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Trent Yu April 9, 2023

Behavioral Analysis of Mice on a Treadmill Walking Task and Understanding the ECoG Data Relations with Behavior

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Abstract

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Beta oscillations (13-30 Hz) are a hallmark of sensorimotor cortical activity. Several neurological disorders characterized by severe motor symptoms, such as Parkinson's disease, are known to exhibit altered beta oscillations. Yet, their functional role in motor behaviors remains unclear. In this study, we establish a wheel-running task for head-fixed mice to investigate cortical activity during locomotion and provide a set of metrics to quantitatively describe limb movements. We use electrocorticography (ECoG) techniques to record the electrical activity of neuronal populations, or local field potentials (LFPs), around an electrode in contact with the surface of the brain. In particular, we measure LFPs from the rostral forelimb area (RFA) and from the caudal forelimb area (CFA), two regions of the rodent motor cortex responsible for the planning and execution of movements of the contralateral limb. Focusing on beta oscillations, our analyses did not allow us to establish a clear relationship between their power and the presence or absence of movements, and as such further investigation is needed. Our work is a first step towards a more extensive characterization of neuronal oscillations related to motor behaviors in healthy animals and provides benchmark control data for future studies in mouse models of Parkinson's disease.

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Acknowledgements

I would like to thank Dr. Dieter Jaeger for his endless support and guidance during my time in his laboratory. I would like to thank Dr. Alfonso Delgado Reyes for helping me set up the experimental apparatus, explanations, and assistance in both collecting and analyzing my data. I would like to thank Dr. Aurelie Pala for her help in explanations, writing, and generous emotional support. I'd like to thank Dr. Li Su for his help with conducting surgeries. Finally, I'd like to thank my parents for all the support they have given me throughout this process. I am immensely grateful for all the help I have received.

Table of Contents

Abstract

Beta oscillations (13-30 Hz) are a hallmark of sensorimotor cortical activity. Several neurological disorders characterized by severe motor symptoms, such as Parkinson's disease, are known to exhibit altered beta oscillations. Yet, their functional role in motor behaviors remains unclear. In this study, we establish a wheel-running task for head-fixed mice to investigate cortical activity during locomotion and provide a set of metrics to quantitatively describe limb movements. We use electrocorticography (ECoG) techniques to record the electrical activity of neuronal populations, or local field potentials (LFPs), around an electrode in contact with the surface of the brain. In particular, we measure LFPs from the rostral forelimb area (RFA) and from the caudal forelimb area (CFA), two regions of the rodent motor cortex responsible for the planning and execution of movements of the contralateral limb. Focusing on beta oscillations, our analyses did not allow us to establish a clear relationship between their power and the presence or absence of movements, and as such further investigation is needed. Our work is a first step towards a more extensive characterization of neuronal oscillations related to motor behaviors in healthy animals and provides benchmark control data for future studies in mouse models of Parkinson's disease.

Introduction

The initiation of movement requires coordination in firing between large numbers of neurons in different brain areas. Electrophysiological techniques can record this activity in the form of neuronal oscillations, either through electroencephalographic (EEG) recordings from the scalp or as a local field potential (LFP) via electrocorticogram (ECoG) recordings from the surface of the cortex. A large body of research suggests that in the motor cortex, beta-band frequency oscillations in the 13-30 Hz range are heavily implicated in the modulation of movement by playing an akinetic role. Beta power has been shown to drop during periods of sustained movement (Nakayashiki et al., 2014). In contrast, if a planned movement does not occur, such as during a Go/No-Go signal task, beta power rapidly increases (Alegre et al., 2004). Similarly, beta power increases when muscles contract or posture becomes stable, such as in a grasping/holding task (Spinks et al., 2008). Consistent with this model, a decrease in beta power is observed during the motor planning phase prior to movement onset (event-related desynchronization, ERD), and a rebound above baseline (event-related synchronization, ERS) is observed after movement ends, peaking at about 300 to 1000ms after movement completes (Pfurtscheller and Lopes da Silva, 1999; Yuan et al., 2010; Ghilardi et al., 2021). This is of particular interest in Parkinson's Disease (PD), as a major biomarker associated with parkinsonism is abnormal electrophysiology in multiple cortical and subcortical circuits. Enhanced power of beta oscillations has been to linked motor symptoms in both animal models and humans, suggesting these oscillations may be markers for slowing of movement (Little and Brown, 2014, (Ganguly et al., 2019). Similarly, elevated beta power in the basal ganglia has been shown to be correlated with the severity of clinical symptoms and suppressed by traditional

treatments, including dopaminergic medication (levodopa) and deep brain stimulation (DBS) (Karekal et al., 2022).

In recent years, studies have suggested that rather than a gradual change in beta power, it is instead the occurrence and timing of beta bursts (transient increases in beta amplitude) that is correlated with movement (Feingold et al., 2015; Ganguly et al., 2019). Studies focusing on beta bursts and PD suggest that the elevated beta power seen in the parkinsonian condition is not continuous but instead manifested as an increased frequency and amplitude of beta bursts (Torrecillos et al., 2018). In parkinsonian subjects, beta bursts in the STN preceding a movement are associated with slowed movement (Shin et al., 2017), and research suggests that the pathophysiological symptoms of PD are associated with both longer duration and amplitude of beta bursting (Torrecillos et al., 2018).

Mice are an ideal model organism to examine cortical neuronal oscillations because of their similarity in brain structure to humans and an ample prior research knowledge base to draw from. In rodents, the rostral forelimb area (RFA) in secondary motor cortex, and caudal forelimb area (CFA) in primary motor cortex are areas of the cortex responsible for the planning, initiation, and execution of movements of the contralateral forelimb. These areas are typically considered as the rodent equivalents of the premotor/supplementary motor and primary motor areas of the primate frontal cortex (Morandell and Huber, 2017). In this study, we simultaneously record LFPs from the RFA and CFA of wild-type mice as they perform a wheel-running task to correlate changes in motor behavior with neuronal activity. Further, we establish a quantitative framework for analyzing locomotion-related movements in the wheel-running task. We hypothesize that initiation of different types of movements during the wheel-running task is associated with a reduction of beta power in the LFP. Characterizing movement-related

cortical activity in wild-type animals will provide a benchmark for similar studies conducted in parkinsonian mouse models. In addition, it will lay the ground for future investigations of the real-time control of cortical beta-band oscillations using optogenetic activation of thalamic inputs, a long-time endeavor of the laboratory towards the development of novel therapeutic strategies for PD.

Methods

Animals

All experimental procedures were approved by the Emory University Institutional Animal Care and Use Committee. Mice were kept on a 12h:12h reverse light cycle, with behavioral training and experiments conducted during the dark half of the cycle to allow for behavioral and motor assessment during the mice's waking cycle. Beginning 3 days before handling, training, and experiments, mice underwent water deprivation to better motivate the animals to respond to training and perform behavioral tasks. Mice had access to unlimited food but kept on a 1-1.5 ml/day water restriction, lasting no more than 2 months at a time. Mice were given 10% sucrose solution as reward during experiments and training. Liquid amounts were measured during testing and supplemented with hydrogel to reach the 1-1.5 ml/day mark. Mice were monitored daily for signs of physiological distress and ensure weight of animal did not drop over 20% of baseline. A total of 4 C57BL/6J mice, 2 males and 2 females were used in the study.

Surgery

Mice were placed in a sealed induction chamber and anesthetized with 4% isoflurane (Sigma 792632). Mice were then head-fixed on a stereotactic frame (KOPF Instruments) and maintained at 1.5-2.5% isoflurane at 0.8 - 1.0 liters per minutes of oxygen condenser (DeVilbiss Healthcare Mannheim, Germany). The level of needed isoflurane was monitored by pinching the paw to assess paw withdrawal reflexes. Mice were given analgesic (buprenorphine 1 mg/kg IP and lidocaine 4 mg/kg under the skin of skull) prior to the start of surgery. Hair on the skull was removed using Nair (Church & Dwight Co., Inc). 70% ethyl alcohol and betadine were applied to the scalp three times to clean and disinfect the head area.

Headpost surgery

Mice underwent head-post attachment surgery to accurately collect neurophysiological data while completing the task and standardize procedure for future experiments. A thin layer of Optibond (KaVo Kerr) was applied to the skull. A stainless steel headpost was anchored to the skull with light-cured dental acrylic cement, and the skull sealed with clear dental cement.

Implantation of electrodes for electrophysiology recording

Sterile ink was used to mark the areas for drilling. An Omnidrill (WPI, Inc.) with a heat-sterilized bit was used to drill the holes in the skull together with intermittent cooling with saline. Stainless-steel skull screws (#19010-10, Fine Science Tools) were placed as electrodes to record from the cortex. Three electrodes were implanted total: one in the rostral forelimb area (AP +2.42, ML 1.3 relative to bregma), one in the caudal forelimb area (AP+0.29, ML 1.58 relative to bregma). both on right side, and an additional reference electrode over the cerebellum. A thin steel wire was also soldered between the screw and a gold pin (male) to connect to the acquisition system for recording.

After surgeries the mouse was placed in a clean cage and monitored every 15 minutes until awake. Mice were monitored daily for at least 3 days and allowed to recover for at least 10 days following surgery, during which they were allowed unlimited access to food. Mice were singly housed in cages without wire-mesh to prevent the headpost from accidentally getting caught.

Behavioral Task

Mice were head-fixed and trained to run forward on a low-friction transparent running wheel (Warren et al., 2021). They were conditioned to run in response to an auditory cue and rewarded with 5 µl of 10% sucrose water when they ran a pre-determined distance in a given amount of time.

Training for the behavioral task began at least 10 days following surgery. Mice were placed on water restriction three days before handling. During this period, mice were held and placed onto the wheel to allow them to become acclimated to the gloved hand of the experimenter and wheel environment (15 minutes/day). Sucrose reward was given via syringe while the mouse was in the hand to let the mouse associate handling with reward. After a week of handling, mice were habituated to head-fixation. Mice were secured via the headpost holder and placed into the behavioral setup for gradually increasing durations, during which they were allowed to run freely and intermittently given reward, at first via syringe, and later via optical lickometer (Sanworks). After mice were habituated and consistently licked at the lickometer for reward, they were transitioned into the wheel-running task.

The wheel-running behavioral task was programmed using MATLAB (MathWorks) and executed using the Bpod system (Sanworks), a programmable state machine used to control rodent behavioral tasks. Liquid reward was delivered via an optical lickometer connected to a gravity water system, which recorded lick times through a built-in photogate. A rotary encoder (Inland KS0013) was used to track the movement of the wheel, and a camera (Basler acA1920-150uc, 39fps) was used to record behavioral videos. The camera shows a side view of the wheel and mouse, and a mirror placed underneath the wheel was used to generate an inferior view to observe the general behavior of the animal and track the paws. The Bpod system controlled the

behavioral setup, taking info from the rotary encoder and optical lickometer to record the total distance traveled, success/failure of the trial, position of the wheel at periodic timepoints, start and end timepoints of the trial, and whether the mouse licked or not during the reward interval.

Each trial was composed of five distinct intervals and lasted up to 28.5 seconds (Figure 1). The rotary encoder records the position of the wheel throughout the duration of the trial. During baseline (8.5 seconds), a solenoid brake prevents the wheel from moving. There is no penalty for attempting to run during baseline. After baseline, a sound cue (2 seconds) plays to signal the mouse to start moving. After the sound cue, the solenoid brake disengages, and the wheel is allowed to rotate as the mouse runs. The mouse has up to 10 seconds to run a pre-determined distance. If it has run the full distance, this state ends early, and the mouse is given a reward. If the mouse was successful, 5 μL of reward is dispensed and the mouse has 4 seconds to lick. Otherwise, a short failure auditory cue plays during this duration instead. Trials were separated by an intertrial period (4 seconds).

Each training session lasted 30 trials, with the mice performing the task for up to three sessions per day with a 3-minute wait in between each session. Mice started out with a target distance of 360 rotational degrees, which was gradually increased up to 540 rotation degrees. If performance was not meeting targets, mice were sometimes allowed an additional 5 seconds during the running phase to meet the distance requirements during training. Once the mouse could successfully run 540 rotational degrees with a >70% success rate over one session, training was considered complete and the mouse was ready for recordings.

Electrophysiology

Local field potentials (LFP) are a type of electrophysiological recording that sums the electrical activity of the neurons in an area surrounding an electrode probe. The mice underwent surgery to implant three stainless-steel skull screws (#19010-10, Fine Science Tools) in the rostral forelimb area, caudal forelimb area, and cerebellum to allow for ECoG recordings of LFP during experiments. During experiments, the mice were head-fixed in the recording set-up for data collection. A 32-channel RHD headstage (Intan Technologies, Part #C3314) was connected to a recording board (Intan Technologies, Part #C3100), via a RHD SPI cable (Intan Technologies, Part #C3213). Gold pins (female) were soldered onto a 36-pin wire adapter (Part #C3420) to connect the three stainless-steel skull screws implanted on the surface of the cortex and cerebellum with the headstage. LFP signals were acquired, digitized, and saved as the difference between RFA/ CFA and the cerebellar reference using the RHD2000 Interface Software (Intan Technologies). Signals were collected at a sampling rate of 20kHz.

The Real-Time eXperiment Interface (RTXI), a real-time data acquisition and control application for biological research (Poisot et al., 2017) was connected to the Intan board and used to collect a third signal consisting of a differential trace between the RFA and CFA to minimize artifact and generate a truly local signal.

Data Analysis

MATLAB (MathWorks) was used to deal with large datasets and perform all behavioral and electrophysiological data analysis. The Bpod system returned information from the rotary encoder and optical lickometer to track success/failure of trials, position of the wheel at timepoints through the trial, start and end timepoints for each phase, and licking events. This data

was used to index successful versus failure trials, calculate speed, and determine if the mouse licked or not.

The RHD2000 Interface Software returned LFP signals from the RFA, CFA and reference electrode in the cerebellum. These signals were down sampled to 1kHz and then filtered in the beta range (15-30 Hz) and plotted as power spectral density (PSD) plots. The PSD was separated into behaviors of interest (Baseline, Running, Licking) in order to examine for differences in the LFP across different behaviors.

DeepLabCut (Mathis et al., 2018) is an deep learning network that can automatically extract features out of videos. To determine differences in gait, a DeepLabCut model was trained on the behavioral videos (inferior view of paws) created during experiments to extract the position of the paws of the mouse on the wheel and generate and raw positional data for each paw (Figure 2). Position data was plotted as position of the limb in pixels with (0,0) in the top left corner. Thus, pixel count increases as image height increases (y axis) and pixel count increases as image width increased (x axis). All videos were size 1200 x 1472. In order to convert from pixels to centimeters, a fixed conversion factor of 0.0115 cm/pixel (17 cm/1472 pixels) was used when necessary. The frames were multiplied by the fps in order to convert from frames to seconds when needed. The data was processed to remove outliers by filtering out frames the model had low confidence in, and data points in between were interpolated. The data was parsed to identify strides, shown as peaks and valleys in the data, which were then used to generate stride length, defined as the range of the x-positional values during running, or X_{max}-X_{min}.

Results

To study how brain oscillations are related to motor behavior, we examined four head-fixed mice as they completed a wheel running task. In response to an audio cue, mice were trained to move forward on a transparent running wheel and rewarded once they had run a sufficient distance within a set timeframe (Figure 1). A single trial consisted of five distinct phases, three of which were examined for this study: baseline phase, running phase, and licking phase. During the baseline phase, the mouse was prevented from moving forward though use of a solenoid brake. After a sound cue played, the brake was removed, and the mouse was able to run forward, transitioning into the running phase. The running phase stopped when the mouse had finished running the target 540° of distance or once 10 seconds passed, and transitioned into the reward phase. During the reward/failure phase, the mouse was given 5 μl of sucrose solution as a reward if it successfully ran the target distance. After an intertrial interval, the solenoid brake was re-engaged, and another trial began. We recorded EcoG/LFP activity in the RFA and CFA from the mice (Figure 3) as they completed the task to gather information about the electrical activity of neurons in those areas.

Behavioral Analysis

Figure 4 shows the average speed of the mice over the course of the entire trial separately for successful and failed trials. Different mice ran at different speeds – Mouse ADBS2-1 ran significantly slower than the other three mice and ADBS2-2 ran significantly faster than ADBS2-3 in successful trials ($p<0.00016$, Student's t-test with Bonferroni correction). There were no significant differences in average running speed in failed trials.

To examine how running changed over the course of the trial, we next examined instantaneous speed as a function of time during the running phase only (Figure 5). Across mice, there was a sharp increase in speed during the start of the running period, and all mice reached a max speed between 1.5 and 2 seconds after the start of the run phase. After reaching the peak, running speed on average gradually leveled off afterwards and decreased with time. A close look at a period centered around the onset of the reward phase (Figure 6) suggested that mouse speed seemed to drop upon onset of licking and consumption of water rewards. However, that drop was transient, as on average mice speed during the entire reward phase was not significantly different compared to the two seconds of running phase prior ($p>0.05$, paired t-test).

To analyze motor performance in more detail, the gait of the animals was analyzed using DeepLabCut. Across animals, the analysis of stride length, calculated as X_{max}- X_{min}, or the most anterior and most posterior position reached by each limb during bouts of running (Figure 7), revealed no statistical differences ($p > 0.0083$, paired t-test with Bonferroni correction) between limbs.

LFP Analysis

To examine correlations between ECoG/LFP activity and behavior, we compared changes in the power of neuronal oscillations between baseline, running, and reward phases. Of particular interest was power in the beta oscillations (13-30 Hz), due to its close relationship with movement behaviors. In the example ADBS2-1 mouse, there appeared to be little change in the power of beta-frequency oscillations (13-30 Hz) during the running phase compared to baseline (Figure 3). However, there were additional high beta (25-30 Hz) and low gamma peaks (30-40 Hz) during the reward phase that are not apparent during the baseline and running states. There

were no differences across different phases for individual RFA and CFA traces. All other mice showed no differences across behavior conditions.

Discussion

We are in the process of improving the DeepLabCut output. DeepLabCut, although useful, is ultimately a tool that returns generated data based off of where it best believes the markers of interest (paws) are at each frame. When attempting to analyze gait using other markers, a lack of resolution and error in the automated tracking led to artifact and subsequent faulty analysis. Specifically, the model sometimes confused the hindpaws with the forepaws, leading to false detection of very quick transient peaks. The vast majority of these peaks were filtered out by thresholding, but there were rare occasions where that was unsuccessful. This was not an issue with stride length, as we calculated by using the median peak data over a running period, but on additional measures such as limb velocity and stepping frequency, these errors skew the data. The model used to analyze the videos was not robust enough and needs to be retrained on additional frames in order to provide more accurate positional data and allow us to analyze gait in more depth.

Given that beta power has been demonstrated to decrease during sustained movement, the lack of beta suppression in the mice during the running phase is surprising. One possible explanation for this is that the running phase/distance was not long enough for the mouse to treat it as a sustained movement, and thus remained alert and ready to stop at any time. Another explanation could be because despite the wheel being locked during baseline, the mice are nonetheless still able to physically move their paws, and thus there may not be a big change in beta power comparing baseline to running. The addition of high beta seen during the reward phase is also somewhat surprising, given that most mice did not exhibit changes in speed as they entered the reward phase, suggesting that the mice are not stopping to drink. However, among the four mice, ADBS2-1 had the worst motor performance, which may be an explanation for

why this effect was seen in this mouse only and not others, as it may be more biased towards stopping during the reward period and thus have increased beta power. One expected marker seen in our results was the presence of increased gamma during the reward period. Gamma oscillations in the cortex are thought to be created by the interaction between inhibitory interneurons and pyramidal neurons and are involved in integrating information across different areas of the cortex (Guerra et al., 2020). Furthermore, they have been shown to increase in power during periods of increased perception such as licking for reward (Guerra et al., 2020), and these results are consistent with the increased gamma power we saw in our mice during the reward phase. Thus, our original hypothesis is only partially supported. Although we did not see the expected relationships in beta oscillations and behavior, we did see results in the gamma frequency that are supported by results from previous studies. Our inquiry requires further investigation.

In summary, this study provides the preliminary steps towards correlating changes in motor behavior with changes in neuronal activity in the motor cortex of wild-type mice and examines possible methods of quantitatively analyzing finer analysis of limb movements on a wheel-running task. Our results regarding the correlation between LFP data and motor behavior are inconclusive and will likely require further investigation. However, we do establish the task, training protocol, effectiveness of our quantitative framework in characterizing movement related cortical activity, and control data for future studies involving parkinsonian mouse models on similar apparatus.

Tables and Figures

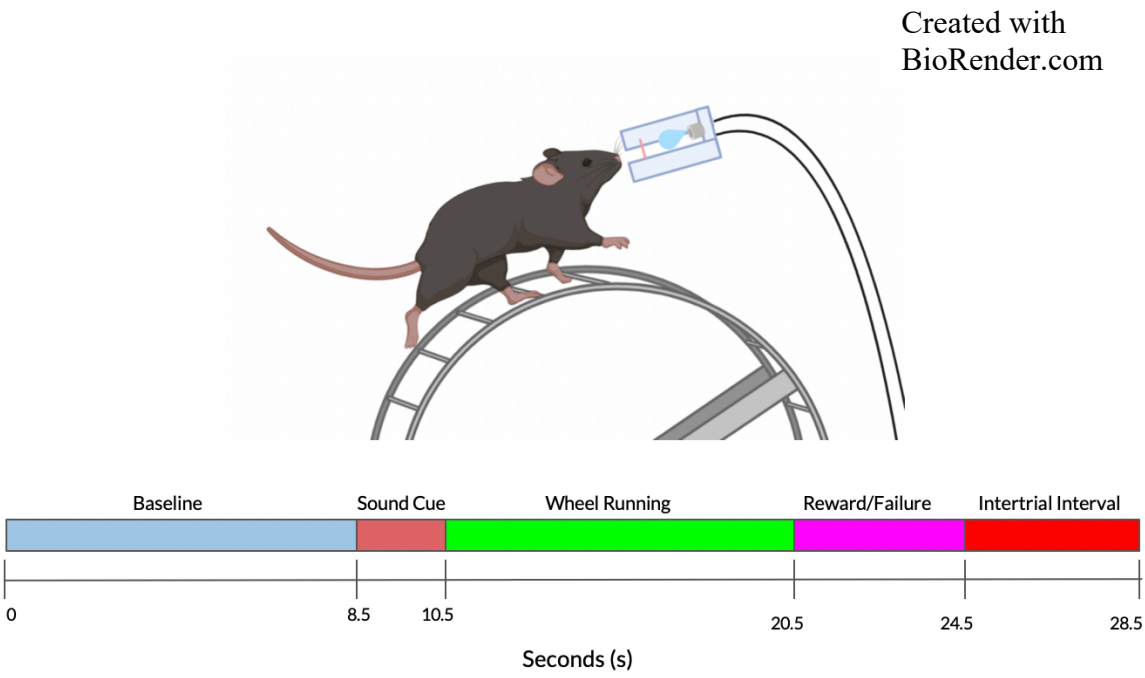

Figure 1: Outline of a Single Trial of Head-fixed Wheel-Running Task.

Head-fixation is not shown in the diagram. Mice are placed on a low-friction transparent running wheel and trained to run a set distance in response to an auditory cue to receive reward. A single trial lasts up to 28.5 seconds, divided into five distinct intervals. During baseline, a solenoid brake prevents the mouse from moving forward. After a 2 second sound cue, the break is disengaged. During the running phase, the mouse has up to 10 seconds to run 1.5 rotations of the wheel (540°). If they are successful, they are transitioned into the reward phase and given sugar water reward. Otherwise, once 10 seconds have passed, they are transitioned into the failure phase and a failure sound cue plays. In between trials, there is a 4-second long intertrial interval. A training session typically consists of 30 such trials at a time.

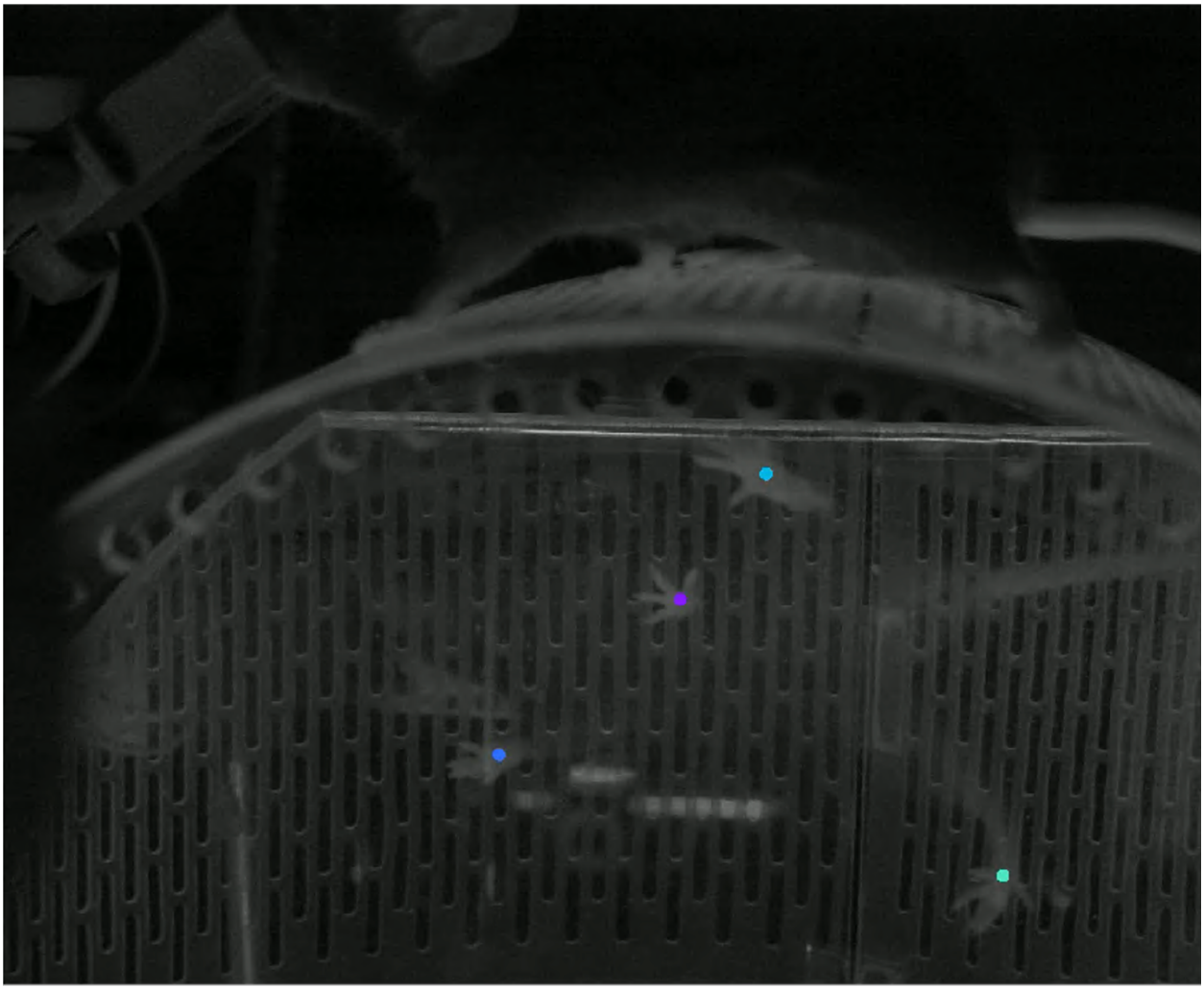

Figure 2: Video Frame Showing DeepLabCut Markers for Position of Each Mouse Limb. Videos were recorded as the mice completed the behavioral task and mirror used to generate inferior view of the mouse. DeepLabCut estimates the position of the paw during behavior. Purple, dark blue, light blue, and green are right forelimb, left forelimb, right hindlimb, and left hindlimb respectively.

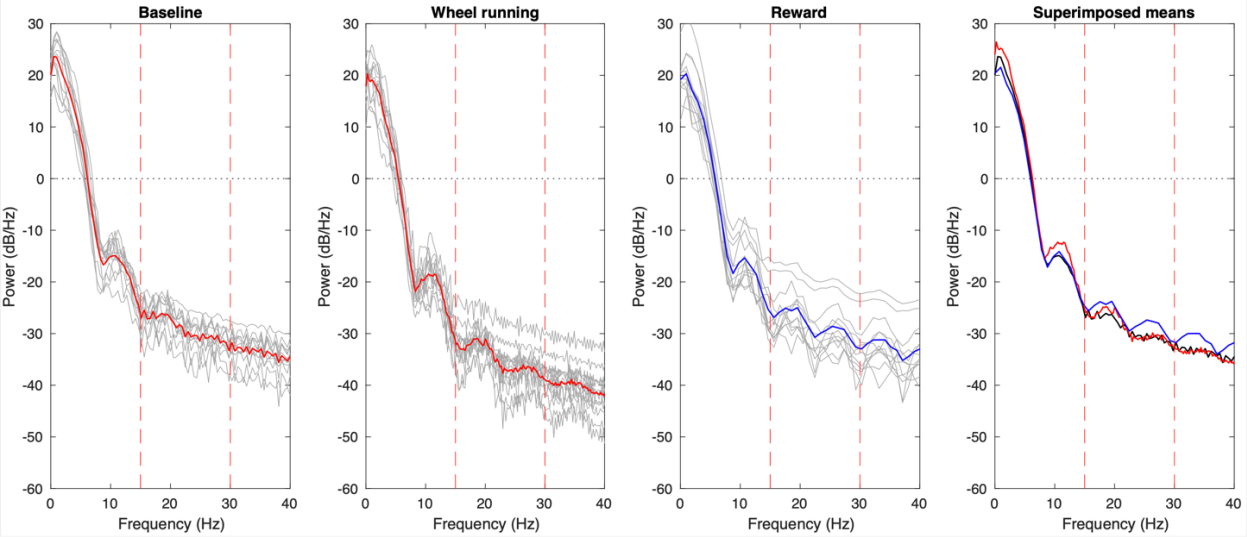

Figure 3: Average Power Spectral Density (PSD) of LFP Signal from CFA – RFA, Mouse ADBS2-1, 12 Trials.

LFP data was averaged from 12 different trials and separated into behaviors of interest: baseline phase (mouse is stopped from moving forward by solenoid brake), running phase (brake is removed, and mouse is able to run), and reward phase (mouse receives sucrose solution reward). Data was aligned and superimposed to examine for differences. Dotted red vertical lines indicate the beta region (15-30 Hz).

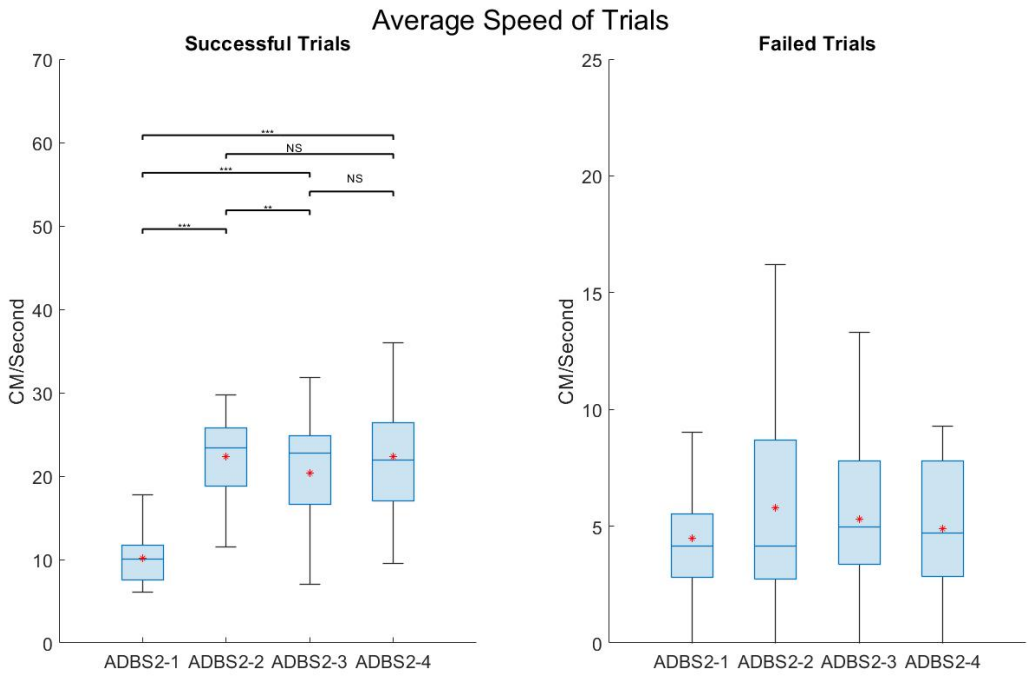

Figure 4: Average Speed of Mice Throughout Entire Trial, Successful and Failed Trials. Means are shown as red stars. In successful trials (left), mouse ADBS2-1 ran significantly slower than all other mice and ADBS2-2 ran significantly faster than ADBS2-3, while no difference in average running speed was found between ADBS2-3 and ADBS2-4. There were no significant differences in average running speed in failed trials (right). *** $p < 0.00016$, NS $p > 0.0083$, Student's t-test with Bonferroni correction for six comparisons. Successful trials n = 80/148, 181/257, 106/144, 106/144.

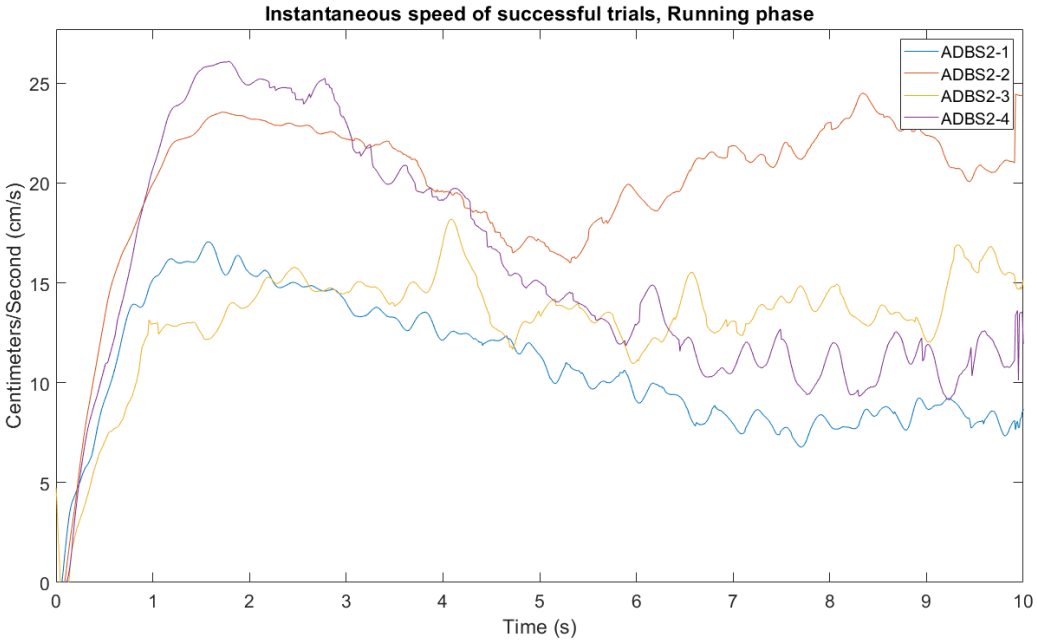

Figure 5: Average Instantaneous Speed of Mice vs Time During Running Phase Only.

T = 0s denotes the start of the running phase, when the brake of the running wheel disengaged and the mouse was able to run. Individual trials that went into the plotted average had different lengths because time that the mice took to run the required distance differed in each trial. Across mice, there was a sharp increase in speed at the start of the running period, and all mice reached a maximum speed between 1.5 and 2 seconds after the start of the run phase. After reaching their peak, running speeds gradually decreased with time before plateauing (number of trials = 130, 218, 159, 159).

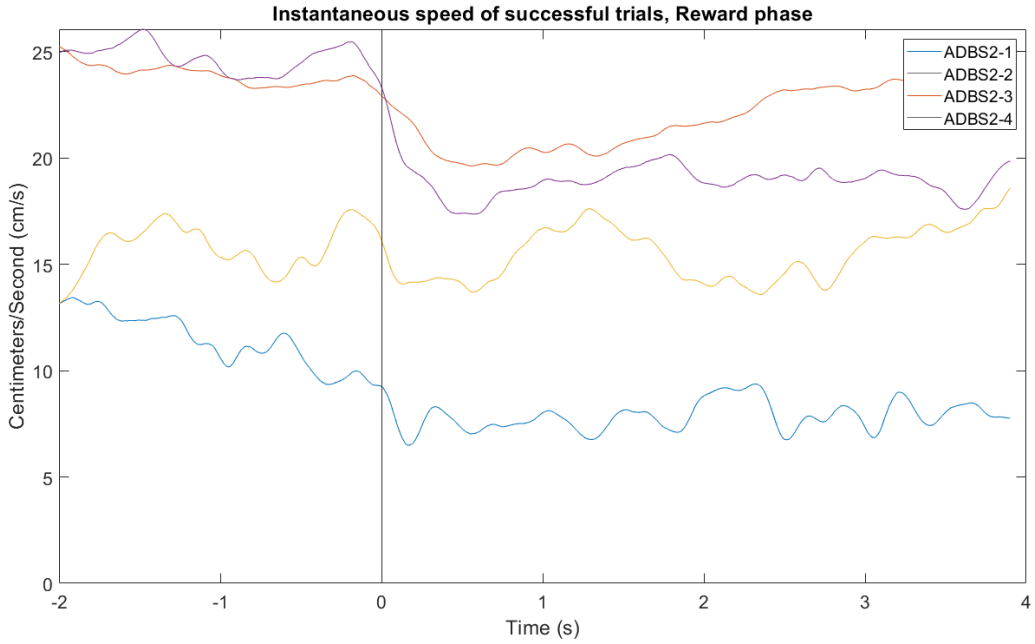

Figure 6: Average Instantaneous Speed of Mice vs Time During Reward Phase.

Vertical bar at t = 0s denotes the start of the licking phase, when the sucrose solution reward was dispensed. Times before t = 0s are from the running phase. Across mice, running speed during the reward phase was not significantly different compared to the two seconds of running phase prior (p= 0.43, paired t-test). (Number of trials = 130, 218, 159, 159).

Figure 7a.

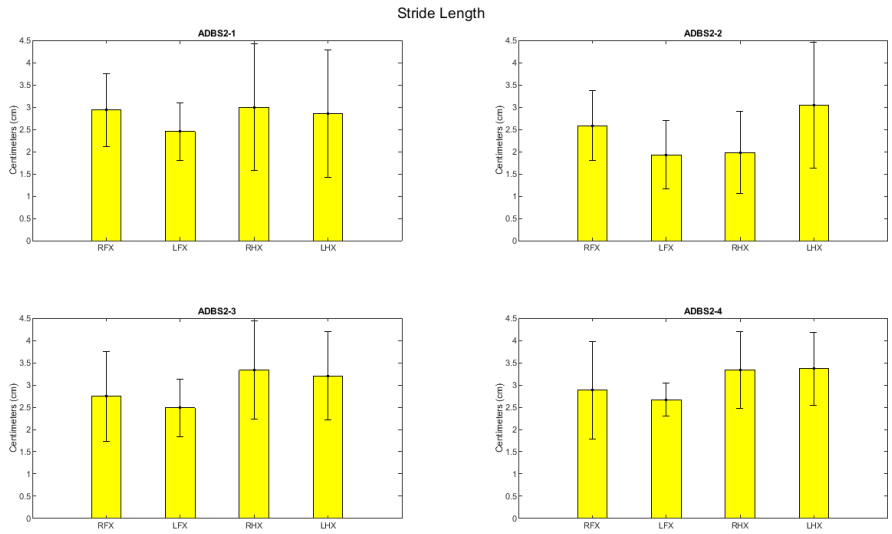

Figure 7b.

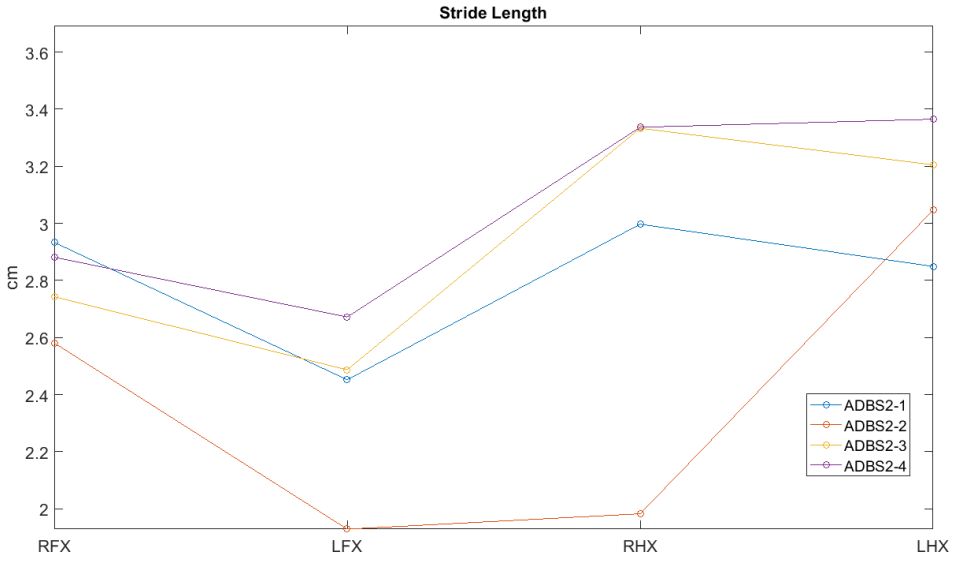

Figure 7: Stride Length of Limbs.

a) Plots of the stride length, calculated as $X_{max} - X_{min}$, for each limb for each individual mouse. Mean +/- Standard deviation errors bars. RFX, LFX, RHX, LHX are right forelimb, left forelimb, right hindlimb, and left hindlimb respectively. 30 trials each.

b) Plot of the stride length for each limb averaged across mice. No difference in stride length was found. (all comparisons $p > 0.0083$, paired t-test with Bonferroni correction for six comparisons).

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