A Mouse Model System for Genetic Analysis of Sociability: C57BL/6J Versus BALB/cJ Inbred Mouse Strains

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Background: *Impairments in social behaviors are highly disabling symptoms of autism, schizophrenia, and other psychiatric disorders. Mouse model systems are useful for identifying the many genes and environmental factors likely to affect complex behaviors, such as sociability (the tendency to seek social interaction). To progress toward developing such a model system, we tested the hypothesis that C57BL/6J inbred mice show higher levels of sociability than BALB/cJ inbred mice.*

Methods: *Mice tested for sociability were 4- and 9-week-old, male and female C57BL/6J and BALB/cJ mice. On 2 consecutive days, the sociability of each test mouse toward an unfamiliar 4-week-old DBA/2J stimulus mouse was assessed with a social choice paradigm conducted in a three-chambered apparatus. Measures of sociability included the time that the test mouse spent near versus far from the stimulus mouse, the time spent directly sniffing the stimulus mouse, and the time spent in contact between test and stimulus mice in a free interaction.*

Results: *C57BL/6J mice showed higher levels of sociability than BALB/cJ mice overall in each of these measures.*

Conclusions: *We propose that C57BL/6J and BALB/cJ mice will be a useful mouse model system for future genetic and neurobiological studies of sociability.*

Key Words: Mouse, model, social, behavior, genetic, autism

Social withdrawal is a highly disabling, treatment-refractory symptom of autism, schizophrenia, and other neuropsychiatric disorders (Hill and Frith 2003; McDougle et al 2005; Pinkham et al 2003; Stein 2003). The biological basis of sociability (defined as a tendency to seek social interaction) is not well understood and has received relatively little attention in the neuroscience literature. To elucidate the neurobiology and genetics of this complex behavior, the development of mouse model systems will be crucial, because of the high level of experimental control that model organisms afford and because of the powerful set of experimental tools available for mouse genetics. Although progress has been made in elucidating the roles of oxytocin, vasopressin, estrogen, and their receptors in various rodent social behaviors (Bielsky et al 2004; Choleris et al 2003; Ferguson et al 2000, 2001; Ferris et al 1997; Lonstein and Gammie 2002; Young 2002; Young et al 1999), there are likely to be many additional genes and gene products that affect sociability (Insel 2001). To identify these genes using mouse genetics, assays for sociability in mice must be developed, and differences among inbred mouse strains in levels of sociability must be identified (Abiola et al 2003; Tarantino et al 2000).

Social choice paradigms, in which a "test" mouse can choose to approach or not approach a "stimulus" mouse that is confined to a restricted area, are being developed for measuring mouse sociability (Brodkin et al 2004; Insel 2001; Moy et al 2004; Nadler et al 2004; Young et al 1999). Social choice paradigms are especially useful for genetic studies, such as quantitative trait locus (QTL) mapping or *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis studies, in which the social behaviors of hundreds of individual F2 or N2 mice towards a standardized social stimulus must be ascertained and compared (Brodkin, in press; Brodkin et al 2002; Hahn and Schanz 1996; Insel 2001).

In an initial study of prepubescent female mice of six different inbred strains, we found that C57BL/6J mice showed the greatest predominance of social approach, whereas BALB/cJ mice showed the greatest predominance of social avoidance, toward prepubescent DBA/2J females (Brodkin et al 2004). In that study, each "test" mouse (from one of the inbred strains) could choose to approach or avoid an unfamiliar "stimulus" mouse (DBA/2J) that was restricted in a cylinder to one side of a three-chambered apparatus (the "social side"). Because our initial study was limited in scope (included only one measure of sociability [time spent in the "social" vs. "nonsocial" sides of the apparatus], only prepubescent [4-week-old] female mice, and only 1 day of behavioral testing), in the present study we sought to more fully characterize the behavior of C57BL/6J and BALB/cJ mice toward a novel social stimulus, in both pre- and postpubescent males and females, using multiple measures of sociability and using 2 consecutive days of testing, to develop this model system for genetic analysis of sociability. Studies of sociability in these two particular mouse strains are especially important because these strains are very widely used in genetic studies, including studies of gene deletions (knockouts), QTL mapping, and mutagenesis (Crawley et al 1997). To our knowledge, the sociability of these two important strains has never been directly and comprehensively compared.

We sought to test the following hypotheses in the current study: (1) overall, C57BL/6J will show a higher level of sociability than BALB/cJ mice, as measured by multiple behavioral variables, including time spent near a stimulus mouse in a three-chambered apparatus ("social approach"), time spent directly sniffing the cylinder in which the stimulus mouse is contained, and the time spent in contact between the test and stimulus mouse during a free interaction; and (2) the observed strain differences in sociability will not be due to differences in locomotor activity. To address these hypotheses, we tested 4-week-old and 9-week-old male and female C57BL/6J and BALB/cJ mice over 2 consecutive days and assessed the effects of strain, age, sex, and day of testing on sociability and locomotor activity.

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Received March 21, 2005; revised June 20, 2005; accepted July 14, 2005.

0006-3223/06/$32.00
doi:10.1016/j.biopsych.2005.07.026

Methods and Materials

Animal Housing Protocol

All mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). Upon arrival at University of Pennsylvania, C57BL/6J mice (to be used as "test" mice) were housed two males or two females per cage; BALB/cJ mice (to be used as "test" mice) were housed two males or two females per cage; and DBA/2J mice (to be used as "stimulus mice") were housed four males or four females per cage. Animals were housed in temperature-controlled rooms with a 12-hour light/12-hour dark cycle (lights on at 7:00 AM). They were given TestDiet 5001 (Purina Mills, Richmond, Indiana) and water ad libitum. Cages were changed weekly. The mice were used for behavioral testing starting 5–7 days after arrival at University of Pennsylvania. Three-week-old mice delivered from The Jackson Laboratory were used for behavioral testing at 4 weeks of age. Eight-week-old mice ordered from The Jackson Laboratory were used for behavioral testing at 9 weeks of age. All animal procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Behavioral Testing

In each test, the degree of social approach of a "test" mouse toward a novel (unfamiliar) "stimulus" mouse was measured. Test mice included 4-week-old C57BL/6J females (n = 19), 4-week-old BALB/cJ females (n = 20), 4-week-old C57BL/6J males (n = 20), 4-week-old BALB/cJ males (n = 20), 9-week-old C57BL/6J females (n = 20), 9-week-old BALB/cJ females (n = 21), 9-week-old C57BL/6J males (n = 21), and 9-week-old BALB/cJ males (n = 20). The 4-week-old mice and 9-week-old mice were separate groups (i.e., the 9-week-old mice had not undergone previous behavioral testing at 4 weeks of age). To minimize variability among stimulus mice, all were 4-week-old DBA/2J mice. The social approach of all male test mice was measured toward male 4-week-old DBA/2J stimulus mice, and the social approach of all female test mice was measureed toward female 4-week-old DBA/2J stimulus mice. Stimulus mice were prepubescent and of the same sex as test mice to minimize aggressive and sexual motivations of the test mouse toward the stimulus mouse. Each test and stimulus mouse was used in only one 2-day testing procedure.

Behavioral testing was conducted between 12:00 PM (noon) and 5:00 PM in a dimly lit (5–7 lux) testing room. Test and stimulus mice were brought to the testing room in their home cages and were allowed to sit undisturbed in the testing room for at least 5 min before the start of behavioral testing. All behavioral testing was videotaped with a Sony Digital Video Camera (Sony Corp., Tokyo, Japan) and the NightShot feature for recording in low light. The behavioral testing apparatus was a black Plexiglas rectangular box (20.5 in long × 10 in wide × 9 in tall), consisting of three interconnected chambers (Figure 1A and C). The two end chambers were of equal size (7.5 in × 10 in), and the middle chamber was smaller (4.75 in × 10 in). This apparatus was slightly larger than that used in our previous study of 4-week-old mice (Brodkin et al 2004), to accommodate the testing of both 4-week-old and 9-week-old mice in the present study. The apparatus did not have a top or a bottom. Before each behavioral test, the apparatus was placed on a clean mat and clean mouse bedding. Two identical clear Plexiglas cylinders (each 3 in in diameter, 4.9 in tall) with removable, black Plexiglas lids were placed in the testing apparatus, one in each end chamber. The diameter of the Plexiglas cylinder was sufficiently large for a 4-week-old stimulus mouse to move around easily on the bottom of the cylinder. The two Plexiglas cylinders had multiple small holes (.5 in diameter), evenly spaced over the entire surface of the cylinder, to allow for air exchange between the interior and exterior of the cylinders (Figure 1B). Auditory, visual, and olfactory investigation between a mouse inside and a mouse outside the cylinder was thus possible. Between each test, the entire apparatus, including the cylinders, was then taken out of the colony room, wiped off with a paper towel moistened with a 30% ethanol solution, rinsed with copious amounts of water, and then dried. The mat and bedding that provided the floor for the

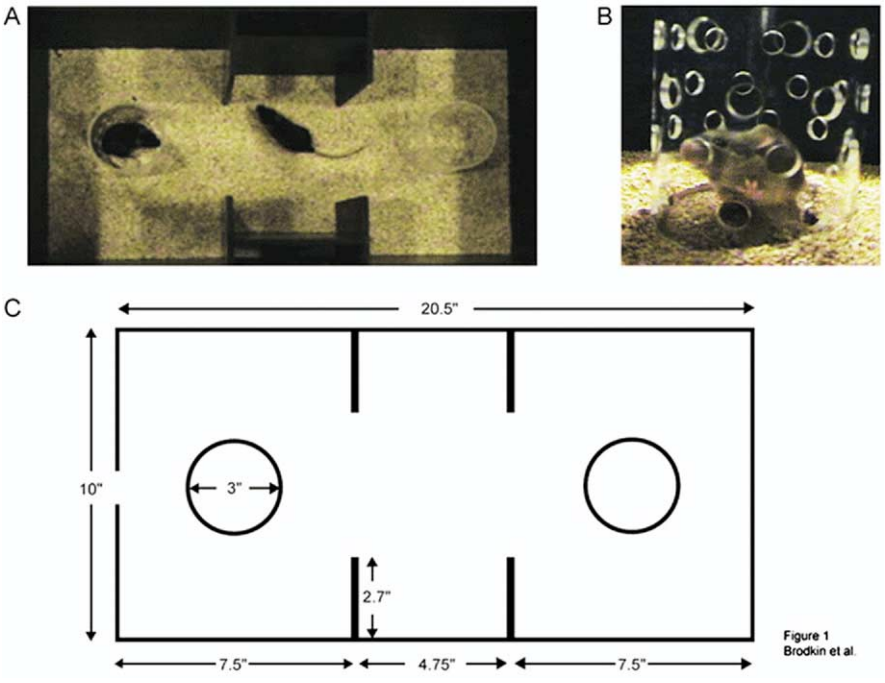

Figure 1. Behavioral testing apparatus. (**A**) The behavioral testing apparatus viewed from above. There is a clear Plexiglas cylinder in each of the two end chambers. Before the start of each test, one of the end chambers was arbitrarily designated the "social side" (the side into which the stimulus mouse would be introduced), and the other end chamber was designated the "nonsocial side." A "test" mouse is shown in the center chamber of the apparatus, and a "stimulus" mouse is shown in the cylinder on the social side of the apparatus (the "social cylinder"). The cylinder on the nonsocial side of the apparatus (the "nonsocial cylinder") is empty. For the purposes of the picture, no lids are shown on the cylinders. (**B**) Multiple holes (.5 in diameter each) are evenly spaced over the surface of the cylinders in each end chamber, and these holes are large enough for a mouse to poke its nose through for olfactory investigation. A stimulus mouse is shown in the cylinder. (**C**) Dimensions of the behavioral testing apparatus.