

## Social Behaviour Tests

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**Note:** The Social Behaviour Tests are directly from an approved SOP.

**Remarks:**

- Allow two hours adaptation to the test room before starting the Habituation or Test
- Lighting condition: Red light (using the heating red light bulb)

### 1 SOCIAL ANXIETY/SOCIAL COGNITION TEST

1. Note

- Stimulus animals: 2 different male controls, called S1 and S2.
  - \* Each stimulus mouse will be confined in a small, overturned circular wire enclosure with bars spaced 1 cm apart. Each enclosure is 11 cm high and 10 cm in diameter at the bottom. A clear glass beaker is placed on top to each wire enclosure to weigh it down and prevent the experimental subjects from climbing on top of it.
  - \* The wire enclosures allow nose and paw contact but prevent fighting.
  - \* Testing is completed in a single session lasting 25 min. The test session comprises 2 consecutive phases following a re-habituation phase.

2. Habituation of stimulus subject

- On the day before testing, each mouse is allowed to freely explore the apparatus for 25 min.
- The 2 small wire enclosures for the stimulus subjects will be present but empty.
- Each stimulus subject will be confined to one of the small wire enclosures for 30 min each day for 7 days prior to the start of the study to habituate them to the confinement procedure.

3. Rehabituation of test subject (5 min):

- The test subject is placed in the center chamber and allowed free access to the entire apparatus for 5 min. The wire enclosures are present but empty.

4. Test - Phase 1 Sociability (10 min)

- An unfamiliar male subject is placed into one of the two wire enclosures while the test subject is confined to the center chamber.
- The left or right location of the stimulus subject is counterbalanced across the 2 genotypes. The wire enclosure in the other chamber is present but empty.
- The test subject is allowed to explore the entire apparatus for 10 min.
- The goal of this phase is to determine whether experimental subject prefers to be with an unfamiliar conspecific or an empty wire enclosure, as well as to observe the nature of the social interaction.

5. Test - Phase 2 Preference for Social Novelty (10 min)

- The second stimulus animal is placed in the wire enclosure on the opposite side of the apparatus, while the test subject is confined to the center chamber.
- The stimulus subject that had been present during Phase 1 is referred to as the "familiar" animal and the one introduced in Phase 2 is called the "novel" animal. For the test subjects exposed to S1 in the sociability phase, S2 is the novel animal and vice versa.
- The test subject is allowed to explore freely for 10 min.
- The goal of this phase is to assess whether the experimental animal prefers to be with a novel or with a conspecific animal.

6. Behaviour coding: all trials will be videotaped for coding.

- The apparatus is divided into 18 identical squares. The four squares surrounding the cage on each side are named "Social" areas and all other squares are "Non-social" areas.
- The coded behaviours include nose contact with cage, rearing on cage, grooming and climbing.
- Square crossings will be recorded to quantify activity levels.