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Research report

Social deficits, stereotypy and early emergence of repetitive behavior in the C58/J inbred mouse strain

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ABSTRACT

Mouse lines with behavioral phenotypes relevant to symptoms in neurodevelopmental disorders may provide models to test hypotheses about disease etiology and to evaluate potential treatments. The present studies were designed to confirm and expand earlier work on the intriguing behavioral profile of the C58/J inbred strain, including low social approach and aberrant repetitive movements. Additional tests were selected to reflect aspects of autism, a severe neurodevelopmental disorder characterized by emergence of symptoms early in life, higher prevalence in males, social deficits and abnormal repetitive behavior. Mice from the C57BL/6J inbred strain, which has a similar genetic lineage and physical appearance to C58/J, served as a comparison group. Our results revealed that C58/J mice display elevated activity levels by postnatal day 6, which persist into adulthood. Despite normal olfactory ability, young adult male C58/J mice showed deficits in social approach in the three-chambered choice assay and failed to demonstrate social transmission of food preference. In contrast, female C58/J mice performed similarly to female C57BL/6J mice in both social tests. C58/J mice of both sexes demonstrated abnormal repetitive behaviors, displaying excessive jumping and back flipping in both social and non-social situations. These stereotypies were clearly evident in C58/J pups by postnatal days 20–21, and were also observed in C58/J dams during a test for maternal behavior. Overall, the strain profile for C58/J, including spontaneously developing motor stereotypies emerging early in the developmental trajectory, and social deficits primarily in males, models multiple components of the autism phenotype.

1. Introduction

Mouse models of behavioral symptoms in human disease can be used to study the underlying neurobiology of aberrant phenotypes and to evaluate potential therapeutic strategies. For this reason, our laboratory has screened a number of inbred mouse strains and mutant lines for the presence of behaviors that may reflect symptoms seen in autism, fragile X syndrome, and other clinical disorders [10,35–37]. In these initial studies, one inbred strain demonstrated a particularly interesting behavioral profile [37]. Male C58/J mice demonstrated a lack of sociability in a three-chambered choice assay, and also showed overt stereotypy in several testing paradigms. Impaired social interaction, communication deficits, and abnormal repetitive behaviors are core features of autism [31]. The goal of this study was to confirm our initial findings, and to determine whether the C58/J mouse model reflects additional components of the autism phenotype.

Our previous study on the C58/J strain examined behavior in adolescent and adult animals. However, autism is a neurodevelopmental disorder with symptoms emerging early in childhood [25,31]. A mouse model of autistic-like behavior would ideally recapitulate the emergence of abnormal behavior early in life. To address this issue, we utilized a neonatal screen to evaluate motor function, activity levels, and the emergence of stereotypy in C58/J pups throughout the lactational period of development, starting at 6 days of age. Both male and female mice were assessed, to determine if the rates of aberrant repetitive behavior reflected the higher prevalence of autism in boys than in girls (with a ratio of approximately 4:1) [25,39]. For these studies, the behavioral profiles of the C58/J mice were compared to profiles of C57BL/6J mice, since this inbred strain shares a common genetic lineage with C58/J [14]. The two strains have an almost identical physical appearance and are available commercially from the same supplier, suggesting that C57BL/6J can serve as an appropriate control for C58/J. In addition to the neonatal screen, measures were taken of maternal responses,

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in order to determine if C58/J dams have an abnormal behavioral phenotype in comparison to control dams.

Male C58/J mice have low sociability in a three-chambered choice task that presents the subject mouse with a choice between spending time with a novel mouse or spending time with a novel non-social object [37]. To extend this earlier finding, we tested both male and female mice in the three-chambered assay and in a social transmission of food preference (STFP) task. The STFP task measures aspects of rodent social communication between conspecifics [6,15,23,34,50,55,59,60]. Rodents are naturally neophobic, and will avoid a completely novel food. However, mice will readily consume food previously smelled and/or tasted on the breath, muzzle, and whiskers of a conspecific. In this assay, the subject "observer" mouse is allowed to freely interact with a "demonstrator" mouse that recently ate a novel flavored food. The subject observer forms a food preference based on cues transferred between the mice during the interaction session.

Olfactory information is a critical component of social preference and interaction in mice [11,28,29,40,51]. The olfactory habituation/dishabituation task [18,33,57] tests the ability of a mouse to discriminate between different odors, including olfactory stimuli collected from unfamiliar mice. We used this assay to determine whether C58/J mice have deficiencies in odor discrimination, in comparison to C57BL/6J mice. A finding of altered olfactory function in C58/J could be relevant to changes in social approach, social transmission of food preference, and maternal behavior in this strain.

2. Materials and methods

2.1. Animals

C57BL/6J and C58/J mice for these studies were offspring of breeding pairs obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained in a vivarium at the University of North Carolina at Chapel Hill. All mice were weaned on postnatal day 21 and subsequently housed with same-sex littermates. Animals were maintained on a light:dark 12:12 circadian schedule with lights on at 7 a.m. The housing room was maintained at 20–24 °C and 40–50% relative humidity. Dams were fed ad libitum on Purina PicoLab Mouse Diet 20. Adults and weaned pups were fed ad libitum on Purina ProLab IsoPro 3000. Mice were housed in standard 20 cm × 30 cm ventilated polycarbonate cages containing laboratory grade Bed-O-Cob bedding and were given water ad libitum. A small section of PVC pipe was present in each cage for enrichment. All breeding and testing procedures were conducted in strict compliance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Research Council, 1996) and approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

Two different cohorts of mice were used in this project. This first cohort consisted of eleven C57BL/6J and seven C58/J dams and litters, which were tested in the neonatal screen, the juvenile motor activity and maternal behavior assays. This testing was completed by the time the pups reached weaning age at postnatal day (PND) 21 and these litters were not used in any other behavioral experiments. The second cohort consisted of C57BL/6J (16 male and 14 female) and C58/J (26 male and 18 female) mice and was tested in the olfactory habituation/dishabituation, three-chambered social choice and social transmission of food preference tasks. Beginning at approximately PND 30, the mice were tested in all three assays in the order described here, with a 1-week break between testing. Light levels for each behavioral test ranged from 570 to 620 lx, except where noted. In every assay, the experimenter was blind to the strain of the animal being tested.

2.2. Neonatal motor screen

Breeding pairs were checked daily for new litters. When a new litter was observed, the number of pups was counted and the litter was considered to be on PND 1. Both the dam and sire were kept in the cage with the pups throughout the neonatal period. On PNDs 6, 8 and 10, the pups were removed from their home cage, placed into a warmed beaker (35 °C; with temperature regulated via a hotplate analgesia meter, IITC Life Sciences Inc, Woodland Hills, CA) and tested in the following neurobehavioral screen (adapted from [8]).

2.2.1. Negative geotaxis

A 20 cm × 20 cm screen with 0.5 cm wire mesh squares was set at a 25° angle. Each pup was placed downward on the screen with its head facing toward the bottom, allowed to grip the mesh, and then released. The time it took for each pup to turn 180° (head and upper torso vertical), was recorded, with a 30 s maximum. This procedure was repeated three times, and the lowest latency to turn was used for the data analysis

2.2.2. Righting

Each pup was placed onto its back on a flat Plexiglas surface. The experimenter gently held the pup in place for 2 s and then released it. The latency to righting (all four feet on the ground) was timed, with a maximum of 30 s.

2.2.3. Forward locomotion

Each pup was placed into the center of a 20 cm × 20 cm Plexiglas chamber with a grid of 2 cm × 2 cm squares drawn beneath the floor. The number of pivots (circling or lateral movement, as opposed to forward locomotion) was recorded. The amount of time spent moving forward and the number of squares crossed after 3 min was also recorded, as was any jumping or rearing.

Each pup was screened through the three assays in the order presented here. After finishing the neonatal motor screen, each pup was weighed, sexed, marked with a non-toxic Sharpie, and placed back into the warm beaker. The wire mesh screen and Plexiglas chamber were wiped down with water and dried with a paper towel between each mouse, and cleaned with alcohol at the end of each testing day.

2.3. Motor activity

On PND 16 or 17 and then again on PND 20 or 21, the litter was removed from the home cage and placed into a beaker. One at a time, each pup was placed into the center of an empty PhenoTyper box (30.5 cm × 30.5 cm × 43.5 cm; Noldus Information Technology, Wageningen, The Netherlands). The PhenoTyper was placed within a sound-attenuating chamber to limit any visual or auditory distractions during the trial. A dim yellow light (30 lx) within the top of the PhenoTyper box was illuminated throughout the trial. The mouse was allowed to acclimate to the apparatus for 30 s and then freely explore for 10 min. The integrated camera system in the PhenoTyper was connected to a computer and the video was scored in real time using EthoVision 3.1 tracking software (Noldus Information Technology, Wageningen, The Netherlands). Using this software, the arena was divided into four different zones: corners (2 cm × 2 cm squares), walls (the outer 2 cm of the arena, not counting the corners), center (4 cm × 4 cm square in the very center of the arena), and the mid-area (the rest of the arena). EthoVision was used to automatically calculate the amount of time spent in each zone and the distance moved during the 10 min trial. In addition to these measures, the experimenter viewed the trial in real time on a computer screen and recorded the number and duration of any self grooming, jumping, or rearing bouts. After finishing the assay, the pup was weighed, sexed, marked with a Sharpie, and returned to the beaker. The PhenoTyper box was wiped down with water and dried with a paper towel after every trial, and cleaned with alcohol at the end of each testing day.

2.4. Maternal behavior

On PND 3, 6, 8, 10, 13, 16 or 17 and 20 or 21, the dam was tested for maternal behavior, for a total of seven maternal behavior tests. The pups were removed from the home cage and placed into a beaker (warmed on PNDs 3, 6, 8 and 10). The dam was also removed from the home cage and placed into a new cage with clean bedding and a nestlet cotton square (Ancare Corp., Bellmore, NY; 5 cm × 5 cm × 0.5 cm), and closed with a wire lid. During the period of separation, the pups either had their motor reflexes tested (PND 6, 8 and 10), had their activity assayed (PND 16 or 17 and 20 or 21) as described above, or remained in the beaker for 10 min (PND 3 and 13). To begin the maternal behavior assay, the pups were gently placed into the new cage with the dam. The pups were arranged in a group as far away from the nestlet material as possible. The experimenter then recorded the amount of time the dam spent in close proximity to the pups and noted the occurrence of multiple behaviors (adapted from [26]), listed in Table 1 (repetitive behaviors are defined in Section 2.8). Following the 15-min observation period, the sire was placed into the cage with the dam and pups and the cage was returned to the housing room.

2.5. Olfactory habituation/dishabituation

Adolescent mice were used in the olfactory habituation/dishabituation assay. Prior to the start of testing, each mouse was placed into a low profile rat-sized cage (30 cm × 60 cm × 15 cm) with clean bedding 30 min prior to testing. After this acclimation period, the olfactory testing began. A 15 cm cotton swab (Fisher Scientific, Pittsburgh, PA) was dipped in water, slid through the top of the cage and held in place with a binder clip. This method positioned the moistened end of the cotton swab approximately 5 cm above the bedding in the center of the cage. The mouse was observed for 2 min and any olfactory investigations of the cotton swab were timed. An olfactory investigation was defined as the mouse lifting and orienting its nose to within approximately 2 cm of the cotton swab tip. Other behaviors such as self-grooming, rearing, jumping, digging in the bedding, eating the bedding, and climbing on or chewing on the cotton swab were manually recorded.

After 2 min, the cotton swab was replaced with a new swab dipped in water. The mouse was again observed for 2 min and this process was repeated a third time with a new swab dipped in water. Following this pattern, every odor was tested in triplicate fashion in the following order: three trials of water alone, three trials with