Automated characterization of naturalistic behaviors in a chronic model of epilepsy

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Abstract

Epilepsy encompasses a set of complex, multifaceted disorders presenting a large panel of disease symptoms. A deeper understanding of their underlying disease mechanisms is likely to be required for the development of disease-modifying therapies (Gschwind et al., 2023; Lignani, Baldelli & Marra, 2020). Several forms of epilepsy are characterized by changes in gene expression profiles of neuronal networks that lead to significant alterations at the neuronal network and behavioral levels. In this study, we investigate the behavioral phenotype of a chronic epilepsy model by leveraging machine learning algorithms to analyze long-term video data of mice in naturalistic settings. We aim to identify behavioral markers beyond seizures and assess the impact of potential therapeutic treatments. Describing their behaviors in terms of behavioral modules will also allow us to better understand behavioral transitions and to capture correlations between neural pathways and behavior in healthy and pathological conditions.

Keywords: epilepsy, behavior, computer vision

Introduction

Automated approaches based on computer vision have become important tools for disease models characterization and biomarker investigation. It allows us to study a wide range of naturalistic behaviors over long periods of time in a minimally stressing environment (home-cage). Here we applied two machine learning-based packages to video recordings of mice infused intrahippocampally with kainic acid (IHKA) to generate a preclinical model of temporal lobe epilepsy. This form of focal epilepsy is often difficult to control with medication; ~30% of patients do not respond to current pharmacological treatment and may require brain surgery. We evaluated the added value of behavioral quantification of this model over long periods of time and validated our pipeline on IHKA videos. This pipeline is currently being applied to other epilepsy mouse models, with the goal to combine it with *in vivo* calcium imaging and capture correlations between neural pathways and behaviors, thereby increasing our understanding of behavioral transitions and the neural pathways involved in different forms of epilepsy.

Methods

Videos were acquired using a top-view camera (1280 x 1024 pixels), then normalized to 30 fps and 128 kbps using ffmpeg (version 4.2.2).

We used DeepLabCut (Lauer et al., 2022; Mathis et al., 2018; Nath et al., 2019) (DLC, version 2.3), a package based on supervised machine learning, to generate keypoints on mice. 15 keypoints were labelled manually in an average of 10 frames per video, and a DLC model was trained using a training dataset of N=28 videos (filtering on single animal videos) on a single GPU (Nvidia GTX 1080Ti). The keypoints generated were then analyzed using keypoint-MoSeq (Weinreb et al., 2024; Wiltschko et al., 2015; Wiltschko et al., 2020) (kp-MoSeq, version 0.4.7) to identify behavioral syllables, which are distinct, recurring patterns of movement that can be used to characterize and quantify the behavior of the mice. The keypoint-MoSeq model was trained using the same training dataset and 8 keypoints (identified as properly detected in a semi-automated analysis) using 2 GPUs (Nvidia GTX 1080Ti). The pipeline was validated on a first dataset of 4-hours videos (N=8) of IHKA and control mice (male, C57BL/6j strain), and on a second dataset of 6-hours videos (N=16) of IHKA mice (male, C57BL/6j strain) undergoing pharmacological treatment (Diazepam 2 mg/kg N=8; Vehicle N=8). All statistical analyses were conducted in python 3.9 using scipy (version 1.11).

Results

Behavioral quantification in long video recordings

DLC is a commonly used package to generate keypoints on mice and it has been extensively adopted in numerous laboratories. However, it is typically employed for either specific tasks or relatively short video recordings. The application of kp-MoSeq to identify behavioral syllables expands the range of possibilities to longer recordings. Those patterns of movement can then be used to characterize and quantify the behavior of the mice in naturalistic settings.

In the first instance, we focused on extending the duration of the videos analysis. We reasoned that the analysis of a longer timeframe may decrease individual variability (fig. 1), such as inter-mice variability of circadian cycles.

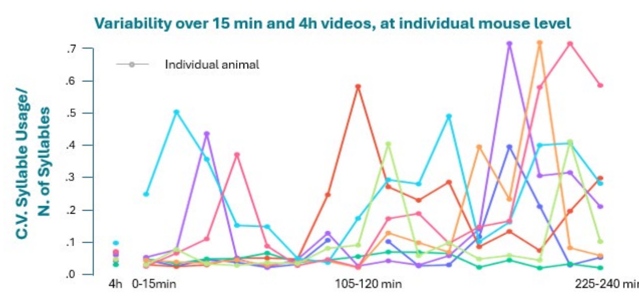

Figure 1 Comparing normalized CV for individual animals between 4-hour and 15-minute observations over time. CV: Coefficient of Variation.

Pipeline validation

We focused our pipeline validation on a well-established model of temporal lobe epilepsy. This approach allowed us to validate our results by comparing them with published data (Gschwind et al., 2023) on the same mouse model of epilepsy, while extending our period of observation to a few hours, better suited for home cage observations over a longer time frame.

We confirmed the previously reported (Gschwind et al., 2023) findings of up and downregulated behavioral syllables in IHKA mice after different treatments (fig. 2). As syllables are identified by kp-MoSeq using an unsupervised approach, expert interpretation is required for the meaningful assessment of behaviors beyond simple measures such as keypoint displacement. The sequence or combination of multiple interpreted syllables is required for the identification of long and/or complex behavior.

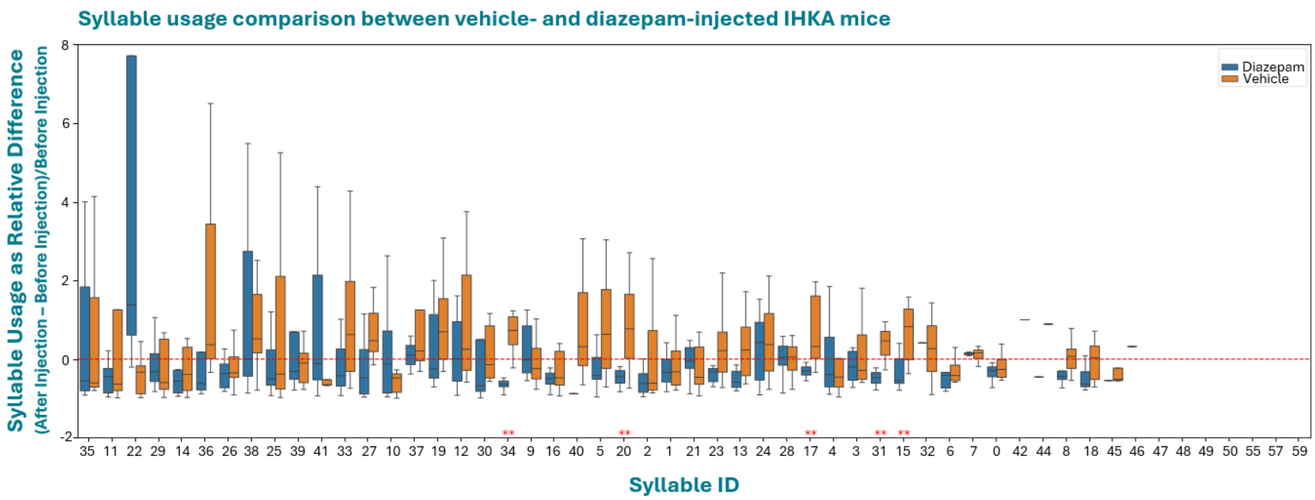

Figure 2 Comparative analysis of syllable usage within animal 3h before and 3h after vehicle and 2mg/kg diazepam injection in a cohort of IHKA mice. The red dotted line represents no change between syllable usage. Double asterisks (**) indicates p-values < 0.01 calculated using a Welch 2-tail t-test, p-value threshold of 0.05 was used; vehicle n=8, diazepam n=8.

Conclusions

In this study, we successfully implemented a pipeline for the automated analysis of mouse behaviors in animal models of epilepsy. This pipeline leverages machine learning algorithms to analyze long-term video recordings of mice in naturalistic settings.

We validated the models on our 2D videos and confirmed previously published findings (Gschwind et al., 2023) that were based on 3D videos. The 2-step approach we selected offers flexibility in our analysis and the possibility to use historical data, by providing keypoints and an intermediate representation of each individual animal. This feature also offers the opportunity to extend this analysis pipeline to multi-animal video recordings.

By comparing the behavioral syllable variability between short and long-term observations, we illustrated the added value of generalizing mouse models behavioral characterization over longer periods of time in naturalistic settings. Keypoint generation using DLC is by far the slowest computational step and currently the main limitation to multi-day analyses. Other avenues are currently being considered. Furthermore, we are also currently working to deepen the interpretation of the identified behavioral syllables.

This behavioral characterization pipeline will next be applied to other epilepsy mouse models, over longer periods of time (multiple days), to analyze the usage of behavioral fingerprints across groups and explore the associations between behavioral transitions and seizures events. The implementation of those automated approaches looking at behavioral transitions offers the possibility to capture correlations between neural pathways and behaviors, to build a map of the brain's trajectories in healthy and pathological conditions, and to better understand the mechanisms driving the disease and the ways to modify them.

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