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## Social Peers Rescue Autism-Relevant Sociability Deficits in Adolescent Mice

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### Abstract

#### Lay Abstract

Limited reciprocal social interactions are central to the diagnosis of autism. Behavioral intervention programs are effective in improving social and communication skills in children with autism. We are employing mouse models to understand the individual components of behavioral interventions which effectively improve social interactions. BTBR T+tf/J (BTBR) is an inbred strain of mice that exhibits multiple social deficits, unusual vocalizations, and high levels of repetitive behaviors, which are relevant to all three diagnostic symptom categories of autism. C57BL/6J (B6) is an inbred strain of mice that exhibits high sociability and low repetitive behaviors. We reasoned that these mouse strains with low versus high social interactions might be useful for evaluating social peer enrichment as a behavioral intervention during adolescence. Juvenile BTBR and B6 of the same sex were placed in a same home cage and lived together as cagemates until they reached young adulthood. The two control groups were juvenile B6 housed together, and juvenile BTBR housed together. B6 controls that lived with B6 cagemates showed their strain-typical high sociability. BTBR controls that lived with BTBR cagemates showed their strain-typical low sociability. Remarkably, BTBR that shared home cages with B6 showed high sociability as young adults. Peer rearing for either 20 days or 40 days were equally effective. These results from a robust mouse model of autism support the strategy of early behavioral intervention for treating the social domain in autism spectrum disorders, including beneficial interactions with social peers.

#### Scientific Abstract

Behavioral therapies are currently the most effective interventions for treating the diagnostic symptoms of autism. We employed a mouse model of autism to evaluate components of behavioral interventions that improve sociability in mice. BTBR T+tf/J (BTBR) is an inbred mouse strain that exhibits prominent behavioral phenotypes with face validity to all three diagnostic symptom categories of autism, including robust and well-replicated deficits in social approach and reciprocal social interactions. To investigate the role of peer interactions in the development of sociability, BTBR juvenile mice were reared in the same home cage with juvenile mice of a highly social inbred strain, C57BL/6J (B6). Subject mice were tested as young adults for sociability and repetitive behaviors. B6 controls reared with B6 showed their strain-typical high sociability. BTBR controls reared with BTBR cagemates showed their strain-typical lack of sociability. In contrast, BTBR reared with B6 as juveniles showed significant sociability as young adults. A 20-day intervention was as effective as a 40-day intervention for improving social approach behavior. High levels of repetitive self-grooming by BTBR were not rescued by peer-

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rearing with B6, indicating specificity of the intervention to the social domain. These results from a robust mouse model of autism support the interpretation that social enrichment with juvenile peers is a beneficial intervention for improving adult outcome in the social domain. This novel paradigm may prove useful for discovering factors that are essential for effective behavioral treatments, and biological mechanisms underlying effective behavioral interventions.

## Keywords

Autism; BTBR inbred strain; mouse model; peer enrichment; social enrichment; behavioral intervention

## Introduction

The first diagnostic symptom category of autism focuses on abnormalities in reciprocal social interactions (American Psychiatric Association, 2000). Prominent deficits in the social domain differentiate children with autism spectrum disorders from those with other developmental disorders (Klin *et al.*, 2007; Reichow & Volkmar, 2009). Compared to typically developing peers, individuals with autism lack social skills that are necessary to interact with others and to form friendships (Causton-Theoharis, Ashby, & Cosier, 2009; Levy, Mandell, & Schultz, 2009). A lack of social experience during critical neurodevelopmental periods may amplify genetic and environmental risk factors, hampering normal development of social behaviors throughout life (Dawson, 2008). Although symptoms of autism tend to be lifelong (Billstedt, Gillberg, & Gillberg, 2005, 2007; Cederlund, Hagberg, Billstedt, Gillberg, & Gillberg, 2008; Nordin & Gillberg, 1998), early intensive behavioral interventions can result in substantial improvements in social and communication skills for a large proportion of children with autism (Dawson, 2008; Geschwind, 2009; Rogers, 2000; Sallows & Graupner, 2005). Behavioral intervention strategies have empirical support as effective treatment options for autism spectrum disorders (National Research Council, 2001; Vismara & Rogers, 2010). There are no effective pharmacological treatments for the social deficits in autism, and intensive research efforts are dedicated to finding drugs that could improve symptoms (Bartz & Hollander, 2008; Bear, Huber, & Warren, 2004; Blundell *et al.*, 2010; Dolen *et al.*, 2007; Ehninger *et al.*, 2008; Hagerman *et al.*, 2009; Silverman, Tolu, Barkan, & Crawley, 2009; West, Waldrop, & Brunssen, 2009; Wirojatan *et al.*, 2009; Zhou *et al.*, 2009)

Behavioral intervention programs based on principles of the Applied Behavioral Analysis (ABA) (Geschwind, 2009; Levy *et al.*, 2009; Volkmar, Chawarska, & Klin, 2005) are generally adult directed (Reichow & Volkmar, 2009; Rogers & Vismara, 2008). Peer-mediated or child-directed intervention programs offer a complementary approach, in which typically developing peers with good social skills are instructed to actively engage in social interactions with children with autism by initiating, prompting, and responding (Goldstein, Kaczmarek, Pennington, & Shafer, 1992; Prendeville, Prelock, & Unwin, 2006; Trembath, Balandin, Togher, & Stancliffe, 2009). Sessions usually take place in natural settings such as school classrooms, playgrounds, or homes. Peer interventions programs such as the Integrated Play Group (IPG); Stay, Play, and Talk, Learning Experiences: An Alternate Program (LEAP); and Floor Time Play Dates have been reported to improve social-communicative behaviors including joint attention, reciprocal play behaviors, language development, and academic performance in many children with autism (Betz, Higbee, & Reagon, 2008; Eikeseth, Smith, Jahr, & Eldevik, 2002; Goldstein, English, Shafer, & Kaczmarek, 1997; Goldstein *et al.*, 1992; Harper, Symon, & Frea, 2008; Hwang & Hughes, 2000; Kamps, Barbeta, Leonard, & Delquadri, 1994; Kohler *et al.*, 1995; Landa, 2008; McGee, Almeida, Sulzer-Azaroff, & Feldman, 1992; Rogers, 2000; Rogers & Vismara,

2008; Strain & Kohler, 1998; Trembath *et al.*, 2009; Wolfberg & Schuler, 1993; T. R. Yang, Wolfberg, Wu, & Hwu, 2003; Zercher, Hunt, Schuler, & Webster, 2001). Characteristics of successful programs are summarized in a number of reviews (Dawson, 2008; Koegel, Koegel, Frea, & Fredeen, 2001; Prendeville *et al.*, 2006; Reichow & Volkmar, 2009; Rogers, 2000). These programs differ greatly in many ways, e.g. the characteristics of subjects, target behaviors, the characteristics of interventionists (e.g. age, sex, familiarity, amount of training received from an expert prior to the intervention sessions) intervention strategies, partner-to-subject ratio, and length of intervention. However, few rigorous randomized controlled studies of peer interventions have been reported (Dawson *et al.*, 2009; Levy *et al.*, 2009; Ospina *et al.*, 2008). Current knowledge about the roles of these factors is based primarily on retrospective reviews which compare features of published studies (Hwang & Hughes, 2000; Koegel *et al.*, 2001; Prendeville *et al.*, 2006; Rogers, 2000). The question of which interventions and factors are most beneficial for improving social behaviors remains to be empirically tested.

While there is no substitute for randomized controlled human trials, a robust mouse model offers a translational tool to evaluate treatments for components of autism spectrum disorders. Mice are a social species which can be experimentally investigated to determine the early developmental factors that affect adult social behaviors (Crawley, 2004, 2007; Silverman, Yang, Lord, & Crawley, 2010; M. Yang, Scattoni, Chadman, Silverman, & Crawley, 2010). Inbred strains of mice are genetically homogeneous populations that exhibit large variations in social behaviors across strains (Bolivar, Walters, & Phoenix, 2007; Brodtkin, 2007; Moy *et al.*, 2008; Moy *et al.*, 2007; Panksepp & Lahvis, 2007; Winslow, 2003). BTBR T+tf/J (BTBR) is an inbred strain that incorporates behavioral traits relevant to all three diagnostic symptoms of autism. As compared to the standard social C57BL/6J (B6) inbred strain, BTBR display low levels of reciprocal social interaction as juveniles, low social approach as adults, low social transmission of food preference, unusual patterns of vocalization as pups, fewer ultrasonic vocalizations in response to social cues as adults, and high repetitive self-grooming that persists throughout development (Bolivar *et al.*, 2007; McFarlane *et al.*, 2008; Moy *et al.*, 2004; Moy *et al.*, 2008; Scattoni, Gandhi, Ricceri, & Crawley, 2008; Silverman *et al.*, 2009; Wöhr, Roullet, & Crawley, 2010; M. Yang, Clarke, & Crawley, 2009; M. Yang *et al.*, 2007a; M. Yang, Zhodzishsky, & Crawley, 2007b). We previously discovered that cross-fostering of BTBR pups with B6 mothers did not improve sociability in BTBR, indicating that early maternal care was not a major factor in determining the social impairments of adult BTBR (M. Yang *et al.*, 2007b). As a second hypothesis, we considered the possibility that the development of social behaviors in mice could be influenced by the interactions of cagemates living together in standard home cages. Specifically, we postulated that BTBR juveniles raised with B6 juveniles might benefit from peer interactions with social partners during adolescence. To directly test this hypothesis, home cages were assembled at the time of weaning to generate social groupings of a) juvenile B6, b) juvenile BTBR, and c) a combination of juvenile B6 + BTBR. Critical length of peer intervention was evaluated by comparing 20 and 40 days of peer rearing. Subject mice were tested as adults for social approach in our automated three-chambered apparatus, as well as an independent measure relevant to the third diagnostic symptom of autism, repetitive self-grooming. Both males and females were used as subjects (Newschaffer *et al.*, 2007; Veenstra-Vanderweele, Christian, & Cook, 2004). Given the evidence that shows normal social and emotional adjustments in some siblings of children with autism (Kaminsky & Dewey, 2002; Macks & Reeve, 2007; Pilowsky, Yirmiya, Doppelt, Gross-Tsur, & Shalev, 2004; Verte, Roeyers, & Buysse, 2003), we did not test B6 cagemates of BTBR subjects.

## Materials and Methods

### Animals

C57BL/6J (B6) and BTBR T+tf/J (BTBR) breeding pairs were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred at NIMH in Bethesda, Maryland. Breeding, housing, and behavioral testing were conducted in accordance 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. 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 maintained on a 12:12 light/dark cycle with lights on at 7:00 AM, at approximately 20°C and 55% humidity.

At postnatal day 20–21, juveniles were weaned and housed with same sex, age-matched cagemates in cages of 4 mice per cage. Cohort 1 consisted of a) 16 experimental cages, 8 male and 8 female, each containing 2 B6 littermates and 2 BTBR littermates; b) 8 control cages, 4 male and 4 female, each containing 4 B6 littermates; and c) 8 control cages, 4 male and 4 female, each containing 4 BTBR littermates. Cohort 2 consisted of a) 16 experimental cages, 8 male and 8 female, each containing 2 B6 littermates and 2 BTBR littermates; b) 8 control cages, 4 male and 4 female, each containing two sets of B6 littermates from two litters; and c) 8 control cages, 4 male and 4 female, each containing two sets of BTBR littermates from two litters.

### Adult social approach task

Adult sociability testing was conducted between 10:00 AM and 4:00 PM. Mice used as the novel targets in the social approach task were 129Sv/ImJ mice of the same gender as the subject, aged 8–14 weeks old, purchased from The Jackson Laboratory. The novel mice were previously habituated to the apparatus but had no prior physical contact with subject mice, as previously described (Chadman *et al.*, 2008; Silverman *et al.*, 2009; M. Yang *et al.*, 2009; M. Yang *et al.*, 2007b).

Social approach behaviors were scored in an automated three-chambered apparatus using methods previously described (Chadman *et al.*, 2008; Crawley *et al.*, 2007; McFarlane *et al.*, 2008; Moy *et al.*, 2009; Moy *et al.*, 2008; Silverman *et al.*, 2009; M. Yang *et al.*, 2009; M. Yang *et al.*, 2007a; M. Yang *et al.*, 2007b). In this test, sociability is defined by the subject mouse spending more time in the side chamber with a novel mouse than in the side chamber with a novel object, and more time sniffing the novel mouse than the novel object, during a ten minute test session. The apparatus was a rectangular, three-chambered box made of clear polycarbonate. Retractable doorways built into the two dividing walls controlled access to the side chambers. Number of entries and time spent in each chamber were automatically detected by photocells embedded in the doorways and tallied by the software. The test session began with a 10 min habituation session in the center chamber only, followed by a 10 min habituation to all three empty chambers. Lack of innate side preference was confirmed during the second 10 minute habituation. The subject was then briefly confined to the center chamber. A novel object (an inverted stainless steel wire pencil cup, Galaxy, Kitchen Plus, <http://www.kitchen-plus.com>) was placed in one of the side chambers. A novel mouse, previously habituated to the enclosure, was placed in an identical wire cup located in the other side chamber. A disposable plastic drinking cup containing a lead weight was placed on the top of each inverted wire pencil cup to prevent the subject from climbing on top. The side containing the novel object and the novel mouse alternated between the left and right chambers across subjects. After both stimuli were positioned, the two side doors were simultaneously lifted and the subject was allowed access to all three chambers for 10 min. In addition to the automatically tallied parameters (time spent in each chamber and

entries into each chamber), an observer with two stopwatches scored cumulative time spent sniffing the novel object and the novel mouse. The apparatus was cleaned with 70% ethanol and water between subjects.

### Repetitive self-grooming

To test for repetitive self-grooming, each mouse was individually placed in a clean empty cage without bedding and with lid on. After a 10 min habituation period in the empty cage, the subject mouse was scored for 10 min for cumulative time spent grooming. The investigator sat approximately 2 m from the test cage and recorded time spent in grooming with a stopwatch.

Cohort 1 was tested for adult social approach and self-grooming on postnatal day 61, i.e. after animals had been weaned from the mother and lived with cagemates for 40 days. Cohort 2 was tested for adult social approach on postnatal day 41, i.e. after animals had been weaned from the mother and lived with cagemates for 20 days. The control groups, B6 living with B6 cagemates and BTBR living with BTBR cagemates, were tested at the same age as BTBR subjects.

### Statistical analysis

For the automated social approach test, time spent in the two side chambers was compared using within-group Repeated Measures ANOVAs, with the factor of chamber side (novel mouse side vs. novel object side). Time spent in the center chamber was included on the graphs for illustrative purposes, but was not included in the statistical analyses, for time in the three chambers adds to 100% and therefore the time spent in the left chamber, center chamber, and right chamber are not independent. Time spent sniffing the novel mouse versus the novel object was similarly analyzed using within-group Repeated Measures ANOVAs, with the factor of chamber side (novel mouse side vs. novel object side). One Way ANOVA was used to analyze total number of entries to the side chambers, and to analyze time spent self-grooming in an empty cage. Scheffe post hoc tests were used to compare individual groups following a significant ANOVA. Significance was defined as  $p < .05$ .

## Results

### Cohort 1. 40-day peer-enriched intervention improved sociability in BTBR male and female mice

Fig 1 A–D shows social approach scores and number of entries of Cohort 1 males. (A) Adult male B6 that had been reared as juveniles with juvenile male B6 displayed typical sociability ( $N=16$ ,  $F_{1,15}=23.79$ ,  $p<.001$ ). Adult male BTBR reared as juveniles with juvenile male BTBR did not display sociability ( $N=16$ ,  $F_{1,15}=0.34$ , NS). Adult male BTBR reared as juveniles with juvenile male B6 displayed significant sociability ( $N=16$ ,  $F_{1,15}=16.02$ ,  $p<.001$ ). (B) Adult male B6 reared as juveniles with juvenile male B6 spent significantly more time sniffing the novel mouse than the novel object ( $F_{1,15}=43.42$ ,  $p<.001$ ). Adult male BTBR reared as juveniles with juvenile male BTBR did not spend more time sniffing the novel mouse than the novel object ( $F_{1,15}=2.12$ , NS). Adult male BTBR reared as juveniles with juvenile male B6 spent significantly more time sniffing the novel mouse than the novel object ( $F_{1,15}=31.89$ ,  $p<.001$ ). (C) The main effect of social housing was significant for the number of total entries to the side chambers during the sociability test phase, ( $F_{2,45}=3.43$ ,  $p<.05$ ). Posthoc Scheffe test showed that male BTBR reared with B6 made fewer entries into the side chambers as compared to male B6 controls ( $p<.05$ ). (D) The main effect of social housing was not significant for the number of total entries to the side chambers during the habituation phase that preceded the social approach task ( $F_{2,45}=1.14$ , NS), indicating no

differences across groups in locomotor abilities or exploration of a novel non-social environment.

Fig 2 A–D shows social approach scores and number of entries for Cohort 1 females. (A) Adult female B6 that had been reared as juveniles with juvenile female B6 displayed typical sociability ( $N=16$ ,  $F_{1,15}=8.94$ ,  $p<.01$ ). Adult female BTBR reared as juveniles with juvenile female BTBR did not display sociability, spending more time in the chamber containing the novel object than in the chamber containing the novel mouse ( $N=16$ ,  $F_{1,15}=5.29$ ,  $p<.05$ ). Adult female BTBR reared as juveniles with juvenile female B6 displayed significant sociability ( $N=16$ ,  $F_{1,15}=12.7$ ,  $p<.01$ ). (B) Adult female B6 reared as juveniles with juvenile female B6 spent significantly more time sniffing the novel mouse than the novel object ( $F_{1,15}=24.76$ ,  $p<.001$ ). Adult female BTBR reared as juveniles with juvenile female BTBR did not spend more time sniffing the novel mouse than the novel object ( $F_{1,15}=0.18$ , NS). Adult female BTBR reared as juveniles with juvenile female B6 spent significantly more time sniffing the novel mouse than the novel object ( $F_{1,15}=21.04$ ,  $p<.001$ ). (C) The main effect of social housing was not significant for the number of total entries to the side chambers during the sociability test phase, ( $F_{2,45}=0.72$ , NS). (D) The main effect of social housing was not significant for the number of total entries to the side chambers during the habituation phase ( $F_{2,45}=0.07$ , NS).

### Cohort 2. 20-day peer-enriched intervention improved sociability in BTBR male and female mice

Fig 3 A–D shows social approach scores of Cohort 2 males. (A) Adult male B6 that had been reared as juveniles with juvenile male B6 displayed typical sociability ( $N=8$ ,  $F_{1,7}=93.77$ ,  $p<.0001$ ). Adult male BTBR reared as juveniles with juvenile male BTBR did not display sociability ( $N=8$ ,  $F_{1,7}=0.046$ , NS). Adult male BTBR reared as juveniles with juvenile male B6 displayed significant sociability ( $N=16$ ,  $F_{1,15}=12.24$ ,  $p<.01$ ). (B) Adult male B6 reared as juveniles with juvenile male B6 spent significantly more time sniffing the novel mouse than the novel object ( $F_{1,7}=117.84$ ,  $p<.001$ ). Adult male BTBR reared as juveniles with juvenile male BTBR did not spend more time sniffing the novel mouse than the novel object ( $F_{1,7}=1.78$ , NS). Adult male BTBR reared as juveniles with juvenile male B6 spent significantly more time sniffing the novel mouse than the novel object ( $F_{1,15}=20.46$ ,  $p<.001$ ). (C) A significant main effect of social housing was found for the number of total entries to the side chambers in cohort2 males ( $F_{2,29}=5.83$ ,  $p<.01$ ). Posthoc Scheffe test indicated that male BTBR reared with B6 made significantly fewer entries than BTBR reared with BTBR ( $p<.05$ ). (D) The main effect of social housing was not significant for the number of total entries to the side chambers during the habituation phase ( $F_{2,29}=2.11$ , NS), indicating no differences across groups in locomotor abilities or exploration of a novel non-social environment.

Fig 4 A–D shows social approach scores of Cohort 2 females. (A) Adult female B6 that had been reared as juveniles with juvenile female B6 displayed typical sociability ( $N=8$ ,  $F_{1,7}=52.20$ ,  $p<.001$ ). Adult female BTBR reared as juveniles with juvenile female BTBR did not display sociability, spending more time in the chamber containing the novel object than in the chamber containing the novel mouse ( $N=8$ ,  $F_{1,7}=0.07$ , NS). Adult female BTBR reared as juveniles with juvenile female B6 displayed significant sociability ( $N=16$ ,  $F_{1,15}=12.18$ ,  $p<.01$ ). (B) Adult female B6 reared as juveniles with juvenile female B6 spent significantly more time sniffing the novel mouse than the novel object ( $F_{1,7}=42.73$ ,  $p<.001$ ). Adult female BTBR reared as juveniles with juvenile female BTBR did not spend significantly more time sniffing the novel mouse than the novel object ( $F_{1,15}=0.06$ , NS). Adult female BTBR reared as juvenile with juvenile female B6 spent significantly more time sniffing the novel mouse than the novel object ( $F_{1,15}=48.28$ ,  $p<.001$ ). (C) The main effect of social housing was not significant for total number of entries during the social

approach task ( $F_{2,29}=1.51$ , NS). (D) The main effect of social housing was not significant for the number of total entries to the side chambers during the habituation phase ( $F_{2,29}=0.24$ , NS).

### **Social peer enrichment did not affect the high levels of repetitive self-grooming in male and female BTBR**

Figure 5 shows time spent in self-grooming in an empty cage over 10 min for cohort 1. The main effect of social housing was significant for adult cohort 1 males ( $F_{2,45}=53.81$ ,  $p<.001$ ). Adult male BTBR that had been reared with either B6 or BTBR as juveniles showed higher levels of self-grooming as compared to B6 control males ( $p<.01$  for each posthoc comparison). Similarly, the main effect of social housing was significant for adult cohort 1 females ( $F_{2,45}=32.70$ ,  $p<.001$ ). Female BTBR reared with either B6 or BTBR as juveniles showed higher levels of self-grooming as compared to B6 control females ( $p<.01$  for each posthoc comparison). Since the 40 day intervention had no effect on repetitive self-grooming in cohort 1 BTBR, self-grooming was not tested after the 20 day intervention in cohort 2 mice.

### **Discussion**

A large body of literature demonstrates that early social environment, including social isolation in monkeys (Harlow and Suomi, 1971) and communal nesting in mice (Branchi, 2009), exerts a profound influence on the development of adult social behaviors. Animal models of autism provide preclinical tools to evaluate treatment interventions, including behavioral and pharmacological therapeutics (Blundell *et al.*, 2010; Chadman, Yang, & Crawley, 2009; Dolen *et al.*, 2007; Ehninger *et al.*, 2008; Silverman *et al.*, 2009; Silverman *et al.*, 2010; M. Yang *et al.*, 2010; M. Yang *et al.*, 2007b; Zhou *et al.*, 2009). In the current study we report the development of a new mouse model of early social enrichment and the novel finding that social deficits in adult BTBR are prevented by social peer enrichment during the period between weaning and sexual maturity. Robust social deficits in adult BTBR were prevented by rearing 21 day old juvenile BTBR with juvenile males of a social strain, B6. Time spent in social approach and social sniffing were both significantly higher in BTBR reared with B6 than in BTBR reared with BTBR, as tested at adult ages. The same beneficial effect of rearing with social cagemates was seen in female BTBR reared with female B6 cagemates. These findings indicate that the social milieu in the home cage during early development has a direct influence on emerging social behaviors in adult mice.

To begin to evaluate the essential components of social peer intervention, we compared 20 days of BTBR+B6 peer rearing with 40 days of BTBR+B6 peer rearing. BTBR males that had spent only 20 days after weaning with B6 cagemates showed significant sociability at age 41 days. BTBR males that had spent 40 days after weaning with B6 cagemates similarly showed significant sociability at age 61 days. The magnitude of sociability rescue was similar after the 20 day and the 40 day intervention periods. This finding indicates that constant interactions with social peers from a juvenile age to a late adolescent/young adult age prevented social deficits in BTBR as effectively as a longer length of home cage interactions from a juvenile age to an adult age. Further experiments will investigate even shorter intervention periods, and will explore whether a critical window exists during a specific age within early development when peer intervention is likely to produce maximal effects. We speculate that it is critical for intervention to begin at an early age. A related interesting question is whether home cage interactions between BTBR and BTBR are strikingly deficient, which could represent a social deprivation environment during early development. Specific qualities of home cage interactions that contribute to the improved sociability in BTBR reared with B6 will require intensive observations of a wide range of home cage behaviors in identified combinations of juvenile mice. Subsequently, it will be

important to investigate long-term outcomes of peer intervention in adult mice that are no longer living with social peers. To further understand which aspects of social behaviors are changed by social peer intervention, it will be important to test reciprocal social interactions between the subject mouse and a freely moving novel partner mouse in various settings, including male-female and male-male pairings.

Unusually low or unusually high levels of general exploratory activity in the three-chambered apparatus would introduce artifacts due to sedation or hyperactivity, that could represent an experimental confound and lead to an overinterpretation of the social approach data. Entries between compartments affords a built-in measure of general exploratory activity in our automated three-chambered apparatus. Number of entries was significantly lower in male BTBR reared with male B6 in both cohorts, but not in female BTBR reared with female B6 in either cohort. To further determine exploratory locomotion in BTBR males reared with B6 males, we analyzed number of entries during the habituation phase that preceded the social approach task. In the novel but non-social test environment, BTBR that had been reared with B6 made similar numbers of entries as compared to B6 reared with B6 and BTBR reared with BTBR. This finding rules out differences in general exploratory tendencies as an explanation for entry differences during the social approach task.

Repetitive self-grooming, one of several other well-replicated phenotypes in BTBR, is relevant to the third diagnostic symptom of autism. High levels of self-grooming in BTBR were present in both BTBR reared with BTBR cagemates and in BTBR reared with B6 cagemates. Thus, the home cage interactions between BTBR and B6 reared together since a juvenile age were beneficial in the social domain but not in the repetitive domain. This finding is consistent with the reported need for different types of behavioral interventions to improve social behaviors and repetitive behaviors in autistic children. A recent randomized control study found that the Early Start Denver Model improved IQ and adaptive behaviors in children with autism but was without effect on repetitive behaviors scores (Dawson et al., 2009). Genetic studies indicate that the symptoms of social-communication deficits and symptoms of repetitive behaviors are regulated by different genetic factors (Happé & Ronald, 2008) and thus may respond differentially to treatments. Previous studies have shown that environmental enrichment was effective in reducing repetitive jumping stereotypies in deer mice (Tanimura, Yang, & Lewis, 2008), that a serotonin reuptake inhibitor was effective in preventing injurious self-grooming in *Sapap3* mutant mice (Welch et al., 2007), and that an mGluR5 receptor antagonist MPEP was effective in reducing repetitive self-grooming, but not social deficits, in BTBR mice (Silverman *et al.*, 2009), indicating a separation of therapeutic responses for the social and repetitive domains.

An important question relevant to the translational value of this mouse model is how the ages of these mice at the beginning and the end of peer intervention translate to human ages. Large species differences in developmental trajectories prohibit direct comparisons between mice and humans. However, reasoning that mice are weaned at 3–4 weeks of age and reach sexual maturity 6–8 weeks of age, it is interesting to speculate that the current intervention period in mice, from approximately 3 to 8 weeks, is roughly equivalent to human ages from approximately 2 to 18 years.

## Conclusions

Structured behavioral interventions through special education programs are currently the most effective treatment available for autistic individuals (Dawson, 2008; Rogers, 2000; Vismara & Rogers). Our findings demonstrate that the profound social deficits in BTBR are significantly improved when BTBR adolescents share home cages with highly social B6 adolescent cagemates. Detailed investigation into the nature of the beneficial peer



interactions between B6 and BTBR may reveal the specific ameliorative components of the juvenile intervention. It will be interesting to compare the nature of social interactions between B6 and BTBR juveniles in the home cage to the types of interactions that occur between children with autism and their siblings at home, and to the types of interactions between children with autism and classmates in a mainstreamed school environment. Our results from a robust mouse model of autism may support the strategy of targeted behavioral intervention with peers for improving social interactions in children with autism spectrum disorders.

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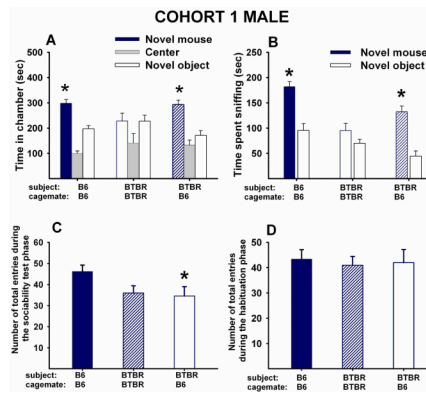
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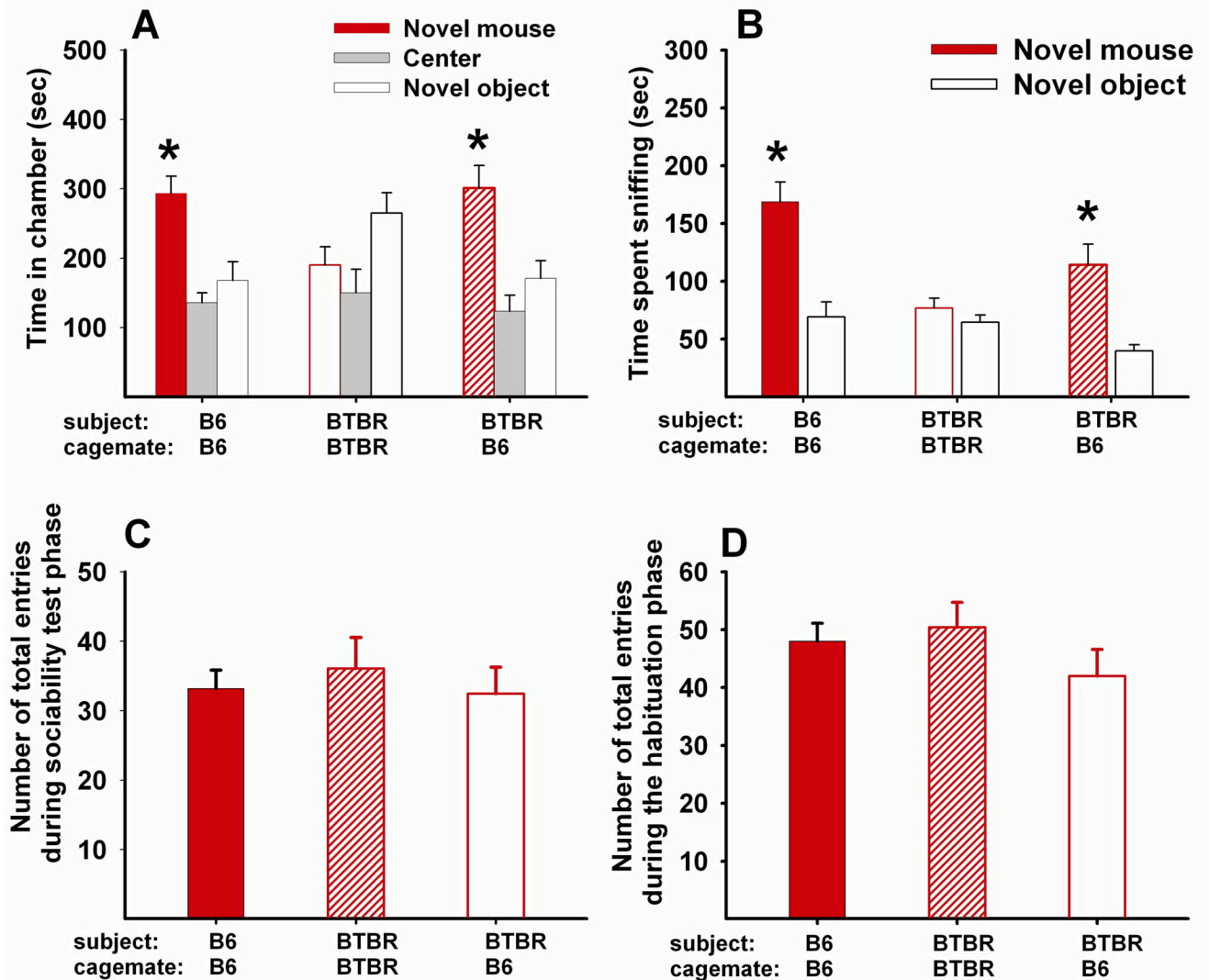
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**Figure 1. 40-day peer-enriched intervention improved sociability in BTBR male mice**  
 Sociability is defined as spending more time in the side chamber with a novel mouse than in the side chamber with a novel object, and more time sniffing the novel mouse than the novel object, during a ten minute test session in the automated three-chambered apparatus. (A) **Time in chambers containing a novel object or novel mouse.** Male B6 that were reared as juveniles with juvenile male B6 for 40 days displayed typical sociability as adults (N=16,  $F_{1,15}=23.79$ ,  $p<.001$ ). Male BTBR reared as juveniles with juvenile male BTBR for 40 days did not display sociability as adults (N=16,  $F_{1,15}=0.34$ , NS). Male BTBR reared as juveniles with juvenile male B6 for 40 days displayed significant sociability as adults (N=16,  $F_{1,15}=16.02$ ,  $p<.001$ ). (B) **Time spent sniffing the novel object and the novel mouse.** Male B6 reared as juveniles with juvenile male B6 for 40 days displayed significant sociability as adults (N=16,  $F_{1,15}=43.42$ ,  $p<.001$ ). Male BTBR reared as juveniles with juvenile male BTBR did not display significant sociability (N=16,  $F_{1,15}=2.12$ , NS). Male BTBR reared as juveniles with juvenile male B6 spent significantly more time sniffing the novel mouse than the novel object as adults (N=16,  $F_{1,15}=31.89$ ,  $p<.001$ ). (C) **Total number of entries to the side chambers during the social approach task.** A significant main effect of social housing was found (N=16 for each group,  $F_{2,45}=3.43$ ,  $p<.05$ ), with male BTBR reared with B6 making fewer entries into the side chambers as compared to B6 control males ( $p<.05$ ). (D) **Total number of entries to the side chambers during the habituation phase that preceded the social approach task.** No significant effect of social housing was found (N=16 for each group,  $F_{2,45}=1.14$ , NS). (A and B)  $*p < .05$  for comparison between novel mouse and novel object sides. Bars for the novel mouse are outlined in color, while bars for the novel object are outlined in black, in panels A and B of Figures 1–4. (C)  $*p < .05$  as compared to B6 reared with B6.

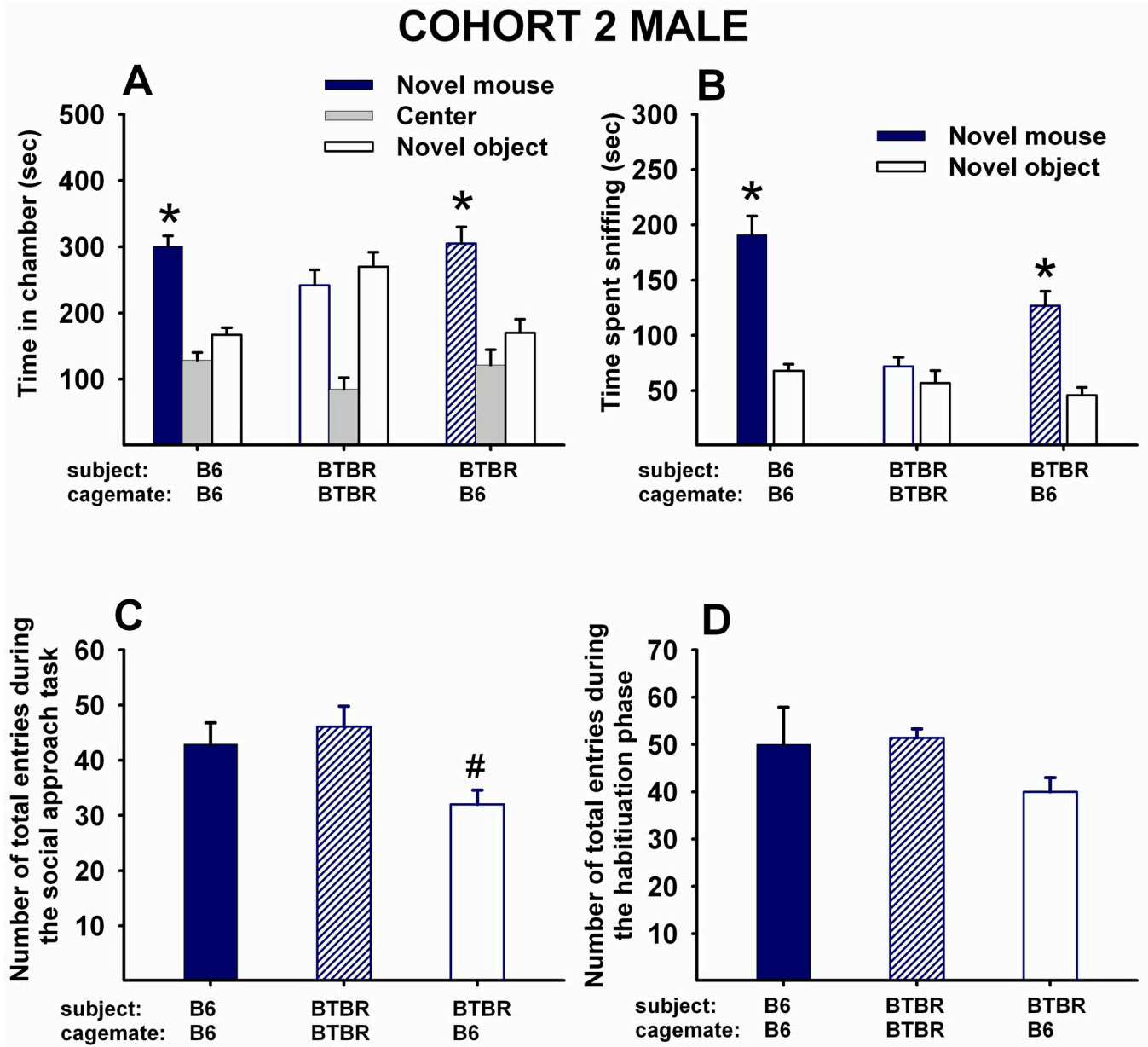
## COHORT 1 FEMALE



**Figure 2. 40-day peer-enriched intervention improved sociability in BTBR female mice**  
**(A) Time in chambers containing a novel object or novel mouse.** Female B6 that were reared as juveniles with juvenile female B6 for 40 days displayed typical sociability as adults ( $N=16$ ,  $F_{1,15}=8.94$ ,  $p<.01$ ). Female BTBR reared as juveniles with juvenile female BTBR for 40 days did not display sociability as adults ( $N=16$ ,  $F_{1,15}=5.29$ ,  $p<.05$ ). Female BTBR reared as juveniles with juvenile female B6 for 40 days displayed significant sociability as adults ( $N=16$ ,  $F_{1,15}=12.7$ ,  $p<.01$ ). **(B) Time spent sniffing the novel object and the novel mouse.** Female B6 reared as juveniles with juvenile female B6 for 40 days displayed significant sociability as adults ( $F_{1,15}=24.76$ ,  $p<.001$ ). Female BTBR reared as juveniles with juvenile female BTBR did not display significant sociability ( $F_{1,15}=0.18$ , NS). Female BTBR reared as juveniles with juvenile female B6 spent significantly more time sniffing the novel mouse than the novel object as adults ( $F_{1,15}=21.04$ ,  $p<.001$ ). **(C) Total number of entries to the side chambers during the social approach task.** No significant effect of social housing was found ( $N=16$  for each group,  $F_{2,45}=0.72$ , NS). **(D) Total number of entries to the side chambers during the habituation phase that preceded the social approach task.** No significant effect of social housing was found

(N=16 for each group,  $F_{2,45}=0.73$ , NS). (A and B)  $*p < .05$  for comparison between novel mouse and novel object sides.

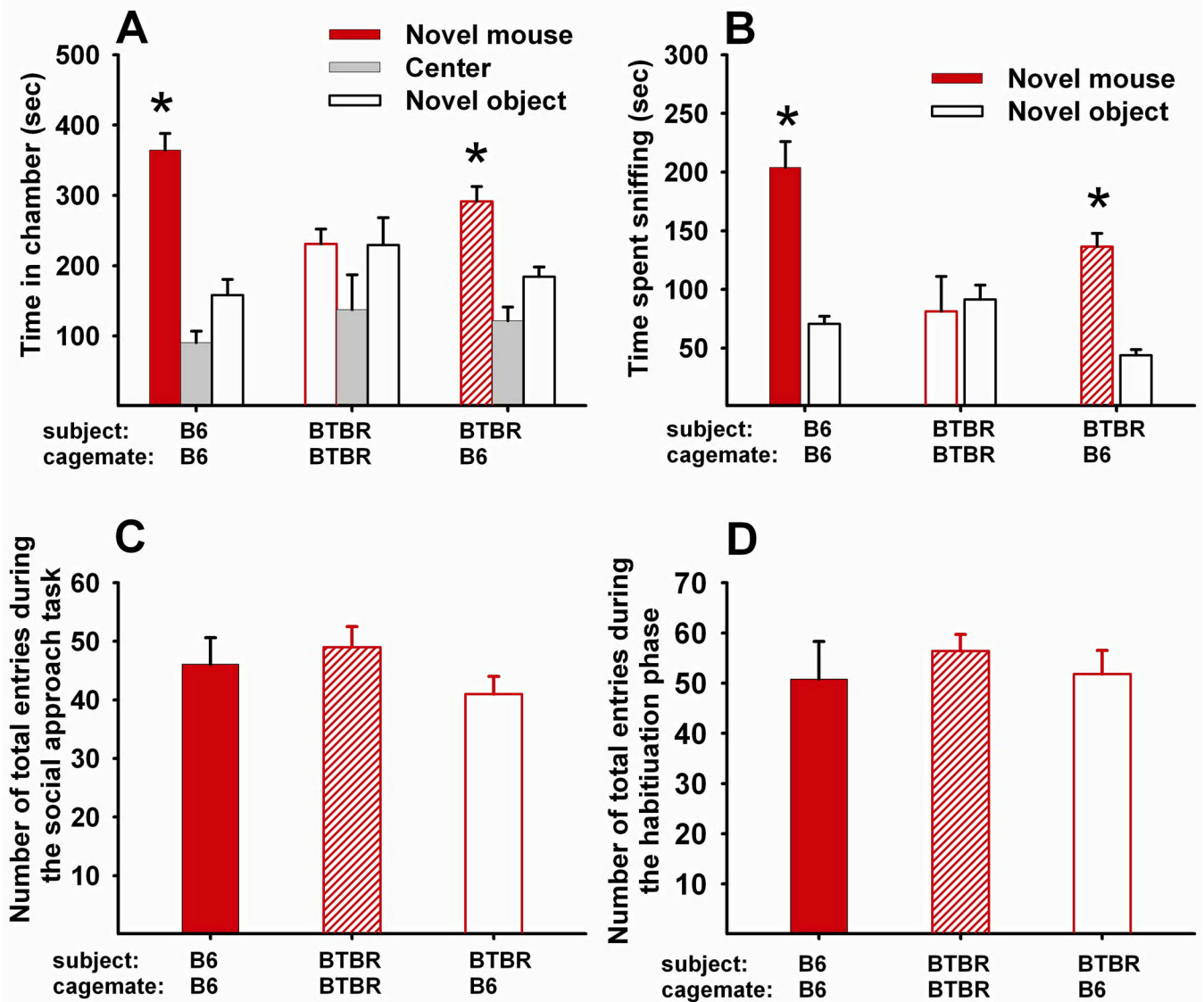




**Figure 3. 20-day peer-enriched intervention improved sociability in BTBR male mice**  
**(A) Time in chambers containing a novel object or novel mouse.** Male B6 that were reared as juveniles with juvenile male B6 for 20 days displayed typical sociability as adults ( $N=8$ ,  $F_{1,7}=93.77$ ,  $p<.0001$ ). Male BTBR reared as juveniles with juvenile male BTBR for 20 days did not display sociability as adults ( $N=8$ ,  $F_{1,7}=0.046$ , NS). Male BTBR reared as juveniles with juvenile male B6 for 20 days displayed significant sociability as adults ( $N=16$ ,  $F_{1,15}=12.24$ ,  $p<.01$ ). **(B) Time spent sniffing the novel object and the novel mouse.** Male B6 reared as juveniles with juvenile male B6 for 20 days displayed significant sociability as adults ( $N=8$ ,  $F_{1,7}=117.84$ ,  $p<.001$ ). Male BTBR reared as juveniles with juvenile male BTBR did not display significant sociability ( $N=8$ ,  $F_{1,7}=1.78$ , NS). Male BTBR reared as juveniles with juvenile male B6 displayed sociability as adults ( $N=16$ ,  $F_{1,15}=20.46$ ,  $p<.001$ ). **(C) Total number of entries to the side chambers during the social approach task.** A significant main effect of social housing was found ( $F_{2,29}=5.83$ ,  $p<.01$ ),

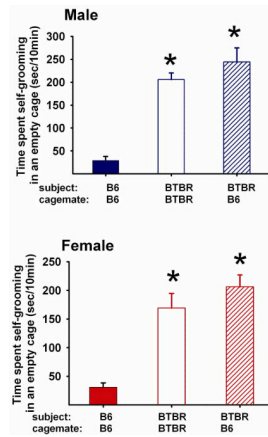
with male BTBR reared with B6 showing fewer entries into the side chambers as compared to BTBR reared with BTBR ( $p < .05$ ). (D) **Total number of entries to the side chambers during the habituation phase that preceded the social approach task.** No significant effect of social housing was found ( $F_{2,29} = 2.11$ , NS). (A and B)  $*p < .05$  for comparison between novel mouse and novel object sides. (C)  $\#p < .05$  as compared to BTBR reared with BTBR.

## COHORT 2 FEMALE



**Figure 4. 20-day peer-enriched intervention improved sociability in BTBR female mice**  
**(A) Time in chambers containing a novel object or novel mouse.** Female B6 that were reared as juveniles with juvenile female B6 for 20 days displayed typical sociability as adults ( $N=8$ ,  $F_{1,7}=52.20$ ,  $p<.001$ ). Female BTBR reared as juveniles with juvenile female BTBR for 20 days did not display sociability as adults ( $N=8$ ,  $F_{1,7}=0.07$ , NS). Female BTBR reared as juveniles with juvenile female B6 for 20 days displayed significant sociability as adults ( $N=16$ ,  $F_{1,15}=12.18$ ,  $p<.01$ ). **(B) Time spent sniffing the novel object and the novel mouse.** Female B6 reared as juveniles with juvenile female B6 for 20 days displayed significant sociability as adults ( $F_{1,7}=42.73$ ,  $p<.001$ ). Female BTBR reared as juveniles with juvenile female BTBR did not display significant sociability ( $F_{1,7}=0.06$ , NS). Female BTBR reared as juveniles with juvenile female B6 displayed significant sociability as adults ( $F_{1,15}=48.28$ ,  $p<.001$ ). **(C) Total number of entries to the side chambers during the social approach task.** No significant effect of social housing was found ( $F_{2,29}=1.51$ , NS). **(D) Total number of entries to the side chambers during the habituation phase that**

**preceded the social approach task.** No significant effect of social housing was found ( $F_{2,29}=0.24$ , NS). (A and B)  $*p < .05$  for comparison between novel mouse and novel object sides.



**Figure 5. Social peer enrichment did not affect high levels of repetitive self-grooming in male and female BTBR**

Time spent in repetitive self-grooming was significantly higher in adult male BTBR that were reared with either B6 (N=16) or BTBR (N=16) as juveniles, as compared to B6 controls (N=16) (Main effect of social housing,  $F_{2,45}=53.81$ ,  $p<.001$ ; posthoc Scheffe test,  $p<.01$  for each comparison). Similarly, adult female BTBR reared with either B6 (N=16) or BTBR (N=16) as juveniles showed higher levels of self-grooming as compared to B6 control females (N=16) (Main effect of social housing,  $F_{2,45}=32.70$ ,  $p<.001$ ; Posthoc Scheffe test,  $p<.01$  for each comparison). \* $p < .05$  as compared to B6 reared with B6.