Standard Operating Procedure
Center for Systems Neurogenetics of Addiction (CSNA)

Holeboard Assay
HB v1.0

Area:	JAX-CSNA-BPC

Controls:	
Superseded Document	N/A, New
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Signatures:	
Author	I indicate that I have authored or updated this SOP according to applicable business requirements and our company procedure: Preparing and Updating Standard Operating Procedures. Name: _Ashley Olson_____ Signature: _____ Date: _____May 1, 2017_____
Approver	I indicate that I have reviewed this SOP, and find it meets all applicable business requirements and that it reflects the procedure described. I approve it for use. Name: __Leona Gagnon_____ Signature: _____ Date: ___1/1/2019 reviewed _____

1. PURPOSE

This SOP addresses the routine procedures used for conducting the hole board assay in mice including methods for analysis of data, and quality monitoring procedures.

2. SCOPE

The SOP applies to laboratories within the JAX CSNA Behavioral Phenotyping Core

3. RESPONSIBILITIES

3.1. Laboratory Staff

3.1.1. Remain up to date in training with this SOP

3.1.2. Comply with this SOP

3.2. Principal Investigator/Core Manager of JAX-CSNA-BPC

3.2.1. Ensures that all personnel involved running this SOP are trained to comply with this SOP

4. GLOSSARY/DEFINITIONS

4.1. Definitions

Item	Definition
Arena Grid	The arena uses a 16x16 grid of infrared beams to measure the locomotor activity of an animal. Each 1x1 square within the grid is equivalent to 1 square inch (2.54cm).
Ambulatory Distance	The total number of centimeters (cm) traveled in the arena throughout the duration of the test as defined by the instrument.
Resting Time	The length of time (seconds) that the subject spent at rest. A resting period is defined as a period of inactivity greater than or equal to 1 second.

Holepokes	The total number of times the mice break the beams in each of the 16 holes of the arena floor.
Time Bin (Sample Duration)	The duration (seconds) for each timepoint in which data is analyzed
Resting Time	Cumulative time (sec) of inactivity through the duration of the entire session
Time Bin (Sample Duration)	The duration (seconds) for each timepoint in which data is analyzed
Total duration of experiment (min)	The duration of the experiment (min)

4.2. Procedure Inputs

Procedure Name	Description	Type	Format
Holeboard	Animal ID	Inputs	(JCMS #)
Holeboard	Group (Start Date-Group)	Inputs	(YYYYMMDD-Group#)
Holeboard	Comments	Inputs	(Experimenter's Initials)

4.3. Procedure Outputs

Procedure Name	Description	Type	ID
Holeboard	Total Entries – Total number of holepokes in all holes. (measured over whole test)	Outputs	Total Entries

Holeboard	Ambulatory Distance- Is total distance travelled in cm. (measured per defined time bin)	Outputs	Ambulatory Distance Bin #

5. MATERIALS

5.1. Instrumentation

5.1.1. Open Field Arena: A square shaped, clear polycarbonate arena (Med-Associates #MED-OFAS-515U) with dimensions 44.5 cm length, 44.5 cm width, 25.4 cm height). Removable clear acrylic, aerated lids were custom manufactured and are placed on top of arenas during testing. External to the perimeter of the arena at the level of the floor, on the left and right sides is a pair of horizontal infrared photobeam sensors (16 x 16 beam array). An additional pair of infrared photobeam sensors raised 3 inches from the arena floor (16 x 16 array) are situated at the front and rear external sides of the arena and used to capture vertical activity. An additional pair of infrared photobeam sensors below the arena floor (16 x 16 array) are situated at the front and rear external sides of the arena and used to capture nose poke activity.

5.1.2. Environmental Control Chamber: Each arena is placed within a sound attenuated, ventilated cabinet with interior dimensions: 26"W x 20"H x 22"D (Med Associates, #MED-OFA-017); Each cabinet containes two incandecent lights, each affixed in the upper rear two corners of the cabinet at a height of ~18.5in from the center of the arena floor which provides illumination of ~14 lux when measured in the center of arena floor.

5.1.3. Activity Monitor software: 7.0.5.10 SOF-812 (Med Associates, Inc.).

5.1.4. Forceps: Metal tongs used to handle and transfer mice by the tail.

5.1.5. Holeboard: Med Associates Holeboard floor consisting of two components, the metal floor on which the mice are in contact with comprised of 16 evenly spaced holes and the under plate containing 16 holes matching those on the metal plate with wire mesh for baiting the holes.

5.2. Consumables

5.2.1. 70% ethanol (ETOH) in water solution: used to sanitize the arena between subjects

5.2.2. Virkon Wipes: 1% Virkon (Virkon S Lanxess in water) working solution used to sanitize the arena at the end of the testing day.

5.2.3. Paper towels

5.2.4. Spor-Klenz: Spor-Klenz working solution (1 part Spor-Klenz Steris Life Science Concentrate to 32 parts water) used to sterilize forceps between animals.

6. PROCEDURE

6.1. Environment

6.1.1. Procedure Room. The dimensions of the procedure room are approximately 20' 9" x 10' 2". Twenty-four arenas, each placed within its own environmental chamber are double stacked and located around the perimeter of the room (chambers # 1-24).

6.1.2. Anteroom. An anteroom located adjacent to the procedure room (~ 15 feet) is used to acclimate mice prior to the test as in 6.3 below.

6.1.3. Temperature. The temperature range in the testing room is 71± 3 F.

6.1.4. Humidity. The humidity range in the procedure room is 50 ± 20%.

6.1.5. Lighting. Room lighting in the testing room is overhead florescent lights with a dimmer switch illuminated to the setting that produces a light level in the testing room of ~ 500 lux. Lighting within the behavioral chambers is provided by two 28 V lamps and measures approximately 14 lux at the testing floor. Chamber lights are observed daily for functionality. Light lux levels are confirmed monthly.

6.1.6. Noise. The ambient background noise level in the procedure room and within chambers is 55-70dB. Fans within the environmental control chambers mask background noise. Audible timers are not used during this test. Noise levels are confirmed monthly.

6.1.7. Visual Cues. No intended visual cues are provided within the testing chambers.

6.1.8. Time of day. The test is conducted during the light phase of the circadian cycle; beginning at least 60 min after the lights on and concluding at least 30 min prior to lights off.

6.2. Subjects

6.2.1. Species. Mice

6.2.1.1. Study specific animals (e.g, strain, sex, date of birth) ordered or bred and documented.

6.2.1.2. Receipt of animals logged (e.g., date of arrival)

6.2.2. Sex. Males or females

6.2.3. Age. The test is validated for mice 8-12 weeks of age.

6.2.4. Housing. Subjects are individually housed for this test with ad lib access to food and water. (View CSNA Housing SOP for more details).

6.2.5. Husbandry. Cage change occurs once a week and is not performed on the same day as testing. The cages are changed on the same day every week. In the current protocol cages are changed on Fridays of each week. Hole Board testing is scheduled on Wednesdays.

6.2.6. Subject Identification. Mice within a cage are marked by ear punches. Ear punches are performed at 6 weeks of age, at the same time that mice are individually housed.

6.2.7. Blinding and counterbalancing. Technicians are blind to strain/genotype during testing. Subjects are pre-assigned a test chamber. Every attempt is made to test mixed batches of strains and/or sexes such that each session does not include only animals from a single strain or sex and counterbalanced across test chambers. A list of subjects IDs, date of test, date of birth, weight, sex, and genotype when available should be prepared prior to testing. Any comments or unexpected observations regarding technical issues during the testsession or in reference to a specific animal, are recorded in the run sheet.

6.3. Testing

6.3.1. Acclimation. Subjects are transported from the housing room to the procedure room on a wheeled rack and left undisturbed to acclimate to the anteroom adjacent to the procedure room for a minimum of 30 minutes. At the conclusion of the acclimation period, the wheeled rack is transported into the procedure room and mice are immediately placed into the chambers as in 6.3.4. below. Test day information is entered into the laboratory notebook in the room.

6.3.2. Sanitization. Prior to the first mouse placed into any arena, and between subjects, the chamber is thoroughly sanitized with 70% ETOH solution (in water), and the box is wiped dry with clean paper towels.

6.3.3. System Set up.

6.3.3.1. Turn on both Computers, the power to the fans and lights (switches are on the back right of each control unit 6 total), and the

Interface Cabinets (Green toggle switch on the front right of the cabinet three total).

6.3.3.2. Activity Monitor Testing. Prior to testing mice, the Activity Monitor settings must be adjusted to run the protocol properly. Click *Activity Monitor* (v7.0.5.10 SOF-812).

6.3.3.2.1. Within Activity Monitor, click *Configure Hardware* under the file tab.

6.3.3.2.2. Click the box next to *Node* so all boxes become checked, Click *Detect*, and confirm all boxes say "Yes" under connected column and "Ok" under status column (This is to ensure all boxes are connected correctly and on).

6.3.3.2.3. Click *Configure* button.

6.3.3.2.4. In the Configure Chambers window, confirm *All Selected Devices* is selected, Type indicates *ENV- 515 -43cm x 43cm*, resolution (ms) is *40*, and Samples Per Second is set to *25*. Confirm the "Hole Board Installed" check box is checked. Click *Apply* and *Ok.*

6.3.3.2.5. Within the window for ENV-520 Device Properties select *Test* and confirm the Status for all chambers is "Ok". Click *Ok* and *Apply.*

6.3.3.2.6. Box Check. Prior to any test session, the open field chambers should be tested to confirm that all infared beams are working properly. In the configure hardware box, click on *Advanced Test*. Click *Start*, confirm that beam breaks start at zero. In addition confirm the test indicates that beams are being broken by running a hand along the floor of each chamber.

6.3.3.3. Activity Monitor Setup. Prior to placing the subjects into the arena, the software must be set up.

6.3.3.3.1. Under File select *Open Project*

6.3.3.3.2. In file menu (This PC > Documents) select the project that contains the Holeboard protocol

6.3.3.3.3. In the Project box click *Acquire Data*

6.3.3.3.4. The Acquire Data window will open. Under Protocol select the protocol that has Holeboard acquisition settings with a duration of 20 minutes with the task floor in use. Verify the time bins are set at 120 sec time bins.

6.3.3.3.5. The acquisition page should be filled out according to the run sheet, with the subject ID typed into the corresponding chamber of the subject column, the session column will read the date and the number test of the day (EX: YYYYMMDD_Test#), and the technicians initials entered into the comment column. Then click *Start*.

6.3.3.4. Follow the steps 6.3.3.2 – 6.3.3.3 for both computers to have all 24 chambers started for testing.

6.3.4. Placement into the arena. At the start of each testing session, subjects are individually placed into the center of the arena, facing the rear of the chamber. The lid is then placed atop the arena and the chamber door is closed. The tracking software detects the mouse in the arena and starts automatically. Load subjects into each chamber in sequential chamber numbering order. As each subject is loaded, the tester should briefly examine each mouse, health concerns should be noted in the run sheet.

6.3.5. Test Duration. The tracking software automatically ends the tracking for the subject 20 minutes after the mouse was initially detected by the software. The environmental chambers are not opened and the subject is not removed from any arena until testing concludes for all arenas in that session.

6.3.6. At the conclusion of the session, the test chamber is opened and subjects are returned to their respective home cages. Arenas are sanitized as described above. Mice are transported back to their housing room. The next set of subjects are not placed into the arenas until all the arenas have been sanitized.

6.3.7. Repeat 6.3.1 through 6.3.6 for subsequent groups of mice.

6.3.8. At the conclusion of all testing for the day, the subjects are returned to the housing room and the arenas are sanitized with Virkon followed by 70% ETOH to remove any Virkon residue.

6.4. Data Analysis and QC

6.4.1. Export. Data are exported from the behavioral tracking software into an excel file.

6.4.1.1. Select Analyze Data under the Project page. Select the desired Analysis, Zones, and Protocol.

6.4.1.2. Select the Group names that were used to indicated the groups that need to be analyzed.

6.4.1.3. When the data is finished being analyzed, select spreadsheet and excel from the drop down menues above the displayed data. Then

click *Open* to open the Excel (CSV) file. Save this file with the name format "Test_CU#_yearmonthday.csv" (HB_CU1_20181119.csv).

6.4.2. Data Review. Data is reviewed as generated for technical issues (e.g. malfunctioning equipment), verification of suject IDs tested, and any other aberrations which are then flagged for QC review. Observed aberrations are noted on the run sheet.

6.4.3. Data Upload. Data uploads are verified and performed as described in the CSNA Data QC and Upload SOP .

6.4.4. Resulted analyzed

6.4.4.1. Data are analyzed over time bins as total distance traveled (cm), and total hole pokes (as defined in the glossary 4.0 above).

6.4.4.2. Data is archived in several locations at minimum: 1) files are preserved on the testing computer; 2) files are saved on the Acronis external hard drive 3) files are saved on a share drive for LIMs QC and upload.