Novel object recognition test
By Xuansong Mao

Necessary pilot experiments

1. Testing for induced reference
2. For habituation, remove the mouse from its home cage and plate it in the middle of the open arena. Allow the mouse to freely explore for 5 min
3. On training day (T1, place 2 different objects in opposite quadrants of the apparatus. Remove the mouse from its home cage and place it in the middle of the open arena. Allow free exploration for 10 min.
4. Calculate the discrimination index. If there is no induced preference, the discrimination index should be at or near zero. Any objects that show preference should not be used for NOR test.

Experiment

1. For habituation session (T0), remove mouse from its home cage and plate it in the middle of the open arena. Allow the mouse to freely explore for 5 min.
2. 24 hrs after T0, on training session (T1), place 2 identical objects in opposite quadrants. Place the mouse in the center of the arena and allow the mouse to explore for 10 min.
3. 60 min after T1, on testing session (T2), place one of the objects used during T1 and one novel object in opposite quadrants. Placing the mouse in the center of the arena and allow free exploration for 10 min.

Note: **Thoroughly clean the apparatus between mice using 70% vol/vol ethanol.** During habituation, anxiety-like behavior can be assessed by calculating time spent in the center. This is a useful metric when considering the length of time for T1. Higher anxiety mice may require a 10 min session to reach the minimum exploration criterion.

4. For both T1 and T2, score the first 5 min. If the mouse does not meet the minimum exploration time of 20 s for both objects, continue scoring past 5 min until total exploration exceeds 20 s.

E1 is the total exploration time during training for 2 identical objects, where a1 and a2 are the identical objects,
E1=a1+a2

E2 is the total exploration time during testing for familiar object (a) and the novel object within 5 min.
E2=a+b

Relative discrimination index= (b-a)/E2