

## Introduction

Mice are a good model organism as mice and humans share greater than 95% of DNA. Researchers studying complex biological systems found in humans use mice as they naturally develop human diseases (Teegarden, 2012).

Additionally, both humans and mice share well conserved brain features and structures that play a role in behavior. Both species have similar gene functions and “share a number of neuroanatomical, neurochemical and behavioral commonalities” (Sartori, Simone B et al, 2011).

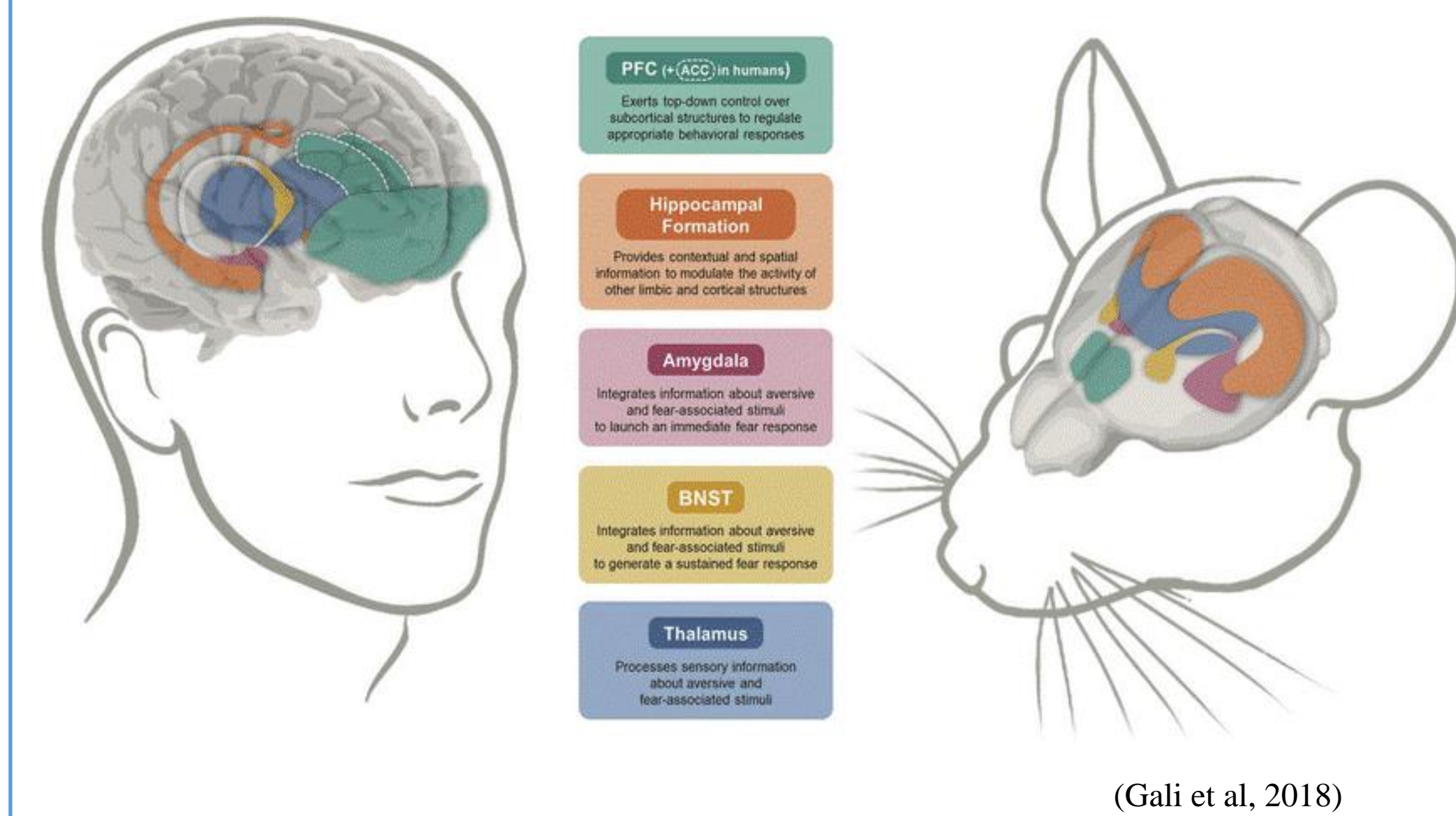


Fig. 1: Key Regions in the Human and Mouse Brain Involved in Fear and Memory.

There are many common methods utilized for the behavioral phenotyping in mice in connection with models of neuropsychiatric disorders. This project introduces a new behavioral study method to our lab: vocal analysis.

Breeding behavior is stronger when there is a territory. The experiment set-up will consist of housing adult C57 male and female mice to help build a territory. After the removal of the female, the male’s instinct to mate will be heightened, resulting in vocalizations when a new female is introduced.

## Research Aims

- Successfully record communications between male and female mice to study mice behavior.
  - Explore different vocal analysis programs.
  - Determine specific parameters for visualization of mice vocalizations during interaction-based tests.
  - Analyze the spectrograms and waveforms of male mice vocalizations when introduced to female mice.

## Experimental Method

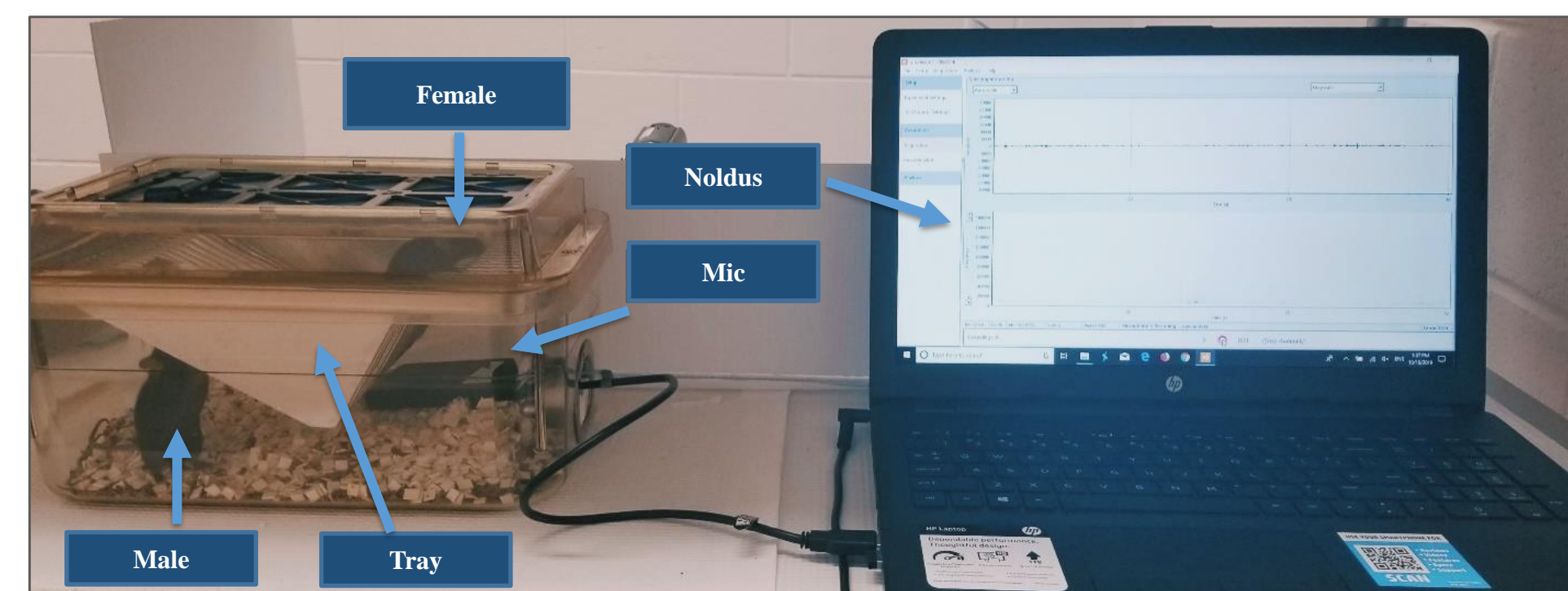
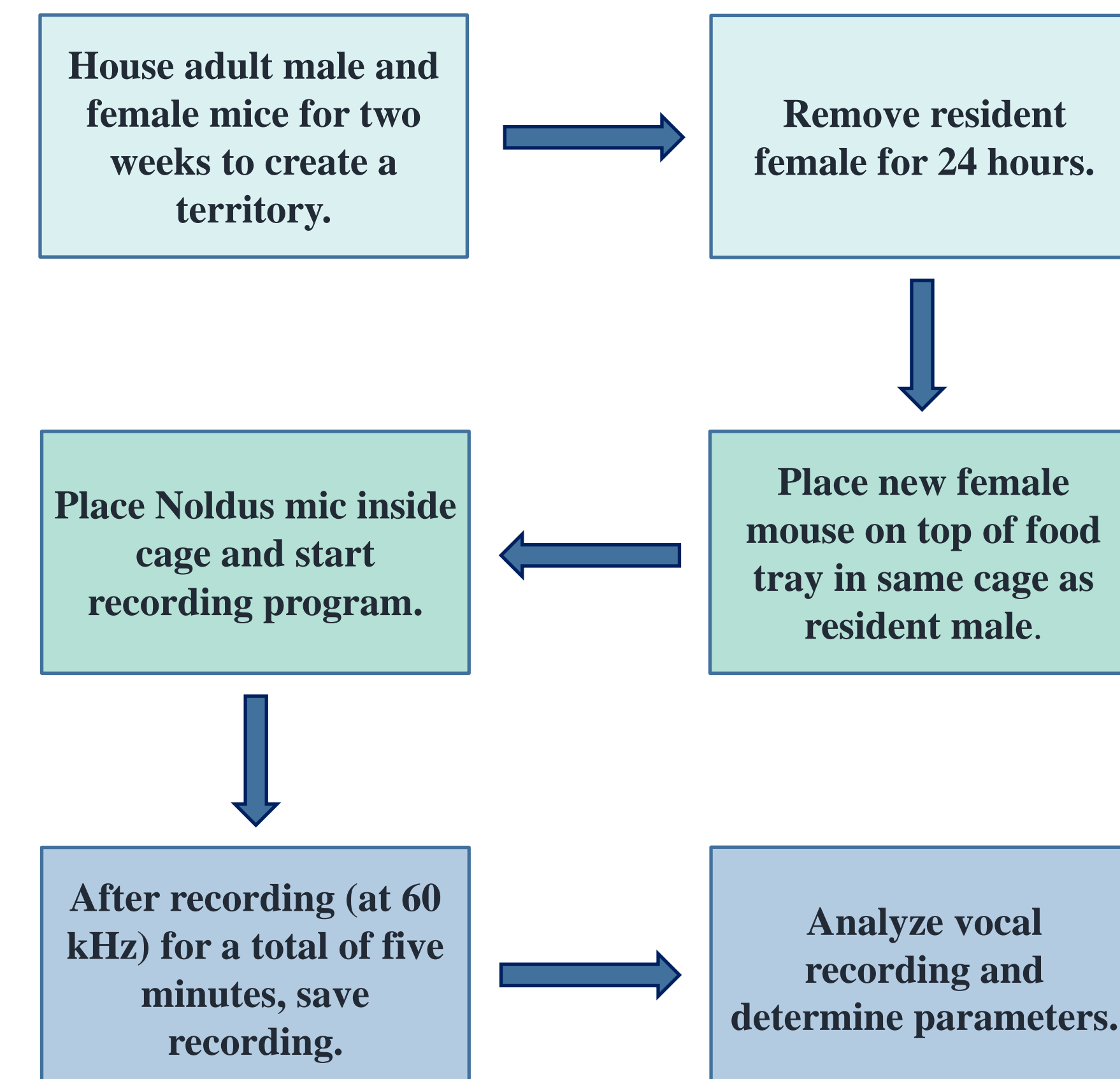


Fig. 2: Experimental Set-up

## Data Analysis Method

- After collecting vocal data, various programs were explored to determine which would best visualize vocalizations.
  - Male to female vocalizations during mating is around 65kHz (Sullivan et al, 2017).
  - Mouse vocalizations have specific shapes, as indicated by the figure below (Keesom et al, 2017).

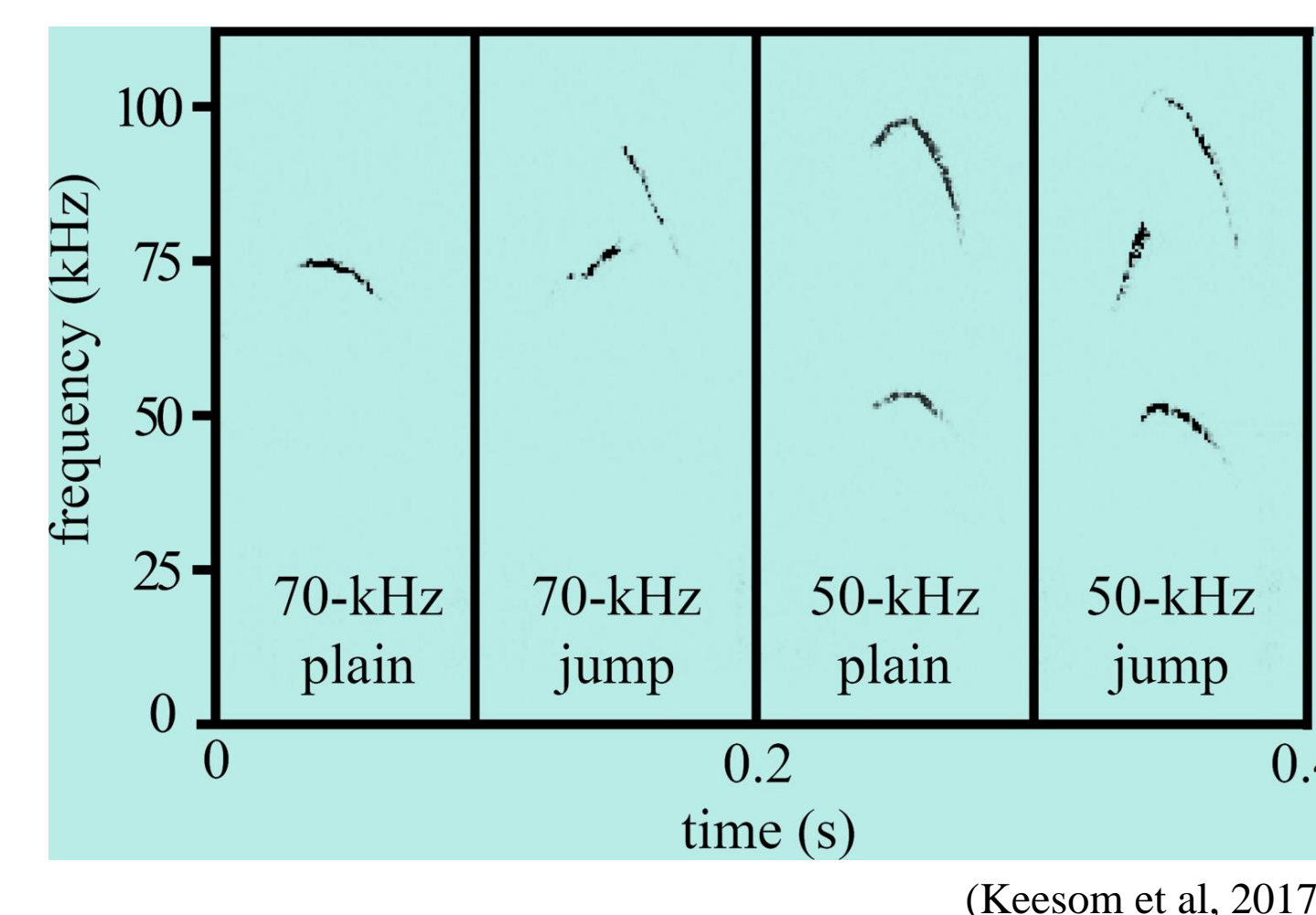


Fig. 3: Spectrograms of representative ultrasonic vocalizations (USVs) produced by male mice during same-sex social encounters.

## Results

- Testing various programs, Raven Lite 2.0 was chosen as the best program.
  - Originally created to analyze bird vocalizations, this program has a simple interface and fast processing speeds.
- Mouse communication detected using specific parameters at 65 kHz.
  - Visualization showed definite peak patterns.

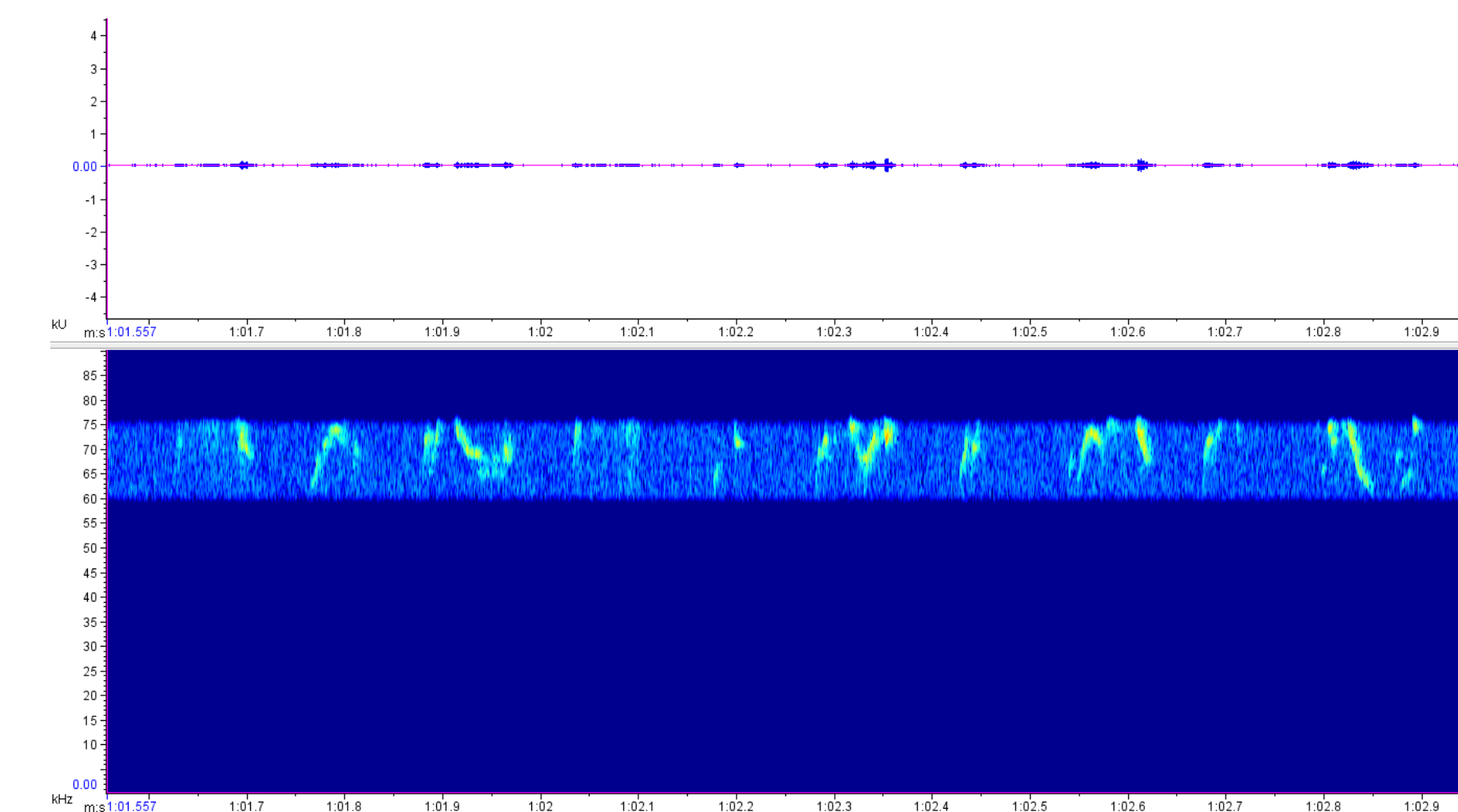


Fig. 4: Filtered spectrogram and waveform of a male mouse's vocalizations when introduced to a female mouse with the above parameters applied.

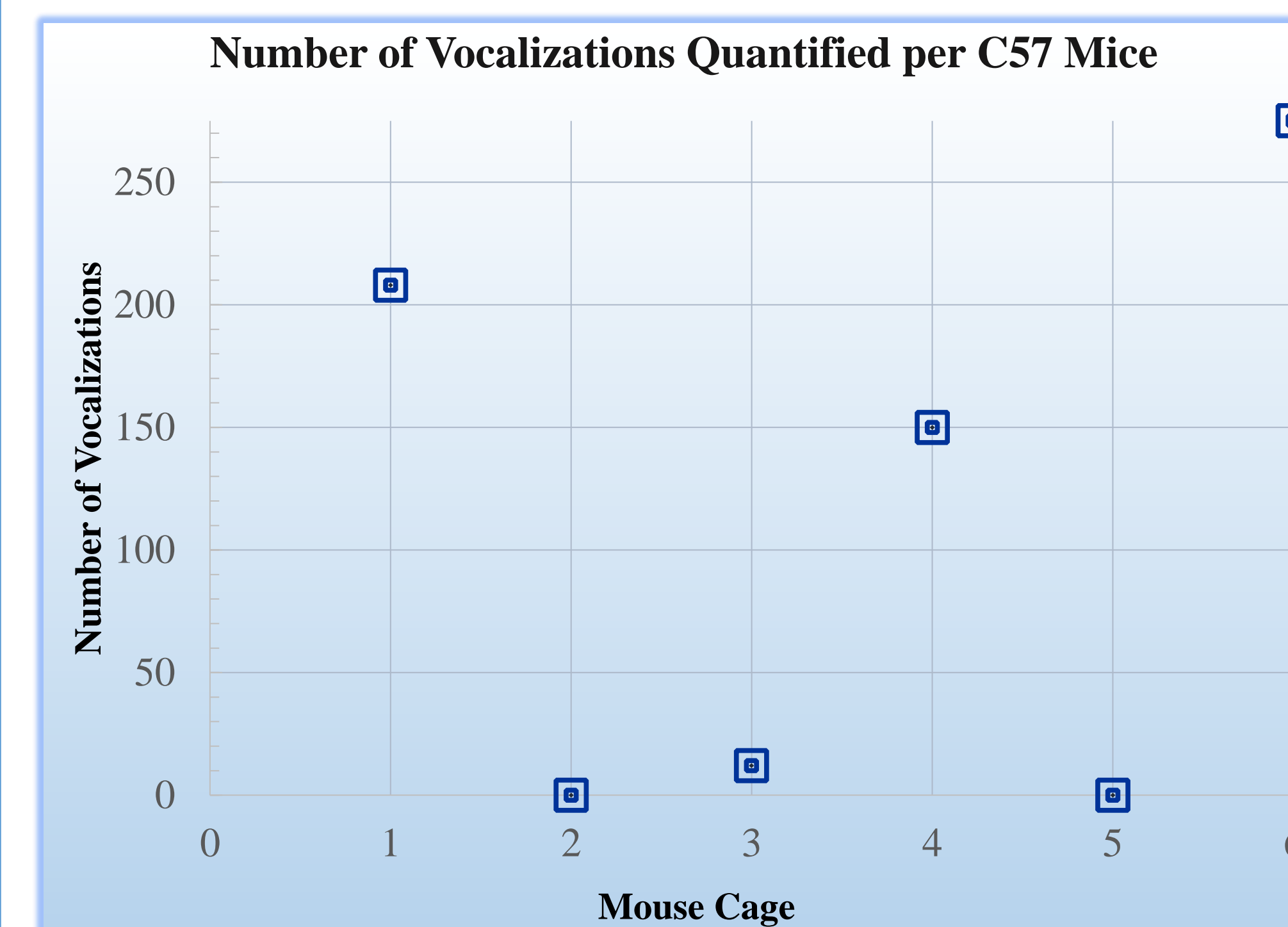


Fig. 5: Quantification of the Number of Vocals per Mice Cage.

Graph shows the quantification of the number of vocalization patterns counted. Six cages of C57 mice were analyzed when male mice were introduced to a female. The average number of vocalizations was 108 vocalizations. The maximum number and minimum number was 275 and 0 vocals, respectively.

## Conclusions

- Mice communication around 65 kHz was successfully visualized using Raven Lite 2.0 and specific parameters.

Parameters	
Amplitude Scale	934 units/line
Time Scale	1.734 sec/line
Frequency Scale	90,001 Hz/line
Lower Limit	60,000 Hz
Upper Limit	75,000 Hz

Fig. 6: Parameters used to produce and visualize mice vocalizations at 65 kHz

- Difference noted in the number of vocalizations produced by different C57 mice.
- If these vocalizations represent the eagerness of the mouse in attracting females, difference in amount of vocalizations could be due to aggressiveness towards mating.
- Hypothesis: Males that produced a fewer amount of vocals are less aggressive in terms of attracting females.

## Future Directions

- Conduct intruder studies (standard method to measure aggressiveness) and compare results to determine if vocal analysis can measure aggression.
- Future studies can implement this technique to test anti-psychotic drugs that target behavioral symptoms in psychiatric disorders, like PTSD.

## References

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## **THE HUMANITY FIRST RESEARCH SYMPOSIUM**

### **Abstract Submission**

#### **Using Vocalizations to Investigate Behavior in Mice**

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Both humans and mice share well conserved brain features and structures that play a role in behavior. These structures include the cerebral hemispheres, forebrain, midbrain, hindbrain, cerebellum, hippocampus, thalamus, and hypothalamus. A popular model organism in neuroscience, the behavioral phenotyping in mice is utilized by researchers in connection with models of neuropsychiatric disorders. The aim of this project is to introduce a new method to our lab, vocal analysis, and investigate its purpose in behavioral studies.

The experiment set-up consisted of housing an adult C57BL/6 male mouse with an adult C57BL/6 female mouse for two weeks to help build a territory. After the removal of the female mice, the male mice's instinct to mate was heightened and resulted in vocalizations when a new female was introduced. After evaluating various vocal programs with test samples, the best program was chosen. Male and female mice communication around 65 kHz was successfully visualized using Raven Lite 2.0 with specific parameters applied. Results showed a difference in vocalizations by different C57 mice. If these vocalizations represent the eagerness of the mouse in attracting females, then the difference in vocalizations could be due to aggressiveness towards mating. We hypothesize that the males that spoke less are less aggressive in terms of attracting females. Further studies, using standard methods to measure mice aggression, will be conducted and compared to this study's results to confirm our hypothesis. If this method is supported, future studies can implement this technique to test anti-psychotic drugs that target behavioral symptoms in psychiatric disorders, like PTSD.