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Techniques

Automated apparatus for quantitation of social approach behaviors in mice

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Mouse models of social dysfunction, designed to investigate the complex genetics of social behaviors, require an objective methodology for scoring social interactions relevant to human disease symptoms. Here we describe an automated, three chambered apparatus designed to monitor social interaction in the mouse. Time spent in each chamber and the number of entries are scored automatically by a system detecting photocell beam breaks. When tested with the automated equipment, juvenile male C57BL/6J mice spent more time in a chamber containing a stranger mouse than in an empty chamber (sociability), similar to results obtained by the observer scored method. In addition, automated scoring detected a preference to spend more time with an unfamiliar stranger than a more familiar conspecific (preference for social novelty), similar to results obtained by the observer scored method. Sniffing directed at the wire cage containing the stranger mouse correlated significantly with time spent in that chamber, indicating that duration in a chamber represents true social approach behavior. Number of entries between chambers did not correlate with duration of time spent in the chambers; entries instead proved a useful control measure of general activity. The most significant social approach behavior took place in the first five minutes of both the sociability and preference for social novelty tests. Application of these methods to C57BL/6J, DBA/2J and FVB/NJ adult males revealed that all three strains displayed tendencies for sociability and preference for social novelty. To evaluate the importance of the strain of the stranger mouse on sociability and preference for social novelty, C57BL/6J subject mice were tested either with A/J strangers or with C57BL/6J strangers. Sociability and preference for social novelty were similar with both stranger strains. The automated equipment provides an accurate and objective approach to measuring social tendencies in mice. Its use may allow higher-throughput scoring of mouse social behaviors in mouse models of social dysfunction.

Keywords: Autism, automated equipment, C57BL/ 6J, DBA/ 2J, exploratory activity, FVB/ NJ, inbred strains, mice, scoring methods, sociability, social behavior, social preference

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Many rodent species, including mice, demonstrate strong social communities in the wild, and easily quantitated social behaviors in the laboratory. Methods for evaluating a large number of social behavior parameters have provided an in-depth ethogram of the rich social repertoire of mice. Social preference tests in rodents have been used to investigate social tendencies of mice, rats and voles, including analyses of pair-bonding, dominance hierarchies and social memory (Blanchard *et al.* 2001; Brodkin *et al.* 2004; Carter *et al.* 1995; Crawley 2000; Dewsbury 1990; Ferguson *et al.* 2001; Gheusi *et al.* 1994; Hahn & Schanz 1996; Insel 2001; Marler & Hamilton 1968; Maxson 1996; Mossman & Drickamer 1996; Nelson & Chiavegatto 2000; Tang *et al.* 2003). Modifications of these procedures can be used to investigate the genetics of mouse social behaviors as models of human disorders involving social deficits (Insel 2001). Progress in behavioral genetics may benefit from an automated approach, to allow rapid evaluation of large numbers of mice from inbred strains, spontaneous mutations, random mutagenesis and targeted gene disruptions.

Here we report a set of simple, easily automated mouse behavioral tasks that can be used to model symptoms of human disorders associated with social approach abnormalities. Autism, for example, is characterized by moderate to severe social interaction deficits, social communication

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abnormalities and ritualistic-repetitive behaviors (Folstein & Rosen-Sheidley 2001; Kanner 1943; Piven 2001; Schloper & Mesibov 1987). The cause of autism appears to be primarily genetic, with a heritability estimate of over 90% (Bailey *et al.* 1995). In contrast, William's syndrome, a disorder associated with a deletion of 1.5 Mb of chromosome 7, includes symptoms of very high levels of social approach behaviors. Children with William's syndrome typically have an 'overfriendly' personality along with strong language skills, but have deficits in visuospatial cognitive skills (Doyle *et al.* 2004; Laws & Bishop 2004; Morris & Mervis 2000). Social phobias and social anxiety are defined by avoidance of social situations (Bell *et al.* 1999; Marcin & Nemeroff 2003; Stein & Deutsch 2003; Tancer & Uhde 1997) and social dysfunction is a component of schizophrenia (Carpenter 1993; Dworkin 1992; Egan & Weinberger 1997; Morrison & Bellack 1987; Pinkham *et al.* 2003; Sams-Dodd *et al.* 1997; Tamminga 2003). Inbred strains of mice present an opportunity to evaluate social approach behaviors relevant to these human diseases and investigate the complex genetics of social behavior. Here we evaluate three inbred strains in two automated tasks, to begin to define the range of genetically influenced social behaviors in mice.

In a companion paper (Moy *et al.* 2004), we describe a three-chambered apparatus designed to quantitate preference for spending time with a conspecific mouse vs. an empty novel environment (sociability), as well as preference for a newly introduced mouse vs. a familiar mouse (preference for social novelty). This method employs human observation and data-entry on a computer. We subsequently designed a more automated version of this task, using photocells across the chamber doorways to record entries and time spent in each chamber. This report describes the components of the automated apparatus and a series of validation experiments, using three inbred strains of mice commonly used in behavioral genetics laboratories. The findings presented herein demonstrate comparable scores on measures of social tendencies, using the automated system vs. the observational hand scoring system. In addition, the present experiments describe the time course of the social approach behavior in both tasks, and the effect of the strain of the stranger mouse on the subjects' social behaviors.

Materials and methods

Animal subjects

Juvenile male mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Target subjects used as 'strangers' were adult C57BL/6J or A/J males, habituated to placement in small wire cages for one five-minute session per day for several days prior to first use. Test subjects used to compare the methods of data gathering (automated vs. human observer), time course and stranger strain were experimentally naïve two-month-old C57BL/6J male mice. Test subjects for the subsequent inbred strain analysis were six-month-old males previously run through a battery of behavioral assays, including the sociability and social novelty preference assays previously conducted at six weeks of age (Moy *et al.* 2004). The three strains were C57BL/6J, from the C57-related mice, DBA/2J, one of Castle's mice, and FVB/NJ, a Swiss line (Beck *et al.* 2000), chosen for their different lineages and frequent use in behavioral genetics research.

All mice were housed four per cage by strain with food and water available *ad libidum*. Cages were kept in a 23 °C room on a 12-h light/dark cycle with the lights off at 19:00. Behavioral experiments were conducted in an adjacent dedicated procedure room. Subject mice were habituated to the test room for at least 20 min prior to start of the behavioral tasks. All animal procedures were approved by the University of North Carolina Institutional Animal Care and Use Committee and were in accordance with the NIH guidelines for the Care and Use of Laboratory Animals.

Test of sociability and preference for social novelty

Apparatus

As illustrated in Fig. 1, the social test apparatus consists of a polycarbonate box with removable partitions separating the box into three chambers. The partitions have openings that allow the animal to move freely from one chamber to another (Fig. 1d). As the animal moves through each opening, it sequentially breaks and unbreaks two infrared beams produced by emitter and detector pairs spanning the opening. An interface box with an embedded real-time controller monitors the beams and determines transitions in and out of each chamber (Fig. 1c). The time spent in each chamber and the transitions between each chamber are recorded and displayed on the user interface (Fig. 2c). The three chambered apparatus is centered on a lab bench to minimize gradients in light, temperature, sound and other environmental conditions that could produce a side preference.

The controller is a National Instruments NI cFP-2000 (Austin, TX) running a Labview Real-Time program. The controller uses a NI cFP-DO-401 output module to turn on the Fairchild Semiconductor F5D1 (South Portland, ME) infrared emitters and to display the status of any blocked beams on the user interface LEDs. The analog levels from the Fairchild L14P1 detectors are digitized with Texas Instruments LM311N comparators (Dallas, TX), and interfaced with a NI cFP-DI-301 input module. Information is displayed to the user with a 3602-105-05220 IEE (Van Nuys, CA) vacuum fluorescent display (Fig. 2c).

The chrome wire cages used to contain the stranger mice were cylindrical, 11 cm in height, bottom diameter 10.5 cm, bars spaced 1 cm apart (Galaxy Cup, Spectrum Diversified Designs, Inc., Streetsboro, OH). A weighted cup was placed on the top of the cage to prevent the test mice from climbing and remaining on the top of the wire cage. The wire cages were placed on lucite disks during testing and both cage and disk were replaced with a clean cage and disk after each

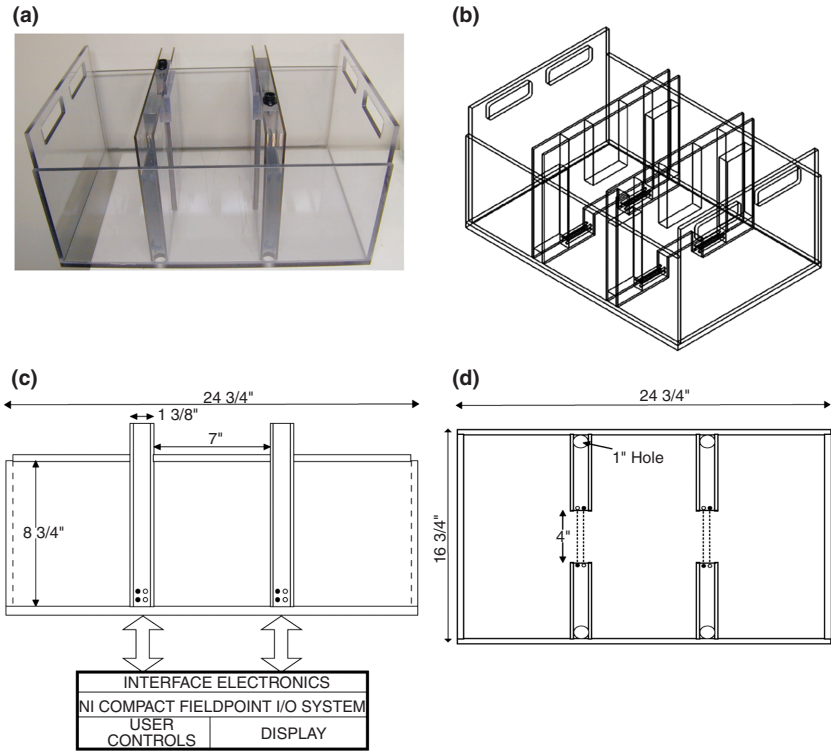

Figure 1: Automated apparatus for social behavior tests. (a) Photograph of polycarbonate apparatus with removable dividers. (b) Angle view schematic showing gates between chambers. (c) Side view showing dimensions, sensor placement and electronics interface. (d) Top view showing dimensions, gates, sensors and drainage holes under removable dividers for cleaning.

subject to minimize scent carryover (Fig. 2a). Similarly, the apparatus was wiped down with water and dried with paper towels for each new test subject. After each testing day, the wire cages, lucite disks and apparatus were wiped down with 70% ethanol and allowed to air-dry.

Sociability test

The animal was placed in the middle chamber with the dividers closed to allow it to explore the middle chamber for five minutes. After this five-minute habituation period, an unfamiliar adult C57BL/6J male (stranger 1) was placed inside a small wire cage in one of the side chambers (Fig. 2a). An identical empty wire cage was placed in the opposite chamber (Fig. 2b). The dividers were then raised, allowing the test subject to move freely throughout all three chambers of the apparatus over a 10-minute test session (Fig. 2b). The thin, widely spaced bars of the wire cage allowed nose contact between the bars, but prevented the stranger mouse from initiating any social contact and limited the possibility of aggressive interactions (Fig. 2a). Thus, initiation of social contact was attributable to the test subject only. This method allowed the test subject to be monitored for lack of initiation of social interaction.

Location of the stranger mouse and the empty wire cage was alternated between left and right chambers on consecutive sessions. Measures were taken of time spent and entries into the chamber containing the unfamiliar mouse in a wire cage (stranger side) and the chamber containing only the empty wire cage on the opposite side of the apparatus (empty side) for 10 minutes. The experimenter simultaneously scored time spent in each chamber and entries between chambers with the use of an event keyboard monitored by data collection software on a computer (Moy *et al.* 2004).

To confirm that time spent in the chamber containing the stranger reflected social behavior directed toward the stranger mouse, a human observer simultaneously scored each test subject for sniffs directed at the wire cage containing the stranger mouse and sniffs directed towards the empty wire cage (Fig. 2a). Sniffing directed at each wire cage was cumulatively scored over each 10-minute test session as total duration in seconds, using the event keyboard.

Preference for social novelty test

A 10-minute test to quantitate preference for social novelty began immediately after the 10-minute test for sociability. The original stranger mouse (stranger 1) remained in its wire cage on one side of the apparatus. A new unfamiliar mouse (stranger 2) was placed in the wire cage on the opposite side, which was previously empty during the sociability test. Identical measures as previously described were scored: time spent in each chamber, entries between chambers and time spent sniffing each wire cage. Stranger 1 and stranger 2 animals originated from different home cages and had never been in physical contact with the subject mice or each other.

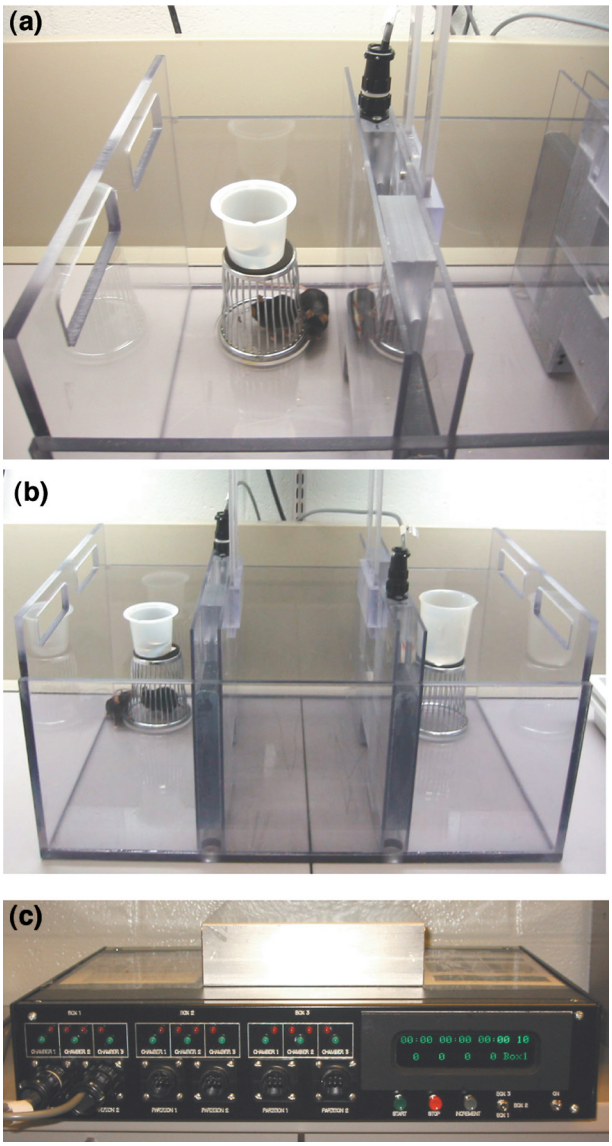

Figure 2: Illustrations of mice engaged in social behaviors within the automated apparatus. (a) Social interaction between test subject and stranger in wire cage. The test subject is sniffing the wire cage containing stranger 1. (b) View of all three chambers of apparatus. Test mouse is interacting with stranger 1 in the left chamber. (c) Electronics interface with outlets for three social test boxes, LEDs to display location of test mouse and digital display of timing and entries.

Time course for social approach behavior in the test of sociability and social novelty preference

Time course analysis was conducted to determine the optimal session length for capturing the majority of social approach behaviors. Mice were tested in the automated apparatus as described above using a 10-minute habituation period in which the subject could move freely throughout the entire chamber. Data were recorded in five-minute time bins across a continuous 20-minute test session, to evaluate the time period during which social approach behaviors were highest. A 20-minute test of preference for social novelty followed directly and was similarly scored in five-minute time bins. Data from subjects in which sociability was significant when the four time bins were pooled were used to analyze the time course.

Comparison of same-strain vs. different strain strangers

To investigate the contribution of the stranger animal to the results of the sociability and preference for social novelty tasks, 20 C57BL/6J subjects were tested, half with C57BL/6J strangers and half with A/J strangers. Assays were performed as previously described with a 10-minute habituation period. A/J was chosen because it is a strain characterized by low exploratory activity, high levels of anxiety-like behaviors and low levels of sociability and preference for social novelty as compared to C57BL/6J (Bouwknecht & Paylor 2002; Cohen *et al.* 2001; Mathis *et al.* 1994; Mathis *et al.* 1995; Moy *et al.* 2004; van der Staay & Steckler 2001).

Data analysis

Statistical analyses were performed using StatView (SAS, Cary, NC). Repeated measures ANOVAs were used to determine level of significance. Independent variables used included side and scoring method. *Post hoc* pairwise comparisons employed the Fisher's Protected Least Significant Difference test to compare individual variables following a significant overall ANOVA. Correlation matrices were used to determine r^2 value and significance was determined by a Fisher's r to z-test. For all comparisons, significance was set at $P < 0.05$.

Results

Comparison of sociability scores for automated vs. observer scoring

Test subjects spent more time in the chamber containing the unfamiliar mouse than in the empty side (Fig. 3a). Subjects generally spent more time in either side of the apparatus than in the middle chamber. An overall repeated measures ANOVA detected a significant effect of side on duration ($F_{1,8} = 5.347$, $P = 0.0495$). No significant overall effect of scoring method was detected ($F_{1,8} = 0.308$, $P = 0.5943$). When scored by an observer, time spent in each side chamber approached significance ($F_{1,8} = 4.764$, $P = 0.0600$). When scored by the automated method, time spent in each chamber reached statistical significance ($F_{1,8} = 5.419$, $P = 0.0449$), perhaps due to decreased variability in the automated data. Despite the significant difference in amount of time spent in each side of the chamber, there was no significant difference in the number of entries made into each side (Fig. 3b, $F_{1,8} = 0.905$, $P = 0.3663$). This result was seen with both the automated and observer scoring methods. Comparison

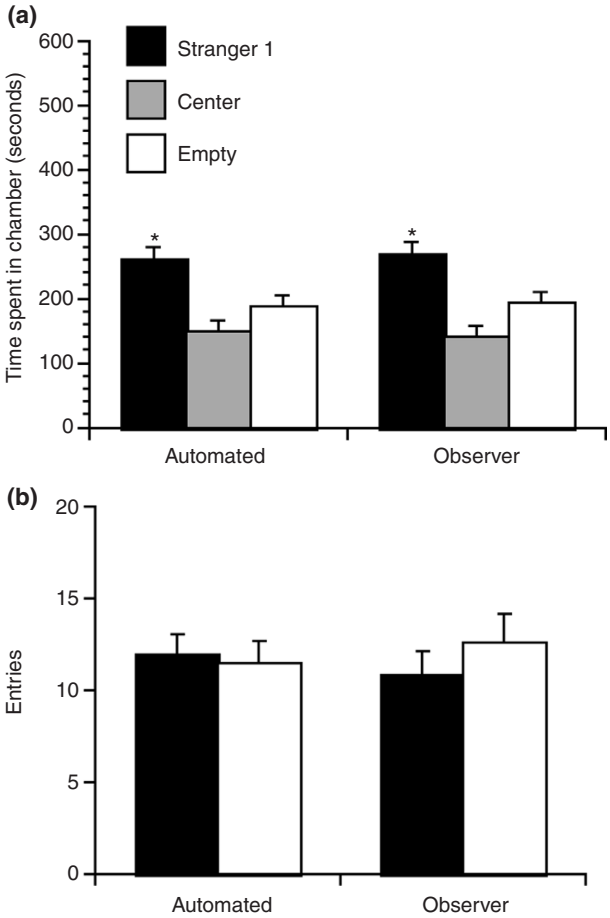

Figure 3: Comparison between observer and automated data collection in the sociability test. (a) Amount of time spent in the chamber containing the stranger mouse in an empty wire cage vs. amount of time spent in the center and the chamber containing an identical but empty wire cage. (b) Number of entries between chambers. Subjects were two-month-old male C57BL/6J mice, $n=9$. Stranger 1 mice were adult male C57BL/6J that had no prior contact with the test subjects. Data shown are mean + SEM for each group. * $P<0.05$, comparison between stranger 1 side and empty side.

of two different investigators simultaneously scoring mice in the sociability test revealed identical scores between observers in greater than 95% of the observations.

Comparison of preference for social novelty for automated vs. observer scoring

Test subjects exhibited a significant preference to spend time in the chamber containing the novel stranger 2, as compared to time spent in the chamber containing the now-familiar stranger 1 (Fig. 4a). No significant effect of scoring method was detected ($F_{1,9}=1.000$, $P=0.3466$). Subjects spent more time in the side chambers than the center of the apparatus. An overall repeated measures ANOVA showed a significant effect of side on duration ($F_{1,9}=19.148$, $P=0.0018$). Time spent in each side chamber reached statistical significance as scored by both the observer ($F_{1,8}=21.761$, $P=0.0012$) and the automated ($F_{1,8}=16.181$, $P=0.0030$) method. Repeated measures ANOVA on number of entries detected no significant effect of side ($F_{1,8}=0.007$, $P=0.9348$) or scoring method ($F_{1,8}=0.455$, $P=0.5190$, Fig. 4b).

Time course of social approach behavior in the automated apparatus

In test sessions for sociability, the majority of the social approach behavior was detected in the first five-minute bin of the time course ($F_{1,11}=16.367$, $P=0.0019$, Fig. 5a). In the last three time bins, the amount of time spent in the chamber with stranger 1 and in the chamber containing the empty wire cage was not significantly different. The amount of time

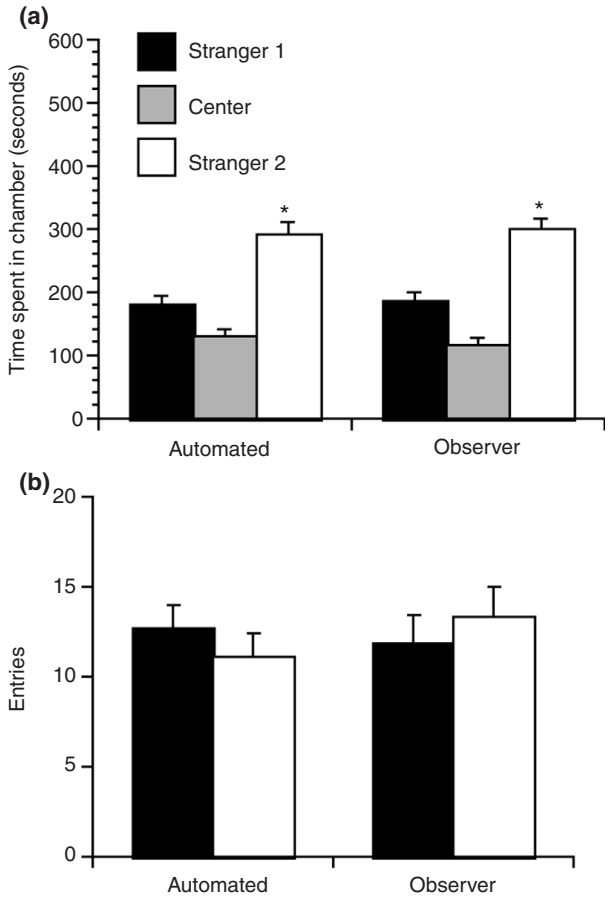

Figure 4: Comparison of observer and automated collection of data in the preference for social novelty test. (a) Amount of time spent in the chamber containing stranger 1, stranger 2 or the center. (b) Number of entries between chambers. Subjects were two-month-old male C57BL/6J mice, $n=10$. Stranger mice were adult male C57BL/6J that had no prior contact with the test subjects. Data shown are mean + SEM for each group. * $P<0.05$, comparison between stranger 1 side and stranger 2 side.

spent in either side chamber vs. the middle was significantly different between the first time bin (0–5 min) and each of the last two time bins (10–15 and 15–20 min) ($P = 0.0254$, $P = 0.0369$, respectively). An overall repeated measures ANOVA showed a significant effect of both time ($F_{1,11} = 10.487$, $P < 0.0001$) and of time bin by chamber side ($F_{1,11} = 4.869$, $P = 0.0065$). *Post hoc* analysis revealed the major effect was the amount of time spent on the side with stranger 1 decreasing over the four time bins. The first five minutes included significantly more time spent with stranger 1 than the other three bins ($P < 0.005$ for all three). The number of entries into the side chambers also decreased between minutes 0–5 and 15–20 ($P = 0.0122$, Fig. 5c). Amount of time spent sniffing the wire cage containing stranger 1 was significantly more than time sniffing the empty cage for the first three five-minute time bins ($F_{1,11} = 17.857$, $P = 0.0018$, $F_{1,11} = 6.056$, $P = 0.0336$, $F_{1,11} = 6.708$, $P = 0.0270$, Fig. 5e). The percentage of time in the chamber spent sniffing the wire cage containing stranger 1 decreased from 43% in the first five minutes to 34% (5–10 min), 38% (10–15 min) and 14% (15–20 min). The percent of time spent sniffing the empty cage was 21–23% for all four time bins.

In the test of preference for social novelty, there was a significant difference in time spent with stranger 1 over stranger 2 in the first five-minute bin ($F_{1,11} = 45.238$, $P < 0.0001$, Fig. 5b). An overall repeated measures ANOVA showed a significant effect of both time ($F_{1,11} = 13.066$, $P < 0.0001$) and of time bin by chamber side ($F_{1,11} = 5.988$, $P = 0.0022$). *Post hoc* analysis revealed the major effect was the amount of time spent on the side with stranger 2 decreasing between the first time bin and the following three. The first five minutes included significantly more time spent with stranger 2 than the other three bins ($P < 0.005$ for all three). Number of entries into the side chambers was significantly different between the first and last time bin ($P = 0.0022$) and third and last time bin ($P = 0.0039$, Fig. 5d). Only the first time bin showed a significant difference in time spent sniffing the stranger cages ($F_{1,11} = 17.857$, $P = 0.0018$, Fig. 5f). Results from the three remaining bins showed no difference between cages, but demonstrated lower amounts of sniffing compared to the first time bin ($P = 0.0035$, $P = 0.0012$, $P = 0.0017$, respectively). In particular, sniffing directed toward stranger 2 fell off significantly after the first bin ($P < 0.0001$ comparison to all other bins). The percentage of time in the chamber spent sniffing the wire cage containing stranger 2 decreased from 47% in the first five minutes to 22% for the next three time bins. The percent of time for the empty cage was 28–35% for all four time bins.

Social behavior tasks in three inbred strains

All three strains spent the majority of the 10-minute sociability session exploring the two side chambers and spent more time in the chamber containing the novel mouse: C57BL/6J ($F_{1,19} = 28.487$, $P < 0.0001$), DBA/2J ($F_{1,19} = 23.972$, $P = 0.0001$) and FVB/NJ ($F_{1,13} = 5.920$, $P = 0.0302$). Two of the three strains showed a preference for social novelty, as measured by duration in the chamber containing the new stranger 2 vs. the now-familiar stranger 1. C57BL/6J ($F_{1,19} = 8.919$, $P = 0.0076$) and FVB/NJ ($F_{1,13} = 12.243$, $P = 0.0039$) spent significantly more time in the chamber with stranger 2. DBA/2J did not show significant preference for social novelty ($F_{1,19} = 1.977$, $P = 0.1759$, Fig. 6). Number of entries was not significantly affected by side in any of the strains in either the test of sociability or preference for social novelty (Fig. 7).

All three strains spent significantly more time sniffing the wire cage containing the unfamiliar mouse than the empty wire cage: C57BL/6J ($F_{1,18} = 62.654$, $P < 0.0001$), DBA/2J ($F_{1,19} = 62.157$, $P < 0.0001$) and FVB/NJ ($F_{1,12} = 23.694$, $P = 0.0002$). All three strains spent more time sniffing the wire cage containing stranger 2 than sniffing the wire cage containing stranger 1: C57BL/6J ($F_{1,19} = 25.463$, $P < 0.0001$), DBA/2J ($F_{1,19} = 4.573$, $P = 0.0457$) and FVB/NJ ($F_{1,11} = 8.931$, $P = 0.0123$) (Fig. 8).

Time spent in the chamber containing stranger 1 correlated with the amount of time spent sniffing the wire cage containing stranger 1. Correlation plots shown in Fig. 9 include data from both sides of the apparatus. Data points represent time spent in the empty side plotted against time spent sniffing the empty cage (+ symbols) and time spent in the stranger side plotted against time spent sniffing the wire cage containing the stranger mouse (● symbols). Time spent sniffing the wire cages correlated significantly with the duration of time spent in each chamber for all three strains: C57BL/6J ($r = 0.703$, $P < 0.001$), DBA/2J ($r = 0.647$, $P < 0.001$) and FVB/NJ ($r = 0.655$, $P < 0.001$). Time spent sniffing the empty cage was much less than time spent sniffing the cage containing the stranger.

Effect of the strain of the stranger mouse on sociability and preference for social novelty

Strain of the stranger mouse had no effect on social approach behaviors. C57BL/6J subjects tested with C57BL/6J strangers showed significant sociability ($F_{1,8} = 7.225$, $P = 0.0276$) and preference for social novelty ($F_{1,8} = 6.367$, $P = 0.0356$, Fig. 10a). Similarly, C57BL/6J subjects tested with A/J strangers also showed significant sociability ($F_{1,8} = 112.393$, $P < 0.0001$) and preference for social novelty ($F_{1,8} = 49.532$, $P = 0.0001$, Fig. 10b). C57BL/6J subjects spent a similar amount of time in the chamber containing C57BL/6J strangers as compared to time in the chamber containing A/J strangers in both the sociability test and the test of preference for social novelty ($F_{1,17} = 0.273$, $P = 0.6082$). Number of entries into either side of the apparatus was not significantly affected by the strain of the stranger animal (Fig. 10c,d). Time spent sniffing the C57BL/6J strangers was not significantly different from time spent sniffing A/J strangers. Data from a single animal were dropped from each group of subject animals due to scores greater than two standard deviations from the mean for time spent with stranger 1 during the test of sociability.

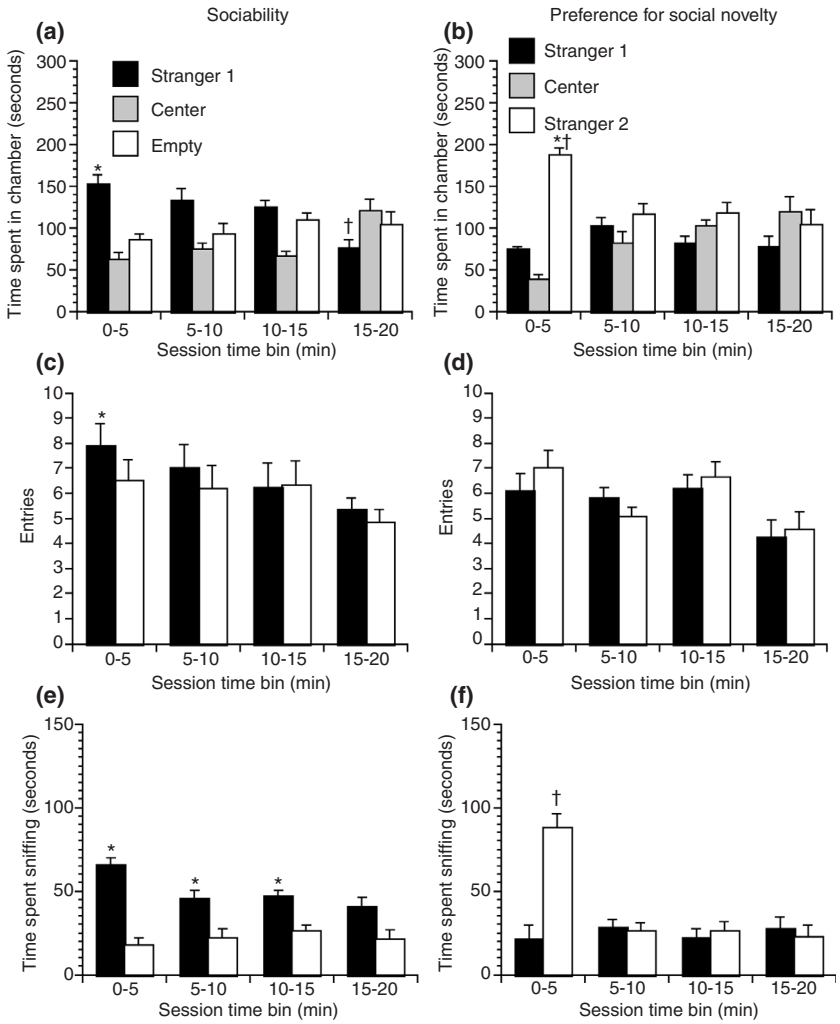

Figure 5: Time course of social approach behavior in the tests for sociability (a,c,e) and preference for social novelty (b,d,f). Data were collected in five-minute time bins across the 20-minute test. Stranger mice were adult male C57BL/6J. Subjects were six-week-old C57BL/6J males, $n = 12$. (a,b) Amount of time spent in each chamber. (c,d) Number of entries between chambers. (e,f) Amount of time spent sniffing the wire cage containing stranger 1, an empty wire cage or stranger 2. Data shown are mean + SEM for each group. * $P < 0.05$, comparison between sides of apparatus. † $P < 0.005$, comparison of one time bin to the other three time bins.

Discussion

The automated system for measuring sociability and preference for social novelty in mice yielded quantitative data comparable to data collected by human observers when scored simultaneously by the two methods. These results support an interpretation that the automated equipment yields data very similar to observer scoring in terms of detection of effects. Both automated and human observer scoring methods detected significant sociability in adult males of three inbred strains: C57BL/6J, DBA/2 and FVB/NJ. The magnitude and direction of effects were analogous to the sociability found in these strains as juveniles by the observer scored method, as described in the companion study (Moy *et al*. 2004). Similarly, preference for social novelty was detected by the automated equipment in juvenile male C57BL/6 and in independent cohorts of adult C57BL/6J and FVB/NJ, analogous to the preference for social novelty found in these strains by the observer scored methods (Moy *et al*. 2004). In addition, the low scores on time spent in the center chamber confirm that all three strains displayed high levels of general exploratory behavior.

The social nature of the time spent in the chamber containing a novel conspecific was confirmed by the strong correlation between time spent in the chamber and time spent sniffing the wire cage containing the stranger mouse. This finding supports the interpretation that time was spent in close proximity to the stranger, rather than elsewhere in the chamber containing the stranger. All three strains spent more time sniffing the wire cage containing the stranger than the empty wire cage. This supports the interpretation that sniffing of the wire cage reflects social approach behavior rather than non-specific exploration of a novel object. The wire cages allowed substantial olfactory, auditory, visual and tactile contact between test subjects and stranger animals, including nose-to-nose and nose-to-tail sniffing. Containment of the stranger in the wire cage prevented fighting that may have been anticipated using males as subjects and strangers.

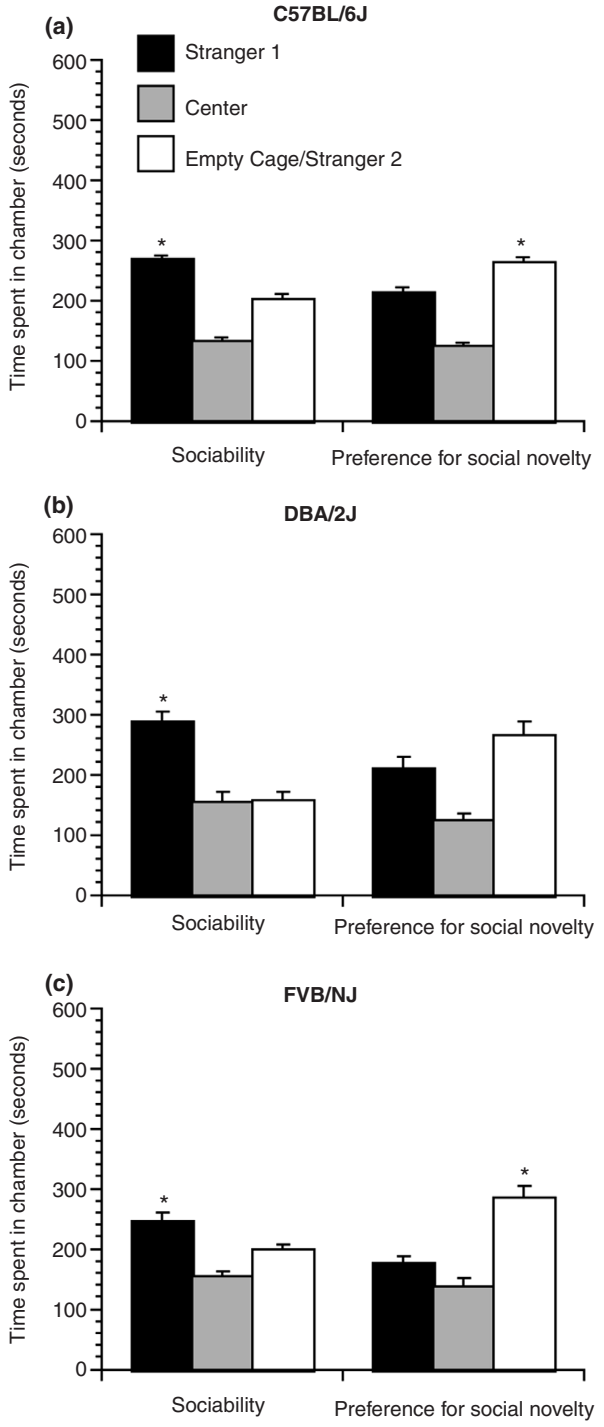

Figure 6: Comparison of three inbred strains on duration in each chamber in the sociability and preference for social novelty tests. (a-c) Subjects were six-month-old male mice. (a) C57BL/6J, $n = 20$. (b) DBA/2J, $n = 20$. (c) FVB/NJ, $n = 14$. Stranger mice were adult male C57BL/6J. Data shown are mean + SEM for each group. $^*P < 0.05$, comparison between stranger 1 and the empty wire cage or between stranger 1 and stranger 2.

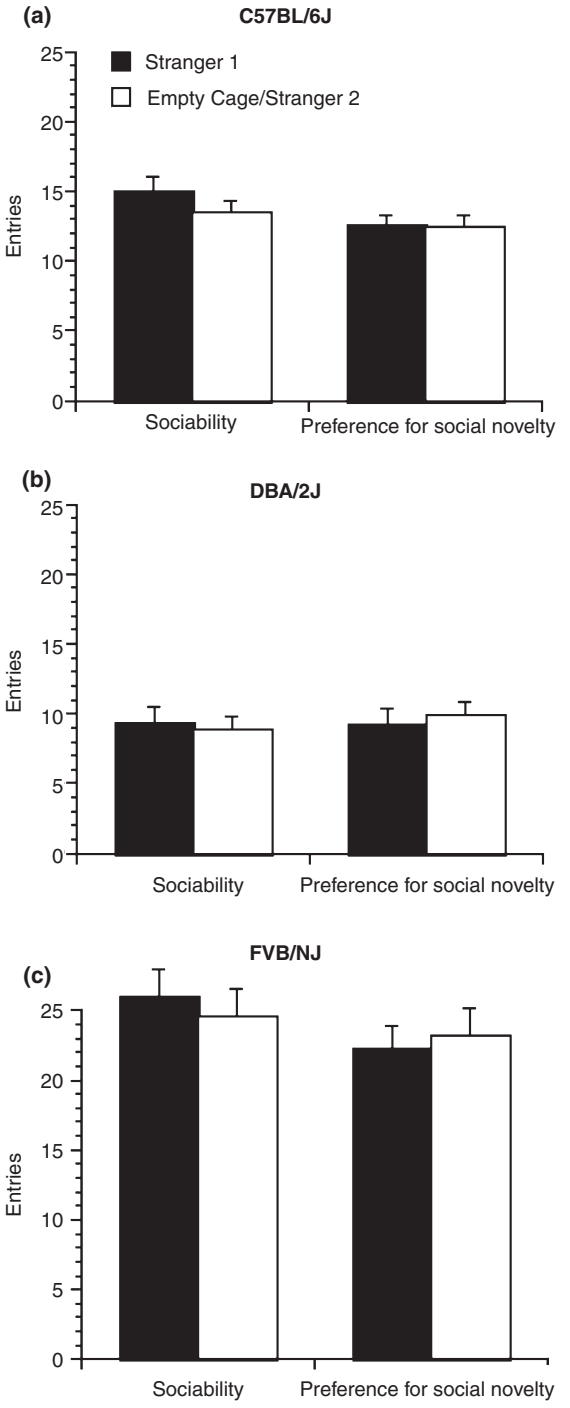

Figure 7: Comparison of three inbred strains on entries into each chamber in the sociability and preference for social novelty tests. (a-c) Subjects were six-month-old male mice. (a) C57BL/6J, $n = 20$. (b) DBA/2J, $n = 20$. (c) FVB/NJ, $n = 14$. Stranger mice were adult male C57BL/6J. Data shown are mean + SEM for each group.

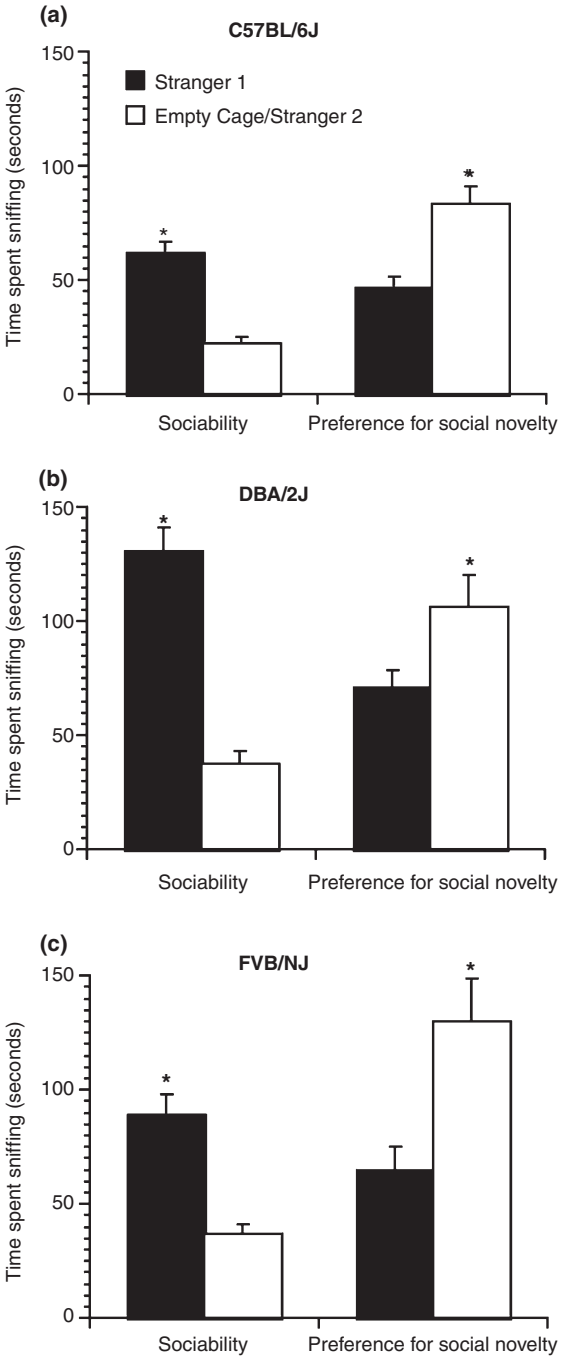

Figure 8: Comparison of three inbred strains on time spent sniffing wire cages in the sociability and preference for social novelty tests. (a-c) Subjects were six-month-old male mice. (a) C57BL/6J, $n=20$. (b) DBA/2 J, $n=20$. (c) FVB/NJ, $n=14$. Stranger mice were adult male C57BL/6J. Data shown are mean + SEM for each group. * $P<0.05$, comparison between chamber side.

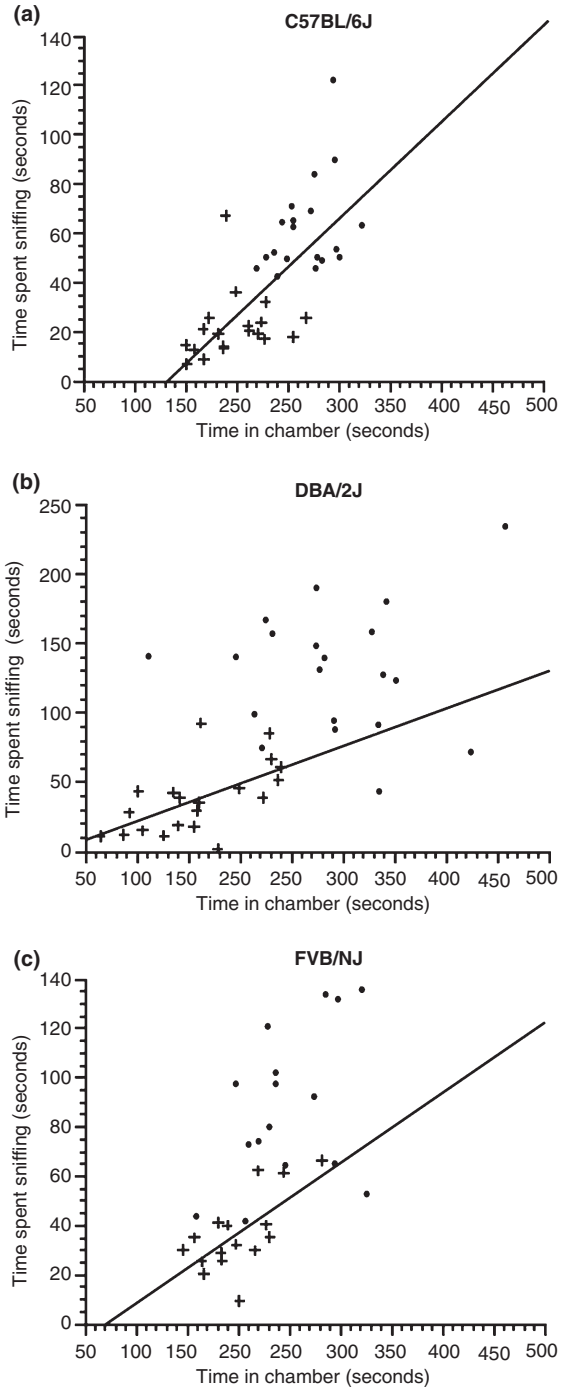

Figure 9: Correlation between the amount of time spent sniffing and time spent in a chamber in the test of sociability presented in Figure 5. *X*-axis represents total time spent in the chamber during the 10-minute test session; *Y*-axis represents total time spent sniffing the wire cage in that side during the 10-minute test session. Data points represent mice in the chamber containing stranger 1 (●) or mice in the empty cage (+). (a) C57BL6/J; $r=0.703$, $P<0.001$. (b) DBA/2J (note change in *Y*-axis values); $r=0.647$, $P<0.001$. (c) FVB/NJ; $r=0.655$, $P<0.001$.

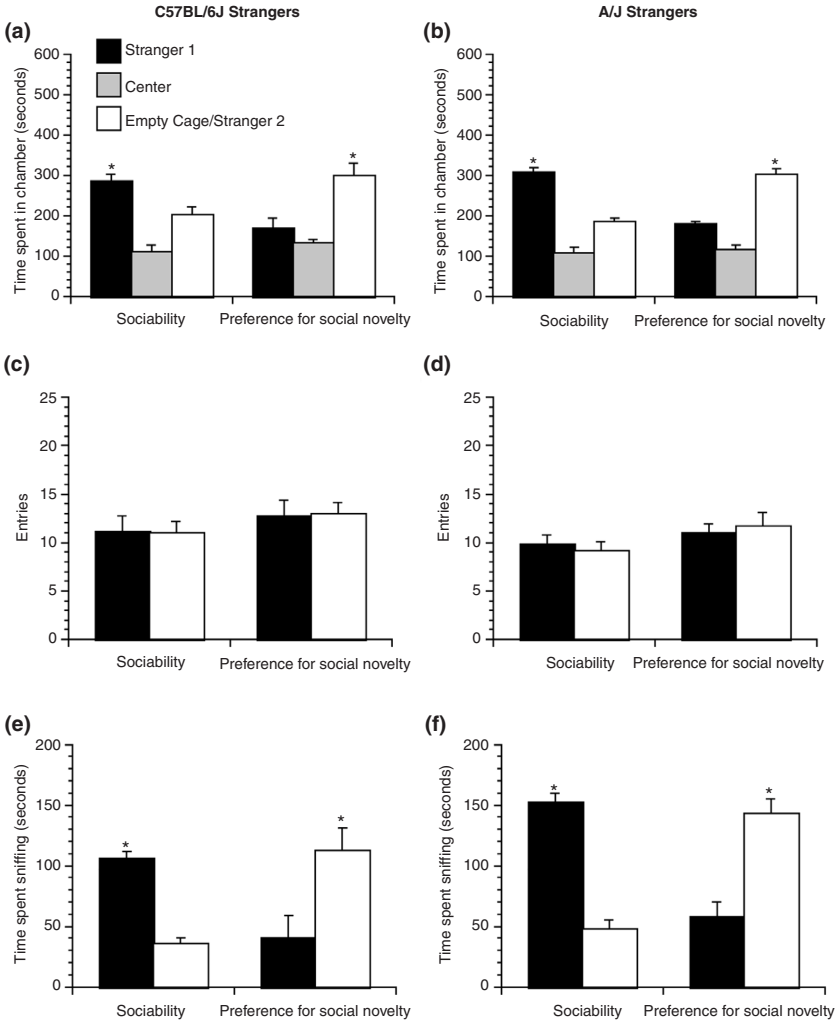

Figure 10: Comparison of two different stranger strains in the sociability and preference for social novelty tests. Subjects were 6-week-old C57BL/6J males. (a,c,e) C57BL/6J stranger animals. (b,d,f) A/J stranger animals. (a,b) Amount of time spent in chambers containing stranger 1 and empty wire cage, or in chambers containing stranger 1 and stranger 2, and in the center chamber. (c,d) Entries into each side. (e,f) Amount of time spent sniffing the wire cage containing stranger 1 or the empty wire cage. * $P < 0.05$, comparison between chamber side.

Number of entries appeared to be independent of time spent in the chambers and number of sniffs directed toward the wire cages. Instead, the entries parameter provided useful control information about general locomotor and exploratory activity. High or low baseline exploratory activity could confound the interpretation of a social deficit in this task. In this regard, it is interesting to note that FVB/NJ mice were significantly more active in terms of number of entries, but showed levels of sociability and preference for social novelty that were similar to C57BL/6J mice. DBA/2J displayed lower levels of activity in terms of number of entries, but retained a significant sociability score and showed a trend toward preference for social novelty. Differences in general exploratory activity therefore did not appear to directly affect sociability and social novelty preference in this initial evaluation of these three inbred strains of mice in the automated equipment.

Time course analysis found that the majority of the social approach behaviors occurred within the first five minutes of the sociability test. The amount of time spent on the side with the stranger animal is high in the first five-minute time bin and steadily decreases as the test continues. In contrast, the amount of time spent in the empty chamber did not vary significantly across the four time bins. The statistical significance of the sociability test is therefore due solely to the change in amount of time spent with the stranger animal. The amount of time spent sniffing the wire cage containing the stranger mouse was similarly highest in the first five-minute time bin and declined over the course of the 20-minute session. There was also a significant increase in the amount of time spent in the center chamber in the last time bin. Further, total number of entries was reduced in the last time bin. Taken together, these results suggest that by the end of the test, subject mice were less engaged in sniffing the wire cages and exploring the side chambers, while spending more time in the center start chamber. The time course obtained justifies the use of 10-minute time bins to capture the majority of the social approach behaviors in this task.

Similar to sociability, the time course of the test of preference for social novelty showed the majority of social

approach behavior occurring in the first five-minute time bin. Amount of time in the side with the now-familiar stranger 1 did not vary across the time bins, while there was a significant difference between the time spent with the novel stranger 2 in the first time bin compared to the other three. Significantly more time was spent in the center chamber in the final two time bins of the test. Time spent sniffing followed the same time course, supporting an interpretation that the majority of the social approach behavior occurred in the first five-minute time bin. Entries also dropped off significantly in the fourth time bin of the novelty preference test, indicating reduced exploratory activity. These time course results further support the use of 10-minute test sessions, as the majority of the preference for social novelty occurred during the first two five-minute time bins of the 20-minute test session.

Using a different strain for the stranger animal had no apparent effect on the sociability and preference for social novelty of test subjects. It is reasonable to suppose that the odors, appearance, behaviors and responses of the stranger will influence the social behaviors of the test subject. We examined this potential effect by substituting the C57BL/6J strangers with A/J animals, a strain which has been previously reported to display higher anxiety-like behaviors, lower exploration and decreased sociability and social novelty preference (Bouwknecht & Paylor 2002; Cohen *et al*. 2001; Mathis *et al*. 1994; Mathis *et al*. 1995; Moy *et al*. 2004; van der Staay & Steckler 2001). Replacing C57BL/6J strangers with A/J strangers produced no significant difference on either social task. This result suggests that the social approach behavior observed in the tests of sociability and preference for social novelty may be inherent in the subject strain, rather than a response to a particular stranger strain.

A major advantage of the automated equipment is the elimination of the labor-intensive and tedious aspects of hand-scoring. The automated apparatus is also likely to minimize observer fatigue and increase the consistency of results across experiments and across laboratories. In addition, automating the basic scoring leaves the observer free to score more interesting and complex behaviors of the test subjects.

The automated apparatus described herein is proposed as a simple, accurate approach to quantitate social behaviors in various strains of mice. A mouse line displaying selective and robust differences in tendencies to initiate social approach behaviors may offer construct validity towards modeling aberrant social approach behaviors in neuropsychiatric diseases such as autism, William's syndrome, social phobias and schizophrenia. Availability of inexpensive automated equipment opens the possibility of higher-throughput experiments that accurately score the tendencies of mice to initiate or avoid social approach. This automated equipment may be useful in future large-scale investigations into the genetic basis of social behavior.

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