The Mechanics of Cochlear Homeostasis:

Re-evaluation of the "Servo-Null" Animal Model Technique for Direct Measurement of Cochlear Chamber Pressures

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Available from: www.mechanicsofcochlearhomeostasis.wordpress.com
Form the strong impression that Reissner’s membrane is quite compliant relative to the compliance of the BM

Alternate hypothesis: **Static pressure** in endolymph may become high relative to perilymph under circumstances of lost homeostasis
PubMed searches for suggestions of static pressure head within the cochlea.

![Graph showing the number of PubMed articles for various topics related to otology and fluid pressures. The topics include Perilymph fistula, Hearing & Shunt surg, HL & LumbarPuncture, Tumarkin or Lermoyez, Stapedect & PerilyLoss, Fullness, CSF Press & orthos_tinnitus, SC Dehiscence & Tullio, Hydrops & SoundExposure, CSF Pressure & Hearing Ios, CSF Press & Meniere's, and Gusher. The x-axis represents the number of PubMed articles, ranging from 0 to 500. The y-axis lists the various topics. Perilymph fistula has the highest number of articles, followed by Hearing & Shunt surg and HL & LumbarPuncture.]
OSMOTIC PRESSURES IN COCHLEA?

- The *mechanics of cochlear homeostasis* is the study of how endolymph volume is regulated and influences hearing sensitivity, vestibular stability.

- This work of The Tübingen group opens a new door because it suggests that the *cochlea may generate pressures spontaneously by virtue of the osmotic gradient driving water into scala media*; regulatory pressures which can be controlled via the *stria vascularis plus aquaporin gating of water flows*.

The highest pressure responses ever measured have been $<< 5$ mmHg

- The data may explain ELH, but seem unlikely to explain ruptures

**Problem with applying servo technique to the cochlea?**
These studies have shown *questionable* behaviours to associate with other data.

The pressure difference between endolymph and perilymph is accepted as close to zero but could mean failure of tracking.

The decline in pressure is not very convincing; it does not time with the other responses, it could be drift.
Yet other data suggest either blockage of cochlear aqueduct or incorrect servo setup.

Marchbanks & Reid 1990

Applied intracranial pressure 50cm water

Thalen Wit et al 2002

Measured intracochlear pressure 2cm water
Principle of the Servo-null
Glass micropipette is filled with \([\text{KCl}] \gg \text{test medium}\)

Changing pressure across the length of a glass pipette causes a rapid change in the electrical resistance of the tip. So in order to keep the electrical resistance constant the servo system matches the tip pressure at the rear of the pipette.
Principle of the Servo-null
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Changing pressure across the length of a glass pipette causes a rapid change in the electrical resistance of the tip. So in order to keep the electrical resistance constant the servo system matches the tip pressure at the rear of the pipette.
EXPERIMENTAL TRAPS
FUNCTION OF CONTROLS NOT INTUITIVE

- The pressure delivery to the micropipette is always active
- Only tracking in AUTO mode
- Electrode resistance is both a readout and a control!
- Sensitivity and damping controls not calibrated, very difficult to test their effects
- Set pressure allows sophisticated experiments

The user instruction manual is a 4 page set up recipe
ALL AIR BUBBLES MUST BE REMOVED

No bubbles are tolerable at any size, particularly not in the tip of the micropipette because, relative to water air is hugely compressible. Bubbles can be judiciously chased out of the tip with localised heat.
What these plots do not show is the regenerative nature of the transitions – they display a switching property and flip to the other state rapidly.
MICROPIPETTE CHARACTERISTICS CRITICAL
(Tip breaks going through the basilar membrane end the experiment)

Electrical ac resistance
2 to 4 \( \Omega \) for 2\( \mu \)m beveled tip

Electrical ac resistance
0.3 to 0.5 \( \Omega \) for 20\( \mu \)m tip

This pipette may still give a good EP >70mV, but pressure will be zero because of catastrophic damage to the BM.
STEREOTAXIC FRAME IS MANDATORY

Gerbil cranium immobilized in 3-D printed stereotaxic frame with cranial canular bar, micropipette attached to micromanipulator

The entry to the cochlear chambers is via the round window, then through BM to scala media
THERE IS NO EQUIPMENT READOUT CONFIRMING SERVO IS TRACKING

Therefore: We use atmospheric pressure pulses (from 60cc syringe) to check servo device is tracking correctly.
CANNOT DELIVER SOUND DURING PRESSURE MEASUREMENT - CM INTERFERES WITH SERVO

Glass micropipettes filled with 2M KCl in bath (0.9M NaCl) (grounded)

Electric DC resistance is strongly polarity dependent

Therefore the WPI-900A measures AC resistance (at 1kHz)

The cochlear response to servo- current injection at 1kHz strongly interferes with the resistance measurement and control of back pressure (readout)
ONLY ONE PENETRATION OF Scala Media IS POSSIBLE PER EXPERIMENT

- A good endocochlear potential +90 mV does not guarantee that the simultaneous pressure measurement is valid!
- Each penetration one may see a fair value of the EP
- However, after the first penetration the integrity of scala media as a pressure vessel appears to be destroyed
- The manufacturer recommends tip sizes for vascular and lung measurements. These are TOO LARGE for cochlear experiments, leading to catastrophic leaks of scala media.
- 2µm to 3µm, bevelled at 45 is near ideal tip size.
- 1 µm too vulnerable to tip breakages
- Leaks through membranes are consistent with the history of low pressures
PRELIMINARY RESULTS
Calibration vs Water Manometer
(calibrated mmHg)

Previous reports lack calibration curves
Delivery of a pressure head to CSF via a cranial canula sealed with glue
GOOD REGISTERING OF APPLIED CSF PRESSURES
HOMEOSTATIC MECHANISM APPEARS TO RESPOND TO INTRACRANIAL PRESSURE MANIPULATIONS (MEASURED IN ENDOLYMPH)
RESPONSE TO FUROSEMIDE SC
(20mg/2ml)
RESPONSE TO KETAMINE-INDUCED HYPOXIA
Summary

• The gerbil preparation seems to be viable/robust for this experiment

• The 900A device has been used for many applications, but its design did not envisage its use with the mammalian cochlea
  – Uncertainty due to the device interfering with cochlear operation
  – Uncertainty because cochlear operation may interfere with the estimation of tip electrical resistance
  – We have not, so far, been able to test whether LOUD SOUND itself causes the pressure to rise.

• Experimental poor yield due to the “single-penetration rule”
  – One of the membranes (the BM) is extremely tough on glass tips
  – Breakage means starting again after a 1hour+ setup time.
  – Yields have improved with attention to pressure seals

• We now routinely see higher pressures than seen in previous mammalian cochlear experiments using servo approach.

• There exists the potential to explain various types ruptures
SUPPLEMENTARY SLIDES
High sound exposure produces ELH displacing Reissner’s membrane

(Flock & Flock 2004)

Just 30 cm water (endolymph) pressure displaces Reissner’s membrane 150 µm
Loud sound >100dB SPL displaces RM by 30 to 100 µm
This suggests that 1) Reissner’s Membrane is rather compliant, and
2) The expansion of SM drives perilymph back to the CSF via the cochlear aqueduct.
Intense tone bursts >100dB produce lasting *displacement shifts* in the position of the basilar membrane in basal turn of guinea pig.

Not OHC motility – but homeostatic response affecting pressures?

LePage, HearRes 1989
Compare difference between applied and measured pressures

- Experiments which apply pressures, e.g. Changing the intra-cranial pressure or CSF pressure, the pressure originates from e.g. A column of saline, and the value of the pressure is unambiguous cm Water
- Experiments which employ a pressure transducer which is calibrated against such a column in a separate, perfectly sealed calibration chamber
- The main problem trying to measure cochlear pressures is the likelihood of interfering with the process under study
- Need a system which does not generate leaks, and thus interfere with volumes
We have two scenarios:

– **Reissner’s membrane is slack** and it only exists to separate perilymph from endolymph
  • It can be displaced by small pressures

– **Reissner’s membrane is normally quite robust/stiff** so when the morphology shows that the membrane has been stretched beyond its elastic limit this can be explained by higher pressures.
  • Indeed if it is much stiffer, then from a generalisation of Hooke’s law we would expect high pressures to be necessary to displace the membrane.
  • If we consider other systems in the body, or in plants small vessels are not necessarily associated with low pressures
    – How high? Suggestion: *Fractions of an atmosphere* (ca 200mmHg)
Literature rife with reports of raised pressures in the endolymphatic system

- Historically both Ter Kuile (1900) and Wittmaack (1931) have considered the possibility that hydrostatic pressure in cochlear chambers may play a functional role.


- There are extensive reports of pressure rise causing Perilymphatic fistula, EVA syndrome, SCC(Tullio) effects.

- We hypothesize that scala media really is a pressure vessel
Reissner’s membrane properties: stiffness
Rupture volume/pressure estimations

  - Estimation $35 \pm 12$ mmHg from 18 measurements in cow

  - normal volume of endolymph is estimated at 4.7 µl
  - “This ‘catastrophe’ occurred when 2.5 to 3.5 µl of artificial endolymph (five to seven injections) was injected.” Yet the pressures registered were inadequate to explain ruptures

  - Extent of the insight has to do with sound processing but suggest the spiral curvature of Reissner’s membrane conveys a form of robustness supportive of stiffness, “NOT SLACK”
LePage & Avan, AROMWM San Diego 2016  Servo-null technique for intracochlear pressure measurement revisited

Range of Static or Acoustic Pressures

- sea level, standard atmosphere
- lungs, extreme exhalation
- air pressure altitude 12,000 m
- blood pressure, systolic
- sustained pressure, eardrum ruptures
- bladder, voiding, maximum
- aircraft shock wave
- blood pressure, diastolic
- intra-ocular pressure, severe glaucoma
- corpus cavernosum, erect
- blowing your nose
- sustained press, eardrum senses pain
- intra-ocular pressure, glaucoma
- bladder, voiding, sustained
- blood pressure, capillary, arterial end
- "medium vacuum"
- bladder, micturition reflex
- intra-ocular pressure, normal
- gastrointestinal tract
- acoustic air pressure 160dB
- 200 daPa, standard tympanometry
- change cerebral pressure, TILT table
- cerebrospinal fluid (CSF)
- blood pressure, capillary, venous end
- blood pressure, venous
- interstitial fluid (osmotic pressure)
- altitude highest manned balloon 35km
- acoust press 120dB, eardrum pain
- "high vacuum"
- acoust press 94dB (1Pa)
- "very high vacuum"
- acoust press 0dB, threshold of hearing
- "ultra high vacuum"
Borosilicate glass tips 1-2µm
Bevelled 45° (preferred)
OTHER IDIOSYNCRASIES WITH SERVO-NUL EXPERIMENTS

• The earlier WPI-900 bellows version was replaced because of zero-crossing (i.e. At atmospheric) errors
• The use of the piezo stack valve demands understanding the difference between absolute pressures and pressure differences
• The manufacturer declined to provide details essential for the science, because of corporate intellectual property issues
• If the Wheatstone bridge zero adjustment for probe tip potential is out of range the probe behaves as if short-circuited
• It risks severe disturbance of the preparation to insert the micropipette through membranes (BM) while servo is active. The device is capable of injecting high pressures
• The inbuilt alarm sounds for two different reasons
Cannot measure 100mmHg if there are ANY leaks in the system; or preparation
Bevelling micropipette tips

Single pull micropipettes are backfilled with 2M KCl,
Ideal AC impedance via normal saline is 2-4MΩ
LePage & Avan, AROMWM San Diego 2016  Servo-null technique for intracoehlear pressure measurement revisted
Design of a new glass micropipette holder (3-D printed) allowing 1) servo pressure control,
2) electric probe connection for the EP and
3) axial illumination of the tip with green laser light.