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Research report

Expression of social behaviors of C57BL/6J versus BTBR inbred mouse strains in the visible burrow system

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ABSTRACT

The core symptoms of autism spectrum disorder (ASD) include deficits in social interaction, impaired communication, and repetitive behaviors with restricted interests. Mouse models with behavioral phenotypes relevant to these core symptoms offer an experimental approach to advance the investigation of genes associated with ASD. Previous findings demonstrate that BTBR T+ tf/J (BTBR) is an inbred mouse strain that shows robust behavioral phenotypes with analogies to all three of the diagnostic symptoms of ASD. In the present study, we investigated the expression of social behaviors in a semi-natural visible burrow system (VBS), during colony formation and maintenance in groups comprising three adult male mice of the same strain, either C57BL/6J (B6) or BTBR. For comparative purposes, an extensively investigated three-chambered test was subsequently used to assess social approach in both strains. The effects of strain on these two situations were consistent and highly significant. In the VBS, BTBR mice showed reductions in all interactive behaviors: approach (front and back), flight, chase/follow, allo-grooming and huddling, along with increases in self-grooming and alone, as compared to B6. These results were corroborated in the three-chambered test: in contrast to B6, male BTBR mice failed to spend more time in the side of the test box containing the unfamiliar CD-1 mouse. Overall, the present data indicates that the strain profile for BTBR mice, including consistent social deficits and high levels of repetitive self-grooming, models multiple components of the ASD phenotype.

1. Introduction

Autism spectrum disorder (ASD) is a highly prevalent and severe neurodevelopmental disorder defined in the DSM-IV by three fundamental symptoms [2,28]. Abnormal reciprocal social interactions include low levels of social approach, and other impairments such as deficits in non-verbal expression (e.g. deficits in eye-to-eye gaze and diminished expression of emotion as measured by lack of gesturing or facial expression) and markedly diminished peer relationships [11,15]. Deficits in social communication include delayed development of speech and poor expressive language [19,29]. Sterotyped, repetitive, and ritualistic behaviors, resistance to change a learned response, compulsions, obsessions, and other persistent behavioral patterns are components of the third diagnostic indicator of ASD [27,41]. While the etiology of ASD is not yet established, support for a strong genetic component is evident from the 90% concordance rate between monozygotic twins [4,5,18]. However, genetic studies to determine specific heritable factors underlying susceptibility for ASD have indicated that the great majority of cases involve the interaction between multiple genes and possible environmental factors [1,20,38].

The primary diagnostic indices of ASD are abnormal behaviors, rather than biochemical, neuroanatomical or other physiological symptoms [2,16,21]. Therefore, suitable animal models for ASD require a relationship to the types of social deficits that are considered the core symptoms of such disorder, including impairments in social interaction and deficiencies in other functional domains [12,31,33]. Advanced transgenic and recombinant technologies, and the sequencing of mouse genome, have made the mouse the species of choice for many researchers in the field of behavioral genetics [8,10,17]. Mice are a social species, displaying social investigation of an unfamiliar conspecific, communal nesting, sleeping in group huddles, aggression directed towards intruders, sexual approach and mating behavior patterns, parental care of the pups and juvenile play [26,39].

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The use of inbred strains of mice provides valuable translational tools to assess the interplay of genetic and environmental factors in neurodevelopmental disorders [13,32,34,35,37]. Results obtained in recent studies reveal that the BTBR T+ tf/J (BTBR) inbred strain displays behavioral traits with face validity for all three diagnostic symptoms of ASD. Compared to the commonly used C57BL/6J (B6) strain, BTBR mice display low reciprocal social interactions, reduced social approach and impaired juvenile play [30], a behavioral phenotype with analogy to the first diagnostic symptom of ASD, social interaction deficits. Similarly, pairs of male BTBR mice spent the least time engaged in social interactions when compared to 129S1/SvImJ (129S1) and B6 inbred strains [8]. The sociability of BTBR pups was not rescued by cross-fostering such mice with B6 mothers, countering the hypothesis that sociability is regulated in this inbred strain by the maternal environment [46]. In regard to the second diagnostic symptom of ASD, communication deficits, a recent study showed that BTBR mice display an unusual pattern of vocalizations consisting of high levels of harmonics, two-syllable, and composite calls, but minimal numbers of chevron-shaped syllables, upward, downward, and short calls, when compared to the B6 strain [40]. With regard to the third diagnostic symptom of ASD, both adult and juvenile BTBR mice exhibit high levels of repetitive self-grooming [30,44,46]. This high level of self-grooming, like the BTBR deficit in sociality, was unaffected by cross-fostering BTBR with B6 mothers [46]. Repetitive behaviors in BTBR mice were also assessed with the use of the exploratory hole-board test [36], with data indicating reduced exploration and high preference for holes located in the corners of the chamber in the BTBR strain. In addition, BTBR was the only strain that did not demonstrate a shift in hole preference towards an appetitive olfactory stimulus, following home cage exposure to the food [36], suggesting that BTBR mice are resistant to change and display restricted interests.

The objective of the present study was to extend the previous findings indicating that the BTBR strain of mice is a useful model for ASD, with focus on the first diagnostic symptom, social interaction deficits. To reach this goal, we investigated BTBR and B6 social behaviors in two tests: the visible burrow system (VBS) and the three-chambered social approach test. The VBS is a semi-natural context which was designed to provide many of the features of the natural habitats of rodents, including multiple tunnels and burrows in addition to an open surface area [6,7,9]. A previous study from our research group investigated the social relationships of B6 mice colonies in the VBS [3], with results indicating that frontal approaches, along with huddling, may constitute particularly appropriate measures of mouse sociability in this context, i.e. pro-social or amicable behaviors that do not involve extrinsic approach motivations such as aggression or sexual interest. In our study, the expression of social behaviors in the VBS was evaluated during colony formation and maintenance in groups comprising three adult male mice of the same strain, either B6 or BTBR. For comparative purposes, an extensively investigated three-chambered test [32] was subsequently used to assess social approach in B6 and BTBR mouse strains. The comparison of strain effects on sociability measures between the VBS and the three-chambered test was used to facilitate an evaluation of the validity of the new ethological measures and enable an assessment of whether these measures offer useful additional information on sociability. Our study also provided additional information on the third diagnostic symptom of ASD, repetitive behaviors, once analysis of the VBS data included scoring the frequency of self-grooming in B6 and BTBR strains.

2. Materials and methods

2.1. Animals

12 B6 male mice aged 10–14 weeks at the first day of colony formation and 12 BTBR male mice, aged 11–14 weeks at this same date were used as subjects. B6 and BTBR mice for this study were offspring of breeding pairs obtained from the Jackson Laboratory (Bar Harbor, ME) and subjects were bred in the animal facilities of the University of Hawaii Laboratory Animal Service. Out-bred CD-1 stimulus mice were purchased from Charles River Labs (Company Location). Breeding pairs from both B6 and BTBR inbred strains were maintained by sibling mating. Subjects and stimulus mice were reared in standard polypropylene cages, 26.5 cm × 17 cm × 11.5 cm (*H*), in a group of three to five male littermates after weaning at the 25 days of age, in a temperature-controlled room (22 ± 1 °C). All subjects were maintained on a 12-h light/dark cycle (lights on at 06:00 am), with free access to food and water in their home cages. All procedures were conducted in accordance with protocols approved by the University of Hawaii Institutional Animal Care and Use Committee.

Fig. 1. Three-chambered apparatus. Three interconnected chambers are separated by two manually operated sliding doors. Side compartments contain inverted wire cups to house a stimulus mouse. A steel weight and a clear Plexiglas cylinder are placed above the inverted cups to prevent lifting or climbing on top. The inset displays a front view demonstrating the clear Plexiglas window on the front of the apparatus.

2.2. Apparatus

2.2.1. VBS

Visible burrow systems were constructed as previously described [3], but with a reduction of the surface area, to improve the clarity of video-records of individual mice. Each colony was housed in a rectangular, galvanized metal bin, 86 cm × 61 cm × 26 cm (*H*). Three chambers, each 12 cm × 7 cm × 6 (H) cm, were positioned behind a barrier wall extending across a short width (61 cm) of the bin, 30 cm from the end wall. This wall separated an open surface area (30 cm × 61 cm × 26 cm (H)) from the chambers in the other compartment. These chambers were connected to the wall via clear Plexiglas tubes 5 cm in diameter. Two of the three chambers, each connected to the surface area via a *Z* shaped tube, were connected to each other via a straight clear Plexiglas tube. The third chamber was connected only to the surface via a straight tube. The animals could pass freely between each chamber and the surface area, or between the two connected chambers, by these tubes. A water spout was located in the left corner of the surface area while food hoppers were located in the frontal wall of the bin. All dividing walls and chambers were constructed of black Plexiglas. A vertical extension of the surface area (30 cm × 61 cm × 30 cm (H)) made of clear Plexiglas was placed above the frontal part of the bin to prevent escape from the apparatus. The floor was covered by a layer of sawdust bedding (1 cm) in all chambers as well as the surface. A video camera was connected to a DVD recorder and mounted on the ceiling over the VBS allowing the recording of subject behavior from an upper view.

The experimental room was maintained on a 12-h light/dark cycle (lights on at 06:00 am), being illuminated by fluorescent lamps during the light period and by infrared light during the dark phase. Temperature (22 ± 1 °C) and humidity (70%) were also controlled in the experimental room.

2.2.2. Three-chambered social approach test

A 41 cm *L* × 70 cm *W* × 28 cm *H* three-chambered arena was used to assess sociability (Fig. 1). 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. The two outside chambers contained an inverted empty black wire cup (Galaxy Pencil/Utility Cup Spectrum Diversified Designs, Inc., Streetsboro, OH) which housed stimulus mice. The experimental room was illuminated by standard fluorescent lamps (120 lx) and the tests were conducted from 10:00 am to 4:00 pm.