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Research report

C57BL/6J mice fail to exhibit preference for social novelty in the three-chamber apparatus

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ABSTRACT

Laboratory models of neurodevelopmental disorders may be useful in assessing investigation and preference for social partners in mice. One such mouse model, the three-chamber test, is increasingly used as an index of social preference. The first phase measures preference for a social stimulus over an identical chamber without a stimulus mouse. The second phase measures preference for a novel mouse compared to the familiar mouse when the latter is presented in the previously empty chamber. In this study we provided an additional analysis of the second phase of the three-chamber test procedure, reversing the typical placement of the novel and familiar stimulus animals. In the first study, male C57BL/6J mice subjects encountered C57BL/6J stimuli and preferred a novel mouse over an empty chamber but failed to show a preference for the novel mouse in Phase 2 when the stimuli presentation was reversed. In an additional study, male C57BL/6J subjects encountered outbred CD-1 mice as stimuli, showing no significant novelty preference in either phase. Specific behavioral indices of investigation were similar to these duration findings with no enhancement of investigation when the novel stimulus mouse was encountered in the chamber in which the initial social stimulus was presented. These data suggest that C57BL/6J mice may show enhanced investigation/preference of novel social stimuli in the three-chamber test only when these stimuli are presented in a relatively novel context.

1. Introduction

Impairments in social behavior are characteristics of autism spectrum disorders (ASD) as well as many other neurodevelopmental and psychiatric conditions [13,17,18,22]. In particular, autism diagnoses reflect deficiencies in reciprocal social interactions, as well as repetitive or stereotyped interests or movements, and/or communication deficits [1]. Increased attention is being directed to the development and refinement of animal models of social deficits [8,12,23]. Nonhuman primate models of autism have shown promise particularly due to the intensity and complexity of primate social interactions, but the cost and ethical issues associated with their use makes this approach problematic for many researchers [2]. Social recognition, maternal behavior, nesting, scent marking, transmission of food preferences, social-conditioned place preference, ultrasonic vocalizations and social approach and interaction have been used to index sociality in mice [20,23,27] and may prove to be useful to evaluate social interactions or preferences in selectively bred or genetically altered mice.

In particular, the Sociality and Social Novelty Approach Task, or Automated Three Chamber Task, has been intensively investigated. This test (hereafter three-chamber task) has proven to provide a standardizable, simple design with a high-throughput approach to compare strains and genotypes, investigate the development of social deficits and to test effects of treatments and other manipulations on proximity and olfactory investigation of stimulus animals. Within this task, most laboratory mouse strains show an initial preference for a conspecific over a novel object, and in a latter phase, a preference for a new mouse placed in the previously empty location [7,9,14–16,19,25]. In this test, several objective behavioral measures are assessed under conditions in which the stimulus animal is restricted, effectively limiting overt forms of aggressive and sexual encounters that are possible in more open, free-interaction paradigms (e.g. [3,6,21]).

A major challenge when interpreting social approach is to precisely identify the motivation involved in preference for a stimulus

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Fig. 1. Three-chamber social approach and social novelty arena. Three interconnected chambers are separated by two manually operated sliding doors. Outside compartments contain inverted wire cups to house a stimulus mouse. A steel weight and a clear Plexiglas cylinder are placed on top of the inverted cups to prevent lifting or climbing on top. The inset shows a front view demonstrating the clear Plexiglas window on the front of the arena which permits videotaping of the dyadic interactions of the mice.

animal. Approach or proximity may reflect aggressive, sexual, novelty seeking, or ideally within these applications, pro-social or affiliative motivation on the part of the subject. Aggressive or sexual motives in this paradigm should be reduced by placement of the stimulus animals in cages to preclude direct contact and by use of juvenile or less-aggressive strains. However, such approach might also reflect investigation of the novel (non-social) context produced when a relatively large unfamiliar object, the stimulus mouse within the cage, is placed in a previously explored situation. Because it is critically important to understand the motivation of the subject animal, additional analyses of which components of the arena are eliciting approach and investigation may be warranted.

The goal of the present study was to determine if the location of the novel and familiar social stimuli in the three-chamber test modulates the apparent preference for the novel mouse in the second phase of the task in C57BL/6J (B6) mice. For half of the animals, instead of placing the novel animal in the opposite chamber, the initial (familiar) stimulus mouse was transferred to the opposite side, and replaced in the chamber in which it had initially been presented by the novel mouse. Specific social and investigational behaviors of the subject mouse were also recorded in order to characterize any differences in investigation, risk assessment, and defensiveness of the subject mouse.

2. Methods

2.1. Animals and housing

Forty 7–8-week-old male C57BL/6J mice were purchased from Jackson Labs (Bar Harbor, ME) for behavioral analysis and were allowed to acclimate to the facility for at least 7 days prior to experimentation. An additional group of 20 naïve C57BL/6J mice of the same age served as inbred stimuli. Finally, 20 adult male CD-1 mice were purchased from Charles River (Wilmington, MA) to use as outbred stimulus mice and utilized at 6–8 weeks of age. Four to five animals of a given strain were housed per cage at a temperature of $72 \pm 2\,^{\circ}$F under a 12:12 h light–dark schedule (lights on at 0600 h) with free access to tap water and lab diet. Mice were transferred to the testing room at least 30 min prior to behavioral testing which was performed between 0900 and 1700 h. All procedures were approved by the University of Hawaii's Institutional Animal Care and Use Committee according to the NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Three-chamber apparatus

A 41 cm L × 70 cm W × 28 cm H three-chambered arena was custom constructed to facilitate videotaping from the front aspect of the arena, in addition to video recordings collected from above (Fig. 1). The bottom 6.35 cm of the front panel was clear acrylic while all other walls were black (Fig. 1, inset). As subject mice were black, white Plexiglas panels were installed on the back walls and the entire arena was placed on a white Plexiglas floor to provide a contrasting background.

2.3. Behavioral testing

Sociability and social novelty preference assessment was conducted as previously described [14]. Briefly, during a 10 min habituation period, a subject mouse was placed in the middle chamber, the sliding doors were opened and the mouse given free access to the entire arena during which the duration of time in each of the two outside stimulus compartments was hand scored with stopwatches. The two outside chambers contained an inverted empty black wire cup (Galaxy Pencil/Utility Cup Spectrum Diversified Designs, Inc., Streetsboro, OH). Following the habituation phase, mice were placed back into the center, the doors were closed and a single unfamiliar male B6 (Study 1) or CD-1 mouse (Study 2) was placed in one of the two cups. The duration of time spent in each chamber was measured in a 10 min session and required all four paws to be in the compartment to be counted. Mice were tested once in Study 1 or Study 2. Between subjects, stimulus mouse placement was successively alternated between trials in this initial phase (Phase 1).

For the social novelty phase (Phase 2), the subject mouse was placed back into the center with the doors closed and a new unfamiliar mouse of the same strain as the initial stimulus was placed either in the previously empty cup (Standard Test) or the familiar stimulus animal was relocated to the previously empty cup while a novel stimulus mouse was placed in the cup the familiar mouse had previously occupied (Reversal Test). The sliding doors were opened and the location and behavior of the subject mouse was recorded for an additional 10 min.

Video collected from the front aspect of the arenas through clear Plexiglas allowed detailed scoring with Noldus Observer software (Noldus Information Technology, Wageningen, The Netherlands) of the frequency and duration of the following behaviors were recorded. Rearing was defined as an upright posture in any portion of the arena. Autogrooming was scored when the subject animal groomed any portion of its own body. Stimulus Contact was recorded when the subject mouse made contact with any part of the wire cup or the stimulus mouse. Stimulus Sniffing refers to sniffing in proximity to, and directed towards the wire cup or the stimulus mouse contained within it. Tail Rattle was defined as rapid shaking of the tail. Stretch-Attend was recorded when the animal adopted a low-back risk assessment posture. Nose-to-Nose was recorded when stimulus mice and subject mice maintained vibrissae and/or nose contact for one or more seconds. Video collected in DVD format was analyzed by a researcher blind to the condition of the mouse.

2.4. Statistical analyses

Unpaired t-tests were used to examine differences in mean duration in each compartment to assess any baseline side preference during the habituation phase, to evaluate sociability in the first phase, and to compare social novelty preference in Phase 2. Frequency and duration of each of the seven behaviors in each stimulus compartment were compared with unpaired t-tests. Two-way analyses of variance (ANOVA) were applied in Phase 2 to compare frequencies and durations of each behavioral form in association with the stimuli under the standard and reversal conditions with condition (standard vs. reversal) acting as the between subject, and stimulus side (familiar vs. novel) as the within subject factor. When significant main or interaction effects were noted, Bonferroni post hoc tests were employed to compare behaviors associated with the familiar and novel mice under the standard and reversal conditions. When frequency or duration scores were zero for a behavioral category within one compartment no statistical test was used but data were included in the graphs for descriptive purposes. All tests were two-tailed and p-values <0.05 were considered significant. Graphs are depicted as mean ± 1 SEM.

3. Results

3.1. Side bias

Across all 40 mice used in the experiment, subjects spent significantly more time in the left stimulus compartment ($t = 3.687$, $p = 0.0004$, data not shown). This effect is likely due to the asymmetric arrangement of overhead lighting creating a small gradient in degree of illumination in the chambers. The influence of baseline side preference on social approach and social novelty preference should equalize as the side of stimulus presentation was systematically counterbalanced between subjects.

3.2. Phase 1: sociability

B6 mice spent significantly more time in the side containing the stimulus animal compared to time spent with the empty cup. This was the case when the stimulus mouse was another B6 mouse ($t = 3.411$, $p = 0.0015$) and when outbred CD-1 males were utilized ($t = 2.286$, $p = 0.0279$) (Fig. 2a and b). B6 subject mice appear to pre-