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INNOVATIVE METHODOLOGY | *Control of Movement*

Individualized tracking of self-directed motor learning in group-housed mice performing a skilled lever positioning task in the home cage

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Silasi G, Boyd J, Bolanos F, LeDue J, Scott SH, Murphy TH. Individualized tracking of self-directed motor learning in group-housed mice performing a skilled lever positioning task in the home cage. *J Neurophysiol* 119: 337–346, 2018. First published October 25, 2017; doi:10.1152/jn.00115.2017.—Skilled forelimb function in mice is traditionally studied through behavioral paradigms that require extensive training by investigators and are limited by the number of trials individual animals are able to perform within a supervised session. We developed a skilled lever positioning task that mice can perform within their home cage. The task requires mice to use their forelimb to precisely hold a lever mounted on a rotary encoder within a rewarded position to dispense a water reward. A Raspberry Pi microcomputer is used to record lever position during trials and to control task parameters, thus making this low-footprint apparatus ideal for use within animal housing facilities. Custom Python software automatically increments task difficulty by requiring a longer hold duration, or a more accurate hold position, to dispense a reward. The performance of individual animals within group-housed mice is tracked through radio-frequency identification implants, and data stored on the microcomputer may be accessed remotely through an active internet connection. Mice continuously engage in the task for over 2.5 mo and perform ~500 trials/24 h. Mice required ~15,000 trials to learn to hold the lever within a 10° range for 1.5 s and were able to further refine movement accuracy by limiting their error to a 5° range within each trial. These results demonstrate the feasibility of autonomously training group-housed mice on a forelimb motor task. This paradigm may be used in the future to assess functional recovery after injury or cortical reorganization induced by self-directed motor learning.

NEW & NOTEWORTHY We developed a low-cost system for fully autonomous training of group-housed mice on a forelimb motor task. We demonstrate the feasibility of tracking both end-point, as well as kinematic performance of individual mice, with each performing thousands of trials over 2.5 mo. The task is run and controlled by a Raspberry Pi microcomputer, which allows for cages to be monitored remotely through an active internet connection.

automation; behavior; motor cortex; RFID; stroke

INTRODUCTION

Motor assessment of rodents has become a mainstay in systems neuroscience, providing a convenient model for studying sensorimotor interactions during learning (Li et al. 2015) or recovery from injury (Jones et al. 2013). A number of tasks have been developed to assess both gross motor behaviors, such as walking and rearing (Farr et al. 2006; Gharbawie et al. 2004; Metz and Whishaw 2002), as well as skilled limb (Ballermann et al. 2000; Guo et al. 2015; Whishaw 2000) or vibrissae movements (O'Connor et al. 2010). Skilled reaching tasks have become especially useful for studies investigating cortical function (Guo et al. 2015); however, it is time consuming to train animals, and studies are limited by the number of trials animals are willing to perform within individual sessions.

More generally, behavioral training of individual animals by experimenters has several disadvantages that can be largely eliminated through automation (Richardson 2015). First, even the most basic tasks, such as skilled locomotion, typically require 3–4 training days (Farr et al. 2006), whereas more complex skills, such as reaching for food, require several weeks of daily training of up to 20 min/subject/session (Gharbawie et al. 2005). In addition to requiring significant human resources, such training may also disrupt the circadian cycle of animals and may induce learning impairments if training is prolonged (Craig and McDonald 2008). A second, related confound is that the quality of training may vary among laboratory personnel and may be difficult to match between laboratories (Fouad et al. 2013; Wahlsten et al. 2003). Even when efforts are made to standardize protocols, subtle differences in training style may impact rodent stress levels and thus the acquisition of new skills (Fenrich et al. 2015; Lewejohann et al. 2006; Sorge et al. 2014). Lastly, many tasks require animals to be deprived of either food or water for several days before training. In addition to being labor intensive, such manipulations alter the motivational state of animals and can introduce unwanted stress that negatively impacts brain physiology (Faraco et al. 2014).

To overcome these limitations, we developed a novel forelimb motor task for mice that can be implemented completely autonomously in the home cage. The task requires mice to

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precisely position a lever attached to a rotary encoder within a rewarded range to dispense a water reward. Animals can engage in the task 24 h/day, 7 days/wk, thus eliminating the disruptive effects of intermittent testing, and it also provides mice with continuous access to water. Mice can be group housed (up to 5 mice/apparatus), which is important for maintaining the natural social environment and it also facilitates high throughput testing. We employ a simple radio-frequency identification (RFID) tagging system (Bolaños et al. 2017) to track the performance of individual mice, over 12 wk of testing. This approach allowed us to autonomously train mice to hold a lever for up to 1.5 s in a narrow rewarded range of 5°, thus providing a sensitive assay for skilled forelimb movement and proprioception.

METHODS

Animals. All procedures were approved by the University of British Columbia Animal Care Committee and conformed to the Canadian Council on Animal Care and Use guidelines. All mice ($n = 24$) were of the C57/Bl6 background strain and 3–6 mo old at the start of the experiment. To identify each mouse within the home cage apparatus, a 12-mm glass-encapsulated RFID tag (Sparkfun, product: SEN-09416) was surgically implanted subcutaneously at the nape of the neck under brief (1–2 min) isoflurane anesthesia (Bolaños et al. 2017). During home-cage training, mice had ad libitum access to standard mouse chow food pellets, but the standard cage-top drinking water bottle was removed. Instead, all water was dispensed from a spout in the training compartment (see below).

Home cage apparatus. Standard mouse home cages (29 cm × 19 cm × 13 cm) were modified by cutting a 1-in. square hole through the end wall (centered 4 cm above the floor of cage) to provide access to a training chamber attached to the outside of the cage (Fig. 1). The training chamber was constructed out of Plexiglas and consisted of a 1-in.^{2} tunnel through which the animals entered. An RFID tag reader (Sparkfun, product: SEN-09963) mounted on the roof of the training compartment identified individual mice as they entered. A 3-mm gap in the floor of the tunnel allowed mice to access a 1.5-mm-diameter metal lever that was mounted on an optical rotary encoder (Robot-Shop, product: RB-Phi-167). The encoder was mounted on an L-bracket attached to the outside of the training compartment and could be lowered relative to the floor of the tunnel to make the lever only accessible with the right forelimb. The travel distance of the lever was limited by two posts that were 2.3 cm apart. This setup allowed a mouse to move the lever a maximum of 16° when grasped at the end. Based on the resolution of the encoder (512 signals/revolution), the precise position of the lever could be resolved at 0.176° increments. To provide a constant resistive force and to return the lever to the start position between trials, a 3-g hanging counterweight was attached with nylon string to the opposite end of the lever. Therefore, at the start of a trial, the lever was 8° anterior of the center position. A water spout made from a blunted 24-G needle was inserted through the end wall of the training compartment (~1.5 cm from lever) and was easily accessible by the mice while actively engaged in the task.

Trial timing and data logging were carried out through custom-written Python software running on a Raspberry Pi computer (https://www.raspberrypi.org). Each Raspberry Pi was set up with a static IP address, allowing us to monitor the task remotely through an active internet connection using a Secure Shell protocol. For each trial, lever position was recorded at ~120 Hz, and correct trials were rewarded by dispensing ~4 μl of water from an elevated water reservoir by actuating a solenoid (Newark, product: MB202-VB30-L203). To monitor the animals while engaged in the task, a small infrared camera and infrared light source (Waveshare, product: 10299) were mounted next to the training compartment. The camera was connected to the Raspberry Pi via a ribbon cable, and frames were only saved when the mice actively moved the lever. A complete bill of materials is

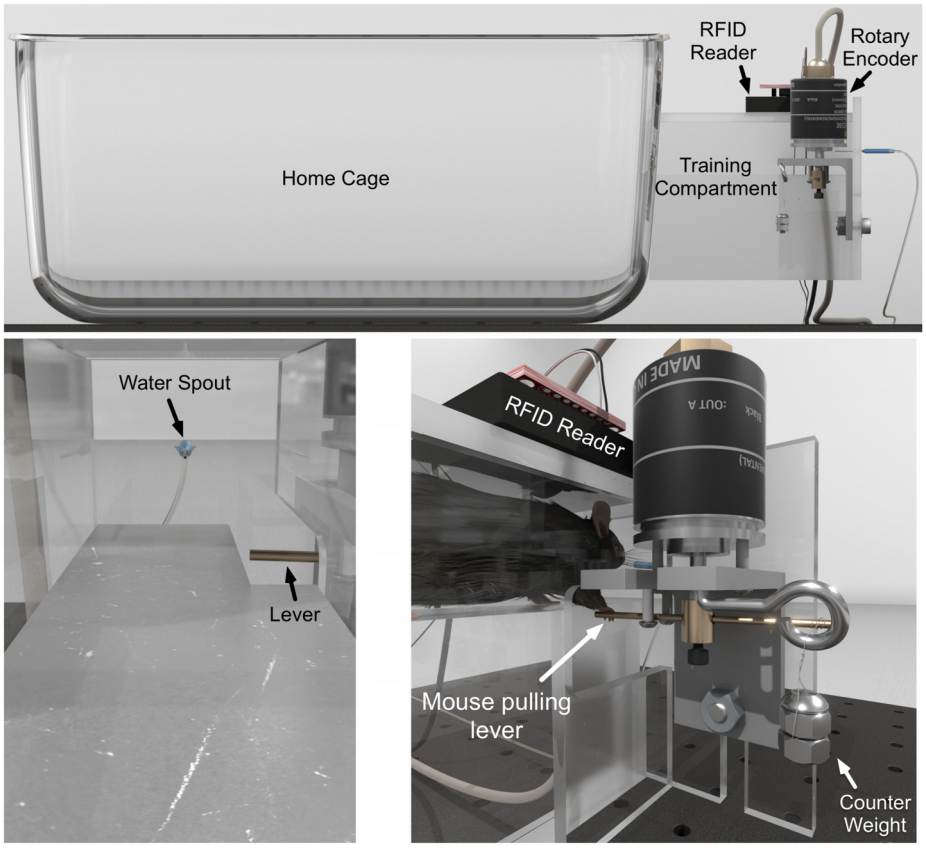

Fig. 1. Apparatus for home cage-based motor training of group-housed mice. A training chamber attached to the home cage allows mice to access a water spout and a metal lever mounted on a rotary encoder. An RFID reader placed on the roof of the training compartment identifies individual mice as they enter. To dispense a water reward, mice have to position the lever within a rewarded range according to user-set task parameters. During a trial, mice grasp the lever and pull it toward themselves in the horizontal plane, thus rotating the shaft of the rotary encoder. Mice have access to the training compartment 24 h/day, 7 days/wk and can initiate trials freely.

Table 1. *Bill of materials*

Component	Vendor
Raspberry Pi + SD	http://canada.newark.com/raspberry-pi/raspberry-modb-8gb-usd/raspberry-pi-model-b-8gb-micro/dp/68X0156
WiFi Module	http://canada.newark.com/webapp/wcs/stores/servlet/ProductDisplay?catalogId=15003&langId=1&urlRequestType=Base&partNumber=53W6285&storeId=10196
Black Enclosure	http://canada.newark.com/raspberry-pi/rpi3-case-blk-gry/for-use-with-raspberry-pi-3-model/dp/80Y1135
Proto-plate	http://canada.newark.com/webapp/wcs/stores/servlet/ProductDisplay?catalogId=15003&langId=1&urlRequestType=Base&partNumber=44W3453&storeId=10196
Decoder Chip (LS7366R)	https://www.amazon.com/SuperDroid-Robots-LS7366R-Quadrature-Breakout/dp/B00K33KDJ2/ref=sr_1_1?s=industrial&ie=UTF8&qid=1418860344&sr=1-1
1440 CPR Encoder	https://www.robotshop.com/ca/en/isc3004-optical-rotary-encoder.html
L293D Darlington	http://canada.newark.com/texas-instruments/l293dne/ic-motor-driver-half-h-600ma-dip/dp/06F9523?ost=L293D&categoryId=800000004663
Solenoid	http://canada.newark.com/gems-sensors/mb202-vb30-l203/solenoid-valve/dp/45M6131
RFID Reader ID-20LA (125 kHz)	https://www.sparkfun.com/products/11828
RFID USB Reader	https://www.sparkfun.com/products/9963
RFID Glass Capsule (125 kHz)	https://www.sparkfun.com/products/9416
RPi NoIR Camera and IR LED	https://www.waveshare.com/product/modules/cameras/raspberry-pi-camera/rpi-camera-f.htm

provided in Table 1, and the Python code running the task can be downloaded here: https://www.dropbox.com/s/wx7aicjh4is81om/HomeCageForelimbTask.py?dl=0.

Lever-pulling task. Group-housed mice (3–5 mice/cage) were placed in the modified cage and provided with standard bedding and nesting materials. Given that the training compartment was directly attached to the home cage, we took advantage of the natural tendency of mice to explore a novel environment when they have a home base (in our case, the home cage) to which they can return at will (Clark et al. 2006). Mice readily entered the training compartment without the need for experimenter coaxing or manipulation (see Table 2). Training was divided into three continuous phases (Fig. 2) that trained the mice to *1*) dispense water by pulling the lever; *2*) hold the lever in rewarded position for an incrementally longer duration; and *3*) hold the lever within an incrementally narrower range. During the first phase, mice received a water reward each time they entered the compartment (up to a total of 1 ml/24 h); however, they could also dispense additional water drops by pulling the lever toward themselves in the horizontal plane. The lever is mounted at 90° to the vertical shaft of the rotary encoder and thus moves in the horizontal plane. To encourage interaction with the lever, it was initially positioned 1–2 mm above the floor of the training compartment, and all pulls that displaced it from the resting position were rewarded. Once all mice were reliably dispensing water by pulling the lever (usually 2–3 days), it was gradually lowered, first to be flush with the floor of the training compartment (for 2–3 days), and then lowered further to be recessed 4–5 mm below the floor surface. The final position of the lever made it inaccessible by the snout, thus ensuring forelimb use. During the second phase of training, entrance rewards were no longer provided, and only trials where the lever was held within a 10° range were rewarded (center of travel range ± 5°). Initially, the lever had to remain within this 10° range for 0.1 s, but was subsequently increased based on task performance. Individualized performance was calculated for blocks of 50 consecutive trials, and task parameters were adjusted according to the following rules. If the hold requirement was met for 75% of the trials, hold duration was increased by 0.1 s, until a maximum of 1.5 s. If the hold requirement was met in <10% of trials, the hold requirement was decreased by 0.1 s, while performance between 10 and 75% produced no change in hold requirement. When each mouse reached its maximal hold criterion (up to 1.5 s), the task was advanced to the third training phase, where the rewarded range was incrementally decreased (in 0.5° increments) from 10 to 5°. To encourage participation in the task, we did not include an aversive stimulus or punishment for incorrect trials. It is important to note that, while progression of individual mice through the three phases required experimenter input, progression through the last two phases was fully controlled by the computer program and was individualized for each mouse based on its performance. In addition, we did not provide any visual or auditory feedback regarding the goal position or trial outcome. The only indication that a trial was completed successfully was the dispensing of the water reward; therefore, the mice had to mainly rely on kinesthetic feedback about limb position when maintaining the lever in the rewarded position. Once mice reached maximal performance in terms of hold duration and accuracy, the task parameters were reset to values used at the start of training (0.1-s hold time; 10° rewarded range) to compare the rate of progression through the task between the two training sessions.

Behavioral analysis. To assess motor learning within the task, mice were first trained to hold the lever in a 10° rewarded range for up to 1.5 s. We then further narrowed the rewarded range to 5°, thus

Table 2. *Heuristics used for shaping mice throughout the training period*

Phase of Training	Experimenter Manipulation	Task Parameters	Heuristic	Duration, days
1	Place mice in cage with training compartment	Lever is readily accessible Dispense entrance rewards Reward all lever movements	Dispense water by pulling lever	4
	Lower lever to make it only accessible by paw	Dispense entrance rewards Reward all lever movements	Pull lever with forepaw	7
2	Set computer program to incrementally increase hold duration (start at 0.1 s)	Entrance rewards are discontinued Hold duration requirement must be met to dispense reward	75% Correct in a block of 40 trials increases hold duration by 0.1 s	21
3	Set computer program to incrementally decrease rewarded range (start at 10°)	Hold duration requirement is maintained at maximum achieved in phase 2 Lever must be maintained within rewarded range for entire hold duration	75% Correct in a block of 40 trials increases hold duration by 0.1 s	8

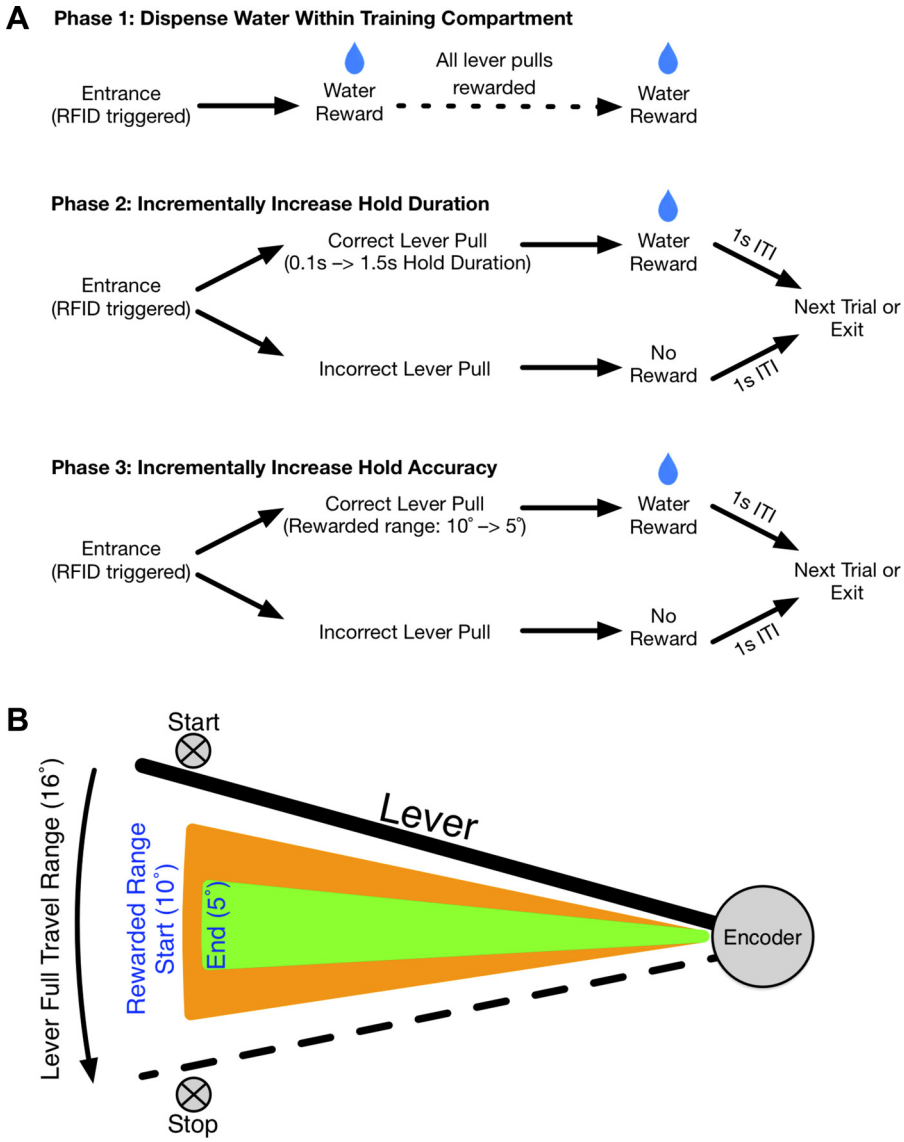

Fig. 2. Training structure for lever positioning task. *A*: the first phase of training encouraged mice to dispense water by interacting with the lever, which was positioned above the floor of the training compartment at the start, and then gradually lowered to be only accessible with the forepaw. *Phase 2* of training incrementally increased the duration for which the lever had to be positioned within the rewarded range. For *phase 3*, the rewarded position was incrementally narrowed from 10 to 5°. *B*: the rewarded range was centered within the full travel range of the lever. At the start of a trial, the lever was 8° anterior of the center position; therefore, mice had to pull the lever toward themselves to correctly position the lever. To complete successful trials, mice had to inhibit their tendency to pull the lever against the physical stop, as this was outside the rewarded range.

requiring more precise arm movements to execute correct trials. Given that we did not provide an aversive stimulus for incorrect trials, there was no incentive for mice to continually increase their success rate; therefore, our main measure of motor learning was the number of successful trials completed in 24 h and the rate at which animals progressed through the task. In order for the task to progress in hold duration or hold accuracy, a 75% success criterion had to be met for a block of 50 trials; however, there was no penalty for subsequent performance <75% (as long as it exceeded 10% success). A trial was defined by any event where the lever was moved at least 1.2°, and mice had to respect a 2-s intertrial interval before having the chance to initiate a subsequent successful trial.

RESULTS

Progressing task difficulty based on individualized performance metrics. The forelimb motor task trained in the current experiment required mice to accurately position a lever within a narrow range for up to 1.5 s to dispense a water reward. The behavior was shaped by first rewarding short (0.1 s) lever pulls and then incrementally lengthening the required hold duration based on individualized performance (Fig. 3*A*). This approach yielded individual learning curves, as well as measures of the number of entries into the training compartment.

When first exposed to the task, mice typically required 4–7 days to reach their maximal rate of successful trial completion (within 24-h blocks); however, their success rate fluctuates around 36 ± 0.9% for the duration of testing (Fig. 4*B*; mean success rate ± SE). In terms of progression through the training phases, all mice (n = 24) learned to dispense water by interacting with the lever (Fig. 3*B*); however, our video recordings showed that a subset of the mice (n = 4) exclusively used their snout to pull the lever throughout *phase 1* training and ceased to dispense water when the lever was lowered beyond the reach of the snout. These mice were not included in subsequent analyses, but were maintained in the cage by providing them with water for just entering the training compartment. Of the remaining 20 mice, 19 progressed to hold the lever for 1.5 s to dispense a reward, and 13 mice reached the final stage of holding the lever within a 5° rewarded range. The general posture of the mice, as well as the movements executed during the task, were highly stereotyped across animals and typically consisted of orienting toward the lever with

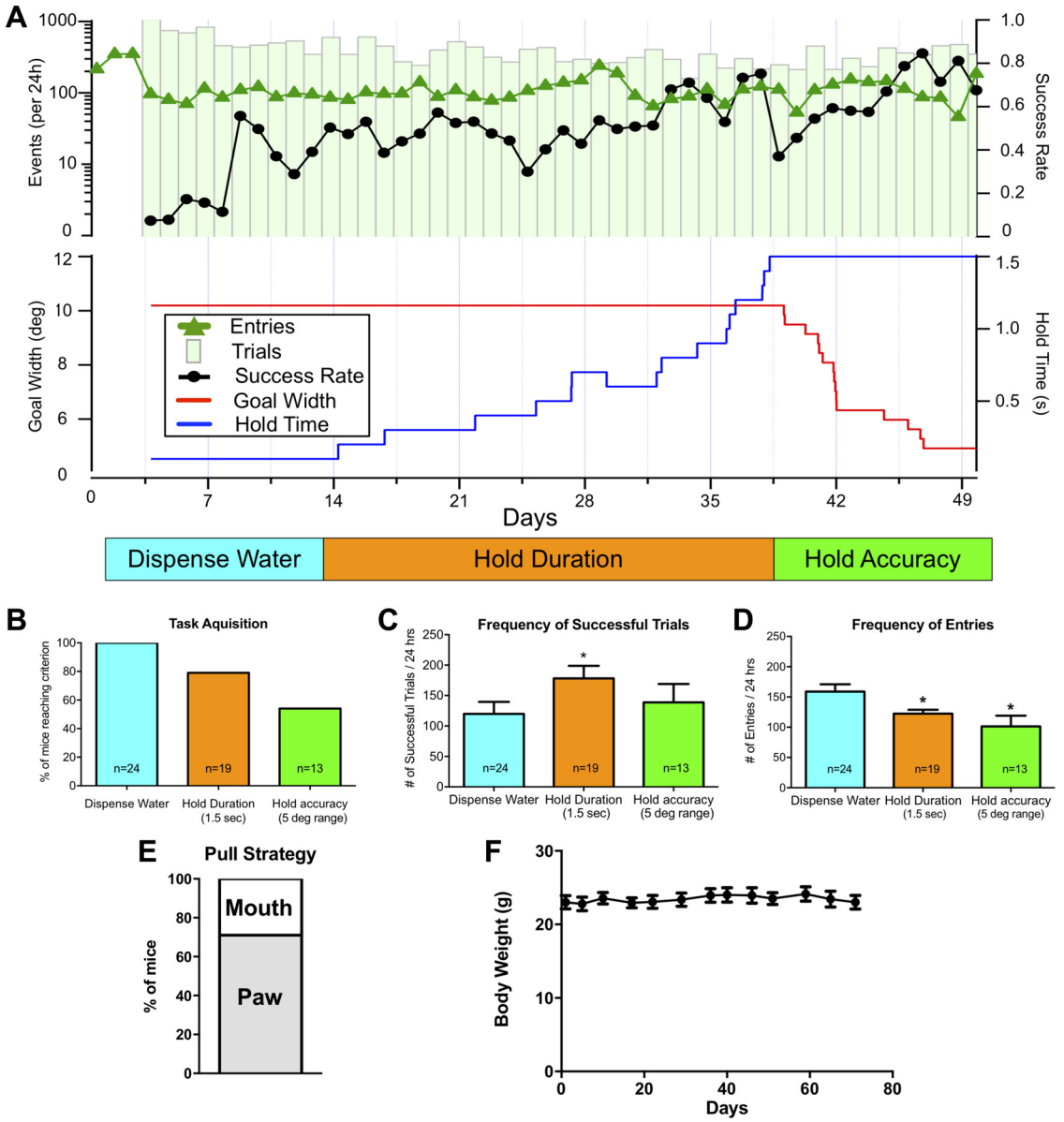

Fig. 3. Tracking task performance during autonomous training. *A*: RFID-based tracking allowed for the number of entries into the training compartment (dark green line), number of attempted trials (green bars; note log scale for *left* ordinal axis), as well as the success rate (black line; *right* linear axis) to be tracked for each mouse. In addition, the task parameters for goal width (*left* axis), as well as required hold duration (*right* axis) could be tracked and modulated for each animal (*bottom*). Graphs show data from an animal performing at a high success rate relative to the cohort. *B*: group quantification showed that all 24 mice learned to dispense water during the first phase of training, whereas only 19 and 13 mice completed the subsequent "hold duration" and "hold accuracy" phases of training, respectively. *C*: the frequency of successful trials was significantly higher in the second phase of training relative to the first (indicated by *), but there was no difference between second and third phases. *D*: however, mice required significantly fewer entries into the training compartment during the second and third phases (relative to first phase) to maintain their level of performance. *E*: a subset of mice (4/24) exclusively used their snout to dispense water and were thus excluded from subsequent analysis. To maintain the social structure within the cage, these animals continued to receive water for just entering the cage. *F*: given that mice had continuous access to water during the task, body weight did not change over the long term (means ± SE).

snout, grasping and pulling the lever with the right forelimb, followed by orientation toward the water spout and liking (Supplemental Video 1; Supplemental Video S1 is available in the data supplement online at the *Journal of Neurophysiology* website).

As a group, there was a significant increase in the mean number of rewarded trials between the first and second training phase (paired *t*-test, $P = 0.008$, Fig. 3*C*; *phase 1*: 119, *phase 2*: 192), but not the third phase (*phase 3*: 139, $P = 0.09$). However, by the third phase, the mice required significantly fewer entries into the training compartment to complete the same number of successful trials (*phase 1*, 159 vs. *phase 3*, 101 entries, $P = 0.0085$, Fig. 3*D*). Given that the mice always had access to water and food, we did not observe a significant drop in body weight during any phases of training ($P = 0.664$).

Lever pulling as an automated tool for assessing forelimb motor learning. Motor learning was assessed using both end-point measures, such as the rate of progression through the task, as well as qualitative measures of movement accuracy, based on lever position dynamics during individual trials. When first presented with the lever, mice showed a significant increase in the number of successfully completed trials [repea-

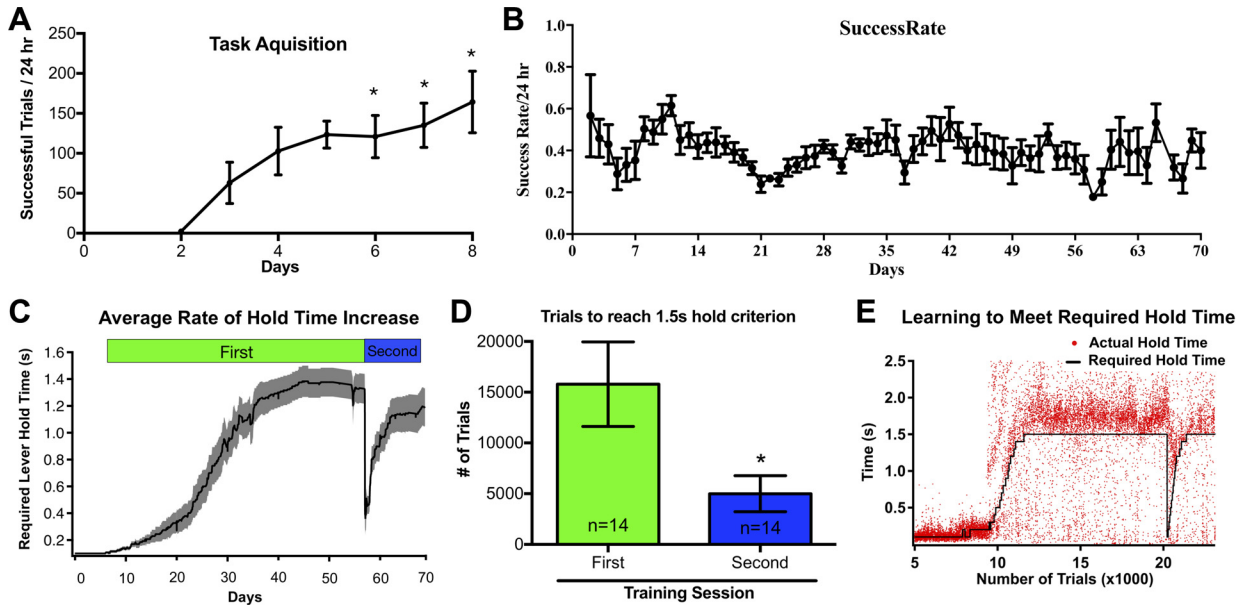

Fig. 4. Measures of task acquisition and motor learning. *A*: mice learned to dispense water by interacting with the lever within ~3 days, and the number of successful trials reached over 150 trials/24 h within the first 8 days of *phase 1* training. *Significant difference from *day 3* performance. *B*: the success rate varied around 36% throughout the experiment. Our primary measure of motor learning was the rate of progression in the task, based on the number of successful trials performed within blocks of 50 trials (see METHODS). *C*: once mice reached a maximal hold duration during the first training session (note that the group average was 1.38 s), the hold requirement was lowered to 0.1 s again to reassess the number of trials required to reach criterion. *D*: mice required significantly fewer trials to reach the 1.5-s hold criterion during the second training session, as they continued to hold the lever for longer than was required by the task. *E*: this facilitated the progression of the task at a faster rate (all trials shown for representative mouse). *Significant difference from first training session. All values are means ± SE.

ted-measures ANOVA ($P = 0.002$), *day 3* vs. *days 6* ($P = 0.038$), *day 7* ($P = 0.042$), *day 8* ($P = 0.023$)], reaching ~150 successful trials/24 h by *day 8* (Fig. 4*A*). We used *day 3* for comparison, as this was the first day when all mice began performing successful trials. The aim of this initial phase of training was to develop an association between pulling the lever and receiving a water reward; therefore, we did not prevent mice from using the snout or mouth. Following this initial acquisition of the task, the lever was lowered beyond the reach of the snout, and we incrementally increased trial difficulty by requiring longer hold durations for dispensing a water reward. On average, it took ~4 wk of training to reach a maximal hold duration of 1.5 s. Given that five mice did not reach the 1.5-s criterion, the group average was 1.38 s at the end of training. Once this target criterion was reached, we then set the hold requirement back to 0.1 s (for all mice within each cage) to reassess the number of trials required to reach the criterion (Fig. 4*B*). Mice required significantly fewer trials to reach the 1.5-s criterion during the second training session (first training session: 15,795 vs. second training session: 4,999 trials, paired *t*-test, $P = 0.0413$; Fig. 4*C*), as they continued to hold the lever for longer than was required by the task at the start of the second training session (Fig. 4*D*).

By continuously tracking the position of the lever during each trial, we were also able to visualize and assess the precision of forelimb movements. Initial phases of training were characterized by the mice maintaining the lever in the rewarded range (10°) for the required hold duration and then releasing it once the water reward was dispensed (Fig. 5). Typically, mice took full advantage of the entire rewarded range (Fig. 5, *A*–*C*), as the lever fluctuated within this range during individual trials. During the third phase of training, we asked whether mice could refine their movement accuracy by incrementally decreasing the rewarded range from 10 to 5°. To quantify movement accuracy, we compared the average distance of the lever from the center of the rewarded position just before the start of *phase 3* training (when rewarded range = 10°) vs. the end of *phase 3* (when rewarded range = 5°). The average error value across 100 consecutive trials (including both rewarded and unrewarded trials) showed a significant decrease by the end of *phase 3* training (Fig. 5*C*) (start = 2.36° vs. end = 1.51° error from target, $P = 0.0019$), indicating that mice had adapted their lever-pull strategy to meet increasing task requirements.

Effects of circadian cycle on task participation and performance. Our home-cage testing apparatus also offers the ability to noninvasively track the activity of individual mice 24 h/day. A histogram of intertrial interval shows that the majority of trials are performed at ~5-s intervals (Fig. 6*A*), as mice often perform multiple trials after entering the training compartment. The timestamp of each entry may also be used to reveal patterns in task participation that may be influenced by the social hierarchy of group-housed mice. For each cage of mice, we examined correlated activity among all cage-mate pairs by plotting the number of entries that occurred up to 20 min within each other (Fig. 6*D*). We see that some animals preferentially enter and exit the compartment after a specific cage mate. For example, mouse EO3 makes the most frequent entries into the training compartment within ~5 min of EO1 entering the compartment. Conversely, EO1 makes the highest number of entries just before EO3 enters, indicating that this particular pair often perform trials together. Other mice within the same cage (e.g., EO5) do not exhibit such behavioral correlations with any of their cage mates. (Fig. 6*E*). This pattern of behavior may be influenced by the social hierarchy within the cage; however, we do not see any evidence for the domination

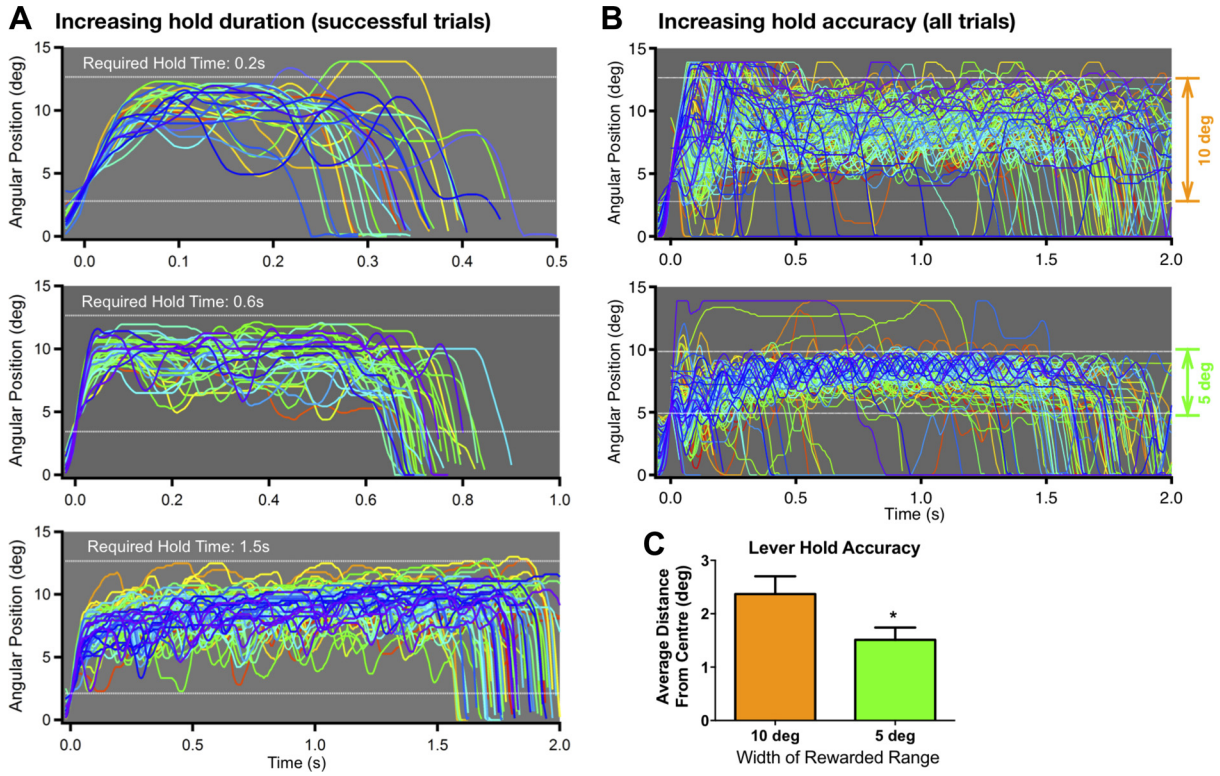

Fig. 5. Lever position dynamics during motor learning. *A*: mice learned to accurately position the lever within the rewarded position for the required hold time, at which point they release the lever to return to the start position. *B*: incrementally narrowing the rewarded position resulted in mice learning to hold the lever more accurately within the rewarded range. *A* and *B* represent individual, consecutive trials from a single mouse. The color of the lines indicates chronological order, with dark blue and purple being the most recent. *C*: comparing a block of 100 trials from each condition, the average distance of the lever from the center of the rewarded position was significantly lower during training with the 5° range (indicated by *), suggesting that mice learned to position the lever more accurately. Values are means ± SE; $n = 10$ mice.

of activity within the training compartment by a single mouse. Instead, we see evidence for certain cage pairs (such as EO1 and EO3) engaging in reciprocal trial participation. Another obvious pattern in activity within our task is the overwhelming preference to perform trials during the dark phase of the circadian cycle (Fig. 6*B*). Once the animals learn to dispense water, 79% of all trials were performed during the dark cycle (Fig. 6*C*; dark = 442 vs. light = 115 trials, paired *t*-test $P < 0.0001$); however, success rate did not differ significantly between circadian phases (dark = 43% vs. light phase = 44%, $P = 0.62$, data not shown).

DISCUSSION

We developed a novel home cage training system that allows high-throughput, autonomous training of mice on a lever positioning task to assess forelimb function. This low-cost apparatus (~$290/cage) can be easily adapted to most types of rodent caging systems and has a low footprint, making it ideal for use within animal-housing facilities, with no disturbance to other animals. Data logging and control of the electronics is performed by a Raspberry Pi microcomputer, which can be accessed remotely through an internet connection.

We chose to train mice on a relatively simple but precise lever positioning task, as it offers several advantages over other motor tasks that are especially important for autonomous training. First, we could ensure that, by the end of the training protocol, all mice were exclusively using the right forelimb to pull the lever. When given free space to execute simple movements, such as pressing a lever (Poddar et al. 2013), rodents tend to employ unique movement sequences that are highly stereotyped for each individual, but vary among animals (Kawai et al. 2015). By limiting the way in which mice can engage with the lever in our apparatus, we constrained the movement sequences that mice could employ, thus making the movement stereotyped across animals (see Supplemental Video S1). We feel this feature makes the task amenable for assessing behavioral compensation and recovery after central nervous system injury or other interventions. Although we did not quantify muscle activity during movement, based on video recordings of grasping and pulling movements, it is likely that a number of extensor and flexor muscle groups are active, including the biceps brachii, brachialis, extensor/flexor carpi radialis, and flexor digitorum. The second advantage of our task is that task difficulty can be systematically varied within experiments. The simplest version of the task requires mice to only minimally displace the lever from the resting position, whereas more stringent paradigms require them to hold the lever in a rewarded position for a set duration of time. Additional paradigms where the rewarded position is indicated by sensory feedback (e.g., visual or tactile cues) could also be easily implemented in future versions of the task. A third major reason for employing lever positioning is that mice continue to perform hundreds of trials each day for at least 2 mo. Reaching for food tasks usually induce satiation after a few dozen trials when training is done in discrete sessions (Erickson et al. 2007); however, by limiting the amount of water dispensed

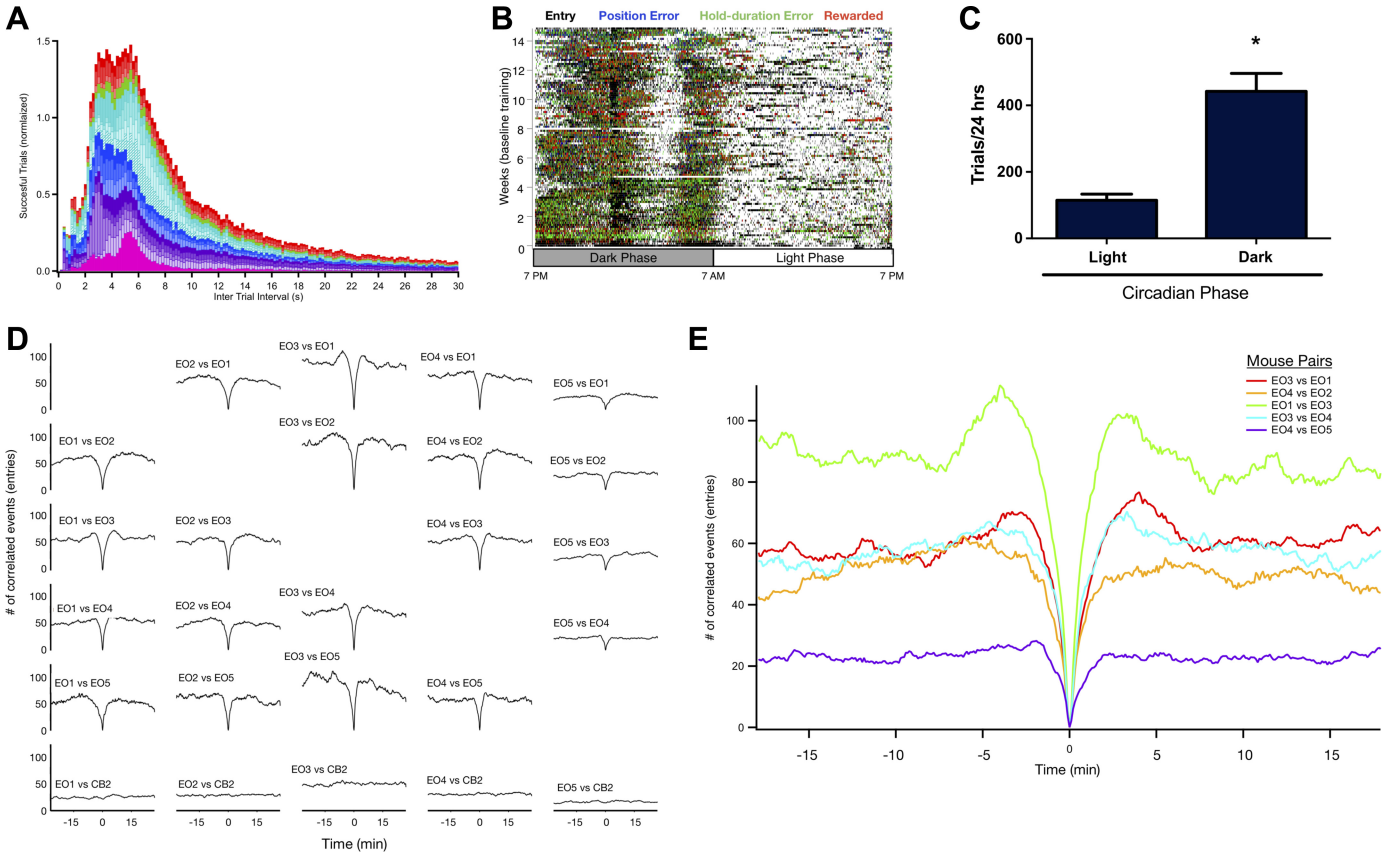

Fig. 6. Tracking circadian activity and the influence of social interactions within group-housed mice. *A*: a stacked histogram of intertrial interval for successful trials among all animals ($n = 19$) shows that the majority of trials are performed between 2 and 6 s apart, indicating that multiple trials are performed during individual entries into the training compartment (colors represent individual mice). *B*: a raster plot of all recorded events for a cage of 5 mice reveals the circadian pattern of activity, with the majority of trials performed during the dark cycle. Each tick represents an event indicated by the colored legend above the graph. *C*: as a group, mice performed the majority of trials during the dark cycle. *Significant difference from light cycle. Values are means ± SE *D*: to examine group dynamics in task participation, we plotted the number of correlated entries into the training compartment between pairs of cage mates ($n = 5$ mice; named EO1–EO5). These plots show that some mice preferentially enter the compartment after a specific cage mate has exited, whereas other mice do not show correlated activity with any of their cage mates (e.g., EO5). A control comparison of pairs of mice housed in different cages, within the same room, showed no correlated activity (*bottom* row). *E*: the strongest correlation for each animal with any of their cage mates is plotted as individual colored lines. Certain pairs of mice showed correlated activity (EO1, EO3), whereas others showed no correlation with any cage mates (EO5).

during each trial, we were able to continuously engage mice for several months.

Our novel home cage motor training paradigm may be applied in a number of ways to complement currently used behavioral tests. For example, it can be used as a high-throughput screening tool for identifying motor deficits in groups of mice with genetic mutations (Azim et al. 2014), neurodegenerative conditions (Fleming et al. 2012), acute injuries, or pharmaceutical treatments (Trueman et al. 2017). Our home cage task makes it possible to include animals from multiple conditions or genotypes (e.g., control vs. experimental) within a single cage, thus avoiding cage or litter effects when assessing therapeutics or other interventions. By individually tracking mice with RFID tags, we can also train a subset of the animals on the skilled lever positioning task, while control mice can continue to receive water rewards for just entering the training compartment. This paradigm may also be used to evaluate the efficacy of rehabilitative therapies after injury, such as stroke, and could even be used to generate much needed dose-response curves for such interventions by limiting the number of trials in which each mouse may participate after an injury. By maintaining mice socially housed within their home cage during testing, our task eliminates the confounding effects, such as stress induced by handling or social isolation, as well as the significant drop in body temperature that can occur from individual housing (Redfern et al. 2014).

Although our task is the first to our knowledge to perform completely autonomous motor training of group-housed rodents in the home cage, several complementary methods have recently emerged that include components of our paradigm. For example, automated training of individual rats on the skilled reaching paradigm has been demonstrated using a specialized training cage into which rats can be placed for part of the day (Ellens et al. 2016; Wong et al. 2015) or that a rat can access 24 h/day from within its home cage (Fenrich et al. 2015). In addition to this training apparatus designed for individual rodents, group-housed rats can also be trained on a cognitive operant task by interacting with a touch-screen within a training chamber (Rivalan et al. 2017). Automated tracking of the general activity of group-housed mice has also been carried out either through video-based tracking (Shemesh et al. 2013) or through RFID implants (Bains et al. 2016; Murphy et al. 2016); however, these paradigms can only be

used to assess general home cage activity and higher-order social interactions.

Our skilled lever positioning paradigm may also be adapted for studies where brain activity is monitored either optically (Murphy et al. 2016; Scott et al. 2013) or with electrodes (Hira et al. 2013a) during behavior. For example, the lever may be incorporated into the home-cage functional imaging apparatus that our laboratory has previously developed (Murphy et al. 2016), thus making it possible to monitor cortical dynamics evoked by lever pulling in an automated fashion. Alternatively, our lever positioning paradigm may also be implemented in mice head-fixed manually outside of the home cage, as has been done previously for a similar paradigm (Hira et al. 2013b). Such experiments could address important questions about differences in both brain activity and behavior when mice are head-fixed by an experimenter vs. voluntary task participation, which our home cage system now makes possible.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.S., F.B., J.L., S.H.S., and T.H.M. conceived and designed research; G.S. and F.B. performed experiments; G.S., J.B., and F.B. analyzed data; G.S., J.B., J.L., S.H.S., and T.H.M. interpreted results of experiments; G.S. and J.B. prepared figures; G.S. drafted manuscript; G.S., J.B., J.L., S.H.S., and T.H.M. edited and revised manuscript; G.S., J.B., F.B., J.L., S.H.S., and T.H.M. approved final version of manuscript.

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