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An automated task for the training and assessment of distal forelimb function in a mouse model of ischemic stroke

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HIGHLIGHTS

- We present an automated assay of distal forelimb function.
- This assay precludes compensation and quantifies multiple aspects of the reach and grasp motion.
- Training, assessment, and analysis on this assay are automated to produce reliable, precise, and richly informative data while requiring relatively little time investment.
- We show that photothrombotic stroke of the cortical motor forelimb representation causes long-term impairment in multiple aspects of this task through 22 weeks of weekly practice and assessment.

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ABSTRACT

Background: Behavioral models relevant to stroke research seek to capture important aspects of motor skills typically impaired in human patients, such as coordination of distal musculature. Such models may focus on mice since many genetic tools are available for use only in that species and since the training and behavioral demands of mice can differ from rats even for superficially similar behavioral readouts. However, current mouse assays are time consuming to train and score, especially in a manner producing continuous quantification. An automated assay of mouse forelimb function may provide advantages for quantification and speed, and may be useful for many applications including stroke research.
New method: We present an automated assay of distal forelimb function. In this task, mice reach forward, grip and pull an isometric handle with a prescribed force. The apparatus partially automates the training process so that mice can be trained quickly and simultaneously.
Results: Using this apparatus, it is possible to measure long-lasting impairment in success rate, force pulled, latency to pull, and latency to success up to 22 weeks following photothrombotic cortical strokes in mice.
Comparison with existing method(s): This assessment measures forelimb function as do pellet reach tasks, but it utilizes a different motion and provides automatic measures that can ease and augment the research process.
Conclusions: This high-throughput behavioral assay can detect long-lasting motor impairments, eliminates the need for subjective scoring, and produces a rich, continuous data set from which many aspects of the reach and grasp motion can be automatically extracted.

1. Introduction

About 80% of people who suffer ischemic strokes incur motor deficits that interfere with quality of life (Langhorne et al., 2009). Developing and refining measures of functional motor impairment and recovery after stroke in mouse models could therefore

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contribute to improved relevance of mouse research. The most promising current motor assays require extensive scoring and subjective evaluation that makes efficient, high-throughput, flexible research challenging.

Since functional impairment of the distal forearm is an important cause of disability in stroke patients, an ideal rodent assay will capture the important aspects of such movements (Klein et al., 2012). It is advantageous to develop such assays particularly for mice given the extensive availability of genetic and pharmacological mouse models. However, assays for mice need to be developed independently from those utilized in rats since neither training nor performance patterns necessarily overlap between the species. Existing tests meeting these criteria such as skilled pellet retrieval reaching tasks (Lai et al., 2015; Clarkson et al., 2013; Lee et al., 2007) can be time consuming to train and score, especially in a manner producing continuous quantification. When behavior is automatically measured rather than scored visually, it can be more easily quantified to a finer degree than is practical with visual scoring. We have developed such an automated assay of skilled forelimb use for mice based on versions published for use in rats (Hays et al., 2013; Wong et al., 2015). Here, we describe this assay, which requires the coordination of several forearm muscles. Like existing skilled reach tasks, this assay requires a mouse to reach through a slit to grasp an object in a manner amenable to automated or hand-scored motion analysis (Lai et al., 2015), however unlike existing tests the subsequent force exertion on the object is highly constrained and isometric. The task precludes behavioral compensation, shows lasting deficits as a result of photothrombotic cortical stroke, allows for flexibility in different aspects of behavioral measurement, can be trained in a partially automated fashion without close attention, and can be consistently applied in large numbers of mice with efficiency and precision. We present and validate this assay primarily for use in a photothrombotic mouse model of stroke and demonstrate its sensitivity to that injury. However, this assay could be useful for any application that requires a sensitive assay of forelimb function.

2. Methods

2.1. Apparatus and procedure

2.1.1. Enclosure, behavior, measurement and analysis

The apparatus has been developed in collaboration with Vulintus, Inc. (Dallas, TX) and resembles a similar design optimized for rats (Hays et al., 2013) (Fig. 1). It consists of a plexiglass enclosure 5.5″ high × 5″ wide × 8″ long. A pattern of square holes in the floor allow waste to fall to the level below. A slot in the right side of the front wall provides access to a vertical handle 3 mm tall, 1 mm wide and 1 mm thick, connected to a force transducer that measures unidirectional horizontal force exerted in the direction of the mouse. The position of the handle and the directionality of the required force constrain the behavior; the mouse cannot succeed by pushing the handle from the sides, top or bottom and must use paw musculature to grasp around the back of it. Additionally, the handle is most easily grasped from the side since a grasp from the top would allow fewer digits to exert force on the back of the handle, providing a natural constraint to the top-down raking motion normally considered compensatory in similar assays. The calibrated transducer measures up to ~70 g with 1 g precision. Accuracy of the force signal is assured through regular calibration and testing with precision weights. Mice can generally pull a maximum of about 35 g on this apparatus. The front edge of the handle is positioned 1 cm from the inner edge of the chamber. Between that edge and the handle, an infrared (IR) slot detector is positioned vertically across the slit to detect reach attempts. Adjacent to the slot, a bracket recessed in the plexiglass wall presents the blunt tip of a feeding needle controlled by a pinch valve. Following a successful pull, the pinch valve emits an audible click and releases approximately 2 μl of peanut oil at the end of the feeding needle. Signals from the infrared beam and the force transducer are sampled every 10 ms using a custom control board and recorded permanently during adjustable trial windows. Trials are initiated by either a break of the IR beam or by a force exerted on the handle greater than an adjustable initiation threshold of 2 g. A trial ends upon the longer of two seconds (also adjustable) or when the IR beam has been unbroken and less than 2 g has been exerted on the handle for at least 1 s. Trial data is written continually during a behavioral session, preventing incidental data loss. Data is streamed by custom MATLAB software, which displays and stores the data as continuous traces. The raw data is used to derive five different measurements, summarized in Table 1 and Fig. 2. Only the first 50 responses of a session are considered in this analysis.

Table 1
Derived behavioral measures. This validation experiment used these five transformations of the raw data returned by the apparatus, though many others are possible.

Derived measure	Description
Success rate	Number of successful trials divided by number of total trials analyzed
Highest force in trial	Highest force measurement within each trial, averaged over trials
Latency to pull	Time between the first moment that the IR beam was broken until the moment that the force reading exceeded the initiation threshold, averaged over trials
Latency to success	Time between the first moment that the IR beam was broken until the moment that the force reading first exceeded 20 g, averaged over trials
Attempts before success	Number of local force peaks above initiation threshold within a trial before the force reading first exceeded 20 g. These "attempts" are caused by grasp-and-pull motions that fall below force requirements

2.1.2. Program

The apparatus is controlled by custom MATLAB software. This software presents a user interface as seen in Fig. 2b. A drop-down menu allows the user to select from variable, customizable program settings. The program specifies the initiation force required to begin a logged pull, the force that must be exceeded to trigger reinforcement (peanut oil delivery), and the manner in which the force required to trigger reinforcement changes throughout the session. "Static" sessions retain a constant force requirement. Adaptive "Linear" sessions increase the requirement by a customizable increment every time a successful pull occurs. Adaptive "Median" sessions set a force criterion as the lower half or quartile of the previous *n* pulls. For experiments reported here, "Linear" was used for training and "Static" for baseline and post-stroke sessions. While a session is running, the MATLAB interface provides a real time list of logged pulls and a plot of trial vs. grams of force, with successful trials in green and unsuccessful in red (Fig. 2b shows a black-and-white image; the on-screen graphical user interface is in color).

2.2. Validation of apparatus in the context of stroke

2.2.1. Subjects

Thirteen adult C57-Bl6 mice weighing approximately 20–30 g were used to assess the behavioral effects of photothrombotic stroke on this forelimb task. Mice ranged in age from 25 to 35 weeks old at the time of stroke; four were female and nine were male. All mice were housed in a temperature and humidity maintained facility on a reverse light cycle to assure that their high-activity periods