Title

Chronic antipsychotic treatment exerts limited effects on the mania-like behavior of dopamine transporter knockdown mice.



Authors

Cope, Zackary
Kenton, Johnny
Minassian, Arpi
et al.

Publication Date

2021-05-07

DOI

10.1016/j.bbr.2021.113167

Peer reviewed

HHS Public Access

Author manuscript

*Behav Brain Res*. Author manuscript; available in PMC 2023 December 19.

Published in final edited form as:

*Behav Brain Res*. 2021 May 07; 405: 113167. doi:10.1016/j.bbr.2021.113167.

# Chronic antipsychotic treatment exerts limited effects on the mania-like behavior of dopamine transporter knockdown mice

**Zackary Cope**[1], **Arpi Minassian**[1,2], **Maureen V. Martin**[1], **William Perry**[1], **Christoffer Bundgaard**[3], **Jørn Arnt**[4], **Jordy van Enkhuizen**[1], **Mark A. Geyer**[1,5], **Jared W. Young**[1,5,*]

[1]Department of Psychiatry, University of California San Diego, 9500 Gilman Drive MC 0804, La Jolla, CA 92093-0804

[2]Center of Excellence for Stress and Mental Health and Research Service, VA San Diego Healthcare System

[3]H. Lundbeck A/S. Neuroscience Research, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark

[4]Sunred Pharma Consulting, Solrød Strand, Denmark

[5]Research Service, VA San Diego Healthcare System, San Diego, CA

## Abstract

**Background:** Bipolar disorder has been linked to dopamine transporter (DAT) polymorphisms and reduced DAT levels in postmortem brains.

**Aims:** The purpose of this study was to examine the effects of $D_2$-family receptor antagonists on DAT dysfunction-mediated mania behavior in mice.

**Methods:** DAT knockdown mice received either $D_2$-family receptor antagonist risperidone or asenapine and mania-related behaviors were assessed in the behavioral pattern monitor.

**Results:** Chronic risperidone did not reverse mania-like behavior in DAT knockdown mice. Chronic asenapine reduced mania behavior but this effect was more pronounced in wildtype littermates than in DAT knockdown mice.

**Conclusion:** Taken together, these findings suggest that while acute antipsychotic treatment may be beneficial in management of bipolar mania, it may not be necessary or effective to continue chronic antipsychotic treatment after an acute mania episode has been reversed. Further, it may be useful to explore whether DAT polymorphisms can predict chronic antipsychotic nonresponse in bipolar disorder mania.

### Keywords

bipolar disorder; mania; dopamine transporter; behavioral pattern monitor; antipsychotic; mice

[*]Correspondence: Jared W. Young, Ph.D., Department of Psychiatry, University of California San Diego, 9500 Gilman Drive MC 0804, La Jolla, California, 92093-0804, Tel: +1 619 543 3582, Fax: +1 619 735 9205, jaredyoung@ucsd.edu.

## Introduction

Bipolar disorder (BD) is a frequently disabling, life-threatening mental illness that occurs in >2.5% of the global population. BD is characterized by striking and persistent mood fluctuations ranging from mania to depression, driving clinically significant impairment (Grande et al., 2016). Current treatments include antidepressants and mood stabilizers, with more recent use of antipsychotics (Sachs, 2003). Today, a majority of patients with BD are prescribed antipsychotics (Ventimiglia et al., 2009). Not all treatments are effective in all patients however, and the neurobiological specificity of actions of these drugs remains to be elucidated. While mood stabilizers can vary in mechanism, the common mechanism for antipsychotics is dopamine $D_2$ receptor blockade (Stroup et al., 2003). Determining whether such blockade remediates specific behaviors associated with mania would prove beneficial to tailor patient-targeted treatments.

Neurobiological and genetic studies have implicated dopamine dysfunction in the etiology of BD. Impaired dopamine homeostatic mechanisms may result in extreme shifts to and from hyperdopaminergia and hypodopaminergia possibly driving mania and depressive states (Ashok et al., 2017). Altered dopaminergic states could arise from altered homeostatic clearance mechanisms, such as reduced levels of the dopamine transporter (DAT), which removes dopamine from the synaptic cleft. Reduced DAT levels were reported in unmedicated people with BD euthymia (Anand et al., 2011), potentially arising from genetic polymorphism-induced reduction in DAT expression (Greenwood et al., 2006; Horschitz et al., 2005). Targeted reduction of DAT expression results in seasonal sensitivity consistent with BD (Young et al., 2018), while DAT knockdown (KD) mice recreate many behavioral aspects of BD mania (Young et al., 2011; Greenwood et al., 2006; Pinsonneault et al., 2011; Zhuang et al., 2001) with a high fidelity of reproducibility such as in the cross-species behavioral pattern monitor (BPM) (Kwiatkowski et al., 2017). Specifically in the BPM, both BD mania patients and DAT KD mice exhibit hyperactivity, increased exploration, and altered locomotor patterns including straighter patterns of movement (reduced spatial d) (Perry et al., 2009), behaviors that are consistent over time (Minassian et al., 2011). Further, acute DAT blockade with GBR12909 consistently induces such behaviors, and the hyperactivity of both models can be reduced with mood stabilizers such as lithium or valproate (van Enkhuizen et al., 2013). Determining whether selective dopamine $D_2$ receptor blockade could more completely remediate such mania symptoms from reduced DAT function is important.

Hence, we tested the effects of chronic treatment with two atypical antipsychotics in the mouse behavioral pattern monitor (BPM) using DAT KD mice. We predicted that blockade of dopamine $D_2$-family receptors would affect the BD mania model mice more strongly than controls, attenuating mania-like exploratory behavior.

## Materials and methods

### Animals

All animals were group housed (max. 4 per cage) and maintained in a temperature-controlled vivarium (21 ± 1°C) on a reversed day-night cycle (lights on at 1900 hours and

off at 0700 hours). All mice had ad libitum access to food and water. Testing was performed during the dark phase of the day-night cycle between 0800 and 1800 hours. All procedures were approved by the UCSD Institutional Animal Care and Use Committee. The UCSD animal facility meets all federal and state requirements for animal care. Male and female adult DAT KD and WT littermates on C57BL/6J background were used to study the effects of chronic risperidone and chronic asenapine in the BPM.

## Drug Treatment

Drug solutions were prepared daily and chronically administered for 28 days, and also 60 minutes prior to testing on the last day. Risperidone (Sigma-Aldrich, St. Louis, MO) was dissolved in water at concentrations of 0.03 mg/kg/day and 0.3 mg/kg/day. Risperidone doses were determined based upon previous research (Duncan et al., 2006). Asenapine maleate powder was obtained from Lundbeck and dissolved in saline at 0.03 and 0.1 mg/kg/day, injected at a volume of 5 ml/kg and administered via osmotic minipumps (Alzet, type 2004). Asenapine doses were determined based on previous studies (Ene et al., 2015). See Table 1 for the experimental design.

## Behavioral Pattern Monitor

The mouse BPM was used with equipment and protocols as described previously by our group (Risbrough et al., 2006) (van Enkhuizen et al., 2013). Briefly, Plexiglas chambers were used to record the animals' movements and exploratory behavior. Chambers consist of a 30.5 x 61 x 38 cm area illuminated by a single light source located above the arena. The chamber contains three floor holes and eight wall holes equipped a grid of 12 x 24 infrared photobeams 1 cm above the floor (2.5 cm apart; 24 x 12 X-T array) that enable the detection of holepoking by the animals. A separate set of 16 infrared photobeams located 2.5 cm above the floor to detect mouse rearings. The location of the mouse is recorded every 0.1 s, with its position defined across nine unequal regions (four corners, four walls, and center). Mice were placed at the bottom left-hand corner of the chamber, facing the corner. Activity measures are obtained from a grid of 12 x 24 infrared photobeams 1 cm above the floor (2.5 cm apart; 24 x 12 X-Y array). The measures recorded during the 60 min session are described in Table 2.

The primary measures investigated in the current study were transitions and center entries representing locomotor activity, holepoking and rearing representing exploratory behavior, and spatial d representing locomotor patterns. Spatial d measures geometric patterns of locomotor activity – i.e., straight line movements versus more circumscribed paths of movement. A spatial d value closer to 1 reflects a straighter path, and values closer to 2 indicating highly circumscribed small-scale movements (Kwiatkowski et al., 2017).

Plasma concentrations of asenapine and risperidone were determined from trunk blood taken after treatment and decapitation using ultra performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS). Plasma samples were precipitated with acetonitrile and DMSO (80:20 v/v) containing internal standard. Following centrifugation, 10 ul was injected onto the chromatographic system (Acquity UPLC system; Waters, Milford, MA). MS detection was performed on a Sciex-API 400 mass spectrometer (AB

Sciex. Foster City, CA). The lower limit of quantification was 5 ng/ml and 1 ng/ml for asenapine and risperidone.

### Data analysis

Data were analyzed using a two-way or three-way analysis of variance (ANOVA) with drug, genotype, and sex as between subject factors. Significant main effects and interactions were followed up with one-way ANOVAs as well as Tukey *post hoc* analyses. The data were analyzed for 60 min testing periods using the BMDP statistical software (Statistical Solutions Inc., USA). The alpha was set at 0.05.

## RESULTS

### Chronic risperidone effects on exploration in DAT KD and WT mice

**Risperidone on activity—**Male and female mice were used in this experiment. A main effect of genotype was observed on all measures of activity, including counts [$F_{(1,79)}$=107.56, p<0.0001], transitions [$F_{(1,79)}$=98.38, p<0.0001], center entries [$F_{(1,79)}$=51.66, p<0.0001], distance travelled [$F_{(1,79)}$=112.81, p<0.0001], with KD mice more active than WT littermates. There was an effect of sex on activity as measured by counts [$F_{(1,79)=}$4.01, p<0.05] and distance travelled [$F_{(1,79)}$=4.05, p<0.05] and a trend toward an effect of sex on transitions [$F_{(1,79)=}$3.39, p<0.1], with males exhibiting higher levels than females. No sex x genotype interactions were observed.

Risperidone treatment exerted a main effect on center entries [$F_{(2,79)}$=3.89, p<0.05], with a trend effect on counts [$F_{(2,79)}$=2.51, p<0.1] and distance travelled [$F_{(2,79)}$=2.63, p<0.1]. No genotype x drug interactions were observed, but there was a significant sex x drug x genotype interaction on transitions [$F_{(2,79)}$=3.25, p<0.05], as well as a trend toward a significant sex x drug x genotype interaction on center entries [$F_{(2,79)}$=5.59, p<0.01] and distance travelled [$F_{(2,79)}$=3.01, p<0.1]. No other interactions were observed. Post-hoc analyses revealed that for center entries DAT KD mice were not different compared to WT vehicle-treated animals at the highest risperidone dose, suggesting possible attenuation of the DAT KD effect at this dose (see Figure 1, Table 3).

**Risperidone on exploration—**We examined the effects of sex, genotype, and risperidone on inspective and diversive exploration. There were significant effects of genotype on all measures of inspective and diversive exploration – holepoking [$F_{(1,79)}$=14.57, p<0.0005], repeated holepoking [$F_{(1,79)}$=13.56, p<0.0005], varied holepoking [$F_{(1,79)}$=13.86, p<0.0005], rearing [$F_{(1,79)}$=30.46, p<0.0001], and center duration [$F_{(1,79)}$=5.31, p<0.05]. There was a trend toward an effect of risperidone on center duration [$F_{(1,79)}$=2.60, p<0.1]. There was a significant effect of sex on rearing [$F_{(1,79)}$=11.09, p<0.005], with males exhibiting more rearing. There was a significant interaction effect of sex x genotype on varied poking [$F_{(1,79)}$=4.72, p<0.05], There were no other significant interaction effects on exploration.

Post-hoc analyses revealed a trend toward an increase in holepoking in DAT KD vehicle and DAT KD 0.03 mg/kg risperidone-treated animals compared to WT 0.3 mg/kg risperidone-treated animals, and a significant increase in DAT KD 0.3 mg/kg risperidone-treated

animals compared to WT 0.3 mg/kg risperidone ($p<0.05$), suggesting risperidone had a larger effect on reducing holepoking in WT animals compared to DAT KD animals. For varied holepoking, there was a trend toward an effect of DAT KD 0.03 mg/kg risperidone compared to WT 0.3 mg/kg risperidone and a significant increase in DAT KD 0.3 mg/kg risperidone compared to WT 0.3 mg/kg risperidone, suggesting high dose risperidone may have opposite effects on varied holepoking in DAT KD mice compared to WT mice, and only decreases varied holepoking in WT mice at the doses studied. For repeated holepoking, DAT KD vehicle, DAT KD 0.03 mg/kg risperidone and DAT KD 0.3 mg/kg risperidone treated mice showed a trend toward an increase compared to WT 0.3 mg/kg risperidone, again demonstrating differential effects of risperidone in WT and DAT KD mice. Post-hoc analyses revealed an increase in rearing in DAT KD mice compared to WT littermates and this effect was most pronounced when comparing DAT KD 0.3 mg/kg risperidone mice to WT 0.3 mg/kg risperidone treated mice ($p<0.01$), suggesting this antipsychotic has a general sedating effect but does not specifically ameliorate the underlying issue of DAT dysfunction (see Figure 1). Center duration was increased in DAT KD 0.03 mg/kg risperidone-treated animals compared to WT 0.3 mg/kg risperidone-treated animals ($p<0.05$), suggesting less aversion to risk in risperidone-treated DAT KD mice compared to risperidone-treated WT mice.

**Risperidone on locomotor patterns—**There were significant effects of genotype on all locomotor pattern variables – spatial d [$F_{(1,79)}=19.53$, $p<0.0001$], entropy h [$F_{(1,79)}=93.97$, $p<0.0001$], spatial CV [$F_{(1,79)}=4.08$, $p<0.05$], temporal CV [$F_{(1,79)}=11.80$, $p<0.001$]. There were no effects of sex on locomotor patterns. There were no significant effects of risperidone on locomotor pattern, nor were there any significant interaction effects on locomotor pattern. Post-hoc analyses revealed a significant decrease in spatial d in KD DAT vehicle treated mice compared to WT vehicle treated mice ($p<0.05$) and WT 0.3 mg/kg risperidone ($p<0.01$). A priori hypothesis testing revealed a trend toward a decrease in KD DAT 0.3 mg/kg risperidone mice compared to WT vehicle-treated and KD DAT 0.03 mg/kg risperidone-treated mice compared to WT 0.3 mg/kg risperidone-treated mice. Post-hoc analyses revealed an increase in entropy *h* in all DAT KD groups compared to all WT groups ($p<0.01$). There were no significant post-hoc results for spatial CV. There was an increase in temporal CV in WT 0.3 mg/kg risperidone mice compared to DAT KD vehicle (trend), 0.03 mg/kg risperidone ($p<0.01$) and 0.3 mg/kg risperidone (trend) treated animals.

## Chronic asenapine effects on exploration in DAT KD and WT mice

**Asenapine on activity—**Only male mice were studied in this experiment. There was a significant effect of genotype on activity as reflected by increased counts [$F_{(1,60)}=134.7$, $p<0.0001$], transitions [$F_{(1,60)}=77.2$, $p<0.0001$] and distance travelled [$F_{(1,60)}=24.64$, $p<0.0001$]. There was a main effect of asenapine on counts [$F_{(2,60)}=11.8$, $p<0.0001$] and transitions [$F_{(2,60)}=4.9$, $p<0.05$] while an interaction between genotype and asenapine was only observed for counts [$F_{(2,60)}=6.1$, $p<0.005$, see Figure 2], but not transitions. Post-hoc analyses revealed that WT mice receiving the highest dose of asenapine (1 mg/kg/day) exhibited fewer counts compared with vehicle-treated WT mice ($p<0.05$).

**Asenapine on exploration—**DAT KD mice in this experiment did not exhibit increased holepoking, repeated holepoking or varied holepoking but reared more [$F_{(1,60)}$=9.7, p<0.005] than WT mice. Asenapine tended to affect holepoking [$F_{(2,60)}$=2.5, p<0.1] and did affect rearing [$F_{(2,60)}$=11.1, p<0.0005]. Genotype and asenapine tended to interact in holepoking [$F_{(2,60)}$=3.0, p<0.1], while significant interactions were observed on rearing [$F_{(2,60)}$=4.7, p<0.05] (see Figure 2). Post-hoc analyses revealed that WT mice receiving the highest dose of asenapine (1 mg/kg/day) tended to exhibit fewer holepokes ($p$<0.1) and exhibited fewer rears (p<0.05) compared with WT vehicle-treated mice.

**Asenapine on locomotor patterns—**DAT KD mice exhibited more linear movements as measured by lower spatial $d$ [$F_{(1,60)}$=9.0, p<0.005] and more erratic patterns of movement as measured by higher entropy h [$F_{(1,60)}$=7.6, p<0.0001] compared with WT mice. Asenapine did not affect spatial d (F<1.8, ns) but did lower entropy h, increasing the predictability of movement [$F_{(2,60)}$=7.6, p<0.005] (see Figure 2, Table 4). Genotype and asenapine did not interact in any measure of locomotor patterns (F<2, ns).

### Plasma concentrations of risperidone and asenapine in DAT KD and WT mice

Plasma levels of risperidone (N = 25) and asenapine (N = 15) were quantified in DAT KD and WT mice. Plasma levels of risperidone and asenapine were not significantly different in DAT KD mice compared to WT mice (p=0.17 and 0.42, respectively; see Table 5). In BD mania when used in concert with other drug treatment, the recommended dose of asenapine is 5 mg twice a day (Marazziti et al., 2016), and plasma asenapine levels in the current study were much higher than what is seen in humans following 5 mg asenapine treatment (below 6 ng/mL) (Gerrits et al., 2012). The recommended dose of risperidone in bipolar mania is 2-3 mg once daily (Sajatovic et al., 2006). In humans, 2 mg risperidone was associated with plasma levels around 3 ng/mL at 8 weeks, which is higher than what we observed in DAT KD mice but lower than what was observed in WT mice (Cardoni, 1995).

## Discussion

DAT KD mice exhibited hyperexploratory patterns consistent with patients with BD mania. Overall, chronic antipsychotic treatment with risperidone or asenapine was ineffective in fully normalizing mania-like behaviors at the doses tested. This finding contrasts with previous findings that acute antipsychotic treatment attenuated hyperactivity, hyperexploration, and lower spatial d of DAT KD mice (Milienne-Petiot et al., 2017) as well as findings that chronic valproate attenuated the hyperactivity of DAT KD mice (van Enkhuizen et al., 2013) and chronic lithium attenuated the hyperactivity and hyperexploratory behavior of GBR12909-treated mice (van Enkhuizen et al., 2015). Our findings are consistent with acute antipsychotic treatment responsiveness in acute mania episodes but suggest chronic antipsychotic treatment may not be efficacious as a long-term prophylactic treatment for mania.

Previous work suggests short-term but not long-term antipsychotic treatment reverses prepulse inhibition deficits and inhibits amphetamine and tail-pinch induced hyperlocomotion in C57BL6 mice (Amato et al., 2018). The authors found the decline in antipsychotic efficacy was correlated to extracellular dopamine levels rather than reductions

in $D_2$ receptor occupancy by antipsychotics. Moreover, antipsychotic treatment failure is associated with a progressively linearized relationship between TH and DAT, which was present after long- but not short-term treatment (Amato et al., 2018). Dopamine receptor densities following chronic treatment also have been observed (Silvestri et al., 2000) and may play a role in changes in efficacy of antipsychotics over time in managing BD mania. Thus, while acute antipsychotic treatment is useful for BD mania, and chronic for maintenance in BD euthymia, chronic treatment-induced dysregulation of dopamine clearance may limit efficacy for mania treatment.

An alternate explanation could be that the compounds administered were biologically inert. This possibility remains unlikely because plasma levels were in a range comparable to those seen in humans at dosages used to manage BD mania. In addition, several main effects of treatments were observed in these studies.

Although asenapine failed to normalize mania-like behavior in DAT KD mice at the doses investigated, asenapine impacted WT behavior. Chronic asenapine treatment reduced activity, exploration, and reduced straight line movements and predictability of movement in WT mice specifically at the highest dose, but this effect was attenuated in DAT KD mice. Therefore, in terms of exploratory behavior, DAT KD mice were less sensitive than WT mice to chronic asenapine treatment. This observation indicates that DAT genotype may predict non-response to antipsychotics. Risperidone partially reduced center entries, increased rearing, and had a small effect in decreasing overall counts. Plasma risperidone and asenapine levels were quantified and although reduced antipsychotic levels were observed in DAT KD vs. WT mice, this effect was not statistically significant. This observation likely rules out differences in drug metabolism between genotypes, although larger sample sizes would be preferable. Nevertheless, higher risperidone and asenapine doses may be warranted to correct mania-relevant behaviors DAT KD mice.

In both experiments DAT KD mice exhibited mania-like behaviors, although these behaviors were more widespread in experiment 1 compared to 2, likely as a result of background strain differences between C57BL/6 and s129 strains (Kwiatkowski et al, 2018). Female DAT KD mice exhibit BD mania-relevant behaviors. Although the estrogen cycle in female mice may affect behavior, these effects are not large enough to justify excluding female mice from mouse behavioral research (Kokras and Dalla, 2014). BD type II affects women more frequently than men, while BD type I affects women and men at equal rates (Diflorio and Jones, 2010). There were no sex x genotype interactions for any variable except for varied poking, in which female DAT KD mice exhibited the most varied poking. This observation is in contrast to previous work in our group showing no sex effects on any measure of the BPM. This inconsistency may be related to use of a different background strain in the previous work (Young et al., 2010), although findings across strains have been consistent for the majority of measures as shown in a meta-analysis (Kwiatkowski et al 2018).

The results of this study indicate that it may be useful to examine whether DAT polymorphisms can predict antipsychotic nonresponse in bipolar disorder. Based on previous work, it is possible that DAT polymorphisms may instead predict response to valproate. Valproate increases DAT mRNA and protein via increased H3K9 and Nurr1 enrichment in

the DAT promoter (Green et al., 2017). In addition, valproate has been shown to reduce hyperactivity and improve restricted locomotor patterns in DAT KD mice (van Enkhuizen et al., 2013). Interestingly, increased dopamine in DAT KD mice has been shown to be associated with a lengthening of circadian rhythm, while valproic acid reverses this pattern (Landgraf et al., 2016). Chronic valproate treatment also has been shown to attenuate GBR 12909-induced hyperactivity, without affecting other behavioral parameters such as specific exploration or sequential organization (van Enkhuizen et al. 2013a).

Dysfunctional dopamine homeostasis has been linked to many diverse psychiatric illnesses and is not limited to BD. Moreover, dopamine dysfunction can be influenced by a variety of factors, including genetic factors such as DAT polymorphisms (Herborg et al., 2018) (Pinsonneault et al., 2011) as well as environmental factors such as childhood adverse events or trauma (Pruessner et al., 2004) (van Winkel et al., 2013). Notably, PTSD comorbidity with BD is associated with poorer clinical outcome (Quarantini et al., 2010). Understanding the contributors to dopamine dysregulation within subjects may be a first step to identifying the correct treatment, which may require multifaceted treatment approaches such as gene-specific pharmaceuticals, nutraceuticals, cognitive behavioral therapy, vagus nerve stimulation, and/or diet. These interventions would be determined at an individual level to best address comorbid conditions and readouts of specific dopamine dysfunction, which could potentially be exacerbated by multiple variables, not simply a single gene.

The results of this study support DAT polymorphisms as a target for pharmacogenetic studies of predictors of drug response in BD. Research on pharmacogenetics of lithium response in bipolar disorder is ongoing (Papiol et al., 2018; Hou et al., 2016; McCarthy et al., 2010), while investigation of pharmacogenetics of antipsychotic response in bipolar disorder has largely been extrapolated from research in subjects with schizophrenia. Importantly, these data support the idea that antipsychotic drugs may provide clinical benefit acutely, but may not provide additional benefit when administered chronically in BD. It is therefore prudent to conduct a benefit-risk assessment of chronic antipsychotic treatment for bipolar disorder mania and to determine if it may be beneficial to cease antipsychotic treatment following the acute mania episode.

## References

Amato D, Canneva F, Cumming P, et al. (2018) A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter. Mol Psychiatry.

Anand A, Barkay G, Dzemidzic M, et al. (2011) Striatal dopamine transporter availability in unmedicated bipolar disorder. Bipolar Disord 13: 406–413. [PubMed: 21843280]

Ashok AH, Marques TR, Jauhar S, et al. (2017) The dopamine hypothesis of bipolar affective disorder: the state of the art and implications for treatment. Mol Psychiatry 22: 666–679. [PubMed: 28289283]

Cardoni AA. (1995) Risperidone: review and assessment of its role in the treatment of schizophrenia. Ann Pharmacother 29: 610–618. [PubMed: 7545034]

Diflorio A and Jones I. (2010) Is sex important? Gender differences in bipolar disorder. Int Rev Psychiatry 22: 437–452. [PubMed: 21047158]

Duncan GE, Moy SS, Lieberman JA, et al. (2006) Typical and atypical antipsychotic drug effects on locomotor hyperactivity and deficits in sensorimotor gating in a genetic model of NMDA receptor hypofunction. Pharmacol Biochem Behav 85: 481–491. [PubMed: 17097724]

Ene HM, Kara NZ and Einat H. (2015) The effects of the atypical antipsychotic asenapine in a strain-specific battery of tests for mania-like behaviors. Behav Pharmacol 26: 331–337. [PubMed: 25590966]

Gerrits MG, de Greef R, Dogterom P, et al. (2012) Valproate reduces the glucuronidation of asenapine without affecting asenapine plasma concentrations. J Clin Pharmacol 52: 757–765. [PubMed: 21628604]

Grande I, Berk M, Birmaher B, et al. (2016) Bipolar disorder. Lancet 387: 1561–1572. [PubMed: 26388529]

Green AL, Zhan L, Eid A, et al. (2017) Valproate increases dopamine transporter expression through histone acetylation and enhanced promoter binding of Nurr1. Neuropharmacology 125: 189–196. [PubMed: 28743636]

Greenwood TA, Schork NJ, Eskin E, et al. (2006) Identification of additional variants within the human dopamine transporter gene provides further evidence for an association with bipolar disorder in two independent samples. Mol Psychiatry 11: 125–133, 115. [PubMed: 16261167]

Herborg F, Andreassen TF, Berlin F, et al. (2018) Neuropsychiatric disease-associated genetic variants of the dopamine transporter display heterogeneous molecular phenotypes. J Biol Chem.

Horschitz S, Hummerich R, Lau T, et al. (2005) A dopamine transporter mutation associated with bipolar affective disorder causes inhibition of transporter cell surface expression. Mol Psychiatry 10: 1104–1109. [PubMed: 16103889]

Hou L, Heilbronner U, Degenhardt F, et al. (2016) Genetic variants associated with response to lithium treatment in bipolar disorder: a genome-wide association study. Lancet 387: 1085–1093. [PubMed: 26806518]

Kokras N and Dalla C. (2014) Sex differences in animal models of psychiatric disorders. Br J Pharmacol 171: 4595–4619. [PubMed: 24697577]

Kwiatkowski MA, Hellemann G, Sugar CA, et al. (2017) Dopamine transporter knockdown mice in the behavioral pattern monitor: A robust, reproducible model for mania-relevant behaviors. Pharmacol Biochem Behav.

Landgraf D, Joiner WJ, McCarthy MJ, et al. (2016) The mood stabilizer valproic acid opposes the effects of dopamine on circadian rhythms. Neuropharmacology 107: 262–270. [PubMed: 27033596]

Marazziti D, Mucci F, Baroni S, et al. (2016) Asenapine augmentation in bipolar disorders: a case series. Clin Case Rep 4: 499–504. [PubMed: 27190616]

McCarthy MJ, Leckband SG and Kelsoe JR. (2010) Pharmacogenetics of lithium response in bipolar disorder. Pharmacogenomics 11: 1439–1465. [PubMed: 21047205]

Milienne-Petiot M, Geyer MA, Arnt J, et al. (2017) Brexpiprazole reduces hyperactivity, impulsivity, and risk-preference behavior in mice with dopamine transporter knockdown-a model of mania. Psychopharmacology (Berl) 234: 1017–1028. [PubMed: 28160035]

Minassian A, Henry BL, Young JW, et al. (2011) Repeated assessment of exploration and novelty seeking in the human behavioral pattern monitor in bipolar disorder patients and healthy individuals. PLoS One 6: e24185. [PubMed: 21912623]

Papiol S, Schulze TG and Alda M. (2018) Genetics of Lithium Response in Bipolar Disorder. Pharmacopsychiatry 51: 206–211. [PubMed: 29579760]

Perry W, Minassian A, Paulus MP, et al. (2009) A reverse-translational study of dysfunctional exploration in psychiatric disorders: from mice to men. Arch Gen Psychiatry 66: 1072–1080. [PubMed: 19805697]

Pinsonneault JK, Han DD, Burdick KE, et al. (2011) Dopamine transporter gene variant affecting expression in human brain is associated with bipolar disorder. Neuropsychopharmacology 36: 1644–1655. [PubMed: 21525861]

Pruessner JC, Champagne F, Meaney MJ, et al. (2004) Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. J Neurosci 24: 2825–2831. [PubMed: 15028776]

Quarantini LC, Miranda-Scippa A, Nery-Fernandes F, et al. (2010) The impact of comorbid posttraumatic stress disorder on bipolar disorder patients. J Affect Disord 123: 71–76. [PubMed: 19732957]

Risbrough VB, Masten VL, Caldwell S, et al. (2006) Differential contributions of dopamine D1, D2, and D3 receptors to MDMA-induced effects on locomotor behavior patterns in mice. Neuropsychopharmacology 31: 2349–2358. [PubMed: 16855533]

Sachs GS. (2003) Decision tree for the treatment of bipolar disorder. J Clin Psychiatry 64 Suppl 8: 35–40.

Sajatovic M, Subramoniam M and Fuller MA. (2006) Risperidone in the treatment of bipolar mania. Neuropsychiatr Dis Treat 2: 127–138. [PubMed: 19412457]

Silvestri S, Seeman MV, Negrete JC, et al. (2000) Increased dopamine D2 receptor binding after long-term treatment with antipsychotics in humans: a clinical PET study. Psychopharmacology (Berl) 152: 174–180. [PubMed: 11057521]

Stroup TS, McEvoy JP, Swartz MS, et al. (2003) The National Institute of Mental Health Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) project: schizophrenia trial design and protocol development. Schizophr Bull 29: 15–31. [PubMed: 12908658]

van Enkhuizen J, Geyer MA, Kooistra K, et al. (2013) Chronic valproate attenuates some, but not all, facets of mania-like behaviour in mice. Int J Neuropsychopharmacol 16: 1021–1031. [PubMed: 23164454]

van Enkhuizen J, Milienne-Petiot M, Geyer MA, et al. (2015) Modeling bipolar disorder in mice by increasing acetylcholine or dopamine: chronic lithium treats most, but not all features. Psychopharmacology (Berl) 232: 3455–3467. [PubMed: 26141192]

van Winkel R, van Nierop M, Myin-Germeys I, et al. (2013) Childhood trauma as a cause of psychosis: linking genes, psychology, and biology. Can J Psychiatry 58: 44–51. [PubMed: 23327756]

Ventimiglia J, Kalali AH and McIntyre R. (2009) Treatment of bipolar disorder. Psychiatry (Edgmont) 6: 12–14.

Young JW, Cope ZA, Romoli B, et al. (2018) Mice with reduced DAT levels recreate seasonal-induced switching between states in bipolar disorder. Neuropsychopharmacology 43: 1721–1731. [PubMed: 29520059]

Young JW, Goey AK, Minassian A, et al. (2010) The mania-like exploratory profile in genetic dopamine transporter mouse models is diminished in a familiar environment and reinstated by subthreshold psychostimulant administration. Pharmacol Biochem Behav 96: 7–15. [PubMed: 20363246]

Young JW, van Enkhuizen J, Winstanley CA, et al. (2011) Increased risk-taking behavior in dopamine transporter knockdown mice: further support for a mouse model of mania. J Psychopharmacol 25: 934–943. [PubMed: 21421642]

Zhuang X, Oosting RS, Jones SR, et al. (2001) Hyperactivity and impaired response habituation in hyperdopaminergic mice. Proc Natl Acad Sci U S A 98: 1982–1987. [PubMed: 11172062]

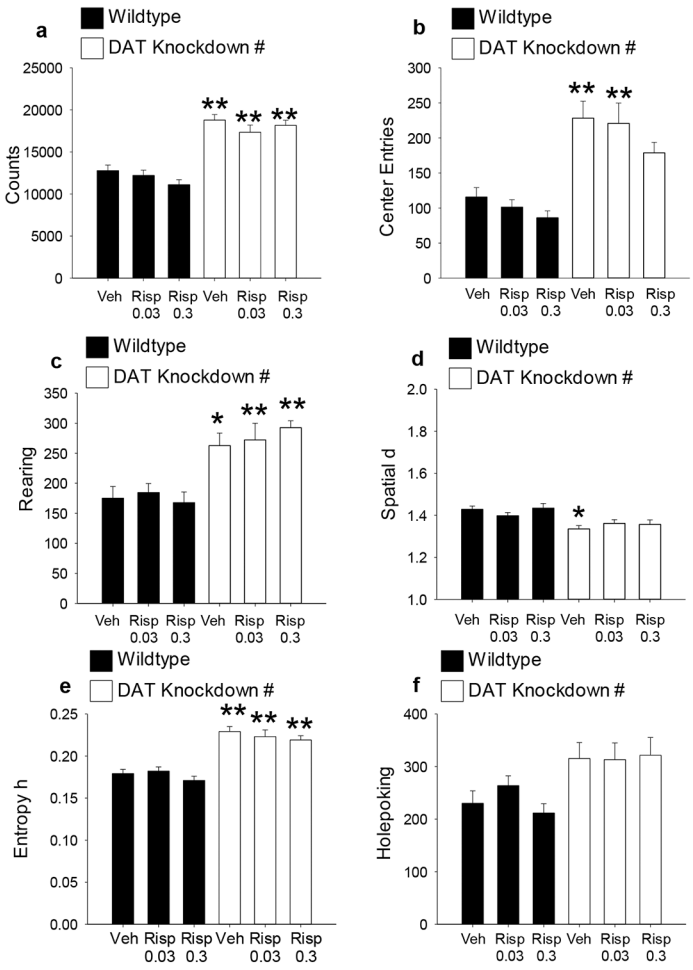


**Figure 1. Risperidone effects on BPM performance in DAT KD and wildtype mice.**

There were significant effects of genotype on all measures. There was a significant effect of risperidone on center entries. There were no significant genotype x risperidone interactions. Abbreviations: WT – wildtype littermates; DAT KD – dopamine transporter knockdown; Veh – vehicle; 0.03 Risp – 0.03 mg/kg risperidone; 0.3 Risp – 0.3 mg/kg risperidone. Data presented as Mean +/− SEM. Statistically significant differences compared to wildtype vehicle controls are denoted with * or ** ($p < 0.05$ and $p < 0.01$, respectively). Statistically significant main effects of gene are denoted with #.

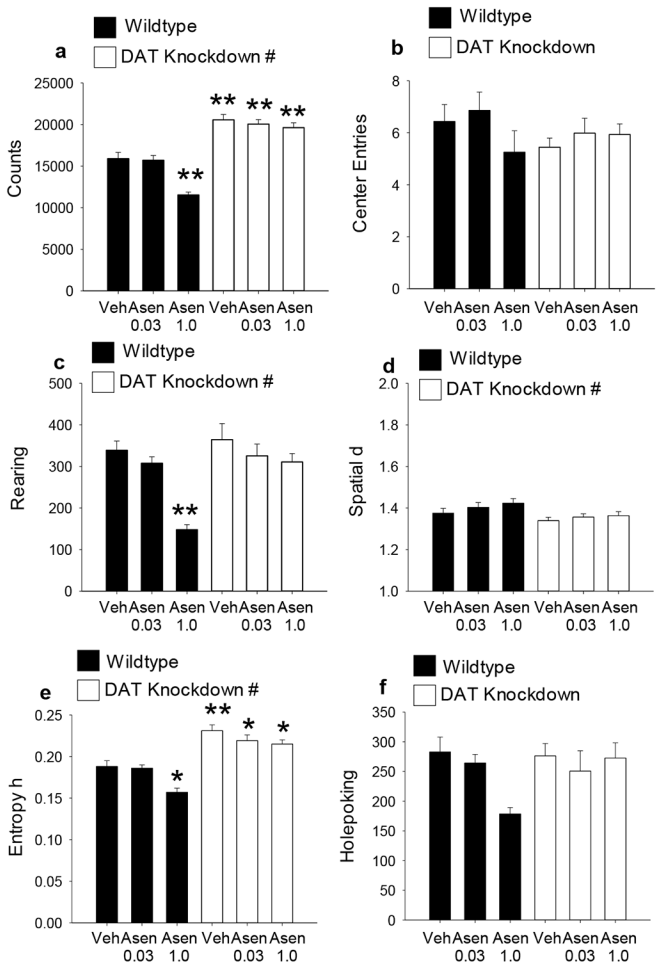


**Figure 2. Asenapine effects on BPM performance in DAT KD and wildtype mice.**

There were significant effects of genotype on all measures except for center entries. There was a significant effect of asenapine on counts, rearing, and entropy h. There were significant genotype x asenapine interactions on counts and rearing, and this was related to a decrease in counts and rearing in wildtype mice. Abbreviations: WT – wildtype littermates; DAT KD – dopamine transporter knockdown; Veh – vehicle; 0.03 Asen – 0.03 mg/kg asenapine; 0.1 Asen – 0.1 mg/kg asenapine). Data presented as Mean +/− SEM. Statistically significant differences compared to wildtype vehicle controls are denoted with * or ** ($p < 0.05$ and $p < 0.01$, respectively). Statistically significant main effects of gene are denoted with #.

**Table 1.**

Experimental design for experiments 1 and 2, DAT=Dopamine Transporter, KD=knockdown

| **Experiment 1 – Chronic risperidone in 5-6 month old DAT KD mice on a C57BL/6J background (male/female)** | | |
|---|---|---|
| **Drug Treatment** | **Wildtype** | **DAT KD** |
| Vehicle | 6/10 | 8/5 |
| Risperidone 0.03 mg/kg | 6/10 | 10/6 |
| Risperidone 0.3 mg/kg | 6/10 | 8/6 |
| **Experiment 2 – Chronic asenapine in 3 month old male DAT KD mice** | | |
| **Drug Treatment** | **Wildtype** | **DAT KD** |
| Vehicle | 9 | 13 |
| Asenapine 0.03 mg/kg | 9 | 12 |
| Asenapine 0.1 mg/kg | 10 | 13 |

**Table 2.**

Measurements and their associated factors in the mouse Behavioral Pattern Monitor.

| Factor | Measure | Description |
|---|---|---|
| **Activity Measures** | **Counts** | The cumulative number of distinct behaviors to occur during testing |
| | **Transitions** | The number of movements from one pre-defined area to another |
| | **Center Entries** | The number of entries into the area defined as the center |
| | **Distance travelled** | Total distance travelled while exploring the chamber measured in cm |
| **Specific Exploration** | **Hole pokes** | The total number of investigatory holepokes across all 11 holes |
| | **Repeated Poking** | The number of times a hole was repeatedly poked before moving to another hole |
| | **Varied Poking** | The number of times the mouse holepoked into different holes |
| | **Rear** | The number of times the mouse reared into the air |
| | **Center Duration** | The amount of time (s) spent in the area designated as the center of the chamber |
| **Locomotor Patterns** | **Spatial d** | Spatial scaling exponent d measures the hierarchical and geometric organization of behavior (Paulus, Callaway, & Geyer, 1993) |
| | **Entropy h** | Quantifies the sequential aspects of sequences of behavior, specifically measuring the degree to which behavior is observed along a continuum between complete order and disorder (Paulus, Geyer, & Braff, 1996) |
| | **Spatial CV** | Measures the consistency by which the animal moves from one region to another (Geyer, Russo, & Masten, 1986) |
| | **Temporal CV** | Measures the consistency of the dwell time in each of the regions (Geyer et al., 1986) |

**Table 3.**

**Risperidone, DAT KD genotype and sex effects on BPM activity.**

Significant findings in bold, *trend toward significance in italics.*

| | Measure | Sex df (1,79) | Genotype df (1,79) | Drug df (2,79) | Sex x Genotype df (1,79) | Sex x Drug df (2,79) | Genotype x Drug df (2,79) | Sex x Drugs x Genotype df (2,79) |
|---|---|---|---|---|---|---|---|---|
| **Activity** | Counts | **F=4.01,p=0.05** | **F=107.56,p=0.00** | *F=2.51,p=0.09* | F=0.01,p=0.92 | F=0.50,p=0.61 | F=1.14,p=0.33 | F=2.23,p=0.11 |
| | Transitions | *F=3.39,p=0.07* | **F=98.38,p=0.00** | F=2.23,p=0.11 | F=0.02,p=0.89 | F=0.75,p=0.48 | F=0.51,p=0.60 | **F=3.25,p=0.04** |
| | Center Entries | F=1.57,p=0.21 | **F=51.66,p=0.00** | **F=3.89,p=0.02** | F=0.00,p=0.98 | F=2.03,p=0.14 | F=0.35,p=0.71 | *F=5.59,p=0.01* |
| | Distance travelled | **F=4.05,p=0.05** | **F=112.81,p=0.00** | *F=2.63,p=0.08* | F=0.12,p=0.73 | F=0.93,p=0.40 | F=0.67,p=0.51 | *F=3.01,p=0.05* |
| **Inspective Exploration** | Hole pokes | F=0.65,p=0.42 | **F=14.57,p=0.00** | F=0.34,p=0.71 | *F=3.07,p=0.08* | F=0.77,p=0.47 | F=0.81,p=0.45 | F=1.39,p=0.25 |
| | Repeated Poking | F=0.54,p=0.46 | **F=13.56,p=0.00** | F=0.36,p=0.70 | F=2.57,p=0.11 | F=0.80,p=0.46 | F=0.68,p=0.51 | F=1.61,p=0.21 |
| | Varied Poking | F=0.99,p=0.32 | **F=13.86,p=0.00** | F=0.14,p=0.87 | **F=4.72,p=0.03** | F=0.42,p=0.66 | F=1.54,p=0.22 | F=0.51,p=0.60 |
| **Diversive Exploration** | Rearing | **F=11.09,p=0.00** | **F=30.46,p=0.00** | F=0.12,p=0.88 | F=0.07,p=0.79 | F=0.85,p=0.43 | F=1.03,p=0.36 | F=0.27,p=0.76 |
| | Center Duration | F=0.51,p=0.48 | **F=5.31,p=0.02** | *F=2.60,p=0.08* | F=0.15,p=0.70 | F=0.81,p=0.45 | F=0.21,p=0.81 | F=1.46,p=0.24 |
| **Locomotor Patterns** | Spatial d | F=0.20,p=0.66 | **F=19.53,p=0.00** | F=0.51,p=0.60 | F=0.48,p=0.49 | F=0.54,p=0.58 | F=1.42,p=0.25 | F=0.27,p=0.76 |
| | Entropy h | F=0.05,p=0.82 | **F=93.97,p=0.00** | F=1.42,p=0.25 | F=0.15,p=0.70 | F=1.11,p=0.33 | F=0.65,p=0.53 | *F=2.57,p=0.08* |
| | Spatial CV | F=0.00,p=0.95 | **F=4.08,p=0.05** | F=2.23,p=0.11 | F=1.98,p=0.16 | F=0.37,p=0.69 | F=0.09,p=0.91 | F=1.03,p=0.36 |
| | Temporal CV | F=0.02,p=0.90 | **F=11.80,p=0.00** | F=0.84,p=0.44 | F=1.46,p=0.23 | F=0.43,p=0.65 | F=0.55,p=0.58 | F=0.62,p=0.54 |

**Table 4.**

Effects of asenapine and DAT KD genotype on BPM activity.

| | Measure | Genotype df (1,60) | Asenapine df (2,60) | G x D df (2,60) |
|---|---|---|---|---|
| **Activity** | Counts | **F=134.65,p=0.00** | **F=11.78,p=0.00** | **F=6.07,p=0.00** |
| | Transitions | **F=77.20,p=0.00** | **F=4.90,p=0.01** | F=1.47,p=0.24 |
| | Center Entries | F=0.70,p=0.41 | F=1.04,p=0.36 | F=1.34,p=0.27 |
| | Distance travelled | **F=24.64,p=0.00** | *F=2.48,p=0.09* | F=0.10,p=0.91 |
| **Inspective Exploration** | Hole pokes | F=1.48,p=0.23 | F=2.45,p=0.10 | *F=3.02,p=0.06* |
| | Repeated Poking | F=1.28,p=0.26 | F=1.95,p=0.15 | *F=2.61,p=0.08* |
| | Varied Poking | F=1.60,p=0.21 | **F=4.13,p=0.02** | **F=3.42,p=0.04** |
| **Diversive Exploration** | Rearing | **F=9.66,p=0.00** | **F=11.14,p=0.00** | **F=4.67,p=0.01** |
| | Center Duration | **F=54.09,p=0.00** | F=1.47,p=0.24 | F=0.43,p=0.66 |
| **Locomotor Patterns** | Spatial d | **F=9.03,p=0.00** | F=1.77,p=0.18 | F=0.17,p=0.84 |
| | Entropy h | **F=75.11,p=0.00** | **F=7.59,p=0.00** | F=1.99,p=0.15 |
| | Spatial CV | **F=102.57,p=0.00** | **F=8.08,p=0.00** | F=2.10,p=0.13 |
| | Temporal CV | F=0.28,p=0.60 | **F=4.31,p=0.02** | *F=2.64,p=0.08* |

**Table 5.**

Risperidone and asenapine blood levels were quantified in WT and DAT KD mice. Means +/− Standard error are shown (ng/mL).

| | Risperidone | Asenapine |
|---|---|---|
| WT | 4.9 +/− 0.6 (n = 23) | 26 +/− 13 (n =7) |
| DAT KD | 2.0 +/− 0.7 (n = 2) | 15.5 +/− 2.9 (n = 8) |