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Research report

Mouse behavioral tasks relevant to autism: Phenotypes of 10 inbred strains

Sheryl S. Moy [a,b,c,*], Jessica J. Nadler [a,b,d], Nancy B. Young [a,b], Antonio Perez [b,1], L. Paige Holloway [b], Ryan P. Barbaro [d], Justin R. Barbaro [d], Lindsay M. Wilson [b], David W. Threadgill [a,b,d], Jean M. Lauder [a,b,e], Terry R. Magnuson [a,b,d], Jacqueline N. Crawley [a,b,c,f]

[a] *North Carolina STAART Center for Autism Research, University of North Carolina School of Medicine, Chapel Hill, NC, USA*
[b] *Neurodevelopmental Disorders Research Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA*
[c] *Department of Psychiatry, University of North Carolina School of Medicine, Chapel Hill, NC, USA*
[d] *Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, NC, USA*
[e] *Department of Cell and Developmental Biology, University of North Carolina School of Medicine, Chapel Hill, NC, USA*
[f] *Laboratory of Behavioral Neuroscience, Intramural Research Program, National Institute of Mental Health, Bethesda, MD 20893-3730, USA*

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Abstract

Three defining clinical symptoms of autism are aberrant reciprocal social interactions, deficits in social communication, and repetitive behaviors, including motor stereotypies and insistence on sameness. We developed a set of behavioral tasks designed to model components of these core symptoms in mice. Male mice from 10 inbred strains were characterized in assays for sociability, preference for social novelty, and reversal of the spatial location of the reinforcer in T-maze and Morris water maze tasks. Six strains, C57BL/6J, C57L/J, DBA/2J, FVB/NJ, C3H/HeJ, and AKR/J, showed significant levels of sociability, while A/J, BALB/cByJ, BTBR *T*$^{+}$*tf*/J, and 129S1/SvImJ mice did not. C57BL/6J, C57L/J, DBA/2J, FVB/NJ, BALB/cByJ, and BTBR *T*$^{+}$*tf*/J showed significant preference for social novelty, while C3H/HeJ, AKR/J, A/J, and 129S1/SvImJ did not. Normal scores on relevant control measures confirmed general health and physical abilities in all strains, ruling out artifactual explanations for social deficits. Elevated plus maze scores confirmed high anxiety-like behaviors in A/J, BALB/cByJ, and 129S1/SvImJ, which could underlie components of their low social approach. Strains that showed high levels of performance on acquisition of a T-maze task were also able to reach criterion for reversal learning. On the Morris water maze task, DBA/2J, AKR/J, BTBR *T*$^{+}$*tf*/J, and 129S1/SvImJ failed to show significant quadrant preference during the reversal probe trial. These results highlight a dissociation between social task performance and reversal learning. BTBR *T*$^{+}$*tf*/J is a particularly interesting strain, displaying both low social approach and resistance to change in routine on the water maze, consistent with an autism-like phenotype. Our multitask strategy for modeling symptoms of autism will be useful for investigating targeted and random gene mutations, QTLs, and microarray analyses.

Keywords: Autism; Locomotion; Sociability; Social preference; Social approach; T-maze; Morris water maze; Reversal tasks

1. Introduction

Autism is a neurodevelopmental disorder, defined in the DSM-IV by three fundamental symptoms [2]. Aberrant reciprocal social interactions include low levels of social approach, and qualitatively unusual modes of social interaction [44,96]. Deficits in social communication include delayed development of speech and poor expressive language [76]. Stereotyped, repetitive, and ritualistic behaviors, narrow restricted interests, insistence on sameness and resistance to change in habit are components of the third defining diagnostic [18,120]. While evidence for neuropathology in autism suggests increased brain volume [6,8,30,37,61,62,94,97,126] and other neuroanatomical changes [7,32,78,95,103,127], and fMRI studies indicate reduced activation of the amgydala and fusiform gyrus during social tasks [43,92,105], there is no consistent neu-

* Corresponding author at: Neurodevelopmental Disorders Research Center, CB#7146, University of North Carolina, Chapel Hill, NC 27599, USA. Tel.: +1 919 966 3082; fax: +1 919 966 5657.
E-mail address: ssmoy@med.unc.edu (S.S. Moy).
[1] Present address: 18 Macomb St. #7, Plattsburgh, NY 12901, USA.

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rological or biochemical marker for diagnosis, and the etiology of autism remains unknown. In addition, there is a lack of effective therapeutic strategies [119]. A significant genetic component for autism is supported by studies of concordance rates between identical twins [13,35,48,70,121], and candidate autism-susceptibility genes have been proposed from linkage and association analyses [11,27,45,87,93,98,129]. These advances in our understanding of the genetic basis of autism are leading to the development of promising mouse models that reflect genetic polymorphisms linked to autism [5,66].

One of the challenges in the evaluation and use of mouse models for autism is to design behavioral tests that reflect the core symptoms of the disease [66,85,88,100]. Without biological markers, behavioral traits with face validity to the core characteristics of autism represent one approach toward evaluating genetic contributions and potential treatments. We and other labs are engaged in developing mouse behavioral tasks with conceptual analogies to the three defining features of autism. The present study addresses the first symptom, low or aberrant social approach, and the third symptom, resistance to change in habit. The goal of the present experiments is to understand the genetic variability across inbred strains of mice on these tasks, which can then be used to identify genes in strains with unusual traits in these behavioral domains.

We have developed a mouse social approach task to assess sociability, the tendency to spend time with another conspecific, and preference for social novelty, the ability to discriminate and choose between familiar and new conspecifics [86,89]. In this procedure, the mouse is placed in the center compartment of a three-chambered test box, and given a choice between spending time in the side containing an unfamiliar (stranger) conspecific mouse, or remaining alone. The stranger mouse is contained within a small wire cage, to allow exposure to visual, auditory, olfactory, and some tactile stimuli, while preventing aggressive or sexual interactions. Measures taken during the test include time spent in each side, entries into each side, and time spent sniffing each cage. An identical wire cage in the opposite side chamber serves as a control novel object, to measure exploration of something new that has no social valence. Adult male mice of three standard inbred strains, C57BL/6J, DBA/2J, and FVB/NJ, and the F1 hybrid B6129, demonstrated a clear preference for spending time in the proximity of another mouse, versus in proximity to a novel object, while the A/J strain did not exhibit significant levels of sociability [86,89]. This social deficit in A/J may result from their general lack of active exploration and anxiety-like phenotype, as observed on the elevated plus maze [23,79,107,115]. Using a similar task, Brodkin and colleagues [24,102] have found low levels of social approach in mice from the BALB/cJ strain, which is also characterized by high levels of anxiety-like behaviors [12,36,40].

A second component of our social behavior task evaluates preference for social novelty in mice. In this phase of the test, a second unfamiliar mouse (stranger 2) is placed into the wire cage that was empty during the assessment of social approach. The test mouse then has a choice between spending time in the side with the now-familiar stranger 1, or investigating the newly-introduced stranger 2. C57BL/6J, DBA/2J, and FVB/NJ, but not A/J, showed significant preference for proximity to stranger 2, versus the already-investigated stranger 1 [86,89].

In addition to deficits in social interaction, children with autism can show cognitive inflexibility, as seen in restricted interests, rigid adherence to schedules, insistence on sameness, and upset at changes in routine and habit. Perseveration and reversal tasks in mice have reasonable face validity to components of these symptoms. We are using reversal learning in T-maze and water maze spatial tasks to examine resistance to change in a learned pattern of behavior in mice. After reaching criterion on acquisition trials to learn the location of a food reward in the T-maze, or the location of the hidden escape platform in the water maze, the reinforcer location is switched to an opposite arm of the T-maze, or opposite quadrant of the water maze. Inbred strains of mice that fail to adapt to the new conditions for reinforcement may provide a model for the insistence on sameness characteristic of the autism phenotype.

The present study replicates and extends the mouse strain distribution on our social tasks to include six new inbred strains, C57L/J, C3H/HeJ, AKR/J, BALB/cByJ, BTBR $T^{+}tf$/J, and 129S1/SvImJ, in comparison to C57BL/6J, DBA/J, FVB/NJ, and A/J. These strains were selected from the top tier of inbred mouse strains recommended by the Jackson Laboratory Mouse Phenome Project (http://www.aretha.jax.org/pub-cgi/phenome/mpdcgi). Young male mice were employed, for consistency with the approximately 4:1 ratio of boys to girls in autism [48,49,87]. After completion of social testing, these 10 inbred mouse strains were evaluated for reversal learning in the T-maze and/or water maze tasks. Evaluation of general health, home cage behaviors, neurological reflexes, activity in an open field, motor coordination, olfactory ability, and anxiety-related behaviors on the elevated plus-maze were conducted to control for procedural abilities necessary for the social and reversal tasks.

2. Materials and methods

2.1. Animals

Twenty male mice from eight inbred strains, C57BL/6J, C57L/J, DBA/2J, FVB/NJ, AKR/J, A/J, BALB/cByJ, and 129S1/SvImJ, 19 male mice from the C3H/HeJ strain, and 24 male mice from the BTBR $T^{+}tf$/J strain were purchased from The Jackson Laboratory, Bar Harbor, ME (JAX). An additional set of 10 male mice from the A/J strain (JAX) was independently tested for elevated plus maze performance. An additional set of 20 male mice from the AKR/J strain (JAX) was tested, due to health problems arising in the older mice (see Section 2.2). Additional sets of C57BL/6J males were independently tested on the social task to confirm consistency of findings across time. Mice were 3–4 weeks of age upon arrival at the University of North Carolina animal facility in Chapel Hill, NC. Animals were housed separately by strain, with three to four mice per plastic tub cage, and provided with Purina 5058 chow and water ad libitum. The housing room was maintained at 23 °C on a 12-h light/dark cycle (lights off at 7 p.m.). 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), and approved by the University of North Carolina Animal Care and Use Committee.