Serotonin 6 receptor blockade reduces repetitive behavior in the BTBR mouse model of autism spectrum disorder

Dionisio A. Amodeo a, *, Brandon Oliver a, Alma Pahua a, Kristianna Hitchcock a, Alexa Bykowski b, Devon Tice b, Aya Musleh b, Bryce C. Ryan b

a California State University San Bernardino, Department of Psychology, 5500 University Parkway, San Bernardino, CA 92407, United States of America
b University of Redlands, Department of Biology, 1200 E. Colton Avenue, Redlands, CA 92373, United States of America

A B S T R A C T

Autism spectrum disorder (ASD) is characterized by the expression of restricted repetitive behaviors (RRBs) and impairments in social recognition and communication. Previous studies have found that specific serotonin (5-HT) receptor modulation can attenuate repetitive behaviors expressed in specific mouse strains. The present study examined how 5-HT6 receptor blockade impacts the expression of repetitive behaviors in two different mouse strains that demonstrate elevated restricted, repetitive behavior and impairments in social behavior. BTBR T+Itrpr3tf/J (BTBR), C58/J (C58) and control C57BL/6J strains were behaviorally tested after acute treatment with the 5-HT6 receptor antagonist BGC 20-761 (BGC) or vehicle. BTBR mice express high levels of self-grooming behavior while C58 mice display high rates of repetitive jumping behavior. Similarly, the effect of 5-HT6 receptor blockade was also tested on social approach behaviors in both strains. BGC significantly reduced repetitive grooming in both female and male BTBR mice compared to vehicle-treated BTBR mice. BGC treatment did not attenuate social approach impairments in either female or male BTBR mice compared to vehicle-treated BTBR mice. Follow-up dose response studies were conducted on repetitive grooming and locomotor activity in BTBR mice. All doses reduced repetitive grooming in female and male BTBR mice. Acute treatment with BGC only reduced locomotor activity with the lower doses. In C58 mice, BGC treatment did not significantly attenuate flipping or general social approach behaviors. Instead, BGC significantly increased social sniff time in female C58 mice. While 5-HT6 receptor blockade did not attenuate the social impairments found in BTBR mice, this treatment did increase sniff time in female C58 mice. Although the lower doses of BGC deduced locomotion, the higher dose attenuated repetitive grooming in BTBR mice while sparing locomotor activity. Together these findings suggest the therapeutic effects of 5-HT6 receptor blockade are complex and may be specific to the types of repetitive behaviors expressed.

1. Introduction

Autism spectrum disorder (ASD) has become a major health priority as the fastest growing neurodevelopmental disorder, with latest estimates at 1 in 54 individuals diagnosed (CDC, 2016). Particularly troubling is the lack of effective therapeutics for ASD, highlighting a pressing need for basic translational research aimed at identifying novel treat

---

* Corresponding author at: Department of Psychology, California State University San Bernardino, 5500 University Parkway, San Bernardino, CA 92407, United States of America.
E-mail address: dionisio.amodeo@csusb.edu (D.A. Amodeo).

https://doi.org/10.1016/j.pbb.2020.173076
Received 21 May 2020; Received in revised form 6 November 2020; Accepted 17 November 2020
Available online 18 November 2020
0091-3057/Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
of higher order repetitive behavior, between C58 and BTBR mice. The autistic-like behaviors displayed by C58 mice have been shown to be sensitive to oxytocin and amphetamine regimens, both of which are modulated by serotonin systems (Moy et al., 2014; Teng et al., 2013). Furthermore, partial serotonin agonists have been shown to improve motor stereotypes in this strain (Canal et al., 2015).

The serotonin 6 (5-HT6) receptor is a more recently discovered receptor with high expression in regions such as the striatum, nucleus accumbens, and cerebral cortex (Plasat et al., 1993; Ruat et al., 1993). Regions such as the amygdala, hippocampus, hypothalamus, thalamus and cerebellum tend to display more moderate 5-HT6 receptor densities (Ward and Dorsa, 1996; Gérard et al., 1996; Gérard et al., 1997). Because 5-HT6 receptors are found to be restricted to the brain and localized to regions that are critical in learning and cognition, this receptor has garnered more interest as a target for novel therapeutics (Woolley et al., 2004; Wesołowska, 2010; Yun and Rhim, 2011).

Recent studies have established that 5-HT6 receptor antagonist treatment can lead to pro-cognitive effects in disease-related amnesia (de Bruin et al., 2016; Liu et al., 2016). Further evidence suggests that this rescued executive function by 5-HT6 receptor blockade may be mediated by increased acetylcholine (ACh) transmission (Marcos et al., 2006). These findings are intriguing because a previous study has demonstrated that treatment with the muscarinic acetylcholine agonist, oxtremorine, can attenuate repetitive behaviors in the BTBR mouse model of ASD (Amodeo et al., 2014). Wang et al. (2015) has also showed a similar reduction in repetitive behaviors and alleviation of social approach deficits with increased nicotinic receptor activation in BTBR mice. Although non-specific increases in SHT with selective serotonin reuptake inhibitors (SSRI) have led to mixed results (West et al., 2009), modulation of 5-HT release may offer a more effective and efficient approach.

To our knowledge, the only study that has examined how the 5-HT system is altered in BTBR compared to C57BL/6J mice is Guo and Commons (2017). This study found a reduction in 5-HT levels in hippocampus of BTBR compared to C57BL/6J mice (Guo and Commons, 2017). In addition, these authors also found an increase in the number of 5-HT neurons of the median raphe, caudal ventral and caudal dorsal portions of the raphe compared to C57BL/6J mice. However, to date, there lacks a fundamental understanding of which specific 5-HT receptor site is the most effective in alleviating RRBs and the cognitive impairments.

The current experiments examined how the 5-HT6 receptor antagonist BGC 20-761 (BGC) attenuates RRBs and social impairments expressed by the BTBR and C58/J mouse strains. BGC has been shown to be highly selective 5-HT6 receptor antagonist, although it does have a low affinity for the 5-HT2A and 5-HT2C receptors (Glenon et al., 2000; Tsai et al., 2000). Because the BTBR and C58/J strain show differing types of repetitive behaviors, such as grooming and jumping, respectively, our findings could potentially generalize to other disorders commonly associated with the expression of repetitive behaviors including obsessive compulsive disorder, Tourette’s and schizophrenia. Previous studies have used the BTBR and C58/J strains together to test the potential ameliorative properties of glutamate (Silverman et al., 2012) and GABA (Silverman et al., 2015) systems on autistic-like behavior. This study expands on this previous work and examines the possible therapeutic effects of 5-HT6 receptor antagonists on attenuating the elevated repetitive behaviors and reduced social approach in these two strains. Although it is important to point out that the social deficits found in BTBR mice are less robust in the C58/J strain (Moy et al., 2007; Ryan et al., 2010; Teng et al., 2016).

2. Methods

2.1. Subjects

A total of 64 female and male C57BL/6J and BTBR mice were bred housed and tested at California State University, San Bernardino (CSUSB). A total of 68 C57BL/6J and C58/J mice were bred, housed and tested at the University of Redlands (UR). All mice were housed in plastic cages (28 cm wide × 17 cm long × 12 cm high) with same-sex littermates. Both vivarium humidity (20–30%) and temperature (20–23 °C) were controlled with a 12-hour light/dark cycle (lights on at 0700). All experimental testing was conducted during the light cycle in separate experimental rooms. Food and water were provided ad lib throughout the experiment. Animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Laboratory Animal Care and Use Committee at CSUSB for the BTBR studies or UR for the C58/J studies. Mice began testing at eight weeks of age.

2.2. Treatment

Mice received either 0 or 2.5 mg/kg of 5-HT6 receptor antagonist BGC 20-761 (Tocris) treatment in 50% DMSO in sterile water. The 50% DMSO vehicle has been used in previous studies without having an aversive effect on locomotion (Amodeo et al., 2018a, 2018b; McDougall et al., 2014; McDougall et al., 2017). This dose was chosen from a previous study using BGC (Mitchell et al., 2006). Mice were injected intraperitoneally (ip) at a volume of 5 ml/kg. Mice received injections 10 min prior to behavioral testing.

2.3. Repetitive behavior

Grooming behavior was tested in female (n = 16) and male (n = 16) C57BL/6J mice and female (n = 16) and male (n = 16) BTBR mice at CSUSB. Jumping behavior was tested in female (n = 18) and male (n = 17) C57BL/6J and female (n = 17) and male (n = 16) C58/J mice at UR. Mice were injected ip with either 50% DMSO or BGC 2.5 mg/kg 10 min prior to initiating the repetitive behavior task. The behavior assay started with an initial 10-minute habituation, followed by a 10-minute testing period where repetitive behavior was measured. Mice were placed individually in a plastic container (28 cm wide × 17 cm long × 12 cm high). During the measurement of behavior at CSUSB, a trained experimenter observed 1.6 m away from the cage for 10 min. At UR, behavior was recorded via a camera positioned above the testing cage and scored later using The Observer software (Noldus, Leesburg, VA). Grooming behavior was characterized as genital/tail grooming, paw/leg licking, body grooming, and head washing. Total grooming time was recorded in seconds using a digital stopwatch. Jumping behavior was characterized as a mouse moving upward with all four paws leaving the ground simultaneously.

A follow up study was also conducted to further examine the effects of 0, 0.25, 2.5, and 5.0 mg/kg BGC on grooming in a separate cohort of female (n = 34) and male (n = 32) BTBR mice.

2.4. Social approach

After a 72-hour drug washout period, the same cohort of female (n = 16) and male (n = 16) C57BL/6J and female (n = 16) and male (n = 16) BTBR mice were tested on a social approach paradigm at CSUSB. A different cohort of male and female C57BL/6J and C58/J were used for the social approach assay at UR. The social approach task was conducted in an acrylic, rectangular three-chambered apparatus. There were two inner walls dividing the apparatus into three identically sized chambers that included centered doorways, which allowed access between all three chambers. Both of the outer chambers contained a stainless-steel wire cup (Galaxy Cup, Spectrum Diversified Designs, Inc., Streetsboro, OH) to house the novel object or the stranger mouse.

There were minor procedural differences in social testing at the two institutions. Testing at CSUSB was conducted in a black acrylic 3-chamber apparatus (80 cm long × 40 cm wide × 28 cm tall). Testing at UR was conducted in a clear acrylic 3-chamber apparatus (60 × 40 × 22 cm).
cm). At CSUSB, a size-matched, novel object (Safari Jeep toy car, Matchbox El Segundo, CA) was placed under the wire cup, in the opposite outer chamber in relation to the stranger mouse. At UR, no such novel object was used. Instead, no wire cups were present during the acclimation period, so the presence of the wire cup during testing was novel. All mice were tested individually and received an ip injection of either 50% DMSO or BGC at 2.5 mg/kg 10 min prior to testing. All mice received a 10-minute habituation session immediately followed by a 10-minute testing session. During the habituation session, mice were placed in the center chamber and allowed to explore all three chambers undisturbed. After 10 min, mice were guided back to the center chamber and the doors were closed. A sex- and strain-matched stranger mouse was pseudorandomly placed under the wire cup in either the left or right chamber, counterbalanced across strains. The inner doors were opened allowing the mouse to explore all three chambers undisturbed for the 10-minute testing session. A video camera located overhead was used to record behavior during testing. Ethovision 3 video tracking software (CSUSB) or The Observer software (UR) (Noldus, Leesburg, VA) was used to determine the total time spent in each of the three chambers and entries into each chamber. During the testing sessions at CSUSB, a trained experimenter scored the time the mouse spent sniffing the stranger or novel object in the wire. Experimenters measuring sniff time were blind to treatment. At UR, sniff time was determined via video. The apparatus was cleaned with a 10% alcohol solution at the end of each testing session.

2.5. Locomotor activity

Locomotor activity was measured in only BTBR (female n = 33; male = 42) mice. Mice were approximately 9 weeks of age before testing. A black acrylic square chamber (60 cm long × 60 cm wide × 30 cm tall) was employed. The testing chamber was divided into four squares with dividers, allowing four mice to be tested at a time in individual open fields. Mice were placed in the apparatus for 60 min. During that time, EthoVision 3 video tracking system (Noldus, Leesburg, VA) was used to measure and record the total distance traveled. Once the trial ended mice were returned to their home cage, and the apparatus was cleaned with a 10% alcohol solution.

2.6. Statistical analysis

For the repetitive behavior task, a two-way analysis of variance (ANOVA) was conducted for each sex to compare mean differences in grooming duration for strain (C57BL/6J & BTBR; C57BL/6J & C58/J) and treatment groups (vehicle & BGC). For the follow-up BTBR studies, a two two-way ANOVA were conducted for grooming duration (female & male BTBR; BGC dose) and locomotor activity (female & male BTBR; BGC dose). For social approach, separate two-way repeated measures ANOVAs were completed for sex to compare strain (C57BL/6J & BTBR; C57BL/6J & C58/J) and treatment (vehicle & BGC) differences in time spent in the novel and social chamber (Yang et al., 2011). Separate two-way ANOVAs were also completed for sex to compare strain and treatment differences in sniff time. Post-hoc Newman-Keuls tests were performed when necessary and statistical significance was set at p < 0.05.

3. BTBR results

3.1. Repetitive grooming

Total grooming duration was recorded during the 10 min testing phase (Fig. 1). In females, a significant main effect of strain [F(1, 28) = 25.8, p < 0.001], main effect of treatment [F(1, 28) = 6.029, p = 0.02],
but no significant interaction \(F(1, 28) = 1.329, p = 0.26\) was found (Fig. 1A). Post hoc comparisons indicate female BTBR mice expressed higher rates of grooming behavior compared to female C57BL/6J mice (\(p < 0.01\)). A significant difference between vehicle-treated and BGC treated female BTBR mice was found (\(p < 0.05\)). Therefore, acute 5-HT6 receptor blockade attenuated repetitive grooming in female BTBR mice. In males, a significant main effect of strain \(F(1, 28) = 12.25, p < 0.01\), main effect of treatment \(F(1, 28) = 4.71, p = 0.04\), but no significant interaction \(F(1, 28) = 1.772, p = 0.19\), was found (Fig. 1B). Post hoc comparisons indicate vehicle-treated male BTBR mice expressed higher rates of grooming behavior compared to vehicle-treated male C57BL/6J mice (\(p < 0.05\)). There was a significant difference between vehicle-treated male BTBR mice compared to BGC treated male BTBR mice (\(p < 0.05\)). Regardless of sex, BTBR mice expressed higher rates of repetitive grooming compared to vehicle-treated male C57BL/6J mice (\(p < 0.05\)). There was a significant difference between vehicle-treated male BTBR mice compared to BGC treated male BTBR mice (\(p < 0.05\)). In males, a significant main effect of strain \(F(1, 28) = 4.71, p < 0.04\), but no significant interaction \(F(1, 28) = 1.74, p = 0.19\), was found (Fig. 1B). Post hoc comparisons indicate vehicle-treated male BTBR mice spent less time in the stranger compartment compared to vehicle-treated female C57BL/6J mice (\(p < 0.01\)). Similarly, BGC-treated female BTBR mice spent less time in the stranger compartment than BGC treated C57BL/6J mice. Most importantly, BGC 20761 did not increase social approach in female BTBR mice.

In males, there was no significant main effect of compartment \(F(1, 56) = 0.30, p > 0.05\), no main effect of treatment \(F(3, 56) = 0.40, p > 0.05\) and no significant interaction \(F(3, 56) = 1.74, p > 0.05\) (Fig. 2B). Although there was a trend for vehicle-treated male BTBR mice to spend less time in the stranger compartment compared to vehicle-treated C57BL/6J mice, this comparison was not significant. Most importantly, BGC treatment did not increase social approach for male BTBR mice. In addition to time spent in each chamber, sniff times were analyzed in both female and male mice separately (Fig. 3). In females, a significant main effect of sniff side \(F(1, 58) = 17.02, p < 0.01\), a main effect of treatment \(F(3, 56) = 3.49, p < 0.02\), and significant interaction \(F(3, 56) = 8.41, p < 0.01\) was found. Post hoc comparisons indicate the vehicle-treated female BTBR spent less time sniffing the stranger compared to vehicle-treated female C57BL/6J mice (\(p < 0.05\)). Similarly, BGC treated C57BL/6J mice spent less time sniffing the stranger compared to vehicle-treated female C57BL/6J mice (\(p < 0.01\)). These findings suggest 2.5 mg/kg BGC is not effective in increasing sniff time in female BTBR mice. Interestingly, 2.5 mg/kg BGC 20761 reduced sniff time in control C57BL/6J mice.

In males, there was a main effect of sniff side \(F(1, 56) = 12.20, p < 0.01\), but no main effect of treatment \(F(3, 56) = 3.39, p = 0.09\), nor a significant interaction \(F(3, 56) = 1.31, p = 0.28\). Post hoc comparisons indicate 2.5 mg/kg treated male C57BL/6J mice spent less time sniffing the stranger compartment than vehicle-treated male C57BL/6J mice. BGC treatment did not increase sniffing in male BTBR mice.

### 3.2. Social approach

Time spent in each of the three social-approach compartments was analyzed (Fig. 2). In females, there was a significant main effect of compartment \(F(1, 56) = 5.91, p < 0.05\), and no main effect of treatment \(F(3, 56) = 0.42, p > 0.05\), but there was a significant interaction \(F(6, 56) = 8.10, p < 0.01\) (Fig. 2A). Post hoc comparisons indicate that vehicle-treated female BTBR spent less time in the stranger compartment compared to vehicle-treated female C57BL/6J mice (\(p < 0.01\)). Similarly, BGC-treated female BTBR mice spent less time in the stranger compartment than BGC treated C57BL/6J mice. Most importantly, BGC 20761 did not increase social approach in female BTBR mice.

In males, there was no significant main effect of compartment \(F(1, 56) = 0.30, p > 0.05\), no main effect of treatment \(F(3, 56) = 0.40, p > 0.05\) and no significant interaction \(F(3, 56) = 1.74, p > 0.05\) (Fig. 2B). Although there was a trend for vehicle-treated male BTBR mice to spend less time in the stranger compartment compared to vehicle-treated C57BL/6J mice, this comparison was not significant. Most importantly, BGC treatment did not increase social approach for male BTBR mice. In addition to time spent in each chamber, sniff times were analyzed in both female and male mice separately (Fig. 3). In females, a significant main effect of sniff side \(F(1, 58) = 17.02, p < 0.01\), a main effect of treatment \(F(3, 56) = 3.49, p < 0.02\), and significant interaction \(F(3, 56) = 8.41, p < 0.01\) was found. Post hoc comparisons indicate the vehicle-treated female BTBR spent less time sniffing the stranger compared to vehicle-treated female C57BL/6J mice (\(p < 0.05\)). Similarly, BGC treated C57BL/6J mice spent less time sniffing the stranger compared to vehicle-treated female C57BL/6J mice (\(p < 0.01\)). These findings suggest 2.5 mg/kg BGC is not effective in increasing sniff time in female BTBR mice. Interestingly, 2.5 mg/kg BGC 20761 reduced sniff time in control C57BL/6J mice.

In males, there was a main effect of sniff side \(F(1, 56) = 12.20, p < 0.01\), but no main effect of treatment \(F(3, 56) = 3.39, p = 0.08\), nor a significant interaction \(F(3, 56) = 1.31, p = 0.28\). Post hoc comparisons indicate 2.5 mg/kg treated male C57BL/6J mice spent less time sniffing the stranger compartment than vehicle-treated male C57BL/6J mice. BGC treatment did not increase sniffing in male BTBR mice.

![Fig. 2. BGC treatment did not increase social approach in male or female BTBR mice. (A) Vehicle-treated female BTBR mice spent more time with the novel object and less time in the stranger compartment in comparison to C57BL/6J mice. BGC treatment was not effective in attenuating this preference. (B) No differences were found amongst male BTBR and C57BL/6J mice. The data is presented as mean ± SEM. *p < 0.05.](image-url)
Fig. 3. BGC treatment does not increase sniff times in BTBR mice. A) Vehicle-treated female BTBR mice spent significantly less time sniffing the stranger mouse compared to vehicle-treated C57BL/6J mice. Treatment with 2.5 mg/kg BGC did not increase this sniff time compared to vehicle-treated female BTBR mice. BGC treatment did decrease the amount of time female C57BL/6J mice spent sniffing the stranger mouse when compared to vehicle-treated C57BL/6J mice. B) A) BGC treatment did not increase social approach in male BTBR mice. BGC treated male C57BL/6J mice spent significantly less time sniffing the stranger mouse compared to vehicle-treated C57BL/6J mice. The data is presented as mean ± SEM. *p < 0.05.

Fig. 4. BGC treatment did not impact elevated jumping in C58/J mice. A) Female C58/J mice demonstrated elevated jumping/flipping compared female C57BL/6J mice. Treatment with 2.5 mg/kg BGC did not reduce jumping behavior in female C58/J mice compared to vehicle-treated C58/J mice. B) Male C58/J mice demonstrated elevated jumping/flipping compared male C57BL/6J mice. Treatment with 2.5 mg/kg BGC did not reduce jumping behavior in male C58/J mice compared to vehicle-treated C58/J mice. The data is presented as mean ± SEM. *p < 0.05, **p < 0.01.
the stranger compared to vehicle-treated male C57BL/6J mice (p < 0.01). These findings suggest 2.5 mg/kg BGC 20761 is not effective in increasing sniff time in male BTBR mice. As found in female C57BL/6J mice, 2.5 mg/kg BGC 20761 reduced sniff time in control C57BL/6J mice.

### 3.3. Locomotor activity

Locomotor activity was measured with 0, 0.25, 2.5 or 5 mg/kg BGC treatment in female and male BTBR mice (Fig. 8). Analysis indicates that there was a significant main effect of sex [F(1, 67) = 16.69, p < 0.01], a main effect of treatment [F(3, 67) = 10.23, p < 0.01], and no significant interaction [F(3, 67) = 0.64, p = 0.59]. Post-hoc Newman-Keuls tests indicate vehicle treated female BTBR mice express greater levels of locomotion compared to vehicle treated male BTBR mice. BGC at 0.25 or 2.5 mg/kg significantly attenuated locomotor activity in female BTBR mice compared to female vehicle treated mice. Similarly, 0.25 and 2.5 mg/kg BGC significantly attenuated locomotor activity in male BTBR mice compared to male vehicle treated BTBR mice. BGC at 5.0 mg/kg reduced locomotor activity in male BTBR mice compared to female BTBR mice, although neither females nor males treated with 5.0 mg/kg BGC differed compared to their respective vehicle control group. Together these findings indicate that BGC at 0.25 and 2.5 mg/kg attenuates locomotor activity in BTBR mice regardless of sex.

### 3.4. C58 results

#### 4.1. Repetitive jumping

Total flips and somersaults were recorded during the 10 min testing phase (Fig. 4). In females, a significant main effect of strain [F(1, 31) = 17.2, p < 0.001], no main effect of treatment [F(1, 31) = 0.10, p = 0.75], and no significant interaction [F(1, 31) = 0.06, p = 0.80] was found. Neuman-Keuls post hoc comparisons of strain indicate vehicle-treated female C58 mice expressed higher rates of jumping compared to female C57BL/6J mice (p < 0.05). Similarly, BGC treated female C58 mice expressed higher rates of jumping compared to female C57BL/6J mice (p < 0.01). Importantly, there was no difference between vehicle-treated female C58 mice and female BGC treated C58 mice. Thus, 5-HT6 receptor blockade with BGC was not effective in reducing the lower order repetitive jumping behaviors in female C58 mice.

In males, a significant main effect of strain [F(1, 29) = 16.29, p < 0.05], no main effect of treatment [F(1, 29) = 0.98, p = 0.33], and no significant interaction [F(1, 29) = 0.06, p = 0.98] was found. Male C58 mice displayed greater rates of jumping compared to C57BL/6J mice. Importantly, there was no difference between vehicle-treated male C58 mice and BGC treated male C58 mice. Thus, 5-HT6 receptor blockade with BGC 271-046 was not effective in reducing the lower order repetitive behaviors in male C58 mice.

### 4.2. Social approach

Time spent in each of the three social approach compartments was analyzed (Fig. 5). In females, a significant main effect of compartment [F(1, 64) = 63.99, p < 0.01], main effect of treatment [F(3, 64) = 5.5, p < 0.01] and significant interaction [F(3, 64) = 3.08, p < 0.05], were found. Post hoc comparisons indicate the vehicle-treated female C58 spent less time with the object compared to vehicle-treated female C57BL/6J mice (p < 0.01). No differences were found between groups in time spent in the stranger compartment. Therefore, BGC was not effective increasing social approach in female C58 mice. Because there was no difference in vehicle-treated C57BL/6J and vehicle-treated female C58 mice, it would be difficult for the BGC treatment have an effect in female C58 mice (Fig. 5A).

In males, there was a significant main effect of compartment [F(1, 29) = 18.59, p < 0.01], and no main effect of treatment [F(3, 29) = 0.89, p > 0.05] but there was a significant interaction [F(3, 29) = 3.62, p < 0.05]. Newman-Keuls post hoc comparisons BGC-treated male C58 mice less time in the object compartment compared to BGC treated male C57BL/6J mice. Importantly, there were no differences in time spent in the stranger compartment compared to vehicle treated male C57BL/6J mice. Therefore, BGC did not increase social approach in C58/J mice.
the stranger compartment amongst all groups. Because there was no difference in vehicle-treated C57BL/6J and vehicle-treated male C58 mice, it would be difficult for the BGC treatment have an effect in male C58 mice (Fig. 5B).

In addition to the time spent in each chamber, sniff times were analyzed in both female and male mice separately (Fig. 6). In females, a significant main effect of sniff side \([F(1, 64) = 39.76, p < 0.01]\), a main effect of treatment \([F(3, 64) = 7.56, p < 0.01]\), was found with no significant interaction \([F(3, 64) = 0.88, p = 0.46]\). Post hoc Newman-Keuls comparisons indicate the vehicle-treated female C57BL/6J spent more time sniffing the object compared to vehicle-treated female C58 mice \((p < 0.05)\). Similarly, vehicle-treated female C57BL/6J spent more time sniffing the stranger compared to vehicle-treated female C58 mice \((p < 0.05)\). Interestingly, BGC treated C58 mice spent significantly more time sniffing the stranger mouse. These findings suggest BGC treatment was effective in attenuating the social sniffing impairment found in female C58 mice (Fig. 6A).

In males, there was a significant main effect of sniff side \([F(1, 56) = 70.04, p < 0.01]\), a main effect of treatment \([F(3, 56) = 2.99, p < 0.05]\), but no significant interaction \([F(3, 56) = 1.39, p = 0.26]\). Post hoc Newman-Keuls comparisons indicate the vehicle-treated male C57BL/6J spent more time sniffing the stranger compared to vehicle-treated male C58 mice \((p < 0.05)\). Although, male C58 mice show a similar reduction in social sniffing of the stranger mouse as found in females, BGC treatment was not effective in attenuating the impairment.
5. General discussion

These experiments examined the impact of 5-HT6 receptor blockade on the expression of repetitive behaviors and social approach behaviors in two mouse models of autistic-like behavior. The current findings demonstrate acute 5-HT6 receptor blockade with BGC was effective in attenuating repetitive grooming behavior in both female and male BTBR mice. This same treatment was not effective in attenuating the repetitive jumping behavior in C58 mice regardless of sex. This suggests that the therapeutic properties are 5-HT6 receptor blockade may be specific to the type of repetitive behavior or strain. Follow up studies in BTBR mice further demonstrate that this reduction in grooming was replicated with the lowdose $0.25 \, \text{mg/kg}$ dose and higher $5.0 \, \text{mg/kg}$ dose in female and male BTBR mice. Because the two lower doses also reduced locomotor activity in female and male BTBR mice, we must take the locomotor reductions into consideration. Although the high dose of $5.0 \, \text{mg/kg}$ BGC reduced grooming duration in female and male BTBR mice, this same dose did not significantly reduce locomotor activity in either sex. This highlights the need to explore the effects of higher doses of BGC on repetitive behaviors in BTBR and C58 mice.

These results together parallel findings with the adenosine 2A agonist CGS21680. Amodeo et al., (2018a) found that CGS21680 attenuated repetitive grooming in BTBR mice, while Lewis et al. (2019) found that CGS21680 treatment was not sufficient to attenuate repetitive flipping in C58 mice. Together these findings support a possible relationship between the altered neural functioning that may contribute to the expression of repetitive behaviors amongst different strains.

Previous studies have focused on the 5-HT6 receptor as a target because of its anxiolytic properties (Jastrzébska-Więcek et al., 2016; Suárez-Santiago et al., 2017; Sun et al., 2018) and as a novel treatment for Alzheimer’s disease (de Jong and Mørk, 2017; Hu et al., 2017; Khoury et al., 2018; Werner and Covenas, 2016). More recent investigations have highlighted 5-HT6 receptor blockade as a target for Parkinson’s medications (Kucinski et al., 2017; Zhang et al., 2016). No studies have examined the effects of 5-HT6 receptor blockade on the expression of core ASD behaviors. To our knowledge, these studies are the first to examine the role of 5-HT6 receptor antagonism on the lower order repetitive behaviors and social approach impairments in both of these mouse strains.

Building evidence supports the theory that 5-HT6 receptor antagonists have pro-cognitive affects (Benhamú et al., 2014; Staroti et al., 2020). These pro-cognitive findings have been linked to 5-HT6 receptor blockade and its resulting increases in acetylcholine release (Herrik et al., 2016; Mørk et al., 2017). Increases in acetylcholine may be the underlying mechanistic change that led to reduction in repetitive grooming in BTBR mice. A previously study has demonstrated that treatment with the muscarinic acetylcholine agonist oxotremorine was effective in reducing grooming rates in BTBR mice (Amodeo et al., 2014). Furthermore, Yoshimura et al. (2017) found that systemic specific positive allosteric modulators of the α7 nicotinic acetylcholine receptor was sufficient in attenuating both the repetitive behaviors and social impairments found in BTBR mice. Similarly, Wang et al. (2015) demonstrated the beneficial properties of nicotine administration on the repetitive behaviors and social impairments expressed by the BTBR mouse. Together, these findings suggest that acetylcholine upregulation may be the mechanism underlying the benefits 5-HT6 receptor blockade.

Fewer studies have directly investigated the neurotransmitter systems involved in the aberrant repetitive and social behaviors present in the C58/J strain (Muehlmann et al., 2012; Lewis et al., 2019; Silverman et al., 2012; Teng et al., 2016; Wilkes et al., 2019). Studies on oxytocin and amphetamines indirectly implicate serotonin, however (Moy et al., 2014; Teng et al., 2013) specific serotonin receptor manipulations have been lacking in the literature. Previous studies have found a high density of 5-HT6 receptors in several portions of the striatum (Brodsky et al., 2016; Hirst et al., 2000; Roberts et al., 2002), a region that has been implicated in the expression of repetitive behaviors. An fMRI study by Wilkes et al. (2019) has shown a correlation between percent total brain volume of striatum and increased repetitive motor behaviors in C58 mice. Interestingly though, Wilkes et al. (2019) found reduced striatum volumes in C58 mice, while Ellegood and Crawley (2015) found reduced striatum volumes in BTBR mice versus controls. Because reduced volume does not imply reduced activity, cortico-striatal circuits may still be hyperactive or dysregulated and thus lead to the expression of elevated repetitive behaviors (Burguéri et al., 2013; Rebec et al., 1997).

Our previous studies have also found that treatment with the 5-HT6 agonist EMD386088 impaired behavioral flexibility in C57BL/6J mice, which is considered a higher order repetitive behavior (Amodeo et al., 2018a, 2018b). These previous findings are in line with our current results demonstrating that the high rates of self-grooming in BTBR mice, a lower order repetitive behavior, are susceptible to 5-HT6 modulation. Specifically, upregulation of 5-HT6 receptors increased a repetitive behavior while blockade lowered repetitive grooming in a strain that expresses innate levels of elevated self-grooming. In addition, blockade of 5-HT2A receptors has also been shown to reduce repetitive grooming (Amodeo et al., 2016) and attenuate behavioral flexibility in the BTBR mouse (Amodeo et al., 2014), further supporting the impact of serotonin on the expression of repetitive behaviors.

Both female and male BTBR mice demonstrated reduced social approach behaviors as expected, but 5-HT6 receptor blockade was not sufficient to rescue those social impairments. This was true for both the time in each compartment and sniff time. In contrast, both female and male C58 mice did not demonstrate reduced social approach behaviors as measured by time spent in each chamber. Instead, female C58 mice...
showed a reduction in sniff time for the stranger mouse. Previous research shows that the CS8/J strain has more drastic impairments in sniff times versus time spent in each chamber in a social approach assay (Ryan et al., 2010). This study shows that the reduction in sniff time was rescued by 5-HT6 receptor blockade in female mice. Similarly, male CS8 also demonstrated a reduction in sniff time for the stranger mouse, but 5-HT6 receptor blockade was not effective in rescuing this impairment. In general, these findings show that both strains show social impairments, but only sniff time was rescued by 5-HT6 receptor blockade in CS8 mice.

Loiseau et al. (2008) injected the 5-HT6 receptor agonist WAY 181187 directly into frontal cortex of healthy rats, which lead to social recognition impairments. Furthermore, de Bruin and Kruse (2015) found that 5-HT6 receptor agonists impaired social recognition tests while co-administration with a 5-HT6 receptor antagonist rescued the impairment. In addition, Mitchell et al. (2006) found that BGC is effective in treating social recognition deficits caused by scopolamine, an acetylcholine antagonist. These findings are consistent with our data in female CS8 mice and show that serotonin modulation can attenuate social impairments in a variety of rodent models. Although we found comparable sniffing of the stranger in C57BL/6J mice at both CSUSB and no studies in C58/J mice have investigated basal monoamine levels, lack of information on the serotonergic systems in these two strains of grooming behavior is observable in the control C57BL/6J strain, we do of BGC in C58 or C57BL/6J strains, this needs to be tested to determine the ameliorating effects of 5-HT6 receptor blockade were not similar amongst the strains and this suggests that the aberrant behavior in the BTBR and CS8/J strains may have different origins, mirroring the heterogeneous nature of ASD in humans. As such, this research suggests that serotonin modulation may benefit some, but not all, individuals displaying social deficits or elevated repetitive behaviors.

**References**

Amodeo, D.A., Jones, J.H., Sweeney, J.A., Ragozino, M.E., 2012. Differences in BTBR T tf/J and C57BL/6J mice on probabilistic reversal learning and stereotyped behaviors. Behav. Brain Res. 227 (1), 64–72.

Amodeo, D.A., Yi, J., Sweeney, J.A., Ragozino, M.E., 2014. Oxtremorine treatment reduces repetitive behaviors in BTBR T tf/J mice. Frontiers in synaptic neuroscience 6, 17.

Amodeo, D.A., Rivera, E., Dunn, J.T., Ragozino, M.E., 2016. M100907 attenuates elevated grooming behavior in the BTBR mouse. Behav. Brain Res. 313, 67–70.

Amodeo, D.A., Cuervas, L., Dunn, J.T., Sweeney, J.A., Ragozino, M.E., 2018a. The adenosine A2A receptor agonist, CGS 21680, attenuates a probabilistic reversal learning deficit and elevated grooming behavior in BTBR mice. Autism Res. 11 (2), 223–233.

Amodeo, D.A., Peterson, S., Pahuja, A., Posadas, R., Hernandez, A., Hefero, E., Qi, D., Vega, J., 2018b. 5-HT6 receptor agonist EMD386088 impairs behavioral flexibility and working memory. Behav. Brain Res. 349, 8–15.

Amodeo, D.A., Pahuja, A.E., Zarette, M., Taylor, J.A., Peterson, S., Posadas, R., Oliver, B.L. Amodeo, L.R., 2019. Differences in the expression of restricted repetitive behaviors in female and male BTBR T tf/J mice. Behav. Brain Res. 372, 110288.

Bales, K.L., Solomon, M., Jacob, S., Crawley, J.N., Silverman, J.L., Larke, R.H., Sahagun, E., Pulger, K.R., Prida, M.C., Mendoza, S.P., 2014. In Long-term exposure to intranasal oxytocin in a mouse autism model (Ed.), Translational psychiatry 4 (11) e480–e484.

Benhamú, B., Martín-Fontecha, M., Vázquez-Villa, H., Pardo, L., López-Rodríguez, M.L., 2014. Serotonin 5-HT6 receptor antagonists for the treatment of cognitive deficits in Alzheimer’s disease. J. Med. Chem. 57 (17), 7160–7181.

Brodsky, M., Gibson, A.W., Smirnov, D., Nair, S.G., Neumaier, J.F., 2016. Striatal 5-HT6 receptors regulate cocaine reinforcement in a pathway-selective manner. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 41 (9), 2397–2407.

Burgué, E., Monteiro, P., Feng, G., Graybiel, A.M., 2013. Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. Science 340 (6137), 1243–1246.

Cai, Y., Wang, L., Natalvate, L., Xiao, R., Li, X., Fan, X., 2019. Citalopram antagonizes social behavior deficits in the BTBR T tf/J mouse model of autism. Brain Res. Bull. 150, 75–85.

Canal, C.E., Felsing, D.E., Liu, Y., Zhu, W., Wood, J.T., Perry, C.K., Vemula, R., Booth, R.G., 2015. An orally active phenylaminolactam-choline serotonin 5-HT7 and 5-HT1A receptor partial agonist that corrects motor stereotypy in mouse models. ACS Chem. Neurosci. 6 (7), 1259–1270.

CDC, 2016. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States. Link below: https://www.cdc.gov/mmwr/volumes/69/sr/pdfs/sr6904a1-hr.pdf.

de Bruin, N.M., Krueger, C.G., 2015. 5-HT6 receptor antagonists: potential efficacy for the treatment of cognitive impairment in schizophrenia. Curr. Pharm. Des. 21 (26), 3779–3795.

de Bruin, N., van Loeweza, A., Wicke, K.M., de Haan, M., Venhorst, J., Lange, J., de Groote, L., van der Neer, M., Prickaerts, J., Andriambeloson, E., Foley, A.G., van Drimmelen, M., van der Wetering, M., Krueger, C.G., 2016. The selective 5-HT6 receptor antagonist SLV has putative cognitive and social interaction enhancing properties in rodent models of cognitive impairment. Neurobiol. Learn. Mem. 133, 100–117.

de Jong, J.E., Mark, A., 2017. Antagonism of the 5-HT 6 receptor—preclinical rationale for the treatment of Alzheimer’s disease. Neuropharmacology 125, 50–63.

Ellegood, J., Crawley, J.N., 2015. Behavioral and neuronalanatomical phenotypes in mouse models of autism. Neurotherapeutics 12 (3), 521–533.

Gérard, C., Mestikawy, S.E., Leboeuf, J., Ruet, M., Traïnoff, E., Martres, M.P., 1996. Quantitative RT-PCR determination of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5, 7-dihydroxytryptamine-treated rats. Synapse 23 (3), 164–173.

Gérard, C., Martres, M.P., Lefebvre, K., Mical, M.C., Vergé, D., Lanfumey, L., El Mestikawy, S., 1997. Immuno-localization of serotonin 5-HT6 receptor-like material in the rat central nervous system. Brain research 746 (1-2), 207–238.

Glenson, R.A., Lee, M., Ragni, B.R., Dukat, M., Roth, B.L., Savage, J.E., McBride, A., Rauzer, L., Hudeisen, S., Lee, D.K., 2006. 2-Substituted tryptamine agents with selectivity for 5-HT6 serotonin receptors. J. Med. Chem. 43 (5), 1018–1021.

Gu, Y.P., Commons, K.G., 2017. Serotonin neuron abnormalities in the BTBR mouse model of autism. Autism Res. 10 (1), 66–77.

Herrick, K.F., Mark, A., Richard, N., Bundgaard, C., Bastlund, J.F., de Jong, L.E., 2016. The 5-HT 6 Receptor Antagonist Idalopirdine Potentiates the Effects of Acetylcholinesterase Inhibition on Neuronal Network Oscillations and Extracellular. Hirsch, W.D., Minton, J.A., Bronmide, S.M., Moss, S.F., Letter, A.J., Riley, G., Routledge, C., Middelminn, D.N., Price, G.W., 2000. Characterization of [(125)I]-SB-258585 binding to human recombinant and native 5-HT6 receptors in rat, pig and human brain tissue. Br. J. Pharmacol. 130 (7), 1597–1605.
Rebec, G.V., White, I.M., Puotz, J.K., 1997. Responses of neurons in dorsal striatum during amphetamine-induced focused stereotypy. Psychopharmacology 130 (4), 163–171.

Hu, L., Wang, B., Zhang, Y., 2017. Serotonin 5-HT6 receptors affect cognition in a mouse model of Huntington’s disease. Neuropharmacology 120, 117–126.

Liu, K.C., Li, J.Y., Xie, W., Li, L.B., Zhang, J., Du, C.X., Zhang, Y.M., Tan, H.H., Wang, H. & Jastrzebski-Wieczek, M., 2018. Role of 5-HT6 receptor antagonists in Parkinson’s disease: an update. Expert Opin. Investig. Drugs 27 (6), 523–533.

Kucinski, A., de Jong, I.E., Sarer, M., 2017. Reducing falls in Parkinson’s disease: interactions between donepezil and the 5-HT1F receptor agonist idalopirdine on C57 mice. Pharmacol. Biochem. Behav. 181, 110–116.

Liu, K.C., Li, J.Y., Xie, W., Li, L.B., Zhang, J., Du, C.X., Zhang, Y.M., Tan, H.H., Wang, H., Zhang, Y., 2016. Activation and blockade of serotonin1 receptors in the dorsal hippocampus enhance Tmaze and hole-board performance in a unilateral 6-hydroxydopamine rat model of Parkinson’s disease. Brain Res. 1650, 184–195.

Loiseau, F., Dekeyne, A., Millan, M.J., 2008. Pro-cognitive effects of 5-HT6 receptor agonists in the social recognition procedure in rats: implication of the frontal cortex. Psychopharmacology 196 (1), 93–104.

Marco, B., Gill, J., Hirt, W.D., Garcia-Allora, M., Ramirez, M.J., 2006. Lack of localization of 5-HT6 receptors on cholinergic neurons: implication of multiple neurotransmitter systems in 5-HT6 receptor-mediated acetylcholine release. Eur. J. Neurosci. 24 (5), 1523–1533.

McDougall, S.A., Valentine, J.M., Gonzalez, A.E., Humphrey, D.E., Widarma, C.B., Wilkes, B.J., Bass, C., Korah, H., Febo, M., Lewis, M.H., 2019. Volumetric magnetic resonance imaging of the adolescent period: age-dependent changes in dorsal striatal D2 high receptors. Neuropharmacology 130, 1–10.

Muehmann, A.M., Edington, G., Mikhalik, A.C., Buchwald, Z., Koppuzha, D., Korah, M., Lewis, M., 2012. Further characterization of repetitive behavior in C57 mice: developmental trajectory and effects of environmental enrichment. Behav. Brain Res. 235 (2), 143–149.

Pearson, B.L., Pobbe, R.H., Defensor, E.B., Oasay, L., Bolivar, V.J., Blanchard, D.C., Pobbe, R.L.H., Defensor, E.B., Oasay, L., Bolivar, V.J., Blanchard, D.C., 2020. Cross-site reproducibility of social deficits in group-housed BTBR mice using an automated three-chamber social approach task for mice. Current protocols in neuroscience 56 (1), 8 –214.

Puotz, J.K., Rebec, G.V., White, I.M., 2017. Allosteric modulation of nicotinic and GABAA receptors subtypes differentially modify autism-like behaviors in the BTBR mouse model. Neuropharmacology 126, 36–47.

Yun, H.M., Rhim, H., 2011. 5-HT6 receptor ligands, EMD368068 and SB265085, differentially regulate 5-HT6 receptor independent events. Toxicol. In Vitro 25 (8), 2035–2040.

Zhang, Y.M., Zhang, L., Wang, Y., Sun, Y.N., Guo, Y., Du, C.X., Zhang, J., Yao, L., Yu, S., Qi, L., 2016. Activation and blockade of preclinical 5-HT6 receptors produce different effects on depression-like behaviors inulin-injected and 6-hydroxydopamine-induced Parkinsson’s neuropsychopharmacology. Front. Aging Neurosci. 8, 351–361.