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An automated behavioral box to assess forelimb function in rats

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HIGHLIGHTS

- We develop a low-cost automated behavioral box to measure forelimb function in rats.
- We illustrate camera-based automated detection of behavioral outcomes.
- We demonstrate the ability to easily vary task structure and practice schedules.
- Our automated setup is able to monitor deficits after unilateral ischemic stroke.
- We show compatibility with modern chronic electrophysiological approaches.

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ABSTRACT

Background: Rodent forelimb reaching behaviors are commonly assessed using a single-pellet reach-to-grasp task. While the task is widely recognized as a very sensitive measure of distal limb function, it is also known to be very labor-intensive, both for initial training and the daily assessment of function.
New method: Using components developed by open-source electronics platforms, we have designed and tested a low-cost automated behavioral box to measure forelimb function in rats. Our apparatus, made primarily of acrylic, was equipped with multiple sensors to control the duration and difficulty of the task, detect reach outcomes, and dispense pellets. Our control software, developed in MATLAB, was also used to control a camera in order to capture and process video during reaches. Importantly, such processing could monitor task performance in near real-time.
Results: We further demonstrate that the automated apparatus can be used to expedite skill acquisition, thereby increasing throughput as well as facilitating studies of early versus late motor learning. The setup is also readily compatible with chronic electrophysiological monitoring.
Comparison with existing methods: Compared to a previous version of this task, our setup provides a more efficient method to train and test rodents for studies of motor learning and recovery of function after stroke. The unbiased delivery of behavioral cues and outcomes also facilitates electrophysiological studies.
Conclusions: In summary, our automated behavioral box will allow high-throughput and efficient monitoring of rat forelimb function in both healthy and injured animals.

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1. Introduction

Rodent forelimb function is widely studied in the context of motor learning, neural plasticity and recovery from injury (Girgis et al., 2007; Hays et al., 2013; Kleim et al., 2007; Montoya et al., 1991; Ramanathan et al., 2006; Ramanathan et al., 2009; Rioult-Pedotti et al., 1998; Slutzky et al., 2010; Weishaupt et al., 2013; Whishaw et al., 2008, 1986). More specifically, the Whishaw single-pellet reach-to-grasp task is among the mostly commonly used behavioral assessment of forelimb function (Fu et al., 2012; Kleim et al., 2007; Rioult-Pedotti et al., 1998; Whishaw et al., 2008, 1986; Whishaw and Pellis, 1990; Xu et al., 2009). Early variations of this task included the use of trays in the home cage containing multiple pellets simultaneously (Castro, 1972; Whishaw et al., 1986).

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The single-pellet task is more difficult as it requires reaching, grasping and retrieving a single pellet located at a distance outside of the behavior box (Whishaw and Pellis, 1990); inaccurate reaches typically result in the pellet being knocked away. The original version of this task included an acrylic box that biased reaching movements to a single limb and allowed video based monitoring of movements from multiple perspectives. Numerous studies have now shown that the single-pellet reaching task involves the learning and acquisition of a new motor skill (Conner et al., 2003; Francis and Song, 2011; Kleim et al., 2007; Rioult-Pedotti et al., 2000; Rioult-Pedotti et al., 1998); it has become an important focus for studies of the neural substrates of motor learning in both rats and mice (Fu et al., 2012; Kleim et al., 1998; Xu et al., 2009). The same task is also commonly used to study recovery of forelimb function after stroke or brain injury (Ramanathan et al., 2006; Whishaw et al., 2008, 1986). In addition, it may be used to assess motor function in other models of neurological dysfunction (e.g. Parkinson's disease) (Klein and Dunnett, 2012; Vergara-Aragon et al., 2003).

While the single-pellet reaching task is widely recognized as a very sensitive measure of distal forelimb function, it is also known to be very labor and time intensive (Kleim et al., 2007). In a typical reaching session, rats are given the opportunity to obtain 20–25 pellets (i.e. 20–25 trials per day). Traditionally, this requires an experimenter to manually present each pellet and to observe/shape the behavior of the rat by placing a subsequent pellet only when the rat has relocated to the other end of the cage. Such a training paradigm requires ~2 weeks to achieve adequate plateau performance levels (Francis and Song, 2011; Kleim et al., 2007). This is only compounded by the fact that multiple trials are necessary to assess outcomes after injury (i.e. if also used as a serial measure of functional recovery).

The primary goal of this study was to develop and validate a low-cost, automated high-throughput version of this task. Our specific focus was to minimize the need for user input and supervision during the training and assessment of animals. Importantly, the ability to automate assessments has the added benefit of facilitating blinding of assessments (i.e. done automatically without human intervention). We further demonstrated the potential use of such a box in varying the trial structure during motor learning as well as its compatibility with chronic electrophysiological recording techniques.

2. Methods

2.1. Subjects

We used a total of 22 male Long Evans rats weighing approximately 250 g. The rats were housed in a temperature-controlled, 12:12 h light cycle environment in which behavioral testing occurred with lights on during the day. Rats were food scheduled, where they received a part of their food requirements from the reaching task depending on trial structure. Rats following the traditional training paradigm of one 25-trial session per day were given an opportunity to obtain a maximum of 25 pellets in the behavior box, which made up approximately ~1/5 of their daily food intake. They were supplemented with 2 larger 'rodent diet' pellets (2500–3000 mg each; 8640 Tklad 22/5 Rodent Diet, Harlan Laboratories, Indianapolis, IN) in their home cages after task performance. Rats undergoing high-throughput training paradigms obtained food ad lib during the task, which amounted to approximately ~2/3 of their daily intake, and were supplemented accordingly at the end of daily training. We measured body weight on a daily basis to ensure that their weight did not fall below 90% of their initial weight. Rats had free access to water when they were not performing the pellet reaching task. All housing and procedures were approved by the Institutional Animal Care and Use Committee at the San Francisco VA Medical Center (Animal Welfare Assurance Number A3476-01).

2.2. Apparatus

The reach box was made of acrylic sheets ($250 \times 300 \times 200$ mm, 3.175 mm thick [1/8″]; Acrycast, Calsak Plastics, Chino Hills, CA) and constructed with a central 12 mm wide slit in the front (Fig. 1). The centralized position and size of the slit only allowed access using one paw. The two "pellet trays" had a circumference of 7 mm with a 1 mm central depression (Supplementary Fig. 1) and were placed 15 mm in front of the slit and slightly left/right of center, respectively (Fig. 1A and F). The centers of the pellet trays were aligned with each respective edge of the central slit (Fig. 1A). Pellets were dispensed through flexible tubes (silicon tubing with 6.35 mm [1/4″] inner diameter, VetEquip Inc., Pleasanton, CA) (Fig. 1D). The tubing was attached to a front gate that controlled the opening of the slit (Fig. 1A and D). During the inter-trial period, the gate moved down to close the slit; a pellet was dispensed to the appropriate tray. During the following trial period, the gate moved up to allow access to the pellet (Fig. 1E and F).

Both a custom-built pellet dispenser (Fig. 1D, Supplementary Fig. 2) and a commercially available dispenser (Supplementary Fig. 3; Pellet Dispenser with 45 mg Interchangeable Pellet Size Wheel, Lafayette Instrument Company, Lafayette, IN) were tested. The custom-built dispenser consisted of two tubes for pellet placement to either the left or the right tray (or to both simultaneously), which allowed for automatic determination of paw preference. This dispenser consisted of a clear acrylic tube (44.45 mm [1.75″] inner diameter, 4.76 mm [0.1875″] thick, and 63.5 mm [2.5″] height, Small Parts, Logansport, IN) attached with plastic bonder epoxy (Loctite, Westlake, OH) to an acrylic square bottom ($63.5\text{ mm} \times 63.5\text{ mm}$ [$2.5'' \times 2.5''$], 4.76 mm [0.1875″] thick). A 12.7 mm [0.5″] diameter hole was created in the center of the tube/bottom (Supplementary Fig. 1C). The shaft of a stepper motor (Hitec 32645S HS-645MG High Torque, HITEC RCD USA, Inc., Poway, CA) was inserted through the 12.7 mm [0.5″] hole and fixed using epoxy; a 44.45 mm [1.75″] circular plastic motor horn was then pushed to the bottom of the acrylic tube and attached to the stepper motor itself; the horn could then freely rotate. Two 6.35 mm [0.25″] holes were created in both the disc and the plastic bottom; the silicon tubes were attached to the plastic bottom such that when the holes were physically aligned, a pellet dropped through the respective tube onto either the L (left) or R (right) pellet tray (Fig. 1E). The customized dispenser required calibration in order to prevent crushing of the 45 mg pellets (45 mg dustless precision pellet, BioServ, Frenchtown, NJ). In contrast, the commercially available dispenser was readily adapted without any further modifications. With the commercial dispenser, we were only able to deliver a pellet to either the L or R (i.e. required a physical switch of the silicon tubing). Notably, the use of two such dispensers can be used to replicate the two simultaneous outputs achieved using the customized dispenser.

An acrylic sheet gate was placed between the pellet tray and the slit (Fig. 1A, C and E). The dispenser tubes were attached to the gate itself. A second stepper motor (Hitec 32645S HS-645MG High Torque, HITEC RCD USA, Inc., Poway, CA) was used to control the position of the gate; gate opening was used to indicate the start of a trial and to allow access to the pellet trays. Pairs of infrared (IR) LED emitters (Sharp Microelectronics, Camas, WA) and IR detectors (Sharp Microelectronics, Camas, WA) were used to both detect the pellet on the tray (Fig. 1D and F) and the location of the rat between trials (Fig. 1B and C). An Arduino board (Arduino Uno—R3, Arduino, Ivrea, Italy) and motor shield (Arduino, Ivrea, Italy) were used to control both stepper motors described above. The IR emitter and the detector pairs were also monitored using the same board.