

Video Article

Assessment of Social Interaction Behaviors

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Abstract

Social interactions are a fundamental and adaptive component of the biology of numerous species. Social recognition is critical for the structure and stability of the networks and relationships that define societies. For animals, such as mice, recognition of conspecifics may be important for maintaining social hierarchy and for mate choice¹.

A variety of neuropsychiatric disorders are characterized by disruptions in social behavior and social recognition, including depression, autism spectrum disorders, bipolar disorders, obsessive-compulsive disorders, and schizophrenia. Studies of humans as well as animal models (e.g., *Drosophila melanogaster*, *Caenorhabditis elegans*, *Mus musculus*, *Rattus norvegicus*) have identified genes involved in the regulation of social behavior². To assess sociability in animal models, several behavioral tests have been developed (reviewed in³). Integrative research using animal models and appropriate tests for social behavior may lead to the development of improved treatments for social psychopathologies.

The three-chamber paradigm test known as Crawley's sociability and preference for social novelty protocol has been successfully employed to study social affiliation and social memory in several inbred and mutant mouse lines (e.g.⁴⁻⁷). The main principle of this test is based on the free choice by a subject mouse to spend time in any of three box's compartments during two experimental sessions, including indirect contact with one or two mice with which it is unfamiliar. To quantitate social tendencies of the experimental mouse, the main tasks are to measure a) the time spent with a novel conspecific and b) preference for a novel vs. a familiar conspecific. Thus, the experimental design of this test allows evaluation of two critical but distinguishable aspects of social behavior, such as social affiliation/motivation, as well as social memory and novelty. "Sociability" in this case is defined as propensity to spend time with another mouse, as compared to time spent alone in an identical but empty chamber⁷. "Preference for social novelty" is defined as propensity to spend time with a previously unencountered mouse rather than with a familiar mouse⁷. This test provides robust results, which then must be carefully analyzed, interpreted and supported/confirmed by alternative sociability tests. In addition to specific applications, Crawley's sociability test can be included as an important component of general behavioral screen of mutant mice.

Video Link

The video component of this article can be found at <http://www.jove.com/video/2473/>

Protocol

1. Equipment and Room Set Up:

1. The apparatus for Crawley's sociability and preference for social novelty test is comprised a rectangular, three-chamber box. Each chamber is 19 x 45 cm and the dividing walls are made from clear Plexiglas, with an open middle section, which allows free access to each chamber.
2. Use two identical, wire cup-like containers with removable lids that large enough to hold a single mouse. These are placed vertically inside the apparatus, one in each side chamber, and will contain the naïve/unfamiliar mouse. Each container is comprised of metal wires to allow for air exchange between the interior and exterior of the cylinder but small enough to prevent direct physical interactions between an animal on the inside with one on the outside.
3. Behavioral testing should be performed between 9:00am and 6:00 pm.
4. General room lighting is 650 lux.
5. Employ Observer 6.0 (Noldus) program (or a suitable alternative) to monitor and record the observation and follow up parameters, mentioned in section 4.3.
6. After each trial, clean all chambers with 70% ethanol (between mice) and then with Clidox 1:5:1 (between the cages) to prevent olfactory cue bias and to ensure proper disinfection, respectively.

- The person who makes the observation has to be at least 2 meters away from the apparatus.

2. Animal Preparation:

- House three to five mice per cage in a room with a 12 hr light/dark cycle (lights on at 7:00 am) with *ad libitum* access to food and water, according to standards and requirements of local Animal Care Committee guidance and protocols.
- Transfer all the cages containing mice into the behavioral room 30 minutes before the first trial begins.
- There are two classes of mouse required for this experiment, one which acts as a control, naïve or "unfamiliarized" animal and one that is the test subject. For the control mouse use a mouse of the same background (usually C57BL6), age (usually 8-12 weeks old), gender and weight, without any prior contact (not littermates) with the subject mouse. Two control mice are required per experiment, one is used for session I and another for session II. The same control mice may be used between trials.

3. Habituation (adaptation):

- Isolate the right and left compartments by using the dividing Plexiglass walls.
- Place empty wire containment cups in the middle of right and left chamber (one per each site).
- Place the subject mouse at the center of the middle chamber for adaptation.
- Habituate for 5 minutes.

4. Social Affiliation Aspect of the Test (session I):

- Place one of the control mice ("Stranger 1") inside a wire containment cup that is located in one of the side chambers. The placement of Stranger 1 in the left or right side of the chamber is systematically altered between trials.
- Remove the walls between the compartments, to allow free access for the subject mouse to explore each of the three chambers.
- Immediately, begin monitoring and recording the following parameters:
 - duration and
 - number of direct (active) contacts between the subject mouse and the containment cup housing or not housing the Stranger 1 mouse, for each chamber individually. Direct contact between the subject mouse and the containment cup, or stretching of the body of the subject mouse in an area 3-5 cm around the cup is counted as an active contact;
 - duration and
 - number of other behaviors by the subject mouse in each compartment, including walking, self-grooming, lack of any body movements for more than 5 seconds ("freezing"), as well as unusual behavior, such as jumping, repetitive behavior, etc;
 - duration and
 - number of entries to each compartment. The mouse is considered to be in the chamber when its head and four paws have entered into the chamber.
- The duration of session I is 10 minutes.

5. Social Novelty/preference Session of the Test (Session II):

- Place a second control mouse ("Stranger 2") inside an identical wire containment cup in the opposite side chamber (that had been empty during the Session I). Monitor the same parameters described in 4.3, differentiating the behaviors between the subject mouse in the presence of Stranger 1 compared with Stranger 2.
- The duration of session II is 10 minutes.

6. Statistical Analysis:

- By using the Origin 6.0 program (or a suitable alternative) analyze the following parameters, using the recorded data:
 - the total number of contacts and
 - total duration of contacts between experimental mouse and empty containment cup vs or cup housing Stranger 1 (in session I), or between experimental mouse and the cup housing Stranger 1 vs Stranger 2 (in session II);
 - mean duration per contact;
 - total number and duration of other behaviors (freezing, self-grooming, walking)
 - total time spent by the subject mouse in each compartment.
- Analyze the significant differences for each of the aforementioned parameters, by comparing the groups:
 - "Empty" containment cup vs "Stranger 1" for a wild type (WT) subject
 - "Empty" containment cup vs "Stranger 1" for the experimental mouse (e.g. a knockout, transgenic or drug-treated mouse, etc.).
 - "Stranger 1" vs "Stranger 2" for a WT subject
 - "Stranger 1" vs "Stranger 2" for the experimental mouse (knockout, transgenic, or drug-treated, etc.).
- Present each dataset of the aforementioned parameters as Session I: WT and experimental subjects; Session II: WT and experimental subjects, as shown in Figure 1(A and B) and 2(A and B), respectively.

It is also advisable to take into consideration other parameters, which help in standardizing this test, such as prior experience in recognizing specific mouse behavioral patterns, such as sniffing, grooming, rubbing, fighting, mounting, which can be largely dependent on subjective

personal observation. The person acting as the recorder of the behavioral parameters during the test should be blind to the genotype or treatment conditions and this information decoded afterwards.

7. Representative Results:

An example of the two steps of the social interaction test is shown in Figures 1 and 2. The first session of the test allows estimation of social affiliation and motivation of the subject mouse. In this part of the test, the stranger mouse was enclosed in a circular wire cup that allowed nose contact, but prevented fighting between mice. These conditions ensure that social approaches are initiated by the subject mouse and are investigatory only, without direct physical contact. The mouse has the choice to spend more time around the containment cup containing the stranger mouse or to avoid any contact by moving to the compartment with the empty cup. Other parameters, such as number of individual contacts, can be used in addition to duration spent near to the stranger mouse.

Typically, a wild type animal will spend significantly more time in the compartment with Stranger 1 compared to the compartment with empty cup (Figure 1A), indicating normal sociability, social motivation and affiliation. In GSK-3 α knockout mice, for example, there are no significant differences between the time spent with empty cup compared to the cup containing stranger 1 (Figure 1B), indicating impaired sociability in these animal model⁶.

The second session of the test is designed to estimate social novelty and social memory. In this part, the subject mouse has a free choice between the first, already-investigated, unfamiliar mouse (Stranger 1), and a novel unfamiliar mouse (Stranger 2). Usually, a WT animal recalls its previous contact with Stranger 1, and, in this session, tends to spend more time with the newly encountered mouse (Stranger 2), indicating intact social memory and predilection for novel experiences (Figure 2A).

Unlike wild-type mice, GSK-3 α mutant animals do not show preference for the chamber containing a newly introduced mouse (Stranger 2) over a chamber containing a now familiar mouse (Stranger 1) in session II (Figure 2B). Indifferent behavior of KO mice in this test is indicative of decreased social motivation and novelty.

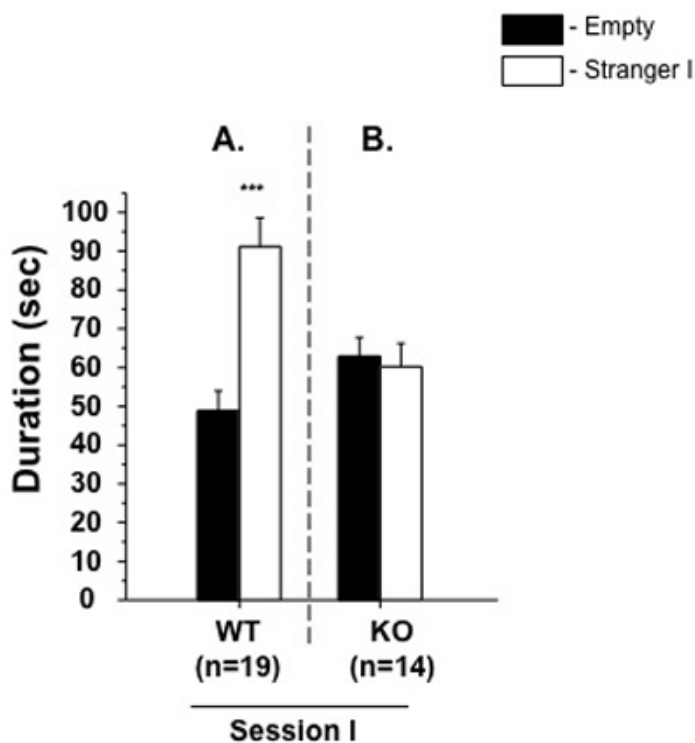


Figure 1. Session I: Social affiliation and sociability. Mean length of time (\pm SEM) in the chamber with the stranger ("stranger side") compared to the opposite chamber ("empty side"). Unlike WT males (A), KO (B) animals failed to demonstrate a preference for social proximity by spending same time in both chambers. *** $p \leq 0.0001$ in WT group.

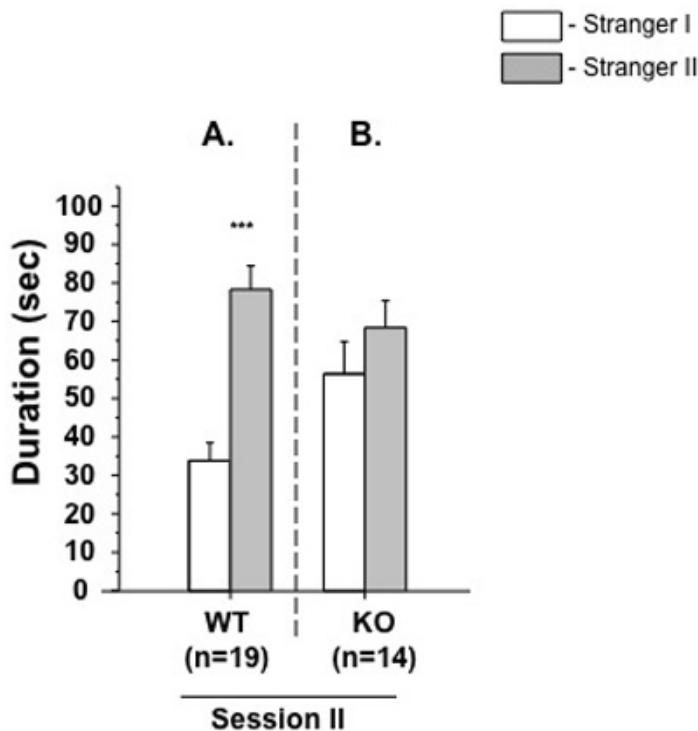


Figure 2. Session II: Social memory and novelty. Mean duration of time (\pm SEM) in the chamber with the unfamiliar mouse from the sociability phase ("Stranger 1") and in the opposite chamber with a new unfamiliar mouse ("Stranger 2"). Unlike WT males (A), KO (B) animals failed to demonstrate a preference for social novelty by spending same time in both chambers. *** $p \leq 0.0001$ in WT group.

Discussion

Mus musculus is a social species that engages in high levels of reciprocal social interactions, communal nesting, sexual and parenting behaviors, territorial scent marking and aggressive behaviors⁸⁻¹⁰. Several tests have been developed to examine social behavioral in mice (reviewed in³). Here, we present a widely employed test to evaluate sociability in mice. Crawley's sociability and preference for social novelty test was originally developed by Crawley and colleagues^{7,11}. There are several advantages to the three-chamber paradigm, which improve data reliability for examination of sociability, social affiliation, social memory and preference, using similar procedures. The main specificity of this test is the design in which a mouse, previously unencountered by the subject animal, is placed under a wire cup and unable to move freely. This prevents direct physical contact, eliminating fighting and/or aggressive behavior but allows sensory interactions (smell, sight, sound, taste). Under these conditions, the experimental mouse initiates and terminates any interactions, always having a choice between unfamiliar mouse vs empty container (in session 1), or between familiar mouse (Stranger 1) vs the novel mouse (Stranger 2). For example, in one-chamber social interaction tests, the subject mouse is exposed to a stranger in the same chamber, and since both mice are able to move freely the aforementioned active physical contact tends to be dominant and cannot be eliminated. The three-chamber apparatus is much larger than a typical single chamber device, which allows the subject mouse to remain in proximity to or move far away from the Stranger mouse. Thus, the stress level of Crawley's sociability test is low and mostly caused by placing the experimental mouse into a novel environment, which allows repetition of this test over time, for example at different ages of the same mouse cohort: juvenile vs adult. By comparing a young group of animals with the same but older cohort, it is possible to follow up and discriminate between impairment in social recognition vs social memory and/or cognitive impairments, which can be observed in Session 2, and be related to age-dependent physiology.

This test is relatively time-consuming (~40 minutes per mouse), which is a disadvantage. However, multiple repertoires of specific parameters can be monitored and recorded (manually and/or video), permitting robust analysis. For example, some of additional parameters, such as freezing and/or self-grooming behavior, may indicate increased anxiety and decreased motivation for social behaviour. By using the same protocol, mice can be retested in the next day with the same Stranger 1 mouse to rule out an effect of social anxiety. Additional parameters (such as tail rattling, biting of the wire cup where Stranger 1 is located) can be helpful in distinguishing aggressive versus unusual friendliness behavior towards the novel animal. Observed anxiety can be due to inappropriate adaptation of the mice to the experimental room, excessive room lighting, poor mouse health, or as a result of genetic modification and/or neophobia (in some KO models). Moreover, impaired habituation may be interpreted as a cognitive impairment, and may affect the results of second session. Before subjecting mice to this test, it is important to subject animals to general health and neurological screening, including body temperature and weight, coat examination, and the series of following reflex examinations: Whisker, Righting, Ear-Twitch, Reaching and Key Jangling (described in¹²⁻¹⁵). Additional behavioral tests can be helpful in discriminating specific responses. An increased number of contacts and/or an increase in the total duration of active contacts maybe associated with hyperactive behavior of specific mouse stains, which can be confirmed by using an open field test (to measure locomotor activity). Decreased duration and/or number of contacts may be associated with depressive- and/or anxiety-like behavior, which can be evaluated by using FST, TST or EPM, zero-maze, light/dark tests, respectively (described in^{6,13,15}).

Once appropriate conditions are applied and significant differences observed, there is a requirement to perform follow-up tests, in order to confirm the findings. Olfaction plays a key role in social recognition in rodents, since either chemically induced anosmia or removal of the

vomeroneasal organ blocks individual recognition^{16, 17}. Mice tend to sniff a novel odour and then quickly habituate to its novelty. The olfactory bulb test can be used for this purpose (described in^{6, 7}).

There are also modifications and alternative usage of three-compartment chamber sociability test, such as estimation of maternal behavior, by examining the time spent by a subject female proximal to a cup containing her pups (reflecting maternal motivation), as well as sexual motivation, by assessing the response of a tested male/female to a female/male under cup instead of Stranger 1 in session 1^{7, 11}.

Further tests can be used to examine sociability in mice by using alternative approaches/paradigms, such as social interaction in the home cage and/or in a novel environment (one-chamber social interaction test), resident intruder, partition test, social approach test, reciprocal social interaction, etc (described in^{3, 18}). Vocalizations in the ultrasonic and sonic ranges, visual cues, gustatory and tactile modalities may also contribute to communication of information and to social bonding (reviewed in¹⁹).

Impaired sociability and dysregulated social behavior has been implicated as a hallmark for many psychiatric disorders. For example, autism spectrum disorder and schizophrenia are both characterized by marked deficits in social cognition and social behavior²⁰, and disruptions of social relationships result in an increased risk of depression²¹. Thus, the described here test can be used in animal models of the aforementioned conditions. Moreover, this test is applicable for assessing potential effects of pharmacological compounds on sociability (reviewed in^{3, 22-24}).

Disclosures

No conflicts of interest declared.

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