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

Novelty Place Preference Assay
NPP v 2.0

Area: G3	JAX-CSNA-BPC

Controls:	
Superseded Document	N/A, New
Reason for Revision Major or Minor changes Effective Date	N/A N/A, Shortened test time (10 Minutes) December 31, 2016

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: ____01/03/2019_____
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: _____01/07/2019_____ Date: _____

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. The floors are barred wired.
Whiteside	Peripheral chamber with white walls. The floors are meshed wired.
Grey chamber	Center chamber in which mice are acclimated to.
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.

4.2. Procedure Inputs

Procedure Name	Variable	Type	comment
NoveltyPlacePreference	Protocol	Input	protocol version
NoveltyPlacePreference	Subject	Input	Subject
NoveltyPlacePreference	StartDate	Input	Date
NoveltyPlacePreference	StartTime	Input	Time bin interval
NoveltyPlacePreference	Experiment	Input	Experiment information - Test or Exposure
NoveltyPlacePreference	Group	Input	Subject Group
NoveltyPlacePreference	Box	Input	Box
NoveltyPlacePreference	MEDState Notation Program Name	Input	Exposure Information
NoveltyPlacePreference	Comment	Input	Comments
NoveltyPlacePreference	Elapsed Session time (sec)	Input	Time elapsed
NoveltyPlacePreference	Adaptation Time (min)	Input	Adaptation time
NoveltyPlacePreference	Test Time (min)	Input	Test duration
NoveltyPlacePreference	Bin Size (sec)	Input	Bin size

4.3. Procedure Outputs

Please see section 8.0

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, aeratedlid (Med Associates, Inc.) 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.

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.)

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

5.1.4. Scale: A scale for weighing animals with 1 gram resolution (if body weights are required as with drug treatment)

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

5.1.1. Sporaklens and water: Used to clean forceps inbetween mice. Foreceps dipped into sporaklens, then dipped in water (sterialized bottles water) to remove excess sporaklens.

5.2. Consumables

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

6. PROCEDURE

6.1.1. Environment

6.1.2. 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). Each set of 8 chambers is controlled by one desktop computer through a KVM switch.

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.

6.1.6. Noise. The ambient background noise level in the procedure room is 55-70dB. No additional or ancillary noise (white noise) is provided. Audible timers are not used during this test.

6.1.7. 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 in the chambers

6.1.8. Complete script should look like the following

```
LOAD BOX 1 SUBJ 1 EXPT NO ENRICH Founders Novelty Group 1-1 - 1 - female - Black Side Exposure PROGRAM Novelty_Preference_EXPOSURE_Black
DELAY 50
LOAD BOX 2 SUBJ 2 EXPT NO ENRICH Founders Novelty Group 1-1 - 2 - male - White Side Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50
LOAD BOX 3 SUBJ 3 EXPT NO ENRICH Founders Novelty Group 1-1 - 3 - female - Black Side Exposure PROGRAM Novelty_Preference_EXPOSURE_Black
DELAY 50
LOAD BOX 4 SUBJ 4 EXPT NO ENRICH Founders Novelty Group 1-1 - 4 - male - White Side Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50
LOAD BOX 5 SUBJ 5 EXPT NO ENRICH Founders Novelty Group 1-1 - 5 - female - Black Side Exposure PROGRAM Novelty_Preference_EXPOSURE_Black
DELAY 50
LOAD BOX 6 SUBJ 6 EXPT NO ENRICH Founders Novelty Group 1-1 - 6 - male - White Side Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50
LOAD BOX 7 SUBJ 7 EXPT NO ENRICH Founders Novelty Group 1-1 - 7 - female - Black Side Exposure PROGRAM Novelty_Preference_EXPOSURE_Black
DELAY 50
LOAD BOX 8 SUBJ 8 EXPT NO ENRICH Founders Novelty Group 1-1 - 8 - male - White Side Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50


SHOWMESSAGE "Start Boxes?"
DELAY 50

START BOXES 1
DELAY 50
START BOXES 2
DELAY 50
START BOXES 3
DELAY 50
START BOXES 4
DELAY 50
START BOXES 5
DELAY 50
START BOXES 6
DELAY 50
START BOXES 7
DELAY 50
START BOXES 8
DELAY 50
```

6.1.9. Save Script as Experiment Group- Subgroup Exposure.Mac (NPP,Date and run: **NPP_20200716_Test_3**) and save as All Files

6.1.10. Creating Test Macro for Novelty CPP

6.1.11. Open notepad and load Test macro script blue print, script blue print is a template of the macro.

6.1.12. Each chamber needs its own line of script to work, they should be formatted as following

6.1.13. Load(BOX#) (SUBJ#) EXPT (Experiment name) GROUP (Group#- SubGroup#) – (Mouse ID #) - SEX - (Exposure Side) Exposure PROGRAM (Script for test) DELAY 50

6.1.14. Example Below

```
LOAD BOX 1 SUBJ 1 EXPT DO IVSA Novelty GROUP  1-1 - 1 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
```

6.1.15. Complete for all boxes

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

6.1.17. For each box write START BOXES 1 DELAY 50

6.1.18. Example seen below:

```
SHOWMESSAGE "Start Boxes?"
DELAY 50

START BOXES 1
DELAY 50
```

6.1.19. Do this for all Chambers

6.1.20. Complete script should look like the following

```
LOAD BOX 1 SUBJ 1 EXPT DO IVSA Novelty GROUP  1-1 - 1 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 2 SUBJ 2 EXPT DO IVSA Novelty GROUP  1-1 - 2 - Female - White Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 3 SUBJ 3 EXPT DO IVSA Novelty GROUP  1-1 - 3 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 4 SUBJ 4 EXPT DO IVSA Novelty GROUP  1-1 - 4 - female - White Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 5 SUBJ 5 EXPT DO IVSA Novelty GROUP  1-1 - 5 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 6 SUBJ 6 EXPT DO IVSA Novelty GROUP  1-1 - 6 - female - White Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 7 SUBJ 7 EXPT DO IVSA Novelty GROUP  1-1 - 7 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 8 SUBJ 8 EXPT DO IVSA Novelty GROUP  1-1 - 8 - female - White Side Test PROGRAM Novelty_Preference_TEST
DELAY 50


SHOWMESSAGE "Start Boxes?"
DELAY 50

START BOXES 1
DELAY 50
START BOXES 2
DELAY 50
START BOXES 3
DELAY 50
START BOXES 4
DELAY 50
START BOXES 5
DELAY 50
START BOXES 6
DELAY 50
START BOXES 7
DELAY 50
START BOXES 8
DELAY 50
```

6.1.21. Save Script as Experiment Group- Subgroup Test.Mac (DO IVSA 1-1 Test.Mac) and save as All Files

6.1.22. Subjects

6.1.23. Species. Mice

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

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

6.1.24. Sex. Males or females

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

```
LOAD BOX 1 SUBJ 1 EXPT DD IVSA Novelty GROUP 1-1 - 1234 - male - BLACK Side Exposure PROGRAM Novelty_Preference_EXPOSURE_BLACK
DELAY 50
```

6.1.26. Housing. Subjects may be group housed or individually housed for this test. Subjects are housed in the housing room for a minimum of 5 days prior to behavioral testing.

6.1.27. Husbandry. The test should not be conducted on the same day that a cage change has occurred.

6.1.28. Transport to Procedure Room. Subjects are transported in their home cages from the housing room to the procedure room on a wheeled transport rack. The procedure room is located ~ 100 feet from the housing room on the same floor. The transport rack is placed in the center of the procedure room, adjacent to the rear wall and not moved until all testing has been completed.

6.1.28.1. Acclimation. Prior to the start of testing, upon transport to the procedure room, subjects are briefly handled and assessed for welfare concerns that may result in exclusion from testing (e.g., fight wounds or bite marks), and then left undisturbed to acclimate to the procedure room environment for at minimum 60 min prior to testing.

6.1.28.2. Randomization and counterbalancing. Subjects are randomized and counterbalanced across all available arenas and across sessions within the testing day. A run sheet in excel format should be created prior to the test that lists the test date, environmental variables, subject IDs, test sessions, run times, arenas with subject IDs, weights if required, and a comments session to note any unexpected observations.

6.1.28.3. Pre-testing macro script creation. 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:

6.2. Create Exposure script

6.2.1. Open notepad and load “Exposure” macro script blue print, this blue print is a template of the macro.

6.2.2. For each chamber format the script as follows:

6.2.3. Load(CHAMBER#) (SUBJ#) EXPT (Experiment name) GROUP (Group#-SubGroup#) – (Mouse ID #) - SEX - (Exposure Side) Exposure PROGRAM (Script for exposure side) DELAY 50

6.2.4. Complete above step 6.2.2. for for each chamber

6.2.5. Create section SHOWMESSAGE "Start Box?" DELAY 50

6.2.6. For each chamber write START CHAMBERS 1 DELAY 50

6.2.7. Example

```
LOAD BOX 1 SUBJ 1 EXPT NO ENRICH Founders Novelty Group 1-1 - 1 - female - Black Side Exposure PROGRAM Novelty_Preference_EXPOSURE_Black
DELAY 50
LOAD BOX 2 SUBJ 2 EXPT NO ENRICH Founders Novelty Group 1-1 - 2 - male - White Side Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50
LOAD BOX 3 SUBJ 3 EXPT NO ENRICH Founders Novelty Group 1-1 - 3 - female - Black Side Exposure PROGRAM Novelty_Preference_EXPOSURE_Black
DELAY 50
LOAD BOX 4 SUBJ 4 EXPT NO ENRICH Founders Novelty Group 1-1 - 4 - male - White Side Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50
LOAD BOX 5 SUBJ 5 EXPT NO ENRICH Founders Novelty Group 1-1 - 5 - female - Black Side Exposure PROGRAM Novelty_Preference_EXPOSURE_Black
DELAY 50
LOAD BOX 6 SUBJ 6 EXPT NO ENRICH Founders Novelty Group 1-1 - 6 - male - White Side Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50
LOAD BOX 7 SUBJ 7 EXPT NO ENRICH Founders Novelty Group 1-1 - 7 - female - Black Side Exposure PROGRAM Novelty_Preference_EXPOSURE_Black
DELAY 50
LOAD BOX 8 SUBJ 8 EXPT NO ENRICH Founders Novelty Group 1-1 - 8 - male - White Side Exposure PROGRAM Novelty_Preference_EXPOSURE_White
DELAY 50


SHOWMESSAGE "Start Boxes?"
DELAY 50

START BOXES 1
DELAY 50
START BOXES 2
DELAY 50
START BOXES 3
DELAY 50
START BOXES 4
DELAY 50
START BOXES 5
DELAY 50
START BOXES 6
DELAY 50
START BOXES 7
DELAY 50
START BOXES 8
DELAY 50
```

6.2.7.1. Save Script as Experiment Group- Subgroup Exposure.Mac (DO IVSA 1-1 Exposure.Mac) and save as All Files

6.2.7.2. Creating Test Macro for Novelty CPP

6.2.7.3. Open notepad and load Test macro script blue print

6.2.7.4. Each chamber needs its own line of script to work, they should be formatted as following

6.2.7.5. Load(CHAMBER#) (SUBJ#) EXPT (Experiment name) GROUP (Group#-SubGroup#) – (Mouse ID #) - SEX - (Exposure Side) Exposure PROGRAM (Script for test) DELAY Example Below:

```
LOAD BOX 1 SUBJ 1 EXPT DO IVSA Novelty GROUP  1-1 - 1 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
```

6.2.8. Complete this for all chambers

6.2.9. Create section SHOWMESSAGE "Start Chambers?" DELAY 50

6.2.9.1. For each chamber write START CHAMBERS 1 DELAY 50 Example seen below:

6.2.9.2. Complete this step for each chamber

6.2.9.3. Example of script:

```
LOAD BOX 1 SUBJ 1 EXPT DO IVSA Novelty GROUP  1-1 - 1 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 2 SUBJ 2 EXPT DO IVSA Novelty GROUP  1-1 - 2 - Female - White Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 3 SUBJ 3 EXPT DO IVSA Novelty GROUP  1-1 - 3 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 4 SUBJ 4 EXPT DO IVSA Novelty GROUP  1-1 - 4 - female - White Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 5 SUBJ 5 EXPT DO IVSA Novelty GROUP  1-1 - 5 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 6 SUBJ 6 EXPT DO IVSA Novelty GROUP  1-1 - 6 - female - White Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 7 SUBJ 7 EXPT DO IVSA Novelty GROUP  1-1 - 7 - male - BLACK Side Test PROGRAM Novelty_Preference_TEST
DELAY 50
LOAD BOX 8 SUBJ 8 EXPT DO IVSA Novelty GROUP  1-1 - 8 - female - White Side Test PROGRAM Novelty_Preference_TEST
DELAY 50


SHOWMESSAGE "Start Boxes?"
DELAY 50

START BOXES 1
DELAY 50
START BOXES 2
DELAY 50
START BOXES 3
DELAY 50
START BOXES 4
DELAY 50
START BOXES 5
DELAY 50
START BOXES 6
DELAY 50
START BOXES 7
DELAY 50
START BOXES 8
DELAY 50
```

6.2.10.

6.2.11. Save Script as Experiment Group- Subgroup Test.Mac (NPP,Date and run: **NPP_20200716_Test_3**) and save as All Files

6.2.12. Testing

6.2.13. Sanitization. Prior to the first mouse placed into any arena, and between subjects, the maze is thoroughly sanitized with 70% ETOH solution (in water), gross urine and feces are removed, and the chamber is wiped dry with clean paper towels. Ensure that urine has also been removed around and under the edges of the dark chamber insertStart Med computer attached to CPP chambers and turn on fans.

6.2.14. Setup Med PC IV: Double click Med PC IV icon on desktop. Med PC will open. Click “Macros” → “Play Macros” from drop down lists at the top of the program. A chamber will pop up asking for a macro file. 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. Double click the macro file. The details of the group to be tested (e.g., chamber, subject id, etc.) will automatically populate MED PC IV.

6.2.15. Double check and confirm: Check this information against printed out sheet and cage cards to confirm alignment of all data. Click “yes” into the pop-up chamber requesting “Start Box?” This will automatically start the program

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

6.2.16.1. 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. 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.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.

6.2.17. Immediately upon conclusion of exposure program as in 6.3.5 above, place mouse back into center compartment.

6.2.17.1. If mouse was in the center chamber at the conclusion of the exposure program as above, the mouse is handled and removed from the center chamber than replaced back in the center chamber in order to mirror the handling of all subjects between exposure and test programs.

6.2.17.2. Upon all subjects being returned to the center grey compartment, start the macro corresponding to the cohort and group with “TEST” in the name.

6.2.17.3. Upon the start of the macro, mice will immediately be detected, the ceiling light in the chamber will turn off, and a 5 minute acclimation timer will start.

6.2.17.4. 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.

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

6.2.19. Remove mouse by tail with forceps and place back in home cage

6.2.20. Repeat steps 6.3.1 through 6.3.7 for the next subsequent group.

6.2.21. At the conclusion of all testing for the day, the subjects are returned to the housing room and the arenas are sanitized.

7. Data Analysis and QC

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

7.2 Open Med PC to Excel. Open excel file you wish to save to, then select first available cell.

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

7.4 Click transfer and select the CPP data you wish to convert to excel. Save the file to the designated location.

7.5 Data QC.

7.6 Confirm all chambers recorded data as expected for all endpoints and time bins. Identify any technical issues with chambers not reporting data. Remove data from chambers that did not contain subjects “empty chambers” which would output “0” values. Identify

and reconcile errors (e.g., subject placed in wrong chamber or incorrect subject ID number was identified with incorrect chamber).Results analyze

7.7 Data are analyzed over time bins as zone time(s), entrance counts, and related AUC (as defined in the glossary 4.0 above Data Archived per SOP-JAX-CSNA

7.1. Data Review.

7.1 1.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.

7.1.2 Data Upload. Data uploads are verified and performed as described in the CSNA Data QC and Upload SOP .Results analyzed 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)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.

8. Variables

8.1. Derived variables : The following variables are calculated based on output variables generated following 7.0 using a custom python script, novelty_pref.py (https://bitbucket.jax.org/projects/CSNA/repos/csna-data-filtering/browse/src/novelty_preference). Variables in MPD are stored by mouse population used in the study (in: Inbred, cc:Collaborative Corss, do: Diversity Outbred)

CSNA Variable	MPD Variable Name(s)	Description	Units	Upload to MPD	Required for analysis	Required for QC
percent.novel.exploration	exploration_wo_gray_in exploration_wo_gray_cc exploration_wo_gray_do	Percent exploration in the novel zone	%	Yes	No	Yes
percent.novel.entrances	entrance_wo_gray_in entrance_wo_gray_cc entrance_wo_gray_do	Percent entrances in the novel zone	%	Yes	No	Yes
percent.novel.time	time_wo_gray_in time_wo_gray_cc time_wo_gray_do	Percent time in the novel zone	%	Yes	Yes	Yes
percent.novel.activity	activity_wo_gray_in activity_wo_gray_cc activity_wo_gray_do	Percent activity in the novel zone	%	Yes	No	Yes
precent.novel.movement	movement_wo_gray_in movement_wo_gray_cc movement_wo_gray_do	Percent movement in the novel zone	%	Yes	No	Yes
percent.novel.exploration.adjusted	exploration_wo_gray_in exploration_wo_gray_cc exploration_wo_gray_do	Percent exploration in the novel zone adjusted for center zone	%	Yes	No	Yes
percent.novel.entrances.adjusted	entrance_wo_gray_in entrance_wo_gray_cc entrance_wo_gray_do	Percent entrances in the novel zone adjusted for center zone	%	Yes	No	Yes
percent.novel.time.adjusted	time_wo_gray_in time_wo_gray_cc time_wo_gray_do	Percent time in the novel zone adjusted for center zone	%	Yes	Yes	Yes
percent.novel.activity.adjusted	activity_wo_gray_in activity_wo_gray_cc activity_wo_gray_do	Percent activity in the novel zone adjusted for center zone	%	Yes	No	Yes
precent.novel.movement.adjusted	movement_wo_gray_in movement_wo_gray_cc movement_wo_gray_do	Percent movement in the novel zone adjusted for center zone	%	Yes	No	Yes

8.2. Output variables

Variable	Description	Units	Upload to MPD	Required for analysis	Required for QC
ActivityCounts_ BlackZone_Block01	Activity counts in the black zone 0-1 min	counts	No	No	Yes
ActivityCounts_ BlackZone_Total	Total activity counts in the black zone	counts	No	No	Yes
ActivityCounts_ GreyZone_Block01	Activity counts in the grey zone 0-1 min	counts	No	No	Yes

ActivityCounts_ GreyZone_Total	Total activity counts in the grey zone	counts	No	No	Yes
ActivityCounts_ WhiteZone_Block01	Activity counts in the white zone 0-1 min	counts	No	No	Yes
ActivityCounts_ WhiteZone_Total	Total activity counts in the white zone	counts	No	No	Yes
EntranceCounts_ BlackZone_Block01	Entrance counts in the black zone 0-1 min	counts	No	No	Yes
EntranceCounts_ BlackZone_Total	Total entrance counts in the black zone	counts	No	No	Yes
EntranceCounts_ GreyZone_Block01	Entrance counts in the grey zone 0-1 min	counts	No	No	Yes
EntranceCounts_ GreyZone_Total	Total entrance counts in the grey zone	counts	No	No	Yes
EntranceCounts_ WhiteZone_Block01	Entrance counts in the white zone 0-1 min	counts	No	No	Yes
EntranceCounts_ WhiteZone_Total	Total entrance counts in the white zone	counts	No	No	Yes
ExplorationCounts_ BlackZone_Block01	Exploration counts in the black zone 0-1 min	counts	No	No	Yes
ExplorationCounts_ BlackZone_Total	Total exploration counts in the black zone	counts	No	No	Yes
ExplorationCounts_ GreyZone_Block01	Exploration counts in the grey zone 0-1 min	counts	No	No	Yes
ExplorationCounts_ GreyZone_Total	Total exploration counts in the grey zone	counts	No	No	Yes
ExplorationCounts_ WhiteZone_Block01	Exploration counts in the white zone 0-1 min	counts	No	No	Yes
ExplorationCounts_ WhiteZone_Total	Total exploration counts in the white zone	counts	No	No	Yes

MovementCounts_ BlackZone_Block01	Movement counts in the black zone 0-1 min	counts	No	No	Yes
MovementCounts_ BlackZone_Total	Total movement counts in the black zone	counts	No	No	Yes
MovementCounts_ GreyZone_Block01	Movement counts in the grey zone 0-1 min	counts	No	No	Yes
MovementCounts_ GreyZone_Total	Total movement counts in the grey zone	counts	No	No	Yes
MovementCounts_ WhiteZone_Block01	Movement counts in the white zone 0-1 min	counts	No	No	Yes
MovementCounts_ WhiteZone_Total	Total movement counts in the white zone	counts	No	No	Yes
ZoneTime_ BlackZone_Block01	Time spent in the black zone 0-1 min	sec	No	No	Yes
ZoneTime_ BlackZone_Total	Total time spent in the black zone	sec	No	No	Yes
ZoneTime_ GreyZone_Block01	Time spent in the grey zone 0-1 min	sec	No	No	Yes
ZoneTime_ GreyZone_Total	Total time spent in the grey zone	sec	No	No	Yes
ZoneTime_ WhiteZone_Block01	Time spent in the white zone 0-1 min	sec	No	No	Yes
ZoneTime_ WhiteZone_Total	Total time spent in the white zone	sec	No	No	Yes