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Influence of stimulant-induced hyperactivity on social approach in the BTBR mouse model of autism

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ABSTRACT

Translational research is needed to discover pharmacological targets and treatments for the diagnostic behavioral domains of autism spectrum disorders. Animal models with phenotypic relevance to diagnostic criteria offer clear experimental strategies to test the efficacy and safety of novel treatments. Antagonists of mGluR5 receptors are in clinical trials for Fragile X syndrome and under investigation for the treatment of autism spectrum disorders. However, in preclinical studies of mGluR5 compounds tested in our laboratory and others, increased locomotion following mGluR5 modulation has been observed. Understanding the influence of general activity on sociability and repetitive behaviors will increase the accuracy of interpretations of positive outcomes measured from pharmacological treatment that produces locomotor activating or sedating effects. In the present studies, dose–response curves for D-amphetamine (AMPH)-induced hyperlocomotion were similar in standard B6 mice and in the BTBR mouse model of autism. AMPH produced significant, robust reductions in the high level of repetitive self-grooming that characterizes BTBR, and also reduced the low baseline grooming in B6, indicating that AMPH-induced hyperlocomotion competes with time spent engaged in self-grooming. We then tested AMPH in B6 and BTBR on the 3-chambered social approach task. One component of sociability, the time spent in the chamber with the novel mouse, in B6 mice was reduced, while the sniffing time component of sociability in BTBR mice was enhanced. This finding replicated across multiple cohorts treated with AMPH and saline vehicle. In-depth analysis revealed that AMPH increased the number and decreased the duration of sniffing bouts in BTBR, suggesting BTBR treated with AMPH mostly engaged in brief sniffs rather than true social interactions with the novel mouse during the social approach task. Our data suggest that compounds with stimulant properties may have some direct benefits on reducing repetitive behaviors in autism spectrum disorders, particularly in the subset of autistic individuals with hyperactivity.

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1. Introduction

Autism spectrum disorders (ASD) are defined by three diagnostic symptom domains: 1) qualitative impairments in social interaction, 2) deficits in communication, and 3) stereotyped repetitive behaviors with restricted interests (American Psychiatric Association, 1994; Dawson et al., 2010; Krasny et al., 2003; Landa, 2008; Lord et al., 2000; Zwaigenbaum et al., 2009). Recent genetic association investigations have identified a large number of autism susceptibility genes and copy number variants (Abrahams and Geschwind, 2008, 2010; Anney et al., 2010; Bucan et al., 2009; Happe and Ronald, 2008). At present, behavioral intervention is the only effective form of treatment, with many positive outcome measures reported (Dawson, 2008; Rogers and Vismara, 2008; Williams White et al., 2007). The only approved pharmacological treatments for autism are Risperidone (Risperdal®) and Aripiprazole (Abilify®), which target the associated symptoms of irritability that include self-injury, tantrums and aggression (Marcus et al., 2011; McCracken et al., 2002; McDougle et al., 2000; Varni et al., 2012). Translational research is needed to discover pharmacological targets for the diagnostic domains (McPheeters et al., 2011; Veenstra-VanderWeele and Blakely, 2012; Wink et al., 2010). Pursuing the discovery of effective pharmacological interventions requires appropriate preclinical screens. Animal models based on hypothesized causes of autism spectrum disorders, and/or with robust phenotypes of high relevance to diagnostic symptoms,

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offer innovative experimental strategies to test the efficacy and safety of proposed treatments.

Abnormal reciprocal social interactions include reduced interest in peers and difficulty maintaining social interaction, failure to use eye gaze and an absence of facial expressions. Communication deficits present as language delays, failure to respond to voices and lack of prosody or intonation. Repetitive behaviors with restricted interests include motor stereotypies (i.e. hand flapping or toe walking), repetitive use of the same objects, and insistence on sameness (American Psychiatric Association, 1994; DiCicco-Bloom et al., 2006; Landa, 2008; Lord et al., 2000). Several inbred mouse strains display impaired social affiliative behaviors despite normal levels of aggressive, reproductive, and maternal behaviors (Bolivar et al., 2007; Brodkin, 2007; Brodkin et al., 2004; Defensor et al., 2011; Moy et al., 2008; Panksepp et al., 2007; Pobbe et al., 2010; Ryan et al., 2010). BTBR T+tf/J (BTBR) is a commercially available inbred strain of mice that displays multiple behavioral phenotypes relevant to all three diagnostic symptoms of autism. Both male and female BTBR engage in marked low levels of reciprocal social interactions at juvenile and adult ages and lack species-typical sociability in the 3-chambered social approach task (Bolivar et al., 2007; Defensor et al., 2011; Pobbe et al., 2011, 2010; Silverman et al., 2010a; Yang et al., 2012a, 2009, 2007a, 2007b). BTBR emit significantly fewer ultrasonic vocalizations in response to social olfactory cues and during reciprocal social interactions, as compared to other standard inbred strains such as C57BL/6J (B6) (Scattoni et al., 2008, 2011; Wohr et al., 2011). BTBR also produce fewer scent marks in response to social olfactory pheromones, consistent with an interpretation of impaired communication (Roullet et al., 2011). BTBR display significantly higher levels of repetitive self-grooming throughout their lifespan as compared to control strains, replicated in multiple cohorts and across several laboratory environments (McFarlane et al., 2008; Pearson et al., 2011; Silverman et al., 2012, 2010a; Yang et al., 2007a; Yang et al., 2007b). Normal scores on measures of general health, motor functions, stress reactivity, acoustic startle reflex, prepulse inhibition and olfactory abilities (McFarlane et al., 2008; Silverman et al., 2010c; Yang et al., 2012a) support an interpretation of remarkably specific autism-relevant abnormalities in BTBR. Investigation into the background genes responsible for autism-relevant behavioral traits in inbred strains of mice is ongoing (Bolivar et al., 2011; Bothe et al., 2011; Jones-Davis et al., 2011; McFarlane et al., 2008). This genetically homogenous, commercially available strain provides a useful model system for assessing pharmacological therapeutics, with particular relevance to those individuals with autism whose genotypic variant is unknown. It is important to note, however, that BTBR differs from the conventional models of autism that are based on targeted mutations of candidate genes for autism. Despite their unknown genetics, BTBR is among the best animal models of autism in terms of face validity to core symptomatology, robustness and replicability of phenotypes.

We and others have employed experimental interventions to evaluate genetic reversal and pharmacological rescue in mouse models of neurodevelopmental disorders (Cobb et al., 2010; Dolen et al., 2007; Ehninger et al., 2008a, 2008b; Guy et al., 2007; Hayashi et al., 2007; Meikle et al., 2008; Ogier et al., 2007; Penagarikano et al., 2011; Silverman et al., 2012, 2010a; Yan et al., 2005; Zhou et al., 2009). Discovery of elevated mGluR5-mediated signaling and protein synthesis in Fragile X knockout mice provided the rationale for testing mGluR5 antagonists in Fragile X clinical trials (Bear et al., 2004; Dolen et al., 2007; Jacquemont et al., 2011; Krueger and Bear, 2011). We recently reported beneficial actions of mGluR5 antagonists on reducing repetitive self-grooming in BTBR (Silverman et al., 2012, 2010a). Improvements in some parameters of sociability were detected in BTBR mice treated with an mGluR5 negative allosteric modulator, GRN-529 (Silverman et al., 2012). However, a potential confound was noted. Increased entries in social approach accompanied the improved sociability in the automated 3-chambered apparatus. Similarly, in a novel open field test conducted with the same BTBR and B6 mice, increased distance traversed after treatment with mGluR5 antagonists was seen, consistent with other reports of mild hyperactivity after mGluR5 antagonist treatments (Mehta et al., 2011; Montana et al., 2009; Thomas et al., 2012).

Understanding the influence of general activity levels on sociability and repetitive behaviors will enhance the accurate interpretation of positive outcomes measured from any test compound that produces locomotor activating or sedating effects. In the present study, we tested the hypothesis that endogenous and drug-induced hyperactivity have direct effects on social and repetitive behaviors in the BTBR mouse model of autism. Specifically, doses of AMPH that increased open field locomotion were administered to BTBR and B6 mice in the repetitive self-grooming assay, and in our automated 3-chambered social approach assay, to evaluate the possibility that higher general exploration contributes to reduced repetitive and enhanced social behaviors.

2. Materials and methods

2.1. Mice

C57BL/6J (B6) and BTBR T+tf/J (BTBR) mice were the offspring of breeding pairs purchased from The Jackson Laboratory (JAX, Bar Harbor, ME). All mice were housed and bred in a conventional mouse vivarium at the National Institute of Mental Health (NIMH), Bethesda, Maryland, USA, using harem breeding trios. After two weeks with a male, females were separated into individual cages (Tecniplast, USA) before parturition. Pups were kept with the dam until weaning at postnatal day 21. After weaning, juveniles were group housed by sex and strain in standard plastic cages in groups not exceeding four per cage. Cages were maintained in ventilated racks in a temperature (20 °C) and humidity (~55%) controlled vivarium on a 12 h circadian cycle, lights on from 0700 to 1900 h. Standard rodent chow and tap water were available *ad libitum*. In addition to standard bedding, a Nestlet square, shredded brown paper and a cardboard tube were provided in each cage. Light levels measured approximately 325 lux during the light phase. Background noise measured approximately 50–60 dB. All procedures were approved by the National Institute of Mental Health Animal Care and Use Committee.

2.2. Drug treatment

D-amphetamine sulfate salt (AMPH; 1.0, 2.0 and 3.0 mg/kg, Sigma Aldrich, St. Louis, MO) was dissolved in saline (0.9% NaCl). Adult male and female B6 and BTBR mice weighing 25–40 g received an intraperitoneal (i.p.) injection of saline vehicle or AMPH 30 min before the start of behavioral test sessions for the social approach, self-grooming, and open field behavioral tasks. Dose response curves and post treatment interval in open field locomotion were determined using previously published literature (Kelley et al., 1986; Mueller et al., 1989; Papaleo et al., 2008; Sills et al., 1998; Stromberg and Svensson, 1975; Yates et al., 2007).

2.3. Experimental design for behavioral assays

Testing was conducted in dedicated behavioral testing rooms during the standard light phase, usually between 0900 and 1600 h. Prior to each behavioral test, mice were acclimatized to the behavioral testing area for at least 60 continuous minutes. Testing began at ages 6–8 weeks. Treatment groups consisted of 10–16 mice per strain for each dose of drug or vehicle. Previous studies in our laboratory documented no sex differences on either sociability or self-grooming in BTBR or B6 (Silverman et al., 2010a; Yang et al., 2009, 2007a, 2007b). Therefore, male and female mice were used in all studies in approximately equal proportions. A single cohort (Cohort 1) was utilized to collect AMPH dose response curve data in the open field locomotion task. Each additional cohort used a between treatment factor design with a one week washout period, such that each mouse received an acute dose of AMPH or vehicle, and was tested in a behavioral task, one task per week. For Cohorts 2 and 3, each mouse was used for all three behavioral tests, and received a total of three injections randomized across AMPH and vehicle. The behavioral task order was social approach (week 1), self-groom (week 2) and open field (week 3). For Cohort 4, each mouse was used for two behavioral tests (social approach and self-grooming), and received a total of two injections randomized across AMPH and vehicle. The task order was social approach (week 1) followed by self-groom (week 2). Drug doses, toe tattoo patterns, and digital videotapes were coded to ensure that the raters were blind to the treatment condition. All procedures were conducted in