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Evaluating Skilled Prehension in Mice Using an Auto-Trainer

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JOVE manuscript submission
Evaluating Skilled Prehension in Mice Using an Auto-Trainer

Dear Dr. Myer, JOVE editors, and esteemed Reviewers

As discussed, we are excited to share our concept of the mouse auto-trainer. To our knowledge, this is the first auto-training device for mice purposefully designed to train animals in their home cage and thus reduce excitability/anxiety. Importantly, we show that our auto-trainer trains mice to a high level of prehension success, which equals that seen with time-consuming manual training.

In this revision, we have addressed the Editorial and Reviewer issues. We believe that the manuscript is significantly improved.

Sincerely, and on behalf of my co-authors,

Steven R. Zeiler, M.D., Ph.D.
Assistant Professor, Johns Hopkins
Head of Stroke Research Johns Hopkins Bayview Medical Center
Director Vascular Neurology Fellowship Johns Hopkins

TITLE:

Evaluating Skilled Prehension in Mice Using an Auto-Trainer

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KEYWORDS:

auto-trainer, motor training, mouse, behavior, prehension, reach-to-grasp, stroke

SUMMARY:

Method to assess the impact of training on motor skills is a useful tool. Unfortunately, most behavioral assessments can be labor intensive and/or expensive.We describe here a robotic method of assessing prehension (reach-to-grasp) skill in mice.

ABSTRACT:

We describe a method to introduce naïve mice to a novel prehension (reach-to-grasp) task. Mice are housed singly in cages with a frontal slot that permits the mouse to reach out of its cage and retrieve food pellets. Minimal food restriction is employed to encourage the mice to perform the food retrieval from the slot. As the mice begin to associate coming to the slot for food, the pellets are manually pulled away to stimulate extension and pronation of their paw to grasp and retrieve the pellet through the frontal slot. When the mice begin to reach for the pellets as they arrive at the slot, the behavioral assay can be performed by measuring the rate at which they successfully grasp and retrieve the desired pellet. They are then introduced to an auto-trainer that automates both the process of providing food pellets for the mouse to grasp, and the recording of successful and failed reaching and grasping attempts. This allows for the collection of reaching data for multiple mice with minimal effort, to be used in experimental analysis as appropriate.

INTRODUCTION:

Methods to experimentally test a motor skill pre- and post- neurological injury as well as modulate the timing, amount, and type of motor training are important to translational research.

Over the last decade, mice, because of the attendant ease of genetic manipulation, have become a popular model system in which to elucidate the mechanisms of motor learning pre- and post-injury. However, behavioral assays in mice have not been optimized in the same way that such assays have been for other mammals (especially rats). Further, there are important differences between the behavior of a mouse and a rat that strongly suggest training the two species in different manners[1,2].

Skilled prehensile movements use a hand/paw to place food in the mouth, to manipulate an object, or to use a tool. Indeed, reaching to grasp various objects in daily life is a fundamental function of upper limbs and the reach-to-eat act is a form of prehension that many mammals use. Many of the genetic, physiological, and anatomic changes underpinning prehensile skill acquisition have been well defined in the field[3]. In translating preclinical findings to clinical outcomes, one needs a relevant test that is efficient and reproducible. Studies of rodent and human reaching demonstrate that prehension behavior is similar in humans and in animals[4]. Accordingly, these similarities suggest that prehension testing can serve as a translational model for investigating motor learning as well as impairments and treatments of human disease. Therefore, evaluating prehension in mice can offer a powerful tool in translational research studying both health and disease states[4].

Unfortunately, the prehension task in mice, even for a small-scale laboratory setting, can be laborious and time consuming. To alleviate this problem, we describe here an automated version of the prehension task. The described task requires mice to extend a single paw through the mouse's home cage frontal slot, pronate the extended paw, grasp the food pellet reward, and pull the pellet back to the cage interior for consumption. The resulting data is presented as either a prehension success or failure. This automation successfully records the data and reduces the burden and time with which researchers must engage the task.

PROTOCOL:

All methods described here have been approved by the ACUC (Animal Care and Use Committee) of the Johns Hopkins University.

1. Preparing mouse cages for use

1.1. Create a slotted opening with dimensions of 0.8 cm width and 7 cm height from the base at the front end of each cage, as illustrated in **Figure 1**. This slot serves as the opening through which the animal will reach.

NOTE: The auto-trainer was designed for the use with the standard mouse cage dimensions (as shown in **Figure 1**) supplied by most animal research supply vendors. Further, the auto-trainer will easily support other cage-types.

1.2. Inside each individual cage, add a platform adjacent to the slot to allow the mice to stand and reach the presented pellets. Ensure that the platform is located above the cage litter floor, approximately 3 cm in height. Use Petri dishes affixed with superglue and capped by a metal sheet approximately 10 cm x 15 cm, however, any flat surface large enough for a mouse to stand on to reach from will suffice.

1.3. Create a vertical notch through the middle of the front of the cage measuring 0.8 cm across and 7 cm high that will allow a mouse to reach his paw out of the cage.

1.4. From a thin sheet of metal, (approximately 2 mm thick) cut a cage gate into rectangles measuring 5 cm x 10 cm to serve as a uniform opening through which the animal is to reach.

NOTE: Mice may chew on plastic cages which would change the size of the opening. The mouse will reach through this 0.8 cm slot when the metallic cage gate is placed over the cage's slotted opening during testing using tape, maintaining the effective width of the slot between cages.

1.5. Cover each cage's slot with tape when its mouse is not being tested to prevent litter from being expelled from the cage.

2. Introducing mice to the reaching motion

2.1. Record each mouse's starting weight and calculate 85% of that value to find their goal weight, rounding up to 20 g if the result is less. Give them a feeding regime to bring them to and then maintain this goal weight.

2.1.1. Give each mouse 2.5 g of pellets the first day and note any change in their weight 24 h later.

NOTE: Weigh the mice once per day and expect a weight drop of 0.25-1 g per day.

2.1.2. Change each mouse's daily feed as required, based on this initial change and ongoing changes in each mouse's weight, in order to induce gradual weight loss (less than 0.8 g lost per day) and then maintain the resulting goal weight. Vary between three to six 500 mg pellets (1.5 to 3.0 g) per day to be effective.

NOTE: Mice remain on this diet to maintain their goal weight throughout the protocol.

2.2. When a mouse has reached its goal weight, introduce each mouse to the concept of coming up to the gated slot for a supplementary food pellet. Start a training session by placing a 45 mg pellet on the pellet surface, directly in front of the slot, and allow each mouse to retrieve it. Most mice will take to this feeding arrangement within 1-2 days.

2.3. Once the mouse associates an open slot with being fed, encourage them to reach with a paw, rather than the mouth.

NOTE: This is the most complex step, taking 1-2 days, and instilling counterproductive behavior in mice by mistake is very easy; please refer to the discussion section for further information and advice.

2.3.1. Using a pair of tweezers, hold a pellet in the same position the mouse has retrieved pellets previously. As the mouse begins to bite for the pellet, pull it away approximately half a centimeter such that the pellet is out of reach of its mouth.

NOTE: A mouse at its goal weight will attempt to retrieve the out-of-reach pellet. Whenever the mouse extends a paw through the slot, reinforce that behavior by allowing it to eat the pellet. Some mice may exhibit a preference for one paw over the other when extending for food.

2.3.2. While not instrumental to experimentation, record whether the left or right paw is preferred. This may potentially allow for higher overall success rates in the behavioral assay; alternatively, eliminate a variable by forcing each mouse to reach with the same paw.

NOTE: Better results are obtained if mice use their preferred paw.

2.3.3. As each mouse associates extending a paw with eating a pellet, further reinforce that behavior by withholding the pellet in response to attempts to retrieve the pellet with the mouth and tongue. Mice will start to comply with this arrangement over 2 to 3 days.

2.3.4. Finalize the introduction of the desired paw reaching behavior by placing the 45 mg pellet just under 1 cm from the outer edge of the cage gate, such that the leftmost or rightmost point of the pellet (whether it is to the right or left of the cage slot from the investigator’s perspective, respectively) is tangent to a line extending straight out from the edge of the cage gate’s slot. Allow the mouse to attempt to retrieve the pellet, being vigilant to remove the pellet and prevent its consumption if the mouse should attempt by some other method than paw extension.

NOTE: When a mouse consistently extends a paw to grab at and is able to touch the provided pellet, it is ready for testing using the auto-trainer described below and associated behavioral assay. The time from naïve introductions to being prepared will vary between mice; if there are stragglers that take more than two weeks to understand, they should be excluded from the data set.

3. Using the auto-trainer

NOTE: Please see **Figure 1-3** and the discussion section for a full description of the hardware, software, and the physical actions of the auto-trainer.

3.1. Prepare for the training session.

3.1.1. Calibrate the bait pellet sensor. Click the **Run** arrow in the LabVIEW interface and note the bait pellet sensor reading both with and without a pellet in place. Click the **Stop** button to stop this test run and change the bait pellet sensor target to a value between those two readings (**Figure 3** and **Table 2**). Most lighting conditions provide a reading between 1 and 4.

3.1.2. Place the modified mouse cage on the auto-trainer (**Figure 2**). Affix the cage gate (**Figure 1**) and align the pellet to the edge of the slot as in the manual procedure.

3.2. Run the mouse's training session using the LabVIEW interface.

3.2.1. Input information as required to record data about the training session (**Figure 3** and **Table 2**).

3.2.1.1. Click the **Mouse ID** field and type the filename of each training session using the computer's keyboard.

3.2.1.2. Click the **Total Pellets to Dispense During Routine** field to control how many pellets are dispensed for a single experiment (usually 20 – 30). To do so, click the up and down arrows or input the number using the computer's keyboard.

3.2.1.3 Click the **Pause After Pellet Number** field to set a 5 s pause after the indicated pellet is removed from the diving board. To do so, click the up and down arrows or input the number using the computer's keyboard.

3.2.1.4 Click the **Pause Length** field to set a pause in between the time a pellet is removed from the diving board and the time a new pellet is dispensed. To do so, click the up and down arrows or input the number using the computer's keyboard

NOTE: Usually 1 s is an appropriate pause time. If the mice are anxious after each pellet is dispensed, it is advisable to increase the pause length using the **Pause Length** field to 5 s.

3.2.1.5. Manually record the distance at which the pellet is placed in the **Reach Distance** field. To do so, click the up and down arrows or input the number using the computer's keyboard

NOTE: The **Size of the Acceleration and Time Arrays** is exposed for debugging purposes and may be ignored.

3.2.1.6 Click the **Folder to Contain Logs** field to select the file location to save the collected data.

3.2.1.7. Once the information fields have been filled out, click the **Run** button to begin the training session. The auto-trainer will dispense individual pellets and track whether they fall through the funnel until the total number of pellets has been dispensed, and the last pellet has either been retrieved or dropped by the mouse. The program will stop automatically at this point. If necessary, it can also be stopped prematurely by clicking on the **Stop** button.

3.2.2. Once the software is set up, place the home-cage of the mouse to be tested on the pedestal and observe the mouse so that you might gauge whether the mouse has indeed learned to attempt the required novel reaching behavior. After clicking the **Run** button, allow the mouse to investigate the slot and its new, unfamiliar surroundings.

NOTE: Similar to when introducing mice to the concept of reaching, expect some mice to be more compliant than others. Mice that have grasped the concept should try to reach within 5-10 min and will associate the movement of the auto-trainer with the presented pellet, as when they associate an uncovered slot with food in the initial stages of this protocol.

REPRESENTATIVE RESULTS:

In general, it is recommended that each training session consist of about 20-30 trials, which may be set by the user, run automatically by the auto-trainer and saved into a single log file per session and mouse. Each trial can be run consecutively, right after the other, with 2-5 s of pause. Mice trained on the auto-trainer exhibit an increase in skill over 10 training sessions.

To compare the utility of the auto-trainer to manual training (considered the gold-standard), we trained adult male C57bl/6 mice aged 100 to 140 days old manually and using the auto-trainer. All animal handling and use were performed according to and with approval from the Johns Hopkins University Animal Care and Use Committee. Mice trained with the auto-trainer learned the prehension task and exhibit a clear increase in motor skill (**Figure 4**). This increase in skill is similar to that seen when the animal is trained manually without the use of the auto-trainer (**Figure 4**). For these data, manual prehension was scored as successful when the mouse reached its forelimb through the slit, grabbed the pellet, and ate it without knocking it from its resting space, dropping it, or in any other way losing control. The percent of successful prehension attempts was determined per pellet. A training block consisted of 30 pellets at a distance of 1 cm with each pellet presented one at a time. Mice trained on the auto-trainer were trained per the protocol described above. Each point in **Figure 4** represents a day of training during which the animals reached for 30 pellets and graphed as percent correct. There was no statistical difference between the two lines using a non-parametric t-test with correction for multiple comparisons.

FIGURE AND TABLE LEGENDS

Figure 1: Exemplar pictures of the home cage. (**A**) Birds-eye view of a standard home cage modified with the platform (orange) and slot on the front of the cage. (**B**) Front-view of a home cage modified with a slotted opening with approximately 0.8 cm x 7 cm. (**C**) cage gate cut from a thin sheet of metal and wrapped with tape to protect edges. (**D, E**) cage gate placed in front of the slot to serve as a uniform opening through which the mouse is to reach; front (**D**) and oblique (**E**) views provided.

Figure 2: Exemplar pictures of Auto-Trainer. (**A, B**) Pictured are the auto-trainer without (**A**) or with (**B**) a modified mouse cage in place. (**C-J**) Detailed views of the diving board food pellet holder design viewed either from the front (**C,D,H,I**) or from the side (**E,F,G,J**), with (**D,E,F,I**) or

without (**C,G,H,J**) a food pellet. Note that pellet distance from the animal can easily be modified as cage distance from the diving board is modifiable.

Figure 3: Screenshot of the software. Screenshot of program used to run the auto-trainer. The image shows the important input fields described in the protocol. See **Table 2** for further description.

Figure 4: Representative data. Skilled prehension increases to a similar plateaued level using both auto-trainer and manual training paradigms. Plot shows reach-to-grasp success (mean +/- SEM; manual: gray, n = 14; auto-trainer: black, n=15).

Table 1: Timetable of mouse training using the auto-trainer.

Table 2: Software interface.

DISCUSSION:

Our auto-trainer evaluates forelimb reach-to-grasp (prehension) in an automated manner. To achieve this endpoint, many of the parameters designed for the mouse prehension task, including pellet placement, pellet size, and training criteria, have been iterated over several years and adapted from prior protocols[2,5,6]. The advancement here is the automation of the task using a robot that allows home-cage housing. Home-cage housing allows the mice to remain calm and perform the task with less anxiety. Non-home-cage training is associated with increased stress which can lead to the increased time and decreased precision[7-9]. We demonstrate here precision resembling our own results with manual home-cage training[5,7,8]. Although home-cage training exists for the rat[10], to our knowledge, this is the first auto-trainer that takes advantage of training mice in their home-cage.

Our auto-trainer includes an adjustable platform on which a slotted cage rests and may be lowered or raised to the appropriate height for alignment with a food pellet holder (also referred to as a diving board). A pellet-dispensing system places the food pellet on the diving board holder. The food pellet holder has a bait pellet sensor assembly consisting of a reflective object sensor to detect whether a food pellet is present or not on the diving board holder. Due to the light sensitivity issues, the reflective object sensor may be calibrated at installation to suit the lighting environment of the laboratory. Each mouse's cage is placed on the auto-trainer such that the pellet's inner edge is in line with the outer edge of the cage gate's slot, corresponding to step 2.3.4 of the manual procedure detailed above. Two lost pellet sensors oriented in opposite directions in a funnel beneath the diving board pellet holder detect falling pellets. One benefit of employing two lost pellet sensors is that it ensures high detection accuracy for various food pellets of different sizes and shapes. Both lost pellet sensors consist of a standard transmissive photo-interrupter with a through-hole design, to sense the motion of a falling pellet without requiring contact.

The software consists of a program that runs the auto-trainer and collects data on successes and failures. User input consists of the file location in which the data is recorded, how many pellets are dispensed in one training session, an option to pause the training session after dispensing a particular pellet, a field to record the increased distance (if any) across which the mouse must reach, and a field to control the array size used in the program's calculations (which may be ignored during normal use). Further, the software enables the user to tune the diving board's reflective object sensor in order to recalibrate the light sensitivity as necessary. An output of each trial is displayed to the user as well as recorded and saved in a log file for later retrieval.

A single trial consists of a single food pellet's time spent on the diving board until it is removed by action from the mouse. If a pellet leaves the diving board as determined by the bait pellet sensor and the pellet is detected falling through the funnel shortly afterward by either of the lost pellet sensors, it is recorded as a failed trial by the software. If the bait pellet sensor determines that the pellet leaves the diving board, but no falling object is detected by either of the lost pellet sensors, it is assumed to have been pulled into the cage by the mouse and is counted as a successful trial.

This formulation is used because it is useful to design a behavioral assay in which the task to perform directly, rather than indirectly, provides the reward. In this way, there is no ambiguity on the animal's part on what the task is (e.g., be hungry, locate food, get food, eat food). Among the many tasks that utilize such a paradigm, the prehension task has become quite popular for such assessments. The task merely requires that an animal use a single limb to reach for and grasp a single food item, which the animal subsequently brings to its mouth for consumption. The prehension task assesses a behavior that is very similar to an everyday behavior used by many mammals. Most importantly, the prehension task resembles human motor behavior[4]. This generalizability enhances the expectation that principles derived from the preclinical assessment of the behavior are clinically applicable in disease states. For example, impairments in skilled forelimb and hand use are seen in stroke, Huntington's disease, Parkinson's disease, and multiple sclerosis. Thus, modeling behavioral deficits and subsequent recovery in mice is invaluable to understanding human recovery and how it might be encouraged[2,11-13].

Many aspects of the auto-trainer proposed herein greatly benefit the research process. First, most behavioral assays require an experimenter to closely train and monitor daily sessions, which can be costly, labor intensive and prohibitively time consuming. Our auto-trainer allows for behavioral data to be collected independently of an experimenter. Second, our auto-trainer can be replicated to allow multiple mice to be trained and evaluated objectively, efficiently and concurrently, thus minimizing time and effort. Third, the low-cost of the auto-trainer allows replication and use of multiple auto-trainers concurrently for large scale and efficient testing.

It should be noted that the critical point that requires careful supervision is during the shaping phase of the training. Notably, this protocol's chief weakness is the risk of bad use becoming fixed in some mice[11]. The protocol aims to mimic tests like the ladder rung test in that succeeding in the task provides the reward. However, the task itself still must be taught to the mice in step 2.3 of the protocol, unlike the ladder rung test. The concept most likely to cause a mouse to stumble in learning this task is from extending a paw out of the cage to using the paw to actually grasp the pellet. In the first session of step 2.3.1, mice should be rewarded simply for extending a paw out of their cage. However, over the following few days, investigators should take care to reward mice less for just extending the paw, and more for extending the paw and touching the pellet, as we describe in step 2.3.3.

Please note that approximately 5% of mice will fail to progress past this stage, typically because of limited extension of their digits to pull in the food pellet. Such mice will fail with one or both paws with little consideration of the actual location of the pellet, providing little or no useful data. To minimize a mouse's potential for failure at this stage, caution is strongly recommended when pulling away the pellet with the tweezers during the learning process. In particular, the mouse should be rewarded with food not only when it extends a paw, but also when that paw grips the pellet and applies enough force sufficient to investigator's satisfaction. A similar risk to potential failure at this stage is posed by mice using their tongue to lick pellets towards them. When training mice that tend to lick, place the pellet further laterally away from the slot. Mice will find it difficult to reach with the tongue over a greater sideways distance, but the range of motion of the arm and paw are more capable of closing the distance.

Our described protocol is readily extended to different lab environments or different methods of data collection. The auto-trainer, for example, is very useful as a labor-saving device, but is not strictly required for data collection, as pellets can be provided and successes/failures can be recorded by hand. Individual reaches can also be categorized based on more detailed information than simply success/failure, for example by considering the angle of approach of each mouse, the number of reaching attempts that do not touch the pellet, or the mechanics of the retrieval motion, which has received more attention in recent years[14]. The ability of the animal to retrieve a pellet is but one measure. Using additional hardware, we will also be able to measure the velocity, angle, and trajectory of the animal's limb movements. This kinematics is an important aspect of motor learning both before and after a neurological injury. To this end, we are currently incorporating various novel means of analyzing the locomotion and kinematics of the mouse's grasping action. We are exploring using high-speed cameras to obtain kinematic measurements of the grasp and attaching pressure transducers and accelerometers to the food pellet holder to measure force and mass data associated with the grasp. These new features will enhance the functionality of the auto-trainer to collect significant data passed a simple pass or fail trial and help illustrate the gait of the mouse's grasp through disease progression. In the future, we will be using the robot assisted prehension task as a platform to evaluate type, dose, and timing of rehabilitation after neurological injury. Moving forward, we will continue to improve the task, with refinements to help lessen incorrect behaviors and improve task acquisition rate and training time.

In summary, we have developed a new auto-trainer for assessing upper forelimb prehension skill in mice. The task requires mice to reach their paw through a slit, grasp a small food pellet, and pull the pellet in the direction of their body so that they may eat the pellet. The task setup is mechanically constrained to assure dominant paw usage. Mice can be trained quickly and simultaneously, with only the shaping process requiring manual input. The test can be administered efficiently and analyzed automatically. This high throughput behavioral assay quantifies success rate and is easily modified for future analysis of kinematics and force dynamics.

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The auto-training device was constructed by Jason Dunthorn, Uri Tasch, and Dan Tasch at Step Analysis, LLC, with design input support and instructions provided by Robert Hubbard, Richard O'Brien, and Steven Zeiler.

Teresa Duarte of the Champalimaud Centre for the Unknown provided valuable insight and ideas about describing and categorizing mouse reaching actions.

DISCLOSURES:

Dan Tasch and Uri Tasch of Step Analysis, LLC have manufactured auto-trainer device with payment from Richard J. O'Brien and Steven R. Zeiler.

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Figure 1

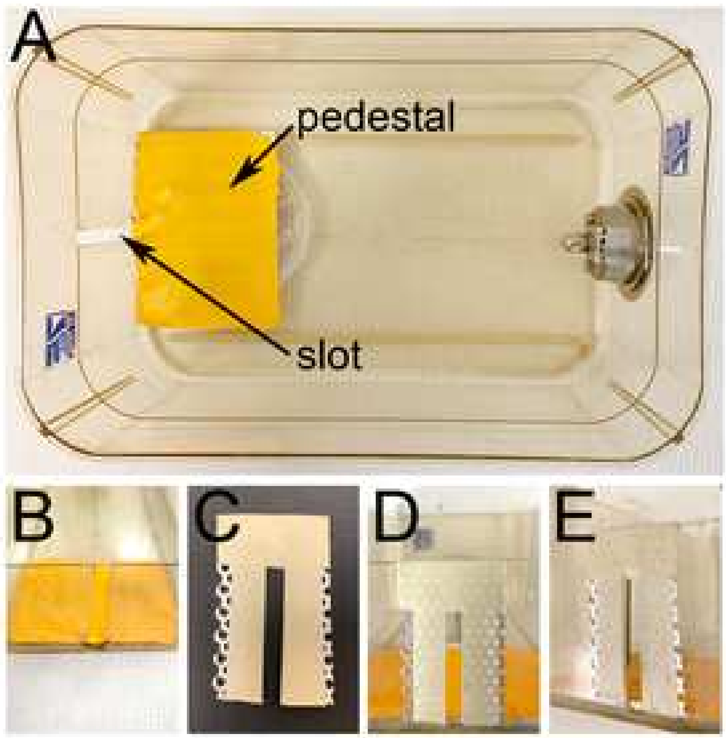

Figure 2

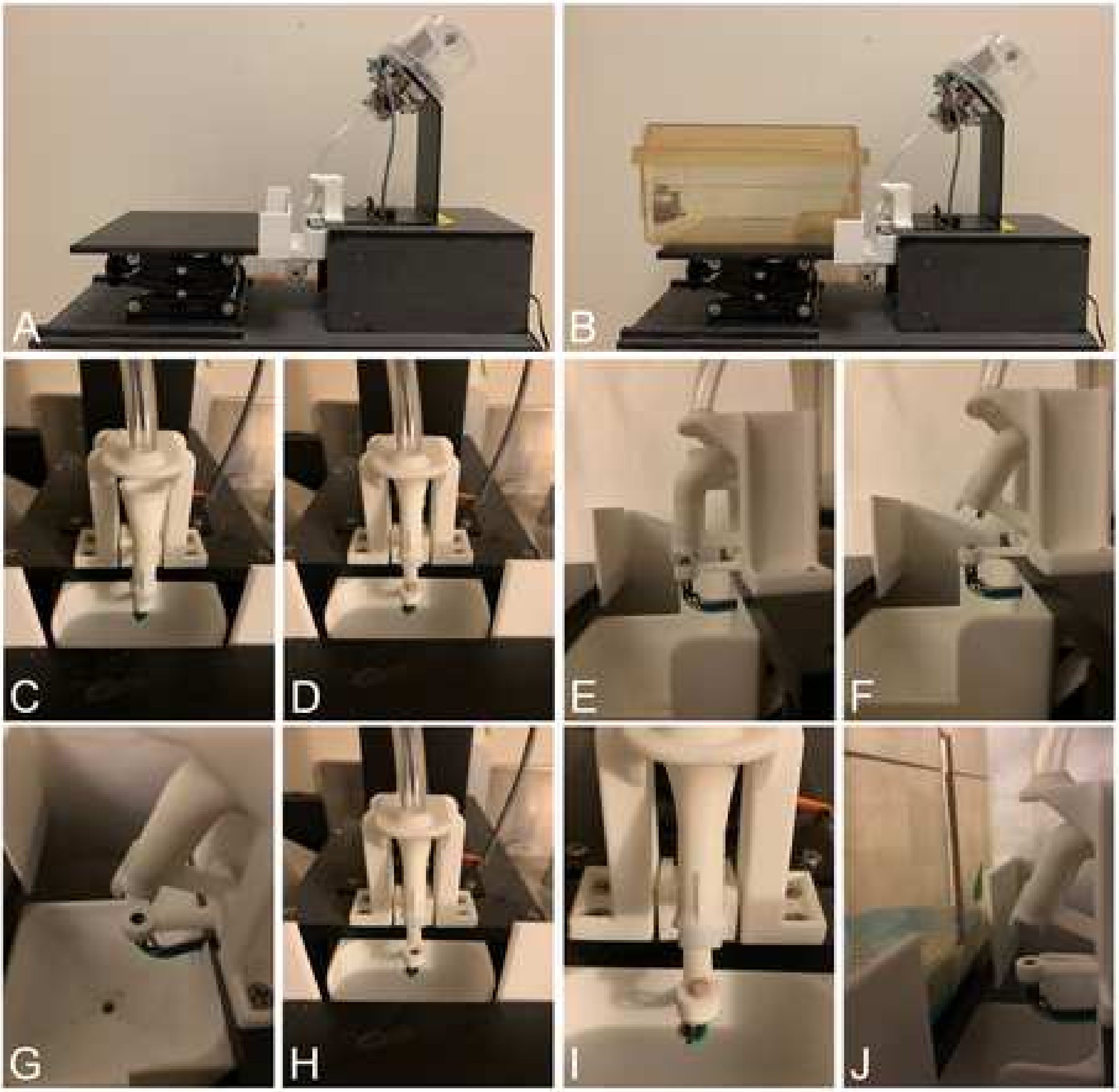

Figure 3 Click here to access/download;Figure;figure 3.psd

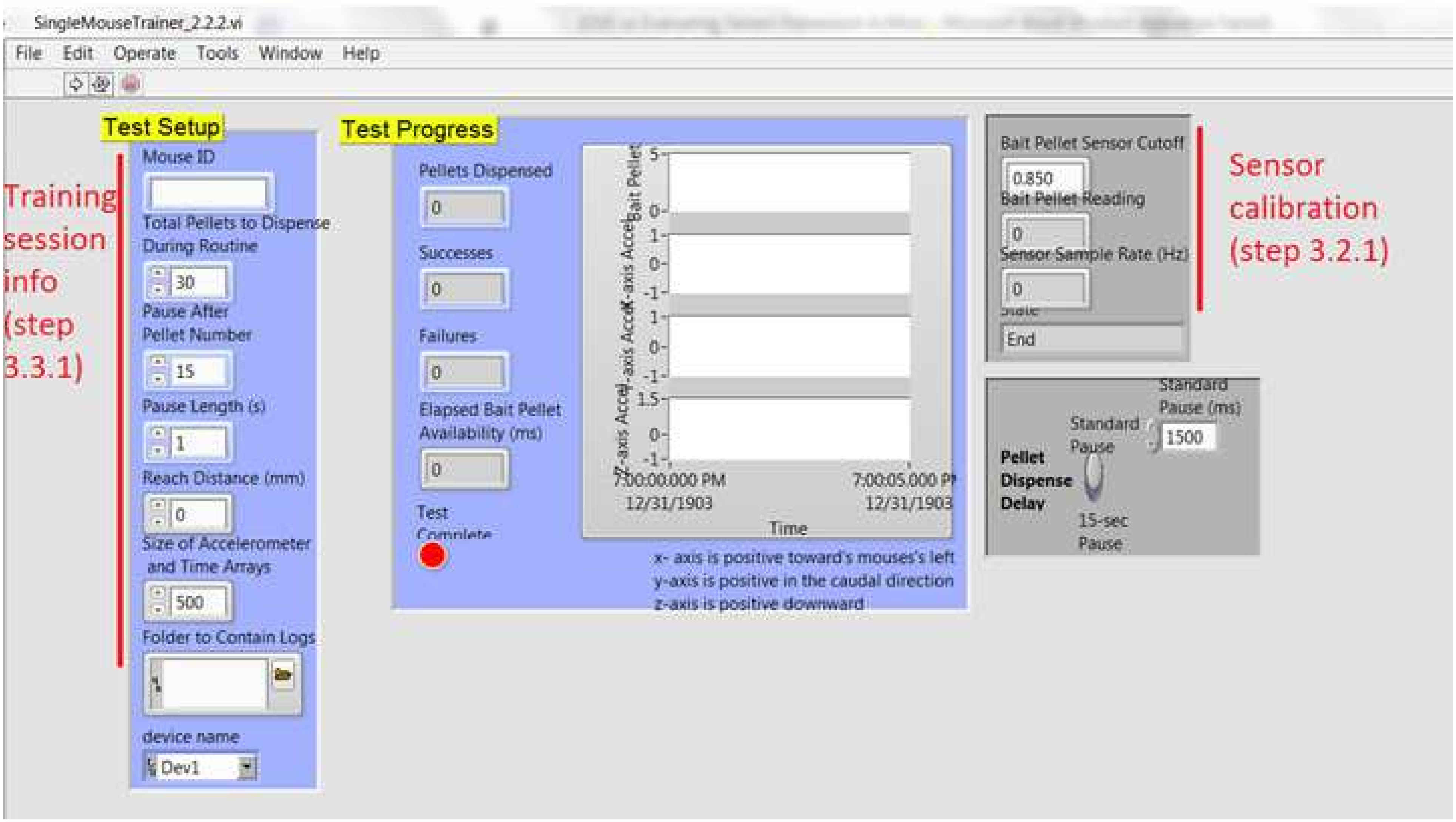

Figure 4

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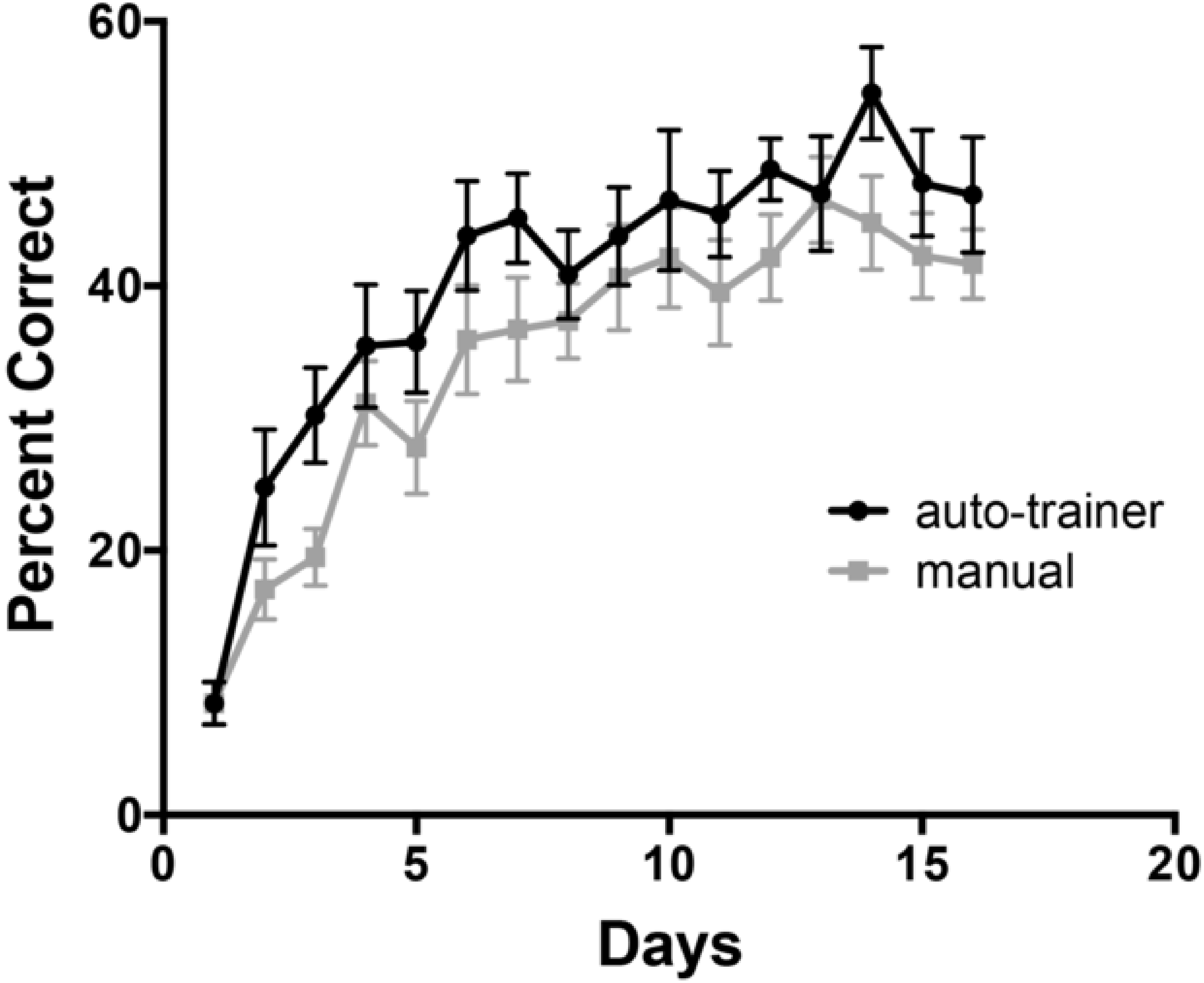

Step	Estimated Duration (in days)	Comment
2.1 Weight Loss	3 to 5	Depending on intial weight and, therefore, how much weight to lose until at goal
2.2 Slot training	1	Mice learn to feel confortable approaching the open slot for food
2.3 Shaping	4 to 8	
2.3.1 Paw use	1	Success here depends on quickly providing the pellet after the mouse, denied its food, paaws for the pellet.
2.3.2 Paw Preference	1	Ascertain if the mouse prefers left or right paw.
2.3.3 Curtailing bad paw use	2 to 3	As in previous step, it is critical to prevent retrieval with the mouth and tongue.
2.3.4 Tweezers	1	Some mice will stumble at taking the pellet by themselves rather than from the tweezers, feed them a little less
3. Auto training	10 to 15	Days until asumptote.

INPUT FIELD
Mouse ID
Total Pellets to Dispense During Routine
Pause After Pellet Number
Pause Length (s)
Reach distance (mm)
Size of Accelerometer and Time Arrays
Folder to Contain Logs
Device name
Arrow button, top left
Stop sign button, top left

USE
Input the filename under which the collected data will be saved.
Input the total number of pellets that will be dispensed during the training session.
Deprecated function. May be used to pause the training session after the specified pellet is dispensed.
Length of time the pause lasts.
Record the distance above minimum over which the mouse must reach to retrieve the pellet. Zero by default.
Function exposed for debugging purposes. Keep at default value of 500.
Click the folder icon to choose where collected data is saved.
LabVIEW function that connects hardware to software. Defaults to Dev1. Depending on USB connections, hardwar
Click to run the program, whether for a training session or for calibration.
Stop the program prematurely.

re may appear in the dropdown menu under another number; choose devices until one works.

Name of Material/Equipment	Company	Catalog Number
ABS Filament	Custom 3D Printed	N/A
ABS Sheet	McMaster-Carr	8586K581
Adruino Mini	Adruino	A000087
Bench-Top Adjustable-Height Positioning Stand	McMaster-Carr	9967T43
Clear Acrylic Round Tube	McMaster-Carr	8532K14
Low-Carbon Steel Wire	McMaster-Carr	8855K14
Pellet Dispenser	Lafayette Instrument: Neuroscience	80209-45
Photointerrupter Breakout Board	SparkFun	BOB-09322 ROHS
Reflective Object Sensor	Fairchild Semiconductor	QRD1113
Servo Motor	SparkFun	S8213
Transmissive Photointerrupter	Sharp	GP1A57HRJ00F

Comments/Description
utilized for pellet holder, frame, arm and funnel
3/8" thickness; used for platform compononents, positioning stand guides and base
nano version also compatiable as well as other similar microcontrollers
35 lbs. load capacity
ID 3/8"
0.148" diameter
with 45 mg interchangeable pellet size wheel and optional stand
designed for Sharp GP1A57HRJ00F
phototransistor output
generic metal gear (micro size)
gap: 10 mm, slit: 1.8 mm

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Title of Article:	Evaluating Skilled Prehension in Mice Using an Auto-Trainer
Author(s):	Robert Hubbard, Jason Dunthorn, Richard J. O'Brien, M.D., Ph.D., Dan Tasch, Uri Tasch, Ph.D., Steven R. Zeiler, M.D., Ph.D.

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Signature: [signature] Date: 1/21/19

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JOVE Manuscript
Title: "Evaluating Skilled Prehension in Mice Using an Auto-Trainer"
Manuscript #: JoVE59784
Authors: Robert Hubbard, Jason Dunthorn, Richard J. O'Brien, M.D., Ph.D., Dan Tasch, Uri Tasch, Ph.D., Steven R. Zeiler, M.D., Ph.D.

Dear Editors and Reviewers,

We appreciate the Editor's comments, which have allowed us to significantly improve the manuscript. Most importantly, we have responded to the Editor comments by changing the manuscript and figures. We have added two new figures. Please note that in one of the figures, we have included a screen shot from LabVIEW. We have edited out the company logo; nevertheless, we want to make sure that this does not infringe upon any copyright parameters. The product webpage is here: http://www.ni.com/en-us/shop/labview.html

When we make mention of line numbers below, they are in reference to the original line numbers in Edit you sent us. The line numbers in the manuscript no longer perfectly correlate since our text is now different.

Editorial comments:

1. The editor has formatted the manuscript to match the journal's style. Please retain the same.
RESPONSE: We have retained the formatting as provided.

2. For the protocol section, please use imperative tense throughout. Each step should consist of an action item that directs the reader to do something. Please also include how is the step performed.
RESPONSE: We have done this.

3. Once done please highlight 2.75 pages of the protocol including headings and spacings with the yellow highlight. This will be used for generating the script for the video.
RESPONSE: we have done this

4. Please ensure live animal will be available for filming purpose.
RESPONSE: We will.

5. Please ensure that all the steps in the protocol is written in imperative tense throughout providing every specific detail. Any text that cannot be written in the imperative tense may be added as a "Note."
RESPONSE: We have done our best to use imperative voice in all steps.

6. Please include a figure to show the prepared mouse cage and label different parts of it as well.
RESPONSE: We have made a new Figure 1 to demonstrate the cage. The figure legend labels each part of the cage.

7. Line 99: This is not an action step, hence converted to a note instead. Please check.
RESPONSE: this is perfect.

8. line 112: How many times a day do you perform the feeding? Include a note after this step stating how much weight change is observed in your experiment.
RESPONSE: In response to the query, we have added the following:

“Note: we weigh the mice once per day and expect a weight drop of 0.25-1 gram per day.”

9. line 166: This section is lacking details. Please exactly write how you perform your experiment and provide all specific details. Please use imperative tense throughout.

RESPONSE: We have added detail throughout this section. Please see our answers to #10 – 12.

10. line 181: This step needs clarity. How is this done. Please provide button clicks, graphical user interface, scripts/codes used for doing the same. Specific codes can be included as a supplementary file. Also, for the sub-steps please write exactly how you do the experiment being as specific as you can with respect to your experiment as this part will be used for filming purpose. Please use imperative tense throughout. As for showing the screenshot cover the logo and show the screenshots as supplementary files.

RESPONSE: We have clarified each step and made reference to an added figure and table.

11. line 192: What is the appropriate field? How is this done?

RESPONSE: we have added a more descriptive adjective and made reference to the figure.

12. line 199: Do you set up the software first and then introduce the mouse? Once the mouse is introduced, how long do you leave it to for learning? Do you turn on the start button? When is the stop button turned on?

RESPONSE: we have modified this description to say the following:

“3.2.1.8. Once the information fields have been filled out, click the **Run** button to begin the training session. The auto-trainer will dispense individual pellets and track whether they fall through the funnel until the total number of pellets has been dispensed, and the last pellet has either been retrieved or dropped by the mouse. The program will stop automatically at this point. If necessary, it can also be stopped prematurely by clicking on the **Stop** button.

3.2.2. Once the software is set up, place the home-cage of the mouse to be tested on the pedestal and observe the mouse so that you might gauge whether the mouse has indeed learned to attempt the required novel reaching behavior. After clicking the **Run** button, allow the mouse to investigate the slot and its new, unfamiliar surroundings.

NOTE: Similar to when introducing mice to the concept of reaching, expect some mice to be more compliant than others. Mice that have grasped the concept should try to reach within five to ten minutes, and will associate the movement of the auto-trainer with the presented pellet, as when they associate an uncovered slot with food in the initial stages of this protocol.”

13. line 215: Please expand the result section to include how this was analyzed? How was the graph generated? How was this conclusion derived? Comment on how many manual trial vs auto trainer trails were required to before the actual session. How many sessions per mouse was conducted for each experiment?

RESPONSE: we have modified the lines 211 – 224 to read:

“To compare the utility of the auto-trainer to manual training (considered the gold-standard), we trained adult male C57bl/6 mice 100 to 140 days old manually and using the auto-trainer. All animal handling and use was performed according to and with approval from the Johns Hopkins University Animal Care and Use Committee. Mice trained with the auto-trainer learned the prehension task and exhibit a clear increase in motor skill. This increase in skill is similar to that seen when the animal is trained manually without the use of the auto-trainer (**Figure 2**). For these data, manual prehension was scored as successful when the mouse reached its forelimb through the slit, grabbed the pellet, and ate it without knocking it from its resting space, dropping it, or in any other way losing control. The percent of successful prehension attempts was determined per pellet. A training block consisted of 30 pellets at a distance of 1 cm with each pellet presented one at a time. Mice trained on the auto-trainer were trained per the protocol described above. Each point in Figure 2 represents a day of training during which the animals reached for 30 pellets and graphed as percent correct. There was no statistical difference between the two lines using a non-parametric t-test with correction for multiple comparisons."

14. Line 206: How long is the pause period between the trials?

RESPONSE: we have added the sentence: Each trial can be run consecutively one right after the other with 2-5 seconds of pause.

15. Line 241: Few paragraphs can be moved to the introduction section. Details about the auto-trainer, the associated software and the meaning of single trials can be moved to the introduction to bring out clarity for understanding the protocol.

RESPONSE: we are uncertain which paragraphs can be moved to the Introduction. It seems to us that moving paragraphs beginning at line 251 or line 266 to the Introduction would be confusing to the reader since the reader otherwise would not have a working knowledge of the auto-trainer. We are happy to re-consider.

16. Line 242: This sentence needs rewording as the protocol does not presents the auto-trainer but the prehension task using the auto trainer previously made.

RESPONSE: we have reworded the sentence to say:

"Our auto-trainer evaluates forelimb reach-to-grasp (prehension) in an automated manner"

17. Please remove the embedded Tables from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.

RESPONSE: we have removed the tables from the manuscript and placed each table into a separate file. We kept them as MSWord documents since the tables are based in Word. We feel that Excel makes horrible looking text-tables.