EXPLORATION OF NEURONAL RESPONSES TO AUDITORY STIMULI IN DRAGONFLIES

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INTRODUCTION

- To date there is no published scientific evidence that dragonflies (*Odonata)*, have a nervous system equipped to process auditory stimuli

- However last year student Andrew Hamlin and Professor Robert Olberg stumbled on neuronal responses in the dragonfly that responded to auditory stimuli of 100-2000Hz sounds (Olberg and Hamlin, unpublished)

- This year our research was aimed at understanding a sensory modality that was previously unknown in dragonflies, the sense of hearing

WHY WOULD THIS BE DISCOVERED NOW?

- Dragonflies are known for their incredible flying mechanics and exceptional sight

- Research in entomology has grown due to new and changing technologies and many insects that were thought to not have a sense of hearing have been found to actually have one
  - Example: In 1989 the preying mantis, which was never thought to have had any auditory sense was found to be sensitive to ultrasound. Free flying and field experiments proved that the preying mantis responded and had specific avoidance patterns to ultrasonic sound waves (Yager and Hoy, 1989).

- We were to conduct electrophysiological and behavioral experiments similar to those that had been done prior in finding audition in insects

ELECTROPHYSIOLOGY / BEHAVIOR: THE BASICS

- Neurons fire using electrical signals
- These signals are currents that can be monitored by an electrode
- By monitoring the currents neurons produce one can deduce nervous system responses to varying stimuli, like sound stimuli
- Behaviorally if an animal is moving or showing responses to varying stimuli, then the something in the nervous system is responding

METHODS

- Electrophysiology = used ice to anesthetize dragonflies and then used a simple dissection method to expose the pro- and mesothoracic ganglion along with the ventral nerve cord
- Behavioral = dragonflies were tethered to an upright pole on a table and video recorded
- Sound Production = LabChart7 was used with an amplifier, waveform generator, and two types of speakers to produce sound stimuli from 100Hz – 16KHz

Electrophysiology recording set up

Under the microscope post dissection

Behavioral experiment set up

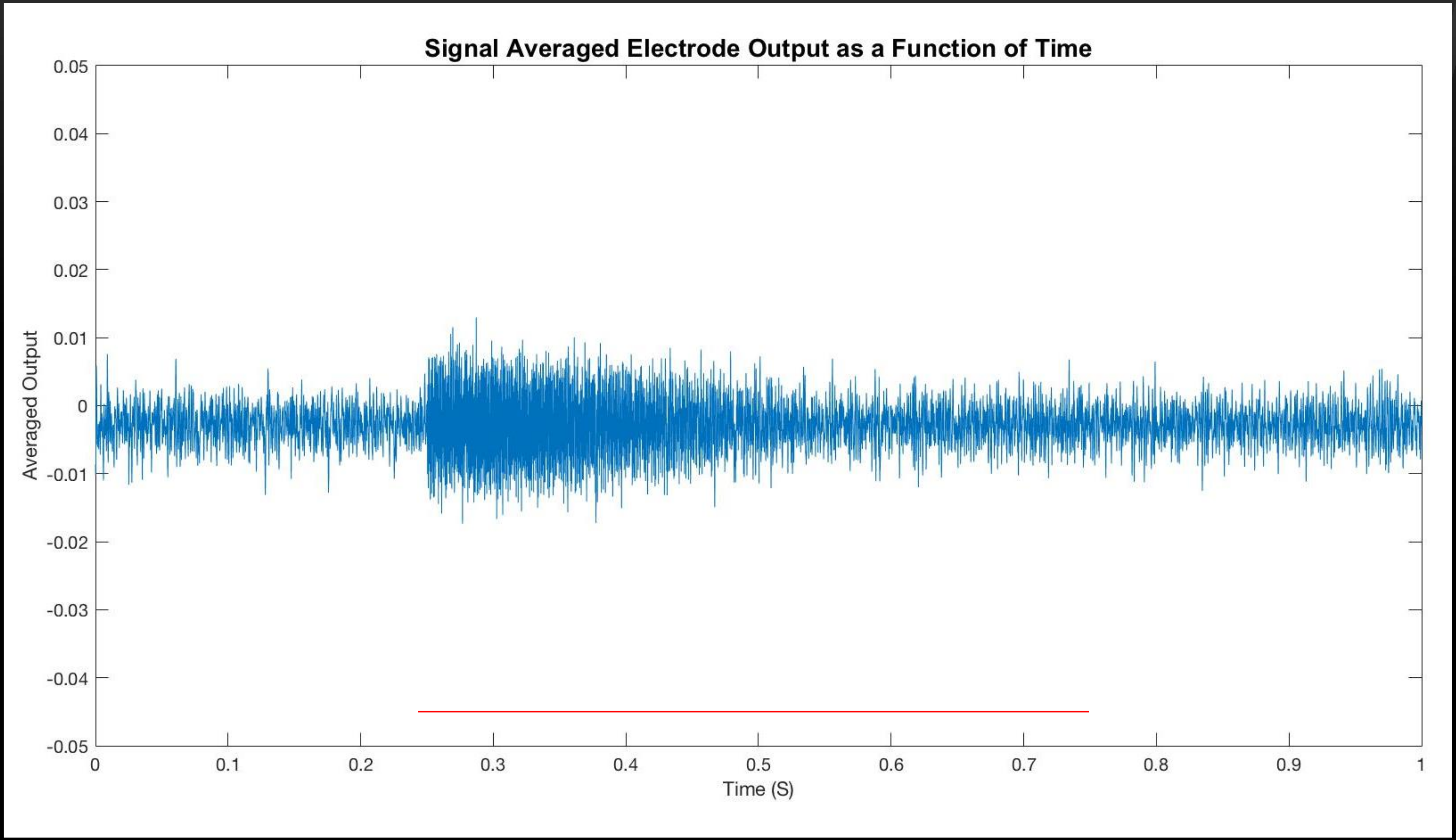


Figure 1: Signal averaged data from a silver wire electrode recording from the left connective of an *Anax junius* dragonfly at 2000Hz on 9/26/2017. The speaker was placed 19cm away from the head of the dragonfly at 180 degrees. A 500ms sound signal was produced by an amplifier, frequency modulator, and LabChart7 for 150 repeats with a 4 second delay between each stimulus. The 500ms sound signal along with the 250ms before and after the signal were recorded by LabChart7 for comparison. This graph was generated by originally written MatLab scripts for signal averaging. As seen by the graph the output from the electrode increases at the 250ms mark when the sound stimulus starts and decreases as the sound plays out. This is characteristic of a neuronal response.

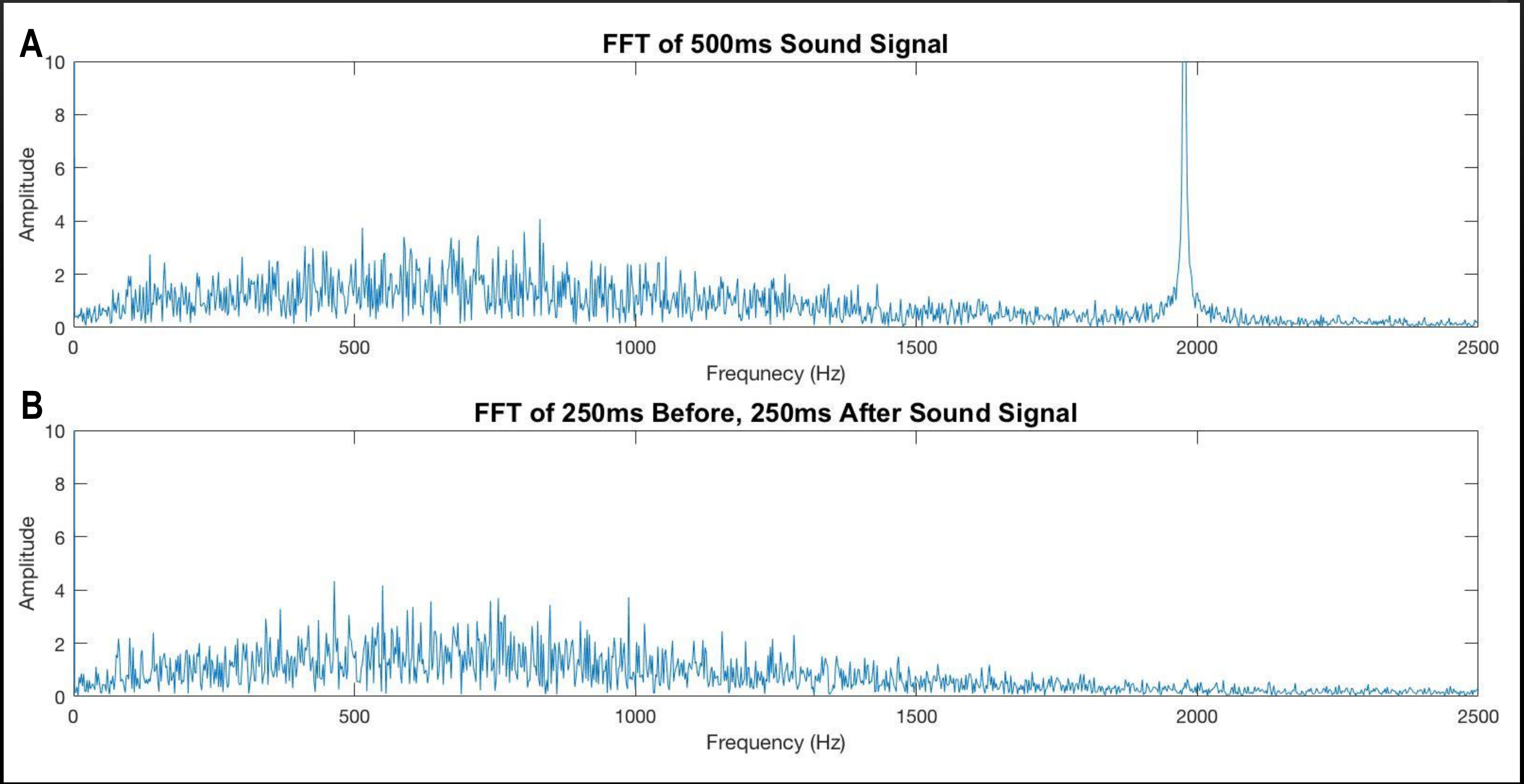


Figure 2: Two Fast Fourier Transforms of silver wire electrode output data from the left connective of an *Anax junius* dragonfly stimulated with a 2000Hz sound stimulus on 9/26/2017. (a) Represents the FFT of the middle 500ms recording when the sound stimulus is on. A large peak at 2000Hz represents the exact frequency of the sound being played. (b) Represents the FFT of the before 250ms and after 250ms of the recording when the sound stimulus is off. These FFT's break up the input and output into their underlying frequencies. There is no obvious difference in these graphs so an integration for the areas under the curve was done. The integration value for (a) was 1.2245 x 10^3, and the integration value for (b) was 1.0687 x 10^3. Mathematically there was no significant difference meaning the output signal was not actually different when sound was on versus off.

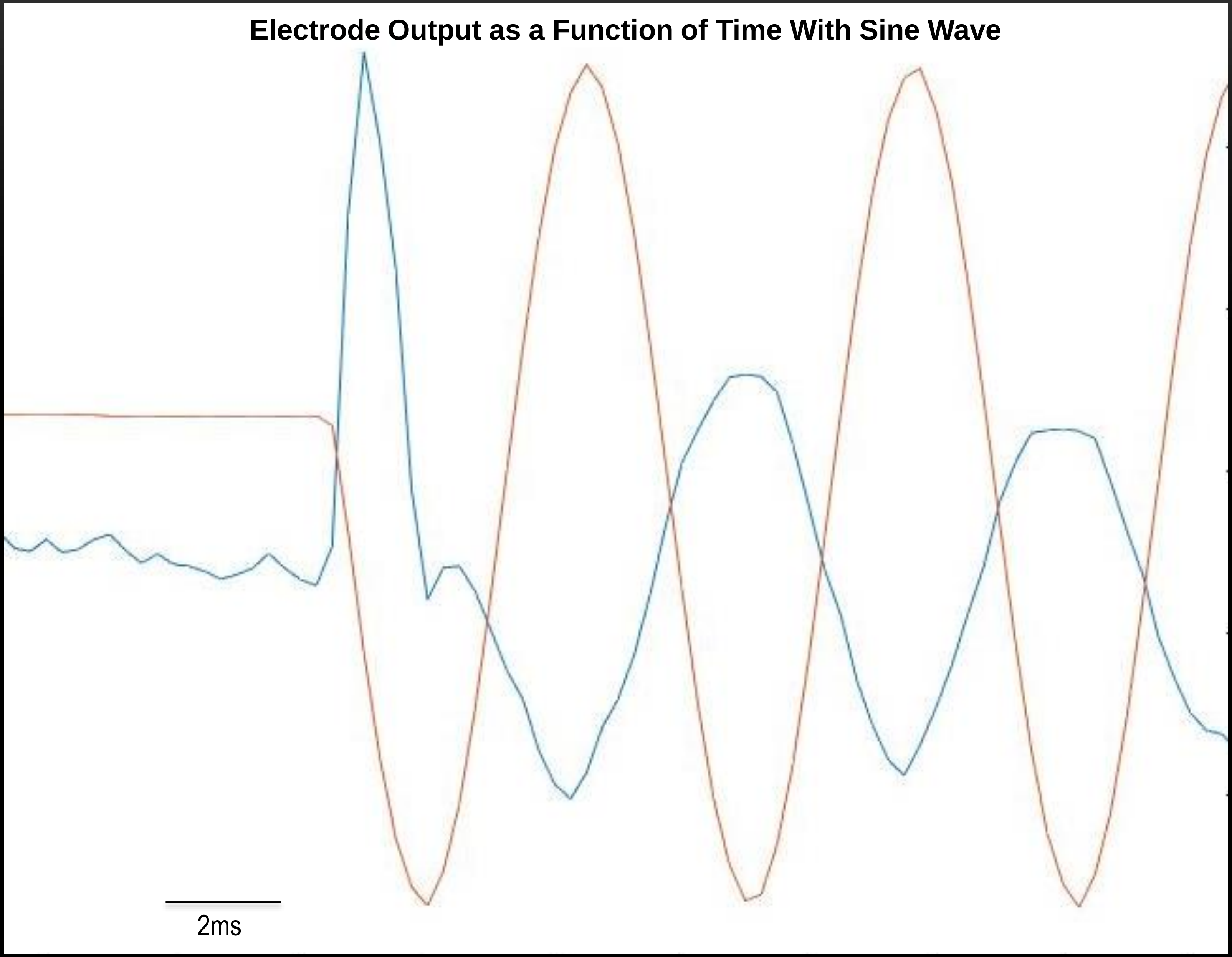
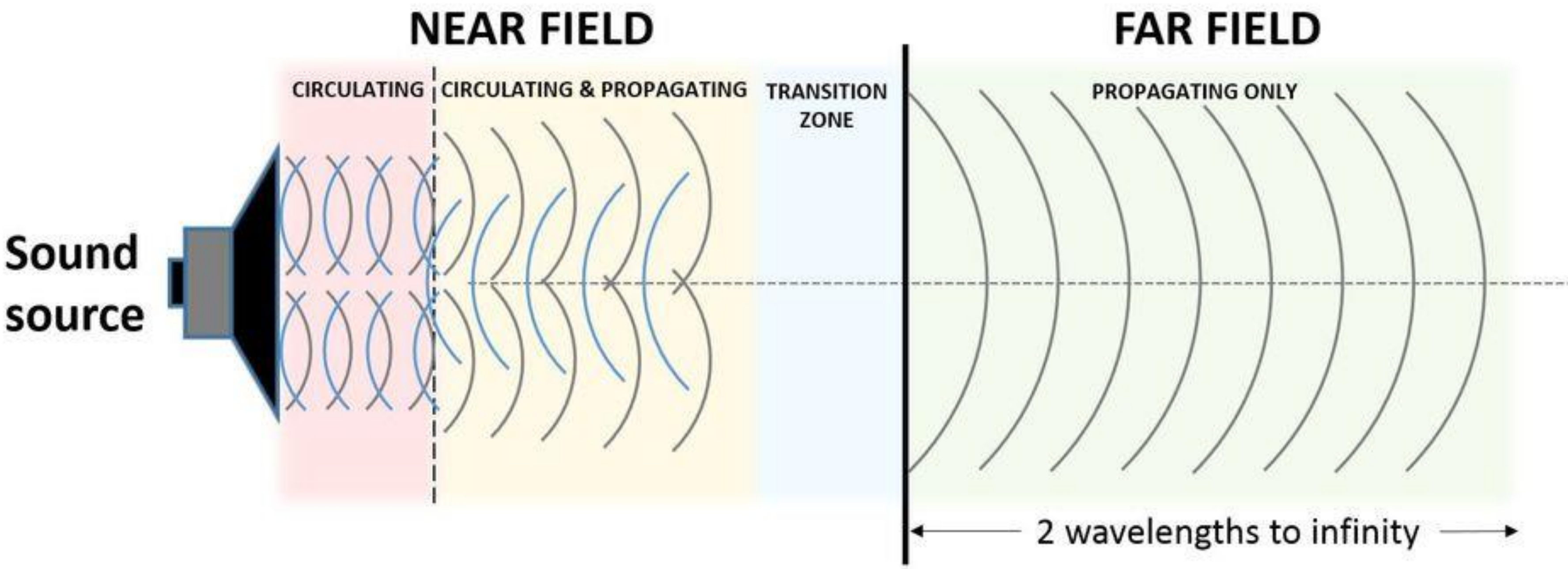


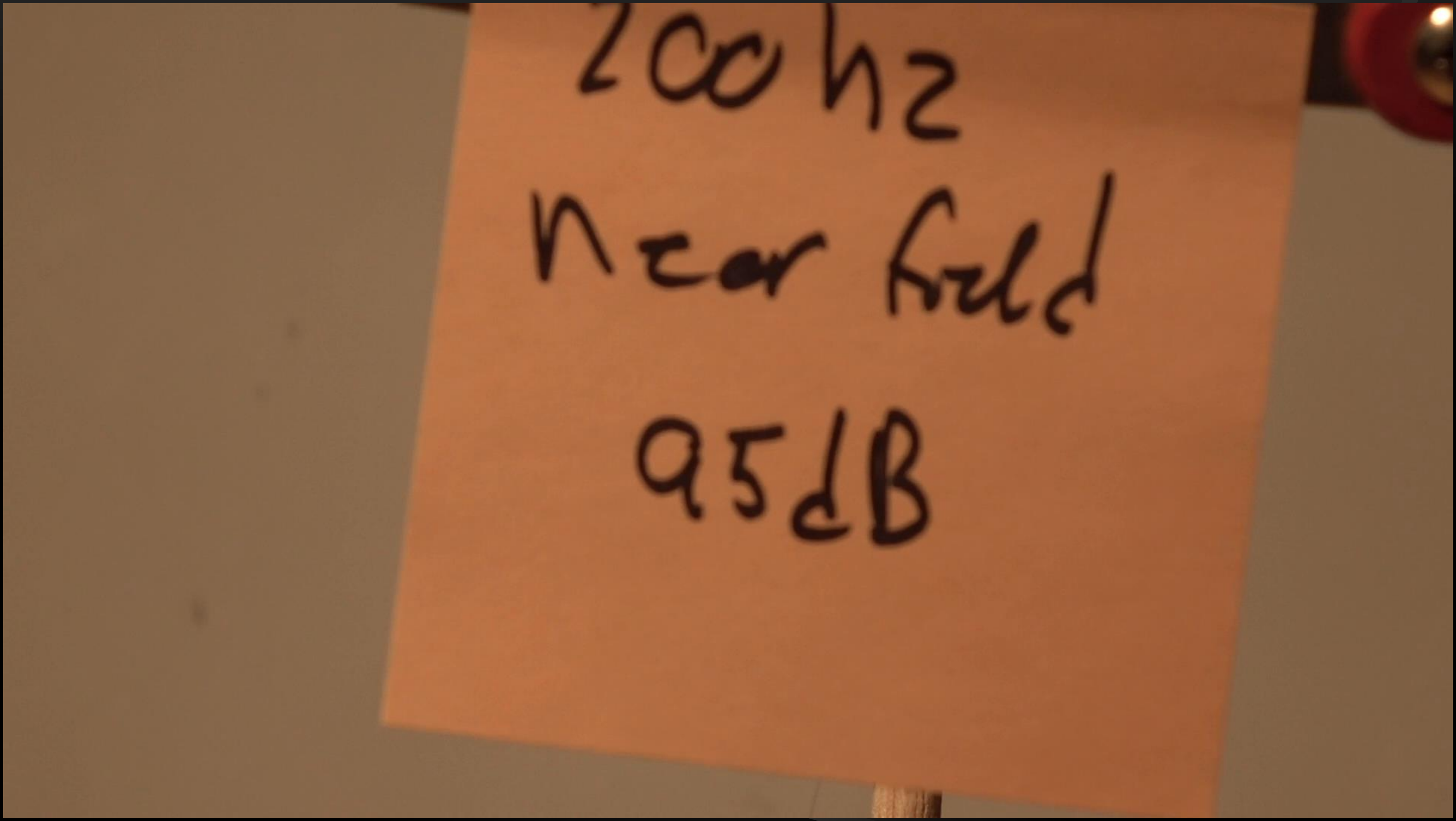
Figure 3: Signal averaged data from a silver wire recording of the left connective of a dead *Anax junius* dragonfly to a 2000Hz sound stimulus with the stimulus sine wave superimposed on the graph to a near-field sound on 10/10/2017. The tan line represents the sine wave and the blue line represents the electrode output. This graph shows that when the sound signal is on the electrode output is essentially phase locked with the sound meaning that the electrode is acting as an antenna and picking up signal directly from the speaker as well as the neural activity in the dragonfly connective.

PHYSICS WAS THE PROBLEM

- A sound wave travelling through a medium has two distinct areas, the near field and the far field of the sound wave.
  - The near field is where a sound wave is circulating and propagating in the medium where as the far field is where the sound wave is just propagating in one direction.
  - Where the sound wave transitions from near field to far field is approximately one wavelength from the sound source of a particular frequency.
  - Wavelength = speed of sound / frequency
  - Our speaker was originally 20cm away from the animal which for most frequencies being used was in the near field + the electrical communication between the speaker and the electrode

- Also: The speaker itself produces an electrical field due to its coil and magnet. This field can be picked up an electrode within a certain distance


NEAR FIELD
FAR FIELD
CIRCULATING
CIRCULATING & PROPAGATING
TRANSITION ZONE
PROPAGATING ONLY
Sound source
2 wavelengths to infinity



Example of Behavioral Test with Tail Movement

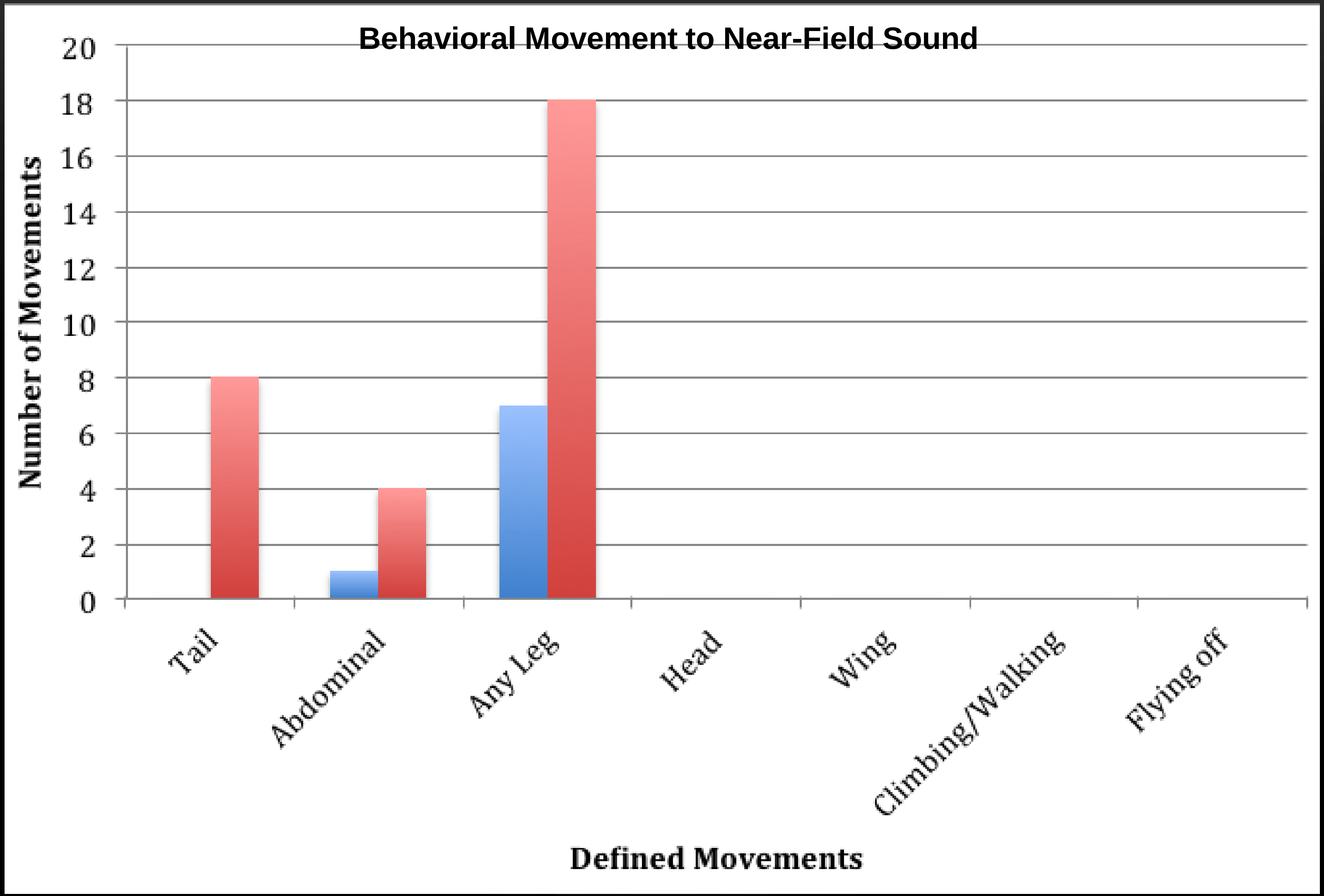


Figure 4: Behavioral movements to near-field sound stimuli in an *Anax junius* dragonfly to 200Hz and 100Hz sound on 1/5/2018. The dragonfly was tethered to an upright pole on a table with the speaker approximately 1 meter away. The whole procedure was recorded on a video camera and analysis of movements to the 3 second sound stimuli compared to the 3 seconds prior to the sound stimuli were done frame by frame using the video recording. The sound was repeated 10 times for each condition with 20 seconds in between repeats. The red bars indicate movements during the sound stimulus and the blue bars represent movements without the sound stimulus. Movements were operationally defined prior to the experiment. This data set shows that movements during the sound were much more common than without the sound. The TTest was 0.000513117 ($P < 0.05$) meaning that this difference was significant.

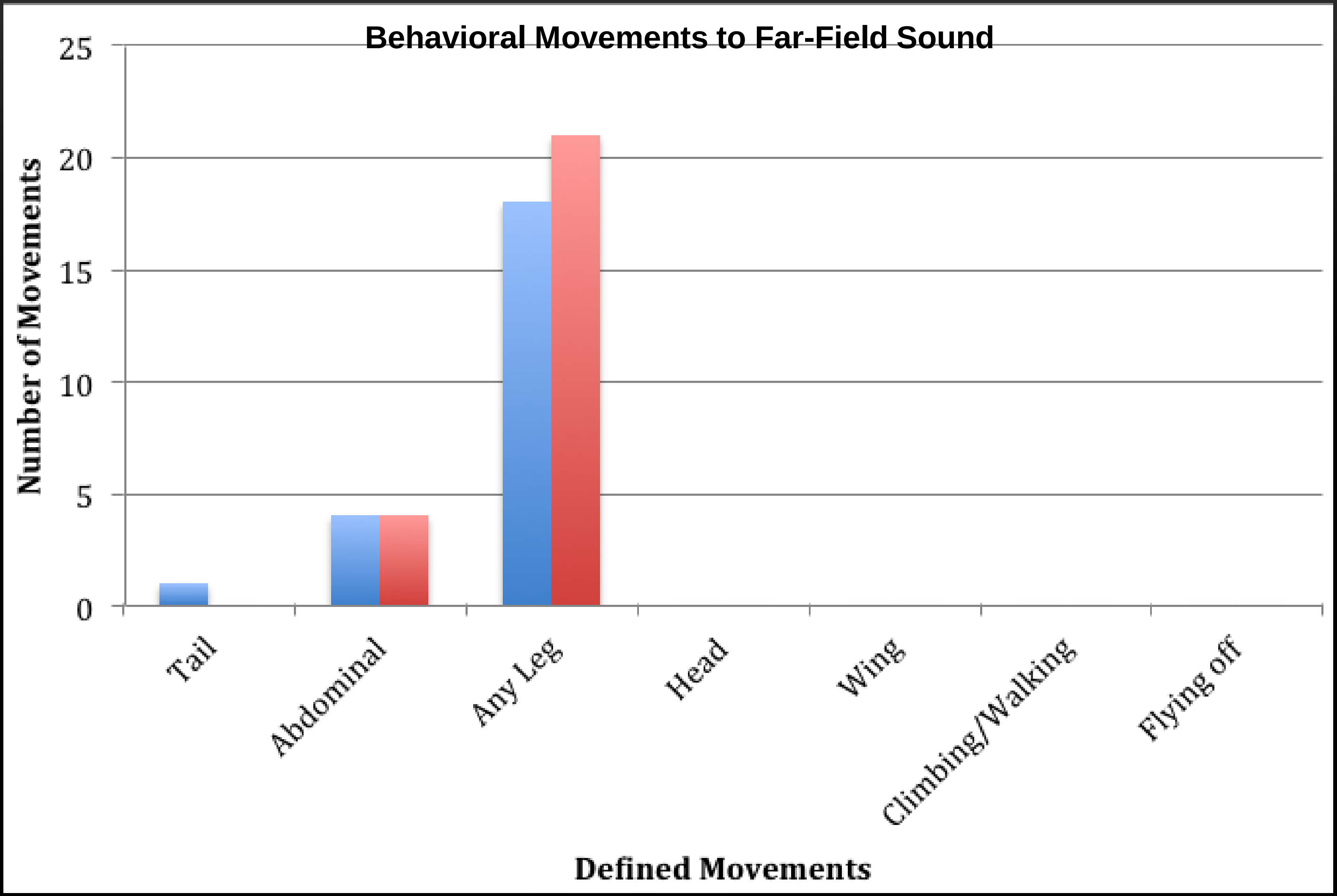


Figure 5: Behavioral movements to far-field sound stimuli in the same *Anax junius* dragonfly to 200Hz sound for comparison of movements on 1/5/2018. The same set up was used as before except the speaker was placed 2 meters away from the animal to get out of the near-field for the 200Hz sound wave (100Hz could not be used because its wavelength is too long). This data set shows a much more even distribution of movements of sound on versus sound off in the far-field. The TTest value was 0.192293379 ($P > 0.05$) meaning that the difference was not significant. This further indicated that dragonfly hearing may be mechanosensory and regulated by the physics of sound. A chi-squared test was also run for this data set compared to the near-field data and the value was 0.00 for near-field, and 0.736 for far-field. This suggest that for this one animal it is definitely significant that it moves to near-field sound more than far-field sound.

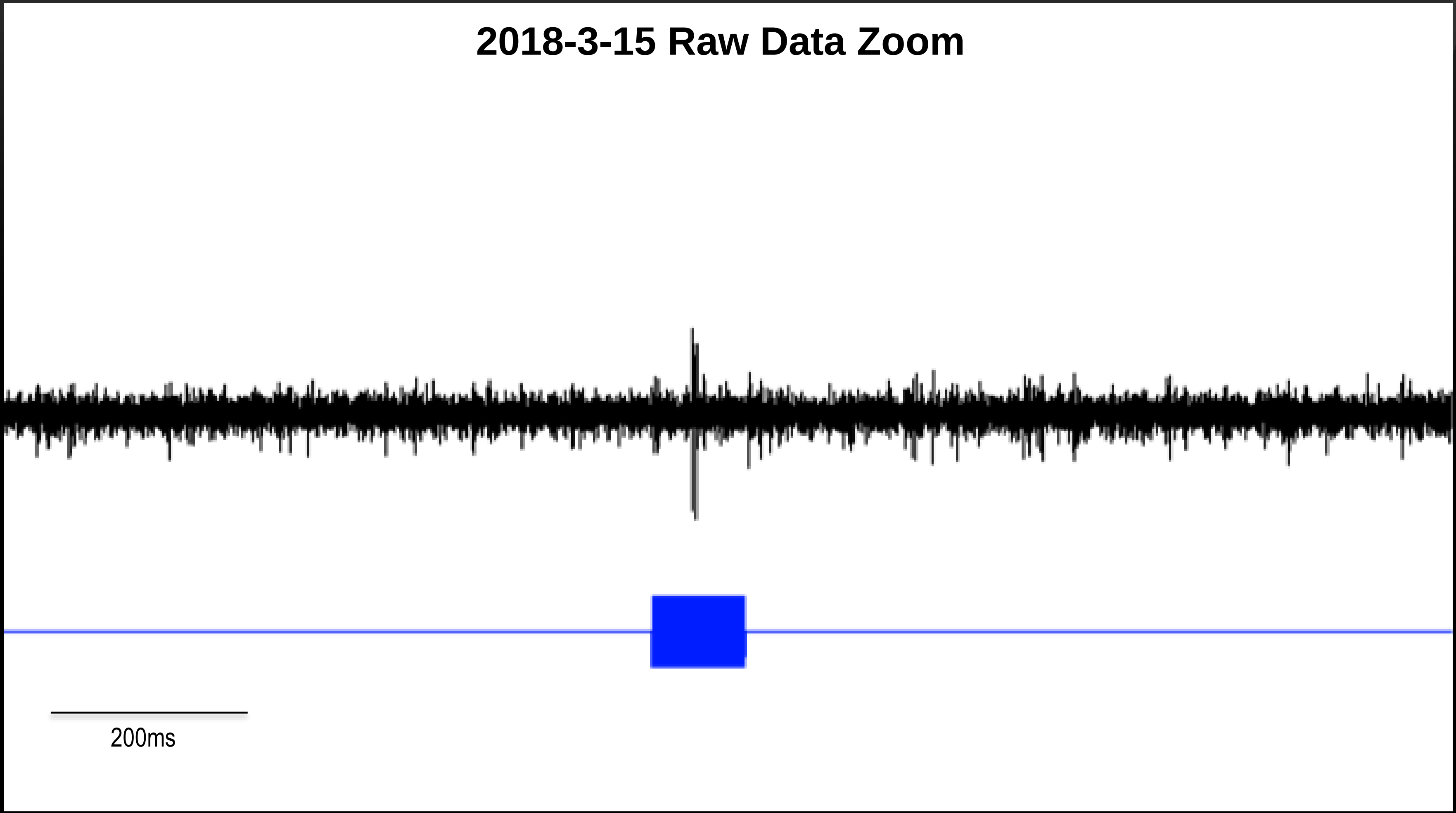


Figure 6: Silver wire electrode recording of the left connective in an *Anax junius* dragonfly with 500Hz sound in the far-field on 3/15/2018. The same set up was employed except we moved to manual stimulation using the stimulator panel in LabChart7 so we could control when the sound was played so the animal was not moving when the stimulus was played. The small speaker was placed 40cm behind the animal at an amplitude of 0.15V. The sound level at the head of the animal was approximately 75dB. This is much less than some of the pervious experiments so the sound response does not have a very high threshold. This raw data shows a very characteristic neural response to a 100ms sound stimulation. Upon further zooming and using the marker the response was found to have a 40ms latency, which is characteristic of dragonfly neural activity.

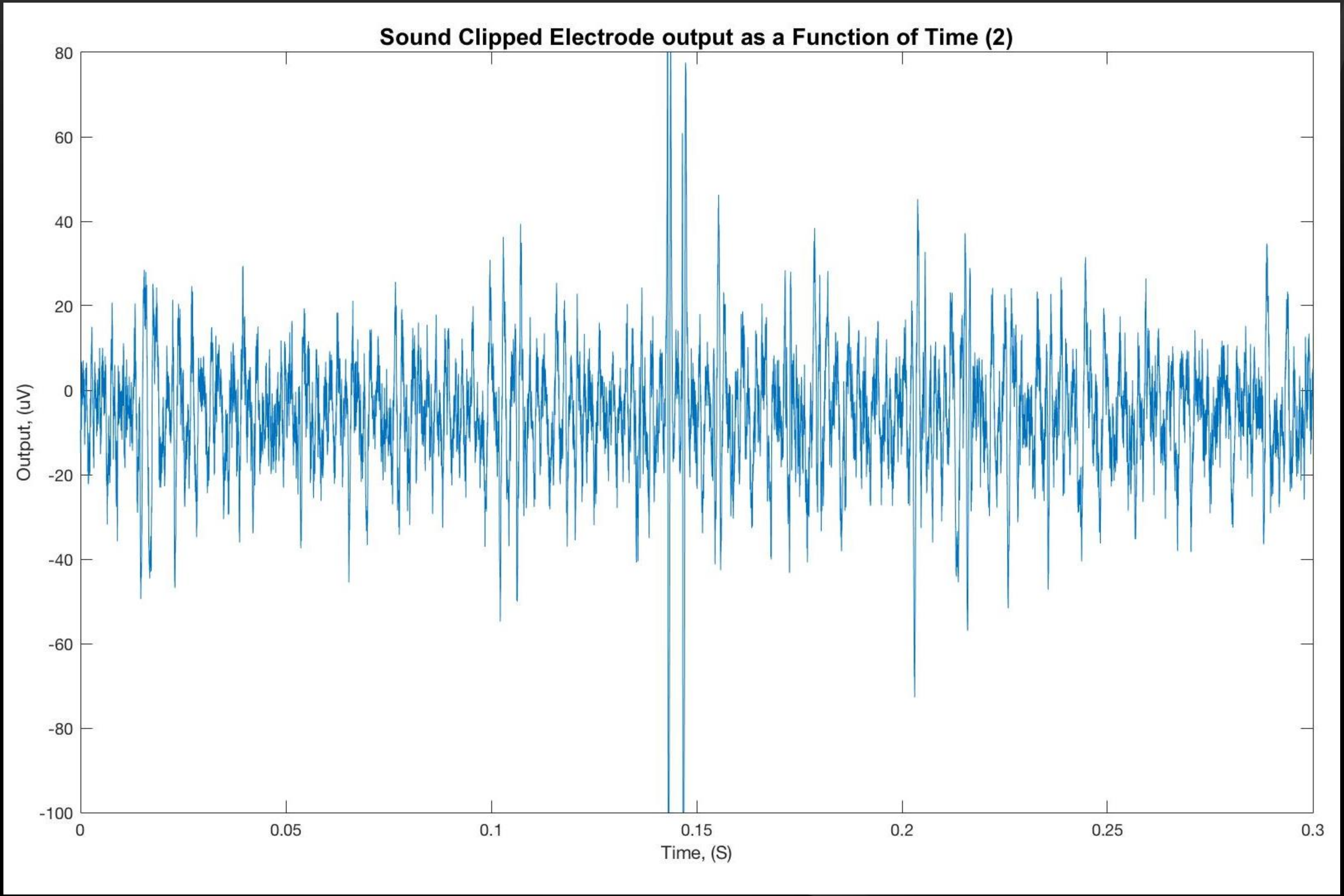


Figure 7: Sound clipped data from a silver wire electrode recording of the left connective in an *Anax junius* dragonfly on 3/15/2018. This graph correspond to the raw data in figure 9. (a) Graph of raw data obtained using an originally written MatLab script. The sound stimulus was 100ms and started at 0.1S on the graph and went until 0.2S. This data shows a clear sound stimulus just like the raw data but with an arbitrary threshold selected showing that this response is clearly different from anything else in the plot.

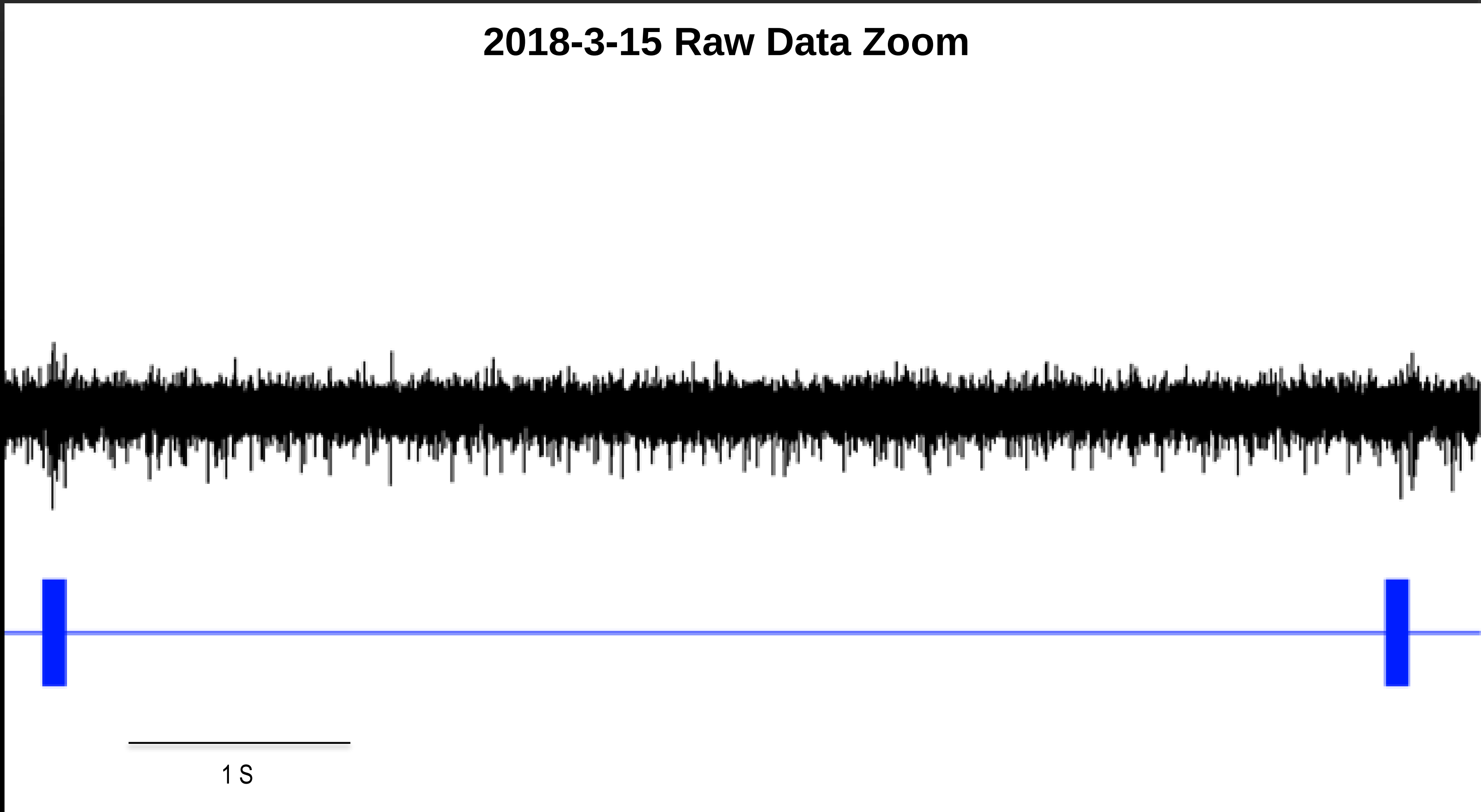


Figure 8: Raw data section from a silver wire electrode recording from the left connective of an *Anax junius* dragonfly to 500Hz sound on 3/15/2018. This zoomed in data shows two manual stimulations in succession and the clear increase in the baseline of neural activity when those stimulations happen. This raw data just further shows how the neural signal is occurring due to sound.

CONCLUSIONS AND FURTHER STUDY

- Our data suggests that dragonflies do have an auditory sense using an external hearing organ like humans and the ones found in other insects like the preying mantis

- This is a subtle sense in these highly visual insects and finding it takes a near perfect set up and analysis

- We started isolation studies to locate the external ear and our preliminary data suggested that it may be on the thorax of the dragonfly

- Future work on further locating the part of the body that dragonflies may use for an auditory sense is needed to continue to investigate this new sensory modality in these insects

A SPECIAL THANKS

- To Professor Robert Olberg for all his guidance, assistance, and allowing me to conduct research under him in his laboratory
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Questions?