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

Light Dark Assay
LD v1.0

Area: G3	JAX-CSNA-BPC

Controls:	
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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: _____5/1/2017_____
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: _____reviewed 1/1/2019_____

The Jackson Laboratory –Center for Systems Neurogenetics of Addiction – Behavioral Phenotyping Core

1. PURPOSE

This SOP addresses the routine procedures used for conducting the light dark 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/Technical Manager of CSNA

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
Light Dark Arena	An open field measuring 44.5 cm x 44.5 cm x 25.4 cm with an insert constructed of black polycarbonate that divides the field into half with a dark zone and light zone
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).
Dark Zone	The half of the arena covered with a black polycarbonate insert that reduces the light levels.

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Light Zone	The half of the arena not covered with a dark insert where light level is approximately 14 lux.
Ambulatory Distance	The total number of centimeters (cm) traveled in the arena throughout the duration of the test as defined by the instrument.
Time In the Light Zone	The amount of time (seconds) spent in the light zone of the arena
Time in the Dark Zone	The amount of time (seconds) spent in the dark zone of the arena
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.
Zone Latency	Time (seconds) of the subject mouse to the first transition into the dark side from the light side
Vertical Activity	Cumulative vertical beam breaks for the duration of the entire session.
Zone Entries	Number of transitions between the light and dark. (measured per defined time bin)
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). For the present protocol the total experiment duration is XX minutes

4.2. Procedure Inputs

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

4.3. Procedure Outputs

Procedure Name	Description	Type	Format
LightDark	Zone Latency– Time to the first transition to the dark (s)	Outputs	Zone Latency Bin # Zone #
LightDark	Zone Entries – number of transitions between the light and dark. (measured per defined time bin)	Outputs	Zone Entries Bin # Zone #
LightDark	Time in the Light	Outputs	Duration Bin # Zone 1
LightDark	Time in the Dark	Outputs	Duration Bin # Zone 2

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 in length, 17.5 in width, 10.0 in 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 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 illuminate 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 all mice transfers.

5.1.5. LightDark Box: An insert constructed of black polycarbonate material that does not interrupt infrared beams (Med Associates) and is placed into the back half of the open field arena with the entry door opening positioned toward the front center of the arena; thereby reducing the light levels within the dark side of the box.

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". 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± 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 two 28 V lamps and measures 14 lux at the testing floor in the light zone. Light lux levels are validated monthly.

6.1.6   Noise. The ambient background noise level in the procedure room and within the chambers 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. 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

6.1.1   Species. Mice

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

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

6.1.2   Sex. Males or females

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

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

6.1.5   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. Light Dark testing is scheduled on Tuesdays.

6.1.6   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.

6.1.7 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.

6.2 Testing

6.2.1 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 6.2.4. below. Enter test day information in the laboratory notebook in the room, as indicated in the lab notebook.

6.2.2 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.

6.2.3 System Set up.

6.2.3.1 Power on both computers, fans, lights, and the interface cabinets (note that the power switches to the fans and lights are located on the back right side of each control unitand the Interface Cabinets green toggle switch is located on the front right side of thecabinet).

6.2.3.2 Dark Box Inserts. Place a dark box insert into the back half of each chamber, with the entry door to the dark box facing the center of the chamber. The walls should be aligned with the chamber walls to ensure that beam breaks are accurately recorded.

6.2.3.3 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).

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

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

6.2.3.3.3 Click *Configure* button.

6.2.3.3.4 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*. Confirm the "Hole Board Installed" check box is <u>not</u> checked. Click *Apply* and *Ok.*

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

6.2.3.3.6 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. Then ensure the test indicates that beams are being broken by running a hand along the bottom of each chamber.

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

6.2.3.4.1 Under File select *Open Project*

6.2.3.4.2 In file menu (This PC > Documents) select the project that contains the LightDark protocol

6.2.3.4.3 In the Project box click *Acquire Data*...

6.2.3.4.4 The Acquire Data window will open. Under Protcol select the protocol that has LightDark acquisition settings with a duration of 20 minutes with the task floor not in use. Verify the time bins are set at 120 sec time bins.

6.2.3.4.5 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*.

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

6.2.4   Placement into the arena. At the start of each testing session, subjects are individually placed into the center of the arena, on the light side, facing the rear of the chamber (facing the dark side). 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.

6.2.4.1   Subjects should be placed into chambers in sequential chamber numbering order. As each subject is handled just prior to placement, the tester should briefly examine each mouse and health concerns should be noted in the run sheet.

6.2.5   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.

6.2.6   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.

6.2.7   Repeat 6.2.1 through 6.3.6 for subsequent groups of mice.

6.2.8   At the conclusion of all testing for the day, the subjects are returned to the housing room and the arenas are sanitized with Virkon with a 10 minute contact time, followed by 70% ETOH to remove any Virkon residue.

6.3   Data Analysis and QC

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

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

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

      6.3.1.3   When the data is finished being analyzed, select spreadsheet and excel from the drop down menues above the displayed data. The click Open to open the Excel (CSV) file. Save this file with the name format "Test_CU#_yearmonthday.csv" (LD_CU1_20181119.csv).

    6.3.2   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.

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

    6.3.4   Resulted analyzed

      6.3.4.1   Data are analyzed over time bins as total distance traveled (cm), , and related AUC (as defined in the glossary 4.0 above).

      6.3.4.2   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.

7.0 **Variables.**

    7.1   The following variables are identified per bin (2 minute time bin).

Trait	Description
total.distance.traveled.light	Light-Dark Box total distance traveled in light compartment
total.distance.traveled.dark	Light-Dark Box total distance traveled in dark compartment
total.distance.traveled	Light-Dark Box total distance traveled
pct.distance.traveled.in.light	Light-Dark % distance traveled in light compartment
total.ambulatory.time.light	Light-Dark Box total seconds spent in light compartment
total.ambulatory.time.dark	Light-Dark Box total seconds spent in dark compartment
pct.time.in.light	Light-Dark Box % time in light
total.transitions	Light-Dark Box transitions
Ambulatory.Time.Bin.1.Zone.1	Light-Dark total time spent traveling 0-2 min in light compartment

Ambulatory.Time.Bin.1.Zone.2	Light-Dark total time spent traveling 0-2 min in dark compartment
Ambulatory.Time.Bin.1	Light-Dark total time spent traveling 0-2 min
Ambulatory.Counts.Bin.1.Zone.1	Light-Dark total number of beam breaks 0-2 min in light compartment
Ambulatory.Counts.Bin.1.Zone.2	Light-Dark total number of beam breaks 0-2 min in dark compartment
Ambulatory.Counts.Bin.1	Light-Dark total number of beam breaks 0-2 min
Ambulatory.Distance.Bin.1.Zone.1	Light-Dark total distance traveled 0-2 min in light compartment
Ambulatory.Distance.Bin.1.Zone.2	Light-Dark total distance traveled 0-2 min in dark compartment
Ambulatory.Distance.Bin.1	Light-Dark total distance traveled 0-2 min
Ambulatory.Episodes.Bin.1	Light-Dark total number of beam breaks before coming to rest 0-2 min
Ambulatory.Episodes.Average.Speed.Bin.1	Light-Dark total number of beam breaks before coming to rest Average Speed 0-2 min
Stereotypic.Time.Bin.1.Zone.1	Light-Dark total time spent in stereotypic behaviors 0-2 min in light compartment
Stereotypic.Time.Bin.1.Zone.2	Light-Dark total time spent in stereotypic behaviors 0-2 min in dark compartment
Stereotypic.Time.Bin.1	Light-Dark total time spent in stereotypic behaviors 0-2 min
Stereotypic.Counts.Bin.1.Zone.1	Light-Dark total number of stereotypic behaviors 0-2 min in light compartment
Stereotypic.Counts.Bin.1.Zone.2	Light-Dark total number of stereotypic behaviors 0-2 min in dark compartment
Stereotypic.Counts.Bin.1	Light-Dark total number of stereotypic behaviors 0-2 min
Jump.Time.Bin.1.Zone.1	Light-Dark total time spent jumping 0-2 min in light compartment
Jump.Time.Bin.1.Zone.2	Light-Dark total time spent jumping 0-2 min in dark compartment
Jump.Time.Bin.1	Light-Dark total time spent jumping 0-2 min
Jump.Counts.Bin.1.Zone.1	Light-Dark total jumps 0-2 min in light compartment
Jump.Counts.Bin.1.Zone.2	Light-Dark total jumps 0-2 min in dark compartment
Jump.Counts.Bin.1	Light-Dark total jumps 0-2 min

Resting.Time.Bin.1.Zone.1	Light-Dark total time spent resting 0-2 min in light compartment
Resting.Time.Bin.1.Zone.2	Light-Dark total time spent resting 0-2 min in dark compartment
Resting.Time.Bin.1	Light-Dark total time spent resting 0-2 min
Vertical.Time.Bin.1.Zone.1	Light-Dark total time spent rearing 0-2 min in light compartment
Vertical.Time.Bin.1.Zone.2	Light-Dark total time spent rearing 0-2 min in dark compartment
Vertical.Time.Bin.1	Light-Dark total time spent rearing 0-2 min
Vertical.Counts.Bin.1.Zone.1	Light-Dark total rears 0-2 min in light compartment
Vertical.Counts.Bin.1.Zone.2	Light-Dark total rears 0-2 min in dark compartment
Vertical.Counts.Bin.1	Light-Dark total rears 0-2 min
Duration.Bin.1.Zone.1	Light-Dark total time spent 0-2 min in light compartment
Duration.Bin.1.Zone.2	Light-Dark total time spent 0-2 min in dark compartment
Duration.Bin.1	Light-Dark total time spent 0-2 min
Zone.Entries.Bin.1.Zone.1	Light-Dark total zone entries 0-2 min in light compartment
Zone.Entries.Bin.1.Zone.2	Light-Dark total zone entries 0-2 min in dark compartment
Zone.Entries.Bin.1	Light-Dark total zone entries 0-2 min
Zone.Latency.Bin.1.Zone.1	Light-Dark latency to enter zone 0-2 min in light compartment
Zone.Latency.Bin.1.Zone.2	Light-Dark latency to enter zone 0-2 min in dark compartment
Zone.Latency.Bin.1	Light-Dark latency to enter zone 0-2 min