Optimizing Training Protocols for Testing Motor Coordination in a Mammalian Hibernator

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Introduction

Hibernation is an evolutionary adaptation that enables animals to endure prolonged periods of starvation or resource scarcity. In thirteen-lined ground squirrels (13LGS), hibernation is characterized by week-long torpor bouts, which include periods of reduced body temperature, metabolic rate, and physical activity that are interrupted by interbout arousals (IBAs). IBAs are marked by a return to normal body temperature and are believed to help maintain muscle, remove waste, and decrease sleep debt. It has been previously reported that 13LGS have the remarkable ability to maintain skeletal muscle mass in specific muscle groups. This is contrasted by rapid muscle degeneration seen in other mammals during similar disuse. The ability to maintain muscle is vital to ensure sufficient strength for post-hibernation foraging and territorial disputes. Despite extensive research on muscle size preservation, few studies have examined how motor coordination is preserved in these squirrels, particularly through the use of the Rota-Rod apparatus. Commonly used in mice and rats to assess muscle coordination, adapting the Rota-Rod to 13LGS presents its own challenges. Characterizing motor coordination in 13LGS can provide valuable insights into the mechanisms underlying muscle preservation during hibernation.

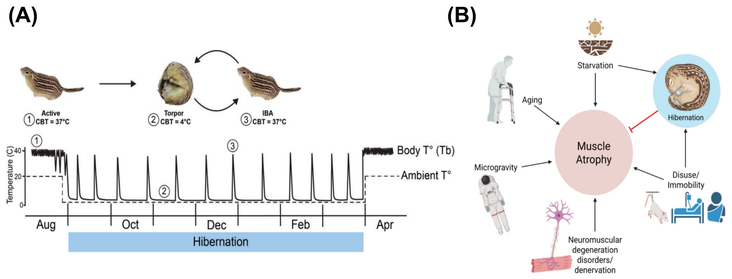
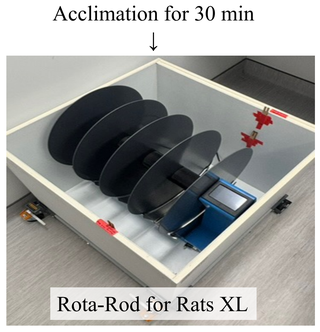


Figure 1: Hibernation protects against muscle atrophy. (A) Hibernation is characterized by alternating physiological states. (B) Non-Hibernators experience muscle atrophy from aging, microgravity, disuse, and starvation.

Methods and Materials

The primary tool used was the Rota-Rod for Rats XL, designed to measure the latency to fall from a rotating rod. The trial was conducted using a group of three males and three females. Subjects were acclimated to the behavior room for 30 min in their home cages. Squirrels were transferred from home cages in PVC tubes and placed on the Rota-Rod axis. Testing occurred three times per session with 10 min intervals between trials. Trials were recorded and the video was reviewed at a later time. To ensure that any falls were due to coordination deficits rather than unfamiliarity with the machine, subjects had to undergo training first. Squirrels underwent a structured training protocol with progressive checkpoints, with the goal of training them to certain checkpoint stages before increasing the difficulty. After 8 weeks of training, the highest performing squirrels were selected to complete a final trial consisting of a ramp from 0 to 40 Revolutions Per Minute (RPM) over 60s. The goal for future testing is to obtain reliable data in summer active and IBA squirrels.

Checkpoints:
1) Standing on a stationary Rota-Rod for 20s
2) Walking at a constant 2 RPM for 15s
3) Walking for 20s after being accelerated from 1 to 8 RPM over 20s.
4) Walking while accelerating from 0 to 40 RPM over 60s.



Results

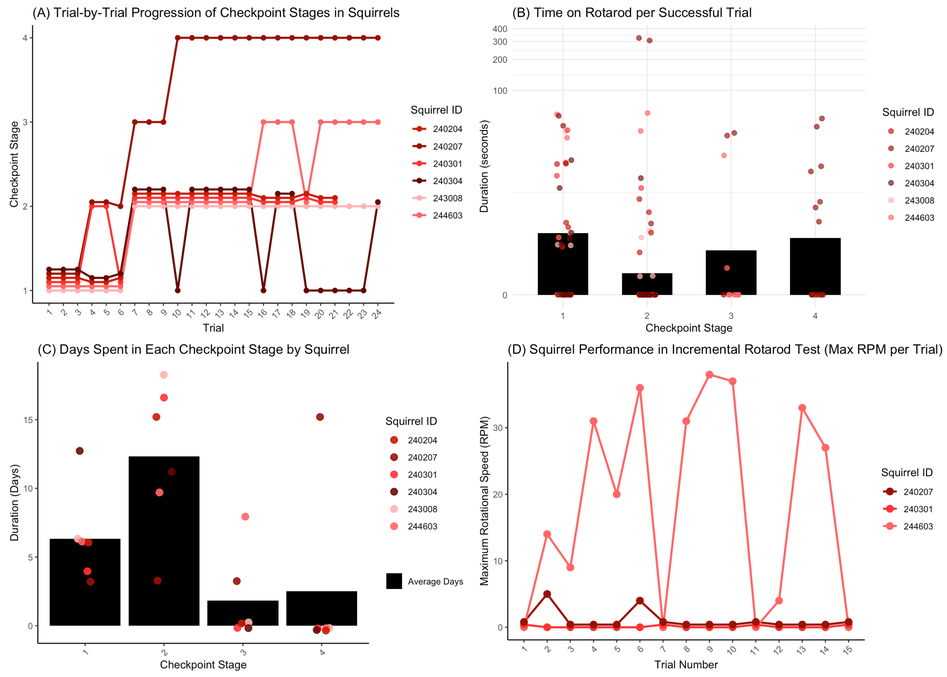


Figure 2: Graphs summarizing behavioral and performance data from the initial squirrel cohort and the three squirrels selected for 0–40 RPM training. (A) This graph shows each squirrel's checkpoint stage during progressive trials. (B) Time spent on the Rota-Rod during successful trials is shown for each squirrel across checkpoints, with average times shown as bar graphs. (C) The number of days each squirrel spent at each checkpoint is presented, with average days per checkpoint displayed as bar graphs. (D) The maximum RPM reached by the three selected squirrels over 15 trials.

Problem Solving

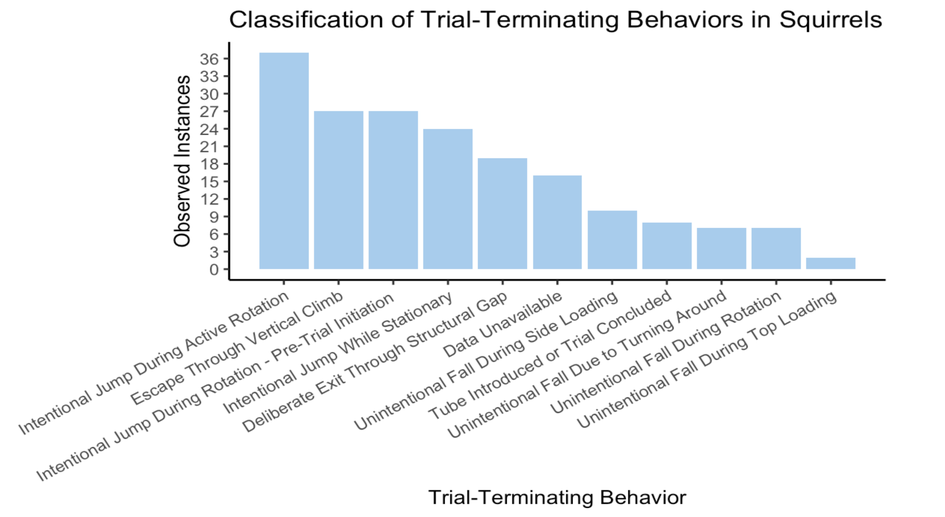
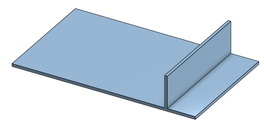
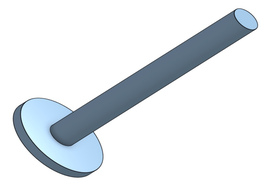
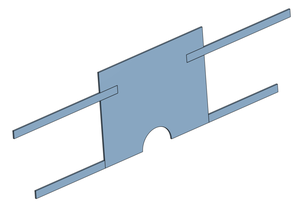
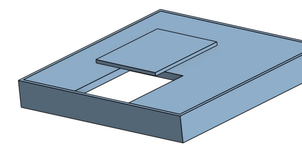


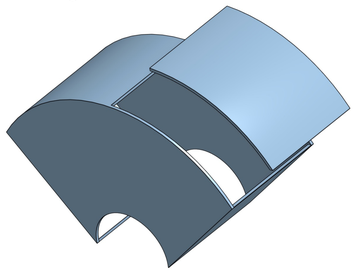
Figure 3: Summary of reasons for trial termination during Rota-Rod testing. We identified factors contributing to unsuccessful trials and used the information to improve training protocols, with the goal of minimizing premature stops and ensuring that falls occur primarily due to speed limitations.

Problem Solving

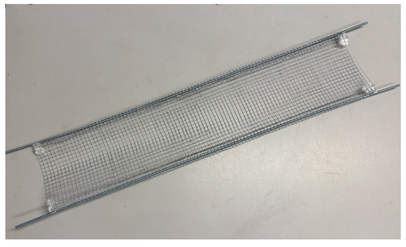
Problem	Solution
• Loading squirrels onto the Rota-Rod was difficult as they frequently fell off during placement. • These planks served as barriers, helping squirrels stay on the Rota-Rod.	
• Top-loading via a PVC tube may have contributed to excess stress. • We switched to a side-load approach, allowing squirrels to enter the Rota-Rod voluntarily. • Squirrels preferred staying in the PVC tube, avoiding the Rota-Rod, so a plunger was added to gradually move squirrels onto the Rota-Rod.	
• As trials progressed, squirrels began walking for longer periods. • A major cause of trial termination was squirrels turning around and falling off when attempting to walk backwards on the moving Rota-Rod (Fig. 3). • An adjustable wall was added to prevent them from turning around.	
• Surprisingly, 13-lined *ground* squirrels, not known as good jumpers, often jumped off the Rota-Rod and out of the enclosure. • A transparent plexiglass cover was added to block escape while allowing video recording.	

Future Directions

- Training without positive or negative reinforcement led to inconsistent results and frequent intentional terminations by the squirrels.
- Future improvements should include reinforcement strategies to discourage falling off the Rota-Rod.
- The ultimate goal is to create a training protocol and environment conducive to using the Rota-Rod as a test for motor coordination in 13LGS.



(A) Rota-Rod Fall Guard



(B) Chicken Wire Catch Platform

Acknowledgments

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