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Fluoxetine but not risperidone increases sociability in the BTBR mouse model of autism

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ABSTRACT

Autism, a neurodevelopmental disorder, is characterized by abnormal social interactions, impaired social communication and repetitive behaviors and/or restricted interests, along with several associated symptoms including irritability and anxiety. Risperidone is approved for the irritability and self-injurious behaviors found in autism. Fluoxetine is under evaluation for the repetitive behaviors and anxiety associated with autism. These two drugs were evaluated in the BTBR T + *tf*/J (BTBR) mouse model of autism and C57BL/6J (B6) mice by using the three-chambered social approach test and elevated plus maze to determine effects on sociability and anxiety. Fluoxetine increased sociability, defined as time spent with a stranger mouse, in the BTBR mice without affecting anxiety-like behavior in the elevated plus maze. Fluoxetine did not significantly change either behavior in the B6 mice. Risperidone did not affect sociability or anxiety-like behaviors and had a sedative-like effect at higher doses. These findings suggest that fluoxetine may have some therapeutic efficacy for treating the social behaviors in autism.

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1. Introduction

In the absence of consistent, certain biomarkers, diagnosis of autism is based on well-defined core behavioral symptoms: abnormal social interactions and social communication, and repetitive behaviors and/or restricted interests. Many drugs, including fluoxetine and risperidone, have been used to treat symptoms associated with autism. Risperidone, an atypical antipsychotic that blocks D_2 and $5HT_{2A}$ receptors, has been approved by the United States Food and Drug Administration (FDA) to reduce the repetitive behavior and self-injurious behavior in children with autism. Fluoxetine, a selective serotonin-reuptake inhibitor (SSRI), is being evaluated by the FDA for anxiety and repetitive behaviors in individuals with autism. Serotonin dysregulation is one theory of the etiology of autism (reviewed by Pardo and Eberhart, 2007) and has been linked with comorbid behaviors associated with autism such as depression, anxiety, mood, impulsivity and aggression (reviewed by Soorya et al., 2008; West et al., 2009). Both risperidone and fluoxetine act in the serotonin system. Risperidone antagonizes the serotonin 2A receptor, and fluoxetine blocks the serotonin transporter, increasing the amount of serotonin available in the synapse. Pharmacological manipulation of the serotonin system may positively affect the core symptoms found in autism.

Animal models are a useful tool in the search for pharmacological treatment for the core symptoms of autism. One approach is to select inbred strains of mice that demonstrate behavioral characteristics that have face validity for autism. The social approach test has been developed to identify deficits in social interaction, whereby a subject mouse has the choice between a social and a non-social environment (Moy et al., 2004, 2007, 2009; Nadler et al., 2004; Yang et al., 2007a,b; McFarlane et al., 2008; Scattoni et al., 2008a; Chadman et al., 2008). Inbred strains of mice differ in their levels of sociability, and several strains have reduced social interactions (Moy et al., 2004, 2007). The BTBR T + *tf*J (BTBR) mice have demonstrated low levels of sociability compared to the C57BL/6J (B6) mice (Bolivar et al., 2007; Moy et al., 2007; McFarlane et al., 2008; Silverman et al., 2009). The BTBR mice exhibit an unusual pattern of ultrasonic vocalizations during development (Scattoni et al., 2008b), and adult males display fewer ultrasonic vocalizations in response to female urine compared to B6 mice (Wohr et al., 2010) that may be homologous to the communication deficits observed in autism.

BTBR males also display lower scent marking in a social setting than B6 males (Wohr et al., 2010). Additionally, BTBR mice exhibit high levels of self-grooming (Yang et al., 2007a,b; McFarlane et al., 2008) that may represent the repetitive behaviors found in autism. Therefore, the BTBR strain of mice model several behavioral symptoms of autism and can be used to test putative treatments for the disorder. Moreover, their neuroanatomy and neurobiology favor them as an autism model. A common finding in autistic brains is underdevelopment of the corpus callosum, and the BTBR mice have almost complete agenesis of the corpus callosum (Wahlsten et al., 2003a; Kusek et al., 2007). Also, the BTBR mice have higher circulating levels of corticosterone, progesterone and its metabolite, 5α-pregnan-3α-ol-20-one, which also functions as a

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neurosteroid, suggesting neuroendocrine dysregulation in the BTBR mice (Frye and Llaneza, 2010). The current study addressed the effects of these two drugs on social and anxiety-like behaviors in the BTBR mouse model of autism, with B6 mice as a control.

To gain an indication of the specificity of the pharmacological effect on social behavior requires measurement of drug effects on other behavior. We chose anxiety-like behavior since anxiety is commonly associated with autism (Skokauskas and Gallagher, 2009), although it is not part of the core symptoms. Inbred strains of mice display different levels of anxiety-like behavior as measured using the elevated plus maze (Mineur and Crusio, 2002; Ducottet and Belzung, 2005; Belzung et al., 2001; Benno et al., 2009). Although neither BTBR nor C57BL/6J (B6) mice display high levels of anxiety-like behavior on the elevated plus maze (Mineur and Crusio, 2002; Ducottet and Belzung, 2005; Benno et al., 2009), fluoxetine has been shown to cause an acute anxiogenic effect in B6 mice (Liu et al., 2010) as well as other strains (Kurt et al., 2000), which could negate its effectiveness in treating the core symptoms of autism.

Mouse strains vary in their sensitivity to neuropsychiatric drugs. For example, when fluoxetine was compared in seven inbred mouse strains in the forced swimming test, it produced an antidepressant-like effect in three strains that did not include the B6 mice (Lucki et al., 2001). Several classes of drugs were compared between B6 and 129/SvJ mice, and the B6 mice were more responsive to midazolam, zolpidem and propofol but less sensitive to ethanol and etomidate than the 129/SvJ mice (Homanics et al., 1999). In BTBR and B6 mice, risperidone did not increase sociability or reduce repetitive behaviors (Silverman et al., 2009). However, the doses of risperidone used in that study also significantly lowered activity levels, potentially masking any effects on sociability. The current study used lower doses of risperidone to decrease the sedative effects. This study is the first to evaluate the effects of fluoxetine and non-sedating doses of risperidone on social behavior and anxiety-like behavior in the BTBR mouse model of autism.

Fluoxetine decreases anxiety and repetitive behaviors and risperidone reduces irritability and self-injurious behaviors found in ASD. The hypothesis for these experiments is that in addition to the effects described above, both of these drugs will increase social behavior in the BTBR mice without affecting anxiety-like behavior. Fluoxetine and risperidone are not expected to affect social or anxiety-like behavior in the control B6 mice.

2. Methods

2.1. Subjects

Male C57BL/6J (B6) (n = 60), BTBR T+*tf*/J (BTBR) (n = 60) and 129S1/SvImJ (n = 10) mice were obtained from Jackson Laboratories (Bar Harbor, ME). There were 12 mice per drug/strain/group with the same vehicle group in each strain used for both drugs as the experiments were all run intermixed and blind to drug and dose. 129S1/SvImJ mice were used as the stranger mice because they have very low levels of activity so that all interactions were initiated by the subject mice. Because autism affects a higher percentage of males than females, only male mice were used in the current study. Mice were housed 5 to a cage with ad lib food and water and 12-h light/dark cycle. Mice were between 8 and 15 weeks of age at testing. All experiments were conducted during the light phase between 10 am and 5 pm. All procedures were conducted in compliance with the NIH Guidelines for the Care and Use of Laboratory Animals and approved by the New York State Institute for Basic Research in Developmental Disabilities' Institutional Animal Care and Use Committee.

2.2. Drugs

Fluoxetine (vehicle,10 and 30 mg/kg) and risperidone (vehicle, 0.03 and 0.30 mg/kg) were administered in a 10 ml/kg volume as an intraperitoneal injection 1 h before testing for both the social approach and elevated plus mazes. Both of these drugs have demonstrated behavioral effects with a 1-h pretreatment (Bruins Slot et al., 2008), and using the same pretreatment time for both drugs allowed the experimenter to be blind to the treatment condition. The same mice were used for both social approach and elevated plus maze and were tested at least 1 month apart.

2.3. Order of testing

Subjects were run in two cohorts of 60 mice each, 30 per strain and 6 for each drug/dose. Each cohort had the same number of subjects for each strain and drug dose, with the second cohort being a replication of the first cohort with naïve mice. Both cohorts of mice were run first in the social approach test followed by the elevated plus maze.

2.4. Social approach test

This experiment has two habituation phases (center and all 3 chambers) followed by two testing phases (sociability and novelty). The first test compares the preference for a social stimulus versus an inanimate object. The second test, or social novelty phase of the test, compares the preference for a now familiar social stimulus to a novel social stimulus. Social approach behaviors were tested in an apparatus with 3 chambers in a single 40-min session, divided into 4 phases, as previously described (Moy et al., 2004; Nadler et al., 2004; Chadman et al., 2008; Silverman et al., 2009). The subject mouse was acclimated to the apparatus for 10 min in the center chamber (phase 1), and then for an additional 10 min with access to all 3 empty chambers (phase 2). The subject was then confined to the middle chamber, while the novel object (an inverted wire cup, Galaxy Cup, Kitchen Plus, Streetsboro, OH) was placed into one of the side chambers, and the stranger mouse (stranger 1), inside an identical inverted wire cup, was placed in the opposite side chamber. Male 129S1/SvImJ mice were used as the stranger mice. The location (left or right) of the novel object and stranger mouse alternated across subjects. The chamber doors were opened simultaneously, and the subject had access to all 3 chambers for 10 min (phase 3). After this, the fourth 10-min session provided a measure of preference for social novelty (phase 4). The subject mouse was gently guided to the center chamber, the doors closed, and the novel object removed, and a second novel mouse (stranger 2) was placed in the side chamber. The chamber doors were opened simultaneously, and the subject again had access to all 3 chambers for 10 min. The fourth 10-min phase provided a measure recognition and discrimination and is used to confirm olfactory abilities for detection and discrimination of social odors. Video tracking with ANYmaze (Stoelting, Inc.; Wood Dale, IL) automatically scored the time spent in each of the 3 chambers, time spent sniffing, and number of entries into each chamber during each 10-min phase of the test.

Animals used as strangers were male 129S1/SvImJ mice habituated to the testing chamber for 30-min sessions on 3 consecutive days and were enclosed in the wire cup to ensure that all social approach was initiated by the subject mouse. An upright plastic drinking cup weighed down with a lead weight was placed on top of each of the inverted wire cups to prevent the subject mouse from climbing on top. Both end chambers maintained a lighting level of 26-27 lux with 2 desk lamps angled away from the maze.

2.5. Elevated plus maze

Anxiety-like behavior was tested in the elevated plus maze as previously described (Holmes et al., 2002; Bailey et al., 2007). The elevated (95 cm) plus maze consists of 2 open arms (30 × 5 cm) and 2 closed arms (30 × 5 × 15 cm) extending from a central (5 × 5 cm) area. A raised lip (0.25 cm) around the open arms minimized falling off the edges of the open arms. Mice were placed in the central area facing an open arm and allowed to traverse the maze freely for 5 min. Arm entries (70% of mouse in the arm) and time spent in the open and closed arms

were tracked and scored using ANYmaze software (Stoelting, Inc., Wood Dale, IL). The center of the maze was lighted to 26 lux with 2 desk lamps angled away from the maze.

2.6. Statistical analysis

For the social approach task, repeated measures analysis of variance (ANOVA) using Statistica 7.1 (StatSoft, Inc., Tulsa, OK) was used to compare time spent in the chamber. However, the times spent in each of the 3 chambers were not independent; for the analysis, only times spent in the side chambers (containing the stranger mouse and novel object) were compared. Time spent in the center chamber is shown in the graphs to illustrate where the subject mouse spent time during the entire 10-min phase. Chamber time, time spent sniffing the novel object versus the stranger mouse, and number of entries to the side chambers in the social approach test were analyzed. The main factors were strain (B6 vs. BTBR), drug dose (3 levels each drug) and cohort (2 levels), with stranger mouse or novel object as the repeated measure. Fisher's LSD *post-hoc* analysis was run when the repeated measure (stranger mouse or novel object) was significant to determine the group differences. Elevated plus maze measures were analyzed using ANOVA and Fisher Least Significant Difference *post-hoc* tests.

3. Results

3.1. Social approach test

Overall there was no effect of cohort in the social approach test: chamber time ($F=1.29$, no significance (NS)); sniff time ($F=0.40$, NS); and entries ($F=0.76$, NS).

3.2. Fluoxetine

3.2.1. Sociability

Mice treated with fluoxetine exhibited more social approach behavior compared to the vehicle groups in the BTBR, but not B6 mice. Fig. 1, panels A–C, illustrate social approach behaviors in BTBR and B6 mice after treatment with 0, 10 or 30 mg/kg fluoxetine. There was a significant main effect of fluoxetine ($F_{2,60}=6.05$, $p<.01$). All of the mice spent more time in the chamber with the stranger mouse (chamber, $F_{1,60}=16.13$, $p<.001$). Paired comparisons revealed that the BTBR mice did spend significantly more time in the chamber with the stranger mouse after the 10 mg/kg fluoxetine dose, but not after vehicle or 30 mg/kg fluoxetine (NS). However, the B6 mice spent more time with the stranger mouse after the vehicle and 10 mg/kg doses of fluoxetine, but not the highest dose of 30 mg/kg. The center time for the BTBR mice is significantly different between the vehicle and 30 mg/kg dose of fluoxetine ($p<.001$), but not the 10 mg/kg dose (NS). There were no significant differences in center times in the B6 mice. After fluoxetine, BTBR mice spent more time sniffing the stranger mouse compared to the vehicle group (Fig. 1B). There was a significant effect of fluoxetine ($F_{2,60}=13.10$, $p<.001$), whereby sniffing time decreased with increasing doses of fluoxetine. There was also a significant difference of the time spent sniffing (sniff, $F_{1,60}=52.27$, $p<.001$), whereby the subject mice spent more time sniffing the stranger mouse than the novel object. Paired comparisons revealed that the BTBR mice failed to sniff the stranger mouse more after administration of vehicle; however, after 10 mg/kg and 30 mg/kg doses of fluoxetine, the BTBR mice did spend significantly more time sniffing the stranger mouse (p's$<.05$). The B6 mice spent significantly more time sniffing the stranger mouse regardless of the fluoxetine dose (p's$<.01$). The number of entries into both chambers decreased with increasing doses of fluoxetine. Fig. 1C illustrates the entries into each chamber made by the BTBR and B6 mice during the test. There was a significant effect of fluoxetine ($F_{2,60}=8.65$, $p<.001$), lowering the number of entries. More entries were made into the chamber with the stranger mouse than the novel object chamber (entries, $F_{1,60}=13.33$, $p<.001$). Paired comparisons revealed that both the 10 mg/kg and 30 mg/kg doses of fluoxetine significantly decreased the number of entries made overall during the test (p's$<.001$). There was a trend towards the interaction of entries,

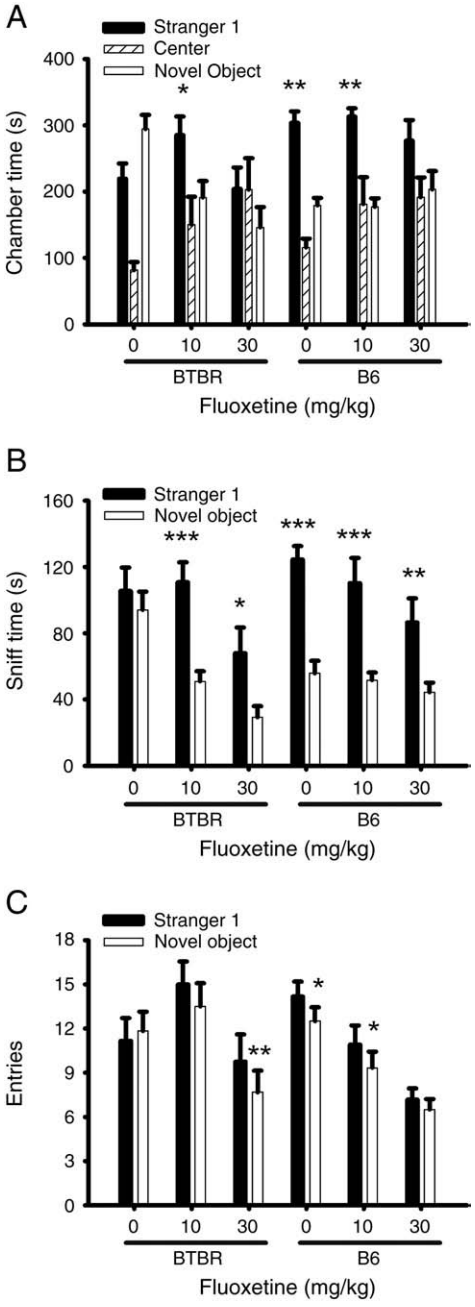


Fig. 1. Fluoxetine increases sociability in the BTBR but not B6 mice. Social approach was assayed in a three-chambered apparatus with video-tracking during the session for time spent in each chamber, sniffing (if the nose was close to the stranger mouse/object) and entries into each chamber. A) Chamber time: BTBR showed no preference for either side chamber after vehicle administration but did show a preference after 10 mg/kg fluoxetine. B6 mice showed a preference for the side chamber with the stranger mouse than the novel object after vehicle and 10 mg/kg fluoxetine but not 30 mg/kg fluoxetine. B) Sniff time: Both doses of fluoxetine increased time spent sniffing the stranger mouse in the BTBR. B6 spent more time sniffing the stranger mouse than novel object after vehicle and both doses of fluoxetine. C) Entries: The B6 mice made significantly more entries into the chamber with the stranger mouse after vehicle and 10 mg/kg fluoxetine ($p<.05$), and the BTBR mice made more entries following 30 mg/kg fluoxetine ($p<.01$). In Figs. 1–6, all data are shown as mean ± standard error of the mean, and $^{*}=p<.05$; $^{**}=p<.01$, and $^{***}=p<.001$. N = 12 all groups.

fluoxetine and strain ($p<.06$), which is illustrated by the differences in the number of entries made by each strain after the 10 mg/kg dose of fluoxetine. The 10 mg/kg fluoxetine BTBR group made more entries compared to vehicle BTBR group, while the 10 mg/kg fluoxetine B6 group made fewer than the vehicle B6 group. The B6 mice made significantly more entries into the chamber with the stranger mouse after vehicle and 10 mg/kg fluoxetine ($p<.05$), and the BTBR mice did following 30 mg/kg fluoxetine ($p<.01$).

3.2.2. Novelty

Fig. 2, panels A–C, illustrate the preference for novelty behaviors in BTBR and B6 mice when the stranger 2 mouse (novel) has been substituted for the novel object. Overall, the mice spent more time in the chamber with stranger 2 (Fig. 2A, chamber, $F_{1,60}=13.83$, $p<.001$). There was a significant effect of fluoxetine ($F_{2,60}=5.64$, $p<.01$) and cohort ($F_{1,60}=6.74$, $p<.05$). The second cohort spent more time in the side chambers than the center chambers compared to the first cohort. There was also a significant interaction of fluoxetine dose with the strain of mice ($F_{2,60}=6.12$, $p<.01$). Paired comparisons revealed that the BTBR mice administered vehicle and 10 mg/kg doses of fluoxetine spent significantly more time in the chamber with stranger 2 (p's$<.05$), but none of the other groups showed a preference (NS). The time spent in the center chamber (i.e., center time) for the BTBR mice is significantly different between the vehicle and 30 mg/kg doses of fluoxetine ($p<.001$), but not the 10 mg/kg dose (NS). There were no significant differences in center times in the B6 mice. All of the mice spent significantly more time sniffing the stranger 2 than stranger 1 (Fig. 2B, $F_{1,60}=39.69$, $p<.001$), although the B6 mice spent more time sniffing overall than the BTBR ($F_{1,60}=5.91$, $p<.05$). Fluoxetine did not affect overall sniffing ($F_{2,60}=2.33$, NS) but did interact with the two strains of mice differently ($F_{2,60}=3.87$, $p<.05$). Paired comparisons showed that the BTBR mice that were administered vehicle and 10 mg/kg doses of fluoxetine, and the B6 vehicle group sniffed stranger 2 significantly more than stranger 1 (p's$<.01$). None of the other groups showed this preference. Increasing doses of fluoxetine lowered the number of entries into each side chamber (Fig. 2C, $F_{1,60}=9.02$, $p<.001$), although it had an effect at lower doses on the B6 mice than the BTBR mice (strain by fluoxetine, $F_{2,60}=3.32$, $p<.05$). Overall, the mice made more entries into the chamber with stranger 2 than stranger 1 ($F_{1,60}=12.27$, $p<.001$), although the number of entries differed by fluoxetine dose (entries by fluoxetine, $F_{2,60}=4.45$, $p<.05$). Paired comparisons revealed that both the BTBR and B6 mice that were administered 10 mg/kg fluoxetine made more entries into the novel mouse chamber than the chamber with stranger 1(p's$<.01$), but none of the other groups entered one chamber more than the other.

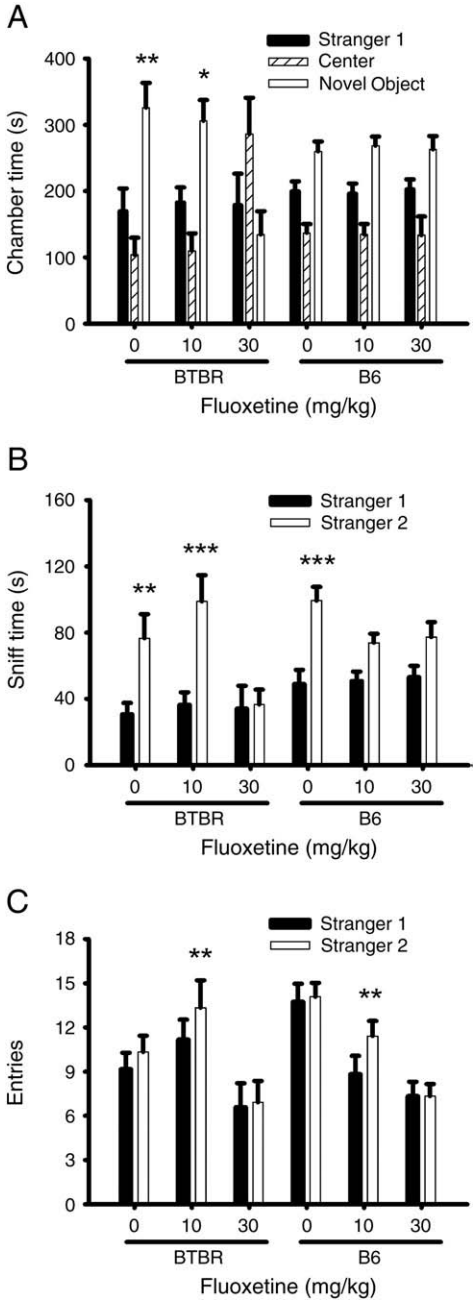
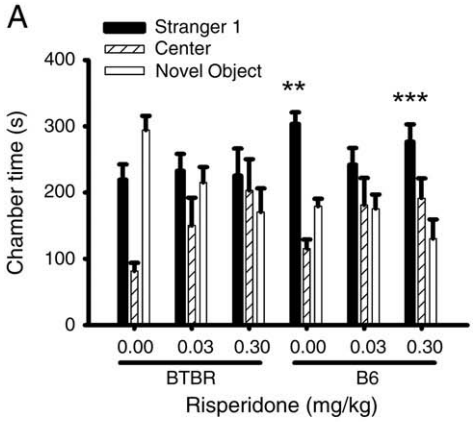


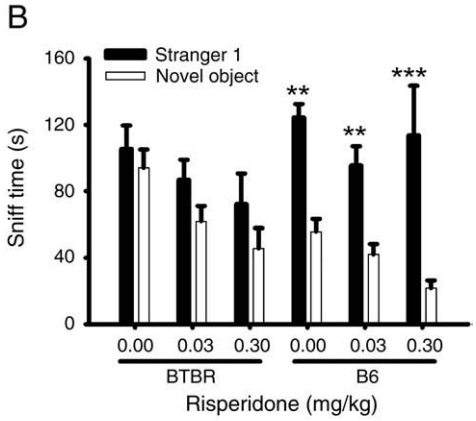
Fig. 2. Fluoxetine reduced preference for social novelty in BTBR but had no effect in B6 mice. A) Chamber time: BTBR spent more time in the side chamber containing the stranger 2 mouse after vehicle and 10 mg/kg fluoxetine administration, but not 30 mg/kg. B6 did not show a significant preference at any dose. B) Sniff time: BTBR spent more time sniffing the stranger 2 mouse than stranger 1 after vehicle and 10 mg/kg fluoxetine, but did not after 30 mg/kg fluoxetine. B6 spent more time sniffing the stranger 2 than stranger 1 after vehicle but not after either dose of fluoxetine. C) Entries: Both the BTBR and B6 administered 10 mg/kg fluoxetine made significantly more entries into the chamber with the stranger 2 mouse compared to the chamber with stranger 1, but neither the vehicle nor 30 mg/kg groups did. N = 12 all groups.

3.3. Risperidone

3.3.1. Sociability

Risperidone did not affect sociability in either strain of mice but did lower exploration as the dose increased. Fig. 3A illustrates social approach behaviors in BTBR and B6 mice after treatment with 0, 0.03 or 0.30 mg/kg risperidone. There was a significant effect of risperidone ($F_{2,60}=4.3$, $p<.05$) and of chamber ($F_{1,60}=11.95$, $p<.01$). Paired comparisons revealed that the BTBR mice failed to spend significantly more time in the stranger mouse chamber after any dose of risperidone (NS), while the B6 mice did prefer to spend more time in the chamber with the stranger mouse after vehicle and 0.30 mg/kg (p's$<.01$) risperidone but not 0.03 mg/kg risperidone (NS). Fig. 3B illustrates sniffing in BTBR and B6 mice during the test. Risperidone significantly lowered the amount of time spent sniffing ($F_{2,60}=6.40$, $p<.01$), although overall, there was a preference for sniffing stranger 1 over the novel object ($F_{1,60}=32.72$, $p<.001$). Paired comparisons revealed that risperidone had no effect on the time spent sniffing the stranger mouse in the BTBR mice (NS). The B6 mice spent significantly more time sniffing the stranger mouse regardless of the risperidone dose (p's$<.01$). Fig. 3C illustrates the entries into each chamber made by the BTBR and B6 mice during the test. There was an effect of entries ($F_{1,60}=4.88$, $p<.05$) for more entries into the chamber containing stranger 1 compared to the novel object, but not of risperidone ($F_{2,60}=1.76$, NS) on the number of entries. Paired comparisons revealed that the only group with significantly more entries to the stranger 1 chamber compared to the novel object chamber was the B6 group that was administered 0.30 mg/kg risperidone.





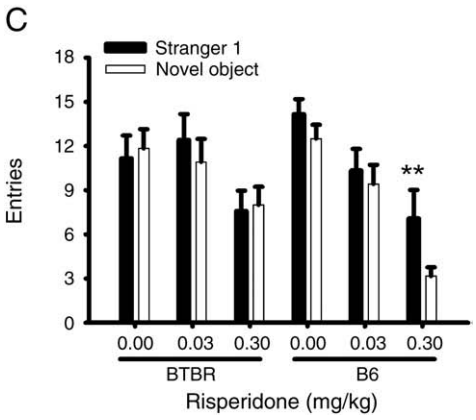
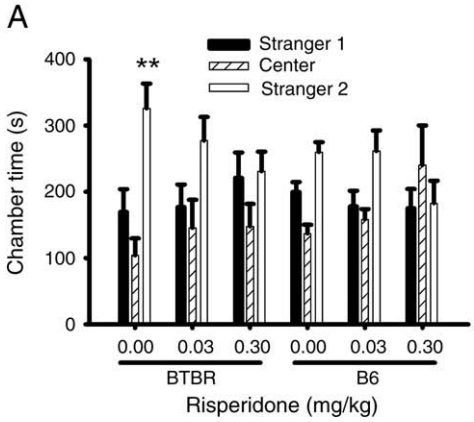
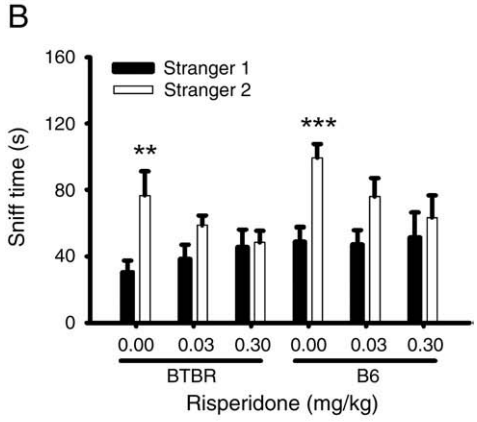


Fig. 3. Risperidone had no effect on sociability in the BTBR or B6 mice. A) Chamber time: BTBR showed no preference for either side chamber after vehicle or either dose of risperidone. B6 mice showed a preference for the side chamber with the stranger mouse over that with the novel object after vehicle and 0.3 mg/kg risperidone but not 0.03 mg/kg risperidone. B) Sniff time: BTBR did not spend more time sniffing the stranger mouse than the novel object after vehicle administration or either dose of risperidone. B6 spent more time sniffing the stranger mouse than novel object after vehicle and both doses of risperidone. C) Entries: Only the B6 mice administered 0.30 mg/kg risperidone made significantly more entries into the chamber with the stranger mouse compared to the chamber with the novel object. N = 12 all groups.





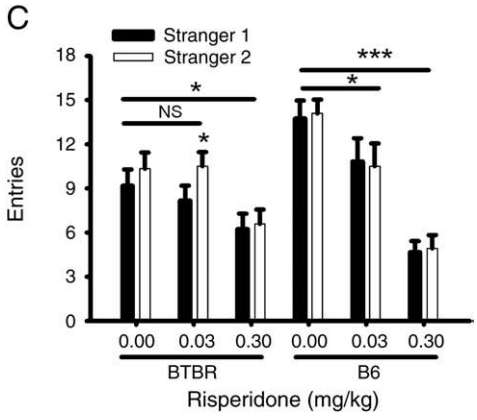


Fig. 4. Risperidone reduced preference for social novelty in BTBR but had no effect in B6 mice. A) Chamber time: BTBR showed preference for the side chamber containing the stranger 2 mouse after vehicle administration but not 0.03 or 0.3 mg/kg risperidone. B6 did not show a preference at any dose. B) Sniff time: BTBR spent more time sniffing the stranger 2 mouse than stranger 1 after vehicle, but not after either dose of risperidone. B6 spent more time sniffing the stranger mouse than novel object after vehicle but not after either dose of risperidone. C) Entries: Risperidone reduced the overall number of entries in both strains of mice. BTBR made fewer total entries (black and white bars combined) at the 0.3 mg/kg dose of risperidone compared to vehicle. B6 made fewer total entries (black and white bars combined) at both 0.03 and 0.3 mg/kg risperidone compared to vehicle. BTBR made more entries into the chamber with stranger 2 after 0.03 mg/kg risperidone. N = 12 all groups.

3.3.2. Novelty

Fig. 4, panels A–C, illustrate the preference for novelty in the BTBR and B6 mice after administration of risperidone. Overall, the mice preferred the chamber containing stranger 2 (unfamiliar mouse) over the chamber with stranger 1 (familiar mouse) (Fig. 4A, $F_{1,60} = 11.27$, $p < .01$). Risperidone dose ($F_{2,60} = 2.10$, NS), strain ($F_{1,60} = 11.27$, NS) and cohort ($F_{1,60} = 1.62$, NS) did not affect the time spent in the each of the chambers. Paired comparisons revealed that only the BTBR mice administered vehicle spent significantly more time in the chamber with stranger 2 ($p < .01$). Overall, the mice spent more time sniffing stranger 2 compared to stranger 1 (Fig. 4B, $F_{1,60} = 20.58$, $p < .001$). The B6 mice spent more time sniffing overall compared to the BTBR mice (strain, $F_{1,60} = 5.23$, $p < .05$); risperidone dose ($F_{2,60} = 1.20$, NS) and cohort ($F_{1,60} = 0.35$, NS) did not affect time spent sniffing. At 0.3 mg/kg risperidone, the mice spent less time sniffing stranger 2, but the time spent sniffing stranger 1 did not change ($F_{2,60} = 4.12$, $p < .05$), regardless of strain and cohort. Paired comparisons show that both

the BTBR and B6 vehicle groups had a preference for sniffing stranger 2 (p's<.01), but none of the other groups showed this preference. Risperidone decreased the number of entries made by the BTBR and B6 mice into both chambers (Fig. 4C, $F_{2,60}=20.67$, $p<.001$), but it affected the strains differently (strain by risperidone, $F_{2,60}=4.23$, $p<.05$). In the B6 mice, there was a dose-dependent decrease in the number of entries, while in the BTBR mice, the reduction was limited to the highest dose, 0.3 mg/kg of risperidone. Overall, the mice did not make more entries into either of the side chambers ($F_{1,60}=3.57$, NS). Paired comparisons revealed that only one dosing group, BTBR 0.03 mg/kg risperidone, made more entries into the chamber with stranger 2 ($p<.05$).

3.4. Elevated plus maze

3.4.1. Overall

The same mice were tested in both the social approach and elevated plus maze tests with a month in between. The mice were run in 2 cohorts, and cohort was a significant variable for the mice treated with either fluoxetine or risperidone on measures of percent open arm time, percent open arm entries and total entries (not risperidone) detailed below. The mice in cohort 1 spent more time in the open arms and made more open arm entries than cohort 2. Though the cohorts were significantly different, there were no interactions with either strain or drug in any measure (see below for statistics); therefore, for simplicity, the data in the graphs will not be divided by cohort. No cohort effects were observed in the social approach test.

3.4.2. Fluoxetine

Fluoxetine did not affect the percentage of time spent in the open arms ($F_{2,60}=1.68$, NS) of the elevated plus maze in either the BTBR or B6 mice illustrated by Fig. 5A . There was no difference between the BTBR and B6 mice ($F_{1,60}=2.39$, NS). There was a significant cohort effect ($F_{1,60}=27.07$, $p<.001$) that did not interact with strain (strain by cohort, $F_{1,60}=1.30$, NS) or fluoxetine (dose by cohort, $F_{2,60}=0.43$, NS). Fluoxetine dose did affect the percent open arm entries ($F_{2,60}=3.48$, $p<.05$, Fig. 5B) as did cohort ($F_{1,60}=25.10$, $p<.001$). Strain ($F_{1,60}=2.71$, NS) did not have an effect. Cohort did not interact with strain (strain by cohort, $F_{1,60}=1.01$, NS) or fluoxetine (dose by cohort, $F_{2,60}=0.84$, NS). *Post-hoc* analysis determined that fluoxetine significantly reduced the percent of open arm entries between the vehicle and the 30 mg/kg dose ($p<.05$), but not the 10 mg/kg dose (NS) overall. The total entries (Fig. 5C) into the open and closed arms of the maze were not affected by fluoxetine dose ($F_{1,60}=0.93$, NS) or strain ($F_{1,60}=0.86$, NS), although there was an effect of cohort ($F_{1,60}=5.88$, $p<.05$). Cohort did not significantly interact with strain (strain by cohort, $F_{1,60}=0.99$, NS) or fluoxetine (dose by cohort, $F_{2,60}=0.54$, NS). There were no overall differences between strain, drug dose or cohort in the time spent in the center of the maze (data not shown). There was an interaction of drug and strain where the B6 mice had decreased center time at 30 mg/kg fluoxetine but the BTBR mice did not (data not shown).

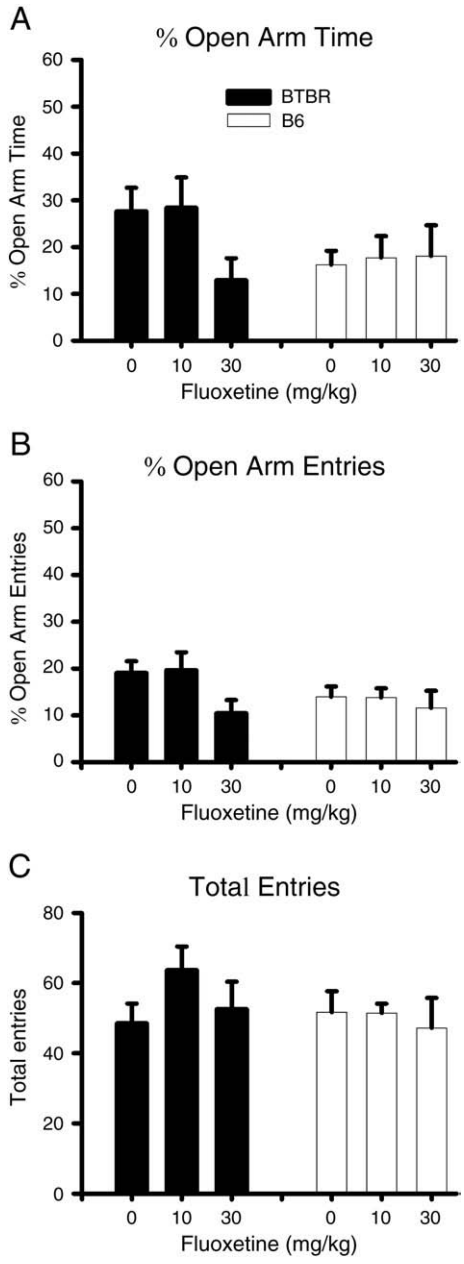


Fig. 5. Fluoxetine did not affect anxiety-like behavior in BTBR and B6 mice. Anxiety-like behavior was assessed in the elevated plus maze using video tracking to score percentage of time spent in the open arms, percentage of entries into the open arms and total entries. A) Fluoxetine did not affect the percentage of time that either the BTBR or B6 mice spent in the open arms; B) the percentage of entries into the open arms made by either the BTBR or B6 mice; or C) the total entries made by either BTBR or B6. N = 12 all groups.

3.4.3. Risperidone

Risperidone did not have an effect on the percentage of time spent in the open arms ($F_{2,60}=0.06$, NS) of the elevated plus maze in either the BTBR or B6 mice. There was no difference between the BTBR and B6 mice ($F_{1,60}=0.15$, NS, Fig. 6A). A significant cohort effect ($F_{1,60}=22.52$, $p<.0001$, data not shown), did not interact with strain (strain by cohort, $F_{1,60}=0.03$, NS) or risperidone dose (dose by cohort, $F_{2,60}=1.14$, NS). Risperidone did not have an effect on the percentage of entries to the open arms ($F_{2,60}=0.48$, NS) of the elevated plus maze in either the BTBR or B6 mice. There was no difference between the BTBR and B6 mice ($F_{1,60}=0.08$, NS, Fig. 6B). There was a significant cohort effect ($F_{1,60}=30.50$, p<.0001), that did not interact with strain (strain by cohort, $F_{1,60}=1.74$, NS) or risperidone dose (dose by cohort, $F_{2,60}=2.53$, NS, data not shown). Risperidone reduced the total entries ($F_{2,60}=26.08$, p<.001) into the elevated plus maze of both the BTBR and B6 mice. There was no difference between the BTBR and B6 mice ($F_{1,60}=1.96$, NS, Fig. 6C) or between the two cohorts ($F_{1,60}=1.65$, NS, data not shown). There was no interaction of risperidone dose with strain (strain by dose, $F_{2,60}=1.36$, NS) or cohort (dose by cohort, $F_{2,60}=0.36$, NS). *Post-hoc* analysis of risperidone dose determined that the total entries with both the 0.03 mg/kg ($p<.05$) and 0.30 mg/kg ($p<.001$) doses were significantly lower than in the vehicle group overall. In the BTBR strain, the total entries were not reduced at the 0.03 mg/kg dose (NS) but were at the 0.3 mg/kg dose ($p<.001$). In the B6 strain, the total entries were reduced at both the 0.03 mg/kg dose ($p<.05$) and 0.3 mg/kg dose ($p<.001$). There were no overall differences or interactions between

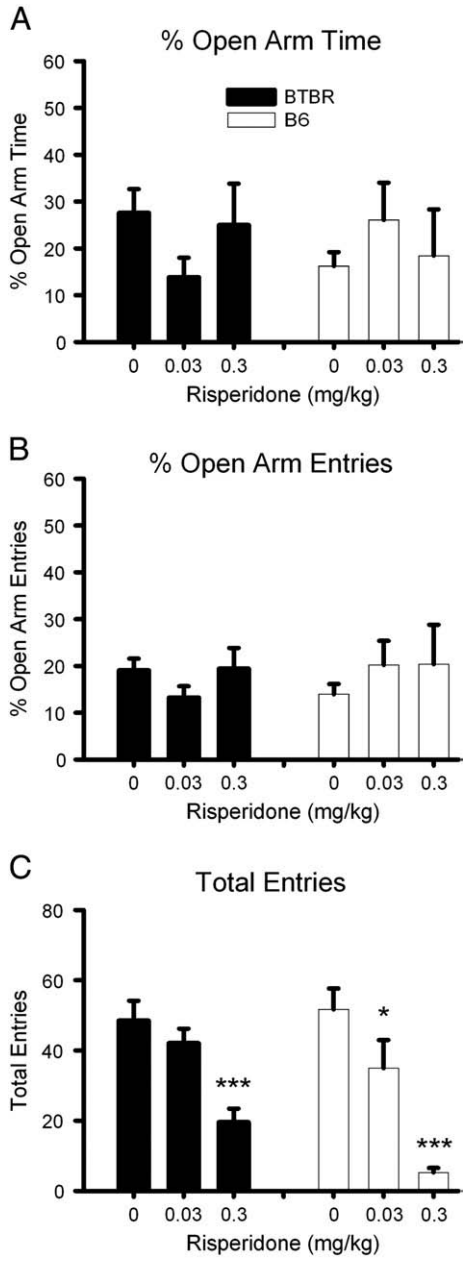


Fig. 6. Risperidone did not affect anxiety-like behavior in BTBR and B6 mice. A) Risperidone did not affect the percentage of time that either the BTBR or B6 mice spent in the open arms or B) the percentage of entries into the open arms made by either the BTBR or B6 mice. C) Risperidone did lower the total entries made by both the BTBR at 0.30 mg/kg and B6 at the 0.03 and 0.3 mg/kg doses. N = 12 all groups.

strain, drug dose or cohort in the mice administered risperidone for time spent in the center of the maze (data not shown).

4. Discussion

These experiments evaluated the effects of fluoxetine and risperidone in the BTBR mouse model of autism compared to the more social B6 strain of mice. These experiments are the first to show that the low level of sociability inherent in the BTBR strain can be increased with fluoxetine. It validates the use of the social approach test to examine the effects of drugs on social behavior in mice. The effects of fluoxetine were specific to increasing sociability; preference for novelty was impaired, and anxiety-like behavior was unaffected in BTBR mice. Fluoxetine also specifically affected the BTBR mice with low levels of sociability, and not the B6 mice with higher levels of sociability.

Risperidone did not affect sociability in either strain of mice. The BTBR mice showed a significant preference for novelty, which was inhibited by risperidone. The B6 mice did not show a chamber preference but did prefer sniffing the novel mouse, and both fluoxetine and risperidone inhibited this preference. Sociability and preference for novelty are thought to be mediated by different background genes, as inbred strains have different rank orders for these behaviors (Moy et al., 2007). Neither drug had an effect on the anxiety-like behaviors of the mice.

Fluoxetine increased sociability in the BTBR mice but not the control B6 mice. Citalopram is an SSRI similar to fluoxetine that is used in the treatment of depression. In the tail suspension test for depression, the BTBR mice were more responsive to citalopram than B6 mice (Crowley et al., 2005), suggesting that there may be differences in the serotonin transporter between these strains. While the BTBR mice were more responsive to fluoxetine in the current study, 30 mg/kg fluoxetine did have a sedative effect in the B6 mice, as shown by the decreased number of overall entries made during the sociability phase. It is unlikely that fluoxetine has an effect on sociability in the already sociable B6 mice. Though, both fluoxetine and citalopram, another selective-serotonin reuptake inhibitor (SSRI), have been found to decrease the the time spent in social interaction, decrease locomotor activity and increase grooming in rats (Dekeyne et al., 2000; Bagdy et al., 2001). The BTBR mice spent a significantly greater amount of time in the center chamber of the social approach apparatus following the 30 mg/kg dose of fluoxetine in both the sociability and novelty phases of the experiment. This most likely reflects some sedation in the BTBR mice from fluoxetine, although it is manifesting in a different manner than in the B6 mice, in which sedation is shown by the decreased number of entries, indicating a decrease in exploration.

Avoiding sedation was the rationale for the lower dose and longer pretreatment time used in the current study to determine if risperidone would affect social behavior. Previous research has suggested a possibly confounding sedative effect of risperidone at 0.125, 0.25 and 0.5 mg/kg (Silverman et al., 2009) with a 30-min pretreatment time. These doses are all higher than one of the doses used in the current study, 0.03 mg/kg risperidone. Exploratory behavior was also used as a measure of sedation by the number of entries into each chamber, which were not significantly affected by risperidone for sociability. Both risperidone and fluoxetine have been shown to be effective at lowering the number of marbles buried with a 1-h pretreatment (Bruins Slot et al., 2008). Despite the lower doses and longer pretreatment time, risperidone still did not affect sociability in either strain of mice. During the preference for novelty part of the experiment, BTBR mice had a significantly reduced number of entries at 0.30 mg/kg risperidone, and the B6 mice had reduced entries at both 0.03 and 0.30 mg/kg risperidone. Risperidone also reduced the total number of entries made in the elevated plus maze test at 0.3 mg/kg in the BTBR, and both 0.03 and 0.3 mg/kg in the B6. Results from the current study support the previous findings in the open field test (Silverman et al., 2009), suggesting that B6 mice seem to be more sensitive to the sedative effects of risperidone than the BTBR mice, as evidenced by reductions in exploratory behavior in both behavioral tests.

Preference for social novelty is defined as the subject mouse spending more time in the chamber or sniffing a novel mouse than a familiar one. This implies that the mouse is capable of social recognition and can differentiate between a novel and familiar conspecific. To differentiate, an intact olfactory sense and short term memory for the smell and location of the stranger 1 (familiar) mouse is required. Object memory has been shown to be similar in both the B6 and BTBR strains of mice (MacPherson et al., 2008), so it is not surprising that both show a preference for social novelty in the current study. B6 mice have shown a preference for social novelty by increased time in the chamber and sniffing the novel mouse, while the BTBR mice have also shown the

preference, though only with chamber time and not time spent sniffing (McFarlane et al., 2008; Moy et al., 2007). In the current study, the BTBR mice spent more time in the chamber with stranger 2 compared to the first stranger mouse as is consistent with previous reports (Moy et al., 2007; McFarlane et al., 2008). The BTBR mice in this study also showed the preference by spending significantly more time sniffing the second stranger mouse which differs from Moy et al. (2007) and sniffing was not reported in McFarlane et al. (2008). It is unclear why the BTBR mice in the current study spent more time sniffing the novel mouse, though it is consistent with chamber time data. The B6 mice did spend more time in the chamber with the novel mouse in the current study, but the data did not reach statistical significance and this may be in part due to the smaller n's used in the current experiments (n = 12) than that found in the literature (n's = 18-20) (Moy et al., 2007; McFarlane et al., 2008).

The BTBR mice showed increased sociability at 10 mg/kg fluoxetine and this dose had no effect during the novelty part of the experiment. The 30 mg/kg dose of fluoxetine decreased the preference for novelty in the BTBR mice which may most likely be attributed to the sedative effect of fluoxetine as there is also a significant decrease in the number of entries made by both strains of mice administered this dose.

Anxiety-like behaviors were also not affected by either fluoxetine or risperidone in either strain of mice. In the elevated plus maze, there was a significant cohort effect for percent open arm time and percent open arm entries that did not interact with either strain or the drug administered to the mice. The first cohort was run in the winter (January), and the mice spent less time moving around the maze and in the open arm of the maze compared to the second cohort run in late spring (May/June). The differences between the cohorts were only observed in the elevated plus maze experiment and not the sociability experiment. It is important to note that even though the cohorts differed, the treatment groups for each strain and drug dose did not differ, suggesting that results are reliable in terms of drug effects in these 2 strains. The variability has also been observed when results from the elevated plus maze were compared across labs and environmental conditions (Wahlsten et al., 2003b). The cohort 2 open arm time for the BTBR and B6 mice is similar to that reported by Benno et al., 2009, whereas the cohort 1 time is much lower and closer to that reported by Moy et al. (2007).

The reports of fluoxetine's effects on anxiety in rodents are mixed. For example, fluoxetine has been found to have an anxiogenic-like effect when administered acutely to B6 and other strains of mice (Kurt et al., 2000; Liu et al., 2010; Mombereau et al., 2010) and rats (Drapier et al., 2007; Griebel et al., 1994; Silva et al., 1999) in the elevated plus maze and zero maze. Also, fluoxetine or citalopram, another selective-serotonin reuptake inhibitor (SSRI), have been found to decrease the the time spent in social interaction, decrease locomotor activity and increase grooming in rats (Dekeyne et al., 2000; Bagdy et al., 2001). Others have found no effect of fluoxetine or citalopram in the elevated zero maze in mice (Troelsen et al., 2005) or the elevated plus maze in mice or rats (Holmes and Rodgers, 2003; Rodrigues-Filho and Takahashi, 1999). Fluoxetine did not affect the amount of time spent in the open arms by the B6 mice in the current study, which is similar to the other studies in mice where fluoxetine had not effect (Holmes and Rodgers, 2003). One possible reason for the lack of effect in the current study may be the slightly longer pretreatment time used than in Liu et al. (2010) administered 10 mg/kg fluoxetine with a 30 pretreatment time whereas in the current experiments there was a 60 min pretreatment time. This was not expected to affect the results based on previous work where fluoxetine was effective in modifying marble burying behavior at 60 min (Bruins Slot et al., 2008), and it had the advantage of allowing more rigorous control of the experiment, as the experimenter was blind to both drug and dose. However, it may be that the longer pretreatment time decreased the anxiogenic effect of fluoxetine in the B6 mice.

This study is among the first to use a mouse model of autism to examine potential pharmacological therapeutics to modify social behavior. Acute fluoxetine increased sociability in the BTBR mouse model without increasing anxiety-like behavior. Behaviors exhibited by mice that have face validity for autism can be used to test therapeutic agents. There are a plethora of mouse models that allow for testing of specific genes and/or teratologic events that may cause autism, and now these models can also be used to determine a drug's potential for alleviating the symptoms of autism. Future research will be needed to determine which serotonin receptor subtypes play a role in social behavior. Additionally, fluoxetine and other SSRIs must be tested in other measures of social behavior and in other mouse models of autism.

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