Published in final edited form as:

Behav Brain Res. 2010 December 1; 213(2): 189–194. doi:10.1016/j.bbr.2010.04.054.

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 two 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.

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Keywords

Sociality; Autism; Social Novelty; Social Approach; C57BL/6J

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 [7,9,14,15,16,19,25]. 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 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.

Methods

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 twenty naïve C57BL/6J mice of the same age served as inbred stimuli. Finally, twenty 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 $72\pm 2^{\circ}\text{F}$ under a 12:12h light-dark schedule (lights on at 0600h) with free access to tap water and lab diet. Mice were transferred to the testing room at least 30 minutes prior to behavioral testing which was performed between 0900 and 1700 hours. 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.

Three-Chamber Apparatus

A 41 cm L $\times$ 70 cm W $\times$ 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 (Figure 1). The bottom 6.35 cm of the front panel was clear acrylic while all other walls were black (Figure 1 inset). Since 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.

Behavioral Testing

Sociability and social novelty preference assessment was conducted as previously described [14]. Briefly, during a ten minute 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 minute 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 minutes.

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.

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 less than 0.05 were considered significant. Graphs are depicted as Mean $\pm$ 1 SEM.

Results

Side Bias

Across all 40 mice used in the experiment, subjects spent significantly more time in the left stimulus compartment ($t=3.687$, $p=.0004$, 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 since the side of stimulus presentation was systematically counterbalanced between subjects.

Phase One: 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 ($t=2.286$, $p=0.0279$) (Figure 2a,b). B6 subject mice appear to prefer proximity to another mouse over a compartment lacking social stimuli.

Behavioral analysis of videotape revealed that subject mice displayed increased frequency of contact with the stimulus cup associated with the unfamiliar B6 mouse compared to the empty cup ($t=2.333$, $p=0.0253$) but not when the CD-1 mouse was the stimulus ($t=0.9617$, $p=0.3423$) (Figure 3a,c). Mice engaged in higher frequencies and durations of sniffing both the cup containing the B6 stimulus mice (Frequency $t=6.045$, $p<0.0001$; Duration $t=6.415$, $p<0.0001$) and the CD-1 stimulus mice (Frequency $t=5.897$, $p<0.0001$; Duration $t=7.716$, $p<0.0001$) (Figure 3a,b,c,d). No other significant differences were shown in behaviors during Phase 1. Nevertheless, B6 mice spent more time in association with and engaged in more investigatory behavior in the presence of the unfamiliar conspecific of the inbred and outbred strains.

Phase Two: Social Novelty

When B6 mice were applied as stimuli, there was a significant main effect revealed for stimulus across the conditions ($F_{(1,36)}=5.20$, $p=0.0287$) and a significant interaction between the factors ($F_{(1,36)}=5.10$, $p=0.0301$). There was no significant main effect for stimulus when CD-1 mice were stimuli ($F_{(1,36)}=1.05$, $p=0.314$) but there was a significant interaction ($F_{(1,36)}=5.31$, $p=0.0270$). Post-hoc analyses revealed that under the standard arrangement of novel subject presentation in Phase 2, B6 mice showed significantly increased duration of time in the compartment containing the unfamiliar B6 ($t=3.209$, $p<0.01$) but not CD-1 mouse strain ($t=0.9072$, $p>0.05$). For the subjects exposed to the stimuli in the reversal condition, no significant difference was noted in post-hoc analyses for time spent in the compartment containing the novel or familiar B6 stimuli ($t=0.01502$, $p>0.05$) but increased duration of time spent in the chamber associated with the familiar CD-1 mouse ($t=2.353$, $p<0.05$) (Figure 4).

Behavioral analysis during phase 2 revealed a significant main effect of stimulus for mean frequency of sniffing for B6 stimuli mice ($F_{(1,34)}=5.326$, $p=0.0272$) but not for CD-1 mice ($F_{(1,36)}=2.300$, $p=0.1381$) across the stimuli and their arrangement conditions (Figure 5a,c). Post-hoc analysis indicated that B6 subject mice showed elevated sniffing of a novel B6 stimulus mouse when it was presented under the standard condition ($t=2.938$, $p<0.05$) (Figure 5a). No other significant main effects were noted in the frequency or duration of behaviors in the familiar and novel mouse compartment for either strain.

B6 mice had increased sniffing in the compartment associated with an unfamiliar mouse compared to a familiar animal for both inbred and outbred stimulus mice, but only when the novel animal was placed in a previously empty location.

Discussion

The automated three chamber apparatus [19] has been widely utilized in the study of social investigation and preference as it provides a high-throughput method for assessing variation among and between strains for propensity to approach novel social partners. By directly manipulating the standard mouse social novelty test, we have demonstrated a lack of investigation to the novel stimulus. These results suggest that the mice either have an inability to distinguish subject animals in either or both conditions or are not expressing a preference specific to the familiarity of the stimulus mouse. Though this test has previously revealed consistent preferences for a novel stimulus animal over a novel object/familiar mouse, the arrangement of the stimuli appears to explain some, if not all, of the anticipated novelty preference.

Our social approach findings in phase one are in accordance with other works demonstrating that B6 mice prefer to investigate a novel mouse over a novel object. Ryan et al (2008) specifically addressed which sensory systems are utilized by B6 mice and noted that in the sociality phase, substrate containing animal scent was investigated as much as an awake, behaving animal [24]. We propose that previously published phase two results may be explained as a preference for a novel context. When B6 mice were stimuli, any novelty preference was lost under the reversal condition. This may reflect general uncertainty about the identity of the two stimulus mice, no preference whatsoever, or a range of effects reflecting both the stimulus animal's identity and its placement in the arena. In particular, it is conceivable that interaction between the olfactory stimuli from the previous occupant and the new occupant in the reversal condition leads to identification difficulties or obscured preference in the social novelty phase. However, these results may well be considered indicative of a lack of a demonstration of social novelty preference which may or may not exist in these mice. Behavioral analyses confirm the notion that the location of the novel

stimulus animal will determine if any apparent novelty preference can be demonstrated. The novel behavioral indices applied to this established paradigm may assist in clarifying the motivational state of subject mice. In particular, contact and sniffing appear to be important behavioral categories differentially displayed in response to relevant social stimuli in the three-chamber arena.

An important consideration when assessing social motivation in mice is the ability of subject mice to discriminate the individual identity of conspecifics. Social recognition is applied as an index of sociality and is most commonly assessed through demonstration of habituation [5,26,28]. Considerable attention has been paid to characterizing the abilities of B6 mice to recognize and distinguish individual identity [11]. We have previously determined that B6 mice fail to indicate recognition through dishabituation to a novel B6 mouse, but do recognize and scent mark more to a novel CD-1 stimulus in a scent marking-based habituation-dishabituation paradigm [4] suggesting that outbred mice may be more appropriate than inbred mice as stimuli for tests of novelty preference, individual recognition, and social memory in mice. It does not necessarily follow that this applies to all outbred strains applied as stimuli or to the discriminatory abilities and preferences of other inbred or mutant mice. Further work should characterize which, if any strains can discriminate and display preference towards a novel mouse.

We advocate counterbalancing the standard and reversal condition in the social novelty-seeking phase to distinguish any apparent novelty preference from that due to contextual novelty. Researchers may also consider the utility of performing an additional free-interaction paradigm [10] between subject mice and those differing in familiarity to eliminate the influence of potentially confounding contextual changes.

Acknowledgments

Mr. Ted Murphy constructed behavioral testing arenas. Amy Vasconcellos and Mary Rowlett assisted in data collection.

Funded by NIH MH081845 to RJB

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Fig 1.

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.

Fig. 2.

Phase one sociability preference for a stimulus mouse of an unfamiliar B6 mouse (a) or for an unfamiliar CD-1 mouse (b) over an empty wire cup. * $p < 0.05$, ** $p < 0.005$, $n = 20$ /group. Data are expressed as Mean $\pm$ 1 S.E.M.

Fig 3.

Increased frequency of contact (a) and increased frequency and duration of sniffing an unfamiliar B6 mouse (a,b) compared to the empty cup control. Increased frequency (c) and duration (d) of sniffing within the compartment with an unfamiliar CD-1 mouse compared to the empty cup control. White bars indicate the empty cup and black bars are the unfamiliar mouse stimulus. * $p < 0.05$, ** $p < 0.001$, $n = 20/\text{group}$. Data are expressed as Mean $\pm$ 1 S.E.M.

Fig. 4.

Mice spent significantly more time in the chamber associated with the novel B6 mouse over the familiar one but only in the standard condition (a). When CD-1 mice were used as stimuli, there was a significant increase in the duration spent in the chamber associated with the familiar mouse under the reversal condition (b). White bars represent familiar while black bars are novel stimuli. * $p < 0.05$, ** $p < 0.01$, $n = 10/\text{group}$. Data are expressed as Mean ± 1 S.E.M.

Fig. 5. Behavioral data collected during phase 2 indicated a significant main effect for stimulus condition for sniffing frequency for B6 stimuli and post-hoc analyses revealed that under the standard presentation, mice spent more time sniffing the novel B6 mouse (a). * $p < 0.05$, $n = 10$ /group. Data are expressed as Mean $\pm$ 1 S.E.M.