

Application of force to the cochlear wall: effect on auditory thresholds, OHC transduction, and DPOAEs

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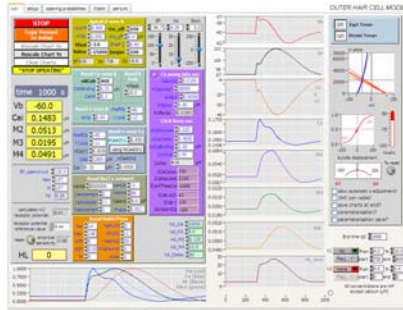


Good morning, my name is Greg O'Beirne. I'm currently at the Department of Communication Disorders at the University of Canterbury, New Zealand, but this morning I'm going to be talking about the work I have done at the University of Western Australia with Dr Robert Patuzzi, to **examine the application of force** to the guinea-pig cochlear wall (or the “push” as we call it), and the effects that this has on auditory thresholds, outer hair cell **mechanoelectrical transduction**, and distortion-product otoacoustic emissions.

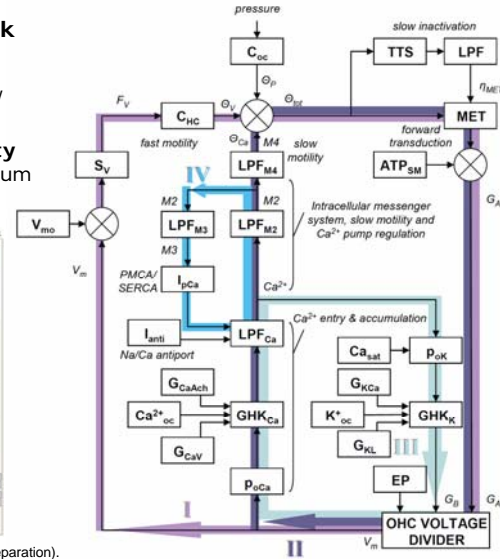
To provide some background, my PhD work has investigated cochlear regulation using a **combination** of mathematical modelling of the **ionic** and **mechanical** interactions likely to exist within OHCs, and electrophysiological **experiments** conducted in guinea pigs.

Homeostatic regulation of the OHC operating point and basolateral permeability

- Interlocking **negative feedback loops** within the OHCs
 - i) control **operating point** via slow motility and fast electromotility,
 - ii) control **basolateral permeability** via the effect of intracellular calcium on Ca^{2+} -gated K^+ channels.



OHC mathematical model (O'Beirne, 2005; O'Beirne and Patuzzi, in preparation).



Now on the right here we have a rather **complicated** looking diagram, which shows the **interactions** between the **ionic transport pathways** and **motile mechanisms** of the outer hair cells, and the coloured circuits are negative feedback loops we've identified over the past few years which help the outer hair cells maintain their exquisite auditory sensitivity, in spite of the daily perturbations they're exposed to (usually by people like us!).

One key feature of the outer hair cells is the **displacement-coupling** of the outer-hair cell stereocilia – the fact that the hair bundle actually inserts into the overlying tectorial membrane.

This DC-coupling creates **some** of these negative feedback loops (shown in this diagram in light and dark purple), whereby the modulation of the electrical properties of the hair cell via the apex (that is, a movement of the hair bundle turning on or off the current through the top of the cell) results in a compensatory change in **cell length** (and therefore hair bundle angle) via prestin-mediated electromotility and calcium-dependent slow-motility. These loops help maintain the resting hair bundle angle (or the "operating point") close to the most-sensitive region for transduction.

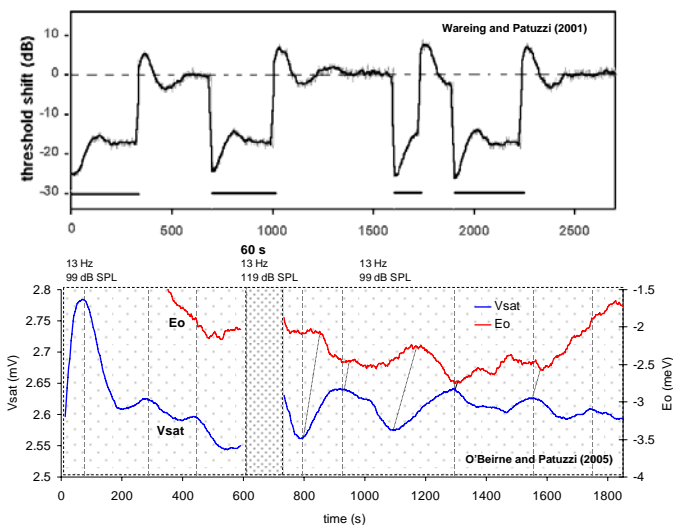
Also shown in this diagram (Loop III in pale blue) is the effect of intracellular calcium and calcium-sensitive potassium channels on basolateral permeability, and therefore resting membrane potential. Changing this permeability or resistance also affects the **magnitude of the small-signal AC receptor potential**, with elevated intracellular calcium resulting in a reduction of the drive to prestin and therefore reduction of the active process.

Over the past five years or so, we have created a **functioning mathematical model** of the outer hair cell, shown here on the left, that is capable of reproducing many of responses we've obtained experimentally, and I presented details of the model at the Inner Ear Biology meeting last year.

These homeostatic regulation systems within the outer hair cell are prone to oscillation, albeit extremely slowly. By slowly I mean a cycle time of several minutes. These oscillations come in many varieties, but...

The low-frequency “bounce phenomenon” – an example of cochlear oscillations


- Mechanical in origin (Kemp, 1982, 1986; Kirk and Patuzzi, 1997; Kirk et al., 1997)
- **Top:** Oscillation of psychophysical thresholds in a human subject (Patuzzi and Wareing, 2002; see also Hirsch and Ward, 1952; Zwicker and Hesse, 1984)
- **Bottom:** Oscillations in the Boltzmann parameters extracted from 200 Hz cochlear microphonic waveforms (O’Beirne, 2005; see also Kirk and Patuzzi, 1997; Kirk et al., 1997)



... the best known example of this is the so-called “**bounce phenomenon**”, whereby exposure to an intense low-frequency tone elicits oscillations in a **number of measures** of cochlear function.

These oscillations have been found to be *mechanical* (rather than strictly neural) in origin, and are visible in measures of:

- * **auditory threshold** (as shown here in this example of Békésy audiometry).
- * otoacoustic emissions
- * the **endocochlear potential**
- * and in measures of outer hair cell **mechanoelectrical transduction**, such as **Boltzmann analysis** of the low-frequency cochlear microphonic waveform, shown here in the bottom panel, which I’ll talk more about shortly.



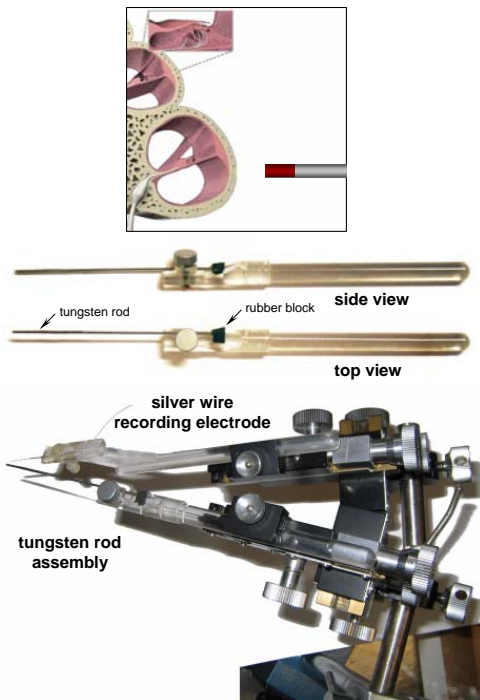
Aim

- Our aim was to develop a method of delivering a large step-perturbation (a mechanical bias) to the cochlea that would elicit some form of regulatory oscillation.

We have studied and modelled these slow oscillations resulting from a number of different cochlear perturbations, such as low-frequency tones, perilymphatic perfusions, and scala tympani current injection, but at the time we started this we were looking for a mechanical method of creating a large step change that could be used to elicit some of this oscillatory behaviour.

Method

- Force applied to the cochlear wall using a 1-mm-diameter insulated thoriated-tungsten rod.
- Reinforced rubber block placed in series. 1 mm displacement produced 452 g force.
- Results presented here derived from pushes of less than 320 g.
- Surgical assistance from Dr Peter Sellick.
- Round-window ECochG recordings made using a Teflon-coated Ag/AgCl wire electrode.
- Preliminary results presented by O'Beirne and Patuzzi at the 1st Australasian Auditory Neuroscience Workshop, Melbourne, 31st January 2004.

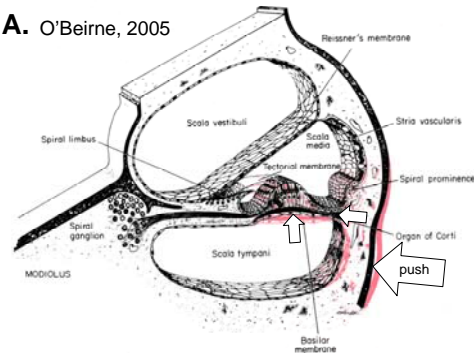


The method we came up with was to apply mechanical force to the wall of the cochlea using a 1-mm-diameter tungsten rod, which we advanced with a micromanipulator through a hole in the guinea pig's bulla into the middle-ear. The forces we applied were well below those required to actually crack the otic capsule. Using the set-up you see here, we were able to apply this force to the bony shell while making a range of electrocochleographic measurements from the nearby round window. We presented preliminary data using this technique in Melbourne in January 2004.

Mechanical changes with the push

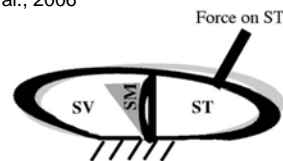
- Our experimental results (which I'll discuss shortly) indicated that the application of force over ST most probably caused a SV displacement of the basilar membrane, as shown in A.

A. O'Beirne, 2005



- Recent paper by Zou et al. measured the displacement of the cochlear wall by a similar probe and proposed a similar mechanism

B. Zou et al., 2006



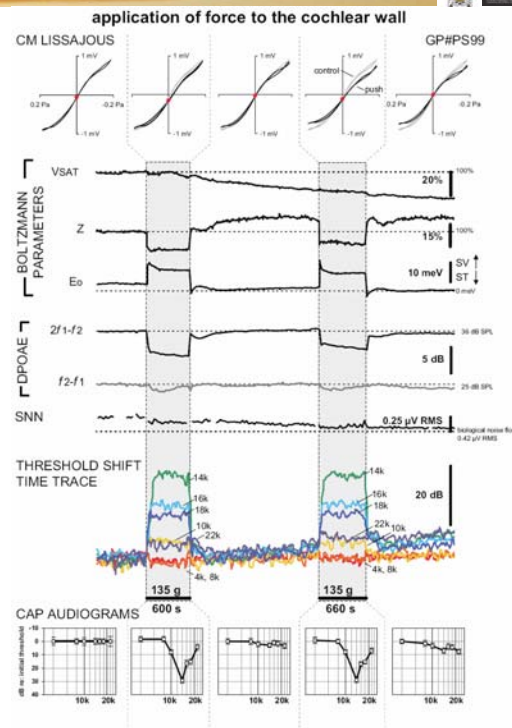
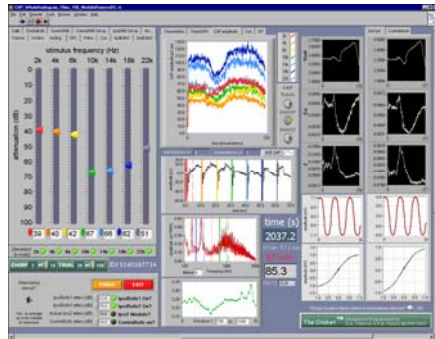
As I'll explain shortly, the electrophysiological data we recorded in response to this perturbation **all seem to indicate** that the application of force to the cochlear wall overlying scala tympani (here) **results in a movement of the basilar membrane** (in the **region of the push**) towards scala vestibuli (here), which causes a **number of changes in cochlear potentials**, and results in a fairly **localized hearing loss**, as I'll show you in a moment.

The recent paper in JASA by Zou, Zheng, Ren and Nuttall, who have also used this technique, also proposed this basilar membrane shift as a likely mechanism.

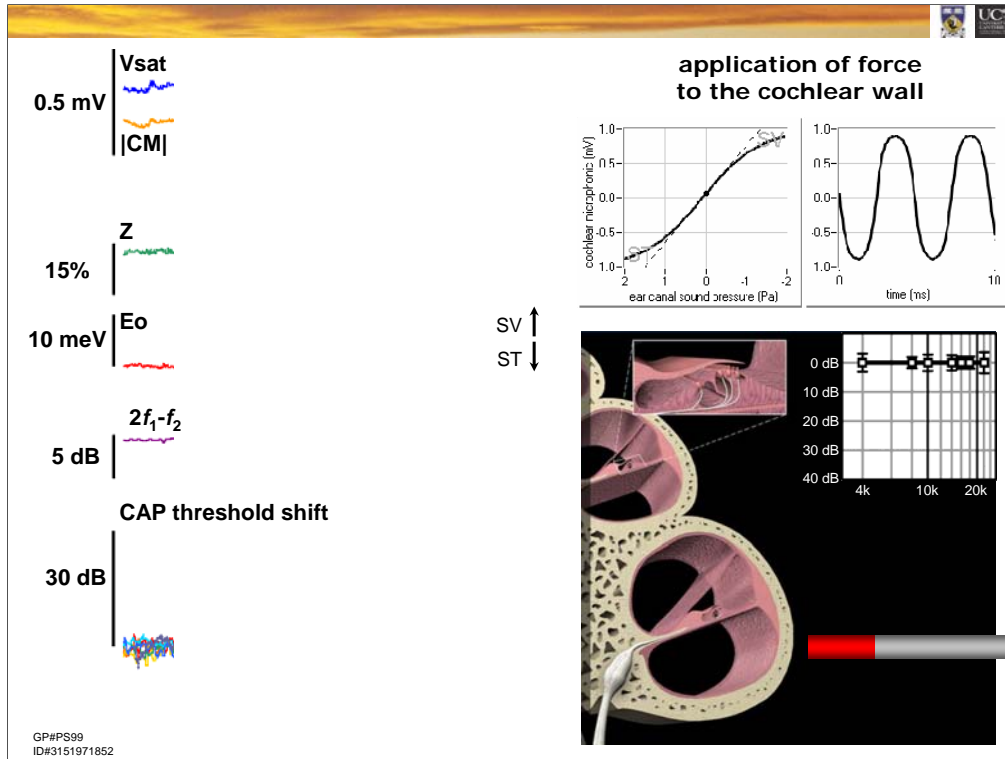
To arrive at our conclusions, we would need to integrate the results of a number of different measurements of cochlear function, preferably all recorded simultaneously, so we could more easily compare the time courses of the changes.

Method

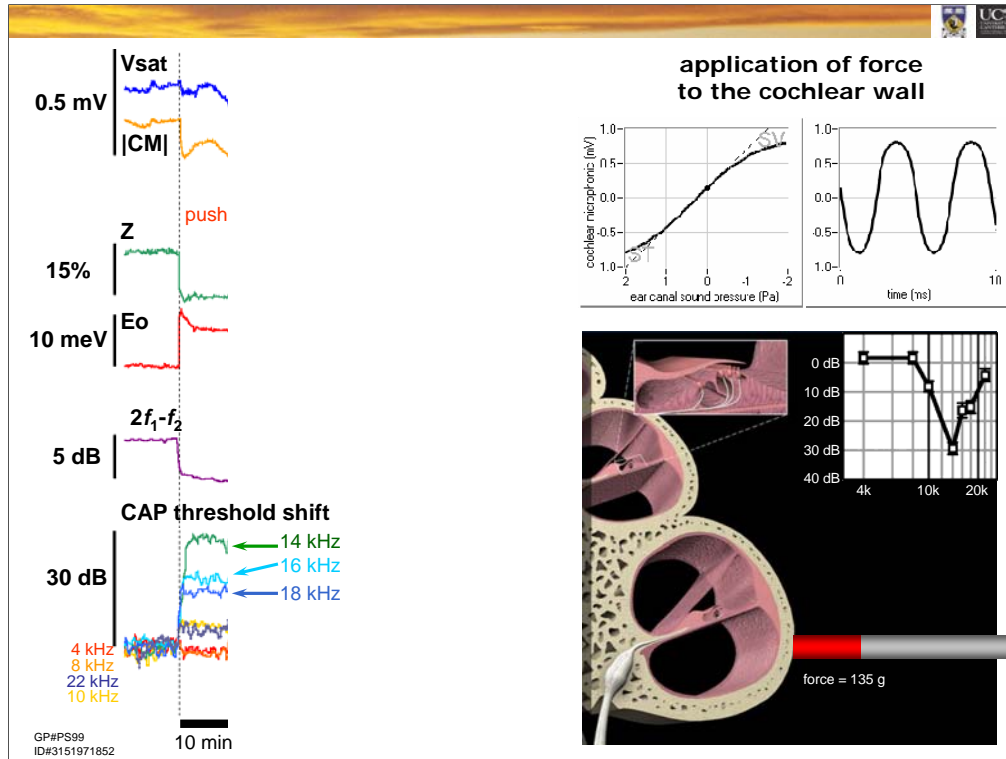
- Custom-written software allowed near-simultaneous measurement of:
 - CAP thresholds and waveforms at seven different frequencies
 - Boltzmann analysis of 200 Hz CM
 - Distortion-product OAEs
 - Endocochlear potential (EP)
 - Spectrum of neural noise (SNN)



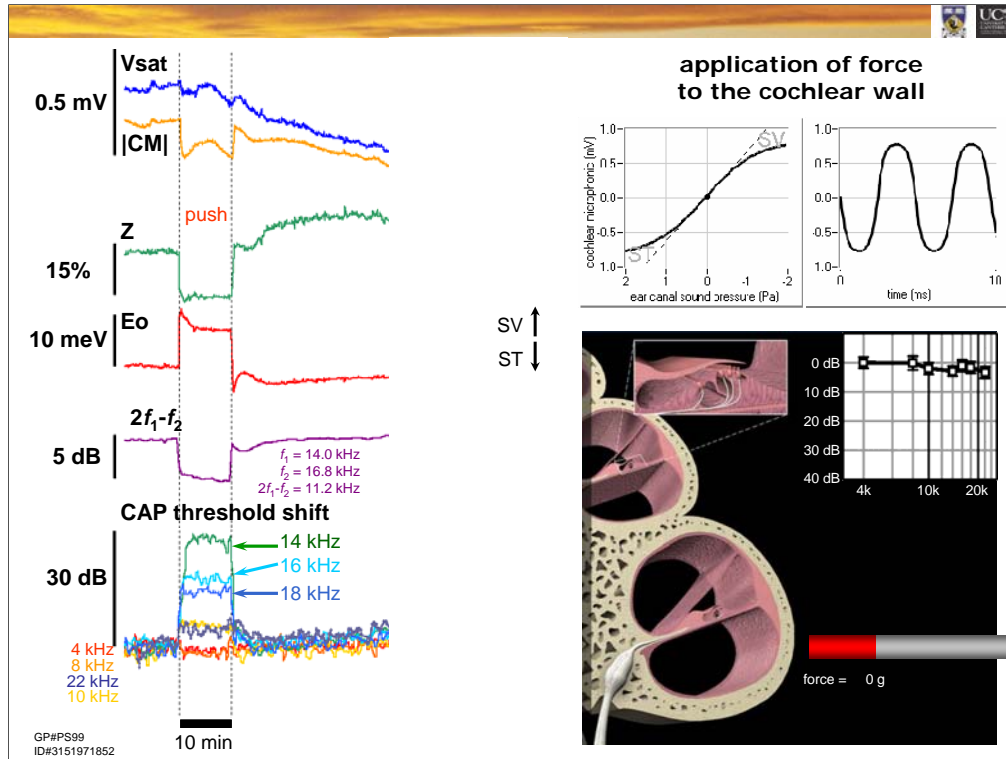
We wrote our own data acquisition software, shown here on the left, with the aim of obtaining as broad or as panoramic a view of cochlear function as possible. The software was capable of carrying out rapidly interleaved and near-simultaneous measurement of compound action potential thresholds and waveforms at seven representative frequencies, Boltzmann analysis of the CM, DPOAEs, the endocochlear potential, and the spectrum of the neural noise recorded in silence. To give you an idea of how all these measures look, on the right we have a typical set of data recorded using this software, for two applications of force (or pushes) of roughly 10 minutes each. The duration of the whole trace shown here is 83 minutes. If we look at this data more closely...



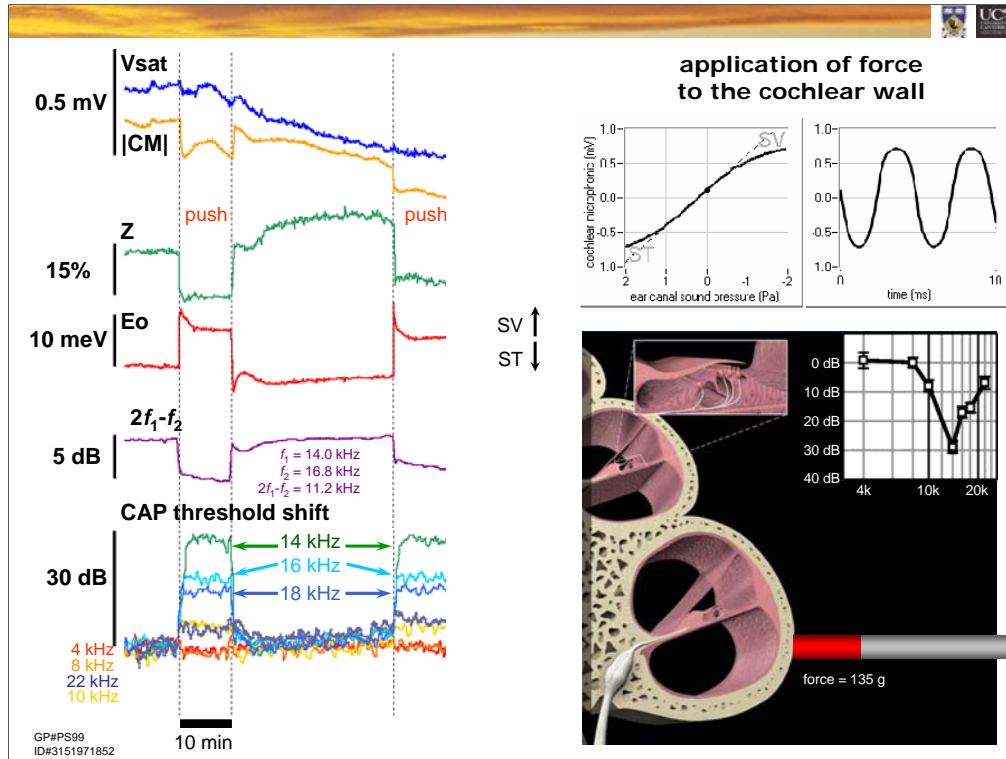
...we see that the application of...



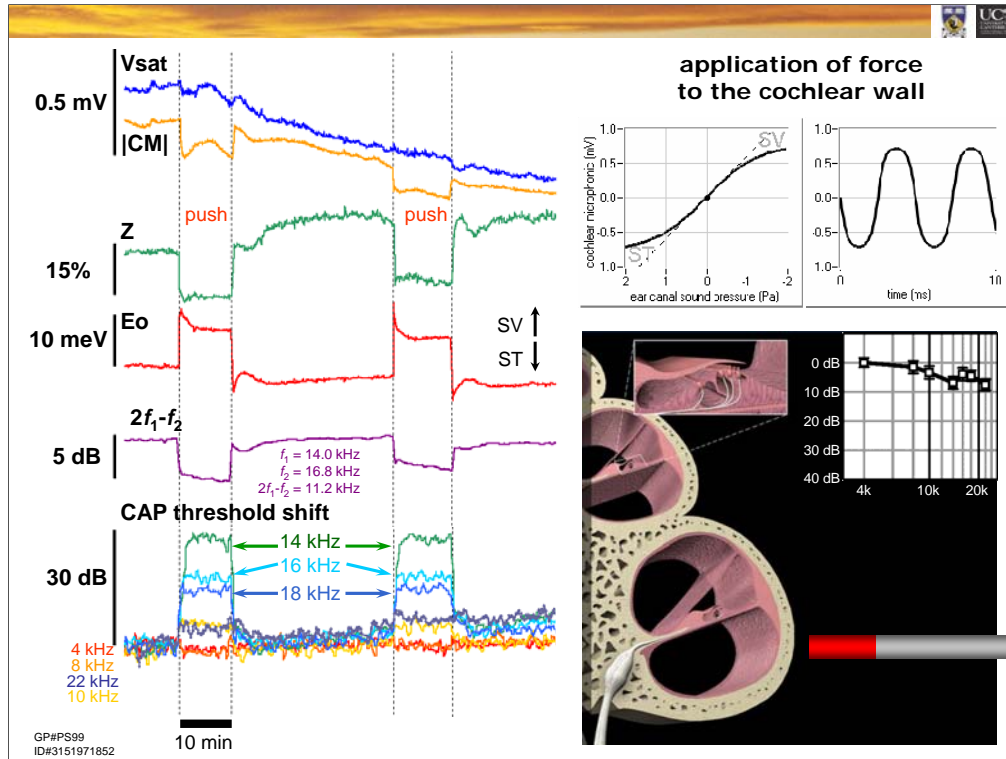
135 g of force to the otic capsule overlying scala tympani in the first turn causes a maximal **30 dB hearing loss**, in this case **centred at 14 kHz**, accompanied by a set of rapid step-changes in our other recordings, which I'll explain shortly. For the moment I want you to concentrate on the bottom set of CAP threshold traces, and the audiogram on the right.



When the force is removed after 10 minutes, the thresholds recover rapidly to within a few dB of their initial values...

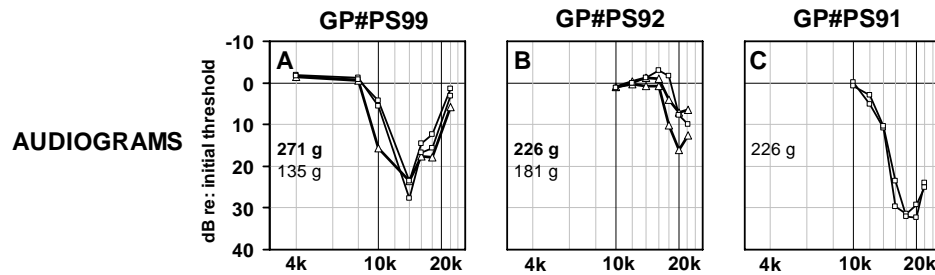


and when repeated using the same amount of force...



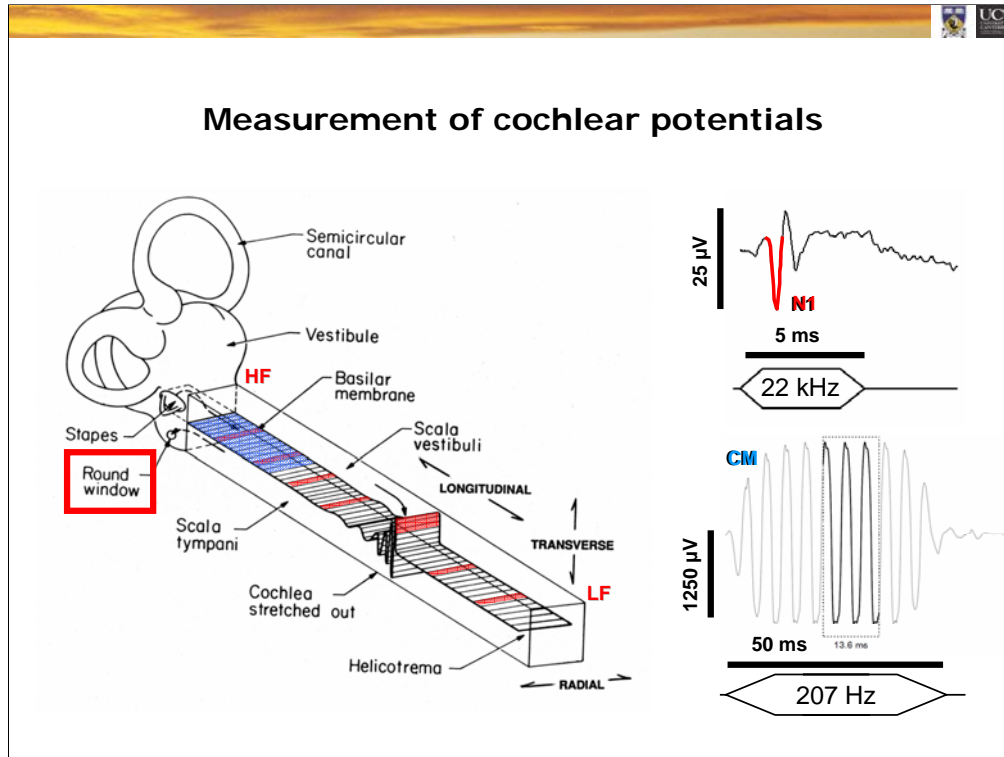
...are quite reproducible.

Results: Changes with auditory sensitivity with application of force to the cochlear wall

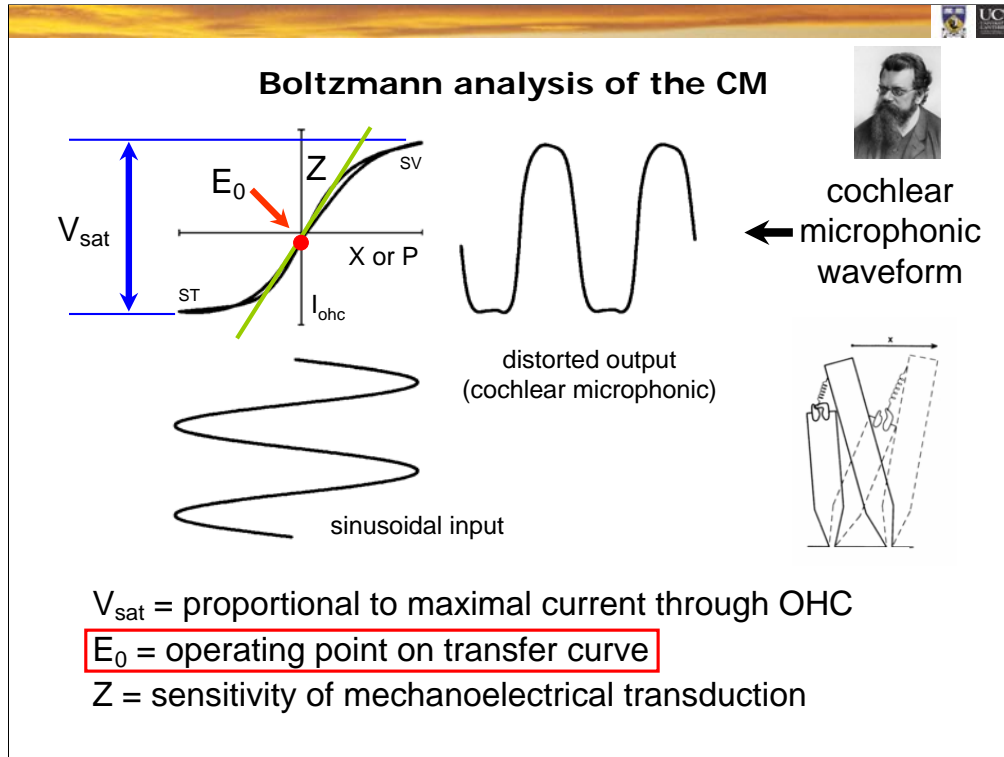


- Hearing loss from application of force largely dependent on point of contact between tungsten rod and otic capsule
- Larger applications of force with same rod alignment caused threshold shift over a broader range of frequencies.

When we look at results from a number of animals, we see that the frequency at which the maximum hearing loss occurred essentially depended on where we were pushing, and in general if we were to push harder at a particular point on the otic capsule, the hearing loss became broader, as the thresholds at adjacent frequencies were affected. And if we look at Panels A and B, we can see that the tracked thresholds actually improved at some frequencies more apical to the push location.



So while this CAP threshold data was obtained at different frequencies along the cochlea, we also used Boltzmann analysis of the cochlear microphonic waveforms, which, despite being a 200 Hz stimulus, actually probes the basal turn of the cochlea, near where our maximal hearing loss occurred.



For those who are unfamiliar with Boltzmann analysis, it is a technique whereby an intense, non-traumatic, low-frequency tone (for example, around 200 Hz) is used to drive the basal-turn OHCs into partial saturation, enabling us to use a curve-fitting process to analyse the characteristics of the nonlinear transfer curve.

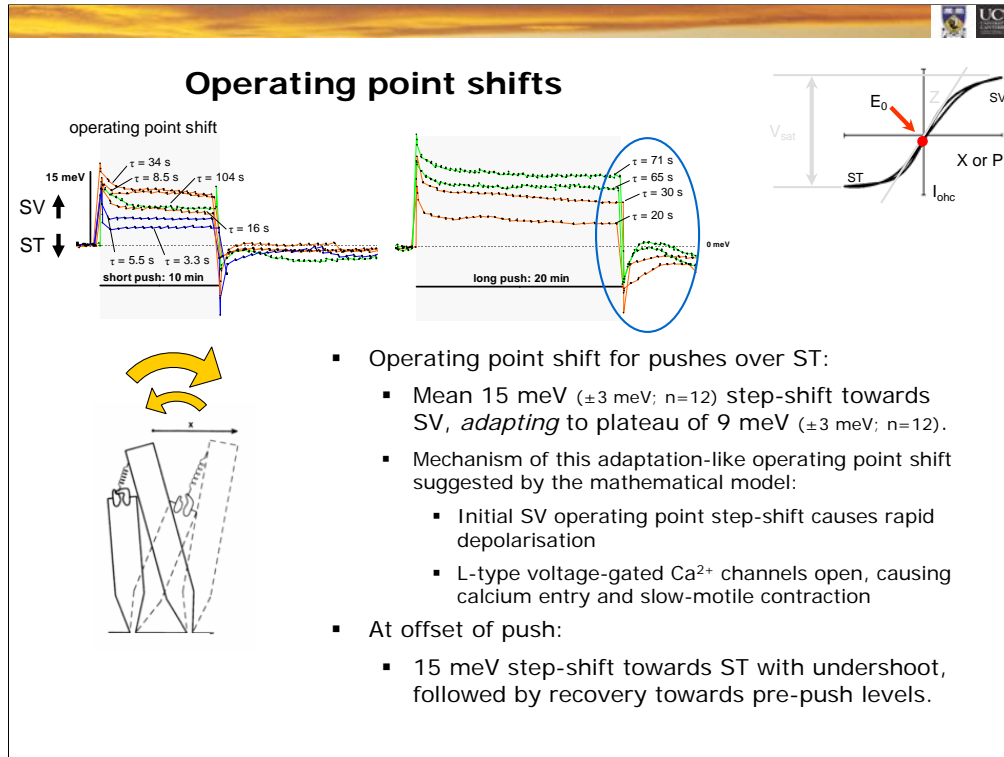
The parameters we extract are:

V_{sat} – which gives the maximal current through the OHC (dominated by the cell's basolateral permeability)

the **operating point** I mentioned earlier – E_0 – which provides an indication of the resting angle of the stereocilia, which is partially determined by the degree of contraction or elongation of the hair cell; and

Z – the overall sensitivity of the mechanoelectrical transduction process.

The technique allows us to probe changes in the basal-turn OHCs over several hours. In the limited time I have available, I'm going to show you the results for the operating point or hair bundle angle.



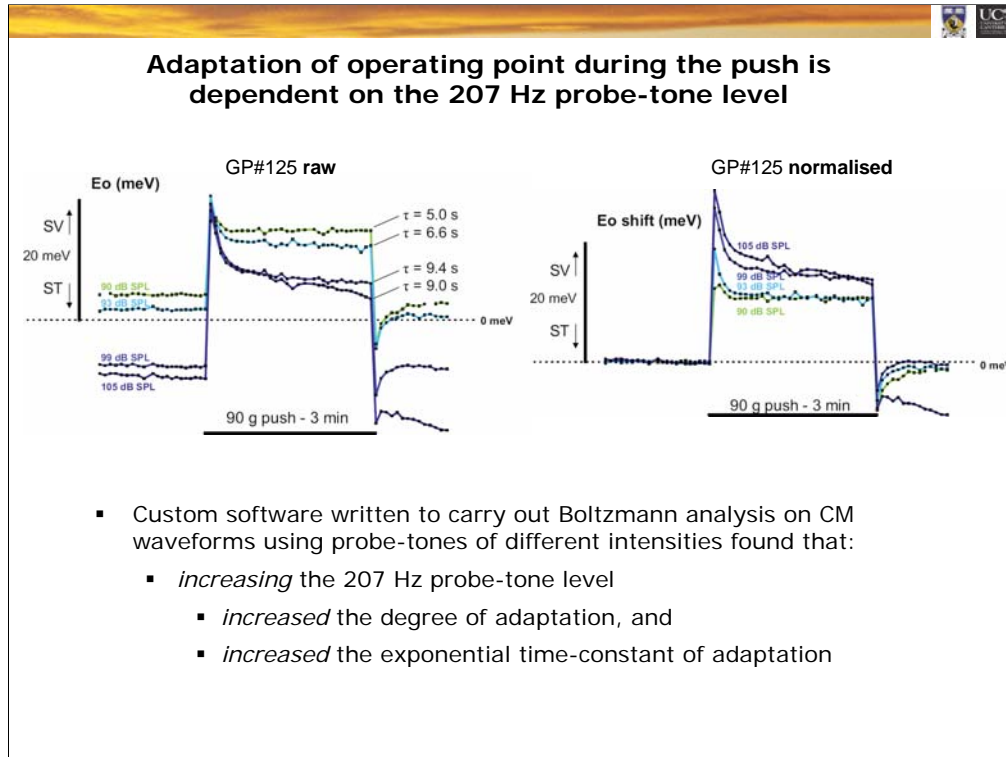
Where we see that, at the onset of the push, the operating point shows a rapid step shift towards scala vestibuli, followed by a slow adaptation. This adaptation can be fitted by a single exponential with a wide range of time constants as shown here.

The average magnitude of the step shift was around 15 mV, adapting to a plateau level of 9 mV above the baseline. Of the many possible mechanisms for these changes, the results of our analysis, and our mathematical modelling work, suggest that the primary cause of the observed pattern is likely to be as follows:

Firstly, as the basilar membrane is displaced by the push, the hair bundle is moved rapidly towards scala vestibuli, which opens the MET channels, allowing more potassium to enter the top of the cell. This **depolarises** the hair cell quite rapidly, causing voltage-gated calcium channels to open. The increase in intracellular calcium concentration then causes a slow motile contraction which partially restores the hair bundle angle toward the more sensitive region of the transfer curve.

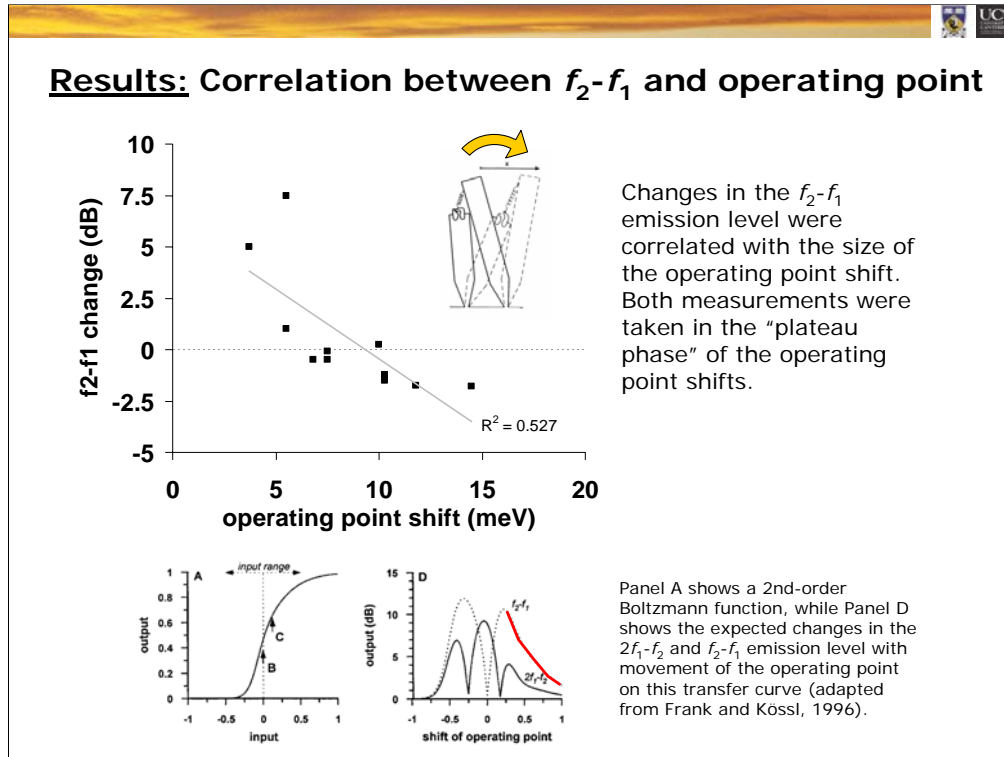
We can tell that the adaptation isn't a result of the force stimuli leaking away, because at the offset of the push we observe a step-shift in the opposite direction, towards scala tympani, of the same magnitude as the initial SV shift.

This is then followed by a half-cycle oscillation as the hair bundle angle recovers.



We also found that the adaptation components were influenced by the level of the 207 Hz tone used to evoke the CM for analysis, whereby increasing the probe-tone level increased both the degree of adaptation, and the time constant of the adaptation.

So, the push didn't produce the huge ringing oscillation we were hoping for, but it does demonstrate the operation of the outer hair cell regulatory mechanisms that we have modelled and characterised. While they are capable of compensating for part of this change, they cannot completely eliminate it.



In our concurrent recordings of distortion product otoacoustic emissions during the push, we found a nice correlation between the size of the change we observed in the f_2-f_1 emission, and the size of the operating point shift we produced using the push. Both of these measurements were taken from the “plateau”, after any adaptation effects had run their course.

This is of course consistent with what we’ve known for a long time – that the f_2-f_1 emission being sensitive to asymmetry in the distortion, and our results are in agreement with the work of Frank and Kössl (shown at the bottom here), as well of those of Shera and Guinan, Kim and Neely, and Zwicker and Manley among others.

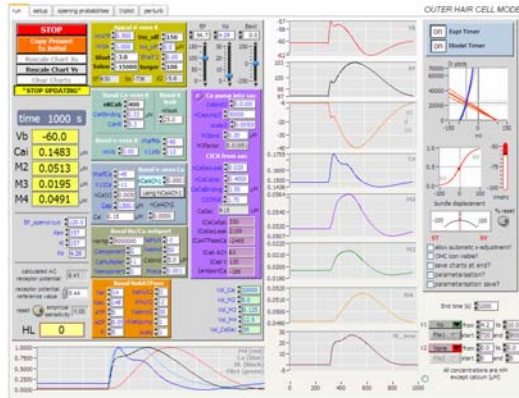
On the other hand, our $2f_1-f_2$ results were extremely complicated, and are beyond the scope of this presentation.

Conclusions

- The application of force to the cochlear wall is not a simple perturbation! In addition to the results presented here, we also observed complex changes in the EP, DPOAEs (particularly $2f_1-f_2$), the Z parameter of the Boltzmann analysis, and the CAP/SP waveshape.
- Not particularly effective at generating large cochlear oscillations, but appears to be one of the few mechanisms for generating a lasting shift in operating point.
 - Most likely due to a geometric change in the organ of Corti, rather than an imbalance in fluid pressures within the cochlea
 - OHC adaptation mechanisms are able to absorb some of the change, but not all of it.
 - Mathematical modelling has provided insights into this process.
- “The push” caused a localised, reversible reduction in BM vibration, causing a threshold shift of around 15 to 35 dB, and minor changes to CAP waveforms.
- Data demonstrate the extreme resistance of the cochlea to DC stimuli
 - *Despite application of forces a quarter of that required to crack the cochlear wall, only observed maximal hearing losses of 35 dB!*

In conclusion...

Acknowledgements



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