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

**Cocaine Locomotor Sensitization Assay
(Sens v1.0)**



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| Area: G3 CSNA | JAX-CSNA-BPC |
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|--------------------|---|
| Signatures: | |
| Author | <p>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.</p> <p>Name: _____ Ashley Olson _____</p> <p>Signature: _____</p> <p>Date: _____ 5/1/2017 _____</p> |
| Approver | <p>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.</p> <p>Name: _____ Leona Gagnon _____</p> <p>Signature: _____</p> <p>Date: _____ reviewed 1/1/2019 _____</p> |

1. PURPOSE

This SOP addresses the routine procedures used for conducting cocaine locomotor sensitization 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. |
| Time Spent at Center | The total time (seconds) spent in the center portion of the open field arena. This area is defined as the center 10x10 square matrix. Increases in center time may be indicative of anxiolytic-like activity. |

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| Time in perimeter zones | Time spent (seconds) by the animal in proximity to the walls of the arena. This area is defined as the exterior 3x16 and 16x3 matrices for the left right bottom and top regions. |
| 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. |
| Vertical Activity | The total number of vertical beam breaks. |
| Time Bin (Sample Duration) | The duration (seconds) for each timepoint in which data is analyzed. In the current protocol, time bins are set at 300 seconds (5 minutes). |
| Total duration of experiment (sec) | The duration of the experiment (sec). In the current protocol the duration of the experiment is 3600 sec (60 minutes). |
| Zone Top Left Corner | The upper left corner 3x3 matrix of the arena floor. |
| Zone Top Right Corner | The upper right corner 3x3 matrix of the arena floor. |
| Zone Top Perimeter | The upper perimeter 3x10 matrix of the arena between the Zone Top Left Corner and Zone Top Right Corner. |
| Zone Right Perimeter | The right perimeter 3x10 matrix of the arena floor between the Zone Bottom Right Corner and Zone Top Right Corner. |
| Zone Bottom Right Corner | The lower right corner 3x3 matrix of the arena floor. |
| Zone Bottom Left Corner | The lower left corner 3x3 matrix of the arena floor. |
| Zone Bottom Perimeter | The upper perimeter 3x10 matrix of the arena floor between the Zone Bottom Left Corner and Zone Bottom Right Corner. |
| Zone Left Perimeter | The right perimeter 3x10 matrix of the arena floor between the Zone Bottom Left Corner and Zone Top Left Corner. |

4.2. Procedure Inputs

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

4.3. Procedure Outputs

| BEHAVIOR | SYMBOL | DESCRIPTION | CALCULATION |
|-------------------------------------|--------|---|-----------------------------|
| Novelty-induced locomotor activity | NILA | Distance traveled in open field at first exposure | Day 1 Distance |
| Baseline locomotor behavior | BLB | Distance traveled on Day 2 of sensitization procedure | Day 2 Distance |
| Acute Locomotor Response to Cocaine | ALRC | Distance traveled on Day 3 of sensitization procedure after 1 st exposure to cocaine | Day 3 minus Day 2 Distance |
| Initial Sensitization Response | ISR | Distance traveled on Day 5 of sensitization procedure after 2 nd exposure to cocaine | Day 5 minus Day 3 Distance |
| Cocaine Sensitization | CSENS | Area under the curve of locomotor distance across all 5 days of cocaine exposure | AUC using trapezium method |
| Conditioned Activation | CONACT | Locomotor response to saline after repeated exposure to cocaine | Day 12 minus Day 2 Distance |

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| Cocaine Sensitization Expression | SENSX | Locomotor response to cocaine after 1 week withdrawal | Day 19 minus Day 11 Distance |
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5. MATERIALS

5.1 Instrumentation

5.1.1. Open Field Arena: A square shaped, clear polycarbonate arena (Med-Associates #MED-OFAS-515U) with dimensions 17.5 inches length x 17.5 inches width x 10.0 inches height (44.5 cm x 44.5 cm x 25.4 cm). 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.

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 contains two incandescent lights, each affixed in the upper rear two corners of the cabinet at a height of approximately 18.5 inches from the center of the arena floor which provides an illumination of 60±10 lux when measured in the center of arena floor.

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

5.1.5 Forceps: Metal tongs used to handle all mouse transfers.

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 between test cohorts of mice.

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.

5.2.3. 1.0 mL syringes

5.2.4. Needles (28G)

6. PROCEDURE

6.1. Environment

- 6.1.1. Procedure Room. The dimensions of the procedure room are approximately 20' 9" x 10' 2". 24 arenas, each placed within its own environmental chamber are double stacked and located on both sides 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 $71 \pm 3^\circ$ 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 maximal setting to produce a light level in the testing room of ~ 500 lux. Lighting within the behavioral chambers is provided by two 28 V lamps and measures 14 lux at the testing floor. Light lux levels are validated monthly.
- 6.1.6. Noise. The ambient background noise level in the procedure room is 55-70dB. Fans within the environmental control chambers mask background noise. Audible timers are not used during this test. Noise levels are validated 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. Open field testing is scheduled on Mondays.

6.2.6. Body Weights. Body weights are collected each day that cocaine is administered, and the first (saline) day of testing. Weights are obtained immediately before habituation starts.

6.2.7. 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 they are individually housed.

6.2.8. Randomization and counterbalancing. 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 the session should be noted either generally or in reference to a specific animal, in the run sheet.

6.3. Testing

6.3.1. Mice are tested on 3 consecutive days and then every other day until day 11. There is an additional test day on day 12. The final test is conducted on day 19. Mice receive IP cocaine injections on days 3, 5, 7, 9, 11 and 19. Mice receive IP saline on days 1, 2 and 12.

6.3.2. 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. Mice are weighed. After the last mouse has been weighed, mice are acclimated 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. Enter test day information in the laboratory notebook in the room, as indicated in book.

6.3.2.1. During the time the mice are in the acclimation anteroom, the syringes for that run are prepared based on mouse weight to create a 10mg/kg dose.

6.3.3. 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.

6.3.4. System Set up.

6.3.4.1. 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).

6.3.4.2. 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).

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

6.3.4.2.2. Click the box next to *Node* so all boxes become checked, Click *Detect*, make sure all boxes say “Yes” under connected column and “Ok” under status column (This is to ensure all boxes are connected correctly and on).

6.3.4.2.3. Click *Configure* button.

6.3.4.2.4. In the Configure Chambers window, ensure *All Selected Devices* is selected, Type indicates *ENV- 515 -43cm x 43cm*, resolution (ms) is *40*, and Samples Per Second is set to *25*. Make sure the “Hole Board Installed” check box is not checked. Click *Apply* and *Ok*.

6.3.4.2.5. Back in ENV-520 Device Properties window select *Test* and make sure the Status for all chambers is “Ok”. Click *Ok* and *Apply*.

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

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

6.3.4.3.1. Under File select *Open Project*

- 6.3.4.3.2. In file menu (This PC > Documents) select the project that contains the Sensitization protocol
 - 6.3.4.3.3. In the Project box click *Acquire Data...*
 - 6.3.4.3.4. The Acquire Data window will open. Under Protocol select the protocol that has Sensitization acquisition settings with a duration of 90 minutes with the task floor not in use. Verify the time bins are set at 300 sec time bins.
 - 6.3.4.3.5. 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 testers' initials entered into the comment column. Then click *Start*.
- 6.3.4.4. Follow the steps 6.3.4.2 – 6.3.4.3. for both computers to have all 24 chambers started for testing.
- 6.3.5. Placement into the arena. At the start of each testing session, subjects are individually placed into the front left corner of the arena, facing the center 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, with a brief pause between each mouse to allow ample time for dosing (dosing described in following steps). As each subject is loaded, the tester should briefly examine each mouse, health concerns should be noted in the run sheet.
- 6.3.6. Dosing Procedure. When the session duration on Activity Monitor reaches 30 min the box is paused. The mouse is then restrained and dosed with either 10 mg/kg cocaine solution or 10mg/kg saline administered with an IP injection. The mouse is then loaded back into the front left corner of the arena. The box is immediately resumed in Activity Monitor.
- 6.3.6.1. Repeat 6.3.6. until all mice have been dosed. If a mouse is dosed +/- 30 sec from the 30 min mark the actual dose time must be marked in the run sheet.
- 6.3.7. Test Duration. The tracking software automatically ends the tracking for the subject 90 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.

6.3.8. 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.

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

6.3.10. 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 Novelty Tests Project page.

6.4.1.2. Select OpenField under the Analysis, Zones, and Protocol.

6.4.1.3. Select the Group names that was used to indicate the groups that need to be analyzed.

6.4.1.4. When the data is finished being analyzed, select spreadsheet and excel from the drop down menus above the displayed data. Then click “Open” to open the Excel (CSV) file. Save this file with the name format “Test_CU#_yearmonthday.csv” (Sensitization_CU1_20181119.csv).

6.4.2. Data Review. Data is reviewed as generated for technical issues (e.g. malfunctioning equipment), verification of subject 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), perimeter time, center time, and related AUC (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.