THE PERIPHERAL AUDITORY SYSTEM OF THE HARP SEAL, *PAGOPHILUS GROENLANDICUS*, (ERXLEBEN, 1777)

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INTRODUCTION

In terrestrial mammals the anatomy of the hearing organ is quite uniform, with homologous structures serving homologous and well understood functions. The hearing organs in aquatic mammals are aberrant, difficult to identify functionally and consequently subject to a wide range of interpretation (de Haan, 1957; Fraser and Purves, 1954; van Heel, 1962; Purves and van Utrecht, 1963; Bullock et al., 1968; Mc Cormick et al., 1970). The main reason for these difficulties is that the auditory impedances of water and of the auditory anatomical structures are similar.

Investigations on humans underwater have shown that they suffer a loss of sensitivity up to 30 dB compared to in-air hearing (threshold levels expressed in watts/cm²), and there is little, if any, sound direction discrimination (Hamilton, 1957; Wainwright, 1958; Montague and Strickland, 1961; Feinstein, 1966; Smith, 1969; Andersen, 1969). This decrease in sensitivity is assumed to be caused by the inefficiency of the air-adapted middle ear apparatus to conduct sound from water to the inner ear. Sound is presumed to be perceived by massive bone conduction, the entire skull being exposed to the sound field as the surrounding tissues are considered sound transparent (de Haan, 1957).

Behavioural experiments in phocid seals have shown that these mammals possess hearing well adapted for underwater use, as evidenced by good sensitivity and directional discrimination (Mohl, 1964; 1968 a; Terhune and Ronald, 1971, 1972). However, nothing is known of the pathway of sound in either air or water from the medium to the cochlea of the seal. As the seal’s in-air hearing is relatively more sensitive than that of the human’s underwater, it has been proposed that seals may have separate inputs for hearing in the two media, or alternatively there may be an impedance switching mechanism (Mohl, 1968 b).

The experiments reported here were designed to provide evidence regarding the nature of the sound conducting system in the seal ear by (1) measuring relative sensitivity of the seal ear in the two media (2) establishing the presence or absence of middle ear reflexes (3) determining the locus of sound entrance of the auditory system (4) examining the theory of a sound opaque versus transparent head by measuring the effective interaural distance. The technique used was that of recording cochlear microphonic potentials (CM). CM are AC potentials generated by the hair cells on the basilar membrane in response to sound stimulation. The CM thus portray the sound stimulus at the very end of its travel from the source to the hair cells; consequently, any changes in the sound path will appear in the CM response. This technique has proved extremely useful in the investigations on the middle ear mechanics in terrestrial mammals (Weyer and Lawrence, 1954), and has recently been successfully applied to anaesthetized, submerged cetaceans (Mc Cormick et al., 1970).

MATERIALS AND METHODS

Electrode implantation was attempted in 5 animals but two did not survive the operation. Of the remaining 3, a successful implantation was made on a 10-month old male, but the electrode failed after two days. A 2-month old female developed a severe infection as a consequence of the surgery. It did, however, produce useful cochlear microphonics for a period of 12 days. Finally, a 3-month old male pup was successfully implanted; cochlear microphonics were recorded from this animal over a period of 2 months, after
which the electrode failed. The animal is still alive three years after implantation.

The pup's head, which yielded data on interaural time differences, bore the following dimensions: circumference of head at level of meatal orifices 44 cm; distance between orifices over the vertex 14 cm; distance between orifices in a direct line 11.5 cm; length of cartilaginous meatus, post mortem, 7 cm; ascending part of meatus 4.2 cm; the skin and blubber covering meatus 1.0 cm; distance between left and right bony cochlear capsule at level of round window 7.5 cm; distance separating drum membranes 8.5 cm.

Surgery was performed under sterile conditions, using halothane for anaesthesia (McDonnell, 1971).

The animals were respired artificially through a tracheal tube. The incision was made on the lateral side of the head, from about 5 cm behind the meatus and down past the bulla, which was approached ventro-laterally by splitting the m. biventer.

When the bulla was reached, a small hole was drilled through the bony wall and a slender probe-electrode was used to explore the roof of the tympanic cavity. The probe was connected to an oscilloscope via a differential pre-amplifier. When an area with high CM activity in response to sound stimulation was found, the probe was withdrawn, the hole enlarged and the self threading electrode was screwed into the hole in the direction of the active area until firm contact between the tip of the electrode and the cochlear capsule was established. A small stainless steel screw was screwed into the bulla, serving as an indifferent electrode. The leads were brought to the surface and terminated with water proofed plugs; subsequently the incision was sutured. The electrode positions were identified by X-ray on the anaesthetized animal (Fig. 349).

The electrodes were made from 12 strands of 38 gauge stainless steel suture wire, terminated with a 12 mm piece of number 22 hypodermic needle and isolated with shrinkable teflon tubing. A 3 × 10 mm stainless steel (medical grade) screw was drilled to fit tightly over the isolated needle so that 4 to 8 mm of the needle was free. This design allowed the electrodes to be autoclaved. The electrodes' firm contact with the cochlear capsule meant that it was not necessary to rely on tissue formation for stable response.

Healing was a slow process, probably because of a 1–2 cm thick layer of subcutaneous blubber. Antibiotics were administered at the incision, as an ointment, as well as with the food. The animals were kept out of water for 24 hours after surgery.
The seals were restrained during testing by aluminium hoops attached to a board (Fig. 350). The end of the board was hinged to the inside of a $3 \times 5$ by $1.7$ m polyester coated plywood tank; during testing the water level was regulated to approximately 0.9 m. The hinged board was convenient in getting the seal out of the water.

The transducers used for projection of the sounds were “University” MM 2 PPS underwater dynamic speakers, calibrated against an “Atlantic Research” LC 32 hydrophone. In air sound was produced by a “University” MLC dynamic loudspeaker and an “Armaco” CT-3 Tweeter, calibrated against a “Bruel & Kjaer” (type 4133) condenser microphone. The audio-frequency source was a “Wavetek” 112 function generator, operated as a toneburst generator from an external gate control. The voltage controlled frequency facility of the generator was used to sweep trains of pulses through the audio frequency range. Power amplification was by a Marshland 200 stereo amplifier.

Input to the transducers was measured by a “Bruel & Kjaer” 2409 voltmeter; this, with the factory calibrated hydrophones and condenser microphone, defined the overall accuracy of the system.

The CM response was amplified by “Grass” P 15 differential amplifiers and, when needed, filtered by a “Dawe” 1417 filter before being displayed on a “Tektronix” 422 oscilloscope.

In most of the experiments the CM response as well as the input to the transducer was recorded on a “Uher” 4400 taperecorder or a “Pemco” model 110 instrumentation recorder. Subsequent analyses were on a “Hewlett-Packard” 1201 A storage oscilloscope, a “Buel & Kjaer” 2010 heterodyne analyzer, 5559 gate, 2425 electronic volt-meter with peak hold function, 2305 level recorder, and a “Krohn-Hite” 3550 filter.

In some of the tests 2-5 msec sound pulses were produced with a toy cricket with an attached piezo-electric device for supplying trigger pulses (Fig. 351). The peak pressures were found to be 34 dB and 72 dB relative to 1 $\mu$ bar at 1 m in air and water respectively. This source has the advantage of not being driven by an electrical signal, which can be radiated to the animal and interfere with the CM response.

All other sound stimulations were pulses of 2 to 5 msec duration. This was in order to minimize the effects of the finite limits of the medium set by the walls of the tank and the surface of the water. By keeping a low ratio of time with stimulation to time without stimulation (low duty cycle) the pulse technique had the additional advantage of allowing stimulation with high peak pressures without causing fatigue or middle ear reflexes. Finally, pulse stimulation provides a simple means of detecting interference from the driving voltage of the transducer with the CM response.

The latter is delayed by an amount corresponding to the distance between the source and the ear. With continuous wave stimulation such interference may be a problem (Vernon et al., 1971). However, what is gained by operating in the time domain, is lost in the frequency domain, as the short pulses used have broad spectra with the principal peak being only about 12 to 15 dB above neighbouring peaks (Fig. 352). With non-linearities in the frequency response of the transducers and the preparation, the response to such pulses can be difficult to interpret. Furthermore, short duration pulses call for relatively wide-band recording with a corresponding high noise level and a limited dynamic range. Finally, pulse stimulation makes analyses by meters or graphic recorders rather complicated. Details of the non-standard ways of analysing the data are given in the results and the description of the figures.

RESULTS

The sensitivity function

CM responses, as a function of stimulating sound pressure, were obtained in air at 1, 1.4, 2, 2.8, 4, 5.6, 8 and 11 kHz and in water at 1.2, 2.8 and 5 kHz. Due to the low sensitivity inherent in pulse stimulating techniques, only the upper part of the sensitivity function was obtainable, the dynamic range of the response averaging 25 dB. Usually the response was reproducible.
within 10 dB, but measurements obtained in air occasionally exceeded this limit (see Fig. 353 in which all 4 kHz data, collected over a period of 6 days in both media, are displayed). The displacement to the left of the points obtained in water (indicating higher sensitivity) was found at all frequencies tested. The average displacement was 20 dB, but due to the variability in response, this figure is considered only as an indicator. No change of sensitivity with time was found except in the infected animal.

The level of the maximum obtainable CM amplitude for this electrode was ~75 dB rel. 1 V, with a range from ~69 to ~82 dB in the range 1 to 8 kHz. Stable responses underwater were obtained only when the seal was at least 30 cm below the surface. It was repeatedly found that, when a seal was slowly raised to the surface, the CM response to pulse trains would suddenly decrease when the animal's nose reached the surface, or in some cases slightly before. There is no direct evidence to show that this was not caused by a decrease in sound pressure level due to the upsetting of free field condition by the surface, but the change occurred in an abrupt and somewhat erratic manner, suggesting control by the animal.

Middle ear reflex [MER]

The correct stimulus to elicit the MER is a sound of high power. The effect of the MER is a reduction in sensitivity due to change in the transmission properties of the middle ear, caused by contraction of the m. tensor tympani and m. stapedius.

In Fig. 354 are shown the CM responses to series of test pulses in which a continuous, high power note was suddenly switched on. The graph was prepared by recording on separate tracks of an instrumentation recorder, the voltage to the pulse transmitting transducer, the CM response after suitable amplification and filtration, and the high power tone. After playback at reduced speed the pulses were filtered, rectified and stored in a peak-hold circuit before being displayed on a level recorder. The peak-hold circuit was reset before each pulse. The graph should therefore be considered as a series of samples of the CM sensitivity, rather than as a continuous record. Sensitivity reductions of about 10 dB could be obtained. Maximum reduction level

Figure 353. CM output as a function of sound level at 4 kHz in a 3 month old male harp seal in air (open circles) and water (filled circles). The points connected with lines were obtained in one session; all other points have been measured individually over a period of 6 days.
Figure 354. Upper trace: CM response to a series of pulses in a 3 month old harp seal in water, (frequency: 2-7 kHz, duration: 3 msec, repetition rate: 15 pps) during stimulation concentrated at the contralateral ear with a 300 Hz tone. Lower trace: timing of the 300 Hz tone.

Figure 355. Upper trace: CM response in a 2 month old female harp seal in air to a series of pulses (frequency: 4 kHz, duration: 2 msec, repetition rate: 8 pps) during stimulation of the contralateral ear with a 3 kHz tone. Lower trace: timing of the 3 kHz tone.

was reached in less than 100 msec after turning on the reflex eliciting tone. Recovery lasted several 100 msec. Similar results were obtained in air (Fig. 355).

Entrance of sound

Experiments performed to localize the entrance of the auditory system consisted in (1) using various methods to block the access of sound to the seal's head and (2) moving a point source around the head and finding the position which caused the highest CM response.

In air, closing of the meatus with a finger usually reduced the cochlear potentials; the range extended from 0 to 16 dB. Frequencies around 5 kHz were most affected. A hand pressed against the side of the head in the region of the orifice, especially ventral, usually reduced the CM response by 20 dB or more, and usually below the limits imposed by noise.

Fig. 356 shows the effect of closing the orifice with a finger. The graph was produced by stimulating the seal in air with test pulses of 6-5 msec duration at a repetition rate of 22 pps and linearly raising the frequency. The recorded CM response was played back at reduced speed. The leading and trailing edges of the individual pulses were suppressed by a gating circuit and the remaining part rectified and stored in a peak-hold circuit and subsequently recorded on a level recorder. The peak-hold circuit was reset before each pulse by the gate. All 4 curves were obtained within 1 minute, hence the variability depicted was of a dynamic nature, in contrast to changes in electrode efficiency, etc. The difference in sensitivity was small at low and high frequencies. The former could be partly explained by operating close to the point of maximum CM amplitude (Fig. 353) and partly by the low efficiency of this kind of blocking at frequencies below 1 or 2 kHz. The latter is a trivial consequence of a low signal to noise ratio in the CM output. In the intermediate range, however, it is evident that the seal gains up to 10 dB from the meatal route for aerial sounds.

Under water, the initial exploration was performed using the toy cricket in a styrofoam beaker with a hole of 10 mm diameter, producing a peak pressure of 60 dB rel. 1 μ bar at the hole. The source was held gently against the skin and the CM potentials
were used to find the area of maximum sensitivity. This approach was not very sensitive as basically it depends on square law attenuation, which, combined with a slope of less than 1 on the input/output function of the CM, reduced the useful dynamic range to about 12 dB. However, the method consistently showed a maximum sensitivity for sounds being produced in a narrow area below the meatal orifice (Fig. 357), with lesser sensitivity at the lower jaw and the top of the skull. No response could be detected for stimulation at the tip of the nose, nor from the opposite side of the head.

The second approach, using sound opaque material to shade specified areas from stimulation, confirmed the previous observation and in addition showed the limits of the receptive area to be rather restricted in the anterior-posterior direction. A 15 by 30 cm piece of plastic packing sheet material with enclosed air bubbles was slid forward from behind the skull so that the side of the head from the ventral to the dorsal midline was covered. When the seal was stimulated repetitively with pulses, the CM response would suddenly decrease and disappear. This occurred when the edge of the plastic had passed the meatal orifice. When the plastic was slid from the vertex of the skull in a ventral direction, reduction in CM response occurred just below the orifice. This reduction continued to increase over the next 5 to 6 cm, after which the response was below noise level. Other tests, using a small (5 by 6 cm) piece of plastic containing air bubbles showed that to be effective it had to be placed between the orifice and a position 7 cm ventrally from the orifice. Covering only the orifice usually but not invariably, resulted in a response reduced by a few dB. Underwater there was no effect if the orifice was blocked by a finger.

**Effective inter aural distance**

The effective distance between the ears (i.e. the distance which determines the inter aural time difference) has often been discussed in relation to mammalian underwater hearing (de Haan, 1957; Fraser and Purves, 1954; van Heel, 1962; Mohl, 1964), because it provides a means of testing the hypothesis of a sound-transparent versus a sound-opaque head.

The CM technique lends itself to the direct measurement of this distance, provided bilaterally implanted electrodes are available. In the present investigation, since we did not succeed in establishing this condition, an indirect method was used. As the animal is bilaterally symmetrical, stimuli received from positions equidistant from each orifice and on a line through these two points will measure the effective inter aural time. This will be measured as the difference in arrival time for the sound from the two source positions. The sound source was the toy cricket with an attached piezoelectric transducer. The latter established a point of reference at the time of stimulus onset. The data were recorded on a Uher 4400 stereorecorder and played back at 1/8th of original speed into a Revox stereorecorder with write-before-read facility. The latter recorder served as delay, enabling the entire front of the trigger pulse to be displayed on the storage oscilloscope, which displayed the output of the Revox recorder but was triggered by the input. The time-base error of the oscilloscope was measured to be +1%/s, while the error in the speed reduction was -0.8%/s, as determined by means of a crystal controlled frequency meter (Brueel and Kjaer, 2010) and Lissajous figures.

The results, averaged over 18 pulses from one side and 12 from the other, show time differences from the reference point on the trigger pulse to the first peak of the CM response of 0.47 ms (S.E.: 0.04) at the close side and 0.58 ms (S.E.: 0.02) at the far side. In water, this corresponds to a difference in sound path of 15 cm.

The relatively high standard error indicates that the method under our conditions suffers from lack of accuracy, mainly because of difficulties in establishing a reliable point of reference on the CM potential, which was band-pass filtered between 3 and 10 kHz. As the response had a fairly low signal to noise ratio, the shape of the peaks underwent some "distortion" by the addition of noise to the signal. However, because this mechanism basically conforms to a Gaussian distribution, the statistical approach used here seems justified.
DISCUSSION

The maximum amplitude of the CM of about −75 dB rel. 1 V is in the order of 40 dB below the value obtained at the standard CM recording locus at the round window and is considered a consequence of the placement of the electrode on the surface of the bony capsule of the cochlea. The heavily vascularised lining of the walls of the middle ear cavity (Tandler, 1899; Ramprashad et al., 1971) may contribute to the low efficiency of the electrode by acting as a shorting resistance for the potentials.

Operating near the maximum amplitude of the input/output function complicates the interpretation of the various sensitivity dependent functions, such as middle ear reflexes and occlusion effects. Such functions refer to equivalent changes in sound level. Without correction for the deviation of the 1 to 1 relationship between input and output, changes in sensitivity will be underestimated.

The variability in the response is associated with several sources, of which distortions of the sound field probably are the most important. The recordings took place either slightly (0.5 m) above or below the water surface, and only a few metres away from the tank walls. The use of pulse technique reduced the effects of reflections, but the effects of the water surface, the bottom of the tank and the restraining board could not be excluded. As the sound field was calibrated without the seal, those changes measured in the presence of the seal were considered as part of the properties of its auditory system. This is in accordance with the concept of the minimum audible field. This procedure is quite sensitive to deviations from the free field situation.

Another source of variation to be considered is electrode efficiency. This type of variation can be distinguished from sound field and conduction variations by changes in the absolute value of the maximum amplitude and was found to be relatively small, possibly due to the firm mechanical contact between the electrode and the bone.

The measurements of absolute CM sensitivity in the two media do not allow new conclusions to be drawn because of the variability, but they are largely consistent with behavioural findings (Terhune and Ronald, 1971; 1972) in the harp seal, in which hearing is better underwater than in air. The same relationship was found in the harbour seal (Møhl, 1968a). While in behavioral experiments, seals might be expected to be motivated to concentrate on their listening task and maximise their sensitivity (which minimises variability), no such cooperation could be expected from the restrained, forced-dived seals in the present study. It is known from studies on the cat that the CM sensitivity is greatly influenced by the activity of the middle ear muscles even at moderate sound levels (Simmons, 1959). Stable and high sensitivity responses were obtained only with anaesthetized or demuscled preparations. We have no reason to believe the seal should behave differently. However, an additional factor controlling sensitivity may be the closing of the meatal lumen, both at the level of the tragal cartilage and at the membranous part of the meatus. Muscles performing these actions have been described by Rosenthal (1825) and Ramprashad et al. (1971). The finding that blocking the meatal orifice with a finger occasionally had no effect can be explained by assuming that it was already closed by the seal itself. This internal closure cannot be determined by visual inspection.

The expected difference in sensitivity between an ear adapted to only one medium when exposed to air and water is 30 dB. This value has been approached in several experiments on human underwater hearing for the frequency range over which the human ear has its best adaption to aerial sound (Smith, 1969).

Comparison between the behaviourally determined underwater threshold for seals with that of humans in air has led to the conclusion that the seal ear is fully water-adapted (Møhl, 1964; 1968; Terhune and Ronald, 1971; 1972). The better than expected hearing of the seal in air has led to speculations on solutions of impedance switching or two input terminals, in response to the amphibious way of life (Møhl, 1968b).

The present findings show that the meatal orifice is of importance for hearing in air. Using this route the gain at medium frequencies is sufficient to explain the better than expected hearing in air (Fig. 356). When the orifice is blocked, aerial hearing, like underwater hearing, is via the superficial tissues ventral to the orifice. This was demonstrated by the great reduction in CM sensitivity when this area was covered with a hand.

In contrast, underwater sound was not received particularly well through the meatal orifice, but rather through the area ventral to the orifice (Fig. 357). When the orifice was blocked, this area was also found to be sensitive to aerial sound. The data are thus consistent with the model requiring two parallel inputs. The underwater input being best adapted. Where and how the two inputs are summed is a subject of speculation, as nothing is known of the fate of the sound from the moment of entry until the point where the middle ear reflex starts to operate. The present work, however, indicates that inputs merge in front of the middle ear complex.

According to the sound-transparent model of underwater hearing first proposed for cetaceans (de Haan, 1957), the distance determining the binaural time differences for sound sources displaced from the medial plane is that between the two petro-tympanic bones. If this model is applied to seals, the inter aural distance should be that between the medial walls of the bullae.
(3 to 4 cm) or that between the two tympanic membranes (8-5 cm). The finding of 15 cm as the maximum interaural distance in the present experiment can hardly be reconciled with the hypothesis of a sound-transparent head. The sensitivity measurements for a source at different places on the head also did not support the model. However, for the most distal parts of the sound path, the shading experiments with the air-bubble sheets indicate tissue transparency.

Therefore, a model of the seals auditory system, to conform to our results should employ both sound-transparent and sound-opaque tissues.

The finding that the CM sensitivity to short pulses was reduced in the presence of a powerful tone is, as in terrestrial mammals, interpreted as evidence for middle ear reflexes. Moller (1962) reported changes in input impedance of the human ear in response to sound stimulation, which showed the same properties on onset and relaxation of the MER as seen for the submerged seal (Fig. 354). The important implication of the demonstration of the MER in the diving seal is that the middle ear system must be part of the sound route. This evidence is not sufficient in itself to rule out the possibility of stimulation by some kind of bone conduction. In one of the modes proposed for bone conduction (the translatory or inertial mode, Weaver and Lawrence, 1954), the differential motion between the ossicles and the cochlear capsule drives the cochlear fluid. This is the kind of stimulation proposed for dolphins (McCormick, Weaver and Palin, 1970). Changes in stiffness of the ossicular system, such as that effected by the MER, conceivably could change the sensitivity of this type of stimulation. A search for published evidence related to this question has been unsuccessful. It has been found however that, in humans exposed to underwater sound, activity of the middle ear muscles had no effect on sensitivity of hearing (Mohl, 1964). Accordingly, the most simple model to account for the evidence available appears to include the standard mammalian ossicular route of sound in the diving seal.

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