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The supination assessment task: An automated method for quantifying forelimb rotational function in rats

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HIGHLIGHTS

- Here we describe the supination assessment task, a novel automated method of quantifying forelimb supination in the rat.
- Animals are trained to reach out of a cage, grasp a spherical manipulandum, and supinate the forelimb.
- A rotary encoder provides a high-resolution, quantitative dataset of turn angle over time.
- Ischemic lesions of primary motor cortex significantly impair multiple measures of task performance.

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ABSTRACT

Background: Neurological injuries or disease can impair the function of motor circuitry controlling forearm supination, and recovery is often limited. Preclinical animal models are essential tools for developing therapeutic interventions to improve motor function after neurological damage. Here we describe the supination assessment task, an automated measure of quantifying forelimb supination in the rat.
New method: Animals were trained to reach out of a slot in a cage, grasp a spherical manipulandum, and supinate the forelimb. The angle of the manipulandum was measured using a rotary encoder. If the animal exceeded the predetermined turn angle, a reward pellet was delivered. This automated task provides a large, high-resolution dataset of turn angle over time. Multiple parameters can be measured including success rate, peak turn angle, turn velocity, area under the curve, and number of rotations per trial. The task provides a high degree of flexibility to the user, with both software and hardware parameters capable of being adjusted.
Results: We demonstrate the supination assessment task can effectively measure significant deficits in multiple parameters of rotational motor function for multiple weeks in two models of ischemic stroke.
Comparison with existing methods: Preexisting motor assays designed to measure forelimb supination in the rat require high-speed video analysis techniques. This operant task provides a high-resolution, quantitative end-point dataset of turn angle, which obviates the necessity of video analysis.
Conclusions: The supination assessment task represents a novel, efficient method of evaluating forelimb rotation and may help decrease the cost and time of running experiments.

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1. Introduction

Many neurological injures or diseases impair the function of motor circuitry, which can lead to permanent physical disability (Jaquet et al., 2001; Anderson, 2004; Walker and Pickett, 2007; Langhorne et al., 2009). One common manifestation of motor dysfunction is impairment of forelimb supination, a fine motor skill critical for object manipulation (Braendvik et al., 2010; Lambercy et al., 2011; Klotz et al., 2013). Preclinical animal models are essential tools for developing therapeutic interventions to improve motor function after neurological damage. Given the prevalence of deficits in forearm rotation, it would be valuable to efficiently quantify this motor function in rats.

Motor assays designed to measure forelimb function in the rat, including pellet retrieval and pasta handling tasks, have provided important insight into motor learning and recovery after neurological damage (Whishaw et al., 1986; Montoya et al., 1991; Ballermann et al., 2001). While powerful, many of these tasks lack automation and require qualitative scoring of end-point measures. High-speed video analysis techniques provide valuable analysis of individual components of complex movements, including forelimb supination, that are too fast to measure in real-time (Whishaw et al., 1993; Alaverdashvili and Whishaw, 2010; Carmel et al., 2010). However, video analysis greatly amplifies the cost and time to run experiments and precludes high-throughput testing.

We have developed a novel, automated method to quantitatively assess forelimb supination in rats. The task requires rats to reach through a narrow slot, grasp a spherical manipulandum similar to a doorknob, and rotate the manipulandum by supinating the forelimb to receive a reward pellet. The task is fully automated and allows testing of multiple animals simultaneously. Furthermore, this operant task provides a high-resolution, quantitative end-point dataset of turn angle, which obviates the necessity of video analysis. Assessment of various physical measures of forelimb rotational function can be calculated, including peak turn angle and rotational velocity.

Here we demonstrate that the supination assessment task can measure long-term deficits across multiple measures in two models of ischemic stroke. These results indicate that this task provides an efficient, sensitive measure of forelimb supination function, and may be useful to accelerate the preclinical development of therapies to improve complex aspects of motor function.

2. Methods

2.1. Subjects

Fifteen adult female Sprague-Dawley rats weighing approximately 250 g throughout the study were used in this experiment. All rats were maintained above 85% of their ideal body weight for their specific age. The rats were housed in a 12:12 reversed light cycle environment and behavioral training was performed during the dark cycle to increase daytime activity levels. All handling, housing, surgical procedures, and behavioral training were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee.

2.2. Behavioral apparatus

The behavioral chamber consists of a clear acrylic cage (12″ × 4″ × 10″) with a 0.5″ wide slot on the right edge of the front wall (MotoTrak Base Cage Rat Model, Vulintus, Inc., Dallas, TX) (Fig. 1A). The slot restricts use to the right forelimb while allowing full range of movement during interaction with the device (Fig. 1B). The spherical manipulandum is 0.375″ in diameter and has miniature grooves to facilitate grip (Fig. 1C). An optical rotary encoder measures turn angle of the manipulandum with a 0.25° resolution. The encoder is mounted on a metal slide allowing the device to be placed at various fixed distances relative to the inside wall of the cage (Fig. 1D & E). A pulley provides counterweight to the manipulandum, limiting animals to clock-wise rotation (supination) while providing a constant torque and returning the manipulandum to the original location once released (Supplementary Video 1). Three pulley configurations with differing counterweights were used in this study: no counterweight, a 6-g counterweight enacting 0.29 mN m of torque on the manipulandum, and a 7.5-g counterweight enacting 0.37 mN m (MotoTrak Behavior Module, Vulintus, Inc., Dallas, TX).

2.3. Software and behavioral training

Custom MATLAB software was used to control the task (MotoTrak Software, Vulintus, Inc., Dallas, TX). The GUI displays real-time turn angle of the device in degrees with a 100 Hz sampling frequency and performance over the course of the behavioral session. Data were collected and stored on a trial-by-trial basis for each animal. Trial initiation occurred when the animal rotated the device a minimum of 5°. Animals were required to rotate the pre-determined turn angle threshold within two seconds of trial initiation to receive a reward pellet and record a successful trial (Fig. 2D). If the turn angle did not exceed the threshold within the two seconds, the trial was recorded as a failure and no reward pellet was given. An additional two-second timeout window followed in which no pellet rewards were delivered to the animal. All activity one second prior and four seconds following trial initiation was recorded for analysis (Fig. 2D & E). Reward pellets were delivered from pellet dispensers (Pellet Dispenser, Vulintus, Inc., Dallas, TX), delivering a 45 mg pellet (dustless chocolate precision pellet, BioServ, Frenchtown, NJ) upon successful completion of a trial.

Animals underwent two 30-min behavioral training sessions daily, five days per week, with at least a 2-h interval between training periods (Fig. 2A). During the initial phases of shaping, the manipulandum was placed 0.5″ inside from the cage wall, a position that allows animals to easily interact with it (Fig. 1D). The reward threshold was set to 5° and no counterweight was attached, allowing the device to freely spin. During the initial phases of training, the experimenter encouraged animal interaction with the manipulandum by using ground pellet dust. When association between device rotation and pellet rewards was made, the device was retracted outside of the cage in 0.25″ increments to a final location of 0.50″ outside of the inner cage wall (Fig. 1E). The counterweight was then added and animals began the adaptive training program.

A training algorithm that uses adaptive success thresholds was utilized throughout this study. The algorithm uses the median of the peak turn angle of the previous 10 trials to calculate the current trial success threshold, with programmable minimum and maximum adaptive threshold bounds (Fig. 2B). The present study consisted of two cohorts (Cohort A and B), with both cohorts training with a minimum adaptive threshold bound of 15° (i.e., the success threshold was never lower than 15°). Cohort A was trained with a 7.5-g counterweight and a 60° maximum adaptive threshold, and Cohort B was trained with a 6-g counterweight and a 75° maximum adaptive threshold. Success rate on adaptive stages is defined as the percentage of trials greater than the maximum threshold (Fig. 2B). In both cohorts, once animals recorded four consecutive behavioral sessions with at least a 50% success rate, animals progressed to the pre-lesion baseline phase of training. Pre-lesion baseline was conducted on a static (i.e., non-adaptive) threshold stage, with the threshold fixed at the maximum adaptive threshold (Fig. 2C; Cohort A: 60°; Cohort B: 75°). Training continued until animals achieved a 75% success rate or greater averaged across six consecutive training