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Direct Testing of the Biasing Effect of Manipulations of Endolymphatic Pressure on Cochlear Mechanical Function

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Abstract. The history of cochlear mechanical investigations has been carried out in two largely separate sets of endeavours; those interested in auditory processing in animal models and those interested in the origin of adverse vestibular symptoms in humans. In respect of the first, mechanical vibratory data is considered pathological and not representative of pristine behaviour if it departs from the reigning model of sharp tuning and high hearing sensitivity. Conversely, when the description of the pathological behaviour is the focus, fluid movements responsible for hearing loss and vestibular symptoms dominate. Yet both extensive sets of data possess a common factor now being reconsidered for its potential to shed light on the mechanisms in general. The common factor is a mechanical bias — the departure of cochlear epithelial membranes from their usual resting position. In both cases the bias modulates hearing sensitivity and distorts tuning characteristics. Indeed several early sets of guinea pig mechanical data were dismissed as "pathological" when in hindsight, the primary effect influencing the data was not loss of outer hair cell function per se, but a mechanical bias unknowingly introduced in process of making the measurement. Such biases in the displacement of the basilar membrane from its position are common, and may be caused by low-frequency sounds (topically including infrasound) or by variations in fluid volume in the chambers particularly applying the case of endolymphatic hydrops. Most biases are quantified in terms of visualisation of fluid volume change, electric potential changes and otoacoustic emissions. Notably many previous studies have also searched for raised pressures with negative results. Yet these repeated findings are contrary to the widespread notion that, at least when homeostasis is lost, it is a rise in endolymphatic pressure which is responsible for membrane rupture and Meniere's attacks. This current investigation in Mongolian gerbils is aimed at quantifying hydrostatic pressures in cochlear chambers by direct measurement using a null-flow micropipette pressure measurement system, while simultaneously quantifying electric potentials and distortion products to provide indirect measures of displacement bias and hair cell integrity. We now suspect that during any experiment obtaining of good pressure seals is critical. Secondary penetrations, such as occur in neural recordings, are contra-indicated. When we address the issue of seals we see raised pressures in response to manipulations known to disturb homeostasis, viz. diuretics and hypoxia.

INTRODUCTION

The field of cochlear mechanics, particularly in respect of signal processing, has largely developed in the absence of experimental data concerning any systematic hydrostatic pressure variation in the three scalae of the mammalian cochlea or any of its evolutionary predecessors. The theory underlying the notion of the cochlear amplifier obtained its substantial foundations [5] a quarter-century before the technology was refined to make such pressure measurements without unduly interfering with the process being measured. Until then the attempt to measure pressures in tiny cells and vessels as small as capillaries would unavoidably necessitate an exchange of fluid which could substantially alter the dimensions of the vessel and by implication the resulting pressure, introducing intolerable uncertainty in the measurement. The situation improved when Wiederhielm (see [4]), capitalised on the change in micropipette electrical resistance with tip pressure to develop the zero-exchange or "servo-null" method. The micropipette can be inserted and sealed into the membrane of plant or animal vessels not much larger than the tip of the micropipette. If intracellular fluid is forced into the tip of the pipette (or the reverse), the system responds by applying an equal and opposite back pressure to the micropipette so as to keep the impedance constant. The value of the tracking pressure constitutes the desired readout which is recorded.

This measurement approach has been applied in over 40 studies on the cochlea, e.g., [2, 11, 12], primarily trying to understand the origin of endolymphatic hydrops, the condition in which scala media may swell to many times its normal volume (ca 5μl), testing the expectation that such swelling is likely associated with a rise in endolymphatic pressure. The consensus of all these studies is that the pressure differential between the outer and middle chambers

is virtually zero – evidently pressures do not appear arise in scala media to account for ruptures of Reissner's membrane characteristic of post-mortem morphology in Meniere's patients [11]. Indeed, as observed by Flock [3] very high pressures may not be essential since regions of weakness in the membrane may exist in patients with this condition. Nonetheless prominent symptoms of the condition are a "feeling of fullness", a fluctuating hearing loss and a low-frequency tinnitus. They may have many origins, yet are consistent with the existence of a mechanical bias acting on the basilar membrane [7] thereby producing asymmetric distortion of the cochlear microphonic plus concomitant changes in distortion products measured in the ear canal [1].

Recently it was shown that the walls of scala media possesses two new anatomical features, viz. tight junctions and aquaporins suggesting that the chamber may be specialised for control of pressure via water transport [6]. This idea supports the hypothesis that the mammalian cochlea also possesses the ability to regulate the mean position of the basilar membrane and thus the operating points of the hair cells. The idea of gain control by bias adjustment is not new. It arose from direct measurements of basilar membrane displacement by two different techniques [9, 10]. The possibility that sound might trigger a pressure change was explored in a mathematical model last meeting [8]. This investigation is aimed at understanding homeostatic regulation using known ways of disturbing homeostasis.

APPARATUS AND METHODS

We report preliminary pressure measurement data from 40 Mongolian gerbils of preferred weight of 70g. The animals are anaesthetised with Ketamine chlorohydrate (Virbac 65mg/kg) and mounted in a stereotaxic head-holder in which the right ear bar contained a Etymotic ER-10B system for measuring distortion products. Ac potentials are also recorded with a silver electrode adjacent to the round window using a Grass amplifier. Both these signals are recorded using a Cambridge Electronic Design (CED) 1401 4-channel sampling system at 50kHz sample rate. Glass micropipettes were pulled to a diameter of 2-5μm and filled with 2M KCl. The tip of the micropipette is inserted first through the right ear round window, to record simultaneously pressures and dc potential on the other two channels at 1kHz sample rate. When reference potential is established and nulled, the micropipette is advanced through the basilar membrane until the endocochlear potential is recorded, signifying that the tip is in scala media. Spike2 software is used to display the data as a chart record, while MATLAB is used to analyse cochlear microphonics, summating potential and distortion products.

The pressure and endocochlear potential (EP) data are obtained using a World Precision Instruments (WPI-900A) "micropressure measurement system" based upon the Wiederhielm methodology. The calibration of micropipettes to obtain these data has been the subject of extensive investigation using both water and electronic manometers. It is relatively easy to calibrate pressures over the range of (-55, +120) mmHg in a special calibration chamber. However, *in vivo* measurements have revealed numerous subtleties in the use of this equipment. For this report two methodological challenges stand out which may be relevant to the failure of early reports to show significant pressures. The first is related to the fact that the device is supplied with no operational characteristics. The user's job dealing with significant subtleties is largely heuristic, e.g., it is possible for the user to believe that *in vivo* one is actually measuring pressure as for the calibration. The device uses phase-lock as part of the feedback loop for tracking pressure, but there is no panel indicator that it is locked and tracking. Without any control on that status, the pressure record may not deviate much from zero. Our *ad hoc* solution is to create a local transient in atmospheric air pressure with a syringe. If a transient appears on the chart record we have some confidence we are registering real pressure effects. If not, we must finesse the controls until we see response to atmospheric change. Until that is found the device can fail to register or *underestimate* even high pressures delivered for calibration. Conversely, and importantly, if correctly adjusted, the device will not seriously *overestimate* any static pressure due to the factory calibration of the internal pressure transducer. The second methodological issue may subtly be tied to the long-established expectation that scala pressures are low. The use of identical micropipettes is standard for recording single neurons – where multiple penetrations increase one's chances of obtaining good data. It is certainly possible to penetrate scala media several times in different places and still find the endocochlear potential. However, it does not follow that, if scala media normally functions as a pressure vessel, its integrity would be retained, particularly if the pressures were high, e.g. intraocular pressure in the case of glaucoma (>21mmHg).

RESULTS

We find all pressure seals are important – the seal of the tip through cochlear membranes, the seals on all pressure tubing for the device and particularly the rubber seals at the rear end of the micropipettes. There was a significant increase in the occurrence of registering pressures greater than 60mmHg after we decided to renew the micropipette holder seal for each experiment.

Figure 1 shows the pressure calibration for the apparatus, but not shown is also the calibration of the dc potential simultaneously obtained from the tip of the micropipette. A significant constraint of the method is the existence of pressure limits (rails) defining the linear range of the device. When correctly configured according to manufacturer specification this range is considerably in excess of the range of arterial pressures. Perilymph pressures are available through penetration of the round window membrane, and these can be manipulated by artificially raising CSF pressure through a cranial canula so that pressures are transmitted via the cochlear aqueduct.

The micropipette may then be advanced through the basilar membrane. At the base this is physically tough and the penetration is liable to break the tip, generally ending the experiment. Typically, during this advancement the device is taken out of feedback mode because the force which the basilar membrane applies to the tip changes its electrical resistance and the device may respond by injecting 2M KCl into the preparation. Adding methylene blue dye to the electrolyte can assist discern if this happens. When successfully traversed we see a rapid rise to the endocochlear potential (EP) which in gerbils may be +90mV or higher, indicating that the tip is in scala media. At this point the device is manually switched back to servo pressure-tracking mode. Figures 2 and 3 show preliminary attempts to measure hydrostatic pressures in scala media. The experimental protocol adopted for these recordings is rendered more meaningful by perturbing the system in ways well established to strongly influence homeostatic behaviour viz, through injection of a diuretic (Fig. 2) and anaesthetic (Fig. 3).
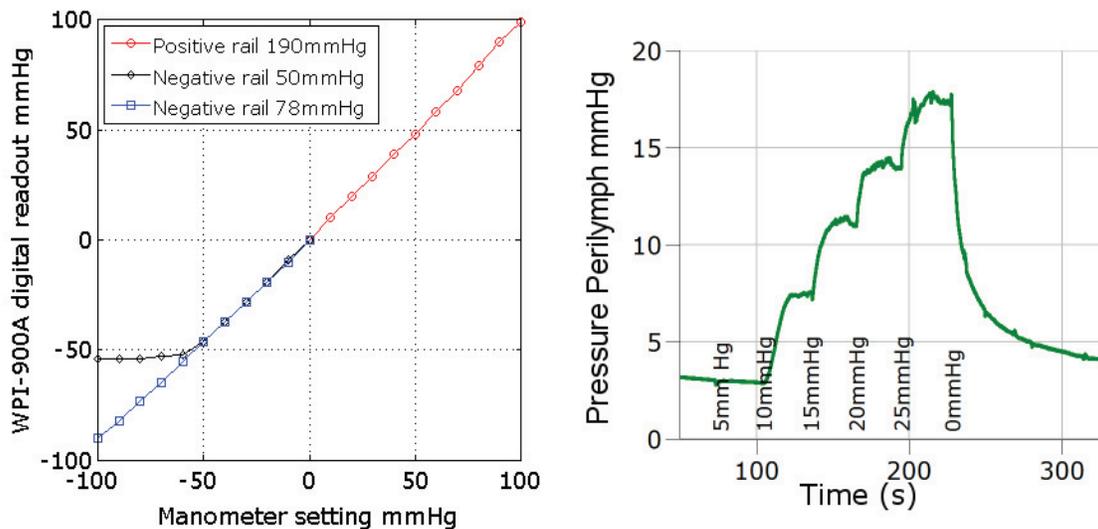

FIGURE 1. The left panel shows calibration of the pressure measurement system against a water manometer (marked as for mmHg) monitoring pressures delivered from a 60 cc syringe. The system has linear range of pressures beyond which the signal saturates. These limits are defined by pressure and vacuum sources needed to supply the positive or negative backpressures to the micropipette. So, for example here, if the negative limit is set to -55mmHg the signal will saturate for lower pressures in the test vessel. For historic reasons initially set by expectation of not seeing high pressures these limits were set to -50 and +80 mmHg (see Fig. 2 and 3). The right panel shows pressures measured in perilymph in response to steady CSF pressures delivered from a water column raised to the appropriate heights, calibrated in mmHg. Not all of the pressure delivered to the cranial catheter is seen in the perilymph presumably due to variable compliance of the cochlear duct and cochlear membranes.
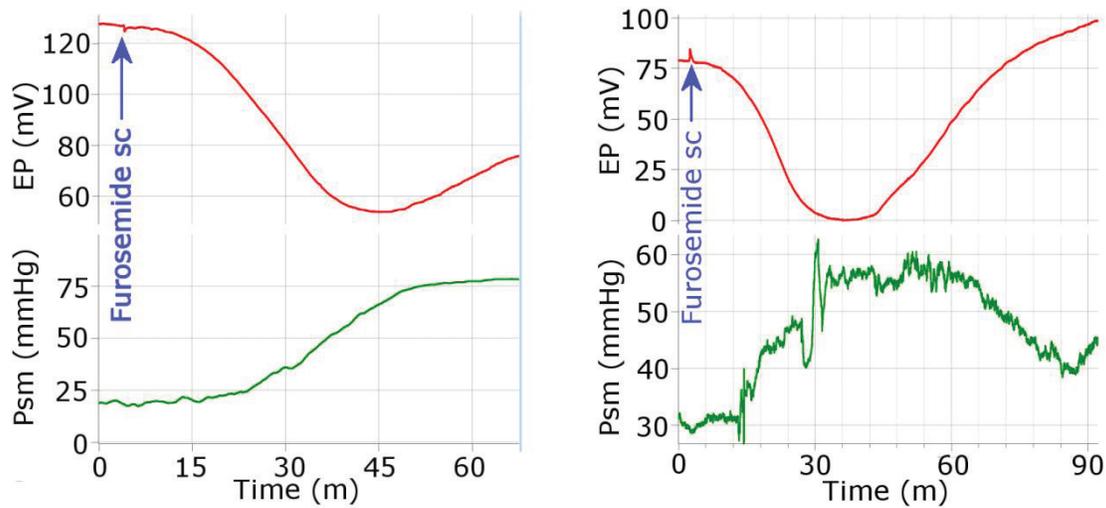
FIGURE 2. Manipulation of cochlear homeostasis using the diuretic furosemide 0.5cc (10 mg/ml) which is delivered subcutaneously to the hind leg. In this supra-clinical dose this typically produces a drop in the endocochlear potential (EP) beginning after a delay of 15 minutes and the effect washes out after about 45 minutes, resulting in a not-always-complete restoration of the EP. In the left panel (GE84R2) is seen a marked drop from of an initially high EP with a coincident rise in scala media pressure in excess of 60mmHg change. The saturation at 80mmHg (ca. diastolic blood pressure) shows the pressure is being underestimated after 50 minutes. The right panel (GE85R2) shows a rather noisier change in pressure of some 25mmHg showing better recovery in both the EP and the scala media pressure.
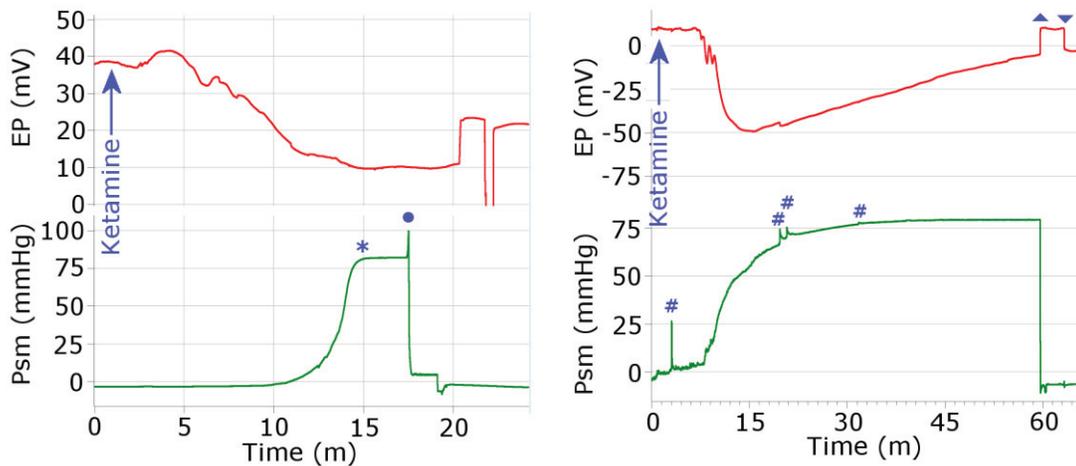

FIGURE 3. Here the manipulation is 0.1cc Ketamine (IP) to produce hypoxia and to euthanise the preparation. These examples show several features mentioned in the text. In these 40 gerbils, loss of homeostasis appears to be associated with considerable rise in endolymphatic pressure exceeding 80mmHg. In both cases the full rise is not seen because at the time the linear range had been underestimated in the setting of the pump supplying pressure and vacuum. In the left panel (GE89R4) the asterisk (*) indicates the time at which hard saturation occurred, but the positive rail was then increased (filled circle) allowing the pressure estimate to rise momentarily to 100mmHg. In the right panel (GE90R3) the hash (#) marks indicate when atmospheric pressure transients were introduced to cross-check that the 900A system was indeed tracking the pressures. It is consistent that these pressure transients were having less effect as the pressure in scala media rose. At the up arrow, the micropipette was withdrawn some 200μm, evidently resulting in a loss of pressure, while at the down arrow the pipette was reinserted 200μm whereupon the previous EP was re-established, but not the high pressure value.

CONCLUSIONS

These are preliminary results, thus far minus the sound-response data collected, from our first series of experiments. They were designed to test the notion that static pressure in scala media may be important to understand the origin of endolymphatic hydrops and further elucidate mechanical processes responsible for homeostatic regulation in mammalian cochlear mechanics. Hence, we have aimed to obtain normative pressure data and monitor the effects of deliberate disturbance of aerobic mechanisms upon which homeostasis depends.

As cochlear mechanical investigations go, this has been one of the more difficult endeavors, because of uncertainties with the technique. If progress has been made, it is as a result of the determination that when the apparatus is correctly configured and stable in performance, the pressures measured repeatedly are not being overestimated. Indeed, they are not inconsistent with high pressures seen using the method in other tiny vessels [4].

The history of cochlear mechanical investigations has largely focused upon the notion that the basilar membrane remains at some mean working position and that the cellular processes are required to enhance the vibrations. In normal individuals and quiet conditions hair cell operating points somehow remain located stably at their maximum sensitivity despite changes in posture, heavy exercise or atmospheric pressure. Hi-pass filtering by the helicotrema has traditionally been used to explain this. However, this is not true of Meniere's patients [1]. Our object is therefore to discover not just what causes failure of homeostasis, but what is responsible for it in the first place. It is well known that a variety of bias effects, particularly due to low frequency sounds, modulate cochlear sensitivity [7], but the tradeoff between the size of the bias effect and compensation for the loss of sensitivity is also unknown.

It is of considerable surprise value to discover that the initial protocol appears to have greatly underestimated scala media pressures which at up to 100mmHg can readily suggest how rupture of Reissner's membrane can occur in susceptible individuals [11]. Previous studies have shown significant dc-shifts of the basilar membrane [9,10] now recognised as consistent with such pressure changes, e.g. the cochlear partition in the basal turn has a stiffness of around 1Nt/m. A pressure rise of just 5mmHg would displace the basilar membrane 1.7μm towards scala tympani.

ACKNOWLEDGMENTS

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