Comparing external tympanum vibration and spontaneous otoacoustic emissions

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Abstract. Generated inside the inner ear, spontaneous otoacoustic emissions (SOAEs) are the most salient evidence for an "active" ear. These emissions propagate back through the middle and external ears, setting the tympanic membrane in motion such that it effectively acts as a "speaker". Better characterization of this motion would be useful for the quantification of power produced by the associated active processes. Towards this end, we took a comparative approach, examining spontaneous activity in both a lizard (green anole, *Anolis carolinensis*) and an invertebrate (tree cricket, *Oecanthus nigricornis*). Using a laser Doppler vibrometer and sensitive OAE probe, we measured both tympanum motion and the acoustic output. For lizards, using a single point measurement system (whose focal point was not well localized), roughly similar behavior was observed in both the mechanical tympanum motion and the acoustic SOAE. For a relatively large spontaneous vibration peak (at 3.9 kHz) on the lizard tympanum, the amplitude of displacement was approximately 60 pm. Differences were however noted between SOAE and vibration, and several possible explanations for such are discussed. For the cricket, only "open" coupling of the microphone could be implemented. It was observed that while spontaneous vibration on the tympanum could be detected above the noise floor (single peak about 3-4 kHz, with displacements of 10-35 pm), SOAEs could not. When a stimulus was presented to a elicit a stimulus frequency emission (SFOAE) using a suppression paradigm, no residual could be detected either in the vibration or acoustic response. Lastly, when two tones were presented, distortions were readily observable in the tympanum motion but no acoustic DPOAEs were detected. It is suggested that the relatively small size of the cricket tympanum and open coupling employed could explain these inconsistencies (e.g., it is too small to effectively radiate acoustic power above the noise floor).

INTRODUCTION

There is compelling evidence that an active mechanism is at work in the inner ear. Low level acoustic signals cause small mechanical perturbations (e.g., picometer displacement) of the external tympanum (**TyM**), and the active mechanism(s) of the inner ear add power to amplify this motion. One of the most salient forms of evidence for such is the presence of spontaneous otoacoustic emissions (**SOAE**) [16], which strongly correlate to perceptual measures of auditory function [8]. Models of SOAE generation implicitly assume something is exhibiting a mechanical spontaneous oscillation (**SO**) to generate the observed acoustic response, such as a stereociliary hair cell behaving as an limit-cycle oscillator [6] or a standing wave along the length of the cochlea [15]. Such an assumption dates back relatively far [5], however empirical evidence of SO is scant.

Physiological observation have suggested SO could arise in hair cell bundles [2, 9], although those sensory cells examined had a relatively low characteristic frequency (CF) of 5-300 Hz. For auditory hair cells at higher CFs (e.g., several kHz), a different relationship between inertial and viscous forces could be at work [4], a biomechanical consideration that could have significant implications for mechanisms of SO. One study [14] reported evidence for mammalian basilar membrane SO and demonstrated correlations to SOAE. Furthermore, studies in invertebrate ears, which lack stereociliary hair cells altogether, have shown the presence of SO on the external tympanum [10]. We note however that SOAEs have not yet been reported for invertebrate ears (only SOs).

The goal of the present study was to empirically characterize mechanical responses associated with the manifestation of SOAEs. Specifically, we aimed to examine vibrational correlates of SOAEs (i.e., SO) on the TyM. We hypothesized that we would observe similar spectral characteristics between SO and SOAE and that vibrational measurements might yield relatively improved signal-to-noise ratios (SNR) given the high sensitivity of laser-Doppler

vibrometry (**LDV**) systems (e.g., tens of picometers). We explored this connection comparatively with two different animal models: lizard and tree cricket. Lizards were chosen because they exhibit relatively robust SOAE behavior and have accessible TyMs. Similarly, tree crickets have a highly accessible TyM (appearing on the front legs), which has been demonstrated to show SO at $\approx$ 3 kHz [10].

METHODS

Measurements were obtained at two different locations. At York University, measurements were made in several green anoles (*Anolis carolinensis*). Lizards were lightly anesthetized. For acoustic-based SOAE measurements, output from a sensitive microphone (Etymotic ER-10C) was digitized using a customized data acquisition system. For mechanical-based SO measurements, a single-point LDV (Polytec OFV 511 and 3001) was used. At the University of Toronto Scarborough, several tree crickets (*Oecanthus nigricornis*) were examined. Animals were awake, but mounted to a holder with wax as shown in Fig.1B. Furthermore, vibrational measurements were made via a scanning system (sLDV; Polytec PSV 400), which more readily allowed for evoked responses to be measured as well (e.g., distortion products). At both locations, animals were placed in an acoustic-isolation booth. For the lizard, the OAE probe could be directly coupled to the external meatus on the side of the animal's head (Fig.1E). However, for the cricket such coupling was not readily feasible (see Fig.1D) and an "open" coupling configuration was used as shown in Fig.1E.

For spectral recording and averaging, cricket TyM SO measurements were done via the Polytec sLDV system (e.g., Fig3A). All other recordings (lizard SO and OAE, cricket OAE; including distortion product emissions or DPOAEs) were done using customized software. For SOAE and SO measurements, two types of spectral measures were obtained. First, buffers of 32768 points were obtained at a sample rate of 44.1 kHz with a 24 bit depth. The fast Fourier transform (FFT) was computed and the magnitude extracted. This was repeated 60 times in succession and the magnitudes averaged (e.g., Fig.2B). The second method entailed recording a 120 s waveform, and performing the analysis offline after the fact. There, 400 successive 8192 point buffers were spectrally averaged in a similar fashion (e.g., Fig.2A).

RESULTS

For lizards, robust SOAE activity was apparent in all ears examined, as long as the coupling between the probe and meatus was tightly sealed. In the condition where the coupling was wide open (but the probe tube tip within 1 mm of the meatus), there was a precipitous drop in spectral amplitude although most peaks were still apparent. A limited set of LDV measurements off the TyM were obtained, presumably due to relatively poor reflectivity or focus of the laser. Several spectral responses were obtained, as shown in Fig.2. While differences are apparent (e.g., the LDV measurements are relatively noisier), there was reasonable agreement between the two independent measurements in terms of peak location. The largest peaks in the two LDV spectra of Fig.2 correspond to roughly 60 pm.

A qualitatively different result was obtained for the cricket. In several animals, SO was clearly seen in the TyM vibration. Chiefly it manifested as a single peak in the range of 3-4 kHz with an amplitude as small as 10 pm (close to the LDV noise floor). The SO was not always stable, in that they would sometimes disappear into the noise floor for several minutes. The SO appeared highly confined to a small portion of the TyM, the majority of the tympanum not exhibiting any coherent sustained motion. However, no SOAE activity was measurable in any animal examined even when SO was simultaneously present. An illustrative comparison is shown in Fig.3. As an aside, for data collected from a closely related cricket species (*Oecanthus henryi*), detailed temporal analysis (via filtering in the spectral domain and subsequently computing the analytic signal) revealed characteristics of a sinusoid for the SO peak. This observation indicates evidence for a self-sustained oscillation, as opposed to (passively) filtered noise. Similar analysis for *O. nigricornis* was less clear, presumably due to a poorer signal-noise-ratio.

To explore this discrepancy further, we measured evoked "OAEs", both as acoustic responses and as TyM-based vibrations. First we attempted to measure a stimulus frequency OAEs (SFOAEs) using a 40 dB SPL stimulus tone via a suppression paradigm (suppressor was 15 dB higher in level and 40 Hz higher in frequency). No residual was seen above the noise floor in the either the LDV of acoustic responses, except for one animal where a weak signal was observed in the LDV response close in frequency to where the SO peak was. A rough estimate of the phase-gradient delay yielded approximately 2 ms, comparable to that seen for lizards. Second, we looked for distortion products using equal level primaries at 65 dB SPL separated by a fixed ratio of 1.07. No acoustic distortion (i.e., DPOAE) was detected, however numerous distortion products (DPs) were readily apparent in the TyM motion.

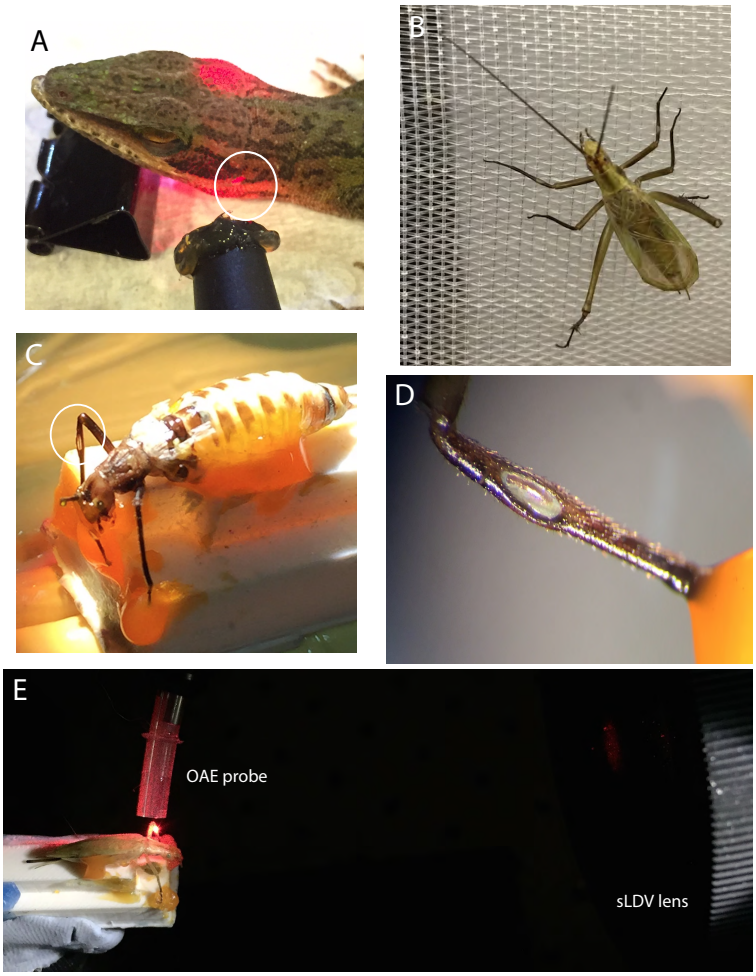

FIGURE 1. Panel A shows the setup for the anole, when set up to measure OAEs in the left ear (probe uncoupled here, white circle indicates location of the meatus) and vibration from the right ear (LDV out of field-of-view). For the cricket (panel B), panels C and D show the position of the TyM on the front leg (just below the "knee", white circle in C). Panel E shows the relative placement of sLDV and OAE probe (which allowed for simultaneous measurements).

DISCUSSION

The cricket tympanum could exhibit small, coherent spontaneous oscillations, consistent with a self-sustained sinusoid (e.g., [15]) if sufficient signal-to-noise was present. However, no acoustic counterpart was correspondingly observed as an SOAE (Fig.3). Several explanations could account for this. One is that the relatively small size of the TyM does not allow it to effectively radiate acoustic power, presumably due to its small surface area and non-isotropic nature (i.e., only a small surface fraction appears to move; see comment below by AT Christensen). The other relates to the "open" coupling of the OAE probe. As argued in previous studies (e.g., [7]), proper coupling is crucial, as such may affect the load impedance the TyM "sees" looking back out. Specifically, "*it is essential to seal the ear with the coupler in position and hence produce a closed system*" [7] and "*a nearly closed acoustic system* [$\cdots$] *is essential for sensitive DPOAE measurements especially in the low frequency range*" [11]. We note that this is in contrast to findings for SOAEs produced by humans [1], as well as subsequent insect OAE experiments (e.g., [13]) that also utilized "open" coupling. We attempted to vary the positioning of the OAE probe, to little effect, though we were not able to achieve a "closed coupling".

Our observations for evoked responses of the cricket TyM appear to be essentially the opposite of a previous study [12]: we observed DPs but no DPOAEs (they did not observe DPs, but did not measure for DPOAEs, a facet

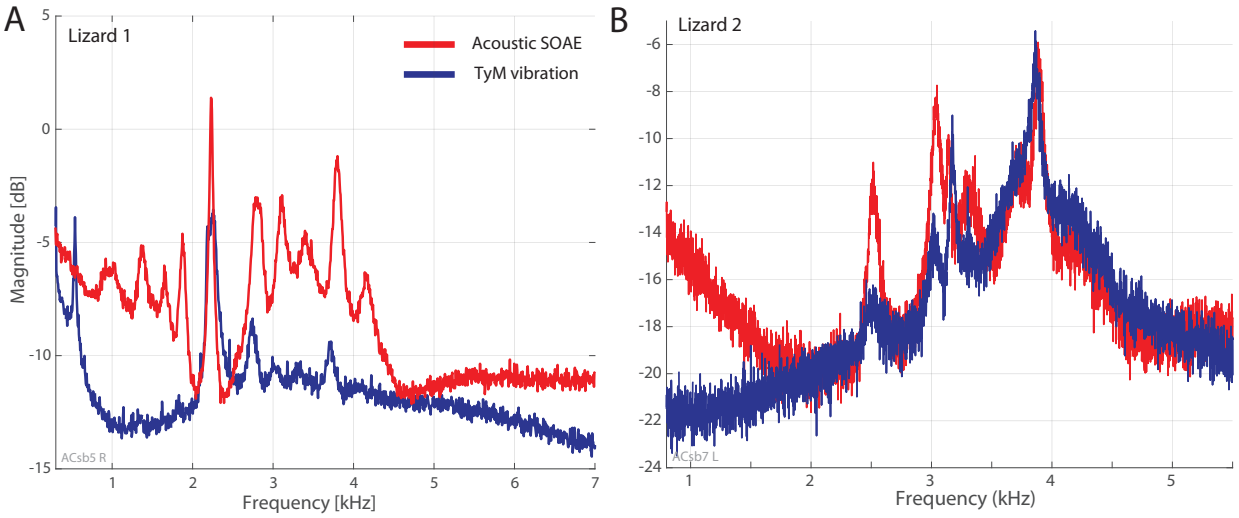

FIGURE 2. For two different lizards, a comparison of LDV-based vibration SO (blue) and acoustic-based SOAE (red) in shown. For the SOAEs, the scale is in dB SPL, while for the LDV the reference is dB re 0.13 nm. Thus the 3.9 kHz peak would correlate to a displacement of ~64 pm. Note the different axis limits between animals. Peaks associated with 60 Hz harmonics (i.e., electrical noise) have been removed for clarity, except for the peak in panel A at ≈0.54 kHz.

that induced criticism [7]). We note that a previous study [13] found good (but not perfect) correlation between DPs and DPOAEs for the TyM of a moth. The present data can thus further help inform the debate about "the location of the mechanical generation of" OAEs from insect ears.

For the lizard, we found reasonable correlation in the spectral amplitudes between acoustic-based SOAE and LDV-based vibrational SO (Fig.2). The displacements measured are relatively small (e.g., 60 pm, approximately the same as the Bohr radius for a hydrogen atom), especially when compared to spontaneous (saccular) hair cell bundle movements which are typically reported as being several orders of magnitude larger [9]. However, our measured SO displacements appear comparable to those reported for the mammalian basilar membrane [14]. We note that there are differences between the SOAE and SO spectra, and several (not mutually exclusive) possibilities exist to explain such. First, the drive from the inner ear may set the TyM into motion in a "discordant" fashion [3] (i.e., different spatial locations show strongly different spectral peaks, somewhat analogous to modal motion in a circular membrane). Coupled to that is the vector-nature of the velocity measurements (i.e., we made measurements at a single point, from a single incident direction). Second is that the effective radiation impedance was different between conditions (i.e., open-coupled LDV measures versus closed-coupled acoustic measures), thereby changing the membrane's vibrational profile (as similarly suggested above regarding the lack of cricket SOAE).

Looking ahead, future steps to study spontaneous vibrations include using an sLDV system for the anole to map out the SO vibrational amplitude across the entire TyM surface and ascertain if/how modal motion plays a role. Furthermore, imaging modalities such as optical coherence tomography (OCT) should be useful to examine papilla and hair cell motion in the Anolis inner ear (the optical path appears not to be relatively dense/complicated) as well as the underside of the cricket TyM where the relative motions of the cuticular plate, schlopedia, and ciliary cells are not well characterized.

ACKNOWLEDGMENTS

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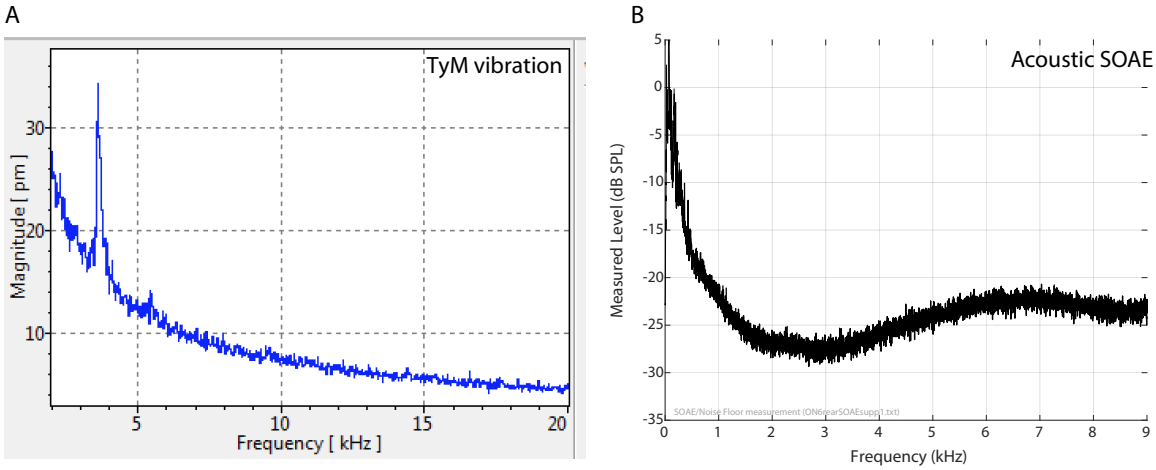

FIGURE 3. Comparison of LDV-based vibration SO (left) and acoustic-based SOAE (right) from a representative cricket, indicating the presence of SO but a lack of SOAE. The measurements were made simultaneously. Note the different axis limits between plots, including the linear ordinate on the left. Peaks associated with 60 Hz harmonics (i.e., electrical noise) have been removed for clarity.

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COMMENTS & QUESTIONS

[*Online Forum*]

Christopher Shera: Nice! Given the measured TyM velocity, an estimate of the area of the TyM that moves, and knowledge of the volume enclosed by your OAE probe, can you estimate the sound pressure level you would have expected to observe had you been able to achieve the closed configuration?

Response: An initial answer is that we need to give more careful thought as to how best to model the situation. For example, the load impedance presumably is pretty important and needs to be carefully considered. A recent review paper [Hambric, SA, & Fahnline, JB (2007) Acoust. Today, 3(2), 9-27] likely provides some relevant pieces that can be incorporated to help form a (reasonable) quantitative answer to this question (*see also next comment by AT Christensen*).

Anders T. Christensen: Hello, very interesting stuff..! The wavelength of that spontaneous cricket emission around 3.7 kHz in free air is 92 mm ($k = 2\pi/92\,[\mathrm{mm}^{-1}]$). And from what I can see radius of those cricket TyMs is on the order of a = 0.2 mm. Radiating such a long wavelength from such a small source is very inefficient.

For simplicity, we could pretend its TyM is a tiny pulsating sphere in free air going at an average speed of U_0 = 34 pm × 3.7 kHz = 13×10^{-5} mm/s. Then from the associated radiation impedance there's this formula for the pressure amplitude at a distance r from the center of it, say 2 mm away where we might put a microphone:

$$\begin{aligned} |p(r)| &= \rho_0\, c\, U_0\, (a/r)\, k\, a \\ &= 0.4133\,[\mathrm{Pa\,s/mm}] \times 13 \times 10^{-5}[\mathrm{mm/s}] \times (0.2\,[\mathrm{mm}]/2\,[\mathrm{mm}]) \times (2\pi/92\,[\mathrm{mm}^{-1}]) \times 0.2\,[\mathrm{mm}] \\ &= 0.070\ \mathrm{uPa} \\ &\approx -49\ \mathrm{dB\ re}\ 20\,\mu\mathrm{Pa} \end{aligned}$$

If you can find a cricket with an emission closer to 10 kHz, you're closer to the noise floor of the measurement. Anyway, I think you're right a closed coupling would make all the difference.

Response: As a tangent to this comment, we note that relatively (extremely) large evoked emissions (e.g., SFOAEs, DPOAEs) have been observed in small lizards such as the side-blotched lizard (*Uta stansburiana*; unpublished), whose inner ear contains only approximately 60 hair cells and the TyM is small compared to most other vertebrates. For example, equal level primaries at 65 dB SPL (with ratio 1.07 or 1.22) evoked DPOAE cubic intermodulations (i.e., $2f_1 - f_2$ and $2f_2 - f_1$) with amplitudes up to 30-40 dB SPL [compare to Bergevin C et al (2008). J. Comp. Physiol. A, 194(7), 665-683].

locustearman (anon.): Nice to see a bridging of the invertebrate and vertebrate aspects of spontaneous activity in hearing organs. In the cricket tympanum were you able to focus your displacement measurements where the auditory neurons attach to the membrane?

Response: The auditory neurons of crickets do not attach directly to the membrane and so this is not possible. We measured tympanal deflection at the position of maximal displacement during sound driven motion based on previously reported scans. This is also the position from which spontaneous oscillations have been previously reported.

[*Post-talk Q&A*]

Marcel van der Heijden: Very beautiful. I have a comparative question. In the examples you showed across a wide variety of species [*including SOAE data from humans and barn owls*], the intensity of the spontaneous emissions were comparable, say ≈ 10 dB SPL. But the neural and behavioral thresholds across species are highly variable. So the usual connection between these active processes that generate these emissions and sensitivity might be questionable. So could we instead be looking at some sort of side effect of adaptation, and not some sort of sensitivity-enhancing mechanism?

Response: Perhaps the best way to answer this would be to also look at a comparison of the audiograms across these species, although such measurements/plots do not (yet) exist. Inferring from other species, lizards in their most

sensitive frequency range (0.7-5 kHz), which also corresponds to where SOAE activity manifests, have thresholds around 0–20 dB SPL [e.g., Turner RG (1987) Hear. Res., 26(3), 287-299; Brittan-Powell EF et al. (2010) J. Acoust. Soc. Am., 128(2), 787-794.]. We know where humans stand (i.e., 0 dB SPL), and barn owls can exhibit thresholds downwards of -20 dB SPL, though their thresholds are likely more comparable to 0 dB SPL as they get a passive boost due to their external "ears" [i.e., the "facial disk"; Konishi, M. (1973) Am. Sci., 61(4), 414-424]. So to first order, within reason, all of these species show similar sensitivity (at least within their optimal frequency range).

Jont Allen: If you take the spacing between SOAE lines, you get a reciprocal delay, which can yield information about travel time. What does this sort of analysis reveal if you compare those delays across animals?

Response: That sort of analysis has been looked at in some detail [see Bergevin C, Manley, GA, & Koppl, C (2015) Proc. Nat. Acad. Sci., 112(11), 3362-3367; Bergevin, C. et al (2012) Hear. Res., 285(1), 20-28.]. In short, delays inferred from SOAE peak spacing correlate well to SFOAE phase-gradient delays. However, at least for the barn owl, there are some deviations relative to auditory nerve fiber tuning.

John Oghalai: So this is really interesting. Why do you think the bandwidth of the peaks are different?

Response: A substantial body of work has been done by researchers such as Pim van Dijk looking at this sort of question [e.g., van Dijk P, & Wit HP (1990) J. Acoust. Soc. Am., 88(4), 1779-1793]. Presumably the width of SOAE peaks is tied to temporal fluctuations of center frequency and/or amplitude, as well as possible inter-peak interactions. So SOAE peak width is likely telling us something important, but precisely what still remains to be better clarified. We note that just looking at "peaks" alone provides a relatively superficial view of what is going on though. Consider that for a given peak, properties of the (filtered) analytic signal for a lizard clearly show evidence for a self-sustained sinusoid, while for a barn owl you typically see what looks like filtered noise [e.g., Bergevin C, Manley GA, & Koppl C (2015) Proc. Nat. Acad. Sci., 112(11), 3362-3367]. Despite that, owl SOAE peaks tend to be larger/narrower than those of a lizard. (*see also next comment by C Abdala*).

Another caveat is that different people might provide a different response here. For example, Geoff Manley has argued that the tectorial membrane (TM) is key, and that lizards with an overlying TM show fewer, but larger/sharper, SOAE peaks. But I (*CB*) have seen (in species such as Anolis, which lack a TM over most of the papilla) that there is a broad range of SOAE spectral properties: small/wide peaks, tall/narrow peaks, and everything in-between. So while the TM is likely playing some sort of important mechanical role, it in of itself is not the entire story and there are likely a variety of ways that inter-hair cell coupling manifests.

Carolina Abdala: Along the lines of John's question, newborn babies have broader bandwidth SOAEs than young adults. We do not know exactly why, but we have some ideas about intra-cochlear noise (as measurement noise can easily be ruled out). But to Marcel's question, if you look within a species, say humans only, where we looked across the lifespan, changes there do correlate to sensitivity changes. That is, SOAE prevalence and SNR decrease with age in a fashion that correlates with changes in the audiogram [Abdala C, Luo P, & Shera CA (2017) J. Acoust. Soc. Am., 141(3), 1874-1886.].

Eric LePage: Thanks very much, that is a great cross-comparison. What about the case of the dog, where extreme examples can help you and tell you something important. I tested a dog once, which had a 63 dB emission in one ear and a 56 dB one in the other, with a 3.7 kHz frequency modulation. A year later, that frequency modulation had dropped, but the basic emission was still there. What kind of insight can dogs help us with?

Response: Not sure how to best answer within the context of dogs per se, but there are clearly idiosyncratic cases (and likely pathological) that have value in detailed study. But how to best approach those individual (and, again, likely pathological) cases is unclear. Interested readers are also directed towards "Mario's dog story" [Ruggero MA, Kramek B, & Rich NC (1984) Hear. Res., 13(3), 293-296].

Christopher Shera: Very nice. One of the early slides in your presentation, you showed a comparison between the spontaneous oscillations observed both acoustically and mechanically in the anole (*e.g., see Fig.2*). It looked like there were many acoustic peaks that had no corresponding mechanical peak. It did not seem to be an SNR problem. Do you know what the origin of that discrepancy is?

Response: As the TyM is driven from the inner ear, it presumably moves in a complex fashion. The blue curve in Fig.2 represents motion of a single point on the TyM. But the acoustic response is the sum of the motion across the entire TyM. So perhaps this single point just illustrates one "mode" of TyM motion, and we just are not seeing energy tied to the missing SOAE peaks [*see also Fay JP, Puria S, & Steele CR (2006) Proc. Nat. Acad. Sci., 103(52), 19743-19748.*]. Presumably the motion measured at different locations would "fill in" some of those gaps. We aim to look at this using a scanning LDV system, comparing spectral measurements across the surface. We would hypothesize that sum all those responses together would yield something that would look like the SOAE response (red curve in Fig.2). [*see also the Discussion section above*]