Analysis of Anterior Cingulate Cortex Neural Activity during Effort-Based Decision-Making

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Introduction

Effort-based decision-making is a cognitive function observed in animals that involves evaluating the cost-benefit ratio of a certain action and deciding whether or not to perform the action. This cognitive function is generally understood to be performed and regulated by the anterior cingulate cortex (ACC) region in the medial prefrontal cortex (mFPC). However, the mechanism behind cost-benefit analysis computation and subsequent behavior initiation requires further study. Previous experiments conducted in the lab involving the optogenetic inhibition of the ACC resulted in the disruption of effort-based decision-making. Circuits within these structures are disrupted in psychiatric disorders such as depression (which can manifest in a lack of making decisions based on effort), and analysis on neural populations in these structures can shed light on decision-making and by extension the disorders that affect this behavior. Here, we will analyze the activity of individual neurons in the ACC during effort-based decision-making and suggest further analyses to ascertain how specific neurons encode parameters in this function and produce this behavior.

Methods

In order to measure neuron activity during effort-based decision-making, we used the "T-Maze" behavioral assay in which mice are faced with an option to climb a barrier and get a high-value reward (HVR) of three peanut butter chips and an option to take an unimpeded path and get a low-value reward (LVR) of one peanut butter chip (shown in Figure 1). In a set of around 12-20 trials, a mouse is placed in the "start" section, kept there for 6 seconds, and then prompted to travel along the maze and make a decision to go either left or right, one of which has the HVR while the other has the LVR. Which reward it decides to eat is recorded each trial.

During this assay, we employed the usage of miniature head-mounted microscopes ('Miniscopes') in order to measure neural activity in the ACC. During an action potential, a neuron is depolarized, leading to an influx of Ca2+ ions. The calcium indicator GCaMP7f, which fluoresces green due to Green Fluorescent Protein (GFP) and binds to calcium, is injected into the region of interest and can be observed through the Miniscope during an action potential. A Gradient Index (GRIN) lens is implanted in the brain over the injection site and a baseplate is placed so that a miniaturized fluorescent microscope (Inscopix Miniscope) can be mounted on the head of the mouse. Thus, the Miniscope image displays several neurons with varying levels of calcium concentration (i.e. brightness) serving as an indirect measure of individual neuron activity as the mouse freely roams throughout the maze. The setup is shown in Figure 2 and a resulting image is shown in Figure 3. GCaMP7f was inserted into the ACC through viral injection, thus we were able to examine individual neuron activity of 349 in this region throughout the assay.

To start creating a method for analyzing individual neuron data, we used a single video in which a mouse consistently chose the HVR of the T-Maze across 14 trials in one experiment session. In short, we were examining the mPFC neural activity of a mouse that seemed to be consciously making a decision based in spite of the higher effort required. The next step was creating a workflow that in which we transformed the video into a data frame of signals for each neuron, cleaned the signals, normalized them, and conducted statistical analyses on the resulting data. The first step was done by utilizing an open source MATLAB package Min1pipe, which uses recurrent neural networks to detect neurons and measure their activity via brightness across every frame of the Miniscope video. The resulting data was a matrix in which each row was an individual neuron with its corresponding activity level over time. We then created and ran a script to analyze the outputted data frame. First, we found the exact frames for the beginning, choice, and end of each trial (when the mouse reaches the food cup) and selected the signals 2-5 seconds before and after the event for each neuron (each event was found by finding the frame of a

video capturing the entire T-Maze, synchronizing the video frames with the Miniscope video frames, and finding the corresponding Miniscope video frame of the event). We would then normalize the neuron activity in this time period and then average the signal across each trial. The plot of each neuron organized by the frame of maximum normalized activity can be seen in Figure 4.

Results

The next step in our analysis was to find neurons that have statistically significant levels of activity related to the decision-making behavior. To do this, we conducted a permutation test on the mean activity of each neuron. The mean of a random selection of 61 frames of a given neuron's activity was compared to the means of 10,000 other random selections of 61 frames. Using a 95% confidence interval, we deemed a neuron "significant" if its mean was greater than 95% of the 10,000 sampled means. The results of the significance test are shown in Figure 5 (non-significant neurons are given a value of -1.5, therefore we can identify the significant neurons based on color).

Of the original 349 neurons, 88 were deemed significant due to the permutation test. The significant neurons all have high peaks (Z-score of 1.5-3), thus their heightened activity suggests that they specifically play a role in the decision-making process.

Among the significant neurons, a large subset ($\sim$175-200 on the sorted plot) seem to reach their maximum activity shortly before the choice, therefore these may be implicated in making the decision to choose the HVR. A theory for why certain significant neurons reach their peaks before the choice ($<\sim$225 on the plot) is that certain neurons help make the decision and/or encode the hidden parameters (cost/benefit ratio) of the choice and other neurons reinforce the decision and spur the mouse to continue down the HVR path. This can be supported by the fact that the mouse showed no sign of hesitation in each trial (see vicarious trial-and-error (VTE)). Further analyses are required to ascertain if there is a real significance to all of the "significant" neurons and their role in effort-based

decision-making.

Discussion

As of now, we have a preliminary method of measuring "significant" neurons in the ACC associated with effort-based decision making. The permutation test around the mean activity level of a neuron is just one metric of measuring the significance, therefore different parameters and tests should be applied to identify significant neurons. Since this is applied to just one set of trials for one mouse, the logical step forward is to apply this technique to several other sets of trials in which mice displayed effort-based decision-making behavior (choosing HVR in the T-Maze). From the same plots produced, we can label the neurons and find if the same neurons are significant across other trials. Additionally, we should compare the activity of the non-significant neurons and the significant neurons to see if there is a noticeable difference. Comparing the activity of these neurons during several variations of this assay and during VTE will also give valuable insight to the purpose of these neurons. Once we have confirmed which neurons are significant, it may be helpful to label these on the Miniscope image and see if there are any visual patterns within the neurons.

A further experiment would be the optogenetic silencing and activation of certain subsets of ACC neurons during the T-Maze assay and testing if it affects the decision-making process (i.e. comparing the HVR vs. LVR selection ratio). Comparison to fiber photometry analysis of ACC populations may also prove to validate our findings.

In the T-Maze assay, the end result is a binary objective in which the mouse chooses to exert more effort and get the HVR or to exert less effort and get the LVR. The end result of individual neuron analysis is an understanding of how the ACC exactly encodes this decision. Possible goals include computational models of decision-making (see Neural Networks, Reinforcement Learning) with neurons encoding effort and cost as parameters and the output being the binary decision or further research toward psychiatric applications

based on the premise of the implication of the ACC with effort and decision-making.

Figure 1

T-Maze Assay

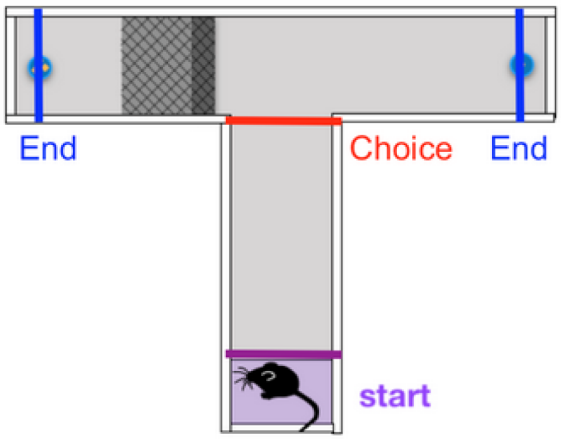

Figure 2

Miniscope Setup

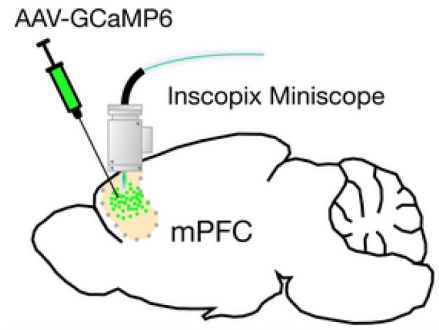

Figure 3

Miniscope Image

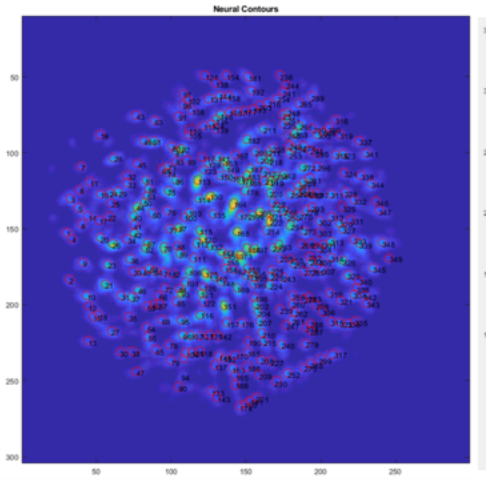

Figure 4

Trial-averaged normalized activity sorted by frame of maximum activity

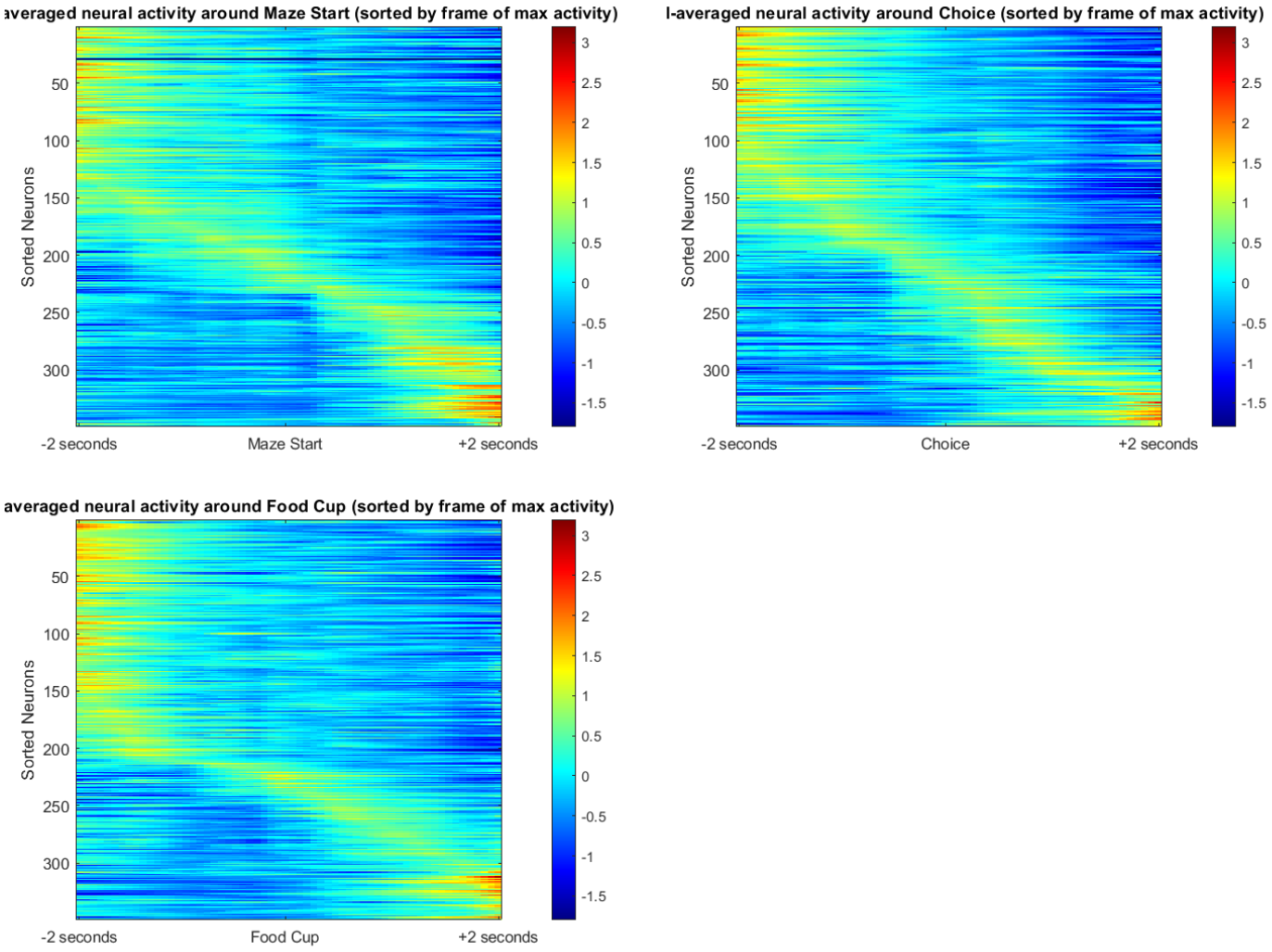

Figure 5

Significant Neuron Activity Based on Permutation Test