



3-chamber Sociability & Social Novelty test

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Ref: Kaidanovich-Beilin JOVE 2011.

Summary:

The test is used to study social affiliation and social memory in mice. The test measures the time a subject mouse spends in any of three box's compartments during two experimental sessions of 10 min. The test evaluates both sociability, defined as the propensity of a typical mouse to spend time with another mouse, as compared to time spent alone in an identical but empty chamber, and social novelty, defined as propensity to spend time with a previously unencountered mouse rather than with a familiar mouse.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Plexiglas arena (40 x 60 cm) with 2 plexiglas divider walls (creating 3 rectangular chambers)		
2 Plexiglas confinement cylinders (10 x 20 cm)		
2 Control mice	See Supplementary Notes	
Holding cage		
Camera (GigE or WebCams)	Bassler or Microsoft, etc	
Tracking Software (EthoVision XT)	Noldus	
Animal marker-black	Stoelting	50451-1
Lab Coat/Gloves/PPE		
Paper towels		
70% ethanol		
Disinfectant	Nolvasan 10%	

Protocol:

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

- a. Acclimate mice (both subjects and 2 control supplementary mice) in the testing room for 30 min but not more than one hour prior to testing; record time of transfer.
- b. Turn the side overhead white lights; center overhead lights should remain off. Turn on/connect the camera to the ethernet port of computer.

- c. Open Ethovision software and the configuration file for the Social Novelty test and place the arena so that the confinement cylinders aligned with the zone map. The arena should be 44 in above ground.
- d. Use balanced test order for the experiment. If more than one mouse per cage is going to be tested, place the mice in a clean cage after testing until all are tested, then return to home cage.

SUPPLEMENTARY NOTES:

- The camera is mounted at an angle of approximately 90 degrees and centered to facilitate the best viewing conditions:
- The 2 control mice should be the same background, age, weight and gender than test mice. These control mice should not be littermates or had any prior contact with the subject mice. They can be used for the whole session as long as they do not have interactions with subject mice.
- If white mice are being run, they need to have a black stripe painted with animal Marker from head to rump so that the tracking will work. If the experiment includes mice of various colors, all mice should go through the marking process even if they don't need the mark for tracking.

2. PROCEDURE

Acclimation (5 min)

- a. Make sure the confinement cylinders are on the left and right sides of the test arena according to the zone map.
- b. Insert the sliding doors in each dividing wall, thereby blocking access of the left and right chambers.
- c. Place the subject mouse in the center arena and allow acclimation for 5 minutes.

Trial 1 Social Affiliation (10 min)

- a. Following acclimation, retrieve 1 control mouse (stranger 1) from holding cage and place in the left cylinder. Right cylinder remains empty.
- b. Remove the sliding doors and immediately click once on **"Start"** to begin video tracking.
- c. Click the red record button to start recording the video.
- d. Leave the test room and make sure the door is closed. Monitor the recording session patiently and quietly.
- e. Once the trial is over, manually stop the video by hitting the same record button and click the save button at the top of the program screen to save data.
- f. Return subject mouse to the center of the arena and replace the sliding doors.

Trial 2 Social Novelty (10 min)

- a. Following trial 1, retrieve the second control mouse (stranger 2) from holding cage and place in the right cylinder. The first mouse (stranger 1) remains in the left cylinder.
- b. Retrieve the subject mouse and place in the middle chamber of the test arena.
- c. Remove the sliding doors and immediately click once on **"Start"** to begin video tracking.

- d.** Click the red record button to start recording the video. When the run is over, manually stop the video by hitting the same record button.
- e.** Leave the test room and make sure the door is closed. Monitor the recording session patiently and quietly.

Clean-up

- a.** Return all mice to their respective home cage.
- b.** Clean the test arena and cylinders with 70% ethanol then with wipe down with nolvasan. Repeat the process for subsequent mice.