EJN fens EUROPEAN JOURNAL OF NEUROSCIENCE

European Journal of Neuroscience, Vol. 29, pp. 1663–1677, 2009 doi:10.1111/j.1460-9568.2009.06714.x

BEHAVIORAL NEUROSCIENCE

Postnatal lesion evidence against a primary role for the corpus callosum in mouse sociability

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Keywords: autism, agenesis of the corpus callosum, BTBR T+tf/J mice, mouse models of autism, repetitive self-grooming, social interactions

Abstract

The BTBR T+tf/J (BTBR) strain is an inbred strain of mice that displays prominent social deficits and repetitive behaviors analogous to the defining symptoms of autism, along with complete congenital agenesis of the corpus callosum (CC). The BTBR strain is genetically distant from the widely used C57BL/6J (B6) strain, which exhibits high levels of sociability, a low level of repetitive behaviors, and an intact CC. Emerging evidence implicates compromised interhemispherical connectivity in some cases of autism. We investigated the hypothesis that the disconnection of CC fiber tracts contributes to behavioral traits in mice that are relevant to the behavioral symptoms of autism. Surgical lesion of the CC in B6 mice at postnatal day 7 had no effect on juvenile play and adult social approaches, and did not elevate repetitive self-grooming. In addition, LP/J, the strain that is genetically closest to the BTBR strain but has an intact CC, displayed juvenile play deficits and repetitive self-grooming similar to those seen in BTBR mice. These corroborative results offer evidence against the hypothesis that the CC disconnection is a primary cause of low sociability and a high level of repetitive behaviors in inbred mice. Our findings indicate that genes mediating other aspects of neurodevelopment, including those whose mutations underlie more subtle disruptions in white matter pathways and connectivity, are more likely to contribute to the aberrant behavioral phenotypes in the BTBR mouse model of autism.

Introduction

Autism is a neurodevelopmental disorder defined by impaired social interactions, communications deficits, and repetitive behaviors (DiCicco-Bloom *et al.*, 2006; Lord *et al.*, 2006). Although the causes are unknown, both genetic and environmental factors have been implicated (Herbert *et al.*, 2006; Abrahams & Geschwind, 2008). Differential concordance rates between monozygotic twins (60–90%) and dizygotic twins and siblings (1–10%), along with a 4 : 1 male/female ratio, lend support to a strong genetic component (Veenstra-Vanderweele *et al.*, 2004; Grice & Buxbaum, 2006; Newschaffer *et al.*, 2007; Abrahams & Geschwind, 2008). Recent evidence indicates a possible association between aberrant patterns of neural connections and autism (Geschwind & Levitt, 2007; Minshew & Williams, 2007; Rippon *et al.*, 2007). Functional imaging studies found less activation of brain regions and reduced functional connectivity in adults with autism during several social and cognitive tasks, suggesting cortical underconnectivity in autism (Castelli *et al.*, 2002; Just *et al.*, 2004, 2007; Kana *et al.*, 2007; Kennedy & Courchesne, 2008; Kleinhans *et al.*, 2008; Koshino *et al.*, 2008). The corpus callosum (CC) is the largest connective structure in the brain, and provides the majority of interhemispherical cortical connections (Paul *et al.*, 2007). Structural imaging studies found reduced sizes of subregions of the CC in some individuals with autism (Egaas *et al.*, 1995; Piven *et al.*, 1997; Manes *et al.*, 1999; Hardan *et al.*, 2000; Brambilla *et al.*, 2003; Boger-Megiddo *et al.*, 2006; Vidal *et al.*, 2006; Geschwind & Levitt, 2007; Kilian *et al.*, 2008). Voxel-based imaging and diffusion tensor imaging indicate abnormal structural integrity of white matter in autism (Barnea-Goraly *et al.*, 2004; Chung *et al.*, 2004; Alexander *et al.*, 2007; Keller *et al.*, 2007; Bonilha *et al.*, 2008; Sundaram *et al.*, 2008). Also, more than one-third of children with congenital agenesis of the CC (AgCC) have social problems, suggesting that a reduction in size of the CC may contribute to abnormalities in social behaviors in some genetically based neurodevelopmental disorders (Badaruddin *et al.*, 2007; Paul *et al.*, 2007).

Animal models offer opportunities to test genetic hypotheses and evaluate proposed treatments. Reverse genetic approaches are elucidating phenotypes of mice with mutations in candidate genes for autism (Young, 2001; Winslow & Insel, 2002; Young *et al.*, 2002; Long *et al.*, 2004; Takayanagi *et al.*, 2005; Cheh *et al.*, 2006; Kwon *et al.*, 2006; Mineur *et al.*, 2006; Crawley *et al.*, 2007; Tabuchi *et al.*, 2007; Chadman *et al.*, 2008; Ehninger *et al.*, 2008; Hung *et al.*, 2008; Jamain *et al.*, 2008; Scearce-Levie *et al.*, 2008;). Forward genetic approaches identify inbred strains of mice with phenotypes relevant to autism, and explore mechanisms responsible for the phenotypes (Brodkin, 2007; Moy *et al.*, 2004, 2007; Panksepp & Lahvis, 2007; McFarlane *et al.*,

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Received 16 October 2008, revised 13 February 2009, accepted 17 February 2009

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2008). Using forward genetics, we discovered an obscure inbred strain, BTBR T+tf/J (BTBR), that exhibits behavioral traits with face validity for the diagnostic symptoms of autism, including aberrant reciprocal social interactions as juveniles and adults, reduced social transmission of food preference, and repetitive self-grooming (Bolivar *et al.*, 2007; Moy *et al.*, 2007; Yang *et al.*, 2007b; McFarlane *et al.*, 2008). Complete congenital AgCC is well documented in BTBR mice (Wahlsten *et al.*, 2003; MacPherson *et al.*, 2008). BTBR males and females are within the normal range on measures of general health, home cage behaviors, home cage nesting, parental care, neurological reflexes, vision, olfaction, hearing, open field locomotion, anxiety-related behaviors, cognitive abilities on acquisition of spatial navigation in the Morris water maze, appetitive learning in a T-maze task, and odor discrimination learning (Zagreda *et al.*, 1999; Bolivar *et al.*, 2007; Moy *et al.*, 2007; MacPherson *et al.*, 2008; McFarlane *et al.*, 2008), ruling out potential confounds from these domains. To experimentally test the hypothesis that CC disconnection leads to autism-like behavioral traits in mice, we lesioned the CC at postnatal day (P7) in C57BL/6J (B6) mice and compared their juvenile and adult social behaviors and repetitive self-grooming with those of sham and non-operated B6 mice. LP/J, the inbred strain that is genetically closest to the BTBR strain (Witmer *et al.*, 2003; Petkov *et al.*, 2004) but has an intact CC, was compared to the BTBR and B6 strains on the same behavioral assays. Findings from these two complementary approaches indicate that factors other than, or in addition to, CC absence mediate social deficits and repetitive behaviors in BTBR mice.

Materials and methods

Animals

B6, BTBR and LP/J breeding pairs were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and bred at NIMH in Bethesda, ME, USA. 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. Subject mice were weaned at P20–22, and then group-housed by sex in standard mouse cages containing two to four mice. 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-h light↔dark cycle with lights on at 06:00 h, at 20°C and 55% humidity. All experiments were conducted between 10:00 h and 16:00 h. Recent experiments from our laboratory showed similar behavioral phenotypes of BTBR and B6 mice tested during the dark and light phases of their circadian cycles (Yang *et al.*, 2007a). Two cohorts of male B6 mice were used as the control groups, one for comparison with sham-operated and CC-lesioned male B6 mice, and one for comparison with male LP/J and BTBR inbred strains. Male non-operated B6, sham-operated B6 and CC-lesioned B6 mice were compared in the first set of experiments. Male B6, LP/J and BTBR mice were compared in the second set of experiments. Female B6, LP/J and BTBR mice were compared in the third set of experiments. Mice used as the novel targets in the social approach test were 129Sv/ImJ mice of the same sex as the subject, aged 8–14 weeks, purchased from The Jackson Laboratory. The novel mice had no previous physical contact with subject mice.

CC transection and histological verification

As both sexes of B6 mice exhibit high levels of social behaviors, we lesioned only male B6 mice to test whether the presence of an intact CC is critical for the expression of sociability. Female B6 mice were compared with LP/J and BTBR mice on juvenile and adult social tasks. Surgical procedures in mouse pups were adapted from previously described methods (Manhaes *et al.*, 2007). CC transection was performed on P7, under a stereoscopic microscope using the freehand method. Pups were separated from the dam one at a time, to reduce maternal stress (Manhaes *et al.*, 2007). General anesthesia was induced by cooling the subject at −10°C for 5 min. Hypothermia was maintained throughout the surgery by placing the pup on wet ice contained in a plastic box covered with dry cotton surgical gauze. The scalp was cleaned with Betadine solution and 70% ethanol. An incision was made in the scalp to reveal the skull surface and visualize lambda. A size 5 scalpel was inserted vertically through the sagittal suture, directly behind the coronal suture, and moved rostrocaudally to transect the CC at a vertical depth of 3.0 mm (Paxinos *et al.*, 2006). The incision was then closed with tissue adhesive (Nexaband; Abbott Laboratories, Chicago, IL, USA). Sham controls underwent the same procedures, except that the scalpel was not inserted into the brain. Following completion of the surgery, which was typically 3 min in duration, each pup was placed in an incubator maintained at 35°C for 10 min or until normal movement was observed. The incubator chamber contained home cage nest bedding placed near the pup to facilitate subsequent acceptance by the mother when the pup was returned to the home cage. Behavioral testing began 14 days after surgery, beginning with the juvenile play test at P21, the day before weaning. After the full sequence of behavioral tests was completed, adult mice (12–16 weeks) were killed by cervical dislocation and decapitated. Brains were removed and stored in 4% formaldehyde solution for at least 48 h before sectioning coronally at 80 μm using a vibratome (OTS-3000-03; Electron Microscopy Sciences, Hatfield, PA, USA). Sections were stained with thionin and examined under a light microscope. The percentage of CC lesion in each mouse was calculated by dividing the rostrocaudal length of CC displaying evidence of lesion by the entire callosal length of 3.64 mm, extending from bregma 1.18 to bregma −2.46 (Franklin & Paxinos, 1997). Any associated lesions of the septum, anterior commissure, hippocampus and hippocampal commissure were noted for each mouse.

Mouse behavioral tests relevant to the behavioral symptoms of autism

Juvenile play

Juvenile play at P21 was conducted in the Noldus PhenoTyper Observer 3000 chamber (Noldus Information Technology, Leesburg, VA, USA), as previously described (Yang *et al.*, 2007b; McFarlane *et al.*, 2008). The floor of the arena was covered with a 0.5-cm layer of clean bedding. Subjects were individually housed in standard mouse cages for 1 h prior to the play session. Two mice of the same age and sex but from different litters were then placed in the arena, and their interactions were recorded for 10 min, the period during which the majority of social interactions occur. For the CC lesion study, mice that had undergone the same treatment were paired, that is, two lesioned juvenile males, or two sham juvenile males. For non-operated mice, age-matched juvenile B6 mice were used as the play partners for B6, BTBR and LP/J subjects. B6 mice were chosen as the partners because this strain is neither unusually high nor unusually low on most behavioral traits, does not exhibit repetitive self-grooming like BTBR mice, and does not exhibit vertical jumping behaviors like LP/J mice. Behaviors were subsequently scored from digital videotapes by a highly trained observer, using Noldus Observer 5.0 software. The observer was uninformed of the sham or lesion condition during scoring. However, the identity of the strains was generally obvious

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from fur color patterns. Behaviors of interest included follow (one mouse walks straight behind its partner, keeping pace with the one ahead), nose-to-nose sniffing (sniffing the nose and face of the partner), and push and crawl (push = pushing head/snout underneath the partner's body or squeezing itself between the wall/floor and the partner; crawl = crawling over or under the partner's body). The last two similar movements were subsequently combined as a single parameter, 'push–crawl', in the present study. These behaviors have been used in previous studies as parameters of play behaviors in juvenile mice (Terranova & Laviola, 2005; Ricceri *et al.*, 2007; McFarlane *et al.*, 2008). Vertical jumping (upward jumping, usually against the wall) and bouts of arena exploration (walking around the arena, sniffing or digging the bedding) were scored as control measures of motor activity and exploratory tendencies. All behaviors were analysed for frequency of occurrence, that is, number of bouts.

Adult social approach test and self-grooming

Social approach behaviors were tested between 8 and 12 weeks of age, in an automated three-chambered apparatus using methods previously described (Nadler *et al.*, 2004; Crawley *et al.*, 2007; Moy *et al.*, 2007; Yang *et al.*, 2007b; McFarlane *et al.*, 2008). 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 chambers. Lack of innate side preference was confirmed during the second 10-min habituation. The subject was then briefly confined to the center chamber while the 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 time spent in each chamber and entries into each chamber, an observer with stopwatches scored time spent sniffing the novel object and the novel mouse and the duration of self-grooming exhibited by the subject mouse during the 10-min test session. The apparatus was cleaned with 70% ethanol and water between subjects. 129Sv/ImJ mice were used as the novel mice, because this strain is inactive and passive, rarely displays irritability when confined under the wire cup, and never exhibits aggressive behaviors towards subject mice. Using a minimally active partner is a strategy that allows all approaches to be initiated by the adult subject mouse only.

Elevated plus-maze, light↔dark exploration, open field locomotion, and rotarod test

To control for potential confounding factors such as anxiety-like traits and unusual motor phenotypes that could confound the interpretation of the social traits, a sequence of control tests was performed when the subjects were 8–12 weeks of age, including: elevated plus-maze (EPM), light↔dark exploration, open field locomotion, and rotarod coordination and balance. In the EPM, the mouse was placed in the center, facing a closed arm, and allowed to freely explore the apparatus for 5 min. Time spent and number of entries into the open arms (20 lux) and closed arms were scored by an investigator, using Noldus OBSERVER software (Karlsson *et al.*, 2008). In the light↔dark exploration test (Crawley & Goodwin, 1980), the mouse was placed in the light compartment facing away from the partition, and allowed to freely explore the apparatus for 10 min. Time spent and number of full-body transitions between the light (300 lux) and dark (3 lux) compartments were automatically scored, as previously described (Mathis *et al.*, 1995; Holmes *et al.*, 2001). In the open field test, the mouse was allowed to freely explore the apparatus (20 lux) for 10 min. Total distance traveled in the arena, vertical activity and time spent in the center were automatically scored by software linked to the photocell detectors (Accuscan, Columbus, OH, USA). Motor coordination and balance were evaluated in male mice on three consecutive 5-min trials on an accelerating rotarod, 4–40 rpm/5 min (Ugo Basile; Stoelting, Wood Dale, IL, USA) (Bailey *et al.*, 2007). The intertrial interval was 60 s.

General health, neurological reflexes, and sensory and motor abilities

To examine physical effects of the CC transection, lesioned and sham B6 mice were tested for general health, as previously described (Bailey *et al.*, 2007; Crawley *et al.*, 2007). General health assessment included scoring fur and whisker condition, as well as limb and body tone. Neurological reflex tests included forepaw reaching, righting reflex, trunk curl, whisker twitch, pinna twitch, eyeblink response, and toe pinch. The reactivity level of the mice was assessed with tests measuring responsiveness to petting, intensity of a dowel biting response, and level of vocalization during handling. Body weight was measured at 10 weeks of age.

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 is included on the graphs for illustrative purposes. Time spent sniffing the novel mouse vs. the novel object was also analysed using within-group repeated measures ANOVAS, with the factor of chamber side (novel mouse side vs. novel object side). Entries into all chambers were summed as 'total entries' and analysed using one-way ANOVA across groups, as a measure of general exploratory activity. Rotarod performance (latency to fall) was analysed with repeated measures ANOVAS followed by a Scheffe test for *post hoc* comparisons. All other behavior assays were analysed using one-way ANOVA. A Scheffe test was used for *post hoc* comparisons following a significant overall ANOVA F-value. Pearson's r was used to evaluate correlations between CC lesion size and social scores. Correlation analyses included lesioned mice only.

Results

Histology

Figure 1 shows representative cross-sections of brains of sham-operated B6, CC-lesioned B6, LP/J and BTBR male mice. The intact CC in a representative sham-operated B6 mouse brain cross-section is illustrated in Fig. 1A and E. A representative CC lesion performed in B6 mice on P7 is illustrated in Fig. 1B. CC lesions remained clearly visible at the time of death, at 3–4 months of age. Light microscopic examination of sections from the 35 lesioned mice showed that the

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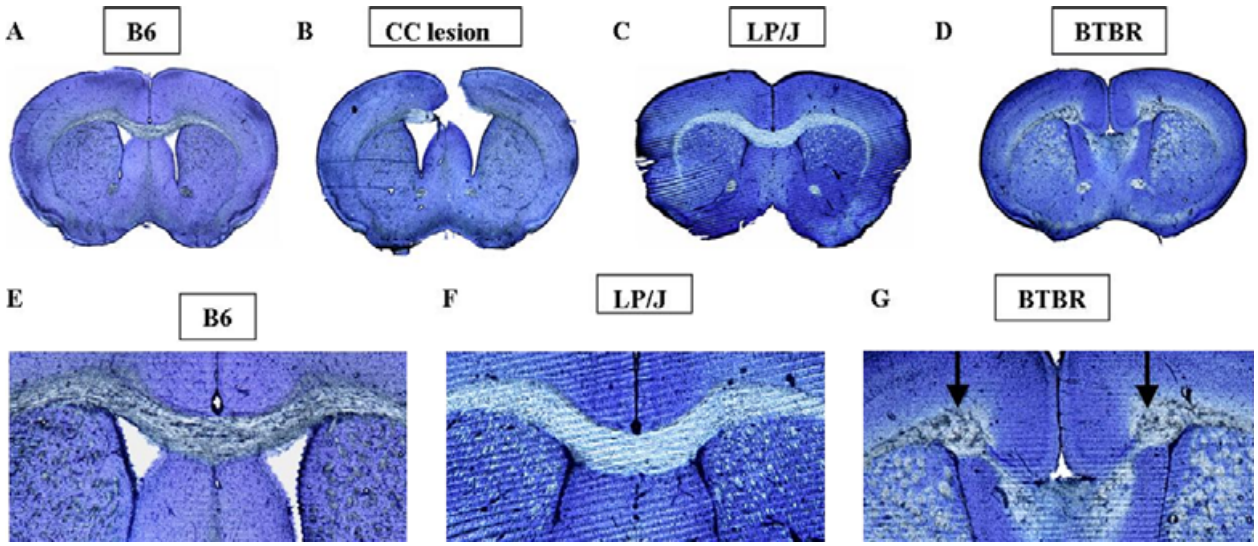

FIG. 1. Photomicrographs of thioin-stained representative coronal sections depicting the corpus callosum (CC) region in representative: (A) C57BL/6J (B6) control mice; (B) B6 mice in which the CC was lesioned on postnatal day 7 (tissue obtained from subjects killed at 3 months of age); (C) LP/J mice with intact CC; and (D) BTBR T+tf/J (BTBR) mice in which the CC is congenitally absent. Close-ups of the CC region are shown for: (E) B6 mice; (F) LP/J mice; and (G) BTBR mice. Probst bundles appear in BTBR mice only (arrows). Scale bar: 80 μm.

most rostral part of the CC was lesioned in all mice, whereas variable portions of the caudal CC remained. Complete transections throughout the rostral–caudal dimension were obtained in seven mice. On average, 70.3% ± 3.5% of the length of the CC was absent, with a range of 33–100% completeness of the lesion in the rostral–caudal dimension. The hippocampal commissure was partially severed in 20 mice, and minor damage was noted in the anterior medial hippocampus in 17 mice. The anterior commissure was intact in all mice.

A representative BTBR brain is shown in cross-section in Fig. 1D and G. Histological analysis showed that the CC was absent in all 10 BTBR brains. A representative LP/J brain is shown in Fig. 1C and F. The CC was present in all 10 LP/J brains that were analysed. The complete absence of the CC in BTBR mice and the normal thickness of the CC in LP/J mice confirm previously published findings (Wahlsten *et al.*, 2003) and information in the database of The Mouse Brain Library (http://www.mbl.org).

General health evaluation in CC-lesioned B6 and sham B6 mice

Table 1 shows that CC-lesioned B6 and sham B6 mice were normal on measures of general health, neurological reflexes, and sensory abilities. No mice exhibited physical abnormalities or aberrant behaviors. Body weights of both groups were within the normal range of adult male B6 mice (http://jaxmice.jax.org).

Juvenile play behaviors in control, sham and CC-lesioned B6 mice

Figure 2 shows parameters of social interactions in 21-day-old B6, sham-operated B6 and CC-lesioned B6 mice. Bouts of nose-to-nose sniffing did not differ across groups [$F_{2,58}$ = 2.11, not significant (NS)]. No group differences were detected for number of bouts of following ($F_{2,58}$ = 0.29, NS), number of bouts of push–crawl play-soliciting attempts ($F_{2,58}$ = 0.66, NS), general exploration of the test arena ($F_{2,58}$ = 1.81, NS), or vertical jumping ($F_{2,58}$ = 1.50, NS; Table 2A).

TABLE 1. General health and neurological reflexes in sham and CC-lesioned adult male B6 mice

	Sham (n = 18)	CC-lesioned (n = 30)
Body weight (g)	28.10 ± 0.52	26.93 ± 0.54
Fur condition (3-point scale)	3.0	3.0
Bald patches (%)	0	0
Missing whiskers (%)	0	0
Piloerection (%)	0	0
Body tone (3-point scale)	2.0	2.0
Limb tone (3-point scale)	2.0	2.0
Positional passivity (%)	27.8	33.3
Trunk curl (%)	100	66.7
Forepaw reach (%)	100	100
Righting reflex (%)	100	100
Corneal (%)	100	100
Pinna (%)	100	100
Vibrissae (%)	100	100
Toe pinch (%)	100	100
Petting escape (%)	88.9	80
Struggle/vocalization (%)	12.5	4
Dowel biting (3-point scale)	0	0

B6, C57BL/6J; CC, corpus callosum.

Adult social approach behaviors and self-grooming behavior in control B6, sham and CC-lesioned B6 mice

Figure 3 shows adult social approach (Fig. 3A–C) and repetitive self-grooming (Fig. 3D) results, obtained simultaneously in 8- to 12-week-old male non-operated control B6 (B6), sham B6 and CC-lesioned B6 mice. Non-operated B6 mice spent more time in the chamber containing the novel mouse than in the chamber containing the novel object ($F_{1,10}$ = 38.11, P < 0.0001). Similarly, sham B6 mice ($F_{1,18}$ = 54.38, P < 0.0001) and CC-lesioned B6 mice ($F_{1,34}$ = 150.35, P < 0.0001) spent more time in the chamber containing the novel mouse than in the chamber containing the novel object. Time spent sniffing the novel mouse showed a similar pattern to time in the side chambers. B6 mice ($F_{1,10}$ = 112.25, P < 0.0001), sham B6 mice ($F_{1,18}$ = 70.72, P < 0.0001) and CC-lesioned B6 mice

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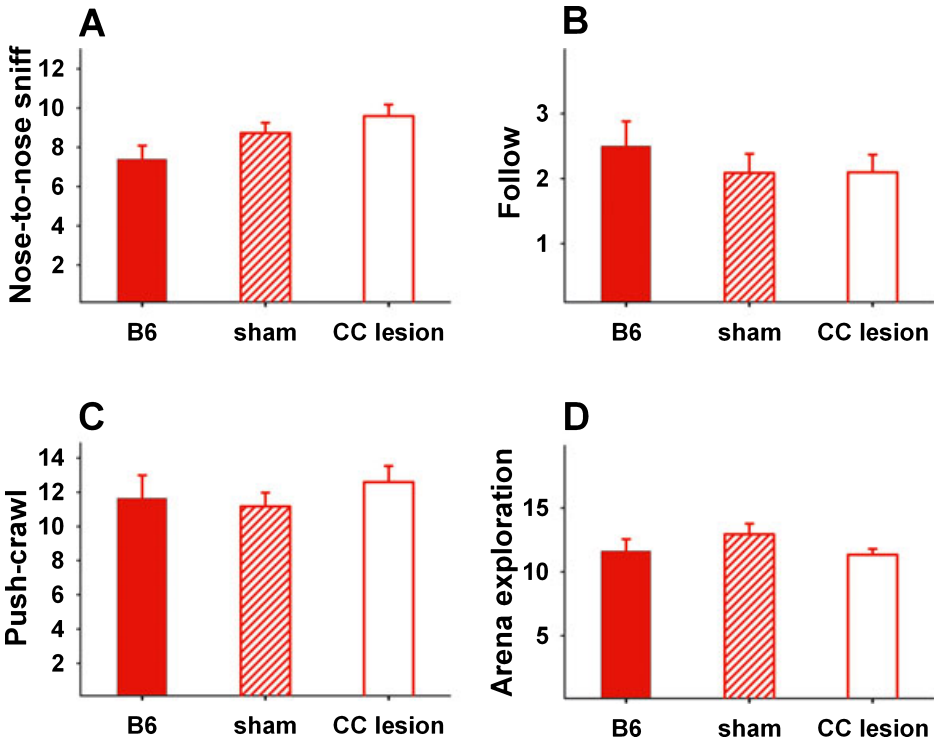

FIG. 2. Juvenile play behaviors were similar in male mice with early postnatal corpus callosum (CC) lesions or sham lesions, and in non-operated C57BL/6J (B6) controls. No significant group differences were detected with one-way ANOVA for: (A) nose-to-nose sniffing; (B) following; (C) push–crawl play-soliciting attempts; and (D) arena exploration. B6, $n = 8$; sham, $n = 22$; CC lesion, $n = 31$. For all bar graphs shown in Figs 2, 3 and 5–8, data are presented as mean + SEM.

($F_{1,34} = 181.50$, $P < 0.0001$) spent more time sniffing the novel mouse than the novel object. A significant main group effect was found for total entries into the side chambers ($F_{2,58} = 4.35$, $P < 0.05$). However, a Scheffe *post hoc* test did not reveal significant differences when sham and lesioned groups were compared with the non-operated B6 group. Cumulative time spent in self-grooming during the social approach test was low in all three groups and not significantly different across groups ($F_{2,58} = 0.23$, NS).

Correlational analysis of extent of CC lesion and behavioral scores in male B6 mice

Figure 4 shows a scattergram plot of the percentage of CC reduction in each male CC-lesioned mouse and its score on measures of juvenile play, adult social approach, and repetitive self-grooming. Only lesioned mice were included in the statistical analysis. Correlation values between percentage of CC lesion and play behaviors were as follows: nose-to-nose sniff, $r = 0.304$, NS; follow, $r = 0.469$, $P < 0.05$; push–crawl, $r = 0.577$, $P < 0.05$. This indicates that the exact length of the remaining posterior CC did not correlate with scores on play behaviors. Correlation values for scores in the adult social approach test were as follows: time spent in the chamber containing the novel mouse, $r = 0.288$, NS; time spent sniffing the novel mouse, $r = 0.216$, NS; cumulative time spent in self-grooming, $r = 0.106$, NS. These findings indicate that the length of the remaining posterior CC did not correlate positively with adult sociability or lack of repetitive self-grooming. The seven mice with complete CC transections scored in the same range as sham controls on all parameters. The 20 mice with partial hippocampal commissure lesions similarly scored in the same range as sham controls on all parameters.

Control measures of motor functions and anxiety-related behaviors in control B6, sham and CC-lesioned B6 mice

Table 2A shows results for adult male B6, sham and CC-lesioned B6 mice on open field exploratory locomotor activity and on rotarod coordination and balance. In the open field, no significant differences were detected across the three groups on total distance traversed in the open field ($F_{2,52} = 2.27$, NS), time spent in the center of the open field ($F_{2,52} = 1.62$, NS), or vertical activity ($F_{2,52} = 1.29$, NS). On the rotarod, no significant differences were detected across the three groups on latency to fall ($F_{2,51} = 1.25$, NS).

Table 2A shows results for adult male B6, sham and CC-lesioned B6 mice on the EPM and light↔dark exploration tests for anxiety-related behaviors. In the EPM test, significant group differences were found for the percentage of time spent in the open arm ($F_{2,61} = 16.62$, $P < 0.001$) and the number of open arm entries ($F_{2,61} = 12.92$, $P < 0.0001$). *Post hoc* Scheffe tests revealed that CC-lesioned B6 mice had higher levels of percentage open arm time and made more open arm entries than non-operated B6 and sham B6 mice ($P < 0.01$ for each comparison). Number of total arm entries did not differ across groups ($F_{2,61} = 3.00$, NS). In the light↔dark exploration test, no significant differences were detected across groups on the number of transitions between the light and dark compartments ($F_{2,61} = 1.52$, NS), the cumulative time spent in the dark chamber ($F_{2,61} = 2.35$, NS), or the latency to first entry into the dark chamber ($F_{2,61} = 1.83$, NS).

Juvenile play behaviors in male B6, LP/J and BTBR inbred mice

Figure 5 shows parameters of social interactions between 21-day-old male B6, LP/J and BTBR mice. Significant strain differences were found for nose-to-nose sniffing ($F_{2,37} = 18.36$, $P < 0.0001$), following

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TABLE 2A. Motor functions and anxiety-like behaviors in male non-operated B6, sham B6 and CC-lesioned B6 mice

	Male B6	Male sham	Male CC lesion
Open field activities			
Total distance (cm/10 min)	1610.0 ± 83.9	2006.3 ± 116.3	2142.2 ± 140.0
Center time (s/10 min)	83.3 ± 15.2	98.1 ± 6.7	78.3 ± 7.4
Vertical activity	96.1 ± 9.1	133.7 ± 16.0	111.1 ± 11.6
Vertical jumping in the juvenile play test	0.75 ± 0.49	1.19 ± 0.47	2.50 ± 0.85
Rotarod, latency to fall (s, average of trials)	208.5 ± 19.61	234.18 ± 13.91	241.25 ± 9.08
Elevated plus-maze			
Open arm time (%)	31.8 ± 5.3	30.5 ± 2.3	48.9 ± 2.4*
Open arm entries	9.6 ± 1.1	12.2 ± 1.9	16.8 ± 0.8*
Total arm entries	21.4 ± 0.80	26.8 ± 1.3	28.3 ± 1.4
Light↔dark exploration			
Number of transitions	37.3 ± 3.3	47.5 ± 3.6	45.8 ± 2.5
Time in the dark chamber (s)	313.9 ± 17.5	295.0 ± 16.8	345.0 ± 16.8
Latency to enter the dark chamber (s)	8.9 ± 2.5	5.0 ± 1.5	4.8 ± 0.7

Data are presented as mean ± SEM. CC lesions did not alter motor functions in male B6 mice; CC lesion reduced anxiety-like behaviors in the elevated plus-maze test but not in the light↔dark test. *$P < 0.05$ vs. B6. B6, C57BL/6J; CC, corpus callosum.

TABLE 2B. Motor functions and anxiety-like behaviors in male B6, LP/J and BTBR mice

	Male B6	Male LP/J	Male BTBR
Open field activities			
Total distance (cm/10 min)	1549.9 ± 114.0	1025.6 ± 125.3	2529.5 ± 223.5*,†
Center time (s/10 min)	93.9 ± 11.5	61.2 ± 37.0	94.2 ± 12.7
Vertical activity	132.3 ± 14.5	24.1 ± 6.8*	84.0 ± 19.2†
Vertical jumping in the juvenile play test	0.29 ± 0.13	8.33 ± 2.93*	0 ± 0†
Rotarod, latency to fall (s, average of trials)	244.901 ± 5.16	159.21 ± 23.87*	95.94 ± 14.54*
Elevated plus-maze			
Open arm time (%)	33.4 ± 2.7	21.1 ± 3.0*	38.3 ± 3.1†
Open arm entries	11.4 ± 1.0	7.3 ± 0.8*	17.2 ± 1.5*,†
Total arm entries	24.2 ± 1.5	18.3 ± 1.3*	29.2 ± 2.1†
Light↔dark exploration			
Number of transitions	43.7 ± 2.9	18.0 ± 3.4*	45.2 ± 3.3†
Time in the dark chamber (s)	331.5 ± 15.1	254.9 ± 44.4*	335.8 ± 25.2
Latency to enter the dark chamber (s)	3.9 ± 1.0	150.3 ± 53.3*	6.9 ± 1.6†

Data are presented as mean ± SEM. *$P < 0.05$ vs. B6; †$P < 0.05$ BTBR vs. LP/J. BTBR, BTBR T+tf/J; B6, C57BL/6J; CC, corpus callosum.

TABLE 2C. Motor functions and anxiety-like behaviors in female C57BL/6J (B6), LP/J and BTBR T+tf/J (BTBR) mice

	Female B6	Female LP/J	Female BTBR
Open field activities			
Total distance (cm/10 min)	1740.5 ± 180.0	805.4 ± 137.8*	2446.5 ± 192.2†
Center time (s/10 min)	105.5 ± 10.6	43.5 ± 10.9*	93.0 ± 18.0†
Vertical activity	121.1 ± 21.8	32.1 ± 8.2*	77.3 ± 16.1
Vertical jumping in the juvenile play test	3.4 ± 2.1	3.8 ± 2.1	0.0 ± 0.0
Elevated plus-maze			
Open arm time (%)	34.1 ± 2.5	16.9 ± 3.3*	38.8 ± 2.7†
Open arm entries	13.1 ± 0.8	5.1 ± 0.8*	13.8 ± 1.2†
Total arm entries	28.0 ± 1.4	13.1 ± 1.8*	24.2 ± 1.6†
Light↔dark exploration			
Number of transitions	43.7 ± 3.5	10.3 ± 2.2*	41.2 ± 4.8†
Time in the dark chamber (s)	386.7 ± 14.0	420.0 ± 57.3	414.2 ± 26.7
Latency to enter the dark chamber (s)	9.9 ± 2.6	82.5 ± 49.2	6.4 ± 1.1

Data are presented as mean ± SEM. *$P < 0.05$ vs. B6; †$P < 0.05$ BTBR vs. LP/J (one-way ANOVA followed by Scheffe *post hoc* test). BTBR, BTBR T+tf/J; B6, C57BL/6J; CC, corpus callosum.

the partner mouse ($F_{2,37} = 8.27$, $P < 0.001$), and push–crawl play-soliciting attempts ($F_{2,37} = 38.10$, $P < 0.0001$). Scheffe *post hoc* tests revealed that LP/J and BTBR mice exhibited fewer bouts of nose-to-nose sniffing, fewer bouts of following and fewer bouts of push–crawl than B6 mice ($P < 0.01$ for each comparison). Strain difference for general exploration of the test arena was significant ($F_{2,37} = 20.41$, $P < 0.0001$). BTBR mice showed more bouts of arena exploration than B6 and LP/J mice ($P < 0.01$ for each comparison). The difference between LP/J and B6 mice was not significant ($P > 0.05$), ruling out an inactivity explanation for the lower level of social interactions in

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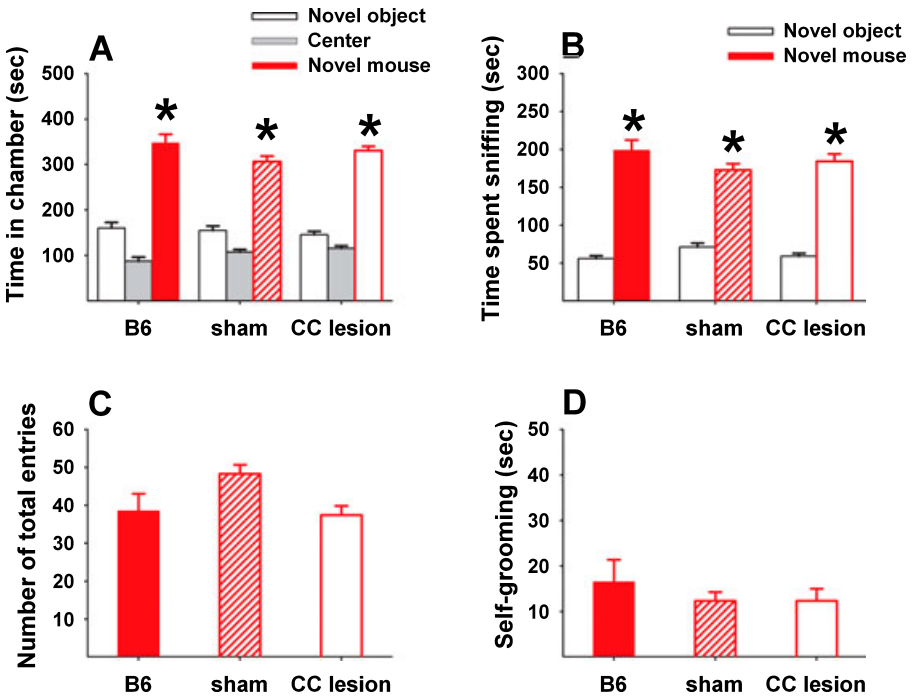

FIG. 3. Adult sociability and levels of self-grooming were similar in male mice with early postnatal corpus callosum (CC) lesions or sham lesions, and in non-operated C57BL/6J (B6) controls. (A) Sociability in the automated three-chambered task is defined as spending significantly more time in the side chamber containing a novel mouse than in the side chamber containing a non-social novel object, an inverted wire pencil cup. Repeated measures ANOVA revealed significant sociability in B6 mice ($F_{1,10}$ = 38.11, P < 0.0001), sham mice ($F_{1,18}$ = 54.38, P < 0.0001), and CC-lesioned B6 mice ($F_{1,34}$ = 150.35, P < 0.0001). (B) Time spent sniffing the inverted wire cup containing the novel mouse vs. sniffing the empty inverted wire cup is a complementary and more sensitive measure of sociability. Repeated measures ANOVA revealed that B6 mice ($F_{1,10}$ = 112.25, P < 0.0001), sham mice ($F_{1,18}$ = 70.72, P < 0.0001) and CC-lesioned B6 mice ($F_{1,34}$ = 181.50, P < 0.0001) all spent more time sniffing the novel mouse than the novel object. (C) One-way ANOVA revealed a significant main group effect for total entries ($F_{2,58}$ = 4.35, P < 0.05). However, Scheffe *post hoc* tests did not reveal significant differences when sham and lesioned groups were compared with the non-operated B6 group. (D) Repetitive self-grooming was simultaneously scored during the social approach test. CC lesion did not alter the amount of self-grooming as compared with sham and B6 mice ($F_{2,58}$ = 0.23, not significant). B6, n = 11; sham, n = 19; CC lesion, n = 35. *P < 0.01 for comparison between novel object and novel mouse.

juvenile BTBR and LP/J mice. Vertical jumping differed significantly among the strains ($F_{2,37}$ = 6.42, P < 0.01, Table 2B), with LP/J mice exhibiting more vertical jumps than B6 and BTBR mice (P < 0.05 for each comparison).

Adult social approach behaviors and self-grooming behavior in male B6, LP/J and BTBR inbred mice

Figure 6 shows social approach and repetitive self-grooming results obtained simultaneously in 8–12-week-old male B6, LP/J and BTBR mice. B6 mice spent more time in the chamber containing the novel mouse than in the chamber containing the novel object ($F_{1,9}$ = 63.01, P < 0.0001). BTBR mice did not spend more time in the chamber containing the novel mouse than in the chamber containing the novel object ($F_{1,11}$ = 3.26, NS). Some of the LP/J mice exhibited very low locomotor activity that confounded the interpretation of scores on the social approach task, and were therefore removed from the statistical analysis. Eighteen LP/J mice with normal locomotion (total entries > 12) spent more time in the chamber containing the novel mouse than in the chamber containing the novel object ($F_{1,17}$ = 18.83, P < 0.001). More time sniffing the novel mouse than the novel object was seen in B6 mice ($F_{1,9}$ = 86.63, P < 0.0001) and LP/J mice ($F_{1,17}$ = 28.44, P < 0.0001), but not in BTBR mice ($F_{1,11}$ = 3.26, NS). Total entries into the side chambers showed a significant main group effect ($F_{2,37}$ = 4.85, P < 0.01). *Post hoc* Scheffe tests revealed that BTBR mice made more entries than LP/J mice (P < 0.05).

Total cumulative time spent in self-grooming during the social approach test was significant across groups ($F_{2,37}$ = 23.47, P < 0.0001). As compared with B6 mice, both LP/J and BTBR mice exhibited higher levels of self-grooming (P < 0.0001 for each comparison). No significant strain differences were detected between LP/J and BTBR mice.

The number of entries in the automated three-chambered social approach apparatus was very low in approximately one-third of LP/J males. Total chamber entries were fewer than 12 in these individuals during the adult social approach test. Instead of exploring the apparatus, as B6 and BTBR strains did, this subset of LP/J mice tended to sit still in one place for a very long time during the test session. Their data were therefore analysed separately. The nine inactive male LP/J mice did not display sociability, that is, did not spend more time in the chamber containing a novel mouse than in the chamber containing a novel object (time in the chamber with the novel mouse, 275.1 ± 72.8s; time in the chamber with the novel object, 174.3 ± 68.1s; $F_{1,8}$ = 0.60, NS), but did spend significantly more time sniffing the novel mouse than the novel object (time spent sniffing the novel mouse, 79.2 ± 11.9s; time spent sniffing the novel object, 22.9 ± 7.1s; $F_{1,8}$ = 17.34, P < 0.01). This subset, whose total entries were 7.56 ± 1.46s, was not included in the data shown in Fig. 3.

Control measures of motor functions and anxiety-related behaviors in male B6, LP/J and BTBR inbred mice

Table 2B shows results for adult male B6, LP/J and BTBR mice on open field exploratory locomotor activity and on rotarod coordination and balance. In the open field test, a significant strain difference was found for total distance ($F_{2,41}$ = 18.80, P < 0.001), with BTBR mice

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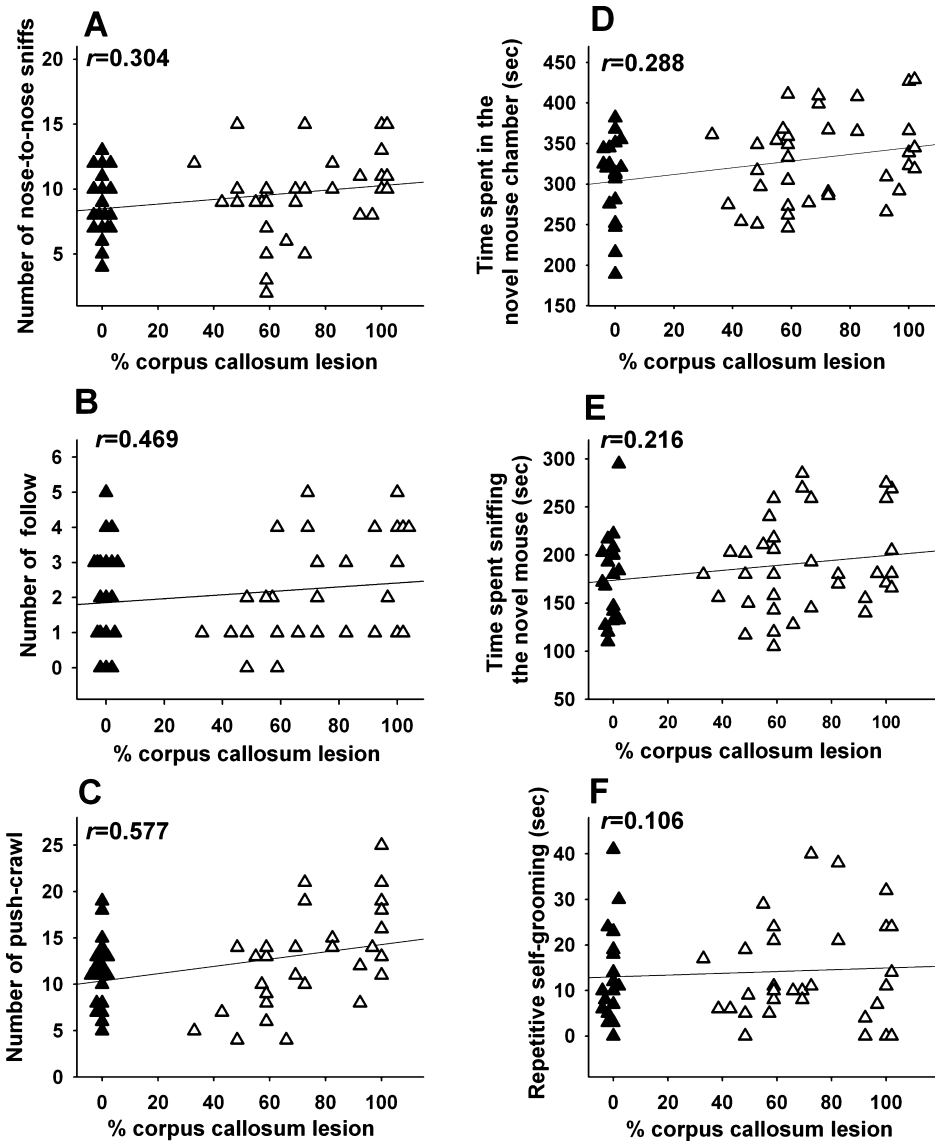

FIG. 4. Lack of correlation between percentage of lesion-induced corpus callosum reduction and scores for juvenile play behaviors (A–C), adult sociability (D and E), and self-grooming (F). Correlation analyses included lesioned mice only, using Pearson's *r*-test. (A) Number of bouts of nose-to-nose sniffing [$r = 0.304$, not significant (NS)]. (B) Number of occasions on which one juvenile male mouse followed the other juvenile mouse ($r = 0.469$, $P < 0.05$). (C) Number of push–crawl interactions ($r = 0.577$, $P < 0.05$). (D) Time spent by the adult male subject mouse in the chamber containing the novel mouse ($r = 0.288$, NS). (E) Time spent sniffing the novel mouse ($r = 0.216$, NS). (F) Time spent self-grooming by the adult male subject mouse in the social approach apparatus ($r = 0.106$, NS). Sham surgery mice (black triangles) were not included in the statistical analyses of *r*-values, but are shown here for illustrative purposes only, as controls with zero for their lesion values. Sham C57BL/6J (B6): $n = 22$ for juvenile play, $n = 19$ for social approach. Corpus callosum-lesioned B6 (white triangles): $n = 31$ for juvenile play, $n = 35$ for social approach. Seven mice displayed complete 100% lesions, with no connections visible between the left and right cerebral cortex throughout the rostral–caudal dimension. Social scores and self-grooming remained within control ranges in mice with complete and partial lesions.

traversing a greater total distance than B6 and LP/J mice ($P < 0.01$ for each comparison). Time spent in the center of the open field did not differ across groups ($F_{2,41} = 0.77$, NS). The strain difference for vertical activity was significant ($F_{2,41} = 15.87$, $P < 0.001$). LP/J mice exhibited fewer vertical movements than B6 mice ($P < 0.01$) and BTBR mice ($P < 0.05$). On the rotarod test, repeated measures ANOVA revealed a significant strain difference for latency to fall ($F_{2,33} = 18.27$, $P < 0.0001$), with both BTBR mice ($P < 0.0001$) and LP/J mice ($P < 0.0001$) falling sooner than B6 mice.

Table 2B shows results for adult male B6, LP/J and BTBR male mice on the EPM and light↔dark exploration tests for anxiety-related behaviors. In the EPM test, significant strain differences were found for the percentage of time spent in the open arm ($F_{2,47} = 8.19$, $P < 0.0001$), number of open arm entries ($F_{2,47} = 17.01$, $P < 0.001$), and number of total arm entries ($F_{2,47} = 9.45$, $P < 0.001$). As compared with B6 and BTBR mice, LP/J mice scored lower on all three measures ($P < 0.05$ or less for each comparison). BTBR mice made more open arm entries than B6 mice ($P < 0.05$). In the light↔dark exploration test, significant strain differences were found for the number of transitions ($F_{2,60} = 24.66$, $P < 0.0001$), cumulative time spent in the dark chamber ($F_{2,60} = 3.46$, $P < 0.05$), and latency to enter the dark chamber ($F_{2,60} = 7.99$, $P < 0.0001$). As compared with B6 and BTBR mice, LP/J mice made fewer transitions between the light and dark compartments and exhibited longer latency to enter the dark chamber ($P < 0.01$ for each comparison). LP/J mice also spent less time in the dark chamber than B6 mice ($P < 0.05$). BTBR

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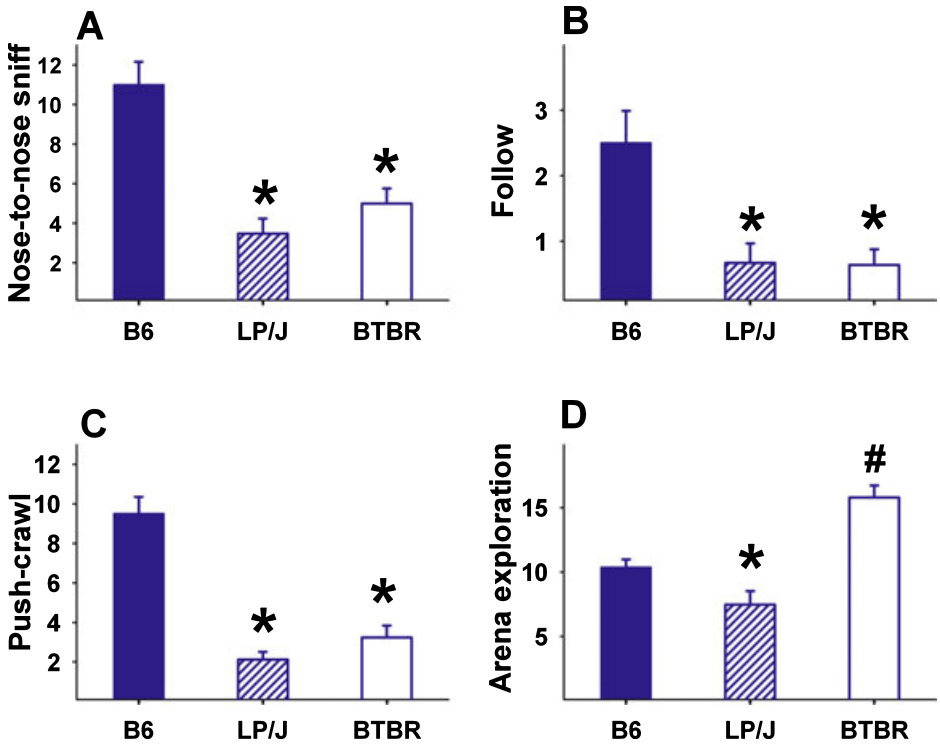

FIG. 5. Deficits in juvenile play behaviors in 21-day-old male LP/J and BTBR T+tf/J (BTBR) mice as compared to C57BL/6J (B6) mice. One-way ANOVA revealed significant strain differences in: (A) nose-to-nose sniffing ($F_{2,37}$ = 18.36, P < 0.0001); (B) following the partner mouse ($F_{2,37}$ = 8.27, P < 0.001); (C) push–crawl play-soliciting attempts ($F_{2,37}$ = 38.10, P < 0.0001); and (D) arena exploration ($F_{2,37}$ = 20.41, P < 0.0001). Scheffe tests revealed that LP/J and BTBR mice exhibited fewer bouts of nose-to-nose sniffing, fewer bouts of following and fewer bouts of push–crawl than B6 mice (P < 0.01 for each comparison), and that BTBR mice showed more bouts of arena exploration than B6 and LP/J mice (P < 0.01 for each comparison). B6, n = 14; LP/J, n = 15; BTBR, n = 11. *P < 0.01 vs. B6; #P < 0.01 for BTBR vs. LP/J.

and B6 mice did not differ significantly on any of the measures in the light↔dark test. Fewer total arm entries in the EPM test and longer latency to enter the dark chamber in the light↔dark exploration test indicated that lower general exploratory activity of LP/J mice may explain their anxiety-like behaviors.

Juvenile play behaviors in female B6, LP/J and BTBR inbred mice

Figure 7 displays parameters of juvenile social interaction in 21-day-old female B6, LP/J and BTBR mice. Significant strain differences were found for nose-to-nose sniffs ($F_{2,30}$ = 18.74, P < 0.0001), following the play partner ($F_{2,30}$ = 7.83, P < 0.01), push–crawl play-soliciting attempts ($F_{2,30}$ = 14.95, P < 0.0001), and arena exploration ($F_{2,30}$ = 20.45, P < 0.0001). LP/J mice scored lower than B6 mice on all four measures (P < 0.01 for each), and lower than BTBR mice on push–crawl and arena exploration (P < 0.01 for each). BTBR mice made fewer nose-to-nose sniffs than B6 mice (P < 0.01) and showed a trend towards reduced following. Arena exploration did not differ significantly between BTBR and B6 mice.

Adult social approach behaviors and self-grooming behavior in female B6, LP/J and BTBR inbred mice

Figure 8 shows social approach behaviors and self-grooming behavior in adult female mice of the B6, LP/J and BTBR inbred strains. B6 and LP/J mice spent more time in the chamber containing a novel mouse than in the chamber containing a novel object (B6, $F_{1,12}$ = 94.93, P < 0.0001; LP/J, $F_{1,20}$ = 6.15, P < 0.05); BTBR mice did not spend more time in the chamber containing a novel mouse than in the chamber containing a novel object ($F_{1,11}$ = 3.26, NS). B6 and LP/J mice spent more time sniffing the novel mouse than the novel object (B6, $F_{1,12}$ = 91.5, P < 0.0001; LP/J, $F_{1,20}$ = 24.94, P < 0.0001); BTBR mice did not spend more time sniffing the novel mouse than the novel object ($F_{1,12}$ = 3.26, NS). Total entries into the side chambers showed a significant main group effect ($F_{2,36}$ = 10.47, P < 0.001). *Post hoc* analysis revealed that LP/J mice made fewer entries than B6 and BTBR mice (P < 0.01 for each comparison). Strain difference for repetitive self-grooming was significant ($F_{2,36}$ = 23.27, P < 0.001). BTBR and LP/J mice spent more time in self-grooming than B6 mice (P < 0.01 for each comparison).

As found in the males, approximately one-third of LP/J females exhibited low levels of activity; that is, total chamber entries were fewer than 12 in the adult social approach test. Their data were therefore analysed separately. The 11 inactive female LP/J mice did not spend more time in the chamber containing a novel mouse than in the chamber containing a novel object (time in the chamber with the novel mouse, 157.5 ± 71.7; time in the chamber with the novel object, 127.0 ± 60.7; $F_{1,10}$ = 0.08, NS) and did not spend more time sniffing the novel mouse than the novel object (time spent sniffing the novel mouse, 62.5 ± 23.0; time spent sniffing the novel object, 17.3 ± 10.0; $F_{1,10}$ = 3.55, NS). This subset, whose total entries were 3.36 ± 1.13, was not included in the data shown in Fig. 8.

Control measures of motor functions and anxiety-related behaviors in female B6, LP/J and BTBR inbred mice

Table 2C shows adult open field activities and bouts of jumping during the juvenile play test in female B6, BTBR and LP/J mice.

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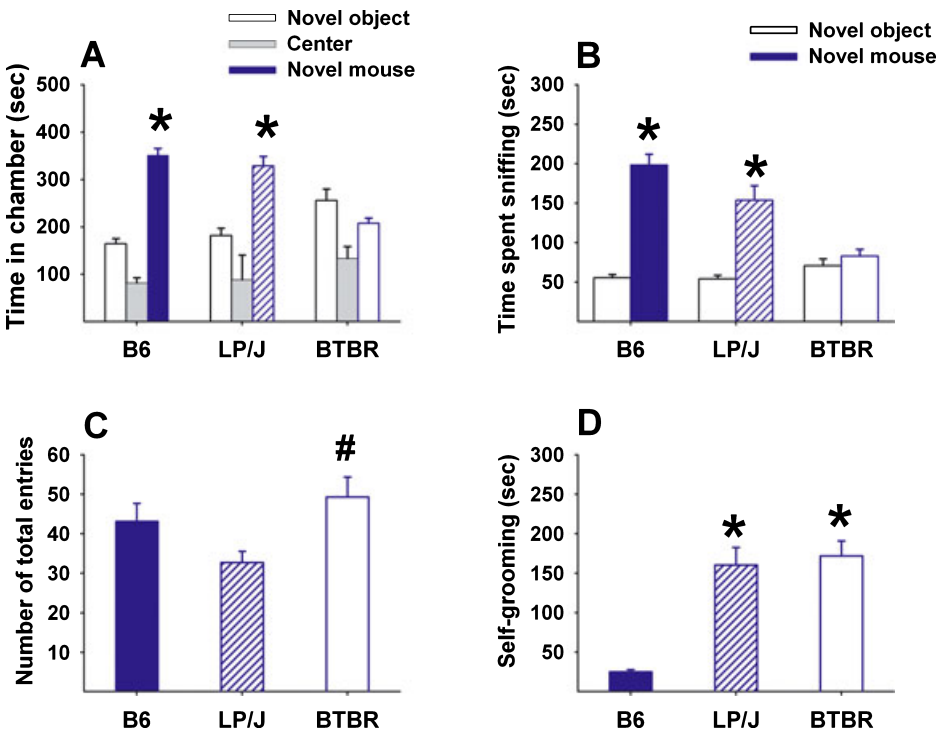

FIG. 6. Deficits in sociability in adult male BTBR T+tf/J (BTBR) mice and repetitive self-grooming in BTBR and LP/J mice as compared to C57BL/6J (B6) mice. Sociability in the automated three-chambered apparatus is defined as spending significantly more time in the side chamber containing a novel mouse than in the side chamber containing a non-social novel object. (A) Repeated measures ANOVA revealed significant sociability in B6 mice ($F_{1,9}$ = 63.01, P < 0.0001) and LP/J mice ($F_{1,17}$ = 18.83, P < 0.001), but not in BTBR mice [$F_{1,11}$ = 3.26, not significant (NS)]. (B) Time spent sniffing the novel mouse vs. the novel object provides a complementary measure of sociability. Repeated measures ANOVA revealed that B6 mice ($F_{1,9}$ = 86.63, P < 0.0001) and LP/J mice ($F_{1,17}$ = 28.44, P < 0.0001) spent more time sniffing the novel mouse than the novel object, whereas BTBR mice ($F_{1,11}$ = 3.26, NS) did not. (C) Total entries into the side chambers showed a significant main group effect (one-way ANOVA, $F_{2,37}$ = 4.85, P < 0.01). Scheffe tests revealed that BTBR mice made more entries than LP/J mice (P < 0.05). (D) Repetitive self-grooming was simultaneously measured during the social approach test. One-way ANOVA indicated that LP/J and BTBR mice spent more time engaged in self-grooming than B6 mice (P < 0.0001 for each comparison). B6, n = 10; LP/J, n = 18; BTBR, n = 12. (A and B) *P < 0.01 for comparison between novel mouse and novel object sides. (C) $^{\#}P$ < 0.05 for BTBR vs. LP/J. (D) *P < 0.0001 vs. B6.

Significant strain differences were found for total distance ($F_{2,46}$ = 25.01, P < 0.001), center time ($F_{2,46}$ = 7.55, P < 0.01), and vertical activity ($F_{2,46}$ = 7.75, P < 0.01). As compared with B6 and BTBR mice, LP/J mice traversed shorter total distances (P < 0.01 for each) and spent less time in the center (P < 0.05 for each). BTBR mice traveled longer total distances than B6 mice (P < 0.01). LP/J mice made fewer vertical movements than B6 mice (P < 0.01). Vertical jumping during the juvenile play test did not differ across strains ($F_{2,30}$ = 1.10, NS).

Table 3 shows anxiety-related tests in adult female B6, LP/J and BTBR mice. In the EPM test, significant strain differences were found for the percentage of time spent in the open arm ($F_{2,43}$ = 16.10, P < 0.0001), number of open arm entries ($F_{2,43}$ = 10.08, P < 0.001), and number of total arm entries ($F_{2,43}$ = 21.049, P < 0.001). As compared with B6 and BTBR mice, LP/J mice had lower percentage open arm time, made fewer open arm entries, and made fewer total entries (P < 0.001 for each comparison). BTBR and B6 mice did not differ significantly on any of these measures. In the light↔dark exploration test, a significant strain difference was found for the number of transitions ($F_{2,43}$ = 20.26, P < 0.001). LP/J mice made fewer transitions than B6 and BTBR mice (P < 0.01 for each comparison). No significant strain difference was found for time spent in the dark chamber ($F_{2,43}$ = 0.72, NS). Strain difference was significant for the latency to enter the dark chamber ($F_{2,43}$ = 3.27, P < 0.05), but Scheffe *post hoc* tests did not reveal significant differences among the three strains.

Discussion

Mice of the inbred BTBR strain display impaired reciprocal social interactions as juveniles, reduced social approach and reduced reciprocal social interactions as adults, less social transmission of food preference, qualitatively and quantitatively unusual vocalizations, and high levels of repetitive self-grooming across developmental ages, as compared with the standard B6 inbred strain (Bolivar *et al.*, 2007; Moy *et al.*, 2007; McFarlane *et al.*, 2008; Scattoni *et al.*, 2008), representing traits relevant to the first, second and third diagnostic symptoms of autism. The currently known major neuroanatomical abnormalities in BTBR mice are: complete abscence of the corpus callosal connections between the right and left cerebral cortex, and a reduced hippocampal commissure, along with a normal anterior commissure (Wahlsten *et al.*, 2003; Kusek *et al.*, 2007). One possible explanation for the behavioral abnormalities in the BTBR strain is its acallosal condition and/or other white matter connectivity abnormalities. We addressed the CC hypothesis using two complementary approaches: (i) experimental lesions of the CC in a standard inbred strain, B6, at P7; and (ii) comparison of the BTBR strain with the inbred strain LP/J, which is adjacent to the BTBR strain on the mouse family tree but has an intact CC. The acallosal BTBR strain displayed lack of adult sociability, low scores on some measures of juvenile play, and high levels of repetitive self-grooming in the present study, consistent with previous reports from multiple cohorts tested in three different laboratories (Moy *et al.*, 2007; Yang *et al.*,

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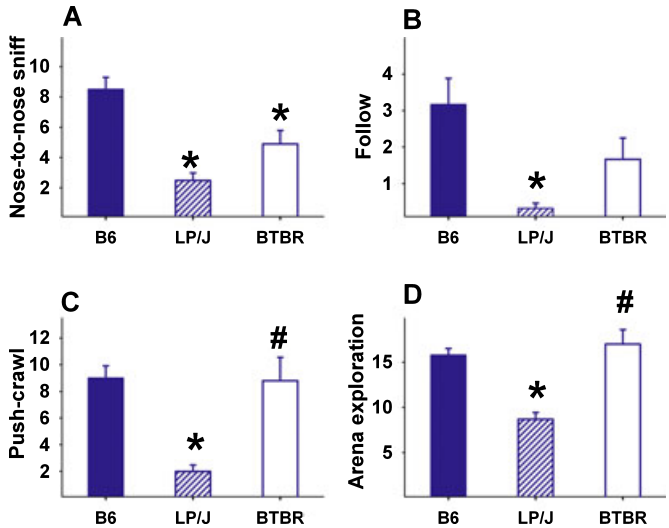

FIG. 7. Juvenile play behaviors in 21-day-old female C57BL/6J (B6), LP/J and BTBR T+tf/J (BTBR) inbred mice. One-way ANOVA revealed significant strain differences in: (A) nose-to-nose sniffing ($F_{2,30} = 18.74$, $P < 0.0001$); (B) following the play partner ($F_{2,30} = 7.83$, $P < 0.01$); (C) push–crawl play-soliciting attempts ($F_{2,30} = 14.95$, $P < 0.0001$); and (D) arena exploration ($F_{2,30} = 20.45$, $P < 0.0001$). Scheffe *post hoc* tests revealed that LP/J mice scored lower than B6 mice on all four measures ($P < 0.01$ for each comparison) and lower than BTBR mice on push–crawl and arena exploration ($P < 0.01$ for each comparison). BTBR mice made fewer nose-to-nose sniffs than B6 mice ($P < 0.01$) and showed a trend towards reduced following. B6, $n = 12$; LP/J, $n = 15$; BTBR, $n = 9$. *$P < 0.01$ vs. B6; $^{\#}P < 0.01$ for BTBR vs. LP/J.

2007a,b; McFarlane *et al.*, 2008). However, postnatal surgical disconnection of the CC did not impair behaviors in either the juvenile social interaction test or the adult social approach test, and did not elevate self-grooming, providing evidence against the hypothesis that CC disconnection is a causal factor for social deficits and/or repetitive behaviors in mice.

Our findings are consistent with human studies reporting that children with congenital AgCC do not uniformly exhibit autism-like symptoms. Although a high percentage (~40%) of children with AgCC are found to have unusual social interactions (Badaruddin *et al.*, 2007), the forms of social abnormalities most frequently cited in AgCC patients are 'emotional immaturity, lack of introspection, impaired social competence, general deficits in social judgement and planning, and poor communication of emotions' (Paul *et al.*, 2006, 2007). Core symptoms of autism, such as aloofness and repetitive/restricted behaviors, are not major characteristics of AgCC children (Badaruddin *et al.*, 2007). The social problems of AgCC individuals may be more closely related to their deficits in cognitive integration, for which the CC might be important. Furthermore, social problems typically do not become obvious in AgCC children until 6 years of age, whereas symptoms of autism are usually detected before age 2 years, and the diagnosis can be reliably made by age 3 years (Volkmar *et al.*, 2005; Geschwind & Levitt, 2007). In addition, human epilepsy patients who received surgical lesions of the CC displayed no adverse consequences on neurophysiological measures, physical health, or motor functions, and remained largely normal in terms of personality and social behaviors (Gazzaniga *et al.*, 1975; Wilson *et al.*, 1978; Sass *et al.*, 1988; Mamelak *et al.*, 1993; Lassonde & Sauerwein,

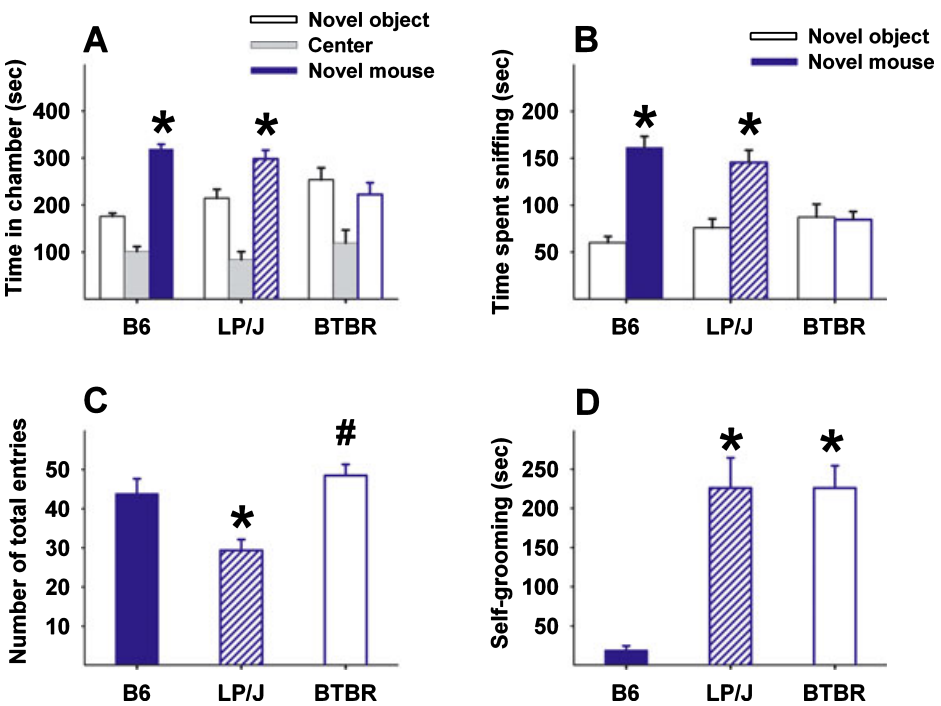

FIG. 8. Deficits in sociability in female BTBR T+tf/J (BTBR) mice and repetitive self-grooming in female BTBR and LP/J mice as compared with C57BL/6J (B6) mice. (A) Repeated measures ANOVA revealed significant sociability in B6 mice ($F_{1,12} = 94.93$, $P < 0.0001$) and LP/J mice ($F_{1,20} = 6.15$, $P < 0.05$) but not in BTBR mice [$F_{1,11} = 3.26$, not significant (NS)]. (B) Repeated measures ANOVA revealed that B6 mice ($F_{1,12} = 91.5$, $P < 0.0001$) and LP/J mice ($F_{1,20} = 24.94$, $P < 0.0001$) spent more time sniffing the novel mouse than the novel object, whereas BTBR mice did not ($F_{1,12} = 3.26$, NS). (C) Total entries into the side chambers showed a significant main group effect (one-way ANOVA, $F_{2,36} = 10.47$, $P < 0.001$). Scheffe tests revealed that LP/J mice made fewer entries than B6 and BTBR mice ($P < 0.01$ for each comparison). (D) Repetitive self-grooming was simultaneously measured during the social approach test. Strain difference for self-grooming was significant ($F_{2,36} = 23.27$, $P < 0.001$). BTBR and LP/J mice spent more time in self-grooming than B6 mice ($P < 0.01$ for each comparison). B6, $n = 12$; LP/J, $n = 21$; BTBR, $n = 13$. (A and B) *$P < 0.01$ for comparison between novel mouse and the novel objects side. (C and D) *$P < 0.01$ vs. B6; $^{\#}P < 0.01$ for BTBR vs. LP/J.

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TABLE 3. Summary of corpus callosum-lesioned, sham surgery and inbred strains of mice on behaviors relevant to the first diagnostic symptom of autism, qualitative impairment in social interaction, and to the third diagnostic symptom of autism, stereotyped and repetitive patterns of behavior with restricted interests

	C57BL/6J	C57BL/6J with CC lesion	LP/J	BTBR T+tf/J
Corpus callosum	Present	Completely or partially disconnected	Present	Congenitally absent
Juvenile play behaviors	High	High	Low	Low
Adult social approach	High	High	High	Low
Repetitive self-grooming	Low	Low	High	High
Open field exploratory behaviors	Medium	Moderately elevated	Low	High
Elevated plus-maze anxiety-like behaviors	Normal	Anxiolytic-like	Anxiety-like	Normal
Light↔dark exploration anxiety-like behaviors	Normal	Normal	Anxiety-like	Normal
Motor coordination	Good	Good	Poor	Poor

Exploratory locomotion and motor coordination are summarized as procedural controls for the social tasks.

1997; Rougier *et al.*, 1997; Devinsky & Laff, 2003). These behavioral differences between callostomy patients and AgCC children vs. autistic individuals support our interpretation that CC disconnection is not the causal factor for autism-like behavioral symptoms in mice.

Although CC-lesioned B6 mice did not resemble BTBR mice in social and repetitive behaviors, they showed phenotypes similar to those of BTBR mice in two non-social tests, namely the EPM anxiety test and the open field exploration test. Both CC-lesioned and BTBR mice displayed high exploration and low anxiety-like scores on some components of these tasks. It is thus instructive to compare the elevated open field exploratory activity and anxiolytic-like scores on the EPM test in intact B6 mice, in CC-lesioned mice, and in the acallosal BTBR strain. One interpretation of the similarities between BTBR and CC-lesioned B6 mice in these tests is that different genes mediate exploratory, anxiety-related and social traits. It is also possible that the CC plays a role in regulating exploratory and anxiety-like traits that are dissociable from sociability. A third interpretation of the anxiolytic-like profile of CC-lesioned mice is that CC-lesioned pups received additional maternal care when returned to the nest, for example extra licking and grooming by their dams, an environmental factor that reduces anxiety-like scores in rodents (Caldji *et al.*, 1998; Meaney, 2001). Furthermore, the required checking of post-lesion mice by the investigators and animal care-takers in our vivarium involved extra handling, a factor that could reduce anxiety-like scores (Vallée *et al.*, 1997).

It is important to recognize that experimental lesions at P7 do not replicate congenital CC agenesis, which begins at an embryonic stage. Therefore, the present neonatal CC lesion is not expected to completely recapitulate the phenotype of the acallosal BTBR strain, in which the CC is absent from the earliest stages of brain development, and which may incorporate other intrahemispheric and long pathway connectivity abnormalities that have not yet been discovered. Furthermore, unidentified genes in the B6 background could conceivably protect B6 mice from the behavioral consequences of CC lesion at P7, thus complicating a direct comparison of behavioral phenotypes in BTBR and CC-lesioned B6 mice.

Another explanation, which will be important for future research, is the possibility that the lack of CC and partial reduction in hippocampal commissure size in BTBR mice (Wahlsten *et al.*, 2003) may represent more pervasive abnormalities in commissural development, white matter pathways, and connectivity. Preliminary examination of the brains of BTBR mice revealed unusual characteristics in several structures other than the CC, for example Probst bundles. It will be interesting to pursue in-depth imaging of white matter pathways in BTBR mice, to determine the scope of connectivity dysfunctions in this mouse strain. Human imaging studies of individuals with autism have found white matter structural deficits and connectivity abnormalities (Castelli *et al.*, 2002; Casanova, 2004; Chung *et al.*, 2004; Just *et al.*, 2004, 2007; Waiter *et al.*, 2005; Boger-Megiddo *et al.*, 2006; Cherkassky *et al.*, 2006; Geschwind & Levitt, 2007; Kana *et al.*, 2007; Keller *et al.*, 2007; Koshino *et al.*, 2008; Sundaram *et al.*, 2008), offering a compelling hypothesis for the cause of autism and other neurodevelopmental disorders. Structural and functional imaging of the BTBR mouse brain across developmental ages will be needed to fully understand the neuroanatomical irregularities in fiber tracts that may underlie its autistic-like behavioral abnormalities.

The LP/J strain is the closest genetic relative to the BTBR strain (Witmer *et al.*, 2003; Petkov *et al.*, 2004). The LP/J strain offers an interesting comparison to the BTBR strain, because the CC is intact in LP/J mice and absent in BTBR mice. Results from the present experiments revealed that LP/J mice are similar to BTBR mice on some traits but differ on others (Table 3). At early juvenile ages, LP/J mice displayed less reciprocal social interactions than B6 mice, consistent with the BTBR phenotype. At adult ages, LP/J mice displayed high levels of repetitive self-grooming, consistent with the BTBR phenotype. In contrast, adult sociability was significant in LP/J and B6 mice but not in BTBR mice. One interpretation is that social deficits are present at young ages in LP/J mice, but are resolved over the course of development by adult ages, whereas repetitive behaviors persist into adult ages. Another interpretation is that unusual phenotypes in other aspects of LP/J behaviors confound the direct comparisons with BTBR mice. Some of the LP/J mice displayed unusual behaviors not seen in BTBR mice, including sporadic vertical jumping during the juvenile play test, locomotor inactivity in the open field test, and anxiety-like traits.

Low exploratory activity, high anxiety-like scores and high adult sociability in LP/J mice are opposite in direction to the BTBR and CC-lesioned phenotypes. However, physical differences in LP/J mice could be the source of anxiety-related behavioral traits, including their smaller body size (Mouse Phenome Database, http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home) and lower rate of reproduction. In our breeding facility, typical litter sizes were 1–4 pups/litter for LP/J mice and 8–12 pups/litter for BTBR mice (unpublished observations). As the strains were tested as groups, rather than identified as individuals, it was not possible to conduct longitudinal comparisons to determine whether the same individuals displayed low open field activity and low entries during adult social approach, and/or were the same individuals with unusual scores on other tasks and physical measures. Comparisons across tests for LP/J individuals will be useful for future studies.

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Although the BTBR and LP/J strains are immediately adjacent on the mouse family tree (Witmer *et al.*, 2003; Petkov *et al.*, 2004), there is still a substantial percentage (~24%) of single-nucleotide polymorphism differences between these two strains (P. M. Petkov, The Jackson Laboratory, personal communication). In addition, the proportion of microsatellite marker alleles that are identical between the BTBR and LP/J strains is 54.7%, indicating substantial genetic differences (Wahlsten *et al.*, 2003). Separate alleles may regulate functions relevant to the CC and other white matter pathways, and relevant to the adult social behaviors, fertility, locomotor activity and anxiety-like behaviors that were found to differ between LP/J and BTBR mice in the present study. Thus, genes that regulate brain development, connectivity, social behaviors, locomotion, anxiety-related traits and reproductive functions could interact with genes responsible for CC development, complicating the unambiguous interpretation of the cause of behavioral similarities and differences between LP/J and BTBR mice.

CC reductions in cross-sectional area have been reported by Wahlsten *et al.* (2003) for several other inbred strains of mice, including 129S1/SvImJ, A/J, BALB/c, C3H/HeJ, NZB/B1NJ, SJL/J, and SWR/J. BALB/cJ mice displayed low sociability at age 30 days (Brodkin, 2007; Fairless *et al.*, 2008), interacted at low levels with former cage mates at 4–6 days post-weaning (Panksepp *et al.*, 2007), and showed low scores on socially conditioned place preference (Panksepp & Lahvis, 2007). BALB/cBy/J males at age 6–7 weeks displayed low levels of social approach (Moy *et al.*, 2007). Similarly, 129S1/SvImJ and A/J mice showed low levels of sociability in several tasks (Brodkin *et al.*, 2004; Bolivar *et al.*, 2007; Moy *et al.*, 2007). However, it must be noted that concomitant low levels of exploratory activity and comparatively high levels of anxiety-like traits have been extensively described for BALB, A/J and 129S1/SvImJ mice (Mathis *et al.*, 1995; Griebel *et al.*, 2000; van Gaalen & Steckler, 2000; Bouwknecht & Paylor, 2002; Cook *et al.*, 2002; Moy *et al.*, 2007), raising potential artifactual confounds for interpreting social approach and social interaction deficits in these strains. Furthermore, some strains with modest CC sizes, including C3H and SWR/J, showed high social approach scores, whereas NOD/LtJ, a strain with a large CC, exhibited low sociability (Wahlsten *et al.*, 2003; Moy *et al.*, 2007, 2008), indicating that reduced CC size is unlikely to be a definitive predictor of social scores in mice. Interactions of multiple genes and other biological, neuroanatomical and behavioral factors are likely to contribute to social traits in mice and other species.

No major sex differences were detected on social scores and repetitive behaviors in B6, BTBR and LP/J mice. BTBR females showed low scores on nose-to-nose sniffing and following during juvenile play, lack of adult sociability in the three-chambered task on chamber time and sniffing time, and high levels of self-grooming, similar to BTBR males. Both sexes of LP/J mice showed low scores on all measures of juvenile play, and exhibited high levels of repetitive grooming. The general lack of sex differences in BTBR and LP/J mice suggests that X-linked genes and sexually dimorphic factors are unlikely to be primary substrates for the social deficits and repetitive behaviors in these two strains. Other hypotheses that have recently been ruled out include the effects of early maternal care (Yang *et al.*, 2007b) and circadian factors (Yang *et al.*, 2007a) on the BTBR behavioral phenotype.

In conclusion, our findings suggest that early postnatal surgical disconnection of the CC fiber tract in B6 mice does not result in the social deficits or repetitive self-grooming that characterize BTBR mice, which have congenital AgCC. In addition, LP/J, the inbred strain that shares the most genes with the BTBR strain but has an intact CC, shares some autism-relevant behavioral traits with the BTBR strain, including juvenile social deficits and repetitive self-grooming. Juvenile reciprocal social interactions, adult social approach, general exploratory activity, anxiety-related behaviors and CC integrity appear to represent discrete traits with separable genetic determinants. Taken together, these several findings suggest that genes regulating other aspects of connectivity and brain development, rather than those regulating CC development specifically, are more likely to be responsible for the social and repetitive abnormalities in BTBR mice. Unusual single-nucleotide polymorphisms in BTBR mice (McFarlane *et al.*, 2008) and genetic loci on the X chromosome linked to CC absence in BTBR mice (Kusek *et al.*, 2007) have been reported. It will be important to conduct a comprehensive investigation of unusual alleles, epigenetic factors and copy number variants in the BTBR background, to increase our understanding of the genetic basis of autism-like phenotypes in the BTBR mouse model of autism.

Acknowledgements

We thank Professor Douglas Wahlsten for his excellent suggestion to compare the LP/J and BTBR T+tf/J strains, Dr Petko Petkov for information on the LP/J genetic background, and Drs Elliorr Sherr, Lynn Paul and Ralph Adolphs for helpful discussion of the human acallosal syndrome. Mr Timothy Sullivan provided assistance with histological analysis. Ms Janet Stephens, NIH Medical Arts, contributed macrophotography expertise for Fig. 1. This work was supported by the National Institute of Mental Health Intramural Research Program.

Abbreviations

AgCC, agenesis of the corpus callosum; B6, C57BL/6J; BTBR, BTBR T+tf/J; CC, corpus callosum; EPM, elevated plus-maze; NS, not significant; P, postnatal day.

References

Abrahams, B.S. & Geschwind, D.H. (2008) Advances in autism genetics: on the threshold of a new neurobiology. *Nat. Rev. Genet.*, **9**, 341–355.

Alexander, A.L., Lee, J.E., Lazar, M., Boudos, R., DuBray, M.B., Oakes, T.R., Miller, J.N., Lu, J., Jeong, E.K., McMahon, W.M., Bigler, E.D. & Lainhart, J.E. (2007) Diffusion tensor imaging of the corpus callosum in autism. *Neuroimage*, **34**, 61–73.

Badaruddin, D.H., Andrews, G.L., Bolte, S., Schilmoeller, K.J., Schilmoeller, G., Paul, L.K. & Brown, W.S. (2007) Social and behavioral problems of children with agenesis of the corpus callosum. *Child Psychiatry Hum. Dev.*, **38**, 287–302.

Bailey, K.R., Pavlova, M.N., Rohde, A.D., Hohmann, J.G. & Crawley, J.N. (2007) Galanin receptor subtype 2 (GalR2) null mutant mice display an anxiogenic-like phenotype specific to the elevated plus-maze. *Pharmacol. Biochem. Behav.*, **86**, 8–20.

Barnea-Goraly, N., Kwon, H., Menon, V., Eliez, S., Lotspeich, L. & Reiss, A.L. (2004) White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biol. Psychiatry*, **55**, 323–326.

Boger-Megiddo, I., Shaw, D.W., Friedman, S.D., Sparks, B.F., Artru, A.A., Giedd, J.N., Dawson, G. & Dager, S.R. (2006) Corpus callosum morphometrics in young children with autism spectrum disorder. *J. Autism Dev. Disord.*, **36**, 733–739.

Bolivar, V.J., Walters, S.R. & Phoenix, J.L. (2007) Assessing autism-like behavior in mice: variations in social interactions among inbred strains. *Behav. Brain Res.*, **176**, 21–26.

Bonilha, L., Cendes, F., Rorden, C., Eckert, M., Dalgalarrondo, P., Li, L.M. & Steiner, C.E. (2008) Gray and white matter imbalance – typical structural abnormality underlying classic autism? *Brain Dev.*, **30**, 396–401.

Bouwknecht, J.A. & Paylor, R. (2002) Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. *Behav. Brain Res.*, **136**, 489–501.

Brambilla, P., Hardan, A., di Nemi, S.U., Perez, J., Soares, J.C. & Barale, F. (2003) Brain anatomy and development in autism: review of structural MRI studies. *Brain Res. Bull.*, **61**, 557–569.

© The Authors (2009). Journal Compilation © Federation of European Neuroscience Societies and Blackwell Publishing Ltd

Brodkin, E.S. (2007) BALB/c mice: low sociability and other phenotypes that may be relevant to autism. *Behav. Brain Res.*, **176**, 53–65.

Brodkin, E.S., Hagemann, A., Nemetski, S.M. & Silver, L.M. (2004) Social approach-avoidance behavior of inbred mouse strains towards DBA/2 mice. *Brain Res.*, **1002**, 151–157.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P.M. & Meaney, M.J. (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc. Natl. Acad.. Sci.. U.S.A.*, **95**, 5335–5340.

Casanova, M.F. (2004) White matter volume increase and minicolumns in autism. *Ann. Neurol.*, **56**, 453. author reply 454.

Castelli, F., Frith, C., Happe, F. & Frith, U. (2002) Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. *Brain*, **125**, 1839–1849.

Chadman, K.K., Gong, S., Scattoni, M.L., Boltuck, S.E., Gandhy, S.U., Heintz, N. & Crawley, J.N. (2008) Minimal aberrant behavioral phenotypes of neuoligin-3 R451C knockin mice. *Autism Res.*, **1**, 147–158.

Cheh, M.A., Millonig, J.H., Roselli, L.M., Ming, X., Jacobsen, E., Kamdar, S. & Wagner, G.C. (2006) En2 knockout mice display neurobehavioral and neurochemical alterations relevant to autism spectrum disorder. *Brain Res.*, **1116**, 166–176.

Cherkassky, V.L., Kana, R.K., Keller, T.A. & Just, M.A. (2006) Functional connectivity in a baseline resting-state network in autism. *Neuroreport*, **17**, 1687–1690.

Chung, M.K., Dalton, K.M., Alexander, A.L. & Davidson, R.J. (2004) Less white matter concentration in autism: 2D voxel-based morphometry. *Neuroimage*, **23**, 242–251.

Cook, M.N., Bolivar, V.J., McFadyen, M.P. & Flaherty, L. (2002) Behavioral differences among 129 substrains: implications for knockout and transgenic mice. *Behav. Neurosci.*, **116**, 600–611.

Crawley, J. & Goodwin, F.K. (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol. Biochem. Behav.*, **13**, 167–170.

Crawley, J.N., Chen, T., Puri, A., Washburn, R., Sullivan, T.L., Hill, J.M., Young, N.B., Nadler, J.J., Moy, S.S., Young, L.J., Caldwell, H.K. & Young, W.S. (2007) Social approach behaviors in oxytocin knockout mice: comparison of two independent lines tested in different laboratory environments. *Neuropeptides*, **41**, 145–163.

Devinsky, O. & Laff, R. (2003) Callosal lesions and behavior: history and modern concepts. *Epilepsy Behav.*, **4**, 607–617.

DiCicco-Bloom, E., Lord, C., Zwaigenbaum, L., Courchesne, E., Dager, S.R., Schmitz, C., Schultz, R.T., Crawley, J. & Young, L.J. (2006) The developmental neurobiology of autism spectrum disorder. *J. Neurosci.*, **26**, 6897–6906.

Egaas, B., Courchesne, E. & Saitoh, O. (1995) Reduced size of corpus callosum in autism. *Arch. Neurol.*, **52**, 794–801.

Ehninger, D., Han, S., Shilyansky, C., Zhou, Y., Li, W., Kwiatkowski, D.J., Ramesh, V. & Silva, A.J. (2008) Reversal of learning deficits in a Tsc2+/− mouse model of tuberous sclerosis. *Nat. Med.*, **14**, 843–848.

Fairless, A.H., Dow, H.C., Toledo, M.M., Malkus, K.A., Edelmann, M., Li, H., Talbot, K., Arnold, S.E., Abel, T. & Brodkin, E.S. (2008) Low sociability is associated with reduced size of the corpus callosum in the BALB/cJ inbred mouse strain. *Brain Res.*, **1230**, 211–217.

Franklin, K.B.J. & Paxinos, G. (1997) *The Mouse Brain: In Stereotaxic Coordinates*. Academic Press, San Diego, CA.

van Gaalen, M.M. & Steckler, T. (2000) Behavioural analysis of four mouse strains in an anxiety test battery. *Behav. Brain. Res.*, **115**, 95–106.

Gazzaniga, M.S., Risse, G.L., Springer, S.P., Clark, D.E. & Wilson, D.H. (1975) Psychologic and neurologic consequences of partial and complete cerebral commissurotomy. *Neurology*, **25**, 10–15.

Geschwind, D.H. & Levitt, P. (2007) Autism spectrum disorders: developmental disconnection syndromes. *Curr. Opin. Neurobiol.*, **17**, 103–111.

Grice, D.E. & Buxbaum, J.D. (2006) The genetics of autism spectrum disorders. *Neuromol. Med.*, **8**, 451–460.

Griebel, G., Belzung, C., Perrault, G. & Sanger, D.J. (2000) Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology*, **148**, 164–170.

Hardan, A.Y., Minshew, N.J. & Keshavan, M.S. (2000) Corpus callosum size in autism. *Neurology*, **55**, 1033–1036.

Herbert, M.R., Russo, J.P., Yang, S., Roohi, J., Blaxill, M., Kahler, S.G., Cremer, L. & Hatchwell, E. (2006) Autism and environmental genomics. *Neurotoxicology*, **27**, 671–684.

Holmes, A., Hollon, T.R., Gleason, T.C., Liu, Z., Dreiling, J., Sibley, D.R. & Crawley, J.N. (2001) Behavioral characterization of dopamine D5 receptor null mutant mice. *Behav. Neurosci.*, **115**, 1129–1144.

Hung, A.Y., Futai, K., Sala, C., Valtschanoff, J.G., Ryu, J., Woodworth, M.A., Kidd, F.L., Sung, C.C., Miyakawa, T., Bear, M.F., Weinberg, R.J. & Sheng, M. (2008) Smaller dendritic spines, weaker synaptic transmission, but enhanced spatial learning in mice lacking Shank1. *J. Neurosci.*, **28**, 1697–1708.

Jamain, S., Radyushkin, K., Hammerschmidt, K., Granon, S., Boretius, S., Varoqueaux, F., Ramanantsoa, N., Gallego, J., Ronnenberg, A., Winter, D., Frahm, J., Fischer, J., Bourgeron, T., Ehrenreich, H. & Brose, N. (2008) Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 1710–1715.

Just, M.A., Cherkassky, V.L., Keller, T.A. & Minshew, N.J. (2004) Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain*, **127**, 1811–1821.

Just, M.A., Cherkassky, V.L., Keller, T.A., Kana, R.K. & Minshew, N.J. (2007) Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. *Cereb. Cortex*, **17**, 951–961.

Kana, R.K., Keller, T.A., Minshew, N.J. & Just, M.A. (2007) Inhibitory control in high-functioning autism: decreased activation and underconnectivity in inhibition networks. *Biol. Psychiatry*, **62**, 198–206.

Karlsson, R.M., Choe, J.S., Cameron, H.A., Thorsell, A., Crawley, J.N., Holmes, A. & Heilig, M. (2008) The neuropeptide Y Y1 receptor subtype is necessary for the anxiolytic-like effects of neuropeptide Y, but not the antidepressant-like effects of fluoxetine, in mice. *Psychopharmacology (Berl)*, **195**, 547–557.

Keller, T.A., Kana, R.K. & Just, M.A. (2007) A developmental study of the structural integrity of white matter in autism. *Neuroreport*, **18**, 23–27.

Kennedy, D.P. & Courchesne, E. (2008) The intrinsic functional organization of the brain is altered in autism. *Neuroimage*, **39**, 1877–1885.

Kilian, S., Brown, W.S., Hallam, B.J., McMahon, W., Lu, J., Johnson, M., Bigler, E.D. & Lainhart, J. (2008) Regional callosal morphology in autism and macrocephaly. *Dev. Neuropsychol.*, **33**, 74–99.

Kleinhans, N.M., Richards, T., Sterling, L., Stegbauer, K.C., Mahurin, R., Johnson, L.C., Greenson, J., Dawson, G. & Aylward, E. (2008) Abnormal functional connectivity in autism spectrum disorders during face processing. *Brain*, **131**, 1000–1012.

Koshino, H., Kana, R.K., Keller, T.A., Cherkassky, V.L., Minshew, N.J. & Just, M.A. (2008) fMRI investigation of working memory for faces in autism: visual coding and underconnectivity with frontal areas. *Cereb. Cortex*, **18**, 289–300.

Kusek, G.K., Wahlsten, D., Herron, B.J., Bolivar, V.J. & Flaherty, L. (2007) Localization of two new X-linked quantitative trait loci controlling corpus callosum size in the mouse. *Genes Brain Behav.*, **6**, 359–363.

Kwon, C.H., Luikart, B.W., Powell, C.M., Zhou, J., Matheny, S.A., Zhang, W., Li, Y., Baker, S.J. & Parada, L.F. (2006) Pten regulates neuronal arborization and social interaction in mice. *Neuron*, **50**, 377–388.

Lassonde, M. & Sauerwein, C. (1997) Neuropsychological outcome of corpus callosotomy in children and adolescents. *J. Neurosurg. Sci.*, **41**, 67–73.

Long, J.M., LaPorte, P., Paylor, R. & Wynshaw-Boris, A. (2004) Expanded characterization of the social interaction abnormalities in mice lacking Dvl1. *Genes Brain Behav.*, **3**, 51–62.

Lord, C., Risi, S., DiLavore, P.S., Shulman, C., Thurm, A. & Pickles, A. (2006) Autism from 2 to 9 years of age. *Arch. Gen. Psychiatry*, **63**, 694–701.

MacPherson, P., McGaffigan, R., Wahlsten, D. & Nguyen, P.V. (2008) Impaired fear memory, altered object memory and modified hippocampal synaptic plasticity in split-brain mice. *Brain Res.*, **1210**, 179–188.

Mamelak, A.N., Barbaro, N.M., Walker, J.A. & Laxer, K.D. (1993) Corpus callosotomy: a quantitative study of the extent of resection, seizure control, and neuropsychological outcome. *J. Neurosurg.*, **79**, 688–695.

Manes, F., Piven, J., Vrancic, D., Nanclares, V., Plebst, C. & Starkstein, S.E. (1999) An MRI study of the corpus callosum and cerebellum in mentally retarded autistic individuals. *J. Neuropsychiatry Clin. Neurosci.*, **11**, 470–474.

Manhaes, A.C., Abreu-Villaca, Y., Schmidt, S.L. & Filgueiras, C.C. (2007) Neonatal transection of the corpus callosum affects rotational side preference in adult Swiss mice. *Neurosci. Lett.*, **415**, 159–163.

Mathis, C., Neumann, P.E., Gershenfeld, H., Paul, S.M. & Crawley, J.N. (1995) Genetic analysis of anxiety-related behaviors and responses to benzodiazepine-related drugs in AXB and BXA recombinant inbred mouse strains. *Behav. Genet.*, **25**, 557–568.

McFarlane, H.G., Kusek, G.K., Yang, M., Phoenix, J.L., Bolivar, V.J. & Crawley, J.N. (2008) Autism-like behavioral phenotypes in BTBR T+tf/J mice. *Genes Brain Behav.*, **7**, 152–163.

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Meaney, M.J. (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu. Rev. Neurosci.*, **24**, 1161–1192.

Mineur, Y.S., Huynh, L.X. & Crusio, W.E. (2006) Social behavior deficits in the Fmr1 mutant mouse. *Behav. Brain Res.*, **168**, 172–175.

Minshew, N.J. & Williams, D.L. (2007) The new neurobiology of autism: cortex, connectivity, and neuronal organization. *Arch. Neurol.*, **64**, 945–950.

Moy, S.S., Nadler, J.J., Perez, A., Barbaro, R.P., Johns, J.M., Magnuson, T.R., Piven, J. & Crawley, J.N. (2004) Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes Brain Behav.*, **3**, 287–302.

Moy, S.S., Nadler, J.J., Young, N.B., Perez, A., Holloway, L.P., Barbaro, R.P., Barbaro, J.R., Wilson, L.M., Threadgill, D.W., Lauder, J.M., Magnuson, T.R. & Crawley, J.N. (2007) Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. *Behav. Brain Res.*, **176**, 4–20.

Moy, S.S., Nadler, J.J., Young, N.B., Nonneman, R.J., Segall, S.K., Andrade, G.M., Crawley, J.N. & Magnuson, T.R. (2008) Social approach and repetitive behavior in eleven inbred mouse strains. *Behav. Brain Res.*, **191**, 118–129.

Nadler, J.J., Moy, S.S., Dold, G., Trang, D., Simmons, N., Perez, A., Young, N.B., Barbaro, R.P., Piven, J., Magnuson, T.R. & Crawley, J.N. (2004) Automated apparatus for quantitation of social approach behaviors in mice. *Genes Brain Behav.*, **3**, 303–314.

Newschaffer, C.J., Croen, L.A., Daniels, J., Giarelli, E., Grether, J.K., Levy, S.E., Mandell, D.S., Miller, L.A. & Pinto-Martin, J. (2007) The epidemiology of autism spectrum disorders. *Annu. Rev. Public Health*, **28**, 235–258.

Panksepp, J.B. & Lahvis, G.P. (2007) Social reward among juvenile mice. *Genes Brain Behav.*, **6**, 661–671.

Panksepp, J.B., Jochman, K.A., Kim, J.U., Koy, J.J., Wilson, E.D., Chen, Q., Wilson, C.R. & Lahvis, G.P. (2007) Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. *PLoS ONE*, **2**, e351.

Paul, L.K., Lautzenhiser, A., Brown, W.S., Hart, A., Neumann, D., Spezio, M. & Adolphs, R. (2006) Emotional arousal in agenesis of the corpus callosum. *Int. J. Psychophysiol.*, **61**, 47–56.

Paul, L.K., Brown, W.S., Adolphs, R., Tyszka, J.M., Richards, L.J., Mukherjee, P. & Sherr, E.H. (2007) Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity. *Nat. Rev. Neurosci.*, **8**, 287–299.

Paxinos, G., Halliday, G.M., Watson, C., Koutcherov, Y. & Wang, H. (2006) *Atlas of the Developing Mouse Brain at E17.5, P0 and P6*. Academic Press, Burlington, VT.

Petkov, P.M., Ding, Y., Cassell, M.A., Zhang, W., Wagner, G., Sargent, E.E., Asquith, S., Crew, V., Johnson, K.A., Robinson, P., Scott, V.E. & Wiles, M.V. (2004) An efficient SNP system for mouse genome scanning and elucidating strain relationships. *Genome Res.*, **14**, 1806–1811.

Piven, J., Bailey, J., Ranson, B.J. & Arndt, S. (1997) An MRI study of the corpus callosum in autism. *Am. J. Psychiatry*, **154**, 1051–1056.

Ricceri, L., Moles, A. & Crawley, J. (2007) Behavioral phenotyping of mouse models of neurodevelopmental disorders: relevant social behavior patterns across the life span. *Behav. Brain Res.*, **176**, 40–52.

Rippon, G., Brock, J., Brown, C. & Boucher, J. (2007) Disordered connectivity in the autistic brain: challenges for the 'new psychophysiology'. *Int. J. Psychophysiol.*, **63**, 164–172.

Rougier, A., Claverie, B., Pedespan, J.M., Marchal, C. & Loiseau, P. (1997) Callosotomy for intractable epilepsy: overall outcome. *J. Neurosurg. Sci.*, **41**, 51–57.

Sass, K.J., Spencer, D.D., Spencer, S.S., Novelly, R.A., Williamson, P.D. & Mattson, R.H. (1988) Corpus callosotomy for epilepsy. II. Neurologic and neuropsychological outcome. *Neurology*, **38**, 24–28.

Scattoni, M.L., Gandhy, S.U., Ricceri, L. & Crawley, J.N. (2008) Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. *PLoS ONE*, **3**, e3067.

Scearce-Levie, K., Roberson, E.D., Gerstein, H., Cholfin, J.A., Mandiyan, V.S., Shah, N.M., Rubenstein, J.L. & Mucke, L. (2008) Abnormal social behaviors in mice lacking Fgf17. *Genes Brain Behav.*, **7**, 344–354.

Sundaram, S.K., Kumar, A., Makki, M.I., Behen, M.E., Chugani, H.T. & Chugani, D.C. (2008) Diffusion tensor imaging of frontal lobe in autism spectrum disorder. *Cereb. Cortex*, **18**, 2659–2665.

Tabuchi, K., Blundell, J., Etherton, M.R., Hammer, R.E., Liu, X., Powell, C.M. & Sudhof, T.C. (2007) A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science*, **318**, 71–76.

Takayanagi, Y., Yoshida, M., Bielsky, I.F., Ross, H.E., Kawamata, M., Onaka, T., Yanagisawa, T., Kimura, T., Matzuk, M.M., Young, L.J. & Nishimori, K. (2005) Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 16096–16101.

Terranova, M.L. & Laviola, G. (2005) Scoring of social interactions and play in mice during adolescence. *Curr. Protoc. Toxicol.*, **13**, 10.1–10.11.

Vallée, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H. & Maccari, S. (1997) Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. *J. Neurosci.*, **17**, 2626–2636.

Veenstra-Vanderweele, J., Christian, S.L. & Cook, E.H. Jr (2004) Autism as a paradigmatic complex genetic disorder. *Annu. Rev. Genomics Hum. Genet.*, **5**, 379–405.

Vidal, C.N., Nicolson, R., DeVito, T.J., Hayashi, K.M., Geaga, J.A., Drost, D.J., Williamson, P.C., Rajakumar, N., Sui, Y., Dutton, R.A., Toga, A.W. & Thompson, P.M. (2006) Mapping corpus callosum deficits in autism: an index of aberrant cortical connectivity. *Biol. Psychiatry*, **60**, 218–225.

Volkmar, F., Chawarska, K. & Klin, A. (2005) Autism in infancy and early childhood. *Annu. Rev. Psychol.*, **56**, 315–336.

Wahlsten, D., Metten, P. & Crabbe, J.C. (2003) Survey of 21 inbred mouse strains in two laboratories reveals that BTBR T/+ tf/tf has severely reduced hippocampal commissure and absent corpus callosum. *Brain Res.*, **971**, 47–54.

Waiter, G.D., Williams, J.H., Murray, A.D., Gilchrist, A., Perrett, D.I. & Whiten, A. (2005) Structural white matter deficits in high-functioning individuals with autistic spectrum disorder: a voxel-based investigation. *Neuroimage*, **24**, 455–461.

Wilson, D.H., Reeves, A. & Gazzaniga, M. (1978) Division of the corpus callosum for uncontrollable epilepsy. *Neurology*, **28**, 649–653.

Winslow, J.T. & Insel, T.R. (2002) The social deficits of the oxytocin knockout mouse. *Neuropeptides*, **36**, 221–229.

Witmer, P.D., Doheny, K.F., Adams, M.K., Boehm, C.D., Dizon, J.S., Goldstein, J.L., Templeton, T.M., Wheaton, A.M., Dong, P.N., Pugh, E.W., Nussbaum, R.L., Hunter, K., Kelmenson, J.A., Rowe, L.B. & Brownstein, M.J. (2003) The development of a highly informative mouse Simple Sequence Length Polymorphism (SSLP) marker set and construction of a mouse family tree using parsimony analysis. *Genome Res.*, **13**, 485–491.

Yang, M., Scattoni, M.L., Zhodzishsky, V., Chen, T., Caldwell, H.K., Young, W.S., McFarlane, H.G. & Crawley, J.N. (2007a) Similar social approach behaviors in BTBR T+ tf/J, C57BL/6J, and vasopressin receptor 1B knockout mice tested on conventional versus reverse light cycles, and in replications across cohorts. *Front. Behav. Neurosci.*, **1**, 1–9.

Yang, M., Zhodzishsky, V. & Crawley, J.N. (2007b) Social deficits in BTBR T+tf/J mice are unchanged by cross-fostering with C57BL/6J mothers. *Int. J. Dev. Neurosci.*, **25**, 515–521.

Young, L.J. (2001) Oxytocin and vasopressin as candidate genes for psychiatric disorders: lessons from animal models. *Am. J. Med. Genet.*, **105**, 53–54.

Young, L.J., Pitkow, L.J. & Ferguson, J.N. (2002) Neuropeptides and social behavior: animal models relevant to autism. *Mol. Psychiatry*, **7**(Suppl. 2), S38–S39.

Zagreda, L., Goodman, J., Druin, D.P., McDonald, D. & Diamond, A. (1999) Cognitive deficits in a genetic mouse model of the most common biochemical cause of human mental retardation. *J. Neurosci.*, **19**, 6175–6182.

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