ABSTRACT: Much of what is known about the generation of otoacoustic emissions (OAEs) derives from mammals and takes account of relevant mammalian anatomy and physiology. Since non-mammals have OAEs, but lack some mammalian features, such as basilar-membrane traveling waves and hair-cell somatic motility, our goal is to learn more about OAE generation through a systematic study of evoked OAEs at lower stimulus levels. We examined four species with very different inner-ear anatomies: humans, chickens, geckos, and frogs. In mammals, the phase gradients (slope of emission phase with respect to frequency) indicate that there are at least two fundamentally different generation mechanisms. Here, the specific question we aim to answer is: In light of anatomical and physiological differences, is there evidence for multiple generation mechanisms in the non-mammalian vertebrates? Our results show many similarities among the four species in emission properties, such as significant level-dependent SFOAE phase gradients on the order of at least 1 ms (significantly larger in humans), non-linear (and sometimes non-monotonic) emission growth with stimulus level for both DPOAEs and SFOAEs, and the presence of spontaneous OAEs. A key difference in our data among species, however, stems from the relation between SFOAE and DPOAE phase gradients as well as gradients between lower (2f1-f2) and upper-sideband (2f2-f1) DPOAEs. Unlike the data in humans and chickens, the gradients provide no evidence of multiple generation mechanisms for lower versus upper-sideband DPOAEs in geckos and frogs. In addition, chickens have a frequency-independent lower side-band DPOAE phase (similar to humans), supporting the presence of a scaling-symmetric traveling wave in the avian ear, in agreement with previous mechanical measurements.

MOTIVATION

- Otoacoustic emissions (OAEs) reveal much about the function of the inner ear, but there is still much we need to understand about how these emissions are being generated and subsequently emitted. By further elucidating the origins of OAEs, we stand to make significant gains in how to better utilize OAEs in the clinic.

- Much of the effort made towards better understanding OAEs has focused on mammals, where certain anatomical and physiological features such as basilar membrane (BM) traveling waves and hair cell somatic motility have played integral roles.

- Numerous studies have examined OAEs in a wide array of non-mammals that lack some of these mammalian features, but have not made a systematic comparison of a number of different OAE properties (such as phase behavior) across species.

GOAL: Our purpose here is to make a comprehensive comparison of OAE properties across wide array of species, whose inner ear anatomy and physiology vary significantly. We hope to gain insight into what features are generic or species-specific and use that to better understand how OAE generation mechanisms are similar and different across ears.

BACKGROUND

We chose four species to examine here:

- **HUMANS** evidence indicates OAEs arise via two fundamentally different mechanisms, one based on a reflection source and one on nonlinear distortion (i.e. proposed *taxonomy*; Shera and Guinan, 1999)
- **CHICKENS** studies have indicated birds lack hair cell somatic motility, but may exhibit a traveling wave along their BM
- **GECKOS** one of the few vocal lizard species, evidence has indicated a lack of BM traveling waves and somatic motility
- **FROGS** this species lacks a flexible BM altogether and hair cells sit atop rigid supporting cells

- Despite widely varying features, all these species exhibit high sensitivity (~0-30 dB SPL at their most sensitive frequencies, with some geckos well below 0 dB SPL!) and relatively sharp tuning. Furthermore, all species exhibit some degree of efferent innervation.

- A common anatomical thread here is that all species possess stereociliary hair cells and some form of overlying tectorium which couples these cells together (in addition to fluid coupling).

- One unique aspect of this study is the emphasis on emission phase behavior and the associated *phase* gradients (sometimes referred to as group delays). These gradients have led to considerable insight into OAE generation and form the basis for classification criteria in the mammalian OAE taxonomy.



- Two different types of emissions were examined:



Simplified schematic of different species' inner ear anatomy. Two perspectives are provided fo each species: a cross-sectional view (top) and a top-down view of the sensory epithelium (bottom). Except for the frog, the arrows in the top-down view represent an individual hair cell (HC), the direction indicating bundle's polarization (pointing from shortest to tallest row). For the frog, the entire longitudinal length of only one auditory organ (AP) is shown and arrows indicate gross trends of the HCs (the finely dashed bounding box corresponds to where the cross-section would lay and the coarsely dashed line represents where the sensing membrane extends down from the roof of the recess). HCs shown to exhibit cell body somatic motility are indicated by a star on their tails. In the cross-sectional view, white regions are fluid-filled grey regions correspond to overlying tectorium (with dark grey lines indicating fibrillar structures), grey striped area represents bone and stippled areas are non-HC cellular regions (i.e. supporting cells, etc.). Legend is as follows: AP- amphibian papilla, AR- amphibian recess, BM- basilar membrane, BP- basilar papilla, FN- fundus, LL- limbic lip, SA- sallet, SC- sallet chain, SE- sensing membrane, SM- scala media, STscala tympani, TC- tunnel of Corti, TM- tectorial membrane.

NEURAI

longitudinal

1. Stimulus Frequency Emissions (SFOAEs) - when ear is stimulated with a single tone (fp) presented at intensity Lp, these OAEs arise at that probe frequency

2. Distortion Product Emissions (DPOAEs) - stimulation with two different tones (f1 and f2) evokes these emissions arising at harmonic and intermodulation frequencies (3f₁, 2f₂ - f₁, etc.)

- We used the same measurement system/paradigms for all species. An Etymotic ER-10C probe containing a microphone and two earphones was tightly coupled to the outer ear and calibrated using flat-spectrum noise. Measurement system intermodulation distortion was close to the acoustic noise floor (or about 100 dB below the primary levels), however the harmonic distortion was not well characterized.

- Two different gecko species were used here. Leopard geckos (Eublepharis macularius), while being ~20 dB more sensitive, lack the wealth of auditory nerve fiber (ANF) data as has been reported for the more vocal tokay gecko (Gekko gecko). Their overall inner ear anatomies are relatively quite similar. Adult leopard frogs (Rana pipien pipiens) were also used as were P14-21 chickens (Gallus domesticus).

- Geckos and frog were lightly anesthetized (Nembutal, 25-45 mg/kg i.p.) for experiments, but all recovered completely and were subsequently used for multiple sessions. Chicken were heavily anesthetized (urethane, 2.5 g/kg i.m.) and tracheotomized, being subsequently sacrificed at the end of the experiment. Awake human subjects sat comfortably in a noise reduction booth.

- SFOAEs were measured using a suppression paradigm in which the OAE was obtained by vector subtraction of the probe frequency components measured in response to the probe alone and in the presence of a nearby suppressor (f_s) and intensity Ls [Shera and Guinan, 1999].

Otoacoustic Emissions in Humans, Birds, Lizards and Amphibians: A Comparative Study Reveals Differences in Emission Generation Mechanisms Christopher Bergevin, Dennis Freeman, and Christopher Shera Harvard/MIT HST Speech and Hearing Bioscience & Technology Program

RESULT 1 - Comparison of Different OAE Phase Gradients

- While studies have examined non-mammalian OAEs, very few have looked at the frequency dependence of emission phase. Fig.2 shows OAE phase curves for three different emissions using a fixed set of stimulus parameters across all species.

- One notable observation is the relative frequency invariance of 2f1-f2 in the human ear (when using a fixed primary ratio during the frequency sweep), as has been described previously. This difference relative to the 2f2-f1 and SFOAE has provided much insight into how emissions are generated in the mammalian cochlea, indicating the presence of two distinct mechanisms and establishing criteria for an OAE *taxonomy*. It is thought this flat 2f1-f2 response results from [1] local scaling and [2] *wave-fixed* generation mechanisms (such as nonlinear distortion).

- A similar observation is made in the chicken, which exhibits a relatively flat 2f1-f2 compared with the other two emissions. This suggests that the mammalian taxonomy is also applicable in the avian ear.

- In contrast to the human and chicken, both the geckos and frog do not show this pattern, with all three phase curves running roughly in parallel (at least using these higher stimulus levels). These gradients provide no evidence that SFOAEs and DPOAEs arise by different mechanisms in the frog and gecko.



FIGURE 3- Comparison of emission phase across different OAE types. NOTE the different scales across species Chickens appears qualitatively similar to humans in that 2f1-f2 exhibits a strong degree of frequency independence. This is in contrast to the other three species. However, all species exhibit significant phase delay (on the order of 1 ms or greater). For each species, multiple individual ears are plotted using varying line thickness, the total number indicated by N (plots also include repeated measurements in some ears at different experimental sessions, as indicated by the bracketed number which shows the total number of curves plotted). Some phase curves were offset vertically by an integral number of cycles for clarity. Stimulus parameters - SFOAE: Lp=40 dB SPL, Ls=55 dB, fs=fp+40 Hz, DPOAE: L1=L2=65 dB SPL, f2/f1=1.22 (constant)

RESULT 3 - Magnitude & Phase Gradient Level Dependence

- Evoked emission dependence upon stimulus intensity has revealed nonlinear behavior (across a number of species) that is not well understood. Fig.4 shows how SFOAE depends upon Lp as fp is swept in an individual ear for a human and gecko.

- In both species, SFOAE magnitude reveals that growth has frequency and leveldependent regions of nonlinear growth, which can also be non-monotonic (i.e. notches with respect to level at a given frequency).

- Phase curves also exhibit a level dependence similar in both species: gradients are independent of level at low intensities (below ~40 dB SPL), and *shift* to a smaller value at stimulus higher intensities.



- Human phase gradient level dependence shown here is consistent with that reported y Schairer et al. (2006), though they did not use stimulus levels below 40 dB SPL.

- Note that the gecko emissions start falling off at higher frequencies (whereas in the human they extend beyond the range

- The similar level dependencies of the SFOAE phase gradients occur despite large differences in anatomy and physiology between the two species.

SFOAE Probe Level
← Lp=50 dB
$\begin{array}{c} & & Lp=20 \text{ dB} \\ \hline & & Lp=10 \text{ dB} \\ \hline & & Lp=0 \text{ dB} \\ \hline & & Noise \text{ Floor} \end{array}$

FIGURE 4 - SFOAE level dependence in an individual humar and gecko (results typical for given species). Note that in both species, phase gradients are independent of Lp at lower intensities, but shift to a smaller value at higher intensities (when Lp is around 40-50 dB SPL). Legend shows probe level used to evoke emission across each sweep. Suppressor level was 15 dB above probe, but never less than 35 dB SPL. Suppressor frequence was always 40 Hz above that of the probe. Error bars show standard error of the mean across 35 averages taken at any given frequency/level. Some phase curves were offset vertically an integral number of cycles for clarity.

RESULT 2 - SFOAE Phase Gradients Across Species

While emission phase behavior can reveal much about generation mechanisms, computing the slope of these curves (hence referred to as the gradient) can tell us about time delays associated with the OAEs.

- Fig.3 shows N_{SFOAE} versus frequency in each of the four species. N_{SFOAE} is the phase gradient expressed in units of stimulus periods (gradient * emission frequency). This dimensionless delay is easier to compare with other dimensionless values of tuning, such as Q (see RESULT 4).

- In all species, N_{SFOAE} generally increases with stimulus frequency (the frog being the slight exception, see **RESULT 4 for discussion**)



Emission Frequency [Hz]

· Comparison of N_{SFOAE} (phase gradient \star fp) across species. All species exhibit significa delays (as indicated by the phase gradients), but they are significantly longer in the human. For a given pecies, multiple individual ears are plotted (each indicated by varied marker size). For the human, lata from Shera and Guinan (2003) are also included (as red dots). Phase gradients are taken as the local derivative of the phase vs. frequency function. N_{SFOAE} values were excluded if the magnitude was not at least 10 dB above the noise floor. Trend lines (loess) are included to help guide comparison. Stimulus parameters: Lp=40 dB SPL, Ls=55 dB, fs=fp+40 Hz.

- Delays associated with the human are significantly longer than those observed in the other species (which can also be seen comparing across species in Fig.2), being ~8-12 ms over the frequency range of 0.5-5 kHz.

- Despite differences, all species exhibit significant phase gradients indicating a delay on the order of at least 1 ms. It is not clear where this delay arises, but it may be a reflection of mechanical frequency tuning (see RESULT 4).

RESULT 4 - Correlation Between SFOAE Delay and Tuning

- It has been proposed that OAEs offer a non-invasive correlate to measures of tuning sharpness, as typically measured via ANF responses. Fig.5 shows how SFOAE delay measurements compare to those tuning estimates across species (various reports are included to indicate the degree of variation across ANF studies).

- Overall trends in both chickens and geckos correlate well to the Q-values, at least at higher frequencies (above ~1 kHz). As indicated in the gecko plot of Fig.5, it is important to use SFOAEs evoked with low intensity stimuli for comparison (also see Fig.4).

- Results in the frog are markedly different from the other species. A major factor to be considered is that frogs have two distinct auditory sensory organs. In the species examined here, one organ (whose anatomy is shown in Fig. 1) responds to frequencies below ~1.2-1.3 kHz while the other to frequencies above this. The comparison for the frog in Fig.5 indicates that there is a significant additional source of delay associated with the frog SFOAEs and that Q does not systematically increase with frequency (as is seen in the other species), suggesting their two organs may process sound very differently relative to the other species.

Frequency [Hz]

- ANF-derived Q-values are not available for humans (in contrast to the other species) so psychophysical estimates obtained using a forward masking paradigm at low stimulus levels are shown for comparison.

FIGURE 5- Comparison of N_{SFOAE} (phase gradient * fp) with that of either psychophysically (human) or ANFderived Q-values across four different species. **NOTE** different scales across species. SFOAE probe levels were chosen to be in the species 'low-level' regime (N_{SEOAE} is shown for two different Lp values in the Tokay to emphasize the level-dependence, see RESULT 3). orrelation is observed in all species except for the frog, which exhibits emission delays significantly longer relative to those expected from ANF measurements. Also, frog Q-values do not systematically increase with frequency as seen in the other species. Trends lines (loess) have been included to help guide the eye. Unless otherwise noted, Lp=40 dB SPL for SFOAE measurements.



Ronken (1991

Frequency [Hz]

1 - DPOAE phase gradients are qualitatively similar in humans and chickens (i.e. flat 2f1-f2), suggesting the presence of a scaling symmetric BM traveling wave. This is in contrast to the results seen in the gecko and frog (at least for the stimulus range used in Fig.2).

2 - SFOAE phase gradients are significantly larger in the human, although gradients in all species indicate a significant delay on the order of at least 1 ms.

3 - Both humans and geckos show a level-dependent transition in their phase gradients at moderate stimulus intensities (~40 dB SPL), gradients becoming smaller at higher levels. This transition occurs despite numerous anatomical and physiological differences between the two.

4 - In chicken and gecko, SFOAE phase gradients and Q_{10 dB} derived from ANF recordings have a similar frequency dependence. This suggests OAEs can provide a non-invasive means of estimating tuning. However, this is not the case for the frog where there appears to be an additional significant source of delay associated with their emissions.

SUMMARY: The comparative study here has revealed that many OAEs properties are common across species (i.e. significant delays, nonlinear growth and level-dependent phase gradients) despite differences in BM tuning and somatic motility. However, some emissions properties do appear unique to certain ears (e.g. a frequency independent 2f1-f2 phase) and suggest that certain physiological features (such as traveling waves) play an important role in OAE generation.

FURTHER QUESTIONS

- What role are generic morphological features (such as stereociliary hair cells and an overlying tectorium coupling nearby *sources* together) playing in OAE generation? [also need to consider OAEs in non-vertebrates who lack these features]

- motility)?
- efferent effects?

value?



The support of James Saunders in acquiring the chicken data is greatly appreciated. Input from P. Fahey, J. Guinan, J. Rosowski, AJ Aranyosi, N. Kiang and B. Peake was of much value. D. Cotanche made insightful suggestions regarding chick TM anatomy. Lastly, the OAE work of G. Manley, and C. Koppl (lizards) as well as that of P. van Dijk, S. Meenderink and P. Narins (frogs) served as an important reference. This work was supported by R01 DC003687 (CAS), R01 DC00238 (DMF), and T32 DC00038 (SHBT training grant) from the NIDCD, National Institute of Health. All experimental protocols were subject to the MIT DCM/CAC, MIT COUHES, MEEI Human Studies Committee and UPenn Animal Studies Committee approval.



MIT Micromechanics Group **EPL Auditory Physics Group**

RECAPITULATION

... and what about species-specific features (such as BM traveling waves and somatic

- What kind of information is the OAE phase gradient level-dependence telling us about how the ear processes lower and higher intensity sounds differently? Possible role of

- What is the source of delay seen for OAEs and how does it compare across species? How does this delay relate specifically to *traveling waves* (or lack thereof)?

- How have mammalian features evolved differently to provide better higher frequency hearing? And how might mechanisms to allow higher frequency detection in chicken and gecko compare, considering their relative absence in the frog?

Would estimates of sharpness of tuning based upon OAE phase gradients be of clinical

ACKNOWLEDGMENTS



The Harvard-MIT Division of Health Sciences and Technology