

Novel Object Location and Recognition Protocol

Objects: 50 mL conical tubes, pyramids and spice jars with plastic cones, OF arena, Blue Strip of plastic

Protocol: Use blue strip standing vertical on one side of arena for days 1-4

Day 1: 1-hour empty arena with no cue - all mice from same cage in one arena

Day 2a: Test one mouse per arena for ten minutes each. Use 2 identical objects: A, A'

Day 2b: 1 hour intersession delay to test Object Location Memory (OLM), i.e. move one of the objects to a new location. Preferably, move the object investigated for less time

Day 3a: 24 hr Novel Object Recognition (NOR) with ten-minute trial: 24-hour testing for NOR (A vs

Day 3b: Then, 1 hour after testing, (optional) using with B vs C, where B is the object less investigated for the 24-hour test, 10 minutes per trial. 3B is testing 1 hour object recognition.

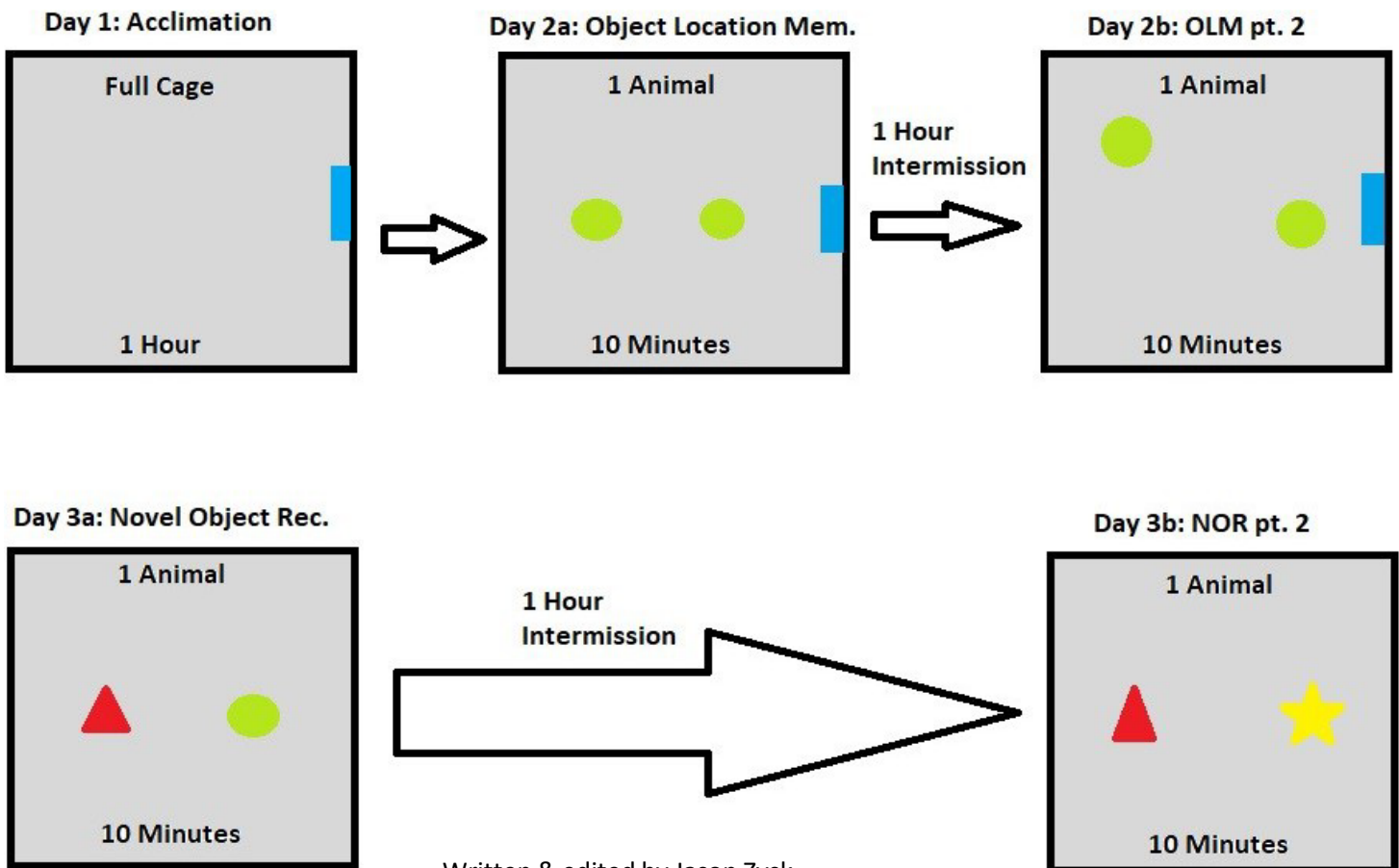
Set up all 4 chambers with appropriate zoom/focus on the cameras.

- Wipe down each chamber before and after each animal with 70% ethanol
- Use white light plus extra LED light bulb and keep the shades up
- Allow 15-60 minutes for animal room acclimation

Open Limelight 4 Software

- File -> Open data file
 - Create a new file
 - Make sure there is space available on the desktop. See bottom right corner for GB available
- Double click on the chamber you want to set up
 - Make sure you zoom in enough where you can see the outline of the OF box and the target is in focus.
 - Set your grid resolution to a 2x2 grid (can be changed during analysis)
 - Click on the find cursors button and set up the [blue grid lines](#)
 - Set the view size:
 - Chamber 1: 85 cm
 - Chamber 2: 84 cm
 - Chamber 3: 80 cm
 - Chamber 4: 81 cm
- Click OK and move onto the next chamber until you are set up to begin your experiments
- Under duration enter appropriate duration for test day
- Enter each animal ID
- Take a reference with no animal inside the chamber box
- Grab your animal and place in the top left corner of the box
- Press Start; Do the same for all chambers

- When the trial is over, the animal ID changes to yellow. Grab the mouse and wait for the rest of the animals to finish their session
 - Wipe down the boxes with ethanol. Reset the boxes in case they moved during cleanup. Enter new animal IDs and repeat for the steps for the set of animals
 - When done with experiment, wipe down all the OF boxes and return in proper place
 - Transfer your data off of the computer and into your BPC server folder
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- **Day 1:** Every mouse in each cage is placed in the box with the blue plastic cue on the right side. The mice are given 1 hour to explore the area with no objects present.
 - **Day 2a:** 1 mouse is placed in each box with 2 identical objects (A, A') aligned with the blue plastic cue. Each mouse is given 10 minutes to investigate the objects.
1 HOUR INTERMISSION
 - **Day 2b:** 1 mouse is placed in each box with 2 identical objects (A, A') where the object farther from the blue plastic cue is moved slightly up in the box. each mouse is given 10 minutes to investigate.
 - **Day 3a:** 1 mouse is placed in each box with 1 original object (A or A') and 1 novel object (B). It should be similar size but distinctly different and aligned similar to day 2a but without the blue plastic cue.
1 HOUR INTERMISSION
 - **Day 3b:** 1 mouse is placed in each box with the added object from 3a (B) and a new novel object (C). Align them the same way as 3a without the blue plastic cue.



Tests for 24hr NOR

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Tests for 1 hour NOR

