Auditory Brainstem Responses and Otoacoustic Emissions in Lizards: Comparisons across Species and Temperatures

Christian Brandt[1], Christopher Bergevin[2], David Velenovsky[3], Kevin Bonine[4], Jakob Christensen-Dalsgaard[1]

[1] Center for Sound Communication, Institute of Biology, University of Southern Denmark, [2] Otolaryngology/Head & Neck Surgery, Columbia University, [3] Speech, Language and Hearing Sciences, University of Arizona, [4] Ecology & Evolutionary Biology, University of Arizona

Introduction

Lizard ears are highly diverse and therefore provide a unique window into how sound is transformed from vibrations in air into neural signals (i.e., forward transduction)..For example, properties such as the number of hair cells (ranging over 50-2000) and tectorial membrane (TM) morphology can vary significantly across species. The relative simplicity of the lizard ears (for example the absence of a basilar membrane) is an advantage for studies aiming at understanding the relationship of inner ear morphology to mechanisms of sensory transduction (Bergevin 2010).

Recent reports have used stimulus-frequency otoacoustic emissions (SFOAEs) (Bergevin et al. 2010) and spontaneous emissions (Manley 1997) to explore how various morphological features, such as presence or absence of a tectorial membrane in addition to temperature variations (lizards are ectothermal), affect certain functional aspects of the lizard ear such as frequency selectivity. Since SFOAEs are an indirect measure of sensitivity, it is desirable to establish a more direct connection between SFOAE generation mechanisms and the actual forward transduction.
To this end, we measured both auditory brainstem responses (ABRs) and SFOAEs in the same animal for several different species: Whiptail lizards (Aspidoscelis), Tegus (Tupinambis), & Alligator lizards (Elgaria) at two temperatures.

Methods

Three species of lizard was studied: Whiptail lizards (Aspidoscelis)(N=3), tegus (Tupinambis)(N=3), and alligator lizards (Elgaria)(N=8) (both Northern and Southern alligator lizards). The whiptail and alligator lizards were anesthetized with Nembutal, 35mg/kg. The dose used for the Tegus was 32mg/kg.

All data (except fig 2) are from the steady-state temperature condition. Lizards were placed on a heating pad (initially turned off) and allowed to settle to ambient room-temperature (~24-26°C). After the COOL recordings were made, the blanket was turned on, after approximately 15-20 min. a stable temperature was reached (~29-32°C), at which point the HOT recordings were made. Temperature was monitored by a calibrated thermocoupler placed in the mouth.

SFOAEs was measured using an Etymotic Research ER-10C probe consisting of an earphone and microphone (+20dB) and custom PC (see Bergevin et al. 2008 for details), and the probe was calibrated in-situ.

Auditory brainstem responses (ABR) were recorded using subdermal needle electrodes, a low-impedance head-stage and preamplifier (Tucker-Davis Technologies, RA4LI + RA4PA) controlled by a digital signal processor (Tucker-Davis Technologies, RM2) and a PC using customized software. The signal was averaged from 400 measurements. Sound stimuli were emitted by the same ER-10C probe as in the OAE measurements. The stimuli used were a broadband click alternated with the click in a pure tone masker. The difference signal (unmasked – masked) is a measure of the sensitivity to the pure tone. Presentations at different levels of the tonal masker were used to determine thresholds and generate audiograms (see Christensen-Dalsgaard et al. 2011 for details). For the latency measurements a tone burst stimulation at 1000Hz (duration 3ms 0.5ms rise fall time) was used. The latency was defined as the delay from sound reaches the eardrum until the first positive peak (P1) response.

Results

**Effect of temperature on ABR Audiograms and SFOAE magnitude.**
For all species in the experiment we saw a change in response with temperature for both ABR audiograms and SFOAEs. For the alligator lizards the change in ABR audiogram was 3-5dB at frequencies above 5 kHz, and the magnitude of the SFOAE was 5-10dB at frequencies above 5 kHz. At frequencies below 5 kHz we saw no clear change with temperature in the alligator lizard. For the Whiptail lizard and Tegu the change was larger., ABR audiograms showed an increased sensitivity at frequencies above 2 kHz with a maximum increase of sensitivity at 4 kHz of 5-20dB. The SFOAE magnitude also changed, mostly above 2 kHz with a maximum increase of sensitivity at 3-4 kHz of 20dB.

**Latency shift for P1 of the ABR signal with temperature.**
For all animals tested the latency of the first peak of the ABR measurement decreased with increasing temperature. For the alligator lizards the decrease was 0.09-0.11 ms/°C. For the Whiptail lizard the decrease was 0.16-0.17ms/C and for the Tegu the decrease was 0.18 ms/°C. The Tegu had the longest P1 latencies between 1.47 and 2.21ms. The alligator lizards had P1 latencies between 0.95 and 1.7ms and the Whiptail lizard had P1 latencies between 0.61 and 1.51ms.

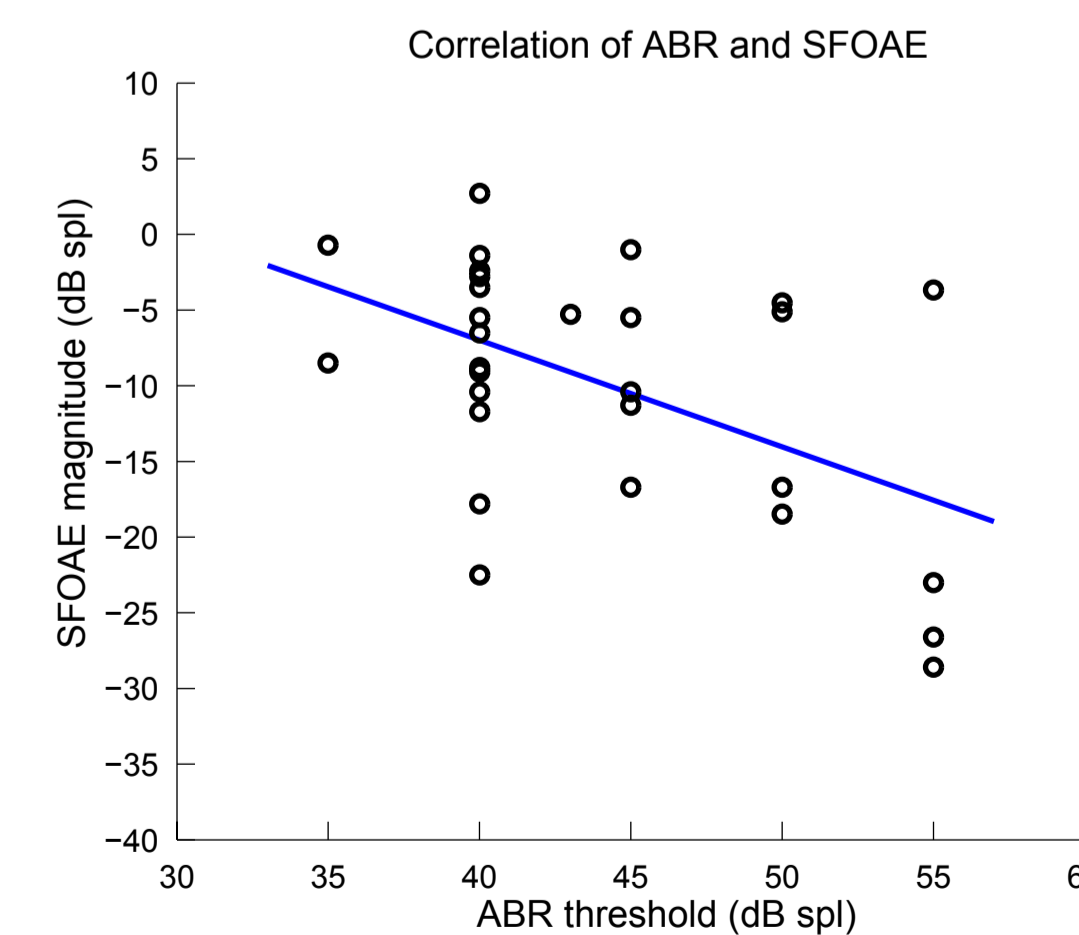**Correlation between ABR thresholds and SFOAE magnitude.**
When comparing the ABR threshold with the magnitude of the SFOAE at the closest measured frequency we found a significant correlation (Pearson Rho = -0.51, Pvalue = 0.0045).
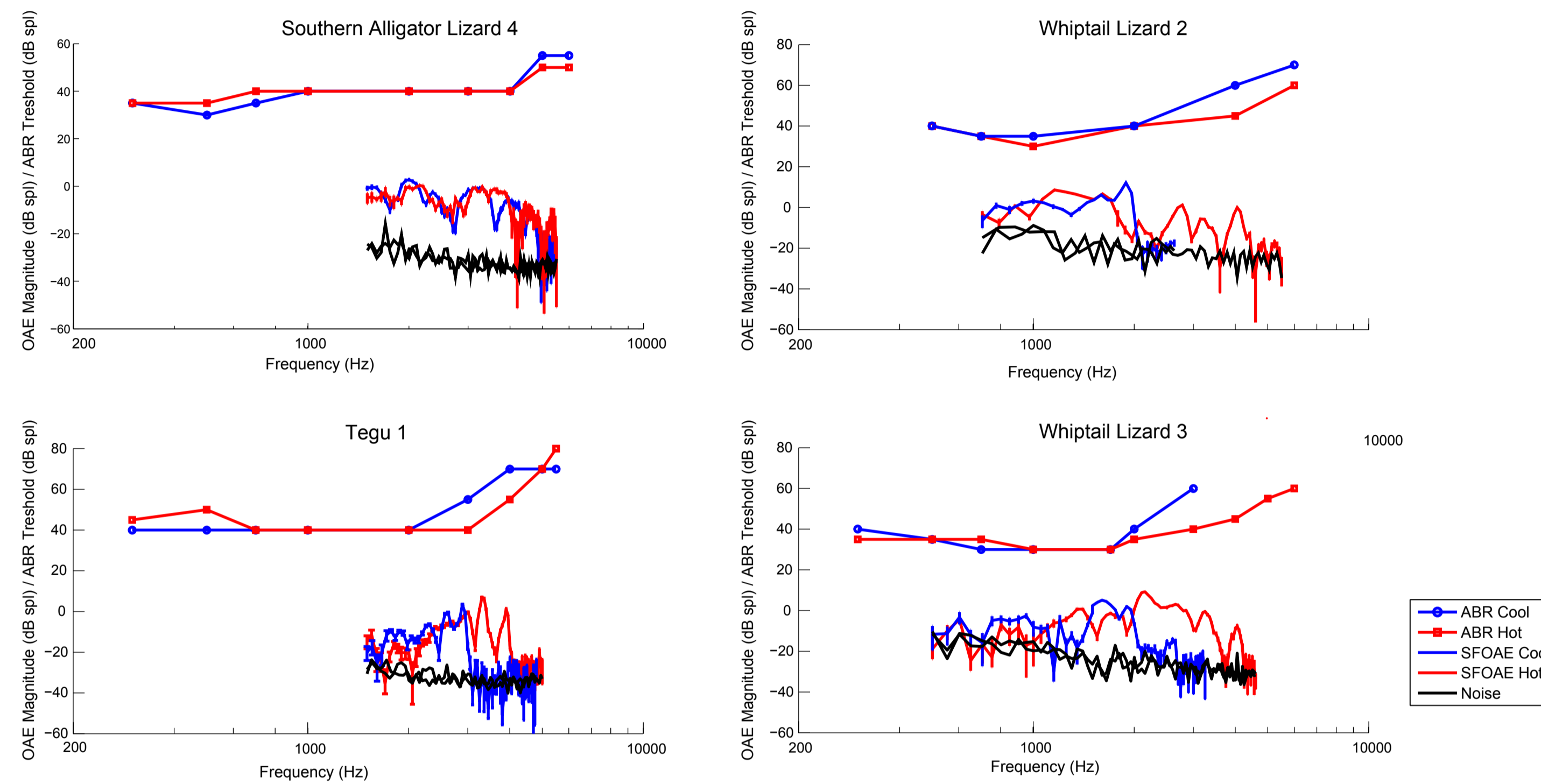
Figure 1. SFOAE and ABR activity shifts in a similar fashion with regard to changes in temperature, 'SFOAE and ABR activity appear broadly correlated in that ABR thresholds are lowest in regions where SFOAE activity is greatest. When SFOAE magnitudes fall off at higher frequencies, where ABR thresholds increase.
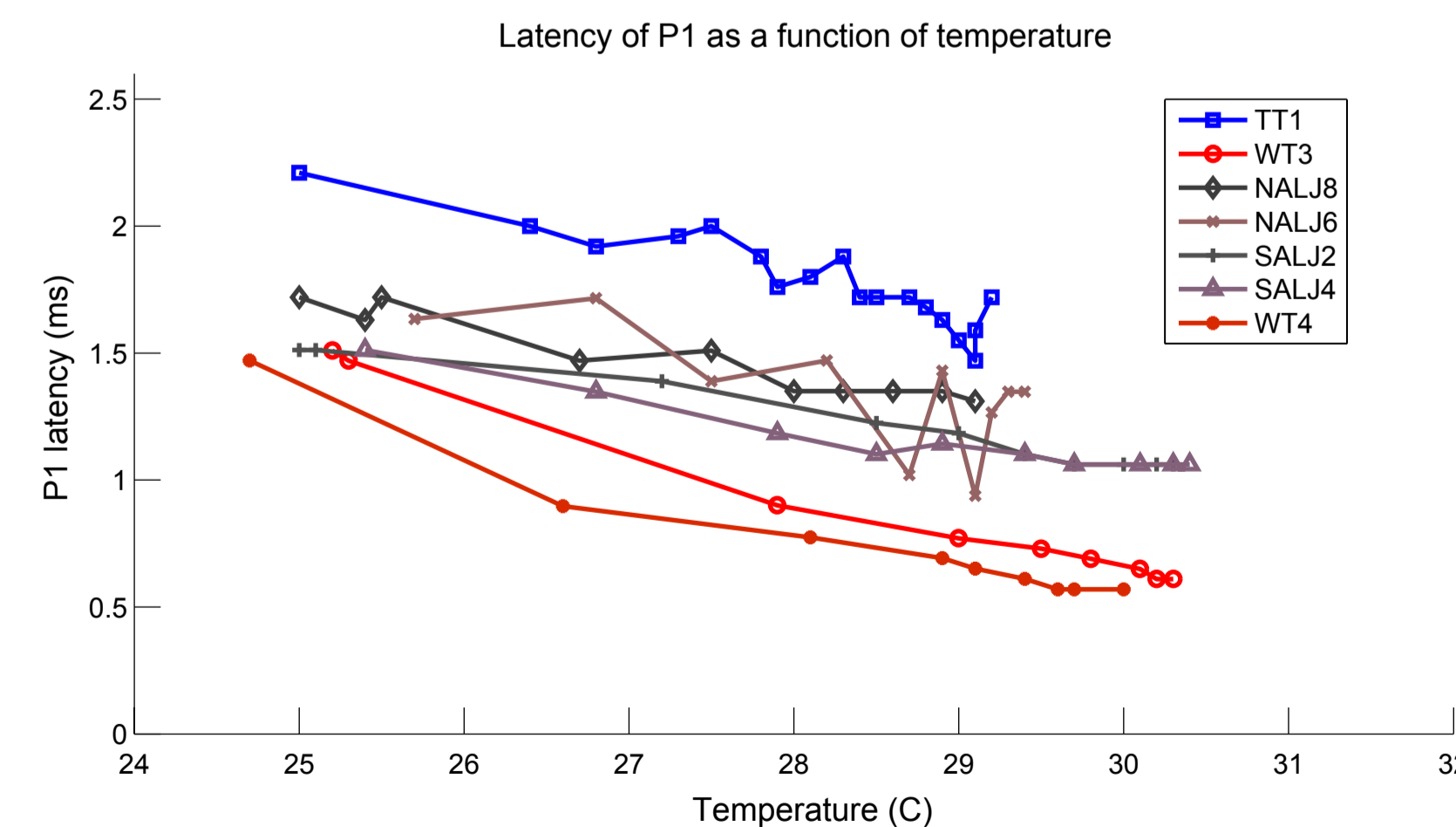
Figure 2. Latency of P1 in the ABR as a function of temperature. The Tegu has the longest latency (1.47-2.21ms) with a slope of 0.18 ms/°C. The Alligator lizards have a shorter latency (0.95-1.7ms) with a slope of 0.09-0.11 ms/°C Whiptailed lizards had the shortest latency(0.61-1.51 ms) and a slope of 0.16-0.17 ms/°C.

Figure 3. Correlation between ABR and SFOAE. There is a significant correlation between the magnitude of the SFOAE and the ABR threshold (Pearson Rho = -0.51, Pvalue = 0.0045).

Discussion

Correlation between ABR and OAE.
We saw a significant correlation between the ABR audiogram and the magnitude of the SFOAE. High-amplitude SFOAEs are correlated with the highest sensitivity in the ABR audiogram, and increased temperature produce both higher sensitivity of the ABR audiogram and increased magnitude of SFOAEs. Interestingly, these effects are confined to frequencies above 1 kHz. . This could indicate that they both reflect the same underlying property, most likely the sensitivity of the inner ear. Thus, it should be possible to correlate SFOAEs with auditory sensitivity and to estimate auditory thresholds based on SFOAE amplitude in lizards.

Latency shift for P1.
We found a decrease in latency with increasing temperature for the ABR measurements. The decrease was larger (0.16-0.18ms/°C) for the Whiptail lizard and Tegu than in the Alligator lizard (0.09-0.11 ms/°C). As in the audiograms we saw that the temperature effects appeared more pronounced in species with a continuous overlying tectorial membrane (Whiptail, Tegu) than those without (Alligator lizard). As previously suggested by Manley,(1997) this disparity likely stems from differences in coupling strength across hair cells,. The latency shift with temperature likely reflects an increase in sensitivity with increasing temperature, since the slope is comparable to the level-latency function reported by Brittan-Powell et al. (2010). The minimal latency ranges from 0.5 to 2 ms, which is comparable to the 1-3 ms SFOAE delays reported for different gecko species by Bergevin (2010) The larger delays in tegu and alligator lizard could be due to a sharper tuning in these animals as is seen with SFOAE measurements (Bergwin 2010).

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