The Effects of Succinylcholine Chloride on the Biomechanics of the Green Treefrog Tympanum

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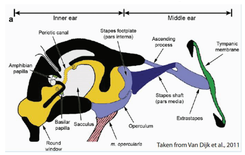
Background

Female Green Treefrogs *Hyla cinerea* rely on hearing to locate the advertisement calls of appropriate mates. During the breeding season frogs collect in large breeding choruses. These animals recognize conspecific calls due to their pulsate nature and spectral frequency peaks at 900 Hz and 2700 Hz.

The biomechanics and neurophysiological aspects of hearing have been extensively studied in anuran amphibians that are awake but restrained by an immobilizing agent. Whether such immobilizing effects reduce the response of eardrum mechanics in the presence of auditory stimuli remains to be examined. There is also little known about how different portions of the tympanic membrane react in response to sound. Mechanosensory transduction starts at the tympanic membrane which relays acoustic input to the frog's two auditory end organs, the amphibian papilla (AP) and the basilar papilla (BP). Since Green Treefrog calls have two spectral frequency peaks, at 900 Hz and one at 2700 Hz, each of these is processed by a separate auditory end organ. The AP correlates to lower frequencies while the BP corresponds to higher frequencies.



Hypotheses

H1: The biomechanics of the frog's tympanum should be unaffected by the use of immobilizing agents

H2: The inner region of tympanic membrane should conduct sound more effectively than the outer region.

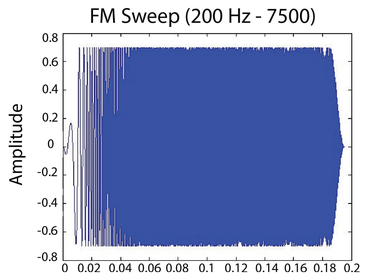
References

1.Caldwell, M. S. et al. Spatial hearing in Cope's gray treefrog: II. Frequency-dependent directionality in the amplitude and phase of tympanum vibrations. J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol. 200, 285–304 (2014).

2.Gerhardt, H. C. Mating Call Recognition in the Green Treefrog (Hyla Cinerea): The Significance of some Fine-temporal Properties. J Exp Biol 74, 59–73 (1978).

Methods

Experiments were conducted using Laser Doppler vibrometry. The auditory stimulus was a "frequency modulated sweep" which swept linearly from 200-7500 Hz over the duration of 175 msec.



Animals which were immobilized were administered an intramuscular injection of succinylcholine chloride. Immobilization lasted for around four hours before the effects of the immobilizing agent wore off. Animals were positioned in the sound chamber at a 90 degree angle relative to the auditory stimulus. The laser was positioned on the animal's tympanic membrane, and the signal was strengthened using microscopic reflective beads.

Each animal was exposed to multiple repetitions of the "frequency modulated sweep. After experiments were completed all data was analyzed using MATLAB.

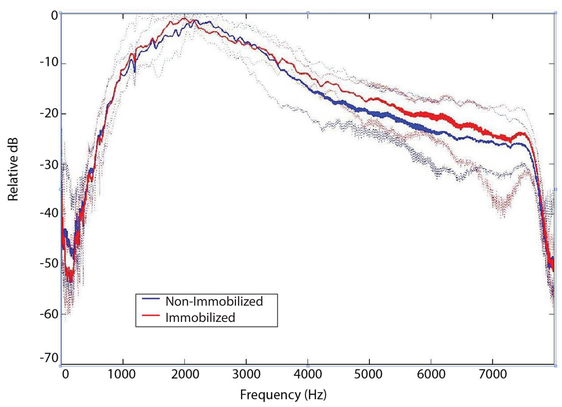
Location of Recordings

Recording Sites

Results

Both immobilized and non-immobilized frogs responded in a similar manner, as was expected. A higher response was found in the 900-3000 Hz range which then tapered off at higher frequencies.

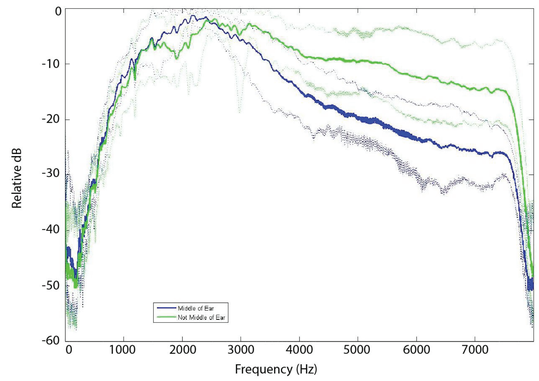
Non-immobilized vs Immobilized



The solid blue line in this figure correlates to the response of the non-immobilized frogs (n = 7). The solid red line corresponds to the immobilized frogs (n=7). The amplitude of the frequency response is normalized to its peak value (so the highest value = 0). The dotted lines blue and red correspond to the highest and lowest response of the non-immobilized and paralyzed frogs respectively.

The differences in the response to the middle and outer recording locations was more pronounced. Both sites responded similarly in the normal call range of 900-3000 Hz, but they differ at higher frequencies. Above 3000 Hz the outer portion of the tympanum responded with higher magnitudes than that of the inner region of the tympanum.

Middle and Outer Tympanum



The solid blue line in this figure correlates to the response of the middle of the tympanum. The solid green line corresponds to the response of the outer tympanum. The amplitude of the frequency response is normalized to its peak value (so the highest value = 0). The dotted lines blue and green correspond to the highest and lowest response of the inner and outer tympanum respectively.

Conclusions

There is no observable difference in the biomechanical response of the tympanum while the frog is chemically immobilized due to succinylcholine chloride. This result was expected but important because it means that studies which focus on the biomechanics of hearing in frogs will be able to use frogs which have been immobilized for data collection without altering the results of the study.

The different regions of the tympanum however provided an unexpected result. The outer region responded much more than that of the inner region. This is unexpected due to the fact that the center of the tympanum is connected to the extrastapes and as a result should provide a more pronounced response. However, this is not the case. The unexpected difference means that the biomechanics of the tympanum create a different response at different parts of the tympanum. Therefore there could be a meaningful difference between the inner and the outer portion of this region when it comes to detecting auditory signals.