Effects of Furosemide on Organ of Corti Vibrations

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Abstract

The endocochlear potential (EP) is necessary for mechanoelectrical transduction and outer hair-cell (OHC) generated force production. Previous work has shown that IV administration of furosemide immediately reduces the EP, which mostly recovers over approximately 40 minutes. Furosemide alters the mechanical response of the basilar membrane (BM), the local cochlear microphonic (LCM) and distortion product otoacoustic emissions (DPOAEs), all of which recover over time.

Using optical coherence tomography) we measured BM and organ of Corti (OoC) vibrations in a region close to the OHCs in vivo in the gerbil cochlea before and after the IV administration of furosemide. The stimuli were multi-tone zwuis complexes containing 60 frequencies presented simultaneously at 40 to 80 dB SPL. DPOAEs were measured, at the same time points, in response to two-tone stimuli at two levels, 50 and 70 dB SPL, and used to ascertain the successful IV administration of furosemide, and monitor recovery.

As previously demonstrated, immediately after administration, the amplitude of BM vibrations was greatly reduced in the peak region near the best frequency (BF), and showed linear, passive-like, growth in response to increasing sound pressure levels. OoC vibrations measured in the OHC region, also showed a decreased amplitude and broad tuning (loss of BF peak) following furosemide injection. In contrast to the BM, however, the vibrations of these internal points retained nonlinearity across the frequency range, especially at the higher stimulus levels. The DPOAEs were also greatly reduced.

Over subsequent recordings, taken at approximately 10 minute intervals, the vibrations of the BM and OoC exhibited gradual recovery as did the DPOAEs. Approximately 70 – 80 minutes post injection, the BM and OoC vibrations begin to recover their peak and compressive nonlinearity near the BF. 120 minutes post injection, vibration amplitudes and DPOAEs had largely, but never fully, recovered with little-to-no further improvements seen at longer time points.

1. Background and Methods

ORGAN of Corti vibrations were measured *in vivo* in gerbils through the round window membrane, near the 25 kHz location, in healthy cochleae with a ThorLabs Telesto III OCT system. Details of the OCT and spectral domain phase microscopy (SDPM) recordings have been previously published [1, 2]. We focused on the vibrations of the basilar membrane (BM) and a region close to the outer hair cells (OHCs) some 60 μm deeper within the organ of Corti [3]. Acoustic stimuli were zwuis [3, 4] tone complexes from 4 kHz to 34 kHz and from 40 to 80 dB SPL (re: 20 μPa). In each experiment, DPOAEs were measured in response to swept two-tone stimuli, f_1 and f_2 where f_2 was swept from 1 to 48 kHz, $f_1 = f_2/1.2$ and the stimulus frequencies were presented at 50 and 70 dB SPL. The effects of furosemide treatment on the EP and local cochlear microphonic (LCM) potential have been previously published [5].

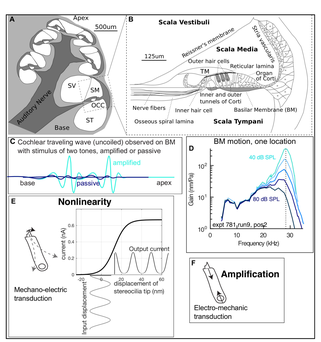

Figure 1: *Cochlear amplification. A and B: Cross-sectional anatomy of the cochlea. C and D: In vivo, one or more active processes amplify the vibrations in a frequency selective manner. E and F: The nonlinearity in the cochlea likely arises from the mechano-electrical transduction channels in the hair cell bundles while the power delivered to the BM, and other structures, likely comes from the somatic motility of the OHCs.*

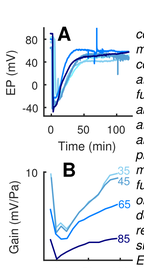

Figure 2: *A: Endocochlear potentials measured in 5 healthy cochleae before and after the treatment with furosemide. The EP is abolished immediately after the drug is injected and stabilizes at a depressed value $\sim$ 30 – 50 mins later. B: Following furosemide, the LCM (gain or mV per unit pressure) decreases and could recover completely on a slower timescale than the EP. Stimulus levels in dB SPL are indicated by the color coded numbers to the right of the curves [5].*

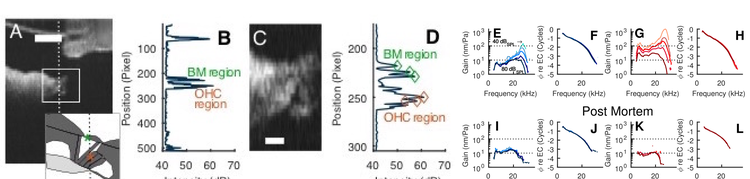

Figure 3: *Basis of OCT and SDPM recordings. A shows a two-dimensional B scan across the organ of Corti. Scale bar: 200 μm. B shows a one-dimensional A scan through the center of A. SDPM was performed on selected pixels corresponding to structures of interest in the organ. 1 pixel = 2.7 μm. E and F show the vibration amplitude (plotted as gain or displacement per unit pressure) and phase along the BM while G and H show the same in the OHC-region. BM vibrations are boosted and compressively nonlinear near the best frequency (BF) but exhibit linear growth for frequencies well below BF. OHC region vibrations exhibit larger amplitudes, broader tuning than the BM vibrations, and show the compressive nonlinearity across all frequencies. I – L post mortem, vibrations are reduced in amplitude, show linear growth, and reduced phase accumulation.*

2. Loss and Recovery of Mechanical Amplification Following Furosemide

WE measured organ of Corti vibrations before and furosemide treatment and also measured distortion product otoacoustic emissions (DPOAEs) in response to swept two-tone stimuli at the same times to assess changes in cochlear nonlinearity and amplification. After a series of baseline recordings, gerbils were given an intravenous injection of furosemide (100 mg/kg) to reduce or abolish the endogenous endocochlear potential (EP). Each set of recordings, DPOAEs and OCT vibrometry, were repeated every 10 minutes for up to four hours post injection.

Prior to the furosemide injection, all gerbils showed robust DPOAEs and the evoked vibrations were typical of healthy cochleae. BM responses were sharply tuned and compressively nonlinear near the BF and exhibited linear growth for frequencies well below BF. OHC region vibrations exhibited larger amplitudes (up to $\sim 10 \times$ larger in the best preparations), broader tuning, and a compressive nonlinearity across the entire frequency band tested.

After treatment with furosemide, BM vibrations resembled those of a passive cochlea showing depressed amplitudes, a loss of tuning and the nonlinearity. OHC region vibrations also showed decreased amplitudes and a loss of the BF peak; but, in sharp contrast to the BM, the compressive nonlinearity in these vibrations persisted. DPOAEs were also greatly reduced following the loss of the EP.

Vibrations, and DPOAEs, recovered slowly with the peak at the BF re-emerging $\sim$ 70 mins after the injection and with recovery largely complete $\sim$ 120 mins post injection. Little to no recovery was observed at further times.

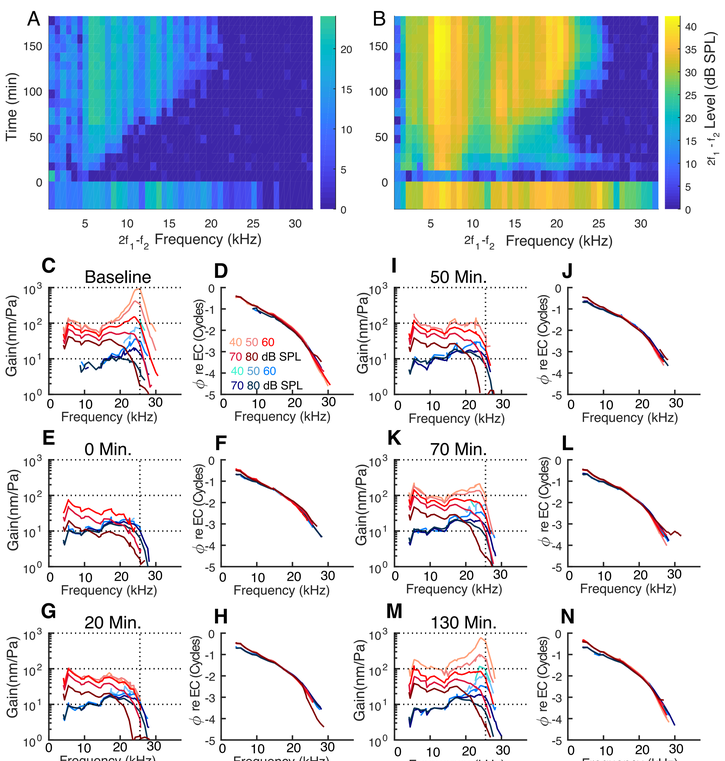

Figure 4: *Loss and recovery of nonlinear amplification in organ of Corti vibrations in response to reduced EP. Panels A and B show mesh plots of DPOAE audiograms measured in response to swept two tone stimuli at 50 and 70 dB primary levels. The times are referenced to the furosemide injection at $t = 0$ min. Immediately after the injection, the DPOAEs were abolished and showed gradual recovery over the subsequent $\sim$ two hours. Panels C – M show BM (blue curves) and OHC-region (red curves) responses at different times across the experiment. Prior to the injection (C and D), all cochleae tested show nonlinear amplification near the best frequency with the OHC region exhibiting higher amplitudes and compressive nonlinearity across all frequencies. Immediately following the injection (E and F), BM responses resembled a passive cochlea showing greatly reduced amplitudes, linear growth, and a loss of the BF peak. In contrast, OoC vibrations, while showing a similarly reduced amplitude, were more low pass in character but retained the low frequency, sub BF, nonlinearity. BM and OHC vibrations recovered gradually over time, with the BF peak beginning to re-emerge $\sim$ 70 minutes after the injection and with recovery largely complete $\sim$120 minutes after the injection and little to no changes seen at further times.*

3. Summary of Effects of Furosemide on Organ of Corti Vibrations

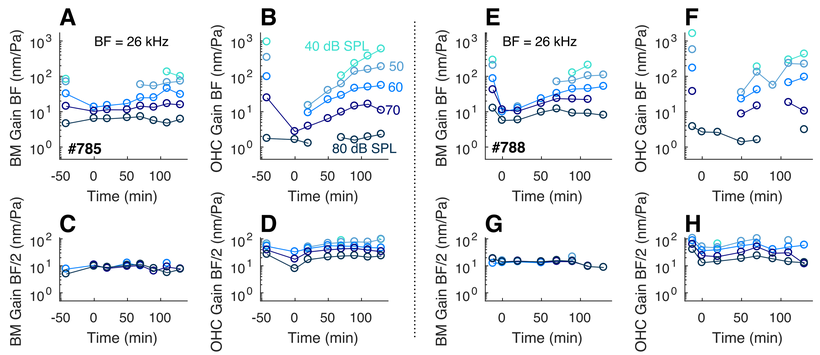

Figure 5: *Summary of the loss and recovery of amplification in BM and OHC-region vibrations from two experiments. Panels A and B show BM and OHC-region vibrations near the BF in one experiment. Time $t = 0$ corresponds to recordings taken immediately after the furosemide injection. C and D show the vibrations in the same regions but at BF/2. At frequencies well below BF, the loss of the EP has little effect on the BM vibration magnitudes. Note that the BM amplitudes grow linearly at this frequency while the OHC-region amplitudes remain nonlinear. The second set of panels, E – H, on the right follow the same layout and are taken from a second preparation. In both plots, gaps in the data occur when the vibrations were not significantly ($\gtrsim$ 3.2 standard deviations) above the noise level in the displacement spectrum.*

4. Summary

- We used OCT to measure vibrations at the BM and OHC-region in gerbil cochleae and used DPOAEs as a real-time measurement of cochlear condition.
- Similar to a number of recent papers, the OHC-region vibrations show higher amplitudes, broader tuning, and are compressively nonlinear over a wider frequency range than vibrations on the BM [3, 2].
- IV injection of furosemide reversibly reduces or abolishes the EP and evoked LCM. While the EP stabilizes to a depressed value $\sim$ 30 – 50 mins. post treatment, the LCM recovers more slowly and more completely [5].
- *With a reduced EP, BM responses resemble those of a passive cochlea showing depressed amplitudes, loss of tuning, and linear growth* [6].
- *In contrast, OHC-region vibrations are reduced in amplitude and show more low-pass like characteristics but remain compressively nonlinear across the frequency band tested.*
- BM and OHC-region vibrations recovered slowly after the reduction in the EP. The peak at the BF began to emerge $\sim$ 70 minutes post injection with recovery largely complete by $\sim$ 120 minutes.
- This timescale is significantly slower than the previously published recovery of the EP itself [5] and similar or slightly lagging the recovery of the LCM.
- *It is possible to separate, in vivo, compressive nonlinearity and active amplification, defined as boosting the response near the BF.*
- *OHC-based electromotility is necessary for amplification but not, itself, sufficient. A number of processes, working in a feedback loop, likely contribute to cochlear amplification.*

5. Acknowledgements

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