Analysing Alcohol Exposure on Ultrasonic Vocalizations (USVs) in Adult Female Mice

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INTRODUCTION

Ultrasonic vocalizations (USVs) are high-frequency sounds that mice use to communicate emotional and social states. In female mice, USV patterns are more complex during interactions with other females and become simpler during mating with males. This project investigated whether alcohol changes USV patterns in adult female C57BL/6J mice, recorded before and after receiving either water or a 20% ethanol solution. Findings suggest that alcohol can acutely alter mouse communication by decreasing calls complexity and transitions between syllables, indicating anxiolytic effects. [2, 4]

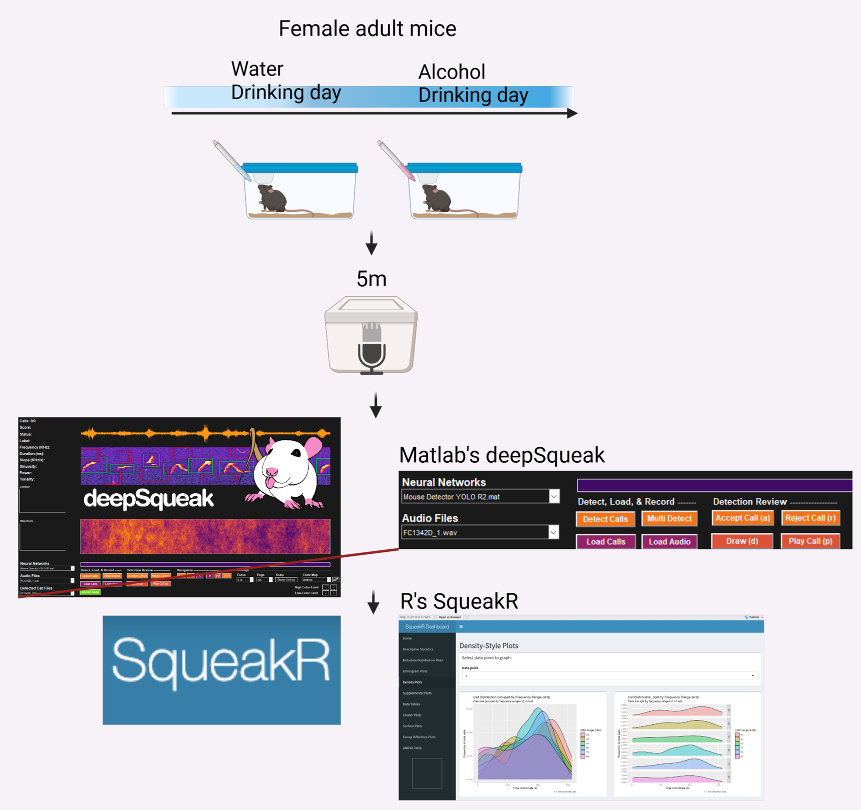

Figure 1. Experimental Setup & Analysis Framework

METHODS

USVs were recorded from 12 adult female mice over three days: day 1 (sober), day 2 (first binge), and day 3 (second binge), with only two cages drinking on the third day. USVs were detected and classified using DeepSqueak, a MATLAB package which applies Faster R-CNN (Faster Region-based Convolutional Neural Network) deep learning for call identification and k-means clustering for call type categorization. [1] Initial data visualization was conducted in R through SqueakR, an experiment interface for DeepSqueak [3]. Further statistical analyses were then performed, including non-parametric tests to account for small sample sizes and unknown distributions. [2]

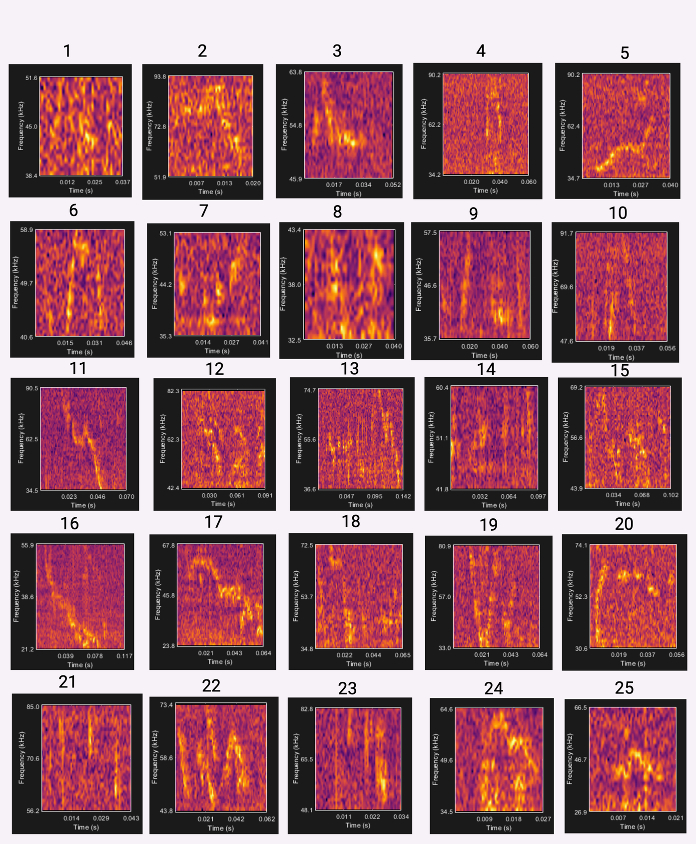

Figure 2. Spectrograms of 25 Mouse Call Types

Example sonograms show the diversity of call categories identified by unsupervised clustering through k-means, based on the slope, frequency, sinuosity and call duration. [1]

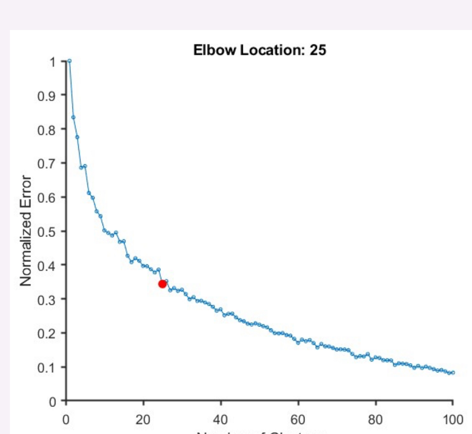

Figure 3. K-means Elbow Plot

The elbow plot helps determine the optimal number of clusters for mouse vocalizations. Feature extraction and clustering rely on detailed analysis of each call's contour.

RESULTS

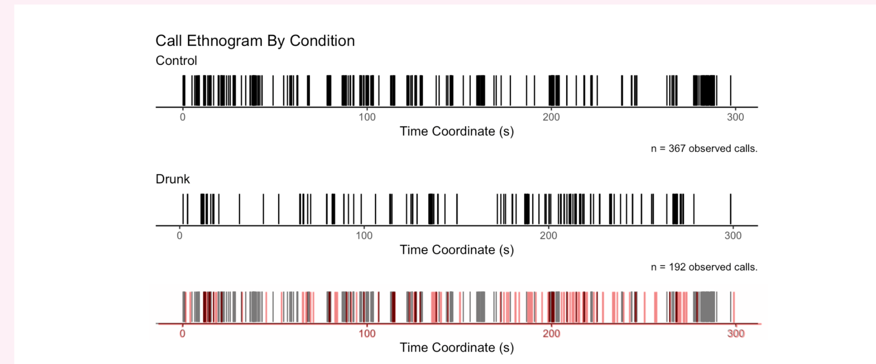

Figure 4. Call Ethogram By Condition

Comparison of vocalization patterns across sober and drunk conditions over a 300-second period. Each vertical line represents a single observed call. [3] The Sober panel shows call timing with a total of 370 observed calls; the Drunk panel shows 193 observed calls. Call frequency is reduced and calls are less clustered in the drunk condition.

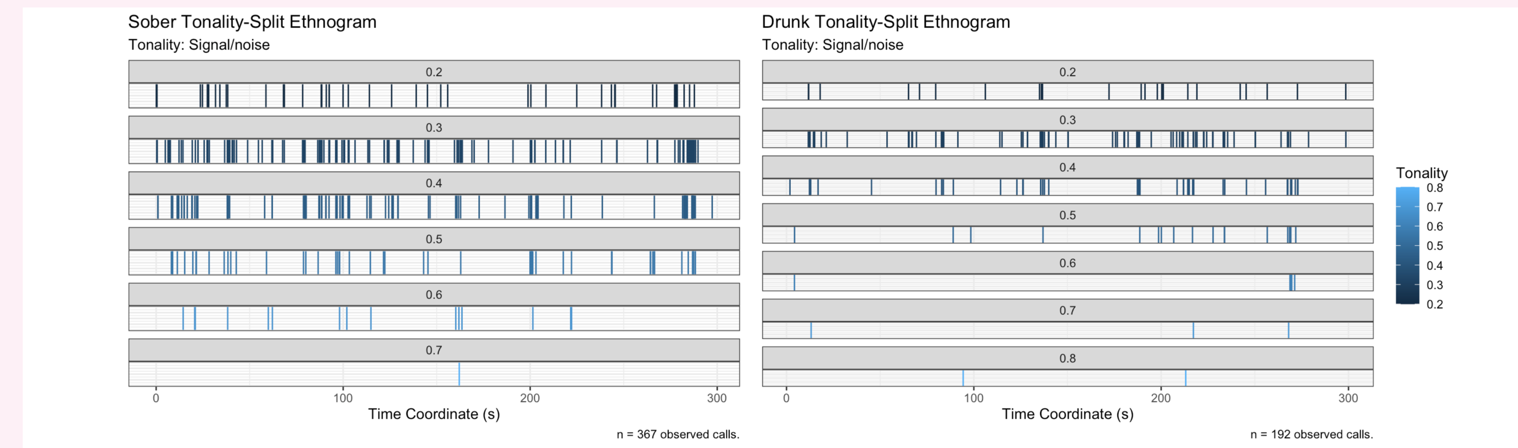

Figure 5. Tonality-Split Ethnograms of Mouse USVs: Sober vs. Drunk

These plots show the timing and tonality of individual ultrasonic vocalizations in mice during sober and drunk conditions, indicating emotional states with higher tonality linking to positive states. Sober mice produce more calls across a wider tonality range, while alcohol exposure reduces both the number and tonal diversity of calls, indicating less varied vocal communication.

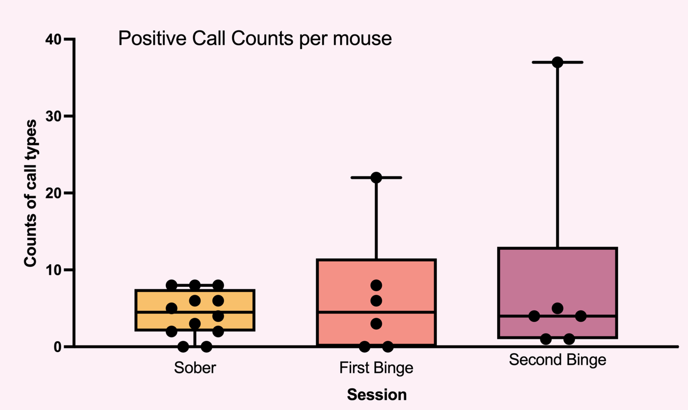

Figure 6. Boxplot of Positive Counts of call types by Session

Boxplots showing the number of USVs are grouped by session and frequency band. Both binge sessions (First and Second) generally show higher and more variable positive call counts compared to the Sober session, indicating an increase of positive-affect calls (40-90 kHz) after alcohol exposure [6]. This suggests an increase towards positive, hedonic emotional states.

Following post-hoc analysis after running a generalized linear model, there is a large significant difference found the in the positive calls between sober and the first binge session. For the negative calls (18-30 kHz), the results did not differ across the binge sessions.

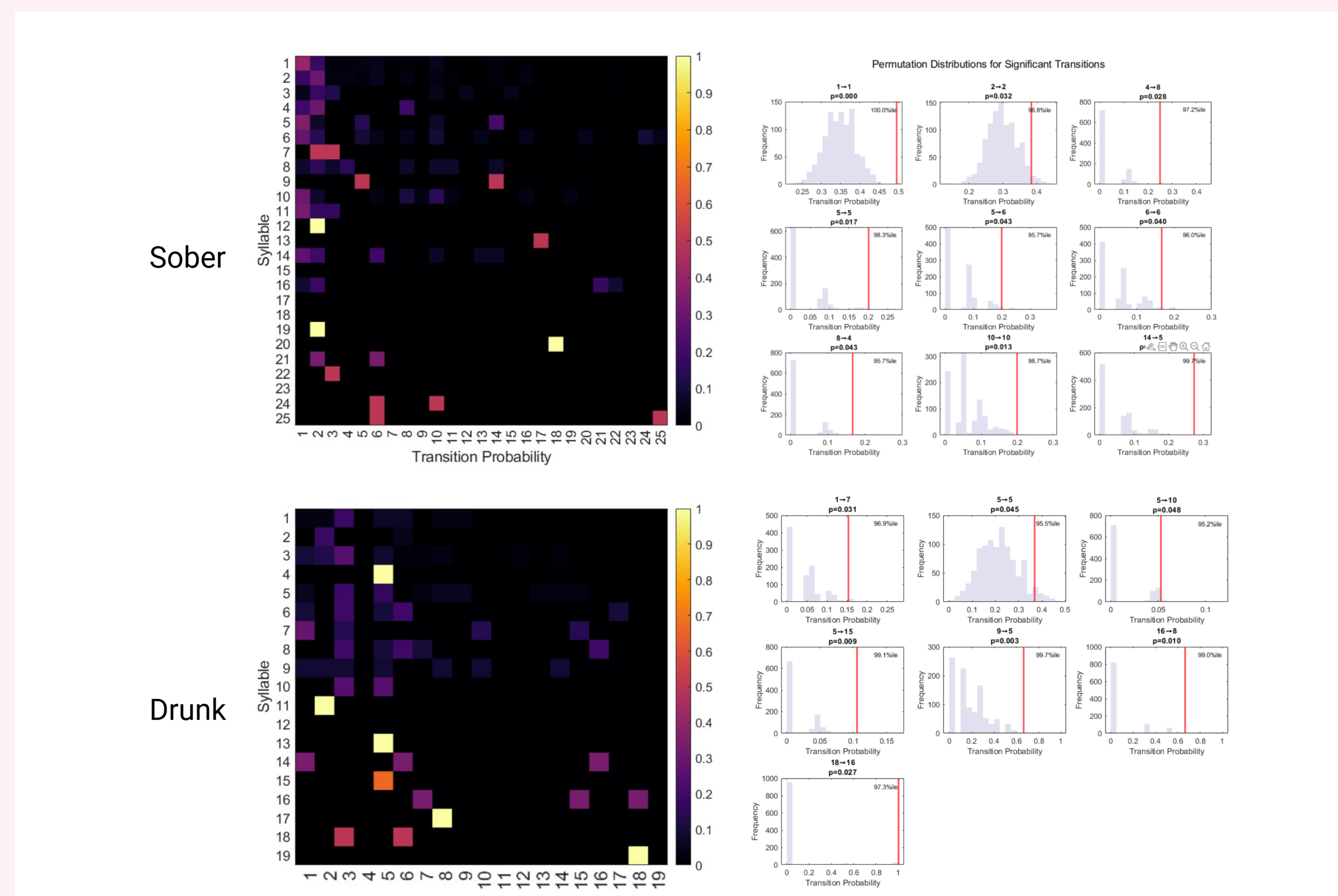

Figure 7. Transition Matrices & Permutation Distributions

Transition probability matrices show how USV call types sequence under sober and drunk conditions. Each cell shows the probability of transitioning from one call type (columns) to another (rows), with lighter yellow indicating higher probability [1]. Sober female mice are shown to be more vocal, indicating higher anxiety.
Right panels depict permutation distributions for transitions found to be statistically significant with large effect sizes, and red lines indicating observed probabilities. This indicates that alcohol exposure may alter call sequence structure, reducing the complexity of significant transitions observed compared to the sober condition.

CONCLUSION

Alcohol exposure in adult female mice was associated with changes in USV patterns, suggesting a reduction in both the number and tonal diversity of calls. However, there is an increase in positive calls toward positive hedonic states when intoxicated. Meanwhile, transition probability analyses indicated changes in vocal sequence structure, with reduced complexity of significant transitions. Further research will be needed to determine the underlying mechanisms and to clarify the potential behavioral significance of the observed changes in USV patterns following alcohol exposure, especiallly in affecting sociability and sex differences.

ACKNOWLEDGEMENTS

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