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Research report

Social approach and repetitive behavior in eleven inbred mouse strains

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ABSTRACT

Core symptoms of autism include deficits in social interaction, impaired communication, and restricted, repetitive behaviors. The repetitive behavior domain encompasses abnormal motoric stereotypy, an inflexible insistence on sameness, and resistance to change. In recent years, many genetic mouse models of autism and related disorders have been developed, based on candidate genes for disease susceptibility. The present studies are part of an ongoing initiative to develop appropriate behavioral tasks for the evaluation of mouse models relevant to autism. We have previously reported profiles for sociability, preference for social novelty, and resistance to changes in a learned pattern of behavior, as well as other functional domains, for 10 inbred mouse strains of divergent genetic backgrounds. The present studies extend this multi-component behavioral characterization to several additional strains: C58/J, NOD/LtJ, NZB/B1NJ, PL/J, SJL/J, SWR/J, and the wild-derived PERA/EiJ. C58/J, NOD/LtJ, NZB/B1NJ, SJL/J, and PERA/EiJ demonstrated low sociability, measured by time spent in proximity to an unfamiliar conspecific, with 30–60% of mice from these strains showing social avoidance. In the Morris water maze, NZB/B1NJ had a persistent bias for the quadrant where the hidden platform was located during acquisition, even after 9 days of reversal training. A particularly interesting profile was found for C58/J, which had low social preference, poor performance in the T-maze, and overt motoric stereotypy. Overall, this set of tasks and observational methods provides a strategy for evaluating novel mouse models in behavioral domains relevant to the autism phenotype.

1. Introduction

Autism is a neurodevelopmental disorder diagnosed on the basis of an aberrant behavioral phenotype, rather than by a physiological biomarker or specific neuropathology. Core symptoms typically emerge early in life, and include deficient social interaction, impaired verbal communication, and stereotyped repetitive or ritualistic behavior, including abnormal motoric responses, resistance to change in routines or schedules, and unusual or obsessional interests [1]. There is significant co-morbidity between autism and mental retardation [15,35], which can include severe language deficits. While the etiology of autism is not well understood, support for a strong genetic component is evident from the 80% to 90% concordance between monozygotic twins [14,32], and association analyses have identified candidate genes for autism susceptibility (e.g. [8,9,43]).

Given the high heritability of autism, the investigation of relevant genetic mouse models can provide a powerful approach to determine the underlying neuropathology and etiology of the clinical disease. One challenge for the evaluation and validation of mouse models has been the ability to assess behavior in mice that reflects autism symptomatology. We have developed a set of behavioral measures for characterizing mouse models of the autism phenotype [30,31,33], based on clinical observations of autistic children and findings from research with human subjects. For example, children with autism have deficiencies in social responses. One study reported that autistic children spend less time in close proximity to other children and are less likely to focus attention on another child, in comparison to typically developing children [27]. More recently, Jahr et al. [19] found significantly reduced frequency of spontaneous social contact in autistic children, both high-functioning or with mild mental retardation. These

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deficits in social approach can be modeled in mice by using social choice tasks, in which mice are presented with a choice between spending time in the proximity of another mouse, or remaining alone [7,30,31,33,42]. Inbred strain distributions using social choice tasks have shown that levels of social approach and preference are dependent upon genetic background, with some strains (AKR/J, C57BL/6J, C3H/HeJ, FVB/NJ) demonstrating high affiliation, and other strains (A/J, BALB/c, BALB/cByJ, BTBR *T+tf*/J, 129S1/SvImJ) having low preference or even avoidance [6,7,30,31,33,42].

Restricted, repetitive behavior is also a core diagnostic indicator of autism [1]. The domain of repetitive behavior can include abnormal or stereotyped motor responses. We use systematic home cage observations to detect the occurrence of unusual motoric responses in mice. Repetitive behavior can also encompass resistance to change a learned response, compulsions, obsessions, and other persistent behavioral patterns, which may be related to deficits in executive function. For example, repetitive behavior in autistic adults was found to correlate with impaired cognitive flexibility and response inhibition [26]. Children with autism have been reported to have deficient performance in a spatial-reversal task, another measure for the ability to change a learned pattern of behavior [11]. To model this task, we have used reversal learning in the Morris water maze or T-maze tasks [31]. In our previous 10-strain comparison, mice from the BTBR T+tf/J strain had a selective deficit in reversal learning in the Morris water maze, but normal performance in reversal learning in a T-maze task, suggesting that measures of quadrant selectivity in the water maze is a more sensitive measure of cognitive flexibility. Deficits in reversal learning have been reported in genetic mouse models relevant to autism, including the fragile X syndrome-model mouse [2,22,38,46] and *Reln*$^{rl/+}$ mice [5], as well as in mice with prenatal exposure to an inflammatory challenge [28].

The present study reports the behavioral phenotype of an additional seven inbred mouse strains (C58/J, NOD/LtJ, NZB/BINJ, PERA/EiJ, PL/J, SJL/J, and SWR/J), and provides a second assessment of social approach in four strains previously evaluated (AKR/J, C57BL/6J, DBA/2J, and FVB/NJ). Strains were also tested for acquisition and reversal learning. Control measures were taken of general health, home cage behaviors, neurological reflexes, olfactory ability, activity, motor coordination, and anxiety-like behavior, to identify potential confounding factors. Subjects were male mice, to reflect the approximately 4:1 higher incidence of autism in males [14,15,32].

2. Materials and methods

2.1. Animals

Sets of male mice (n = 17–22 mice) from 10 inbred strains, AKR/J, C57BL/6J, C58/J, DBA/2J, FVB/NJ, NOD/LtJ, NZB/BINJ, PL/J, SJL/J, and SWR/J, were purchased from the Jackson Laboratory (JAX; Bar Harbor, ME). Due to limitations on available mice, the PL/J and SJL/J mice arrived in two separate cohort groups. Mice were 3–4 weeks of age upon arrival at the University of North Carolina animal facility in Chapel Hill, NC. An additional set of PERA/EiJ mice (11 males), matched in age to the other inbred strains, was derived from breeding pairs obtained from JAX. Mice were housed separately by strain, with three or four animals per plastic tub cage, and given free access to water. Purina 5058 chow was provided ad libitum, except when mice were under food restriction for appetitive tasks. The housing room had a 12-h light/dark cycle (lights off at 7:00 pm). Testing methods were designed to minimize pain and discomfort in the mice. All procedures were conducted in strict compliance with the policies on animal welfare of the National Institutes of Health and the University of North Carolina (stated in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, 1996 edition). All procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

2.2. Test procedures

Order of testing for most strains was: (1) home cage observations at age 3–4 weeks (initiated at least 5 days following arrival); (2) general health and neurological reflexes at age 4–5 weeks; (3) open field locomotion and rotarod at age 5–6 weeks; (4) social behavior test at age 6–7 weeks; (5) olfactory test with buried food at age 7–8 weeks; and (6) elevated plus-maze at age 8–10 weeks. The four inbred strains included in the previously published strain distribution from our laboratory [31] were not re-assessed in every test. Only the seven new inbred strains were evaluated in the Morris water maze task for reversal learning at age 3–6 months. Four of these strains were unable to perform the cued visible platform task on the water maze, and were assessed for T-maze learning and reversal. Across the entire study, mice were periodically observed for the emergence of abnormal behaviors in the home cage or during testing. Unless otherwise indicated, testing was conducted during the light phase of the light cycle, under fluorescent laboratory lighting (180–205 lx for activity and water maze tests, 320–340 lx for social approach and elevated plus maze tests, and 420 lx for the T-maze test).

2.3. Control measures

Several tests were conducted to aid in the interpretation of the results from the social approach and learning tasks, and to identify possible confounding factors. Observational measures were taken of home cage behavior, general health, and neurological reflexes, using direct scoring by a single observer. Exploration in a novel environment was used to assess activity levels (Versamax System, Accuscan Instruments). Motor coordination was assayed with an accelerating rotarod (Ugo-Basile, Stoelting Co., Wood Dale, IL), measuring maximum time for remaining at the top of the rotating barrel. For this test, records were taken of latency to fall off the barrel or to passively rotate, or invert off, by clinging to the barrel. Anxiety-like behavior was evaluated in the elevated plus maze test. Olfactory ability was assessed using a buried-food procedure following food deprivation. These procedures have previously been described in detail [31].

2.4. Sociability and preference for social novelty

The social behavior apparatus, previously described in detail [31,33], was designed to assess whether subject mice prefer to spend time in the proximity of stranger mice. The apparatus was a rectangular, three-chambered box fabricated from clear polycarbonate (42.5 cm $W \times$ 22.2 cm H; center chamber, 17.8 cm L; side chambers, 19.1 cm L). Dividing walls had retractable doorways allowing access into each chamber. Photocells were embedded in each doorway to allow automatic quantification of entries and duration in each chamber of the social test box.

2.4.1. Habituation

The test mouse was first placed in the middle chamber and allowed to explore for 10 min, with the doorways into the two-side chambers open. Each of the two sides contained an empty wire cage (Galaxy Cup, Spectrum Diversified Designs, Inc., Streetsboro, OH). Measures were taken of time spent in each of the side chambers and number of entries into each side chamber by the automated measurement system. The habituation phase was given immediately before the sociability test.

2.4.2. Sociability

After the habituation period, the test mouse was enclosed in the center compartment of the social test box, and an unfamiliar mouse (stranger 1; an adult C57BL/6J male) was enclosed in 1 of the wire cages and placed in a side chamber. The location for stranger 1 alternated between the left and right sides of the social test box across subjects. Following placement of stranger 1, the doors were re-opened, and the subject was allowed to explore the entire social test box for a 10-min session. Measures were taken of the amount of time spent in each chamber and the number of entries into each chamber by the automated testing system. In addition, a human observer scored time spent sniffing each wire cage, using a computer keypad and software [20].

2.4.3. Preference for social novelty

At the end of the 10-min sociability test, each mouse was further tested in a third 10-min session to quantitate preference for spending time with a new stranger. A new unfamiliar mouse was placed in the wire cage that had been empty during the prior 10-min session. The test mouse had a choice between the first, already-investigated mouse (stranger 1) and the novel unfamiliar mouse (stranger 2). Measures were taken as described above.

2.5. Water maze test

The Morris water maze task was based on the standard methods for spatial learning in rodents [29,37,48]. The water maze consisted of a large circular pool (diameter = 122 cm) partially filled with water (45 cm deep, 24–26 °C), located in a room with numerous visual cues. To allow detection by an automated tracking system (Noldus Ethovision), overhead fluorescent lighting was used for dark-pigmented strains, while halogen lighting directed at the ceiling was used for the albino strains (NOD/LtJ, PL/J, SJL/J, and SWR/J). Mice were tested for their ability to find an escape platform (diameter = 12 cm) on three different components: ability to find a cued