An Application of Computer Vision in Automating Behavioral Analysis of Predator Odor Exposure in Rats

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Introduction

In the study of stress-reactive behaviors as predictors of PTSD and the comorbidity of PTSD and alcohol use disorder in animal models, the quantification of these behaviors, specifically digging and immobility, often relies on manual annotation (AnyMaze). Digging is thought to be an active stress-reactive behavior and is defined by actively pushing bedding to form a structure or barrier. Immobility is an inactive stress-reactive behavior defined by a lack of movement for more than one second (Ornelas et al., 2021). This project demonstrates a novel application of machine learning software DeepLabCut (DLC), a deep learning program for pose estimation, and SimBA, an open source toolkit for computer classification of complex behaviors in laboratory animals, to analyze stress-reactive behaviors. The purpose of this project was to implement DLC and SimBA in our research in stress-reactive behaviors by developing a deep learning model that can locate anatomical features and perform accurate pose estimation on laboratory rats using DLC, then identifying and generating descriptive statistics for the stress reactive behaviors of immobility and digging with SimBA.

Methods

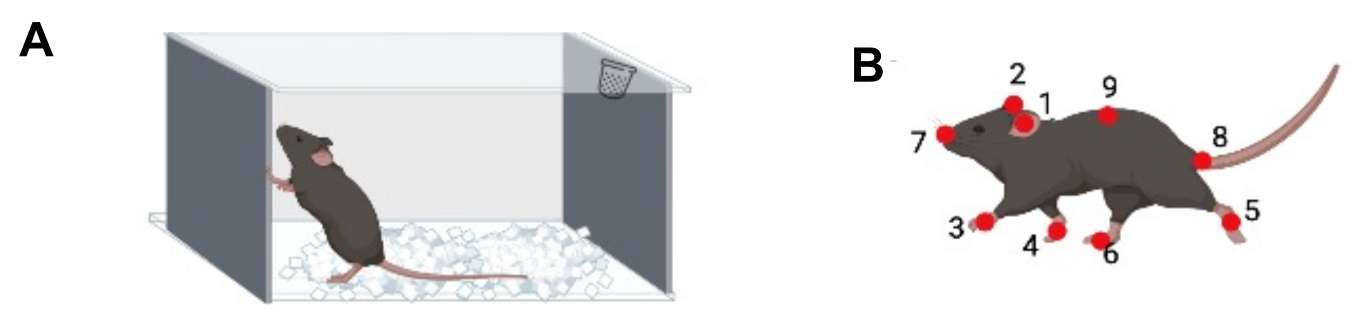

Figure 1: Experimental Setup and Labeling Configuration.
A. Rats are placed into a confined, inescapable exposure chamber and 10 μ TMT (2,5-dihydro-2,4,5-trimethylthiazoline) is pipetted onto filter paper in a metal basket on one side of chamber. Behavior is video recorded for 15 min. **B** Labeling configuration used throughout this project.

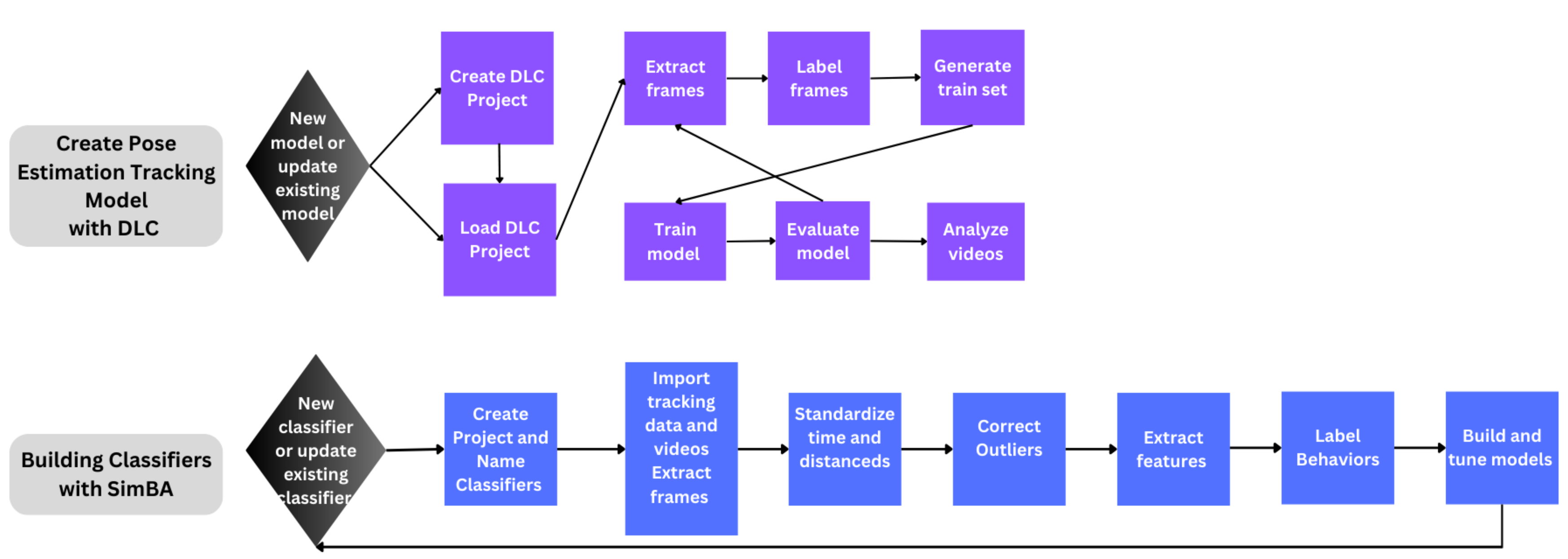

Figure 2: Building and Training the Models
Top. Using DeepLabCut for Multi-Animal Projects, a neural network was developed using 12 training videos from past predator odor exposures. 20 to 30 frames were extracted for each training video, creating an original training dataset of >150 frames. Training was performed on a Windows 10 machine with a NVIDIA GeForce RTX 3090 graphics card, and allowed to run up to 200,000 iterations, or until the loss plateaued.
Bottom. Using SimBA, behavioral classifiers were built for immobility and digging, using the unfiltered tracking data from DLC, and applying Gaussian smoothing when importing the data. 13 videos were used in the training set, and annotated for each behavior of interest.

Figure 3: Validating the Models
The models were validated on novel videos and validation videos with gantt charts were visually inspected for accuracy and specificity, then retrained and refined as needed.

Immobility and Digging at Different Discrimination Thresholds

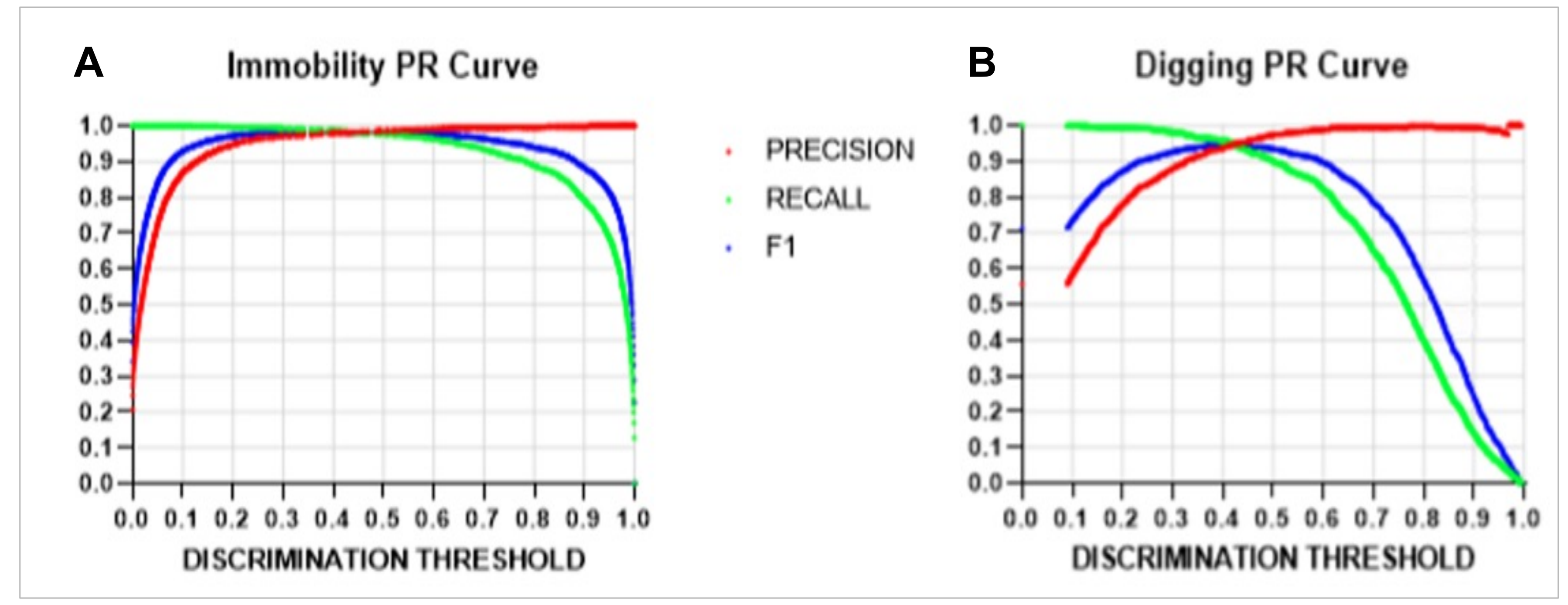

Figure 4: A: PR curve indicates optimal classifier performance as measured by F1 at discrimination thresholds of approx. 0.4-0.5 for immobility (A), and 0.38-.45 for digging (B).

Tuning Parameters on Classifier Performance

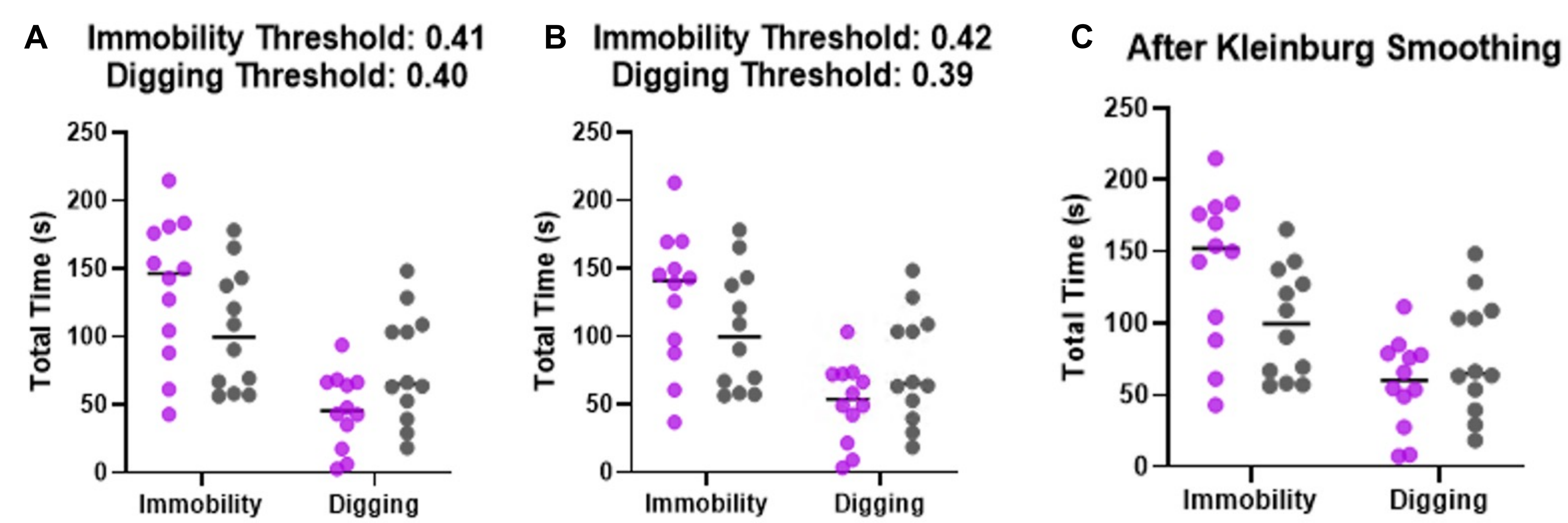

Figure 5: A. SimBA identified a significantly higher total immobility time compared to ANY-Maze, with a mean increase of 31s, and a significantly lower digging time, with a mean difference of about 31s. Min. bout was set at 1000ms. **B.** SimBA identified a significantly higher total immobility time compared to ANY-Maze, with a mean increase of 24s, and a significantly lower digging time, with a mean difference of about 26s. **C.** The predictions after Kleinberg smoothing for immobility and digging were significantly correlated with the manual ANY-Maze annotations. Minimum bout was set at 1000ms. T-tests showed a significant difference between the SimBA predictions and ANY-Maze annotations for immobility, but a nonsignificant difference for digging, with a mean increase of 31s for immobility and 19s for digging.

Conclusions

- Predictions with discrimination thresholds associated with optimal F1 scores are significantly correlated with values obtained by manual annotation, with SimBA overall identifying significantly more time for immobility and significantly less time for digging.
- Kleinberg smoothing resulted in a nonsignificant difference in predictions for digging and significantly more time for immobility, indicating that Kleinberg smoothing is efficient in optimizing some/certain behavioral predictions.
- Overall, the results indicated that the behavioral classifiers are fairly accurate in identifying immobility and digging in predator odor exposure chambers. Thus, the use of DeepLabCut and SimBA, after determining the optimal parameters for our needs, is an appropriate method of automating behavioral analysis in predator odor exposure chambers that is able to generate reasonably accurate predictions for immobility and digging from only experimental videos.
- This project demonstrates the novel application of the open source machine learning softwares DeepLabCut and SimBA in the automation of behavioral analysis of the stress reactive behaviors immobility and digging in male and female Long-Evans rats exposed to TMT. This project creates a framework for the identification and analysis of other behaviors that may be indicative of stress-reactivity in predator odor experiments, as well as other potential applications of behavioral research in and outside of Besheer Lab.

Acknowledgements

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