An Accurate and Markerless Tracking of Multiple Mice Using the Deep Learning Program DeepLabCut

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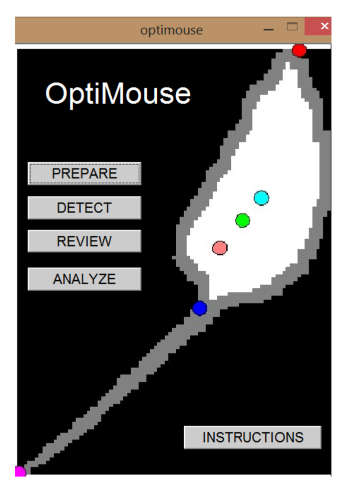
Introduction

The ability to quantify behavior can be a critical component for research in neurosciences. The tracking and analysis of a mouse's behavior through video recordings are often a complicated process that may require specialized equipment such as reflective markers which can affect the mobility of the animal (Mathis et al., 2018). In order to limit the dependency for such gadgets, several markerless tracking programs have been recently developed that have been used in our lab for behavioral analysis. The programs include Optimouse (OM) by Ben-Shaul (2017) and the deep learning program DeepLabCut (DLC) by Mathis et al. (2018). In this study, we compared the reliability and accuracy between DeepLabCut and Optimouse. We hypothesized that DeepLabCut was more accurate and versatile, where it can track a mouse more accurately than Optimouse and can be used under many different conditions.

DeepLabCut



Optimouse

Methods

Chronic Social Defeat Stress

Our behavioral analysis involved using data from chronic social defeat stress which is used to study depression-related behavior. We tested recordings of the social interaction (SI) test involving one mouse in a square open field. The social defeat witnessing (Def) test was also used since it had three mice separated into 2 equal zones: a black mouse was sectioned off into the left zone while the right zone contained a black mouse and a white CD1 mouse.

Testing the Accuracy of Optimouse

In order to test the accuracy of our data, we used an algorithm to randomly extract around 40 frames of each recording. We then manually labeled the head position of the mouse in these frames and assumed that the hand-labeling of each frame is the most accurate method. Afterwards, we computed the average head size of the mouse in each according in terms of pixels which will be the acceptable margin of error that Optimouse can make when predicting the head positions. Finally, we analyzed the recordings through OM, which automatically detected the features (e.g. nose, body, and tail) and processed the recordings. We then extracted the corresponding frames and calculated the percentage of errors produced.

Testing the Accuracy of DeepLabCut

The same method for Optimouse was also used to analyze the accuracy of DeepLabCut. We also used DLC to test three mice simultaneously.

Applying DeepLabCut on egocentric tuning

As an example of an application, we determined the relative positions and angles of a mouse to an object with DeepLabCut. The DLC data was compared to calcium imaging of the mouse which was taken from the miniature, integrated microscope technology of Resendenz et al. (2016).

Results

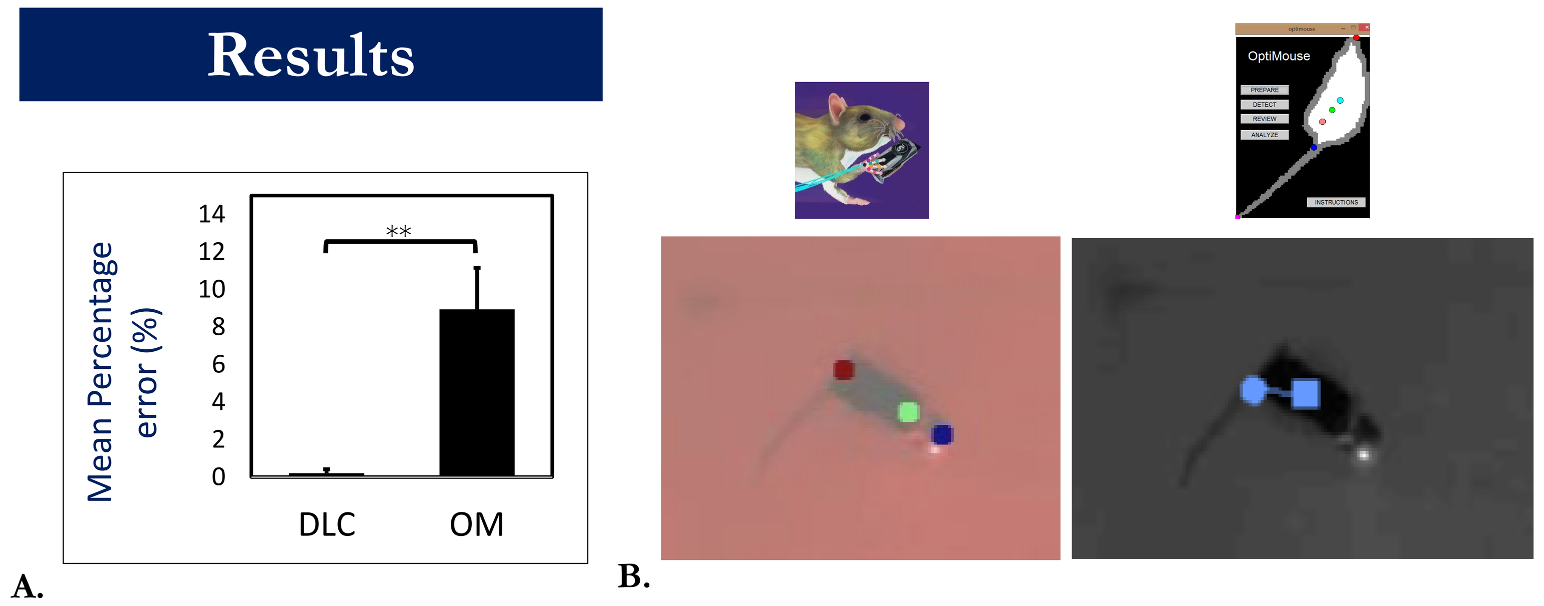


Figure 1. The comparison of DeepLabCut and Optimouse in terms of accuracy
A) The average percentage of frames (mean percentage error) taken from 1 mouse recordings (n=4) where the predicted positions of DLC and OM was outside the acceptable error range of manually labeled frames.
** p<0.05, Student's T-test
B) Examples of an analysis of the same frame by DLC (top-left) and OM (top-right), where DLC correctly labeled the head (blue dot), body (green dot), and tail (red dot). In comparison OM correctly labeled the body (square dot), but not the head (round dot) and did not include any labeling for the tail.

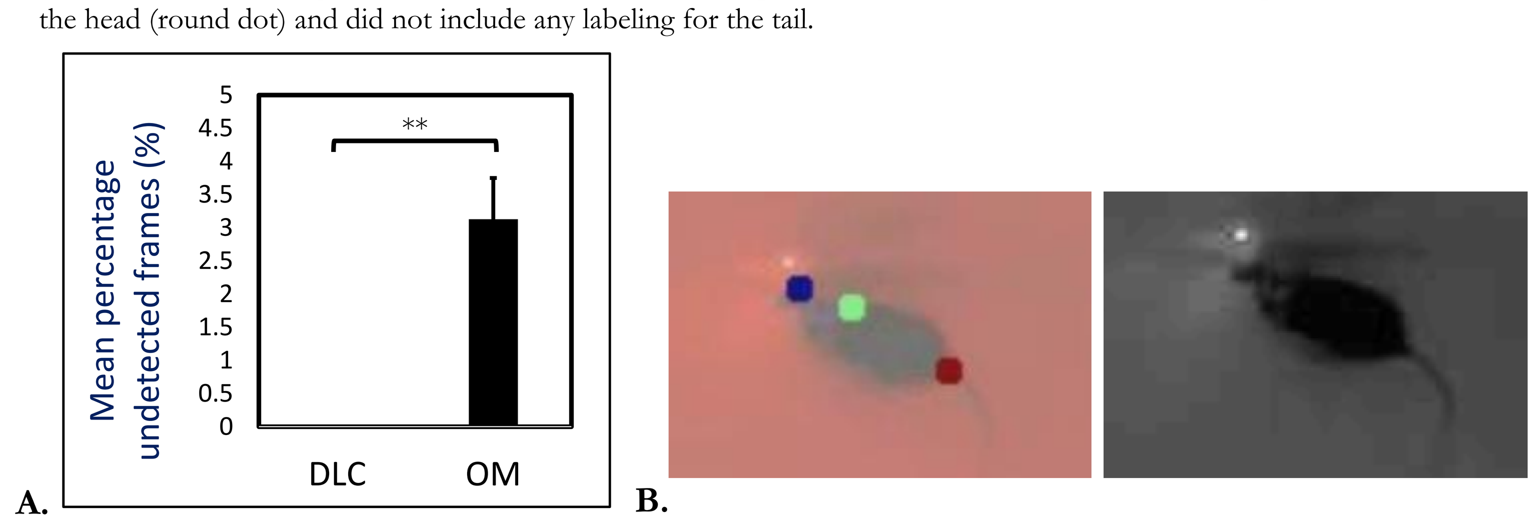


Figure 2. The comparison of DeepLabCut and Optimouse in terms of mouse detection
A) The average percentage of frames (mean percentage undetected) taken from 1 mouse recordings (n=4) where DLC and OM did not detect a mouse even if the mouse was present.
** p<0.05, Student's T-test
B) Examples of an analysis of the same frame by DLC (left) and OM (right) were shown where DLC was able to accurately detect a mouse while OM showed no detection

Figure 3. The mouse detection accuracy of DeepLabCut for analysing 1 mouse in an open field
The accurate detection of various features of the mouse (i.e. head (blue), body (green), and tail (red)) in different movements and shapes during the 1 mouse analysis.

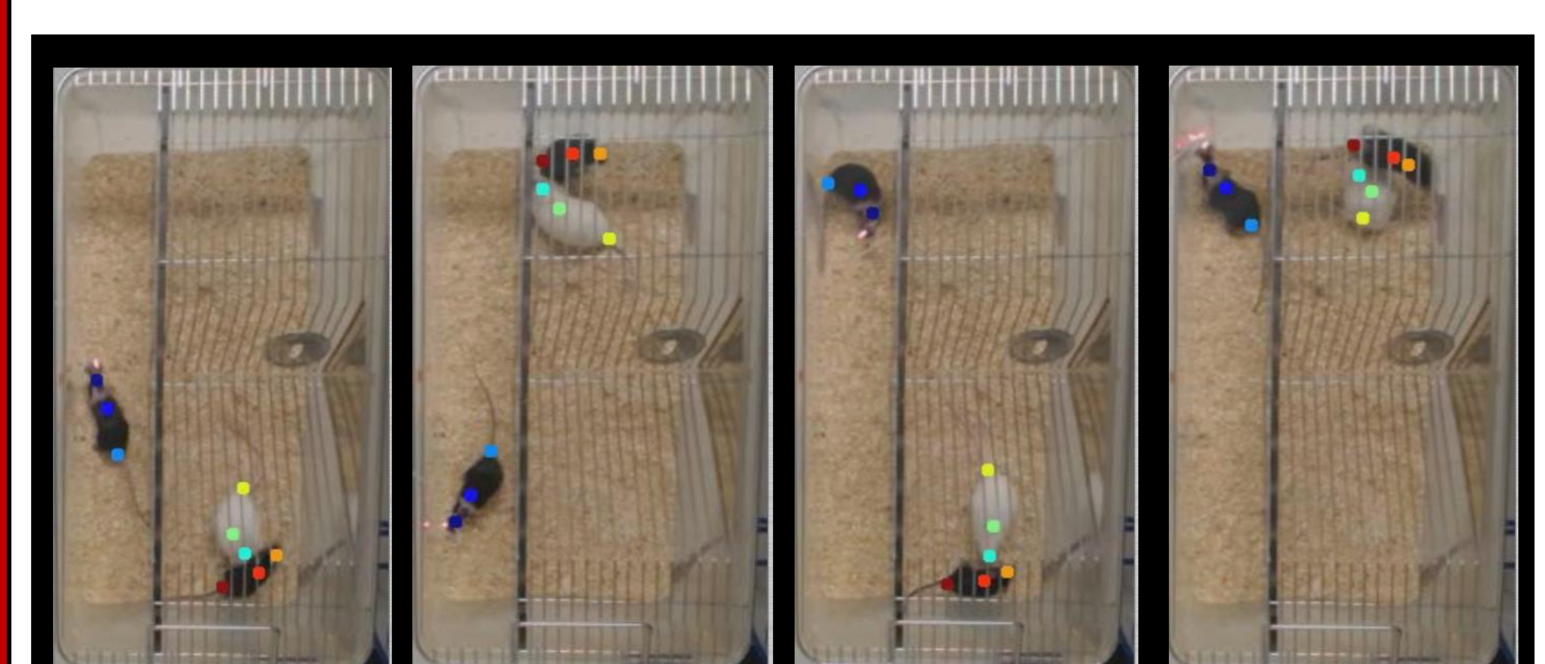


Figure 4. The mouse detection accuracy of DeepLabCut for analyzing 3 mice in social interaction
Examples taken from the recordings of the detection of various features of all 3 mice under the social defeat witnessing condition, where the different movements and forms of the mice were all successfully detected.

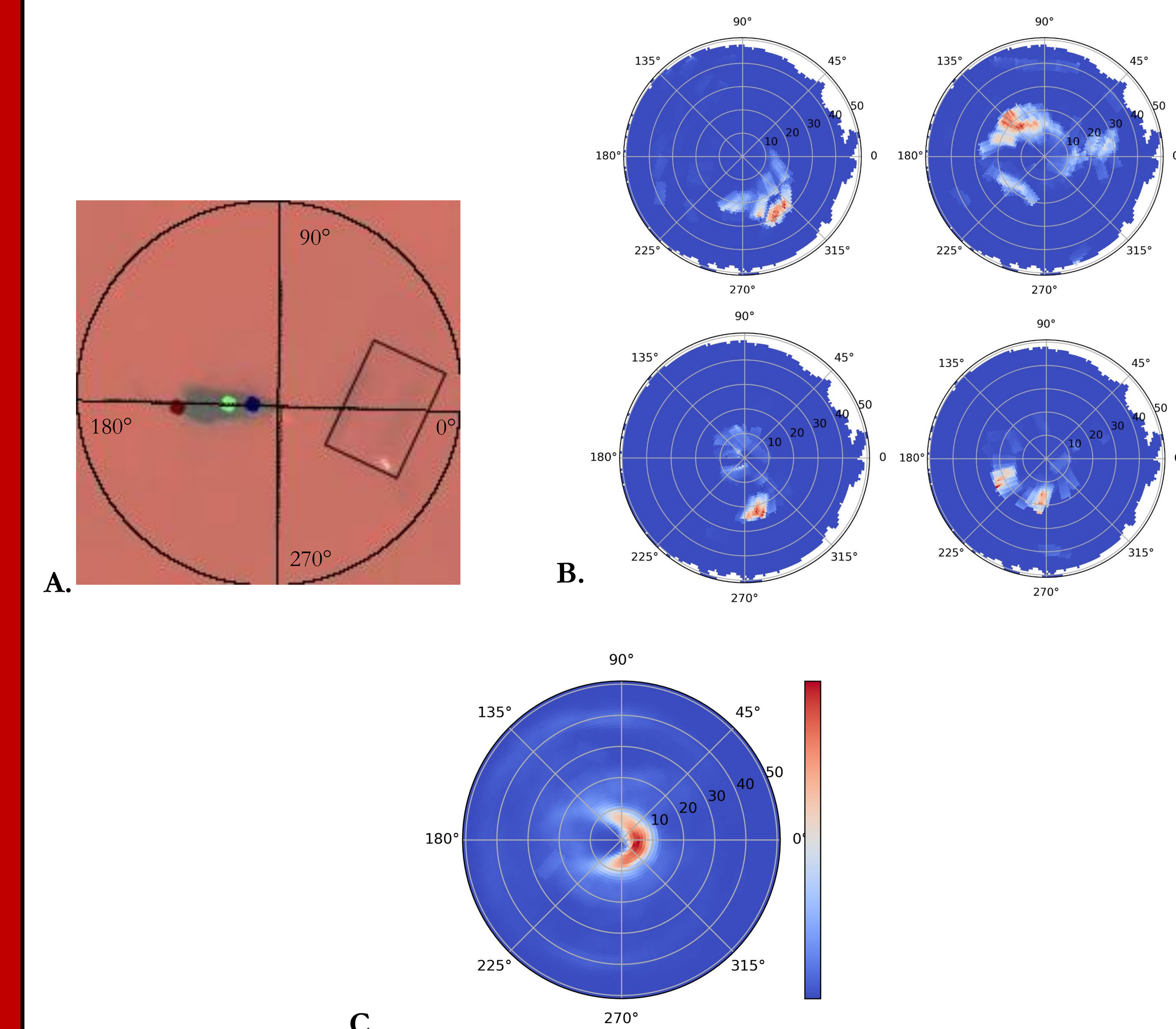


Figure 5. An Application of DeepLabCut for Egocentric Tuning
A) The mapping of the mouse in relation to the social target (i.e. rectangle in front of the mouse) during egocentric tuning, The DLC labels were used to calculate the direction and the location of the moving mouse.
B) Generated heat maps showing the raw data for the frequency of hippocampal CA1 neuron activation (calcium firing) in relation to the position and angle to the social target.
C) Example heatmap of the spatial occupancy of the social target

Conclusion

DeepLabCut and Optimouse can correctly detect behavior with a high precision, but DeepLabCut's higher accuracy and versatility is more useful for highly precise experiments that required behavioral analysis of many different features.

Additionally, Optimouse did not allow the detection of multiple mice simultaneously. Thus, DeepLabCut is also a great tool for the accurate and efficient behavioral tracking of mice in social interactions.

Finally, an application of DLC was also shown, where we were able combine the DLC-predicted positions of the mouse with hippocampal calcium firing to generate data on egocentric tuning.

References

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