Light-Dark Test

Version: 1/Sept 2023
Edited by: Lynette Bower/Louise Lanoue

Summary:

The light/dark transition test is used to monitor anxiety-like behavior in mice. Mice are placed in the light side of an arena that consists of 2 chambers, one light and one dark. The test measures the latency an to enter the dark chamber and the time spent in the respective dark and light chambers over 20 min. Typically, mice will tend to avoid the light chamber and the times spent in each chamber is an index of anxiety-like behavior.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Opto-Varimex 4 Systems w. infra-red beams	Columbus Instruments	
Light-Dark boxes	Columbus Instruments	
Auto Trak 5 Software	Columbus Instruments	
Room with fluorescent lighting		
Lab coats/gloves/PPE		
Disinfectant	Nolvasan 10%	
Disinfectant	Coverage Plus	

Protocol:

1. SET-UP (acclimation of mice, calibration of room light, software setup)

- **a.** Acclimate mice in quite room for 30 min prior to testing. **NOTE: Test males and females separately, running males first.**
- **b.** Insert the Light-Dark boxes onto the open field arena, with the light-side on the left side of the arena and the dark-side on the right side of the arena. Make sure the floor legs are sitting in the designated arenas indentations and the partition inserted into its designated slot in the middle of the box.
- **c.** Set room lights set at 70.00 Lux measured on tabletop. Set OptoVarimex Chamber lights between 150-200 Lux measured with light meter on chamber floor pointing up. Light metering should be performed at each test day.
- **d.** Turn analyzer system on. Be sure all lights are on (green).

e. Configure OptoVarimex program.

2. CONFIGURATION of OPTOVARIMEX

a. Open Auto Track 5 program and select "Setup New Experiment". This should activate the chambers lights blinking).
b. Click on Data filename and enter the file name. Save the file in Project folder.
c. Under Experiment Start Mode, select the "Start on Position" ; this will enable the recording of motion detected inside the arena.
d. Set experiment length to 00 hours, 20 minutes.
e. Enter the mouse id under Subject ID by clicking the box you want to change. Make sure to *uncheck* the "enabled" checkbox for any arenas you are not using if more than one system is run.
f. Press the Start button under "Experiment" when you are ready to start the experiment. After you hit Start, the "Action" column will populate with start buttons if you need to manually start or override an arena.

3. PROCEDURE

a. Take mouse from its cage and place in the light side (LEFT) of the arena facing the left wall. The software should automatically start tracking as soon as it detects a mouse in the arena. Do this for all mice if running more than one system.
b. Check the software "Status" column to ensure timers are running for all the cages and leave the room.
c. Return in 20 minutes when the run is finished.
d. Remove animals and return to home cages. Thoroughly clean the arenas with 10% Nolvasan before the next run. At the end of the testing, clean arenas with Coverage Plus.
e. Click Analyze in the Auto Trak software window.
f. Under the "Map" column, look for any entries that say "No Map", click those and select the "KOMP" map checkbox under the Settings tab.
g. Set the Bin Length to 300 (5 minutes).
h. In the top menu bar, click "File" then "Export All Subjects" then "All Analysis". This will export the CSV in the same folder where you saved the experiment file.