

# Development of Mouse Behavior Detection Software to Enhance Calcium Neuroimaging Analysis for Somatosensory Studies

Quentin Leifer<sup>1,3</sup>, Jonathan Krynitsky<sup>1</sup>, Ana Lourenco Inacio<sup>2</sup>, Soohyun Lee<sup>2</sup>, Tom Pohida<sup>1</sup>

<sup>1</sup> Instrumentation Development and Engineering Application Solutions, NIBIB (Signal Processing and Instrumentation Section, CIT)

<sup>2</sup> Unit on Functional Neural Circuits, NIMH

<sup>3</sup> Trainee; Measurement, Statistics and Evaluation master's student at University of Maryland, College Park, MD



## Introduction:

The Unit on Functional Neural Circuits aims to characterize neuronal activity patterns in the primary somatosensory cortex, modeling after the behavior states of mice. Experiments were conducted recording neuronal activity using two-photon calcium imaging in head-fixed, wake mice in the dark. Near-infrared camera acquisition and illumination is used to record whisker movement and mice are fixed on top a wheel that records locomotion. Prior to this project, only whisker movement and locomotion had been used to characterize behavior. However, several other behaviors are worth studying, such as grooming behavior or a gaping mouth, to better understand and categorize neuronal activity. These behaviors can be identified by repurposing the camera recorded data and analyzing with using machine learning.

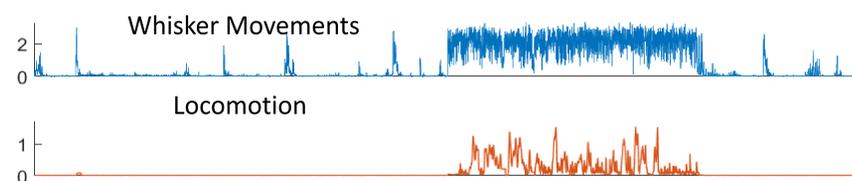


Figure 1: Graph of neuronal firing for whisker movements and locomotion, showing their high correlation along with unexplained remaining activity.

## Objective:

- To identify spontaneous behavioral patterns using video data of the mouse face

## Methods:

### Brightening the Mouse

As the video data was originally meant for whisker movement detection, it was rather variable. A video normalization script was developed in MATLAB using the following steps:

- Find a region of interest (ROI), where the mouse is usually located.
- Calculate the mean ROI brightness of a random frame.
- Brighten until the desired brightness value is reached.

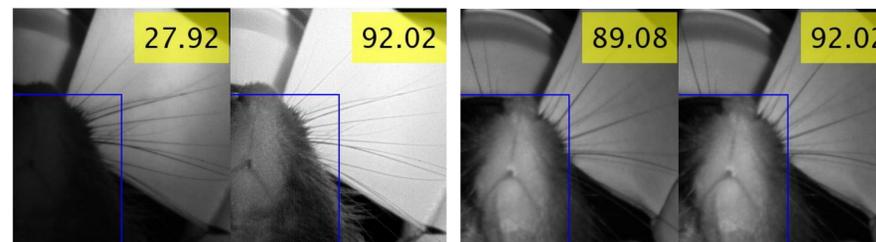


Figure 2: Sample mouse frames of various lighting, darker starting point (Left), brighter (Right), before and after using imlocalbrighten. Brightness values are in the upper left corner.

### Identifying Keypoints

In building the training set, video frames were annotated. Once enough samples were annotated, a DeepLabCut classifier was trained and evaluated on videos outside the training dataset.

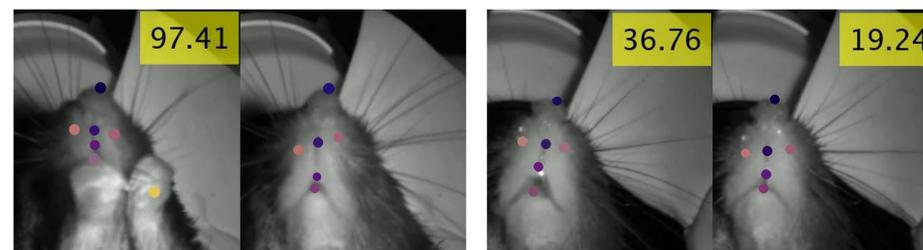


Figure 3: DeepLabCut keypoint frames with detected grooming vs resting state (Left) and mouth gaping vs closed (Right). Keypoint distance values are displayed in the upper right.

### Behavior Detection

This keypoint data is used to infer behavior states by using the following procedure:

- Filter the data to ignore video frames with low keypoint likelihood values.
- Calculate the distance between body part keypoints.
- Use a threshold value to determine where the distance is sufficiently large.
- Create two videos based on the threshold for visual inspection and validation.

## Results/Conclusions:

- A method using imlocalbrighten was developed to successfully brighten videos, overcoming the challenges of variable lighting.
- A DeepLabCut classifier for body part detection was trained and applied to mice videos outside of the training set, effectively identifying keypoints.
- The DeepLabCut classifier failed across images that had yet to undergo the brightening process, showing the importance of the image normalization process.
- A behavior of interest (mouth opening) was successfully detected (despite variable video data), paving the way as a method for future study of additional behaviors.

## Future Developments:

- Detection of additional behaviors and behavioral patterns

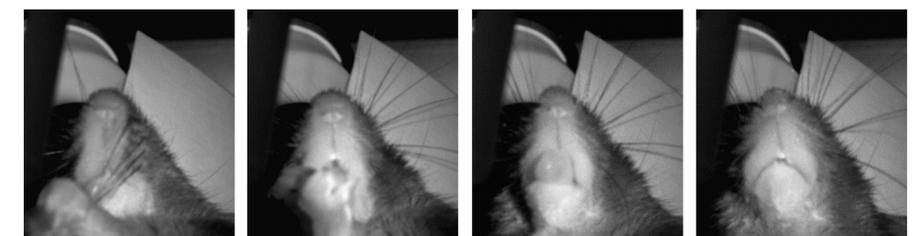


Figure 4: Whisker grooming (Far Left), Paw grooming (Mid Left), Licking (Mid Right), Nose twitch (Right)

- Use of these behaviors to better identify whisking activity and learn more about the functionality of somatosensory cortex.