The Effects of Salicylate on Sound-Evoked Vibrations on the Basilar Membrane and Outer Hair Cell Region in Vivo

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Abstract

Recent optical coherence tomography (OCT) results have shown that the intra-organ of Corti (OoC) vibrations have larger amplitudes and different tuning than the vibrations measured at the basilar membrane (BM). An area we term the "outer hair cell (OHC) region", between the OHC-Deiters' cell connections and the reticular lamina, exhibits a broad-band nonlinearity that is metabolically sensitive and especially pronounced when stimulated with wide-band signals.

Salicylates, such as aspirin, are known to have a number of ototoxic effects including blocking somatic electromotility, a key component of the feedback mechanism which provides amplification in the cochlea and are known to reduce BM vibrations but their effects on the vibrations within the OoC have not yet been explored.

We use OCT and spectral domain phase microscopy (SDPM) to measure BM and OoC vibrations in a narrow radial region close to the outer hair cells in vivo in the base of the gerbil cochlea, close to the 25–27 kHz location. We measured the vibrations before and after a 10 to 30 minute application of sodium salicylate, 50 or 100 mM in artificial perilymph, on the round window membrane followed by a washout with fresh perilymph. DPOAEs measured in response to swept two-tone stimuli at 50 and 70 dB SPL at the same time points were used to assess the efficacy of the salicylate administration and subsequent recovery of cochlear condition.

1. Background and Methods

Salicylates are known to have a number of ototoxic effects some of which act directly on the hair cells including: reduction in the sound-evoked potentials in the cochlea; reduced distortion product otoacousitic emissions (DPOAEs); reduced electrically-evoked vibrations near the reticular lamina; and, in isolated OHCs, inhibition of somatic electromotility a key component of the cochlear amplifier. However, little remains known about the effects of salicylate on sound evoked motion within the ear with only a few studies performed on basilar membrane (BM) vibrations [1, 2] after the introduction of salicylate into the perilymphatic space. Sadreev *et al.* measured the salicylate concentration along the length of the cochlear spiral following the application of a sodium salicylate solution on the Round Window Membrane (RWM) and showed that at basal regions, the concentration quickly reached a steady-state concentration ~ 5 % of the extra-cochlear value [3]. We used a ThorLabs Telesto III optical coherence tomography (OCT) system to measure organ of Corti (OoC) vibrations *in vivo* in gerbils through the round window membrane, near the 25 kHz location, in healthy cochleae before and after introducing salicylate following a similar protocol as Sadreev *et al.*.

Details of the OCT and spectral domain phase microscopy (SDPM) recordings have been previously published [4, 5]. 5 μL of either 50 or 100 μM was placed on the RWM and allowed to passively diffuse into scala tympani for 10 to 30 minutes before a washout. In one set of experiments, 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 [6] at one radial location. Acoustic stimuli were zwuis [6, 7] tone complexes from 5 kHz to 35 kHz and from 40 to 80 dB SPL (re: 20 μPa). Vibration measurements were taken before the salicylate was applied and in ten minute intervals following the washout for up to five hours after the initial introduction.

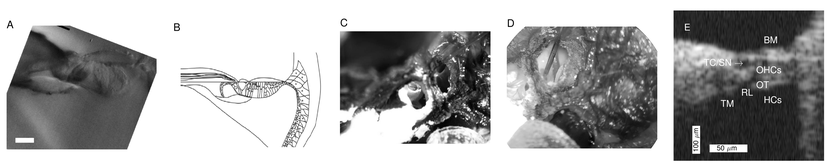

Figure 1: *(A) Micrograph of the basal region from an unfixed gerbil cochlea [8]. Scale bar: 60 μm. (B) Schematic diagram of the cochlear partition. (C) Image of the Round Window Space taken through the open bulla with the Telesto's video camera. (D) View through an operating microscope showing fine needle used to inject the salicylate atop the Round Window Membrane. (E)B-scan of the organ of Corti taken through the RWM. For clarity, this image has been stretched in the horizontal direction. Anatomical features labeled: BM: Basilar Membrane, OHCs: Outer Hair Cells, OT = Outer Tunnel, HCs = Hensen's Cells, TM = Tectorial Membrane, and TC/SN = Tunnel of Corti and Space of Nuell (not well differentiated in this image).*

In the second set of experiments we measured the two-dimensional vibration patterns in a 120 – 160 μm wide region of the organ of Corti. These measurements were taken prior to the salicylate injection and at 20 minute intervals for up to five hours post treatment. In these experiments, the stimuli were a short zwuis complex containing 15 frequencies from 10 kHz to 35 kHz and presented at 60, 70, and 80 dB.

In each experiment, DPOAEs were measured in response to swept two-tone stimuli just before each set of vibration measurements. In these 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. Since salicylate is known to affect the cochlear nonlinearity, the DPOAE audiogram serves as a real-time measurement of the cochlear condition. In particular, the loss of high frequency DPOAEs within 10 minutes of the injection indicated that the "active process" or "cochlear amplier" was suppressed at basal locations close to the recording site.

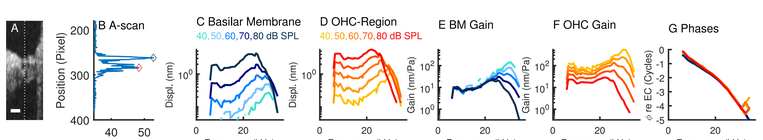

Figure 2: *OCT imaging and vibrometry with SDPM. (A) Two dimensional B scan showing the organ of Corti with the BM at the top of the image. Scale bar 50 μm. (B) Vibrations were measured along this Axial or A-scan through the center of the image. Peaks in the A-scan correspond to the strong reflectors. The BM and OHC-region are labeled with diamonds. The space of Nuel and outer tunnel are visible in both the B- and A-scans. (C) – (F) show the sound-evoked vibrations at the BM and OHC-region from 40 to 80 dB SPL. At the lowest level measured, the OHC-region moved $\sim 2\times$ more than the BM.*

2. Loss and Recovery of Mechanical Amplification at the BM and OHC-region Following Salicylate

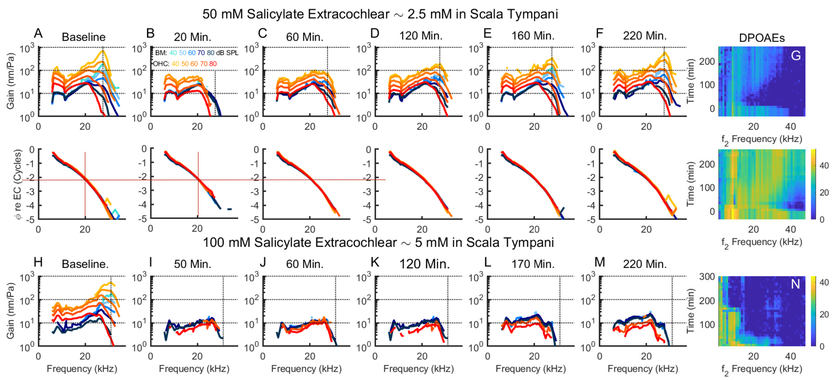

Figure 3: *(A) – (F): Basilar Membrane (blue) and Outer Hair Cell Region (red) responses before (A) and at selected time points after (B) – (F) after the application of 50 mM sodium salicylate on the RWM. (G) Loss and recovery of DPOAEs in the same experiment. Top panel: DPOAEs evoked by 50 dB primaries, bottom panel: DPOAEs evoked by 70 dB primaries. Time $t = 0$ minutes corresponds to the injection on the RWM and the loss of distortion at 50 dB at 10 – 20 minutes indicates that the salicyate diffused into the cochlea. In good preparations, DPs recovered about two hours post treatment, on a similar timescale as the vibrations. Experiment #846, 12 November 2020. Bottom row, (H) – (M). Following the application of 100 mM sodium salicylate on the RWM, the responses resembled those of a passive cochlea and never recovered. The DPOEAs, panel (N) likewise did not recover of the timescale of the experiment. Experiment #810, 15 July 2020.*

3. Two-Dimensional Maps of Organ of Corti Vibrations Before and After Salicylate

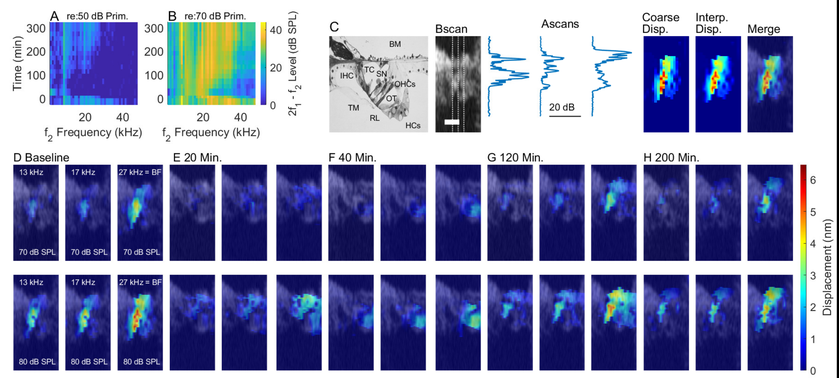

Figure 4: *A and B. Reduction of DPOAEs at 50 (A) and 70 (B) dB SPL following the application of salicylate on the RWM. Images in (C) explain of the method for measuring two-dimensional vibration patterns. The left image shows a photomicrograph of a guinea pig cochlea [9] with important structures labeled. BM = Basilar Membrane, RL = Reticular Lamina, IHCs = Inner Hair Cells, OHC = Outer Hair Cell, HCs = Hensen's Cells, TM = Tectorial Membrane, TC = Tunnel of Corti, SN = Space of Nuell, and OT = Outer Tunnel. The second image shows a raw Bscan, 160 μm wide. Scale bar = 50 μm and three representative A-scans spaced 20 μ apart across the radial direction along the dashed lines in the B-scan. The third image shows the axial motion as measured in 10 μm steps in the radial direction and the next image shows theinterpolated vibrations. The final image is a merge of the vibration patterns overlaid on the structural Bscan. Panels (D) to (H) show the two dimensional maps of the vibrations at selected times evoked at 70 dB (top row) and 80 dB (bottom row). At each time point, vibrations at three frequencies are shown. Experiment #855, 12 December 2020.*

4. Tuning Curves at Selected Radial Locations and Times

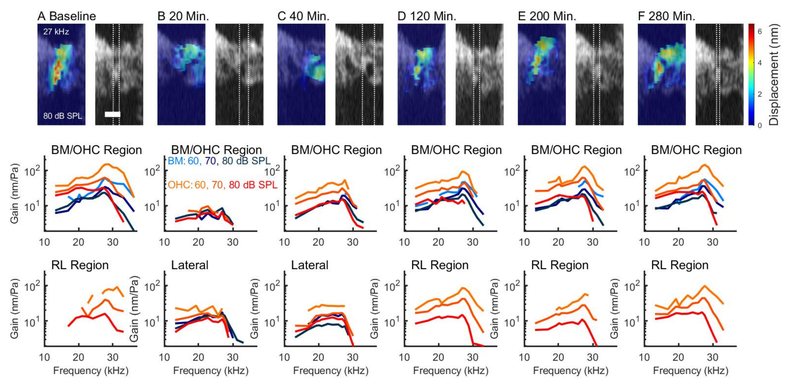

Figure 5: *Tuning curves measured at the BM/OHC-region and other selected radial locations. Top Row: Heat map plots and B-scans showing the radial locations marked. Middle Row: Tuning curves measured at the RM and OHC-region at the same time points. Bottom Row: Tuning curves measured close to the reticular lamina before and well after introducing salicylate and $\sim$ 20 μm lateral to the OHC-region during the times when those structures showed larger amplitudes. Experiment #855, same preparation as the previous figure.*

5. Summary

- Following the application of 50 mM salicylate to the round window membrane (estimated concentration in scala typicani $\sim$ 2.5 mM), vibrations at both the BM and OHC-region showed decreased amplitudes and a loss of the peak at the best frequency.
- BM vibrations were linearized while the OHC region could retain its broad-band nonlinearity.
- Recovery occurred slowly following salicylate delivery, with the vibration amplitudes and tuning largely returning to baseline levels $\sim$3–4 hours post treatment with few changes seen at later times. High frequency DPOAEs, generated near the base of the cochlea, recovered on a similar timescale.
- Following treatment with a higher concentration, 100 mM extracochlear ($\sim$ 5 mM in scala tympani), responses resembled those of a passive cochlea and did not recover over the timescale of the experiment, $\sim$ 5 hours.
- Preliminary experiments suggest that shortly after the introduction of salicylate, structures lateral to the sensory cells, the spiral ligament and Hensen's cells transiently move with greater amplitudes than the sensory portion of the organ of Corti. The amplitudes, however, remain low relative to the baseline conditions.

6. Acknowledgements

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