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Automated Behavioral Analysis Using DeepLabCut for Mouse Behavioral Phenotyping

Background: Attention-Deficit Hyperactivity Disorder (ADHD) is a highly heritable neurodevelopmental disorder characterized by persistent symptoms of inattention, hyperactivity, and impulsivity. Preclinical animal models provide a valuable framework for investigating the genetic and neurobiological mechanisms underlying psychiatric disorders, as behavioral phenotypes represent a measurable output of neural circuit function. Progress into improving our understanding of ADHD is hampered by limitations of existing animal models for ADHD where there is no universal behavioral phenotype for ADHD and most often, the behavioral outcome measures are collected at single timepoints, require subjective observation, and are fragile to behavioral variability. To improve the rigor and translational relevance of preclinical ADHD behavioral studies, continuous, robust, and automated analytical pipelines are required. Such approaches may enhance reproducibility, reduce observer-dependent variability, and facilitate standardized behavioral assessments for comparison across ADHD models. DeepLabCut is a deep learning-based motion tracking platform that enables automatic tracking of user-defined body landmarks from video recordings. We hypothesize that implementation of DeepLabCut will aid in establishing a tractable behavioral phenotype within the *Lphn3* mouse model of ADHD by providing high data throughput, unbiased, and ultimately comprehensive analysis of animal behavior.

Methods: To test our hypothesis, we first set out to establish and develop an automated pipeline, DeepLabCut (DLC), for training and analyzing large-scale behavioral datasets. Behavioral videos were analyzed using DLC within a dedicated Anaconda computing environment. A total of 17 datasets from various behavioral tasks such as novel object recognition and open field tests were processed with approximately one hundred frames extracted from each video. Each video was manually labeled, totaling ~1700 labeled frames for training the pipeline. In each frame, six anatomical landmarks were identified: the mouse's nose, left ear, right ear, center, base of tail, and tip of tail. DeepLabCut2Kinematics was installed within the same environment to analyze locomotive activity using an h5 file obtained during the initial analysis. Through DeepLabCut2Kinematics, we obtained data quantifying distance traveled, percentage of time moving, and mean speed. We proceeded to compare eight videos analyzed by DeepLabCut to the same eight videos scored manually. For a deeper analysis, we took six novel object recognition task (NORT) videos and monitored the percentage of interaction time and compared those against manual scoring for accuracy.

Results: We successfully established a DeepLabCut automated pipeline to train a complex neural network. As far as locomotor activity, our DLC analyzed videos produced similar results to those previously analyzed with Bonsai software. Up until now, NORT interactions were manually counted. Upon implementation of the DLC pipeline, we observed inconsistencies between our manual and DLC behavioral measures and believe implementation of DLC analyses not only improves the objectivity and resolution of existing analyses, but can be used to query more complex behavioral measures relevant to ADHD, and improve the field.

Future Directions: We will continue to optimize and scale our DLC pipeline and behavioral analyses. We plan to next apply DLC to our *Lphn3* behavioral dataset on Lphn3 deletion using a floxed Lphn3 mouseline and crosses to target all neurons (*Syn-Cre*), prefrontal cortex neurons (*PFC-Cre*), glutamate neurons (V*glut2-Cre*), and dopamine neurons (*DAT-Cre)*. This previously collected dataset includes >1200 videos across several tasks used to measure locomotor activity, anxiety-like behavior, spatial recognition memory, working memory, and novelty-induced hyperactivity. This tool is a valuable resource that can improve our understanding of our ADHD model.