MINISCOPE PIPELINE: IN VIVO CALCIUM IMAGING DURING FEAR CONDITIONING

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INTRODUCTION

Fear conditioning is a behavioral paradigm in which a subject is trained to pair an unconditioned stimulus (a foot shock) and a conditioned stimulus (a tone). Very little is known about the infralimbic cortex (IL) in fear conditioning, even though the IL is involved in executive function. By tracking the calcium signaling in the IL, we may be able to gain insight into the region’s role in fear conditioning.

For the fear conditioning experiment, the animal was brought to a habituation room for 30 minutes before testing began. During testing, the animal was placed in an unmodified chamber with metal bars on the bottom and presented with three tones, each of which coterminated with a foot shock.

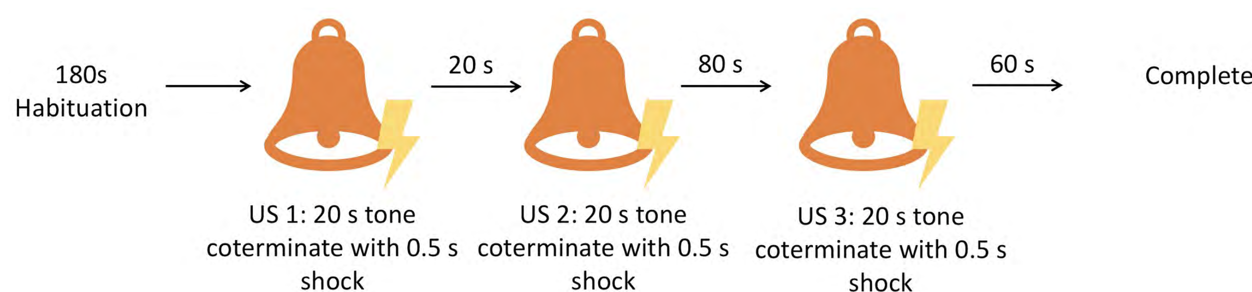


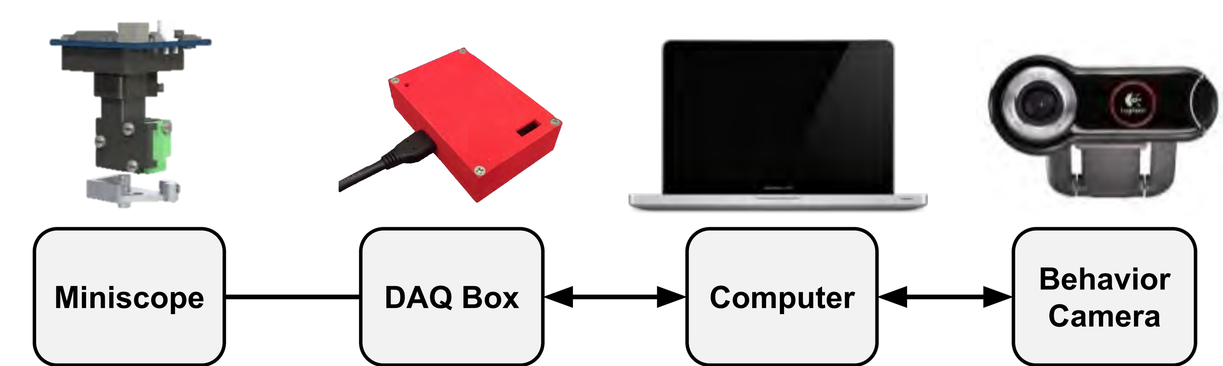
Figure 1: Fear conditioning protocol for the pairing of the conditioned and unconditioned stimulus.

ANALYSIS AND RESULTS

DATA AND INITIAL PROCESSING

Calcium imaging and behavioral videos were recorded simultaneously during the fear conditioning day (day 2) for Mouse 16. The calcium imaging videos were acquired through the miniscope mounted on the mouse’s head; the behavioral videos were acquired through an overhead camera.

The miniscope videos were passed through the pipeline to extract calcium spikes and traces for each identified unit. The behavioral videos were passed through the pipeline to extract the positions of different body parts during the experiment (see sections “Miniscope Workflow” and “Behavioral Workflow” on our first poster)[1, 2].



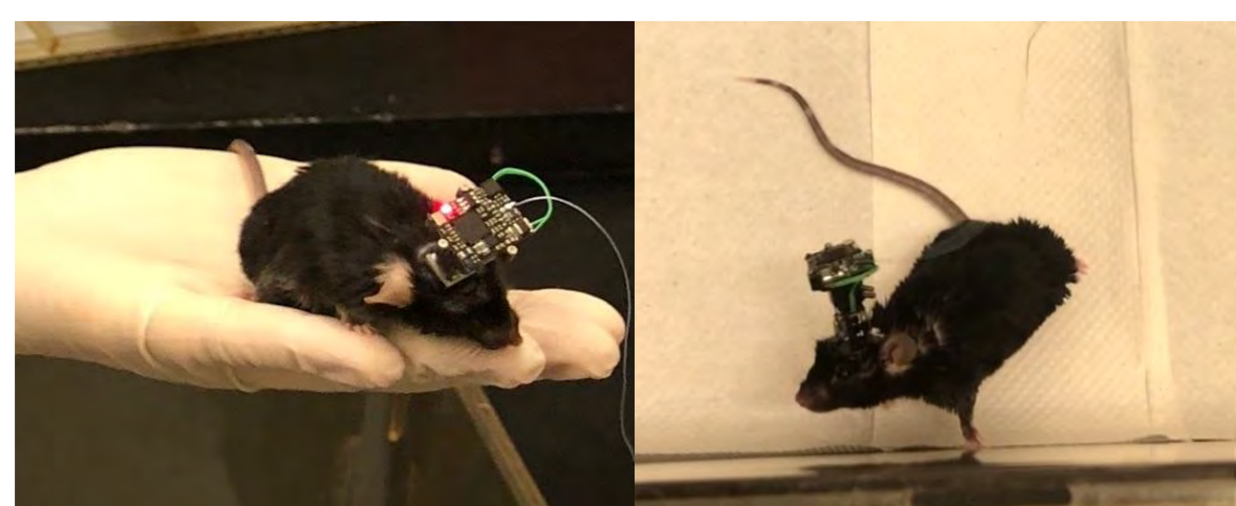


Figure 2: (A) Connection pathway of miniscope to computer (modified from miniscope.org. (B) Mouse with miniscope. (C) A mouse exploring a novel environment with the miniscope recording activity in the hippocampus.

NEURAL ACTIVITY PATTERNS

The resulting data from the MiNiAn pipeline analysis (including pre-processing, motion correction, background removal, initialization, and neural network constrained nonnegative matrix factorization (CNMF) processes) was transformed and visualized using a series of functions created in R. Each of the analyses aimed to track both measured calcium traces and inferred spiking patterns across tone intervals that were hand marked in both the original behavioral and miniscope videos.

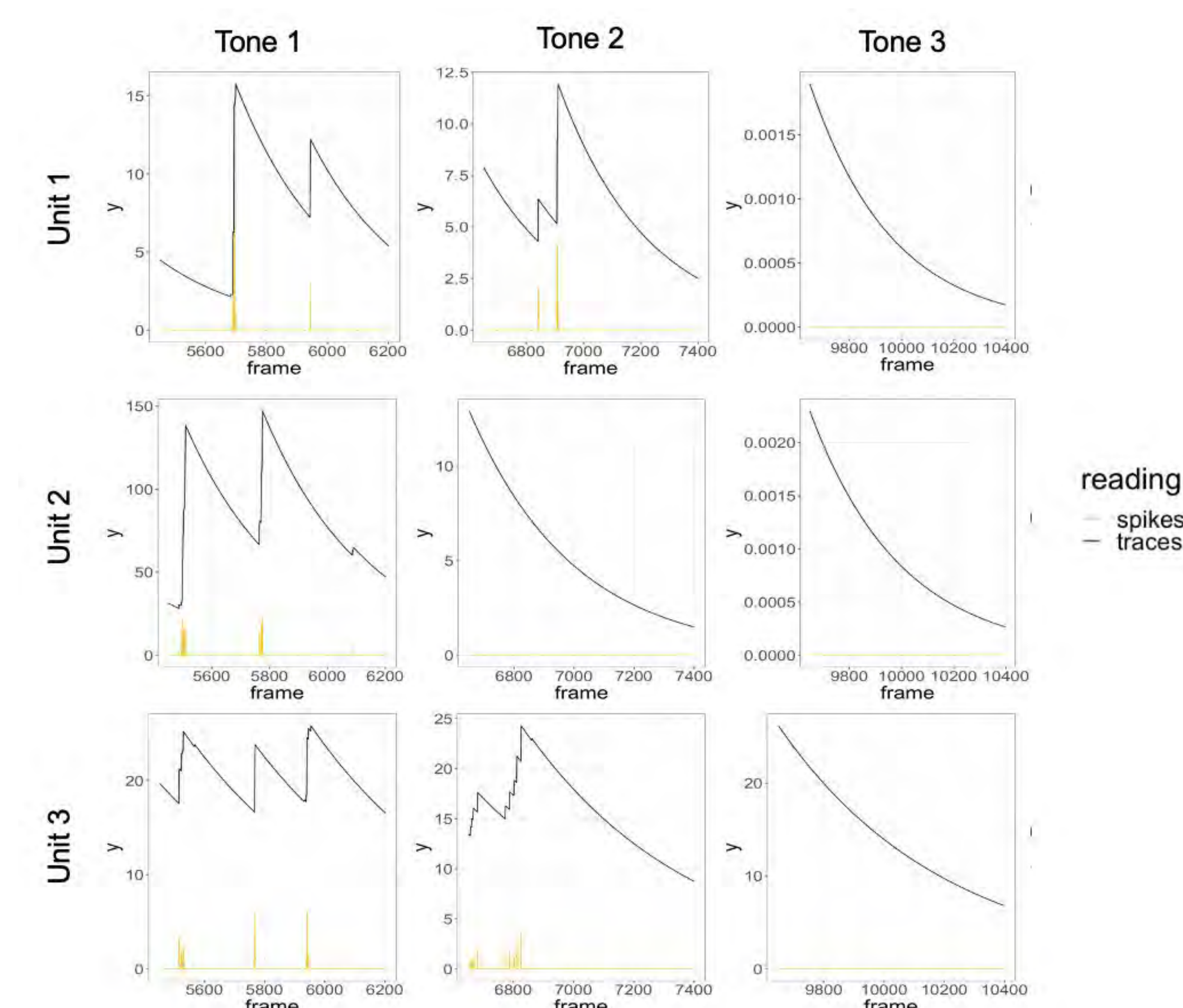


Figure 3: A comparative plot of three different units across the three tones during fear conditioning. Note that the axes dimensions vary in each plot.

In Figure 3, 3 of the best of the original 22 units were manually selected to exemplify units that may be useful in further analyses as they show inferred spiking patterns within the tone intervals while others did not and would not be useful in later analyses.

FREEZING BEHAVIOR

Mice exhibit freezing, a defensive behavior in which the organism is motionless in response to an aversive stimulus, during fear conditioning experiments. We adapted the quantification of freezing from Makino *et al*. (2019) and labeled continuous periods of snout movement less than 4 cm/s for at least 1.28 s as freezing[3]. We

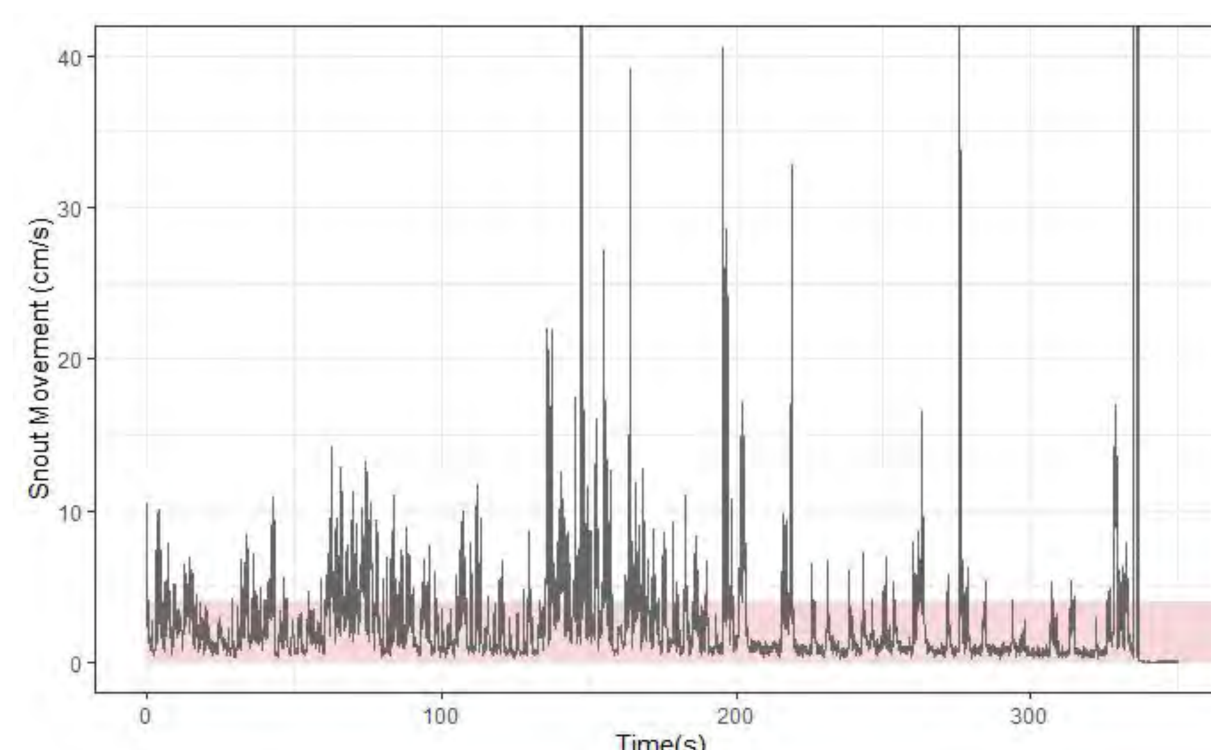


Figure 4: Movement of the mouse’s snout during the experiment. The red shading indicates when the velocity is lower than 4 cm/s.

also cross validated our results with manually identified freezing periods and confirmed that the freezing extraction algorithm yielded matching results ($r^2 = 0.825$). Figure 4 shows the snout movement of the mouse during Day 2 of the experiment. A total of 50 freezing periods with the average duration of 4.4 s were identified by the algorithm.

CORRELATION BETWEEN BEHAVIOR AND NEURAL ACTIVITY

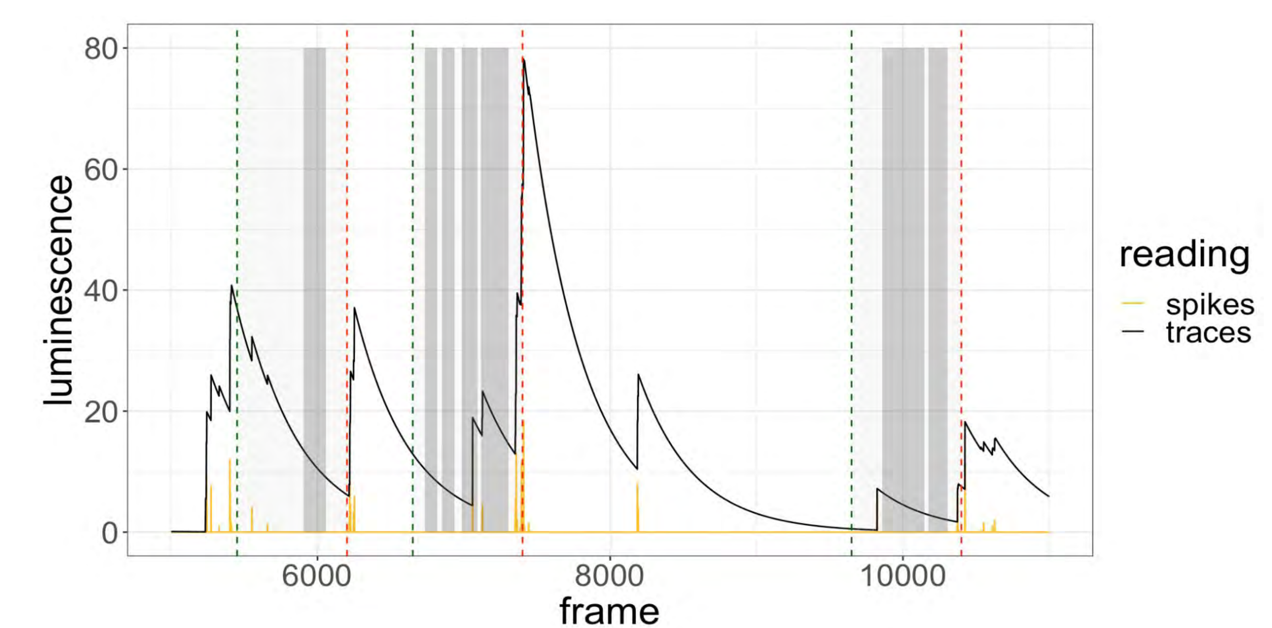


Figure 5: A comparative plot of neural activity and the intervals during which freezing behavior was recorded for a single, highly active neuron from the sample. The dotted green and red lines signify the duration of each of the tones while the dark gray bands within those intervals signify freezing behavior.

Figure 5 shows a simple comparative analysis of neural activity with freezing behavior in a single neuron. With further analysis, the goal is to be able to replicate this figure with an average of the neural activity in the different neurons.

DISCUSSION

Our analysis of the neural activity showed that there were certain neurons that were firing across multiple tones. We were also able to extract the freezing behavior and match these behaviors with neural activity with high accuracy. These findings may suggest that these neurons are involved in the mice’s fear response. The pipeline allowed us to match up neuronal activity and behavior with high accuracy, which would help us better understand what drives behavior. In the future, it would be possible for us to analyze other behaviors and neuronal activity using this method. In later analysis, a statistical approach to determining the most active neural units may be developed.

ACKNOWLEDGEMENTS AND REFERENCES

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1. DeniseCaiLab. (2020). *minian*. Github. https://github.com/DeniseCaiLab/minian
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3. Makino, Y., Polygalov, D., Bolaños, F. *et al.* (2019). Physiological Signature of Memory Age in the Prefrontal-Hippocampal Circuit. *Cell Reports* 29, 3836-3846.