$GABA_{a2}$ Subunit Agonism Improves Social Memory in BTBR T+tf/J Mice

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http://jaxmice.jax.org/strain/002282.html

Introduction

Autism Spectrum Disorder is an illness characterized by aberrant social interactions, communication deficits and repetitive behaviors (McFarlane et al., 2008). The BTBR T+tf/J (BTBR) is an inbred mouse strain that has been demonstrated to possess several hallmark behavioral phenotypes of Autism Spectrum Disorder, including reduced social interactions and impaired social recognition (McFarlane et al., 2008). One of the characteristic hallmarks of this strain of mice is its reduced level of inhibitory neurotransmission in comparison to their C57BL/6J (B6) counterparts (Han et al., 2014). The primary mediator of inhibitory neurotransmission in the brain is the neurotransmitter GABA acting on its receptors, particularly the $GABA_A$. Thus, it is likely that autistic-like behaviors in these BTBR mice could result from these decreased levels of inhibitory neurotransmission (Han et al., 2014). Previous findings in our laboratory have indicated that selective subunit activation of α_2 significantly improves spatial memory in the BTBR mice (Yoseph and McFarlane, unpublished). The current study assesses the hypothesis that α_2 stimulation will improve social memory deficits in the BTBR mouse model as well.

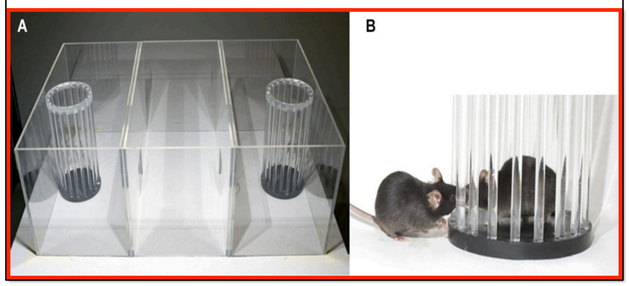
Methods

Drug Administration: TCS-1105 is a non-sedative anxiolytic benzodiazepine that acts as an agonist of the α_2 subunit. TCS-1105 will be dissolved in DMSO and ethanol and administered in 1mg/kg doses through intraperitoneal (i.p) injections 30 minutes prior to behavioral testing. Controls will be treated with a smilar volume of vehicle consisting of DMSO and ethanol solution.

Subjects: All C57BL/6J (B6) and BTBR mice strains will be bred at Kenyon College Research Laboratory (Gambier, Ohio). Mice will be raised in temperature controlled reverse lightning conditions in a 12:12 light/dark cycle; lights will be on at 0800 h and off at 2100 h. Testing will be conducted during the dark phase. Water and food were available *ad libitium*. All experimental procedures will be conducted in strict compliance with the National Institution of Health (NIH) guidelines and the local Institution Animal Care and Use Committee (IACUC) guidelines for the ethical care, treatment and use of laboratory animals.

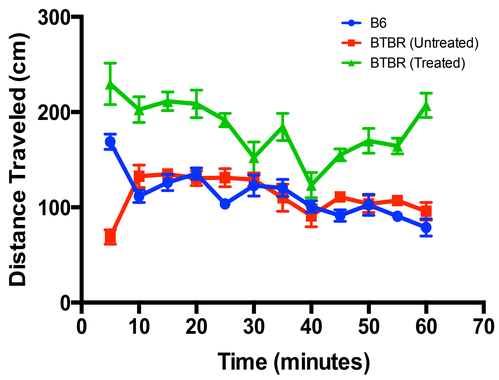
Open Field: In order to assess the locomotion of the test subjects prior to any drug administration, all subjects were placed individually in the center of an open field, where their behavior was recorded for thirty minutes. Subjects will habituate for ten minutes to the testing environment. Exploratory locomotive behavior will be measured and recorded via a VersaMax Animal Monitoring System (AccuScan Instruments, Columbus, Ohio, USA). Data will be analyzed using repeated measures ANOVA

Social Approach Testing: Twenty-four adult male BTBR mice and twelve adult male B6 mice will be used for this experiment. Drug or vehicle will be administered to all subjects ten minutes prior to the first habituation phase of the Social Approach test. Eight adult male BTBR mice and four adult male B6 mice will be used as strangers/familiars for this experiment and will not be treated. An automated three-chambered social approach test was purchased from Noldus (see below, taken from journal.fronteirsin.org). The protocol for the test follows four ten-minute phases. During the first habituation phase, subject will be placed in the center sociability chamber with doors to the side chambers shut. During the second habituation phase, doors of the side chambers will be opened to eliminate the possibility of the subject's preference to one side of the chamber as a confounding variable. Familiar and stranger mice will be encased in wire side chambers to ensure that all social interactions are initiated by the test subject. All behavior was recorded using Noldus Ethovision 9. Data will be analyzed using one-way ANOVA and Fisher's post-hoc test.



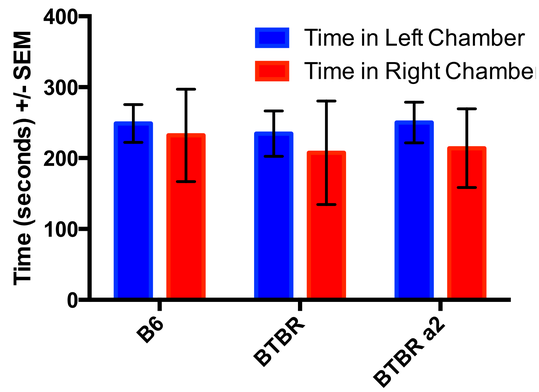
Figures and Results

Total Distance Traveled



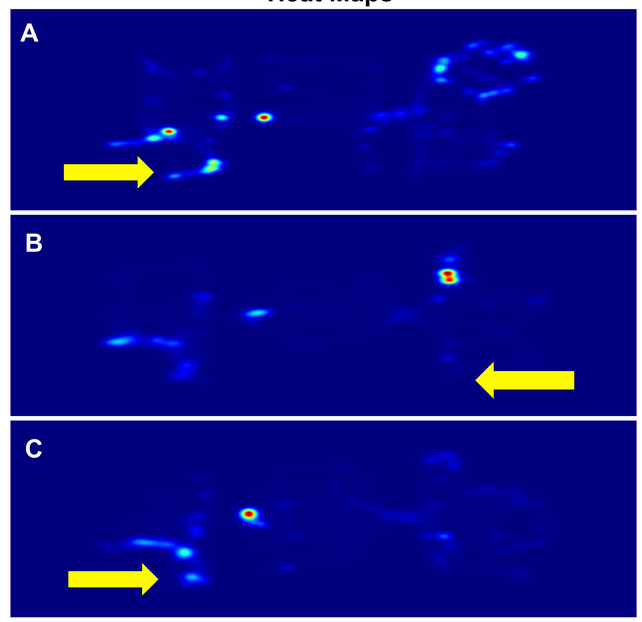
There was no significant difference in the total distance traveled between the B6 and the treated BTBR (M_{diff}=4.218, P=0.008), but there were significant differences found between B6 and untreated BTBR (M_{diff}= 17.99, P<0.0001) and between the untreated and the treated BTBR (M_{diff}= 13.78, P<0.0001).

Habituation



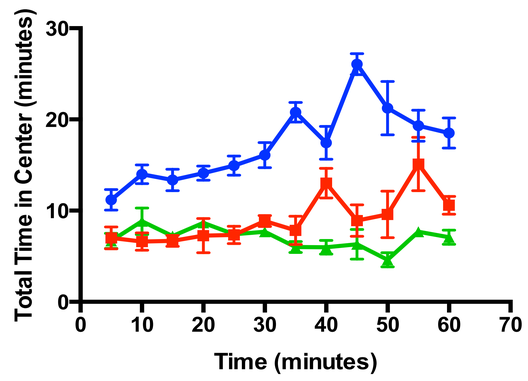
There was no significant difference in the time spent in either the right or left chambers for any of the three experimental groups (P<0.932).

Heat Maps



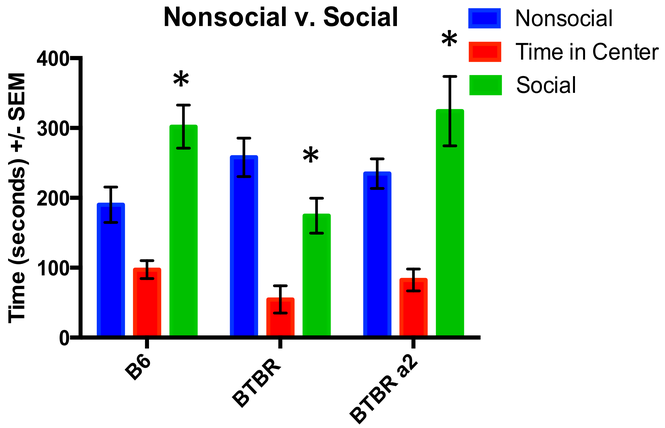
Heatmaps taken from Social Approach test. Yellow arrows indicate location of stranger in trial 4 of social approach test. Red areas indicate where subject has spent the majority of the time during the trial. Figure A is taken from a B6 subject, Figure B from a BTBR, and Figure C from a BTBR treated with a2 agonist.

Time in Center



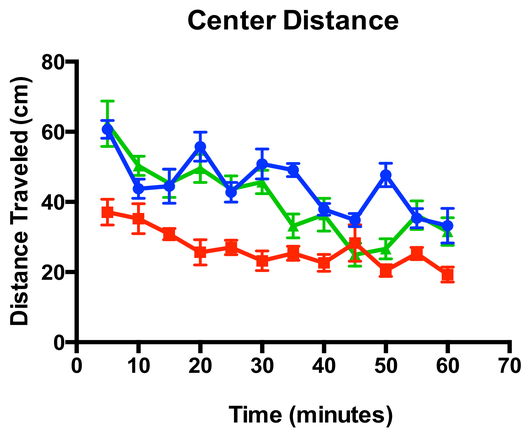
There was no significant difference in amount of time spent in the center of the open field between the B6 mice and the untreated BTBR (M_{diff}=.6417, P=0.1591), but there were significant differences found between B6 and treated BTBR (M_{diff}= 70.60, P < 0.0001) and between untreated and treated BTBR mice (M_{diff}= 71.24, P<0.0001).

Nonsocial v. Social



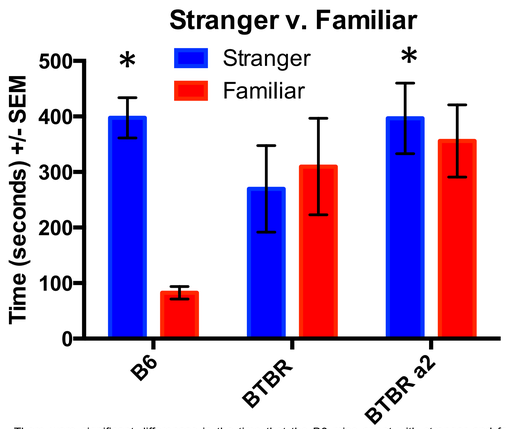
There were significant differences between the time spent in the center chamber and the nonsocial environment (M_{diff}=149.5, P<0.001), time in the social and nonsocial environments (M_{diff}=39.25, P<0.001), and the center and the social environments (M_{diff}=188.8, P<0.0001).

Center Distance



There was no significant difference in the center distance between untreated and treated BTBR (M_{diff}=2.054, P=0.3729), but there were significant differences found between B6 and untreated BTBR (M_{diff}= 8.186, P<0.0001) and between the B6 and the treated BTBR (M_{diff}= 10.24, P<0.0001).

Stranger v. Familiar



There were significant differences in the time that the B6 mice spent with stranger and familiar mice (M_{diff}= 314.9, P<0.0001) as well as the time that the BTBR a2 group spent with the stranger and familiar mice (M_{diff}= 80.14, P<0.001), but there was no significant difference in the time in the untreated BTBR group (M_{diff}=40.16, P=0.1018).

Discussion

BTBR mice treated with the GABA α_2 agonist showed dramatic increases in their social memory (see Fig. 5 & 6) by spending more time in a social environment over a nonsocial environment. Additionally, they showed a preference for social novelty over a familiar conspecific. As shown in the heatmaps, the treated BTBR group spent more time than the untreated group in the same chamber as the stranger, mirroring the findings found in the B6 group. A_2 treatment also increased generalized locomotion in BTBR mice (Fig. 1). There was a significant increase in the total distance traveled (Fig. 1), and while these mice spent less time in the center of the open field (Fig. 2.), they crossed through the center more often than the untreated BTBRs (Fig. 3.), which could indicate an overall decrease in anxiety. However, further testing using an elevated plus maze will be necessary to determine if the drug decreases anxiety levels in these mice. Ongoing research will include coding social approach videos to determine if there is an overall decrease in repetitive self-grooming in the mice treated with TCS-1105. Previous studies (Silverman, et al., 2010) suggest that repetitive self-grooming may be an anxiety-like behavior profile.

References

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