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Inhibition of Amphetamine-Induced Locomotor Hyperactivity by D1 and D2 Dopamine Receptor Antagonists in the C57BL/6J and BTBR T+tf/J Mouse Strains Neuroscience

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Inhibition of Amphetamine-Induced Locomotor Hyperactivity by D1 and D2 Dopamine Receptor Antagonists in the C57BL/6J and BTBR T+tf/J Mouse Strains

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Introduction

The BTBR T+ tf/J (BTBR) mouse strain has been demonstrated to have good face validity for the cardinal symptoms of autism spectrum disorder due to its behavioral phenotypes which include atypical and reduced reciprocal social interactions and increased repetitive grooming (McFarlane et al., 2008). Ongoing research in the McFarlane lab has observed low tissue levels of dopamine in the frontal cortex, hippocampus, and striatum of BTBR. Additionally, BTBR were demonstrated to exhibit potentiated locomotor hyperactivity in response to a low dose of amphetamine, an indirect dopamine agonist, relative to the control mouse strain C57BL/6J (B6). These findings suggest that the dopamine system of BTBR differs in some way from that of B6. The dopamine pathway is involved in mediating locomotion and reward (Carlson, 2010) and may therefore contribute to BTBR's behavioral abnormalities. One possible hypothesis is that the low tissue levels of dopamine in BTBR might result in higher sensitivity of dopamine receptors, greater receptor density, or both (Kim et al., 2000), which might contribute to the potentiation of amphetamine-induced locomotor hyperactivity in BTBR.

The present study proposed to evaluate the involvement of the D1 and D2 dopamine receptor subtypes in the BTBR amphetamine response by administering BTBR and B6 mice with low doses of the D1 antagonist SCH23390 and the D2 antagonist haloperidol prior to amphetamine administration. In the phenotypically normal B6 mouse strain, this was expected to inhibit the increase in locomotor activity that amphetamine normally elicits in rodents (O'Neill and Shaw, 1999). It was hypothesized that the degree of locomotor inhibition produced by the antagonists would be more pronounced in BTBR than B6, due to greater D1 and D2 dopamine receptor sensitivity and/or density.

Methods

Animals:
Adult male C57BL/6J (*n* = 48) and BTBR T+ tf/J (*n* = 48) mice were bred at Kenyon College and housed no more than four animals per cage on a 12/12 hour light/dark cycle with food and water available ad libitum. Twelve animals were used for each drug treatment.

Drug Treatments:
Amphetamine (AMPH) was dissolved in 0.9% NaCl saline, SCH23390 (SCH) was dissolved in distilled water, and haloperidol (HAL), which was dissolved in tartaric acid, diluted with DI water, and brought to a neutral pH with NaOH. All injections were given i.p. at a volume of 3.6µL/g body weight. There were four drug treatments, all of which included a pretreatment injection followed by a second injection, as described below.

	Pretreatment	Treatment
1.	Vehicle	Saline
2.	Vehicle	2.5 mg/kg AMPH
3.	0.06 mg/kg HAL	2.5 mg/kg AMPH
4.	0.0075 mg/kg SCH	2.5 mg/kg AMPH

The doses of HAL and SCH23390 (SCH) used did not significantly inhibit baseline locomotor activity in pilot studies (Figs. 1,2).

Behavioral Testing:
Mice were allowed to habituate individually in VersaMax Animal Activity Monitor Cages (AccuScan Instruments, Columbus, OH, USA) for 30 minutes, received pretreatment injections, and were returned to the Activity Monitors for another 45 minutes to allow the antagonists to reach maximum effectiveness (Cabib). Mice then received injections of saline or amphetamine and locomotor activity was monitored by open field test for the following 60 minutes.

Figures and Results

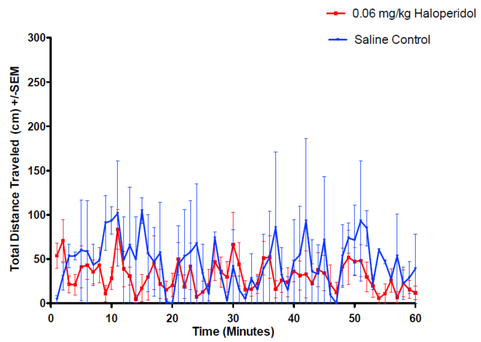

Figure 1. B6 mice administered with 0.06 mg/kg HAL (*n* = 8) did not show significantly diminished locomotor activity compared to saline-injected B6 mice (*n* = 4) [F(1,59) = 0.594, p = 0.4629].

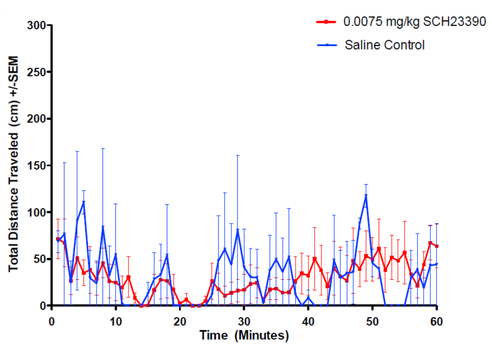

Figure 2. B6 mice administered with 0.0075 mg/kg SCH (*n* = 8) did not show significantly diminished locomotor activity compared to saline-injected B6 mice (*n* = 4) [F(1,59) = 0.591, p = 0.4641].

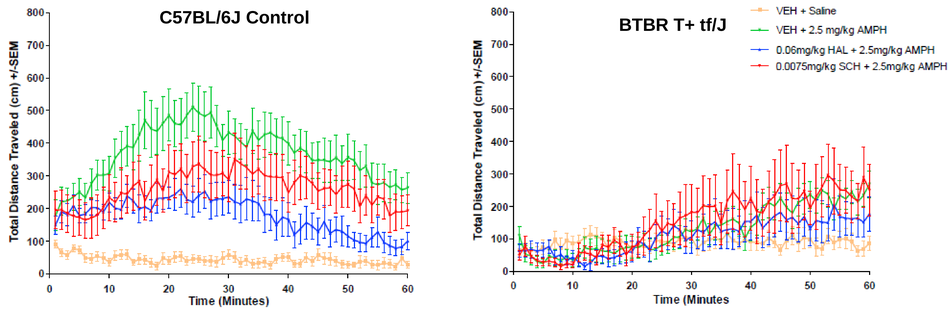

Figure 3. There was a difference between strains in locomotor response to drug treatment over time. The total distance traveled was affected by strain [F(1,88) = 9.552, p = 0.0027], drug treatment [F(3,88) = 7.228, p = 0.0002], time [F(59,5192) = 10.289, p < 0.0001], the interaction between strain and treatment [F(3,88) = 3.893, p = 0.0116], and the interaction between time, strain and treatment [F(177,5192) = 2.273, p < 0.0001]. B6 mice showed an increase in locomotor activity in response to AMPH that was inhibited by both HAL and SCH, whereas there was no significant drug effect for BTBR mice [F(3,44) = 0.523, p = 0.6689].

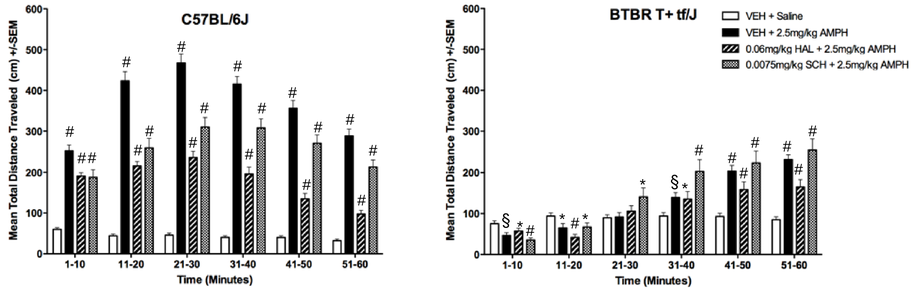

Figure 4. BTBR mice exhibit a delayed response to amphetamine relative to B6 controls. In B6 mice, all amphetamine treatments caused significant increases in mean total distance travelled during each 10-minute time interval. Conversely, BTBR mice only exhibited maximal amphetamine response during the last 20 minutes of the locomotor test. Student's *t*-test between VEH + Saline and Pretreatment + AMPH groups, *p < 0.05, §p < 0.01, #p < 0.001.

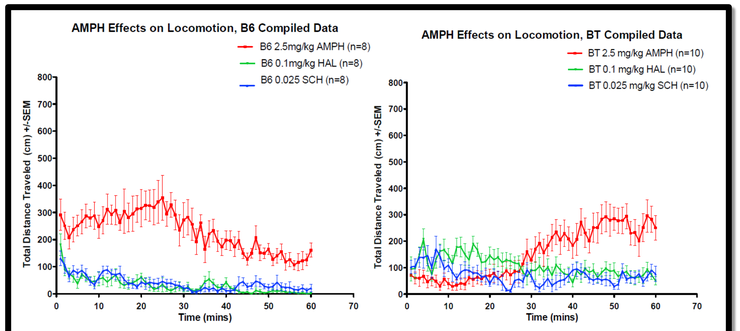

Figure 5. B6 and BTBR mice responded differently to 2.5 mg/kg AMPH and high doses of HAL and SCH without pretreatment. To evaluate the effect of pretreatment on AMPH response and to characterize BTBR's response to higher doses of D1 and D2 receptor antagonists, C57BL/6J (*n*=8 for each treatment) and BTBR T+ tf/J (n=10 for each treatment) mice were administered with either 2.5 mg/kg AMPH, 0.1 mg/kg HAL, or 0.025 mg/kg SCH and tested for locomotor activity for 60 minutes. The total distance traveled was affected by drug treatment [F(2,48) = 28.4765, p < 0.0001], time [F(59,2832) = 2.097, p < 0.0001], the interaction between strain and treatment [F(2,48) = 7.813, p = 0.0012], and the interaction between time, strain and treatment [F(118,2832) = 7.142, p < 0.0001].

Discussion

Conclusion: BTBR mice show an abnormal response to both the dopamine agonist amphetamine and the D1 and D2 antagonists haloperidol and SCH23390, suggesting abnormal dopamine system function. The D1 and D2 antagonists haloperidol and SCH23390 do not reduce amphetamine-induced hyperactivity in BTBR mice. Furthermore, BTBR show an abnormally delayed response to amphetamine, which is unaffected by the pretreatment regimen used in this study.

The failure of D1 and D2 antagonist administration to reduce amphetamine-induced hyperactivity in BTBR mice suggests that their abnormal amphetamine response is not directly mediated by an increased sensitivity of these two receptor types. Furthermore, BTBR did not demonstrate depressed locomotor activity after receiving higher doses of HAL or SCH alone, which indicates that these receptors may be less abundant and/or sensitive, antithetical to our initial hypothesis. However, our findings are suggestive of an abnormal *uninvolvement* of dopamine receptors in BTBR's amphetamine response. Future research regarding the BTBR mouse strain will therefore aim to assess dopamine receptor irregularities, characterize the time course of drug responses, and investigate whether the dopamine transporter (DAT), another target of amphetamine, mediates abnormal amphetamine response.

References

•Carlson, Neil R. 2010. *Physiology of Behavior* (10th ed.) (p 121,625). Boston: Allyn and Bacon.
•Kim, D.S., Szczypka, M.S., and R.D. Palmiter. 2000. Dopamine-deficient mice are hypersensitive to dopamine receptor agonists. The Journal of Neuroscience 20(12): 4405-4413.
•McFarlane, H.G., Kusek, G.K., Yang, M., Phoenix, J.M., Bolivar, V.J., and J.N. Crawley. 2008. Autism-like behavioral phenotypes in BTBR T+ tf/J mice. Genes Brain and Behavior 7: 152-163.
•O'Neill, M.F. and Shaw, G. 1999. Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801, and the dopamine D1 agonist C-APB in mice. Psychopharmacology 145: 237-250.
•Smith, F.L., St. John, C., Yang, T.F.T., W.H. Lyness. 1989. Role of specific dopamine receptor subtypes in amphetamine discrimination. Psychopharmacology 97: 501-506.

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