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The isometric pull task: A novel automated method for quantifying forelimb force generation in rats

Seth A. Hays*, Navid Khodaparast, Andrew M. Sloan, Daniel R. Hulsey, Maritza Pantoja, Andrea D. Ruiz, Michael P. Kilgard, Robert L. Rennaker II

The University of Texas at Dallas, School of Behavioral Brain Sciences, 800 West Campbell Road, GR41, Richardson, TX 75080-3021, United States

HIGHLIGHTS

- ▶ We describe the isometric pull task, a novel fully automated method to measure forelimb strength and function in rats.
- ▶ Several parameters of forelimb strength and function are accurately analyzed.
- ▶ Ischemic lesions of primary motor cortex significantly decrease all measures of performance in this task.
- ▶ The isometric pull task will be useful in assessing function in multiple models of brain damage and motor dysfunction.

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ABSTRACT

Reach-to-grasp tasks are commonly used to assess forelimb function in rodent models. While these tasks have been useful for investigating several facets of forelimb function, they are typically labor-intensive and do not directly quantify physiological parameters. Here we describe the isometric pull task, a novel method to measure forelimb strength and function in rats. Animals were trained to reach outside the cage, grasp a handle attached to a stationary force transducer, and pull with a predetermined amount of force to receive a food reward. This task provides quantitative data on operant forelimb force generation. Multiple parameters can be measured with a high degree of accuracy, including force, success rate, pull attempts, and latency to maximal force. The task is fully automated, allowing a single experimenter to test multiple animals simultaneously with usually more than 300 trials per day, providing more statistical power than most other forelimb motor tasks. We demonstrate that an ischemic lesion in primary motor cortex yields robust deficits in all forelimb function parameters measured with this method. The isometric pull task is a significant advance in operant conditioning systems designed to automate the measurement of multiple facets of forelimb function and assess deficits in rodent models of brain damage and motor dysfunction.

1. Introduction

A wide variety of methodologies are used to assess forelimb movement in rodents. Experiments using these behavioral tasks have provided valuable insight into motor learning and the impairments caused by brain damage or disease. These measures have been used extensively to evaluate therapies designed to improve motor recovery after brain insult.

Pellet retrieval tasks are the most commonly used methods to test forelimb function in rats. There are many variations of these tasks, with animals trained to retrieve an appetitive food reward through a narrow slot outside the cage (O'Bryant et al., 2007; Adkins and Jones, 2005; Whishaw et al., 1991; Gharbawie et al., 2005; Buitrago et al., 2004; Kleim et al., 1998) or from ledges of varying heights (Montoya et al., 1991; Adkins-Muir and Jones, 2003; Whishaw et al., 1997) among others. These methods provide estimations of dexterity as defined by retrieval success rate or qualitative scoring. Another test, the pasta matrix task, provides additional analytical power by including a measure of range of motion (Ballermann et al., 2001; Metz et al., 2001). While these methods have been essential for investigating forelimb function, they are limited in the scope of data that is collected. Qualitative assessment of reach-to-grasp tasks can provide rich data on the biomechanics of forelimb movement, but rating these tasks is time intensive and may potentially suffer from variability in scoring. Quantitation is usually restricted to success rate, with few other measures of performance. Forelimb weakness is associated with many forms of brain damage (Canning et al., 1999; Ada et al., 1996), but there is a paucity of methods for accurately measuring forelimb strength in rodents. Some reach-to-grasp tasks can estimate

* Corresponding author. Tel.: +1 972 883 2376; fax: +1 972 883 2491.
E-mail address: sxh129730@utdallas.edu (S.A. Hays).

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forelimb strength but do not provide quantitative data (Remple et al., 2001, Ballermann et al., 2001). Alternatively, the grip strength task does measure quantitative values of strength, but each trial requires direct handling and administration by an experimenter (Dunnett et al., 1998; Smith et al., 1995). This results in fewer trials in comparison to other tasks and may be more susceptible to trial-to-trial variability. Therefore, it would be valuable to have an automated operant conditioning task that quantifies force generation.

Here we describe a new method that incorporates reach-to-grasp motion and force generation to assess forelimb function in rats. The isometric pull task consists of training an animal to reach out through a slot in a cage wall, grasp, and pull a handle attached to a stationary force transducer. If a predefined force threshold is met when the animal pulls the handle, a food reward is delivered. The task is fully automated, allowing for high-throughput screening of multiple animals simultaneously and real-time analysis of data. Animals initiate the trials, and therefore dictate the total number of trials in a session. The number of trials in this task exceeds that of most pellet retrieval (Montoya et al., 1991; Pagnussat et al., 2009; Buitrago et al., 2004) and grip strength tasks (Smith et al., 1995; Dunnett et al., 1998), resulting in increased statistical power. Multiple aspects of the task can be adapted to tailor the degree of difficulty for specific applications. The distance of the handle from the inside of the cage and the force threshold defined by the experimenter can be adjusted to scale the difficulty of the task. The cage window restricts use to one forearm, facilitating measurement of forelimb function after unilateral damage, preventing compensation with the unimpaired limb. The force transducer provides highly accurate, quantitative, real-time measurement of forelimb strength. Various other quantitative parameters can be derived from the measurements, including success rate, pull attempts before success, and latency to reach maximal force.

We demonstrate that a lesion in the primary motor cortex produces statistically significant deficits in multiple aspects of performance on the isometric pull task. Both success rate and force are reduced, while pull attempts and latency to maximal force are increased. These results indicate that the isometric pull task can be used to quantitatively and efficiently measure multiple facets of forelimb function in rat models of brain damage.

2. Methods

2.1. Subjects

Fifteen adult female Sprague–Dawley rats, approximately 4 months old and weighing approximately 250 g when the experiment began, were used in this experiment. The rats were housed in a 12:12 h reversed light cycle environment so that behavioral testing took place during the dark cycle in order to increase daytime activity levels. Rats were food deprived to no less than 85% of their normal body weight during training as motivation for the food pellet rewards. All handling, housing, surgical procedures, and behavioral training of the rats were approved by the University of Texas Institutional Animal Care and Use Committee.

2.2. Behavioral apparatus and software

The behavioral chamber consisted of an acrylic box (10 in. × 12 in. × 4.75 in.) with a slot (2.5 in. × 0.4 in.) located in the front right corner of the box through which the rats could access the pull handle (Fig. 1A and B and Supplementary Fig. 1). The slot location restricted access such that only the right forelimb could be used to perform the task. The aluminum pull handle was centered in the slot at a height of 2.5 in. from the cage floor and at lateral distances varying from 0.75 in. inside to 0.75 in. outside relative to the inner wall surface of the cage, depending on the training stage (described below). The handle was affixed to a custom designed force transducer (Motor Pull Device, Vulintus LLC, Sachse, TX) located outside the cage. The maximum load capacity of the transducer was 2 kg, and the typical forces generated by the rats fell within the linear range of measurement. Forces readings were sampled at 20 Hz and measured with ±1 g accuracy. Force measurements were calibrated with a force meter at least once per week. Typically, no drift in calibration was observed, but in some cases a deviation never exceeding 5 g was measured and corrected.

Custom software was used to control the task and collect data. A motor controller board (Motor Controller, Vulintus LLC, Sachse, TX) sampled the force transducer every 50 ms and relayed information to a custom MATLAB software which analyzed, displayed, and stored the data. Force values and corresponding timestamps were collected as continuous traces for each trial to allow for the analysis of force profiles over the course of a session (see Fig. 3A). If a trial was successful, the software triggered an automated pellet dispenser (Vulintus LLC, Sachse, TX) to deliver a sucrose pellet (45 mg dustless precision pellet, BioServ, Frenchtown, NJ) to a receptacle located in the front left corner of the cage.

2.3. Behavioral training

Training sessions lasted 30 min and were conducted twice daily, five days a week, with sessions on the same day separated by at least 2 h. During early phases of training, experimenters manually shaped animals by using ground sucrose pellets to encourage interaction with the handle, as olfaction of food rewards is beneficial to direct reaching during training (Whishaw and Tomie, 1989). Rats pulled the handle initially located 0.75 in. inside the training cage to receive a sucrose reward pellet. A trial was initiated when the rat generated a force of at least 15 g on the handle. After trial initiation, the force was sampled for 4 s. If the force threshold was broken within a 2 s window following the initial contact, the trial was recorded as a success and a reward pellet was delivered. If the force did not exceed threshold within the 2 s window, the trial was recorded as a failure and no reward was given. Force on the pull handle was sampled for 2 additional seconds following the 2 s trial window, regardless of the trial outcome, to capture any late attempts which were unrewarded. Following the 4 s of data collection there was a 50 ms pause before rats could initiate another trial. If rats did not receive 50 pellets in a single day, they were given 10 g of pellets after daily training sessions were complete. The task was made progressively more difficult as rats met the criterion for number of successful trials within a session and progressed to the next stage. As the training stages increased, the handle was gradually retracted to 0.75 in. outside the cage and the force threshold progressively increased up to 120 g. The values for criterion, handle location, and pull threshold are detailed for each training stage in Table 1. If an animal exceeded criteria for a proceeding stage, they were automatically advanced to the stage that matched their performance. The prescribed position and threshold values were strictly adhered to for pre- and post-lesion measurements. Rats were held at the pre-lesion stage until they had 10 successive sessions averaging over 85% success rate. The pre-lesion data reported in this study is compiled from these 10 sessions. None of the animals in this study failed to meet this criterion. After this point, the rats were given an ischemic lesion followed by seven days of recovery, after which they returned for post-lesion behavioral testing with the same parameters as pre-lesion allowing for a direct comparison of performance. The post-lesion data presented in this study is collected from the four testing sessions over two consecutive days immediately following the surgical recovery period.