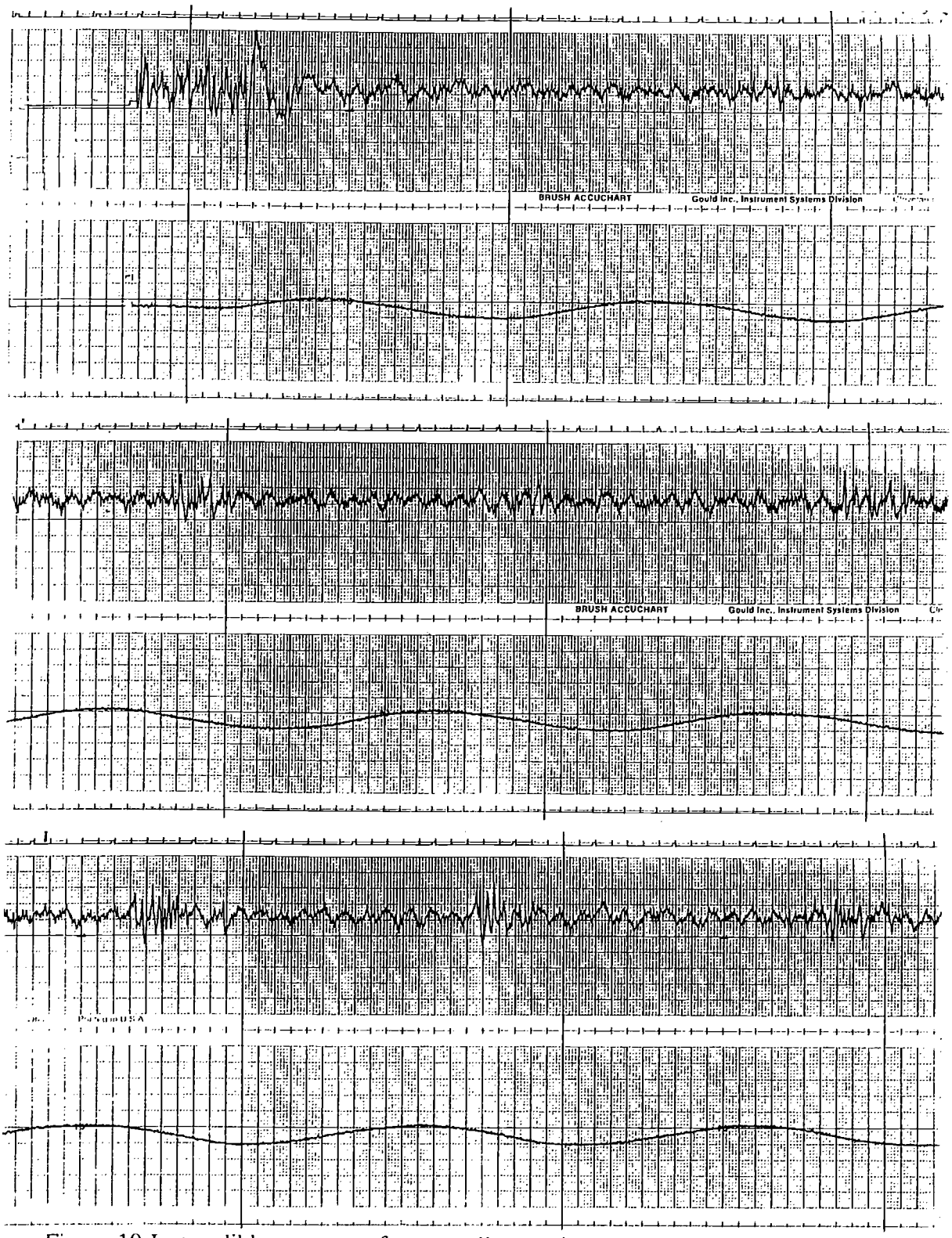

Figure 10 Just audible response of an ampulla to a sine wave of 4 Hz

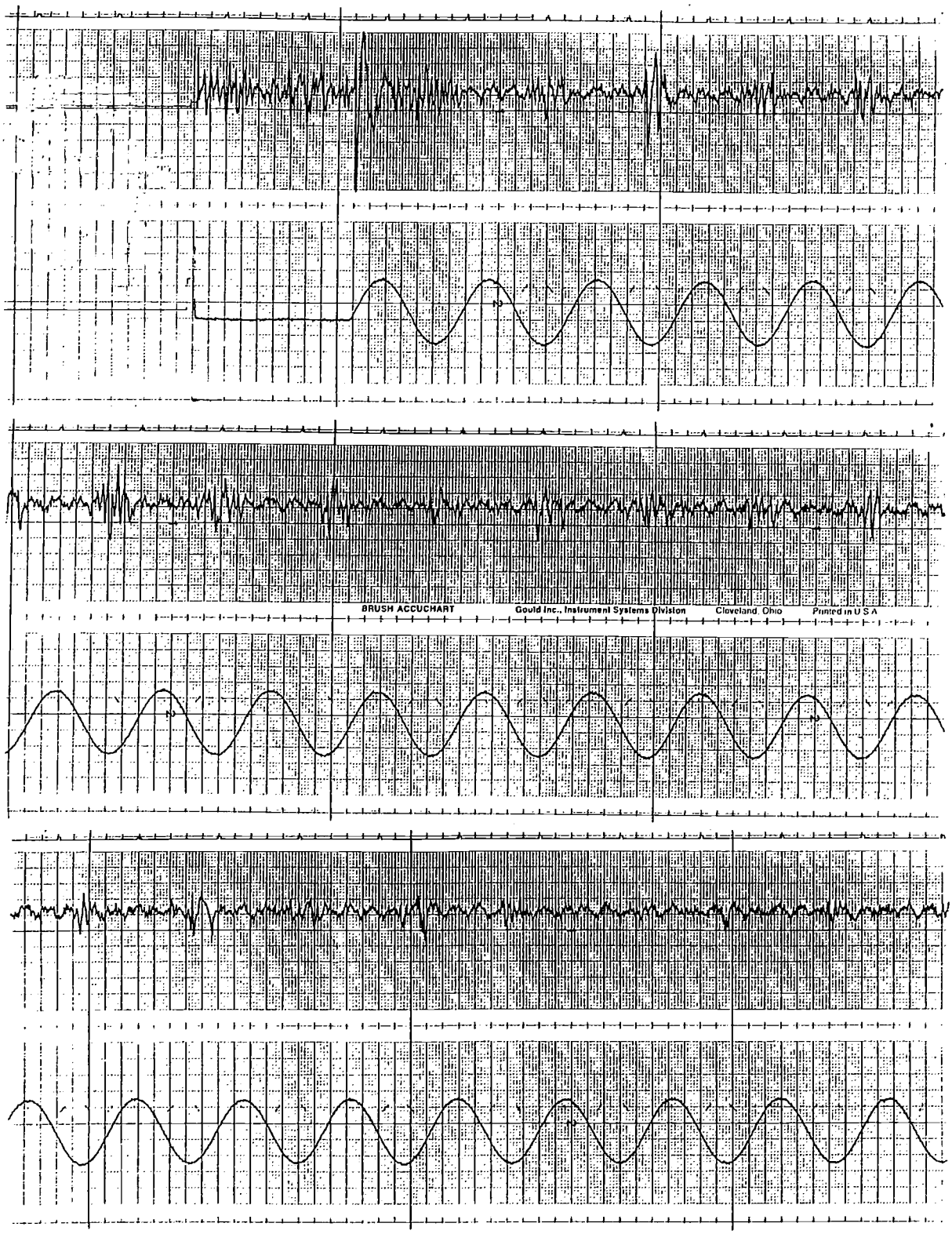

Figure 11 Just audible response of an ampulla to a sine wave of 12 Hz.

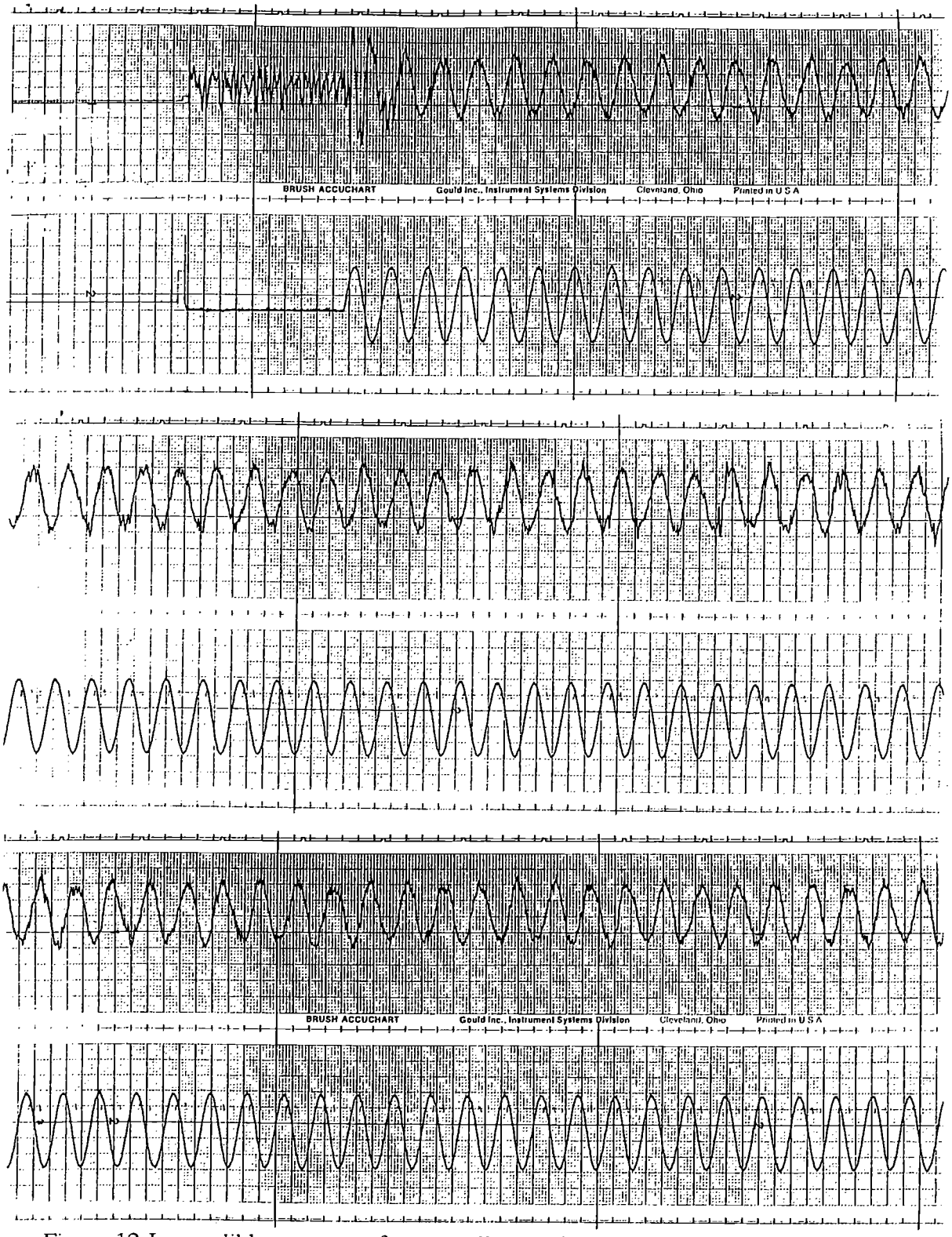

Figure 12 Just audible response of an ampulla to a sine wave of 32 Hz.

3.5 The Ampullary Epithelial Potentials:

The epithelial potentials were measured simultaneously with the action potential recordings, but on a specific DC channel (the spikes could also be measured on an AC channel).

When the spontaneous activity was recorded, the response of the epithelium was usually very flat with some small high frequency oscillations resulting from large spike potentials (see also figure 6). Sometimes however , oscillations of about 20 Hz could be seen, they would come and after a short period of time disappear again.

When a square wave was applied, the ampulla gave a definite active response. When the voltage step was given, there was a short transient (lasting only about 1/16 of a second) and the DC signal would recuperate and become either flat or sometimes, if insulated enough, even become reversed (overcompensation). On the inhibited part (the pore positive part) the high frequency oscillations would disappear, since there was no longer any major spike activity. The 20 Hz oscillations would still come and go, but there was no regularity to be seen in their appearing and disappearing cycle, nor was there any change in regularity from the stimulated part (figure 13).

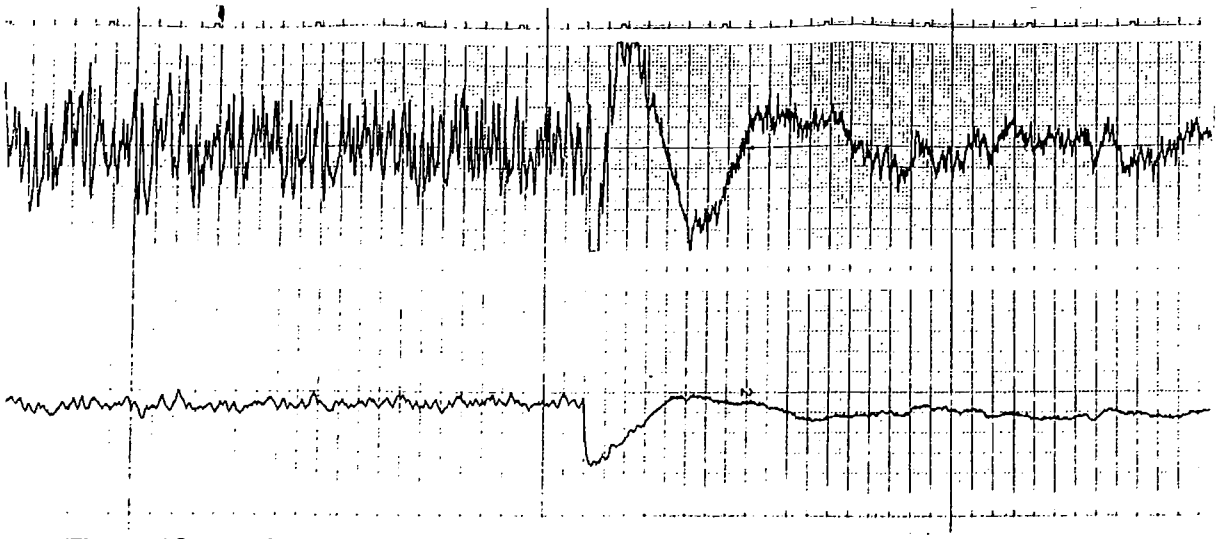

Figure 13. Detail of transient and the active response of the ampullary epithelium.

The application of a step-ramp signal did not show such an active response. The DC epithelial signal just seemed to follow the stimulus signal (see also figure 8). The high frequency oscillations were not visible on the ramp part of the signal, since most of the spike activity was suppressed. The 20 Hz oscillations would come and go just as they did before, they did not respond to the ramp signal whatsoever.

With the application of the superimposed test stimuli on the ramp, there was again an active response of the ampullary epithelium. When a sine wave was applied the DC signal would also show a sine wave, there was however one difference: at higher frequencies (>12 Hz) the response of the ampulla would have a slight delay, a small phase shift would occur. Oscillations were again visible with no apparent reaction to the test stimulus.

3.6 Oscillations:

As previously mentioned we found several types of oscillations on both the spike channel and the DC channel. The most conspicious type had a frequency of about 20 Hz (Figure 14). It did not seem to respond to any kind of stimulus and was sometimes very obviously present and sometimes hardly noticeable. It would come and go, but without any regularity. The spike activity was also completely independent from the oscillations. They could either be on the rising part of an oscillation, the descending part, at its minimum or at its maximum. The amplitude and frequency of the spikes remained uninfluenced by the oscillations.

Another type of oscillations were also sometimes present. They were found mainly on the spike channel and had a frequency of about 60 Hz. This 60 Hz oscillation was also independent of any kind of stimulus, but the amplitudes were usually so small they would disappear in the noise or in the spikes. They dissappeared after switching off a water pump.

The final type of oscillations were found only on the depolarized periods. It consisted of a mixture of high frequency oscillations and was only present when there was much spike activity (see also figure 13).

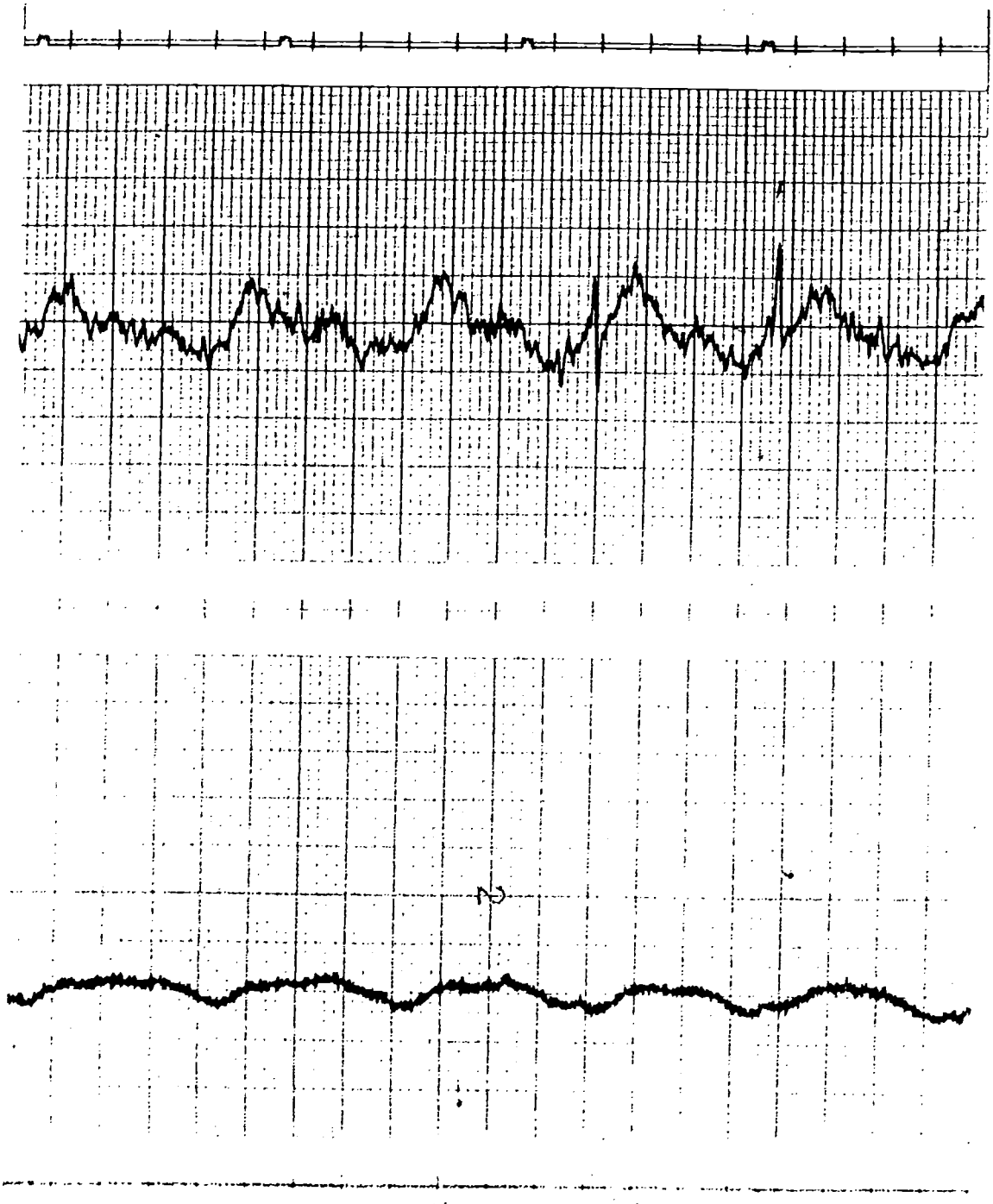

Figure 14. Detail of 20 Hz oscillations on a ramp signal.

4 Discussion:

The greatest improvement we made in our experimental set-up when compared to other workers (Clusin and Bennett, Waltman, Obara and Bennett, Broun) was the fact that we conducted the experiments in a nearly '*in vivo* ' situation. We were able to carry out experiments for up to seven days in the live animal, whereas others only used the excised ampulla or worked on dead animals, which of course changes the responses dramatically. The only drawback we faced was that we were unable to mimic the biological circumstances completely for we could not yet succeed to get any recordings of the nerve signals when the animal was fully submerged.
Another thing we took care of was that the stimuli did not exceed beyond the dynamic range of electroreception (Bennett and co-workers). By giving stimuli outside the dynamic range of the receptors, an unnatural situation was created which gave rise to biologically irrelevant artefacts and did not reflect the true process of electroreception.

This project should be viewed as preliminary research and is just a small step towards the complete understanding of the process of electroreception.
Initially an attempt was made to make the nerve recordings from fully submerged animals. First the nerve signals had to be raised above the noise level. We succeeded in doing this by increasing the capacitive reactance of the canal wall by slipping it into a plastic tube. After rinsing with sucrose (later urea), the insulation of the canal was large enough that the spikes were measurable and the DC-response of the ampullary receptors was flat (no amplification or attenuation of the stimulus signal). As soon as the water level was raised however, the insulation was lost and both signals (DC and spikes) were no longer visible. Attempts to 'waterproof' this construction by covering the tube (inside and out) with silicone-grease, failed.
The decision was then made to temporarily conduct the experiments with the canal lifted out of the water, but with the pore remaining in the water. By placing the canal in a slit made in a silicone-rubber block, the capacitive reactance was increased. Urea was from now on used for the isolating rinsing, because sucrose seemed to be responsible for a bacterial growth in the water system.

This bacterial growth influenced the condition of the animal severely (the animal would already die after three days).
The first thing we did was check if the prepared canal was damaged in any way during the preparation and if any nerve signals could be recorded. This was done by the application of a square-wave stimulus. If the canal was damaged, the nerve spikes were hardly audible and were almost completely lost in the noise. The leakage problem would also show at the DC-signals. If the canal was damaged we would be unable to get the DC-signal flat by insulating the canal more with urea. This meant there was a significant loss of the signal due to leakage. Usually the preparation caused no problems and we were almost always able to get spikes. The DC-signal we would get flat most of the time and sometimes even reversed. This reversal of the signal means that the ampulla actively amplifies the signal it receives. Broun and Gorvardovskii found an amplification of a factor 2. We however, never found an amplification larger than a factor of 1.2. In the opinion of the Russians a factor two of active amplification by the ampulla did not explain the extraordinary sensitivity of the ampullae of Lorenzini. Their hypothesis was therefore that the amplification would largely be at the synapse. We think however that, although we did not get an amplification larger than 1.2, it does not mean the receptorcells do not actively amplify the signals they receive. The best spike recordings we would get when the DC-signal was flat. Even though there was a lack of amplification, the receptor response would be as high or higher then compared to when there was an amplification. Kalmijn suggests that there is an amplification by positive feedback at the basal membrane which has as a result that there is an infinite input impedance. The hypothesis is that besides the amplification between the pore and the ampulla lumen (of a factor two, Broun and Gorvardovskii; a factor 1.2, Kalmijn) there is a much larger intracellular amplification of the signal across the sensory epithelium, resulting in a large signal across the basal membranes of the receptor cells giving rise to synaptic transmission. In an electric model this is already being tested and so far it has proven stable and correct. To prove this hypothesis in the live animal however, intracellular recordings will be necessary.

Already with the first recordings of the spontaneous activity of the nervefibers it became clear that the signals were far too complex for sorting out the action potentials of individual units. To silence all but one or a few of them a pore-positive hyperpolarizing voltage step was applied.
Directly after the transient we could see that all the fibers were completely silenced. After less then one second one or a few fibers started to respond again. Several seconds later almost all the fibers had accommodated completely to the imposed voltage step.
As we wanted to see the reaction of a single unit to a stimulus we had to cancel the accommodation of all the fibers but one. We thought this could be done by giving a pore-positive voltage step followed by a ramp signal. This ramp signal would then go against the accommodation of the fibers. This was however harder then it at first seemed. The first problem was to set the voltage step just right, it should leave one fiber active and suppress the others. Since we could not see the detailed result of the imposed voltage step directly on the oscilloscope but first had to print it on paper it took a lot of effort to get that right. The next problem we faced was to get the ramp that followed the step just right so it would offset the accommodation of the other fibers. The same technical problem of not being able to see the detailed results of the stimuli directly on the oscilloscope occurred. As can be seen in the results we partially succeeded in getting the step plus the ramp right. The prints show that the ramp successfully counteracts the accommodation of the fibers. Only a few fibers remain active and the ramp makes sure the total amount of activity remains approximately the same.
After setting the step plus ramp, partially on audial, partially on visual judgement we now could give the test stimuli to see the response of a single unit to different amplitudes and frequencies. The test stimuli we gave consisted at first of rectangular pulses of various frequencies and amplitudes. Since in the natural surrounding of the animal it would be more likely that it would receive sine waves (for example due to swaying of the head when the animal is swimming) we later switched to giving short trains of sine waves of various frequencies and amplitudes. The frequencies we applied ranged from 2 Hz to 32 Hz (see also Materials and Methods).

The amplitudes also ranged in height, but it was not possible to set the amplitudes at the same position for each frequency (the amplitude knob on the function generator we used for this purpose, did not have set calibrated positions and attempts to make them failed due to shifting.). We therefore decided to set the amplitudes for each frequency separately.

For each frequency three amplitude settings were decided upon. The first setting would be with a clearly audible response of the ampulla. The second setting would give a just audible response and the third setting would give a barely audible response. This is of course a very subjective way of setting the amplitudes, but in future experiments this problem will be solved, because the amplitudes and frequencies will be set by a computer. In this experiment we therefore concentrated more on the effect of different frequencies on the response then on the effect of different amplitudes. The amplitudes will be looked at in a more general way.

We started out with a frequency of 2 Hz. The ampulla follows the test stimulus with a short delay. The problems arose when we looked at the amplitude. Already when the amplitude was just audible, it seemed that more than one fiber was active. When we subsequently lowered the amplitude and the response of the ampulla was only barely audible, it looked like only one fiber was active. However it would no longer follow the test stimulus as precise as before and would sometimes skip a sine, the stimulus we gave was probably just too weak.

Up to a frequency of 8 Hz the results were approximately the same. At higher frequencies (12-32 Hz) the delay between stimulus and response became of a bigger importance. It was also noticeable that if we wanted to get a just audible response in this frequency range, the amplitude of the test stimulus we had to apply would have to be significantly higher as when compared to the amplitude needed to get a just audible response at lower frequencies. The length of the bursts is at higher frequencies also shorter as at lower ones. They do not get shorter however then seconds.

The fact that the bursts of spikes become shorter at higher frequencies, indicates that the ampulla tries to follow the stimulus with its response. The delay of the burst and the skipping of sines at higher frequencies however, shows it is no longer able to keep up with the speed of stimulus changes.

It is possible that it takes some time for the ampulla to respond to the new situation (i.e. sine). At higher frequencies the time it takes for the ampulla to respond is longer than the situation (i.e. sine) lasts. By the time the ampulla starts to fire, the stimulus has already reached another phase.
The amplitude needed for a response in the lower frequency range lies in the order of 0.02 μA, whereas at higher frequencies that may rise up to 10 times as high.

From all this we can conclude that the Ampullae of Lorenzini are better adapted to stimuli with frequencies up to 8 Hz. This is in agreement with the literature which states that the most important bio-electric fields are DC and low frequency AC-fields.

For further and complete understanding as to how the individual nerve fibers respond to electric fields with various frequencies and amplitudes, further experiments are required. It is of great importance to give more controlled stimuli and to visualize the effects of an applied stimulus directly. This can be accomplished by the use of computer controlled stimuli. A computer program for this purpose has recently been completed.
For the understanding as to how the amplification of the signals takes place, single cell recordings are necessary. So far this has proven to be very difficult, although recent experiments have shown to be very hopefull.

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