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Social deficits in BTBR *T + tf*/J mice are unchanged by cross-fostering with C57BL/6J mothers

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Abstract

Inbred strains of mice are useful model systems for studying the interactions of genetic and environmental contributions during neurodevelopmental stages. We recently reported an inbred strain, BTBR *T + tf*/J (BTBR), which, as compared to the commonly used C57BL/6J (B6) strain, displays lower social interactions as juveniles, lower social approach in adult ages, and higher levels of repetitive self-grooming throughout developmental stages. The present study investigated whether the early postnatal maternal environment contributes substantially to the unusually low expression of social behaviors and high self-grooming in BTBR as compared to B6. Within 24 h of birth, entire litters of pups were cross-fostered to either a dam of the same strain or a dam of the opposite strain. Control litters were left with their own mothers. Offspring were tested for juvenile play at postnatal day 21 ± 1, for sociability at 8 weeks of age in an automated three-chambered social approach test, and for self-grooming at 9–11 weeks of age. Results indicate that deficits in play behaviors in juvenile BTBR pups were not rescued by a B6 maternal environment. Similarly, a BTBR maternal environment did not induce play deficits in B6 pups. Cross-fostering had no effect on sociability scores in adults. The high self-grooming in BTBR and low self-grooming in B6 were not affected by maternal environment. These findings favor a genetic interpretation of the unusual social behaviors and self-grooming traits of BTBR, and support the use of the BTBR inbred strain as a mouse model to study genetic mechanism of autism.
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Keywords: Inbred strains of mice; Behavioral development; Maternal factor; Postnatal environment; Social interaction; Juvenile play; Autism

1. Introduction

Accumulating evidence indicates that early postnatal environment can substantially influence a broad range of behavioral traits in the offspring (Meaney, 2001; Kaffman and Meaney, 2007). Human studies showed that childhood trauma and neglect are associated with increased risk of developing various psychiatric disorders later in life (Dube et al., 2001; Kendler et al., 2002, 2004; Chapman et al., 2004; Holmes et al., 2005; Millstein and Holmes, 2007). In rodents, adult rats born and raised by high licking-grooming and arched-back nursing (LG-ABN) mothers exhibited lower levels of novelty-induced fear-like behaviors and milder HPA responses to acute stress, as compared to offspring of low LG-ABN mothers (Caldji et al., 1998). Periodic maternal deprivation during the early postnatal life was associated with increased anxiety-like behaviors (Ogawa et al., 1994; Wigger and Neumann, 1999; Macri et al., 2004) and enhanced hypothalamo-pituitary-adrenal (HPA) axis responses to stress (Plotsky and Meaney, 1993; Suchecki et al., 1995; Ladd et al., 2004). Mice raised in a communal nesting environment, where multiple females share care-giving for their litters, displayed higher levels of social interactions than mice raised in standard laboratory homecages (D'Andrea et al., 2007).

Cross-fostering is a experimental procedure widely used to dissociate relative influences of genetic versus early environmental factors on various neurobiological phenotypes in rodents (Caldji et al., 2004; Priebe et al., 2005; Prakash et al., 2006; Champagne et al., 2007) and primates (Maestripieri, 2003). In an elegant study (Francis et al., 1999) showed that rats born to low LG-ABN mothers but raised by high LG-ABN mothers were more likely to explore a novel open field than rats raised by low LG-ABN mothers, including biological offspring of high LG-ABN mothers, indicating the

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importance of non-genomic factors on anxiety/fear-like behaviors in the rat (Bartolomucci et al., 2004) found that cross-fostered male Swiss CD1 male mice exhibited lower levels of anxiety-like behaviors in the free-exploratory paradigm as compared to non-fostered males, suggesting that the cross-fostering procedure itself could have significant impact on behavioral development.

Inbred strains of mice offer valuable translational tools to study the interplay of genetic and environmental factors in neurodevelopmental disorders. Recent studies (McFarlane et al., 2007; Moy et al., 2007) demonstrated that the BTBR *T + tf*/J (BTBR) inbred strain displays behavioral phenotypes with analogies to all three diagnostic symptoms of autism, which are aberrant reciprocal social interactions, qualitative impairments in communication, and restricted repetitive and stereotyped patterns of behavior, interests and activities (American Psychiatric Association, 1994; Lord et al., 2001, 2006; Volkmar et al., 2004), and thus might be a useful model for the disorder. Compared to the commonly used C57BL/6J (B6) strain, BTBR showed lower levels of play soliciting behaviors at postnatal day 21 (first diagnostic symptom), lower levels of adult social approach (first diagnostic symptom), reduced social transmission of food preference as adults (first and second diagnostic symptoms), and higher levels of repetitive self-grooming throughout developmental stages (third diagnostic symptom). Moreover, comprehensive physical examination and behavioral tests confirmed that BTBR were normal on measures of general health, sensory abilities, motor functions, learning and memory, and anxiety-related tasks These data indicate that the BTBR inbred strain exhibits traits with reasonable face validity to all three of the diagnostic criteria for autism. Following the initial discovery, subsequent research in our laboratory has begun to investigate underlying mechanisms of the autism-like phenotype in BTBR mice.

Cross-fostering studies in rodents have mostly focused on stress and/or anxiety related processes. Data on effects of cross-fostering on mouse social behaviors are scarce. As such, the present study was carried out to investigate the influences of early postnatal maternal environment on subsequent expression of social behaviors in BTBR and B6 mice. Social and grooming behaviors were compared in BTBR and B6 raised by either a dam of the same strain (intra-strain cross-fostering) or a dam of the opposite strain (inter-strain cross-fostering). Control BTBR and B6 mice were raised by their biological mothers. The most critical comparison is between BTBR offspring fostered onto B6 and B6 offspring raised by BTBR mothers. Three behavioral tests were conducted. The juvenile play test was carried out on postnatal day 21 ± 1. Social approach and self-grooming were tested between 8 and 11 weeks of age. This experimental design tests the hypothesis that the unusually low social behaviors and high self-grooming trait in BTBR are shaped primarily by maternal characteristics, observational learning, and/or homecage environment. Alternatively, if inter-strain cross-fostering does not significantly alter social and/or self-grooming behaviors, then it is probable that these traits are attributable to genomic variations between the BTBR and the B6 strain. Given the strong evidence for a genetic component in autism (Wassink and Piven, 2000; Muhle et al., 2004; Bacchelli and Maestrini, 2006; DiCicco-Bloom et al., 2006; Schanen, 2006), a genetic rather than early maternal environment determinant of autism-like traits in BTBR would support the use of this inbred strain in searching for new candidate genes for autism.

2. Materials and methods

2.1. Animals

Breeding, housing, and behavioral testing were conducted in strict compliance with the NIH guidelines for the Care and Use of Laboratory Animals and approved by the National Institute of Mental Health Animal Care and Use Committee. Mice were weaned at postnatal days 21–22, then group housed by sex in standard mouse cages containing two to four mice. Animals were bred and housed in colony rooms with controlled temperature (20 °C) and humidity (∼55%). Standard rodent chow and tap water were available *ad libitum*. In addition to standard bedding, a Nestlet square and a cardboard tube were provided in each cage. The colony room was on a reverse 12:12 light/dark cycle, with lights on at 9:00 p.m. All experiments were conducted between 10:00 a.m. and 4:00 p.m., during the dark phase of the circadian cycle, in a procedure room illuminated by a single 25 W red light. Recent experiments from our laboratory indicated similar behavioral phenotypes of BTBR and B6 tested during the dark and light phases of their circadian cycles (Yang et al., in press).

2.2. Cross-fostering

Cross-fostering was conducted within 24-h of parturition. All pups from the litter were removed from the biological mother and placed in a clean cage containing a small amount of bedding taken from the cage of the foster mother. After 5 min, pups were placed in the cage of the foster mother. With this method, pups born to B6 mothers were cross-fostered to either other B6 mothers (intra-strain cross-fostering) or BTBR mothers (inter-strain cross-fostering). Similarly, pups born to BTBR mothers were cross-fostered to either other BTBR mothers (intra-strain cross-fostering) or B6 mothers (inter-strain cross-fostering). Control B6 and BTBR subjects were raised by their own mothers. Fig. 1 illustrates the six treatment groups. Each experimental group consisted of 8–12 subjects, drawn from 3–5 litters. Litter sizes ranged from 5 to 11 pups per cage. Litters were not culled because litter size could be an important aspect of

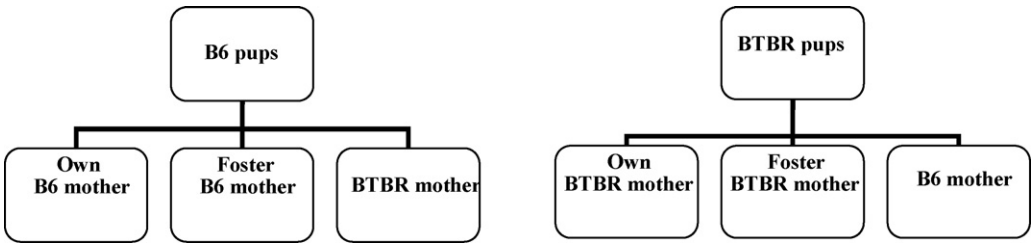

Fig. 1. Schematic illustration of the six treatment groups in the present study. BTBR and B6 pups were either transferred to another female of the same strain (inter-strain cross-fostering) or to a female of the opposite strain (intra-strain cross-fostering). Control animals were raised by their own mothers.