Development of a Self-motivated Treadmill Task that Quantifies Differences in Learning Behavior in Mice for Optogenetic studies of Basal Ganglia Function

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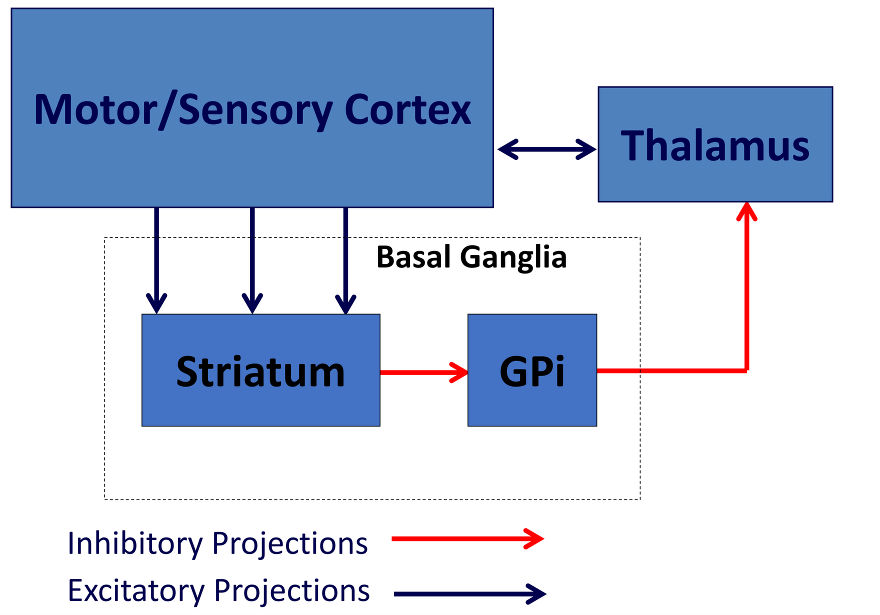
Introduction

The basal ganglia (BG) are involved in various cognitive functions, including stimulus-response associative learning and decision making. One of the major pathways is through the globus pallidus internus (GPi) which sends inhibitory projections providing the final output. Signals through these pathways converge to inhibit the glutamatergic thalamic nuclei, which output onto the cortex[1,2]. GPi activity suppresses inappropriate motor activity that may conflict with the movement being performed, making it an important integrator of learned reward-related behaviors[3].

In our studies we have designed a self-paced treadmill task that will help better our understanding of how movement planning intersects with a self-motivational task. We designed an open field environment with a horizontal treadmill and a simple water delivery system. Sinusoidal sound cues of different frequencies signal the run requirements of the trial, and the mouse can choose to run or let the trial pass. To investigate the motivation aspect, we manipulate the reward sizes, which are proportional to the run distance requirement and are delivered at the end of each period. Ultimately, the goal is to use this task with optogenetic methods to activate GPi[4,5] and determine how it reduces learned reward-seeking behaviors.

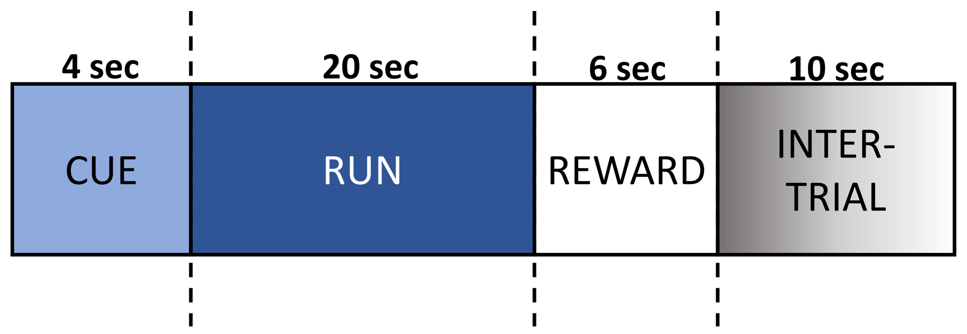


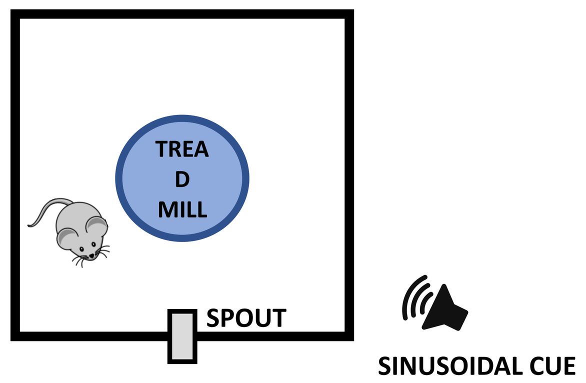
Methods

Animals: All animal procedures complied with Emory IACUC approved protocols. Three male C57BL/6J mice (9 weeks old) were used in this study.

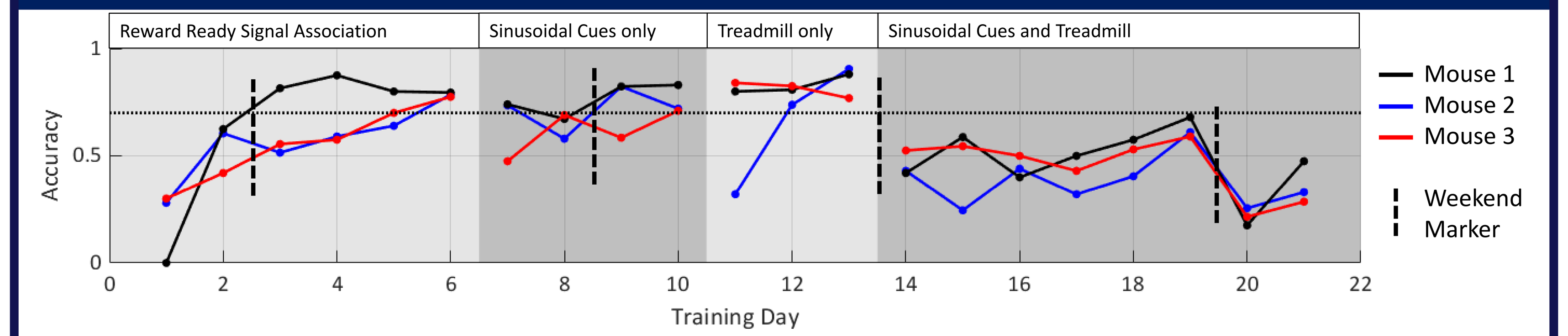
Training: Water restriction of 1 mL per day was instituted to encourage licking for a sucrose reward during training. Behavioral training involved the sounding of either a low (7 kHz) or high (16 kHz) sinusoidal pure tone that signaled the easy or hard run distance thresholds (respectively) of the task. The mouse was required to respond within 2 seconds of the end of the tone by running. Upon fulfillment of the run distance threshold, a light and sound cue were turned on to signal that the water reward was ready. Volume of reward is proportional to the difficulty of the task. The spout delivered a grape flavored sucrose solution (10% w/v) as a reward for run completion.

Materials: A 12x12x14 in box was constructed to house the training setup. Rotations of the horizontal treadmill were detected with Scurry-Trac pad, and an optical lickometer from Sanworks detected the beam breaks that allowed for delivery of sucrose rewards.





Training Progression



Given the relative complexity of this task, training was divided into multiple stages (shades of gray). Each stage introduced a new component of the task. Once proficiency was achieved (greater than 70% success rate), trials moved on to the next level. Total training took 21 days. Weekends are marked, as mice are given free water access over weekends, and thirst levels can affect motivation to run.

Response to the Cue

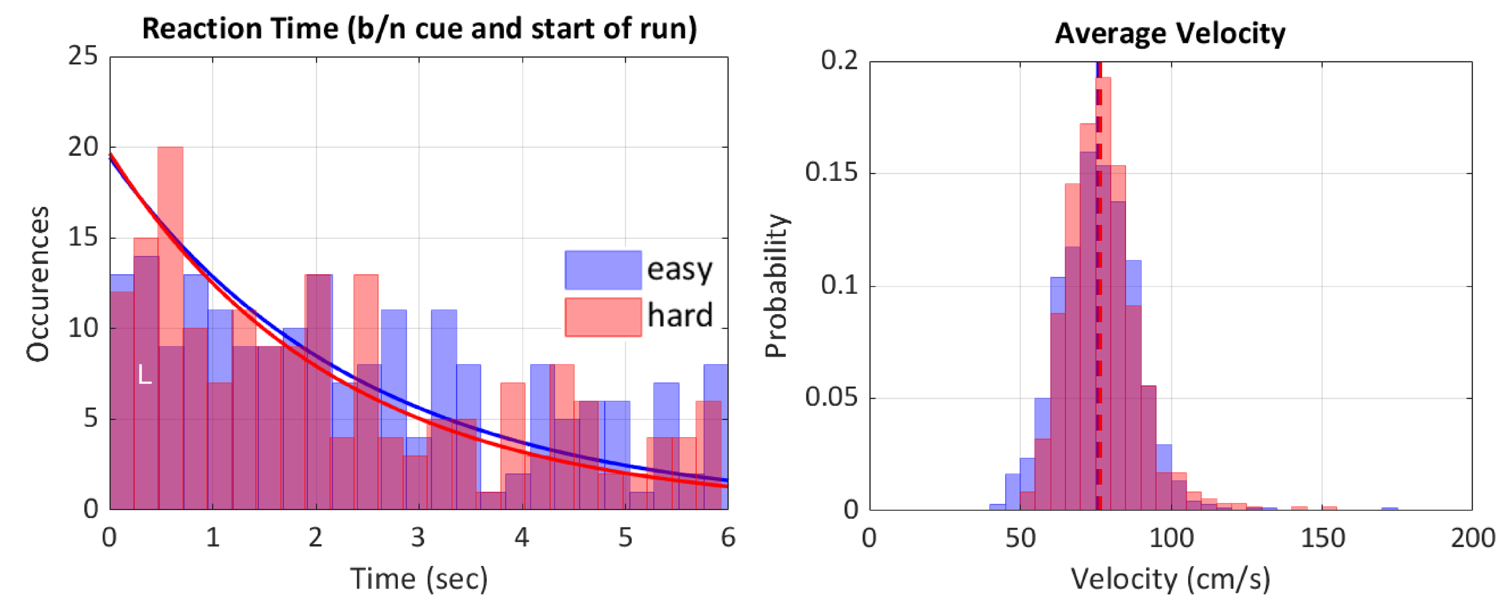


Figure 2: Reaction time to the sound cue and the average run velocity show the vigor that mouse has to complete the task depending on difficulty. Figure (a) shows the time between the start of sound cue and the first turn of the running wheel, aka the reaction time. Figure (b) shows the average run velocities. There is no significant difference in the distribution of reaction times and velocities for the easy and hard run conditions.

Delay in Reward Collection

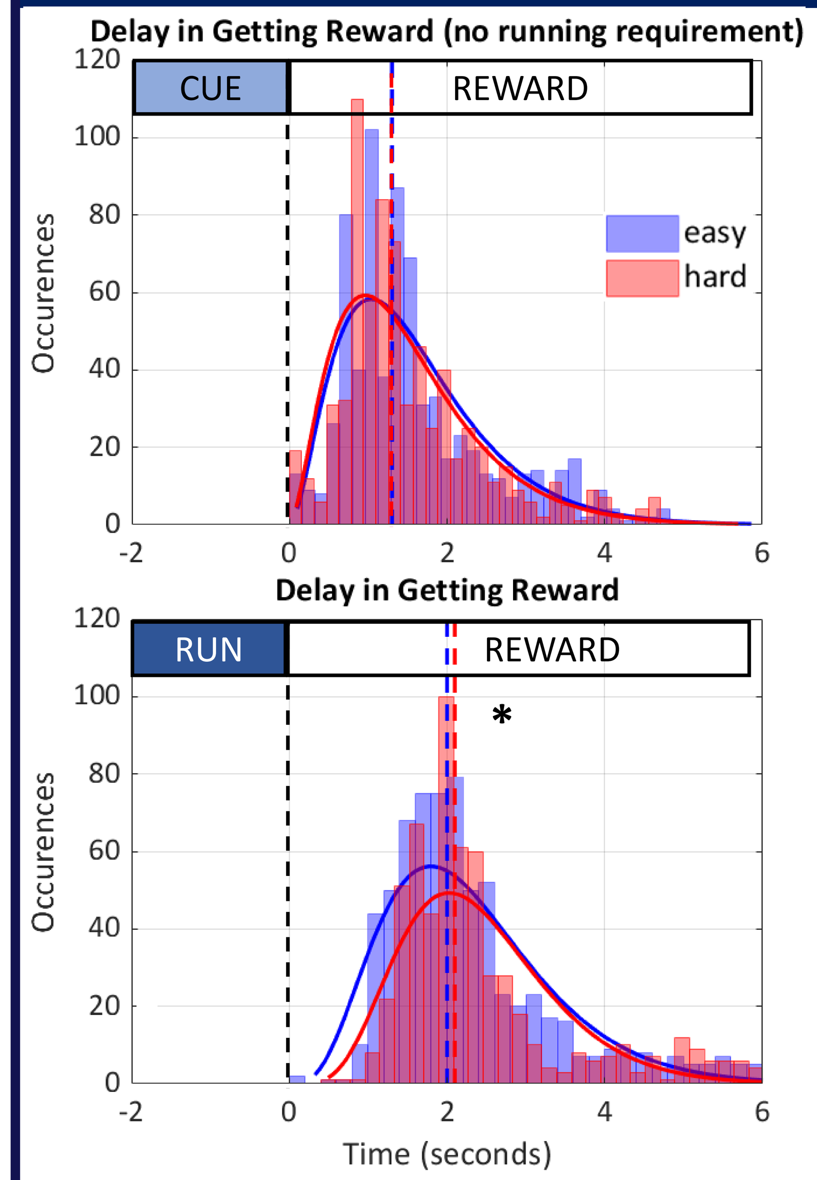


Figure 2: Time delay in reward collection for all mice for trials without and with running requirements. (a) In trials with no running requirement, where sinusoidal sound cues were followed by respective size rewards, there is no significant difference in the distributions of delay in reward collection (n=1435 trials). (b) With the running requirement there was a significant difference in how quickly the mouse left the wheel to receive the reward. There was a median delay of 2.0000 and 2.0985 seconds for the easy and hard conditions respectively (n=1275 trials; Wilcoxon ranksum test; $p<0.05$).

Analysis of Failures

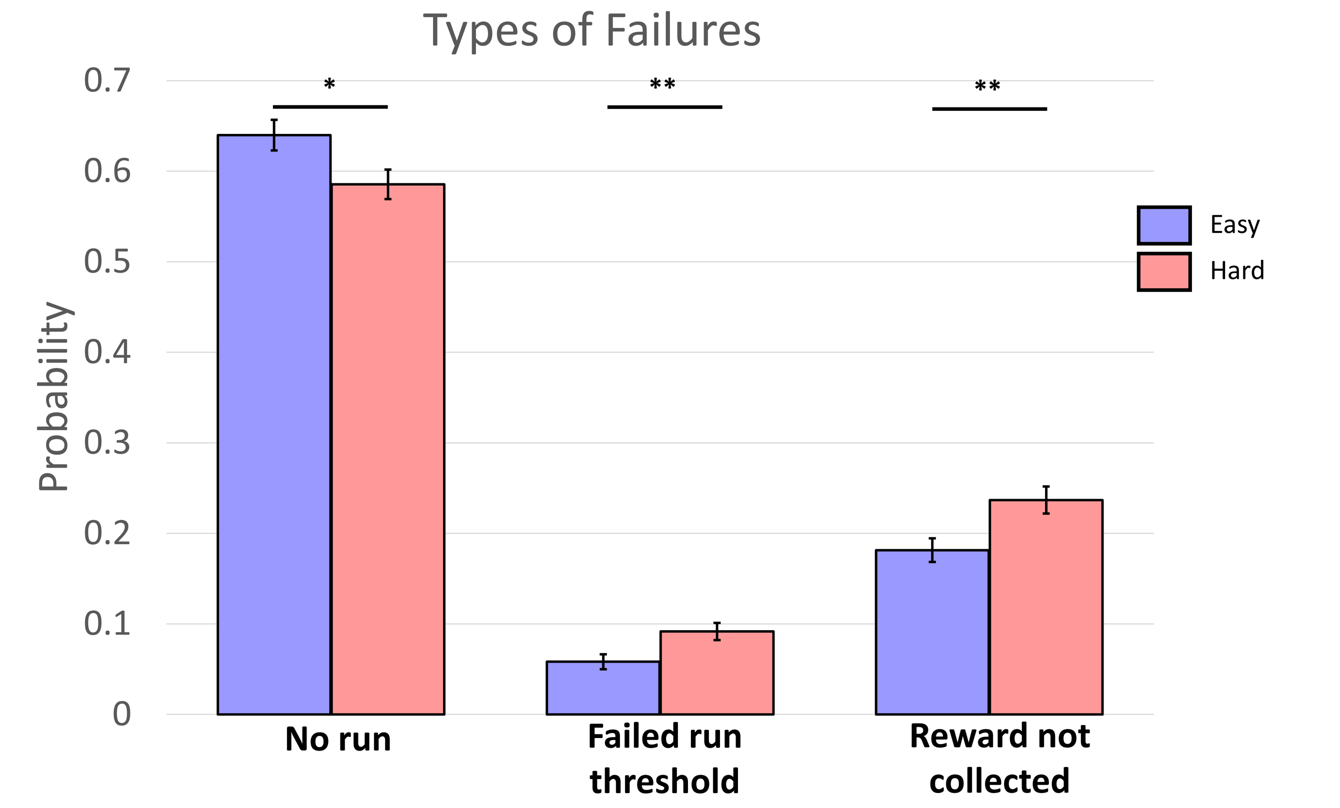


Figure 4: Breakdown of the types of failures. (a) Mice were more likely to fail due to not running after hearing the sinusoidal cue in the cue stage on easy trials than on hard trials ($X^2 = 5.318$, $p<0.05$). (b) They are also more likely to fail a trial if the run condition was hard ($X^2 = 6.842$, $p<0.01$). (c) Given that the mice had fulfilled the run requirement, they were more likely to ignore a reward if the trial was hard than if it was easy ($X^2 = 7.722$, $p<0.01$).

Conclusions

- The faster reward collection for easy trials suggests that mice are more anticipative of a smaller reward earned with low effort, and that they are more likely to ignore a large reward even if they have already completed the task.
- This suggests that the running component is interfering with their response, as running is inherently rewarding for mice. Future studies should implement a wheel that has resistance as an independent variable to ensure mice only run when they need to.
- Even though mice failed more due to not running after hearing the easy cues, the run velocities and reaction times to the cues suggest no difference in vigor between cues.
- Future studies can implement optogenetic manipulation to test the difference in response and vigor to the sound cues.

Acknowledgements

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References

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