DeepLabCut as a tool to investigate olfactory-guided behaviors in mice

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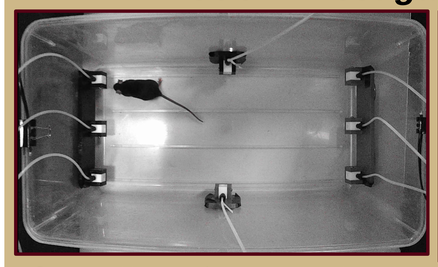
Introduction

Measuring olfactory investigation typically involves the presentation of one or more odors to a freely-moving animal and examining the resultant behavior. This process can be extremely time consuming, as the animal must be observed by a human and manually assessed. DeepLabCut (DLC) is an open source markerless pose-estimation software package that utilizes convolutional neural networks and machine-learning to analyze videos and output coordinates tracking various user-defined points on laboratory animals. Thus, this software allows for the position of specific body parts to be quantified without having to manually observe the animal. In this way, olfactory investigation can be assessed in a high throughput manner.

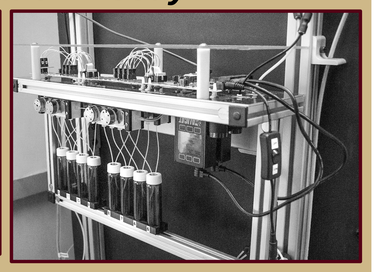
The goal of this project was to establish DeepLabCut in the Dewan lab. We want to analyze how olfactory investigation is influenced by both neuromodulators and the olfactory receptor repertoire. We are also interested in how olfactory investigation is correlated to with neural activity in the olfactory cortex.

Methods

Odor Investigation Assay

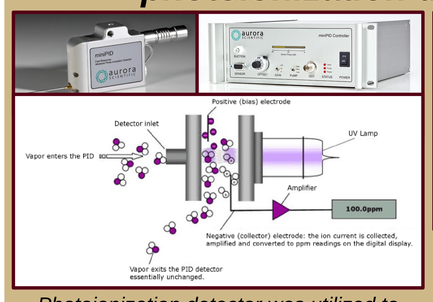


Overhead image of the test chamber with two vacuum ports (center) and two banks of odor ports (left and right)



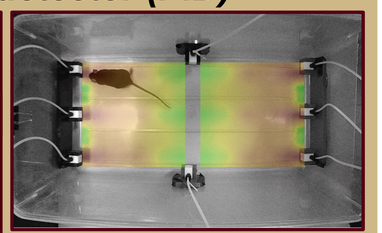
Odors are delivered via a flow dilution olfactometer

Mapping the odor plume using a photoionization detector (PID)



Photoionization detector was utilized to map the odor concentration at each location of the test chamber

Photos from Aurora Scientific and Equipco

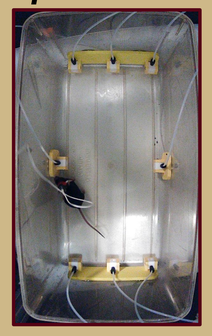


Gradient demonstrating the PID-mapped odor concentration.

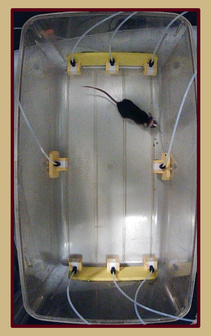
Methods (cont.)

Video acquisition for model

View of an unmanipulated transgenic mouse



View of a mouse with a microendoscope

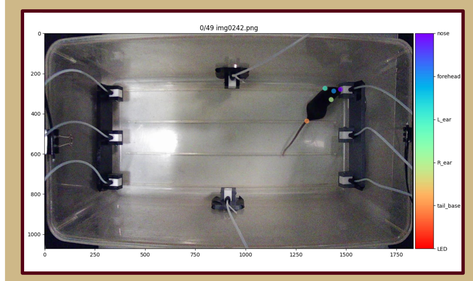


View of a mouse with a cannula targeting the olfactory cortex

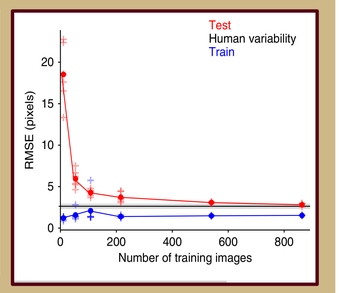
Frame extraction and labeling

DeepLabCut™
a software package for animal pose estimation

DeepLabCut uniformly extracts ~200 frames from 4 videos that are presented to the user for labeling



- *Example of labeling interface within DeepLabCut.*
- *The user views a video frame and places markers manually on the body parts (nose, head, left ear, right ear, and tail base)*
- *Two LEDs are also "tracked" to determine which odor port is currently active.*



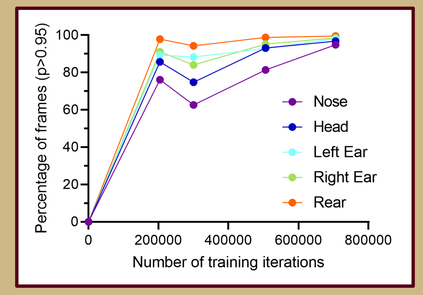
- *Graph from Mathis et al., 2018 that demonstrates the root mean square (RMS) error between model and human performance.*
- *After training the model on ~200 frames the difference in performance is negligible.*

Methods (cont.)

Training the model

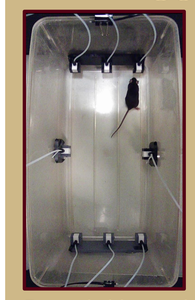
rcc.fsu.edu

We utilized servers and GPUs that were sourced through the Research Computing Center at FSU.

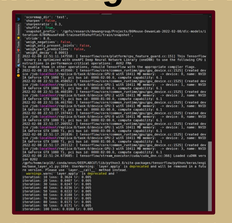


The percentage of frames in which positions were estimated with high confidence (p>0.95) according to the number of training iterations.

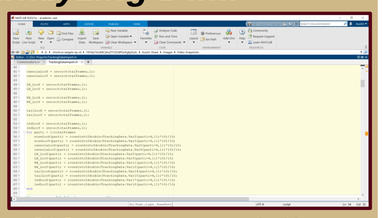
Collecting and Analyzing Data



Record video and present odorants



Use the trained model to determine the position of the mouse in novel experimental videos



- *DLC output is processed with a custom MATLAB script to quantify:*
  - *Position*
  - *Velocity*
  - *Acceleration*
  - *Head Angle*
  - *Specific behaviors*

Summary & Future Applications

DeepLabCut is a robust tool for pose-estimation in laboratory animals without the need for manual behavior classification. The positional output of DeepLabCut can be processed to determine animal behavior in relation to olfactory stimulation. The positional and extrapolated behavioral data can be utilized with other methods in the future, such as calcium imaging or drug manipulations, to further correlate neural activity with olfactory-guided behaviors.

References

- Insafutdinov, E., Pishchulin, L., Andres, B., Andriluka, M. & Schiele, B. DeeperCut: A deeper, stronger, and faster multi-person pose estimation model. In European Conference on Computer Vision, 34–50 (Springer, 2016).
- Mathis, A. et al. Deeplabcut: markerless pose estimation of user-defined body parts with deep learning. Nature Neuroscience 21, 1281–1289 (2018).
- Nath, T. et al. Using deeplabcut for 3d markerless pose estimation across species and behaviors. Nature Protocols 14, 2152–2176 (2019).