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

Novelty Place Preference Assay (NPPv1.1)
Effective Date 11/1/2017

Area: G3	JAX-CSNA-BPC

Controls:	
Superseded Document	N/A, New
Reason for Revision Major or Minor changes	Change in duration of test Reduced to 10 minute test
Effective Date	11/1/2017

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: _____Rainy Dodd_____ Signature: _____ Date: _____11/1/2017_____
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 H Gagnon_____ Signature: _____ Date: _____Reviewed 1/1/2018_____

1. PURPOSE

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

2. SCOPE

The SOP applies to laboratories within the 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
Place Preference Chamber	An arena comprised of 3 distinct compartments (chambers) including a center grey chamber, with doors on left and right sides leading to a chamber with white walls ("whiteside") on the left or a chamber with black walls ("blackside") on the right
Blackside	Peripheral chamber with black walls and stainless steel bar flooring.
Whiteside	Peripheral chamber with white walls and stainless steel wire grid flooring.
Grey chamber	Center chamber in which mice are acclimated to.

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ExplorationCounts	Breaking of the first beam in a zone.
EntranceCounts	Breaking of any beam beyond the first in a assigned zone.
ZoneTime	The amount of time (in seconds) spent in the zone.
ActivityCounts	Any beam break within the current zone.
MovementCounts	A change in the beam broken in the current zone.
Time Block	Designated time (minutes) intervals in which the test is broken up into.

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4.2. Procedure Inputs

Procedure Name	Description	Type
Conditioned Place Pref	Arena ID	Inputs
Conditioned Place Pref	Arena Size	Inputs
Conditioned Place Pref	Cage Distributor	Inputs
Conditioned Place Pref	Cage Material	Inputs
Conditioned Place Pref	White Chamber Size	Inputs
Conditioned Place Pref	Black Chamber Size	Inputs
Conditioned Place Pref	Grey Chamber Size(Center Chamber)	Inputs
Conditioned Place Pref	Cage Type	Inputs
Conditioned Place Pref	Habituation Time	Inputs
Conditioned Place Pref	Color of Arena	Inputs
Conditioned Place Pref	Configuration File/ Software Version	Inputs
Conditioned Place Pref	Date of Birth	Inputs
Conditioned Place Pref	Date of Test	Inputs

Conditioned Place Pref	Disinfectant	Inputs
Conditioned Place Pref	Distance to light source	Inputs
Conditioned Place Pref	Equipment Manufacturer	Inputs
Conditioned Place Pref	Equipment Model	Inputs
Conditioned Place Pref	Equipment Name	Inputs
Conditioned Place Pref	Experimenter ID	Inputs
Conditioned Place Pref	Height of Wall	Inputs
Conditioned Place Pref	Light Level	Inputs
Conditioned Place Pref	Number of animals per cage	Inputs
Conditioned Place Pref	Start time	Inputs

4.3. Procedure Outputs

ProcedureName	Description	Type	ID
Open Field	Center Average Speed	outputs	CSNA_OFT_024_001
Open Field	Center Distance Traveled	outputs	CSNA_OFT_025_001
Open Field	Center Permanence Time	outputs	CSNA_OFT_026_001
Open Field	Center Resting Time	outputs	CSNA_OFT_027_001
Open Field	Clockwise	outputs	CSNA_OFT_028_001
Open Field	Corners Permanence Time	outputs	CSNA_OFT_029_001
Open Field	Counter Clockwise	outputs	CSNA_OFT_030_001
Open Field	Distance Traveled First Five Minutes	outputs	CSNA_OFT_031_001
Open Field	Distance Traveled Fourth Five Minutes	outputs	CSNA_OFT_032_001
Open Field	Distance Traveled Habituation Ratio	outputs	CSNA_OFT_033_001
Open Field	Distance Traveled Second Five Minutes	outputs	CSNA_OFT_034_001
Open Field	Distance Traveled Slope	outputs	CSNA_OFT_035_001
Open Field	Distance Traveled Third Five Minutes	outputs	CSNA_OFT_036_001
Open Field	Distance Traveled Total	outputs	CSNA_OFT_037_001
Open Field	Fecal Boli	outputs	CSNA_OFT_038_001
Open Field	Latency to Center Entry	outputs	CSNA_OFT_039_001
Open Field	Number of Center Entries	outputs	CSNA_OFT_040_001
Open Field	Number of Rears First Five Minutes	outputs	CSNA_OFT_041_001
Open Field	Number of Rears Fourth Five Minutes	outputs	CSNA_OFT_042_001
Open Field	Number of Rears Second Five Minutes	outputs	CSNA_OFT_043_001
Open Field	Number of Rears Third Five Minutes	outputs	CSNA_OFT_044_001
Open Field	Number of Rears Total	outputs	CSNA_OFT_045_001
Open Field	PctTime Center	outputs	CSNA_OFT_046_001
Open Field	PctTime Center Habituation Ratio	outputs	CSNA_OFT_047_001
Open Field	PctTime Center Slope	outputs	CSNA_OFT_048_001
Open Field	PctTime Corners	outputs	CSNA_OFT_049_001
Open Field	PctTime Corners Habituation Ratio	outputs	CSNA_OFT_050_001
Open Field	PctTime Corners Slope	outputs	CSNA_OFT_051_001
Open Field	Periphery Average Speed	outputs	CSNA_OFT_052_001
Open Field	Periphery Distance Traveled	outputs	CSNA_OFT_053_001
Open Field	Periphery Permanence Time	outputs	CSNA_OFT_054_001
Open Field	Periphery Resting Time	outputs	CSNA_OFT_055_001
Open Field	Time Spent Mobile	outputs	CSNA_OFT_056_001
Open Field	Vertical Time	outputs	CSNA_OFT_057_001
Open Field	Whole Arena Average Speed	outputs	CSNA_OFT_058_001
Open Field	Whole Arena Permanence Time	outputs	CSNA_OFT_059_001
Open Field	Whole Arena Resting Time	outputs	CSNA_OFT_060_001

5. MATERIALS

5.1 Instrumentation

5.1.1. Novelty Place Preference apparatus: A rectangular shaped, 3 chambered arena (acrylic or polycarbonate materials) with dimensions (white/black chambers - 5 in length, 6.5 in width, 5 in height grey center chamber – 5 in length, 3.5 in width, 5 in height) with automatic doors between the 3 sections, and a clear, aerated lid (Med Associates, St Albans VT, USA). The bottom of the arena contains pairs of horizontal infrared photobeam sensors at the level of the floor which are not visible to the mouse.

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5.1.2. Environmental Control Chamber: Each arena is placed within a sound attenuated, ventilated cabinet with dimensions 25 in width x 26 in depth x 21 in height (Med Associates, Inc).

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5.1.3. MED-PC IV software: (Med Associates, Inc.)

5.1.4. 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; Lanxess Corporation Pittsburgh, Pensylvania) 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.

6. PROCEDURE

6.1. Environment

6.1.1. Procedure Room. The dimensions of the procedure room are approximately 10 ft. x 11 ft. 16 arenas, each placed within its own environmental chamber are double stacked and located on two walls of the room (chambers # 1-16).

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

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 maximal setting to produce a light level in the testing room of ~ 500 lux. Lighting within the behavioral chambers is provided by overhead lights in each of the three testing compartments. The lights are 28 V lamps, each measuring ~12 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 55-70 dB. 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. There are three compartmetns within the behavioral apparatus. Each are a different color and have a different floor type. (i.e. white walls with a metal wire grid floor).

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 and documented.

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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. 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.

6.2.7. Counterbalance. 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. Macros. Subjects will be tested in two consecutive programs including an initial "exposure" paradigm in which they are randomly assigned and restricted to either the black side or white side and then a subsequent "test" paradigm where they are placed in the center grey chamber and allowed free access to either black or white sides. Prior to testing, the relevant macro scripts are created as follows:

6.3.1. Creating Exposure Macro Scripts

6.3.1.1. Open notepad and load Exposure Macro Script Blue Print, script blue print is a template of the macro.

6.3.1.2. Each subject requires its own line of script, which is formatted as follows: Load(CHAMBER#) (SUB#) EXPT (Experiment name) GROUP (Group#-SubGroup#) – (Mouse ID #) – (Sex) – (Exposure Side) Exposure PROGRAM (Script for test) DELAY 50

6.3.1.2.1. Example Below

```
LOAD BOX 1 SUBJ 1 EXPT Test Novelty Group 1-1 - 16994 - M - White Side
Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50
```

6.3.1.3. Repeat step 6.3.1.2. for each subject

6.3.1.4. Create section SHOWMESSAGE "Start Boxes?" DELAY 50

6.3.1.5. For each Chamber in use write START BOXES 1 DELAY 50

6.3.1.5.1. Example Below

```
SHOWMESSAGE "Start Boxes?"
DELAY 50

START BOXES 1
DELAY 50
```

6.3.1.6. Repeat step 6.3.1.5. for each chamber

6.3.1.7. Save Script as Experiment Group-Subgroup_Exposure_YYYYMMDD.Mac (1-1_Exposure_20181101.Mac) and save as All Files

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6.3.2. Creating Test Macro Scripts

6.3.2.1. Open notepad and load Test Macro Script Blue Print, script blue print is a template of the macro.

6.3.2.2. Each subject requires its own line of script, which is formatted as follows: Load(CHAMBER#) (SUBJ#) EXPT (Experiment name) GROUP (Group#- SubGroup#) – (Mouse ID #) - SEX - (Exposure Side) Exposure PROGRAM (Script for test) DELAY 50

6.3.2.2.1. Example Below

```
LOAD BOX 1 SUBJ 1 EXPT Test Novelty Group 1-1 – 16994 – M – White
Side Test PROGRAM Novelty_Preference_TEST-10 min test
DELAY 50
```

6.3.2.3. Repeat step 6.3.2.2. for each subject

6.3.2.4. Create section SHOWMESSAGE "Start Boxes?" DELAY 50

6.3.2.5. For each Chamber in use write START BOXES 1 DELAY 50

6.3.2.5.1. Example Below

```
SHOWMESSAGE “Start Boxes?”
DELAY 50

START BOXES 1
DELAY 50
```

6.3.2.6. Repeat step 6.3.2.5. for each chamber

6.3.2.7. Save Script as Experiment Group- Subgroup_Test_YYYYMMDD.Mac (1-1_Test_20181101.Mac) and save as All Files

6.4. Testing

6.4.1. 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. Enter test day information in the laboratory notebook in the room, as indicated in book.

6.4.2. 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.

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6.4.3. System Set Up.

6.4.3.1. Turn on both Computers and the Interface Cabinets (Green toggle switch on the front right of the cabinet; two total).

6.4.3.2. Setup Med PC IV: Click *Med PC IV*.

6.4.3.3. Click *Macros* and then *Play Macros* from drop down lists at the top of the program. Navigate to the macro file (.MAC extension) corresponding to the cohort and group you are about to run. Chose the macro with "EXPOSURE" in the name. The details of the group to be tested (e.g., chamber, subject id, etc.) will automatically populate MED PC IV.

6.4.3.4. Under "Start Box?" click *Yes* to start the program

6.4.3.5. Place mouse in center grey chamber and close all hinged ceiling doors.

6.4.3.6. Once is the software detects the placement of the mouse the ceiling light in the chamber will turn off and a 5 minute acclimation timer will start.

6.4.3.7. At the completion of the 5 minute acclimation period, the motorized guillotine doors leading to the pre-assigned exposure chamber (black or white) will open and the ceiling lights will turn on.

6.4.3.8. After 20 minutes have elapsed, the exposure session is complete. The guillotine door will close, the subject information on the screen will disappear, and the data for that mouse is automatically saved.

6.4.3.9. Immediately upon conclusion of exposure program as in 6.4.3.5. above, place mouse back into center compartment. If mouse is in center chamber at the conclusion of exposure program, remove the mouse and then replace into the center chamber.

6.4.3.10. Click *Macros* and then *Play Macros* from drop down lists at the top of the program. Navigate to the macro file (.MAC extension) corresponding to the cohort and group you are about to run. Chose the macro with "TEST" in the name. The details of the group to be tested (e.g., chamber, subject id, etc.) will automatically populate MED PC IV.

6.4.3.11. Under "Start BOX?" click Yes to start the program. The program will immediately detect that all chambers have mice in them. The ceiling light in the the chamber will turn off and a 5 minute acclimation timer will start.

6.4.3.12. At the conclusion of the 5 minute acclimation period, both of the motorized guillotine doors leading to the black and white chambers will open and the ceiling lights will turn on. A 10 minute testing timer will begin.

6.4.3.13. After 10 minutes, the session is complete and doors will close.

6.4.3.14. Remove mouse by tail with forceps and place back in home cage. Cages are returned to housing room.

6.4.3.15. Sanitize arenas thoroughly with 70% ETOH solution (in water) and wipe dry with clean paper towels.

6.4.3.16. Repeat steps 6.4.1 through 6.4.3.15 for the subsequent groups.

6.4.4. 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.

6.5. Data Analysis and QC

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

6.5.1.1. In the Med PC to Excel interface, under row transfer profile click Select. Navigate to and select designated novelty preference script. Ensure Data and (column labels if needed) are checked and that horizontal orientation is selected before you transfer.

6.5.1.2. Click transfer and select the NPP raw data files you wish to convert to excel. Save the file to the designated location with the name format "Test_CU#_yearmonthday.csv" (NPP_CU1_20181119.csv).

6.5.2. 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. Oserved aberrations are noted on the run sheet.

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

6.5.4. Results analyzed

6.5.4.1. 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).

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