Title

Chronic nicotine, but not suramin or resveratrol, partially remediates the mania-like profile of dopamine transporter knockdown mice

Authors

Kwiatkowski, Molly A
Roberts, Benjamin Z
van Enkhuizen, Jordy
et al.

Publication Date

2021

DOI

10.1016/j.euroneuro.2020.11.004

Peer reviewed

HHS Public Access

Author manuscript

Eur Neuropsychopharmacol. Author manuscript; available in PMC 2022 February 17.

Published in final edited form as:

Eur Neuropsychopharmacol. 2021 January ; 42: 75–86. doi:10.1016/j.euroneuro.2020.11.004.

Chronic nicotine, but not suramin or resveratrol, partially remediates the mania-like profile of dopamine transporter knockdown mice

Molly A. Kwiatkowski[1], **Benjamin Z. Roberts**[1], **Jordy van Enkhuizen**[1], **Baohu Ji**[1], **Xianjin Zhou**[1], **Jared W. Young**[1,2]

[1]Department of Psychiatry, University of California, San Diego

[2]Research Service, VA San Diego Healthcare System

Abstract

Bipolar disorder (BD) is a severe mental illness affecting 2% of the global population. Current pharmacotherapies provide incomplete symptom remediation, highlighting the need for novel therapeutics. BD is characterized by fluctuations between mania and depression, likely driven by shifts between hyperdopaminergia and hypercholinergia, respectively. Hyperdopaminergia may result from insufficient activity of the dopamine transporter (DAT), the primary mediator of synaptic dopamine clearance. The DAT knockdown (DAT KD) mouse recreates this mechanism and exhibits a highly reproducible hyperexploratory profile in the cross-species translatable Behavioral Pattern Monitor (BPM) that is: a) consistent with that observed in BD mania patients; and b) partially normalized by chronic lithium and valproate treatment. The DAT KD/BPM model of mania therefore exhibits high levels of face-, construct-, and predictive-validity for the pre-clinical assessment of putative anti-mania drugs. Three different drug regimens – chronic nicotine (nicotinic acetylcholine receptor (nAChR) agonist; 40mg/kg/d, 26d), subchronic suramin (anti-purinergic;20mg/kg, 1×/wk, 4wks), and subchronic resveratrol (striatal DAT upregulator; 20mg/kg/d, 4d) – were administered to separate cohorts of male and female DAT KD- and wildtype (WT) littermate mice, and exploration was assessed in the BPM. Throughout, DAT KD mice exhibited robust hyperexploratory profiles relative to WTs. Nicotine partially normalized this behavior. Resveratrol modestly upregulated DAT expression but did not normalize DAT KD behavior. These results support the mania-like profile of DAT KD mice, which may be partially remediated by nAChR agonists via restoration of disrupted catecholaminergic/cholinergic equilibrium. Delineating the precise mechanism of action of nicotine could identify more selective therapeutic targets.

Correspondence: Jared W. Young, Ph.D., Professor, Department of Psychiatry, UC San Diego School of Medicine, La Jolla, CA 92093-0804, jaredyoung@ucsd.edu; 619-471-0497.

Contributors: Kwiatkowski MA, van Enkhuizen, and Young JW designed studies and conducted experiments, Ji B and Zhou X conducted the western blot study and analyses. Kwiatkowski MA, Roberts BZ, and Young JW managed literature searches and undertook statistical analyses. Kwiatkowski MA wrote the first draft of the manuscript with contributions from Roberts BZ, Ji B, and Young JW. All authors approved the final manuscript.

Conflict of Interest: None.

Introduction

Bipolar disorder (BD) is a life-long and often life-threatening mental illness that occurs in approximately 2% of the global population (Merikangas et al., 2011). BD is characterized by mood fluctuations between mania and depression (Grande et al., 2016) that may reflect extreme shifts between hyperdopaminergia and hypercholinergia, respectively (Ashok et al., 2017; van Enkhuizen et al., 2015). Homeostatic control of dopamine levels is primarily mediated by dopamine transporters (DAT), suggesting that this hyperdopaminergia may be at least partially driven by altered DAT expression/function in patients with BD.

Consistent with this potential for DAT alterations to give rise to BD-related behaviors, gene polymorphisms have been linked to BD pathogenesis (Greenwood et al., 2006, 2001; Pinsonneault et al., 2011), which may result in reduced DAT expression (Horschitz et al., 2005). Indeed, DAT binding and expression is reduced in the BD dorsal caudate (Anand et al., 2011). DAT knockdown (KD) mice, which express DAT at 10% of normal levels (Zhuang et al., 2001), model this hypoexpression phenotype and exhibit reproducible mania-like behaviors in the cross-species translatable Behavioral Pattern Monitor (BPM). The BPM characterizes the unconditioned motor behavior of rodents and humans on three major axes: general activity/locomotion, exploration, and ambulatory path pattern. Acutely manic (Perry et al., 2009), and, to a lesser extent, euthymic-state (Minassian et al., 2011) BD patients exhibit a characteristic motor profile of hyperactivity, increased exploration, and straightened path trajectory in the BPM that is reproduced by the DAT KD mouse with remarkable consistency (Kwiatkowski et al., 2019; Perry et al., 2009; Young et al., 2007; Zhuang et al., 2001). Critically, chronic valproate partially remediates this shared BPM profile in a manner that is consistent across species (Minassian et al., 2011; van Enkhuizen et al., 2013; Young and Dulcis, 2015). This finding establishes the pharmacological predictive validity of the DAT KD model of mania, which, in combination with the robust reproducibility of the hyperactivity profile of the DAT KD mouse (Kwiatkowski et al., 2019) and the construct- and face-validity indicated by the interspecies similarities in DAT expression and locomotor profiles, enables the use of this paradigm to assess putative therapies for aspects of BD mania.

Novel pharmacotherapies are urgently needed for this patient population given the incomplete effectiveness and side-effects of current treatments. Consistent with observations in the BPM (Milienne-Petiot et al., 2017; van Enkhuizen et al., 2013), current medications provide an incomplete remediation of BD mania, with meta-analyses reporting only moderate effect sizes (Cipriani et al., 2011; Yildiz et al., 2011). Efforts to address this need are hindered by a lack of knowledge of mania pathogenesis, regarding which a number of hypotheses have been formulated. The catecholaminergic-cholinergic balance hypothesis, for example, postulates that mania symptoms reflect low cholinergic versus catecholaminergic activity in the brain (van Enkhuizen et al., 2015). This hypothesis is supported by studies demonstrating that increasing cholinergic activation reverses symptoms of mania (Dulawa and Janowsky, 2019). Following this model, it may be possible for a nicotinic acetylcholine receptor agonist (e.g., nicotine, the active component of tobacco smoke) to exert a similar effect. Interestingly, tobacco use is highly prevalent in BD

populations – patients with histories of mania have a 3.9× greater likelihood of tobacco dependence than individuals without psychiatric illness (Grant et al., 2004). Nicotine may therefore play a role in the management and prevention of manic symptoms.

Alternative hypotheses of BD mechanisms include the purinergic hypothesis, which posits that disruption of purinergic signaling gives rise to mania symptoms (Machado-Vieira et al., 2002). Purines play an important role in the regulation of neurotransmission and metabolic processes (Zarate and Manji, 2008). Enhanced purinergic metabolism, as indicated by high levels of uric acid (UA), have been associated with greater impulsivity in humans and mice (Sutin et al., 2014), as well as with severe manic symptoms in patients with BD (Machado-Vieira et al., 2002). Indeed, two placebo-controlled clinical trials reported that reduction of UA formation via the addition of allopurinol, a xanthine oxidase inhibitor, to mood-stabilizing pharmacotherapies significantly reduced manic symptoms in BD patients (Akhondzadeh et al., 2006; Machado-Vieira et al., 2008). Limiting the conversion of xanthine to UA in such a manner leads to elevations in upstream compoundsin the purine degradation pathway, including the purine ribonucleoside adenosine, an agonist at the P1 G protein-coupled purinergic receptor (Cheffer et al., 2018). The A_{2A} subtype of P1 receptors is highly expressed in striatal neurons, and inhibitors of these receptors have been demonstrated to have neuroprotective effects (Boison, 2008). Suramin, which inhibits activation of heterotrimeric G-proteins and thereby blocks downstream signaling (including atthe A_{2A} receptor), normalizes social behavior, novelty preference, and metabolism in a mouse model of autism (Naviaux et al., 2014), and may also prove beneficial to the treatment of BD mania.

Given the construct (Anand et al., 2011) and pharmacological predictive validity (van Enkhuizen et al., 2013) of the DAT KD mouse model of BD, directly addressing the most likely cause of the DAT KD mania-like behavioral profile, DAT hypoexpression, may itself yield important information regarding BD pathogenesis. Subchronic administration of resveratrol, a naturally occurring phytoestrogen with anti-apoptotic, anti-aging, and anti-oxidant properties, increases striatal DAT expression in female wildtype (WT) mice (Di Liberto et al., 2012). A similar treatment regimen may therefore increase DAT levels in DAT KD mice, and thereby normalize their mania-like profile in the BPM.

In order to assess the therapeutic potential of each of these drugs, separate cohorts of DAT KD mice and WT littermates were treated with: a) chronic nicotine, b) subchronic suramin, or c) subchronic resveratrol, and then assessed in the cross-species translatable BPM. Each of these treatments was hypothesized to differentially affect DAT KD versus WT mice in the BPM, resulting in a partial remediation of the mania-like profile of the DAT KD mice in the BPM. The results of these investigations will serve to identify novel pathways and biological processes that may be targeted to ameliorate symptoms of mania.

Materials & Methods

Animals

DAT KD and WT littermates were bred in-house using heterozygous breeding pairs. Mice were housed in tetrads by genotype in transparent plastic boxes in a climate-controlled

room maintained on a 12-hour light/dark schedule (7:00 AM-7:00 PM dark). Testing was conducted during the dark phase of the animals' light/dark schedules. Food and water were available *ad libitum*, except during testing. Mice were bred, raised, and maintained in a dedicated animal facility approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All procedures were approved by the University of California San Diego Animal Care and Use Committee.

Study 1: Chronic Nicotine

Animals—The effects of chronic nicotine on locomotor and exploratory behavior were assessed in male DAT KD- and wildtype (WT) C57BL6/J mice (DAT KD: N=30, 23–32 g; WT: N=27, 26–34 g). Mice were between 50 & 60 weeks of age at time of testing.

Procedure—DAT KD- and WT mice were chronically infused with either vehicle or (−)nicotine hydrogen tartrate at a rate of 40 mg/kg/day for 26 days, at which point they were assessed in the BPM for 60 min. Nicotine was dissolved in sterile 0.9% saline solution and was pH-adjusted to 7±0.5 using sodium hydroxide. Infusions were delivered by ALZET mini-osmotic pumps (Model 2004) at a pumping rate of 0.25 μL/h (±0.05 μL/h). Prior to surgical implantation, pumps were filled and primed in room temperature saline for 40–48 h. Mice were anesthetized with isoflurane (1–3% in oxygen) and were operated on using the following procedure. Ahead of the initial incision, the surgical site (area around the back of the neck) was shaved, and then sterilized with betadine. Once sterilized, an incision was made and subsequently enlarged by blunt dissection to create a pouch large enough to accommodate the pump. The pump (pre-filled) was then inserted into this pouch, oriented such that the flow modulator was directed caudally. The incision was closed using 9 mm wound clips (MikRon Precision, Inc., Gardena, CA). Subcutaneous baytril (5 mg/kg) and flunixamine (2.5 mg/kg) were administered post-operatively in order to prevent infection and minimize pain. All drugs and reagents were acquired from Sigma Aldrich (St. Louis, MO). Drug doses were chosen based on previously reported nicotine effects in mice (Hall et al., 2015; Higa et al., 2017; Portugal and Gould, 2009).

Study 2: Subchronic Suramin

Animals—The effects of subchronic suramin on locomotor and exploratory behavior were assessed in male and female DAT KD- and WT littermate mice (DAT KD: N=30; females=15, 20–24 g; males=15, 23–28 g) (WT: N=33; females=17, 19–24 g; males=16, 24–29 g), all of which were 12–14 weeks of age at time of testing.

Procedure—DAT KD- and WT mice received weekly intraperitoneal injections of either suramin (20 mg/kg; DAT KD: females=6, males=10; WT: females=11, males=6) or vehicle (saline; DAT KD: females=5, males=9; WT: females=10, males=6) for 4 weeks. Mice were assessed in the BPM for 45 min on the day after the final injection. Drug doses were chosen based on previously reported suramin effects in mice (Naviaux et al., 2015, 2013).

Study 3: Subchronic Resveratrol

Animals—The effects of subchronic resveratrol on locomotor and exploratory behavior were assessed in two cohorts each of female DAT KD- and WT C57BL6/J mice, one cohort

aged 50 weeks (N=24; DAT KD=11, 23–32 g; WT=13, 26–34 g) and the other aged 18–20 weeks (N=32; DAT KD=11, 20–22 g; WT=21, 19–23 g).

Procedure—DAT KD- and WT mice received daily intraperitoneal injections of either resveratrol (20 mg/kg; DAT KD=11, WT=18) or vehicle (25% DMSO, 25% ethanol, 50% saline; DAT KD=11, WT=16) for 4 days following a between-subjects design. Injections were administered at a volume of 5 ml/kg. All drugs and reagents were obtained from Sigma-Aldrich (St. Louis, MO). On the 7th day following the final injection, mice were assessed in the BPM for 60 min. The dose selected was the same dose reported to induce DAT upregulation in striatum of female mice (Di Liberto et al., 2012).

Following previously published methodologies (Ji et al., 2014), striata of these DAT KD and WT mice (DAT KD: N=10; WT: N=8) were dissected and homogenized in Passive Lysis Buffer (PLB) (Promega, WI) with 0.2% Sarkosyl and 1× protease inhibitor cocktail (Sigma, MO, P8340). After sonication, solubilized striatum was centrifuged at 10,000xg. The protein concentrations of the supernatant were measured via the Bradford method (Abs. 595 nm). 20μg total proteins from each sample were separated with 4–20% gradient polyacrylamide gel. After electrophoresis, proteins were transferred onto PVDF membranes. The membranes were first blocked with 5% non-fat dry milk in TBST buffer (pH 7.5, 10 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween 20) at room temperature for 1 h. After blocking, the membranes were incubated with the rat anti-DAT monoclonal antibody (diluted 1:1,000; sc-32258 Santa Cruz Biotechnology, CA, USA) at 4°C overnight. After washing three times, the membranes were further incubated with horseradish peroxidase-conjugated anti-rat IgG (1:5,000, sc-2006) for 1.5 h at room temperature before visualization with chemiluminescence (Amersham ECL). DAT expression was quantified with Image J. To normalize DAT expression between different samples, β-actin was measured on the membranes with mouse monoclonal anti-β-actin antibodies (sc-47778, Santa Cruz Biotechnology 1:5000) and secondary HRP-conjugated anti-mouse IgG antibodies.

Behavioral Pattern Monitor (BPM)

The unconditioned motor and exploratory behavior of DAT KD and WT mice was characterized using the Behavioral Pattern Monitor (BPM). The BPM comprised a 30.5 × 61 cm arena that was monitored by two sets of infrared photobeams, disruptions of which were recorded by microcomputer and used to ascertain animals' location and activity from moment to moment. Animals' X-Y coordinates were provided by a 12 × 24 grid of photobeams positioned 1 cm above the floor, while rearing behavior, either against the walls or into the air, was captured by a second array of 16 photobeams traversing the chamber at a height of 2.5 cm. The BPM also contained 11 photobeam-monitored apertures distributed across the walls and floor that mice could investigate via nosepoke. The arena was enclosed by 38 cm-high Plexiglas walls that appeared opaque to the mice but permitted the passage of photobeams. 8 BPM chambers were used in the present studies, each of which was enclosed within a ventilated sound-attenuating cabinet and illuminated by a single light source above the arena (producing 350 lux in the center, 92 lux in thecorners). Photobeam arrays were sampled at 100-msec intervals.

The BPM provided a multivariate readout of general activity levels, specific locomotor behavior, exploration, and path patterns. General activity levels were reported by the *counts* variable – the total number of photobeam disruptions of any kind recorded during the session. Locomotor activity was ascertained from the *total distance traveled* within the session, and from the total number of times mice crossed from one region of the field to another (*transitions*). Exploratory behavior was quantified using the *holepokes* (number of investigatory nosepokes into the apertures) and *rears* measures. Animals' path trajectories were quantified by the *spatial d* metric, a measure of hierarchical/geometric organization of behavior in which values approaching 2 represent highly circumscribed movement patterns and values approaching 1 describe straight-line trajectories.

The effects of chronic nicotine and subchronic resveratrol on locomotor and exploratory behavior were characterized within 60 min BPM sessions, while the effects of subchronic suramin were assessed in 45 min sessions. Water was used to thoroughly clean the floors and walls of the chambers between runs and was subsequently wiped dry in random patterns in order to disrupt any residual scent trails left over from previous mice.

Statistics

Outcome measures of the BPM were analyzed via three- and four factor ANOVA for each of the three studies. Study 1 (chronic nicotine) data were analyzed using 20-min observation bin as a within-subjects factor and genotype and treatment as between-subjects factors. Study 2 (subchronic suramin) data were analyzed using 15-min bin as a within-subjects factor and genotype, sex, and treatment as between-subjects factors. Study 3 (subchronic resveratrol) data were analyzed via four factor ANOVA, with 20-min bin as a within-subjects factor, and genotype, treatment, and age as between-subjects factors. Statistically significant interactions between two or more factors ($p<0.05$) were investigated by follow-up ANOVAs, as were near-significant interactions ($p<0.10$) predicted by the *a priori* hypothesis that each treatment would differentially affect the locomotor and exploratory behavior of DAT KD mice versus WT mice.

Prior to statistical analysis of Western blot data (Study 3), DAT expression levels were normalized to β-actin levels for each sample. These resultant values were then normalized to the group means of the respective vehicle-treated groups and expressed as a percentage, e.g.:

$$\frac{DAT\ Expression_{DAT\ KD+Resveratrol}}{Mean\ DAT\ Expression_{DAT\ KD+Vehicle}} \times 100\,\%$$

These percentages were then averaged across samples, generating mean percent DAT expression values for each genotype/treatment combination. Fold-changes between resveratrol- versus vehicle-treated DAT KD and WT mice were then calculated by dividing mean percent DAT expression values (resveratrol/vehicle) within each genotype. The DAT hypoexpression phenotype of the DAT KD mouse was verified via a one-tailed t-test comparing the initial β-actin-normalized values from vehicle-treated DAT KD mice versus vehicle-treated WT mice. Two-tailed t-tests were used to compare fold-changes between: 1) resveratrol- versus vehicle-treated DAT KD mice; and 2) resveratrol- versus vehicle-treated

WT mice. All data were analyzed using SPSS 25.0 (Chicago, IL) and were represented graphically by mean and standard error of the mean.

Results

Study 1: Nicotine (Chronic)

Outcome variables of the BPM were analyzed via three factor ANOVA using 20-min observation bin as a within-subjects factor, and genotype and treatment as between-subjects factors. Main effects of genotype were observed on overall activity [counts; $F_{(1,48)}=29.0$, $p<0.001$; Fig. 1A] and on both measures of locomotion – transitions [$F_{(1,48)}=21.2$, $p<0.001$; Fig. 1B] and distance traveled [$F_{(1,48)}=29.7$, $p<0.001$; Fig. 1C]. No main effects of chronic nicotine treatment were observed on any of these measures [F's<1.3, n.s.]. Genotype × treatment interactions were observed on counts [$F_{(1,48)}=4.6$, $p<0.05$], transitions [$F_{(1,48)}=4.5$, $p<0.05$], and distance traveled [$F_{(1,48)}=4.7$, $p<0.05$], revealing that chronic nicotine reduced overall activity [$F_{(1,26)}=6.3$, $p<0.05$; Fig. 1A] and tendency for distance traveled [$F_{(1,26)}=3.5$, $p=0.073$; Fig. 1C] in DAT KD mice only.

Genotype did not affect holepoking [F<1.4, n.s.; Fig. 1D], though chronic nicotine tended to non-specifically reduce this behavior across genotypes [$F_{(1,48)}=3.4$, $p=0.070$]. Given that we had hypothesized *a priori* that nicotine would exert genotype-specific effects on specific exploration, planned separate analyses of DAT KD and WT holepoking data were conducted. These analyses revealed a significant reduction of holepoking behavior in DAT KD mice only [$F_{(1,28)}=6.0$, p<0.05]. DAT KD mice reared significantly more frequently than WT mice [$F_{(1,48)}=8.6$, $p<0.01$; Fig. 1E], regardless of treatment [F<1, n.s.]. DAT KD mice demonstrated significantly lower spatial d than WT mice [$F_{(1,48)}=11.4$, $p<0.01$; Fig. 1F], indicating straighter path trajectories. No main or interactive effects of treatment were observed on these measures [F's<1.2, n.s.].

Significant main effects of 20-min observation bin were observed on counts [$F_{(2,96)}=76.5$, $p<0.001$], transitions [$F_{(2,96)}=58.4$, $p<0.001$], distance traveled [$F_{(2,96)}=83.9$, $p<0.001$], holepoking [$F_{(2,96)}=6.6$, $p<0.01$], and rearing [$F_{(2,96)}=18.8$, $p<0.001$], indicating habituation-related decrements in each measure across the testing session. Significant bin × genotype [$F_{(2,96)}=6.2$, $p<0.01$] and bin × treatment interactions [$F_{(2,96)}=3.2$, $p<0.05$], but not a bin × genotype × treatment interaction, were observed on counts; the results of follow-up analyses were inconclusive. Similar interactions of bin and genotype [$F_{(2,96)}=2.7$, $p=0.070$] and of bin and treatment [$F_{(2,96)}=2.6$, $p=0.080$] were observed on transitions and distance traveled [bin × genotype: $F_{(2,96)}=2.7$, $p=0.070$; bin × treatment: $F_{(2,96)}=2.6$, $p<0.080$], though these effects failed to reach statistical significance.

Study 2: Suramin (Subchronic)

Primary outcome variables of the BPM were analyzed via four factor ANOVA, using 15-min observation bin as a within-subjects factor, and sex, genotype, and treatment as between-subjects factors. These analyses revealed the same effects of genotype on counts [$F_{(1,55)}=52.4$, $p<0.001$; Fig. 2A], transitions [$F_{(1,55)}=17.7$, $p<0.001$; Fig. 2B], and distance traveled [$F_{(1,55)}=22.8$, $p<0.001$; Fig. 2C] that were observed in Study 1. No main or

interactive effects of sex or treatment were observed on these measures [F's<2.5, n.s.; Fig. 2A–C]. Significant main effects of sex [$F_{(1,55)}$=6.3, p<0.05] and genotype [$F_{(1,55)}$=7.5, p<0.01], as well as a sex × genotype interaction [$F_{(1,55)}$=5.6, p<0.05], were observed on holepoking, with male DAT KD mice demonstrating significantly less holepoking behavior than females [$F_{(1,28)}$=14.6, p<0.01; Fig. 2D]. Significantly more rears were performed by DAT KD mice than WT mice [$F_{(1,55)}$=15.7, p<0.001; Fig. 2E]. Neither sex nor treatment significantly affected rearing behavior, though a non-significant trend toward sex × genotype interaction was observed on total rears [$F_{(1,55)}$=3.5, p=0.067]; follow-up analyses of this trend were not conducted. As in the nicotine study, DAT KD mice demonstrated straighter path trajectories than WT controls, indicated by lower spatial d values [$F_{(1,55)}$=7.2, p<0.05; Fig. 2F]. No main or interactive effects of sex or treatment were observed on spatial d [F's<1, n.s.].

Main effects of bin were observed on counts [$F_{(2,110)}$=217.6, p<0.001], transitions [$F_{(2,110)}$=118.2, p<0.001], and distance traveled [$F_{(2,110)}$=203.3, p<0.001], indicating motor habituation across the session. Genotype significantly interacted with bin on counts [$F_{(2,110)}$=11.3, p<0.001], transitions [$F_{(2,110)}$=5.5 p<0.01], and distance traveled [$F_{(2,110)}$=5.6, p<0.01]. Follow-up analyses of these bin × genotype interactions were conducted, but the results did not reveal any markedly differential effect of genotype across the session – DAT KD mice exhibited significantly more activity than WT mice within each bin, as measured by each of these three variables [F's>14, p's<0.001]. Treatment significantly interacted with bin on transitions [$F_{(2,110)}$=4.1, p<0.05] and distance traveled [$F_{(2,110)}$=5.6, p<0.01], and non-significantly on counts [$F_{(2,110)}$=2.8, p=0.065]; follow-up analyses failed to identify any significant bin-specific effect of suramin [F's<1.3, n.s.]. Sex also interacted with bin on counts [$F_{(2,110)}$=17.5, p<0.001], transitions [$F_{(2,110)}$=12.8, p<0.001], and distance traveled [$F_{(2,110)}$=23.4, p<0.001]; given that these latter interactions were between sex and bin alone, and did not implicate either genotype or drug, follow-up analyses were not conducted. No three- or four-way interactions between bin and genotype, sex, or treatment were observed on any measures [F's<2.3].

Study 3: Resveratrol (Subchronic)

Primary outcome variables of the BPM were analyzed via four-factor ANOVA, using 20-min observation bin as a within-subjects factor, and genotype, treatment, and age (52 weeks vs 18–20 weeks) as between-subjects factors. Age did not significantly interact with genotype or treatment on any measure (p's>0.10), so mice from both age groups were treated as a single cohort for purposes of interpretation. As in the nicotine and suramin studies, main effects of genotype were observed on counts [$F_{(1,48)}$=24.3, p<0.001; Fig. 3A], transitions [$F_{(1,48)}$=18.0, p<0.001; Fig. 3B], and distance traveled [$F_{(1,48)}$=23.5, p<0.001; Fig. 3C], with DAT KD mice demonstrating higher levels of each measure. No main or interactive effects of treatment were observed on any of these measures, however [F's<1.2, n.s.]. Main effects of genotype [$F_{(1,48)}$=10.5, p<0.01] and treatment [$F_{(1,48)}$=5.3, p<0.05] indicated more frequent holepoking behavior in DAT KD mice and in resveratrol-treated mice, with a weak, non-significant trend toward genotype × treatment interaction [$F_{(1,48)}$=2.8, p=0.098]; follow-up analysis of this interaction revealed that resveratrol increased holepoking in DAT KD mice only [$F_{(1,20)}$=4.8, p<0.05; Fig. 3D]. DAT KD mice also completed more rears than

WT mice [$F_{(1,48)}$=19.9, p<0.001]; no main or interactive effects of treatment were observed on rearing behavior [F's<1.5, n.s.; Fig. 3E]. As in the nicotine and suramin studies, DAT KD mice displayed significantly lower spatial d than WT mice [$F_{(1,48)}$=5.4, p<0.05; Fig. 3F], indicating significantly straighter path trajectories which were not affected by resveratrol.

Main effects of 20-min observation bin were observed on counts [$F_{(2,96)}$=174.7, p<0.001], transitions [$F_{(2,96)}$=139.7, p<0.001], distance traveled [$F_{(2,96)}$=176.2, p<0.001], holepoking [$F_{(2,96)}$=11.8, p<0.001], and rearing [$F_{(2,96)}$=117.0, p<0.001], indicating a decrement in locomotor and exploratory activity across the testing session. Although bin did not significantly interact with genotype on counts [F<2.4, n.s.] or distance traveled [F<2.4, n.s.], a significant bin × genotype interaction was observed on transitions [$F_{(2,96)}$=3.6, p<0.05]; follow-up analyses were inconclusive. No two- or three-way interactions were observed between treatment and bin on these measures [F's<1, n.s.]. Bin did not interact with genotype or treatment on holepoking or rearing behavior [F's<1.7, n.s.].

The DAT hypoexpression phenotype of the DAT KD mouse was confirmed via Western Blot analysis [t(7)=−25.65, p<0.001]; DAT expression in the striata of DAT KD mice was approximately 10% that in WT mice, as has been previously described (Zhuang et al., 2001). Further analysis revealed that subchronic resveratrol treatment increased DAT expression in both DAT KD [1.55 fold-change; t(8) = 2.9, p<0.05] and WT mice [1.13 fold-change; t(6) = 3.6, p=0.01] compared to respective vehicle-treated groups (Fig. 4).

Discussion

When assessed in the cross-species translatable behavioral pattern monitor (BPM), dopamine transporter knock-down (DAT KD) mice reliably demonstrated a pattern of hyperexploration consistent with that of patients with bipolar disorder (BD) mania. Specifically, in each of the three studies, DAT KD mice exhibited increased motor activity, increased specific exploration, and straightened path trajectories relative to wildtype (WT) littermate controls (Figs. 1–3), as do BD mania patients relative to healthy participants (Minassian et al., 2011, 2010; Perry et al., 2009). Chronic nicotine, but not subchronic suramin or resveratrol, partially normalized this hyperactivity (Fig. 1A–C) and reduced holepoking behavior in male DAT KD mice (Fig. 1D). Western blot analysis of DAT KD and WT striata confirmed baseline DAT hypoexpression in the DAT KD cohort, and indicated an upregulation of striatal DAT in both lines following resveratrol treatment. The present findings illustrate the high fidelity of the mania-like profile of DAT KD mice (Kwiatkowski et al., 2019), as well as their utility in testing novel treatments. These data also support a role for long-term cholinergic agonism in the management of BD mania (Dulawa and Janowsky, 2019).

A recent meta-analysis of BPM data from >20 cohorts of DAT KD mice found that these mice reliably demonstrated significantly elevated levels of activity and exploration relative to WT littermates, as well as a significant shift toward straight-line path trajectories (Kwiatkowski et al., 2019). This consistency was observed across each of the three present studies as well, as measured by counts, transitions, distance traveled, rears, and spatial d. Holepoking behavior was somewhat more variable, meanwhile, with main effects of

genotype observed only in the suramin and resveratrol studies, and only in females in the former. The variability observed on this measure is not inconsistent with extant literature, however. Indeed, of the outcome variables examined in the recent meta-analysis, holepoking was found to be the least reliable (Kwiatkowski et al., 2019). Therefore, while male DAT KD mice did not demonstrate increased holepoking activity during the nicotine or suramin studies, the observed genotype effects on rearing, general activity, and spatial d nevertheless indicate robust mania-like profiles within those cohorts. Consistent with previous reports (Milienne-Petiot et al., 2017; Perry et al., 2009; van Enkhuizen et al., 2014, 2013; Young et al., 2010), no main or interactive effects of sex were observed on measures of general activity (counts, transitions, or distance traveled) during the suramin study (Fig. 2A–C), though as noted above, a significant sex × genotype interaction did reveal that only female DAT KD exhibited elevated holepoking.

Chronic treatment with the non-specific nicotinic acetylcholine receptor (nAChR) agonist nicotine reduced counts (Fig. 1A), distance traveled (Fig. 1C), and holepoking (Fig. 1D) in DAT KD mice only, thus partially remediating their manic phenotype and supporting our hypothesis that increasing cholinergic signaling would be beneficial to patients with BD. This remediation is incomplete, however, given that nicotine did not affect rearing (Fig. 1E) or spatial d (Fig. 1F), and that *post-hoc* analysis of a drug × genotype interaction ($p<0.05$) on transitions failed to reveal any significant DAT KD-specific normalization (Fig. 1B). Importantly, the measures of the BPM are dissociable, and it is not uncommon for them to be differentially affected by individual pharmacological manipulations; for example, valproic acid, a front-line treatment for BD, reduced transitions (i.e., hyperlocomotion) in DAT KD mice, but did not affect exploratory holepoking or rearing (van Enkhuizen et al., 2013). Nicotine, which affected holepoking but not rearing behavior, demonstrated a similar selectivity. Holepoking (the human analogue of which is interaction with objects placed around the BPM) is reflective of targeted, inspective exploration, whereas rearing is more closely tied to overall locomotor activity, and considered to reflect nonspecific, diversive exploratory behavior (Tanaka et al., 2012). Taken with the marginality of effect on the two explicit measures of locomotion – distance traveled ($p=0.073$) and transitions (non-significant *post hoc*) – this selectivity of nicotine for inspective versus diversive exploration may indicate that it primarily targets those frontal systems underlying specific exploration, while affecting motor activation secondarily (Young et al., 2016). Interestingly, holepoking behavior in DAT KD mice modestly correlates with risk preference in the Iowa Gambling Task (on which both DAT KD mice and BD patients exhibit deficits) (Young et al., 2019, 2011), and increased object interactions correlates with impaired performance on the Wisconsin Card Sorting Task in methamphetamine-dependent humans (Henry et al., 2011). Such correlations may form the basis of future studies of risk-preference and cognition in nicotine-treated DAT KD mice (see below).

The partial remediation of the DAT KD behavioral phenotype by nicotine is in line with the catecholaminergic-cholinergic balance model of BD pathology, which posits that symptoms of mania result from decreased cholinergic- vs. catecholaminergic activity (van Enkhuizen et al., 2015). Chronic nicotine administration ultimately induces long-term upregulation of most classes of nAChRs (Gentry and Lukas, 2002), which may produce symptom remediation via restoration of catecholaminergic-cholinergic equilibrium.

Indeed, this hypothesis is substantiated by findings that acetylcholinesterase inhibitors (e.g., physostigmine) also mitigate mania symptoms, ostensibly via a similar enhancement of cholinergic signaling (Dulawa and Janowsky, 2019) (such treatments are also likely to induce a depressive episode, however, an effect not observed following chronic nicotine). Importantly, nicotine does not affect all nAChR subtypes in the same manner, raising the possibility that its putative therapeutic effects are mediated by specific receptor subtypes rather than by gross enhancement of cholinergic signaling. For example, chronic nicotine induces *downregulation* of striatal α6-containing (α6*) nAChRs (Lai et al., 2005), a population of receptors involved in dopamine release (Salminen et al., 2004). Mice with gain-of-function mutations of the α6*nAChR exhibit similar characteristics to DAT KD mice, including hyperactivity and enhanced striatal dopamine transmission (Drenan et al., 2008). Though these similarities do not necessarily implicate specific α6*nAChR dysregulation in DAT KD mice, downregulation of this receptor class may nevertheless account for the nicotine-mediated modification of their behavioral profile, possibly via partial normalization of striatal dopamine signaling. Future work with specific nicotinic compounds applied directly to the striatum is needed to address this hypothesis.

Future investigations should also examine the contributions of other neuromodulatory systems to the effect profile of nicotine, particularly serotonin. Accumulating evidence suggests that the attenuation of hyperactivity by psychostimulants is mediated by the serotonin system (Gainetdinov et al., 1999); furthermore, direct enhancement of serotonergic signaling via fluoxetine, quipazine, and brexpiprazole normalized the behavioral phenotypes of DAT knockout and DAT KD mice (Gainetdinov et al., 1999; Milienne-Petiot et al., 2017). Nicotine increases serotonin release in the rat frontal cortex *in vivo* (Ribeiro et al., 1993) and rat striatum *in vitro* via nAChR-dependent mechanisms (Westfall et al., 1983), making this system an enticing target for future study. As mentioned above, however, care must be taken in differentiating between overall locomotion and specific exploration. As indicated by its significant attenuation of holepoking behavior and only borderline effects on transitions and distance traveled, nicotine seems to preferentially target the latter behavioral dimension versus the former. Brexpiprazole, meanwhile, decreased only transitions in DAT KD mice (Milienne-Petiot et al., 2017), and fluoxetine and quipazine (though yet to be assessed in DAT mutant mice in the BPM) mediated major reductions in horizontal movement in DAT knockout mice (Gainetdinov et al., 1999). This apparent difference in effect profile between nicotine and serotonergic drugs is not in itself discouraging, however. The serotonin system comprises many receptor subtypes with different, sometimes opposing, effects in the BPM (Geyer, 1995), none of which have been individually investigated in the context of specific exploration in genetically hyperdopaminergic mice. Therefore, while nicotine primarily attenuates different aspects of hyperactivity than those non-specific and mixed-action serotonergic drugs mentioned above (Maeda et al., 2014; Oosterhof et al., 2014), it remains possible that its effects are mediated via specific serotonin receptor populations.

Studies of the effects of nicotine on cognition in the DAT KD mouse are also warranted. In addition to the robust motor and exploratory profiles discussed above, BD mania is also characterized by inattention and risky decision making, as is readily apparent in the 5-choice continuous performance test (5C-CPT) and Iowa Gambling Task, respectively. Both of these tasks are translatable across species, and indeed, DAT KD mice exhibit performance deficits

that are consistent with those observed in BD mania patients (Young et al., 2019, 2011). Given that nicotine improves CPT performance in healthy humans (Levin et al., 1998) and WT mice (Young et al., 2013), a logical next step would be to determine whether nicotine remediates cognitive deficits in DAT KD mice. Indeed, nicotine has already been noted to exert potentially pro-cognitive and protective effects in BD patients; specifically, cigarette-smoking BD inpatients scored higher on tasks of memory and language than their nonsmoking counterparts at admission and discharge (Caldirola et al., 2013). Nicotine withdrawal, meanwhile, worsens cognitive performance in both humans and animals (Hall et al., 2015; Higa et al., 2017) and places BD patients at an increased risk for symptom recurrence (Thomson et al., 2015). The effects of acute and chronic withdrawal on DAT KD mice is therefore also worthy of study, the results of which may serve to partially explain the lower rates of smoking cessation rates observed amongst BD patients versus the general population (Jackson et al., 2015).

We did not observe any remediation of DAT KD mania-like behaviors following sub-chronic suramin administration (Fig. 2A–F). Although no studies have assessed the effects of suramin on BD patients, suramin has been shown to remediate symptoms of autism spectrum disorder (ASD) in both humans and mouse models. In a small study of male patients with ASD, suramin administration improved language and social interaction, and reduced repetitive behaviors (Naviaux et al., 2017); similarly, suramin restored normal social behaviors and novelty preference in mouse models of ASD (Naviaux et al., 2014, 2013). Although the present data do not suggest a role for suramin in the management of mania, reduction of purinergic signaling via other drugs (e.g., allopurinol) remediated mania symptoms in BD patients (Akhondzadeh et al., 2006; Machado-Vieira et al., 2008). Interpretation of the present data is limited by suramin's lack of receptor specificity – though popularly described as an inhibitor of the purinergic system, suramin also antagonizes dopaminergic (Beindl et al., 1996), adrenergic (Huang et al., 1990), glutamatergic (Peoples and Li, 1998), and opioid (Butler et al., 1988) receptors via destabilization of the ternary complex. The absence of effect on WT and DAT KD behavior may therefore be a cumulative result of suramin simultaneously interacting with opposing receptor classes, rather than of specific purinergic antagonism. Compounds that target specific purinergic receptors will be critical in determining the contribution of purinergic dysfunction to mania-related behaviors.

Subchronic resveratrol, previously demonstrated to upregulate striatal DAT expression in female WT mice (Di Liberto et al., 2012), also confirmed here in DAT KD mice (Fig. 4), did not significantly affect locomotion (Fig. 3A–C), rearing behavior (Fig. 3E), or path trajectory (Fig. 3F) in either WT or DAT KD mice, thought it did increase holepoking behavior in DAT KD mice only (Fig. 3D). The absence of treatment × genotype interactions on locomotion variables – and especially the genotype-specific increase in holepoking – was surprising, as the DAT-enhancing effect of resveratrol was expected to directly address the genetic modification of DAT KD mice and at least partially remediate their behavioral abnormalities. This lack of normalization was not likely a result of sub-optimal study parameters, given that the previously reported effect of resveratrol on striatal DAT was confirmed in both WT and DAT KD mice via Western blot analysis (Di Liberto et al., 2012). It therefore appears that upregulating striatal DAT is not sufficient to remediate mania-like symptoms in DAT KD mice. Given that the DAT gene is non-specifically knocked down in

DAT KD mice, and the consequent reduction in DAT expression therefore not limited to a single structure (e.g., the striatum), the overall absence of effect of resveratrol may indicate that the mania-like profile of DAT KD mice is mediated by altered dopamine dynamics elsewhere in the brain. Furthermore, it remains unknown whether the effects of resveratrol on DAT expression are limited to the striatum, as studies investigating this interaction have so far only examined this one general area. Given the significantly decreased DAT availability observed in the BD dorsal striatum (Anand et al., 2011), more targeted study is certainly justified.

The DAT KD mouse is a robust model of mania-relevant behaviors (Kwiatkowski et al., 2019) that, when assessed in the BPM, provides a pharmacologically valid means by which to screen potential anti-mania therapeutics. The present findings suggest a role for chronic cholinergic agonism in the management of mania symptoms, especially those related to specific exploration. Targeted studies of specific brain regions implicated in BD pathology are needed to elucidate the mechanisms underlying this attenuation. Future studies should also utilize nAChR subtype-specific compounds in order to identify receptor subtypes that may be targeted for the remediation of mania symptoms.

Acknowledgements:

The authors thank Drs. Mark A. Geyer, Arpi Minassian, and William Perry as well as Ms. Mahalah R. Buell and Mr. Richard F. Sharp for their support.

Role of Funding Source:

Funding for this study was provided by NIH R01s MH0104344, DA043535, DA44909, as well as the Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center. These funding sources had no further role in study design, data collection, data analysis, or data interpretation; they had no role in the writing of this manuscript or in the decision to submit it for publication.

References

Akhondzadeh S, Milajerdi MR, Amini H, Tehrani-Doost M, 2006. Allopurinol as an adjunct to lithium and haloperidol for treatment of patients with acute mania: a double-blind, randomized, placebo-controlled trial. Bipolar Disord. 8, 485–489. doi:10.1111/j.1399-5618.2006.00363.x [PubMed: 17042886]

Anand A, Barkay G, Dzemidzic M, Albrecht D, Karne H, Zheng Q-H, Hutchins GD, Normandin MD, Yoder KK, 2011. Striatal dopamine transporter availability in unmedicated bipolar disorder. Bipolar Disord. 13, 406–13. doi:10.1111/j.1399-5618.2011.00936.x [PubMed: 21843280]

Ashok AH, Marques TR, Jauhar S, Nour MM, Goodwin GM, Young AH, Howes OD, 2017. The dopamine hypothesis of bipolar affective disorder: the state of the art and implications for treatment. Mol. Psychiatry 22, 666–679. doi:10.1038/mp.2017.16 [PubMed: 28289283]

Beindl W, Mitterauer T, Hohenegger M, Ijzerman AP, Nanoff C, Freissmuth M, 1996. Inhibition of receptor/G protein coupling by suramin analogues. Mol. Pharmacol. 50, 415–23. [PubMed: 8700151]

BOISON D, 2008. Adenosine as a neuromodulator in neurological diseases. Curr. Opin. Pharmacol. 8, 2–7. doi:10.1016/j.coph.2007.09.002 [PubMed: 17942368]

Butler SJ, Kelly EC, McKenzie FR, Guild SB, Wakelam MJ, Milligan G, 1988. Differential effects of suramin on the coupling of receptors to individual species of pertussis-toxin-sensitive guanine-nucleotide-binding proteins. Biochem. J. 251, 201–5. doi:10.1042/bj2510201 [PubMed: 2839158]

Caldirola D, Daccò S, Grassi M, Citterio A, Menotti R, Cavedini P, Girardi P, Perna G, 2013. Effects of cigarette smoking on neuropsychological performance in mood disorders: A comparison between smoking and nonsmoking inpatients. J. Clin. Psychiatry 74. doi:10.4088/JCP.12m07985

Cheffer A, Castillo ARG, Corrêa-Velloso J, Gonçalves MCB, Naaldijk Y, Nascimento IC, Burnstock G, Ulrich H, 2018. Purinergic system in psychiatric diseases. Mol. Psychiatry 23, 94–106. doi:10.1038/mp.2017.188 [PubMed: 28948971]

Cipriani A, Barbui C, Salanti G, Rendell J, Brown R, Stockton S, Purgato M, Spineli LM, Goodwin GM, Geddes JR, 2011. Comparative efficacy and acceptability of antimanic drugs in acute mania: a multiple-treatments meta-analysis. Lancet 378, 1306–1315. doi:10.1016/S0140-6736(11)60873-8 [PubMed: 21851976]

Di Liberto V, Mäkelä J, Korhonen L, Olivieri M, Tselykh T, Mälkiä A, Do Thi H, Belluardo N, Lindholm D, Mudò G, 2012. Involvement of estrogen receptors in the resveratrol-mediated increase in dopamine transporter in human dopaminergic neurons and in striatum of female mice. Neuropharmacology 62, 1011–1018. doi:10.1016/j.neuropharm.2011.10.010 [PubMed: 22041555]

Drenan RM, Grady SR, Whiteaker P, McClure-Begley T, McKinney S, Miwa JM, Bupp S, Heintz N, McIntosh JM, Bencherif M, Marks MJ, Lester HA, 2008. In vivo activation of midbrain dopamine neurons via sensitized, high-affinity alpha 6 nicotinic acetylcholine receptors. Neuron 60, 123–36. doi:10.1016/j.neuron.2008.09.009 [PubMed: 18940593]

Dulawa SC, Janowsky DS, 2019. Cholinergic regulation of mood: from basic and clinical studies to emerging therapeutics. Mol. Psychiatry 24, 694–709. doi:10.1038/s41380-018-0219-x [PubMed: 30120418]

Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MC, 1999. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science (80-.). 283, 397–401. doi:10.1126/science.283.5400.397

Gentry CL, Lukas RJ, 2002. Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. Curr. Drug Targets. CNS Neurol. Disord. 1, 359–85. [PubMed: 12769610]

Geyer MA, 1995. Serotonergic functions in arousal and motor activity. Behav. Brain Res. 73, 31–35. doi:10.1016/0166-4328(96)00065-4

Grande I, Berk M, Birmaher B, Vieta E, 2016. Bipolar disorder. Lancet 387, 1561–1572. doi:10.1016/S0140-6736(15)00241-X [PubMed: 26388529]

Grant BF, Hasin DS, Chou SP, Stinson FS, Dawson DA, 2004. Nicotine Dependence and Psychiatric Disorders in the United States. Arch. Gen. Psychiatry 61, 1107. doi:10.1001/archpsyc.61.11.1107 [PubMed: 15520358]

Greenwood TA, Alexander M, Keck PE, McElroy S, Sadovnick AD, Remick RA, Kelsoe JR, 2001. Evidence for linkage disequilibrium between the dopamine transporter and bipolar disorder. Am. J. Med. Genet. 105, 145–51. [PubMed: 11304827]

Greenwood TA, Schork NJ, Eskin E, Kelsoe JR, 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, 125–33, 115. doi:10.1038/sj.mp.4001764 [PubMed: 16261167]

Hall FS, Der-Avakian A, Gould TJ, Markou A, Shoaib M, Young JW, 2015. Negative affective states and cognitive impairments in nicotine dependence. Neurosci. Biobehav. Rev. doi:10.1016/j.neubiorev.2015.06.004

Henry BL, Minassian A, Van Rhenen M, Young JW, Geyer MA, Perry W, Grant I, Letendre S, Ellis RJ, Achim C, Woods S, Ortiz S, Sherman M, Schrier R, Toperoff W, Talob A, Potter M, Capparelli E, Pharm D, Marquie-Beck J, Heaton R, Alexander T, Atkinson JH, Cherner M, Marcotte T, Duarte N, Parikh M, Meyer R, Aron A, Dawson M, Brown G, Archibald S, Dale A, Fenema-Notestine C, Jernigan T, Liu T, Scadeng M, Beyenhof B, Walpole D, Masliah E, Everall I, Lipton S, Soontornniyomkij V, Tatro E, Sabouri A, Mante M, Zhang D, Buell M, Grethe A, Paulus M, Morris S, Smith D, Pacheco DD, Semenova S, Markou A, Kaul M, Von Jaeger R, Oyos T, Hendrix T, Seghers R, Gamst AC, Cushman C, Repinski M, Mendez J, Abramson I, Ake C, Deutsch R, Vaida F, 2011. Effect of methamphetamine dependence on inhibitory deficits in a novel human open-field paradigm. Psychopharmacology (Berl). 215, 697–707. doi:10.1007/s00213-011-2170-2 [PubMed: 21279333]

Higa KK, Grim A, Kamenski ME, van Enkhuizen J, Zhou X, Li K, Naviaux JC, Wang L, Naviaux RK, Geyer MA, Markou A, Young JW, 2017. Nicotine withdrawal-induced inattention is absent in alpha7 nAChR knockout mice. Psychopharmacology (Berl). 234, 1573–1586. doi:10.1007/s00213-017-4572-2 [PubMed: 28243714]

Horschitz S, Hummerich R, Lau T, Rietschel M, Schloss P, 2005. A dopamine transporter mutation associated with bipolar affective disorder causes inhibition of transporter cell surface expression. Mol. Psychiatry 10, 1104–1109. doi:10.1038/sj.mp.4001730 [PubMed: 16103889]

Huang RR, Dehaven RN, Cheung AH, Diehl RE, Dixon RA, Strader CD, 1990. Identification of allosteric antagonists of receptor-guanine nucleotide-binding protein interactions. Mol. Pharmacol. 37, 304–10. [PubMed: 2154676]

Jackson JG, Diaz FJ, Lopez L, de Leon J, 2015. A combined analysis of worldwide studies demonstrates an association between bipolar disorder and tobacco smoking behaviors in adults. Bipolar Disord doi:10.1111/bdi.12319

Ji B, Higa KK, Kim M, Zhou L, Young JW, Geyer MA, Zhou X, 2014. Inhibition of protein translation by the DISC1-Boymaw fusion gene from a Scottish family with major psychiatric disorders. Hum. Mol. Genet. 23, 5683–5705. doi:10.1093/hmg/ddu285 [PubMed: 24908665]

Kwiatkowski MA, Hellemann G, Sugar CA, Cope ZA, Minassian A, Perry W, Geyer MA, Young JW, 2019. Dopamine transporter knockdown mice in the behavioral pattern monitor: A robust, reproducible model for mania-relevant behaviors. Pharmacol. Biochem. Behav. 178, 42–50. doi:10.1016/j.pbb.2017.12.007 [PubMed: 29289701]

Lai A, Parameswaran N, Khwaja M, Whiteaker P, Lindstrom JM, Fan H, McIntosh JM, Grady SR, Quik M, 2005. Long-Term Nicotine Treatment Decreases Striatal α6* Nicotinic Acetylcholine Receptor Sites and Function in Mice. Mol. Pharmacol. 67, 1639–1647. doi:10.1124/mol.104.006429 [PubMed: 15681595]

Levin ED, Conners CK, Silva D, Hinton SC, Meck WH, March J, Rose JE, 1998. Transdermal nicotine effects on attention. Psychopharmacology (Berl). 140, 135–141. doi:10.1007/s002130050750 [PubMed: 9860103]

Machado-Vieira R, Lara DR, Souza DO, Kapczinski F, 2002. Purinergic dysfunction in mania: an integrative model. Med. Hypotheses 58, 297–304. doi:10.1054/mehy.2001.1543 [PubMed: 12027524]

Machado-Vieira R, Soares JC, Lara DR, Luckenbaugh DA, Busnello JV, Marca G, Cunha A, Souza DO, Zarate CA, Kapczinski F, 2008. A double-blind, randomized, placebo-controlled 4-week study on the efficacy and safety of the purinergic agents allopurinol and dipyridamole adjunctive to lithium in acute bipolar mania. J. Clin. Psychiatry 69, 1237–45. [PubMed: 18681754]

Maeda K, Lerdrup L, Sugino H, Akazawa H, Amada N, McQuade RD, Stensbøl TB, Bundgaard C, Arnt J, Kikuchi T, 2014. Brexpiprazole II: Antipsychotic-like and procognitive effects of a novel serotonin-dopamine activity modulator. J. Pharmacol. Exp. Ther. 350, 605–614. doi:10.1124/jpet.114.213819 [PubMed: 24947464]

Merikangas KR, Jin R, He J-P, Kessler RC, Lee S, Sampson NA, Viana MC, Andrade LH, Hu C, Karam EG, Ladea M, Medina-Mora ME, Ono Y, Posada-Villa J, Sagar R, Wells JE, Zarkov Z, 2011. Prevalence and Correlates of Bipolar Spectrum Disorder in the World Mental Health Survey Initiative. Arch. Gen. Psychiatry 68, 241. doi:10.1001/archgenpsychiatry.2011.12 [PubMed: 21383262]

Milienne-Petiot M, Geyer MA, Arnt J, Young JW, 2017. Brexpiprazole reduces hyperactivity, impulsivity, and risk-preference behavior in mice with dopamine transporter knockdown—a model of mania. Psychopharmacology (Berl). 234, 1017–1028. doi:10.1007/s00213-017-4543-7 [PubMed: 28160035]

Minassian A, Henry BL, Geyer MA, Paulus MP, Young JW, Perry W, 2010. The quantitative assessment of motor activity in mania and schizophrenia. J. Affect. Disord. 120, 200–206. doi:10.1016/j.jad.2009.04.018 [PubMed: 19435640]

Minassian A, Henry BL, Young JW, Masten V, Geyer MA, Perry W, 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. doi:10.1371/journal.pone.0024185 [PubMed: 21912623]

Naviaux JC, Schuchbauer MA, Li K, Wang L, Risbrough VB, Powell SB, Naviaux RK, 2014. Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy. Transl. Psychiatry 4. doi:10.1038/tp.2014.33

Naviaux JC, Wang L, Li K, Bright AT, Alaynick WA, Williams KR, Powell SB, Naviaux RK, 2015. Antipurinergic therapy corrects the autism-like features in the Fragile X (Fmr1 knockout) mouse model. Mol. Autism 6. doi:10.1186/2040-2392-6-1

Naviaux RK, Curtis B, Li K, Naviaux JC, Bright AT, Reiner GE, Westerfield M, Goh S, Alaynick WA, Wang L, Capparelli EV, Adams C, Sun J, Jain S, He F, Arellano DA, Mash LE, Chukoskie L, Lincoln A, Townsend J, 2017. Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial. Ann. Clin. Transl. Neurol. 4, 491–505. doi:10.1002/acn3.424 [PubMed: 28695149]

Naviaux RK, Zolkipli Z, Wang L, Nakayama T, Naviaux JC, Le TP, Schuchbauer MA, Rogac M, Tang Q, Dugan LL, Powell SB, 2013. Antipurinergic Therapy Corrects the Autism-Like Features in the Poly(IC) Mouse Model. PLoS One 8. doi:10.1371/journal.pone.0057380

Oosterhof CA, El Mansari M, Blier P, 2014. Acute effects of brexpiprazole on serotonin, dopamine, and norepinephrine systems: An in vivo electrophysiologic characterization. J. Pharmacol. Exp. Ther. 351, 585–595. doi:10.1124/jpet.114.218578 [PubMed: 25225185]

Peoples RW, Li C, 1998. Inhibition of NMDA-gated ion channels by the P2 purinoceptor antagonists suramin and reactive blue 2 in mouse hippocampal neurones. Br. J. Pharmacol. 124, 400–408. doi:10.1038/sj.bjp.0701842 [PubMed: 9641559]

Perry W, Minassian A, Paulus MP, Young JW, Kincaid MJ, Ferguson EJ, Henry BL, Zhuang X, Masten VL, Sharp RF, Geyer MA, 2009. A reverse-translational study of dysfunctional exploration in psychiatric disorders: from mice to men. Arch. Gen. Psychiatry 66, 1072–80. doi:10.1001/archgenpsychiatry.2009.58 [PubMed: 19805697]

Pinsonneault JK, Han DD, Burdick KE, Kataki M, Bertolino A, Malhotra AK, Gu HH, Sadee W, 2011. Dopamine Transporter Gene Variant Affecting Expression in Human Brain is Associated with Bipolar Disorder. Neuropsychopharmacology 36, 1644–1655. doi:10.1038/npp.2011.45 [PubMed: 21525861]

Portugal GS, Gould TJ, 2009. Nicotine withdrawal disrupts new contextual learning. Pharmacol. Biochem. Behav. 92, 117–123. doi:10.1016/j.pbb.2008.11.001 [PubMed: 19028516]

Ribeiro EB, Bettiker RL, Bogdanov M, Wurtman RJ, 1993. Effects of systemic nicotine on serotonin release in rat brain. Brain Res. 621, 311–318. doi:10.1016/0006-8993(93)90121-3 [PubMed: 8242344]

Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, Grady SR, 2004. Subunit Composition and Pharmacology of Two Classes of Striatal Presynaptic Nicotinic Acetylcholine Receptors Mediating Dopamine Release in Mice. Mol. Pharmacol. 65, 1526–1535. doi:10.1124/mol.65.6.1526 [PubMed: 15155845]

Sutin AR, Cutler RG, Camandola S, Uda M, Feldman NH, Cucca F, Zonderman AB, Mattson MP, Ferrucci L, Schlessinger D, Terracciano A, 2014. Impulsivity is Associated with Uric Acid: Evidence from Humans and Mice. Biol. Psychiatry 75, 31–37. doi:10.1016/j.biopsych.2013.02.024 [PubMed: 23582268]

Tanaka S, Young JW, Halberstadt AL, Masten VL, Geyer MA, 2012. Four factors underlying mouse behavior in an open field. Behav. Brain Res. 233, 55–61. doi:10.1016/j.bbr.2012.04.045 [PubMed: 22569582]

Thomson D, Berk M, Dodd S, Rapado-Castro M, Quirk SE, Ellegaard PK, Berk L, Dean OM, 2015. Tobacco use in bipolar disorder. Clin. Psychopharmacol. Neurosci. 13, 1–11. doi:10.9758/cpn.2015.13.1.1 [PubMed: 25912533]

van Enkhuizen J, Geyer MA, Halberstadt AL, Zhuang X, Young JW, 2014. Dopamine depletion attenuates some behavioral abnormalities in a hyperdopaminergic mouse model of bipolar disorder. J. Affect. Disord. 155, 247–254. doi:10.1016/j.jad.2013.08.041 [PubMed: 24287168]

van Enkhuizen J, Geyer MA, Kooistra K, Young JW, 2013. Chronic valproate attenuates some, but not all, facets of mania-like behaviour in mice. Int. J. Neuropsychopharmacol. 16, 1021–1031. doi:10.1017/S1461145712001198 [PubMed: 23164454]

van Enkhuizen J, Janowsky DS, Olivier B, Minassian A, Perry W, Young JW, Geyer MA, 2015. The catecholaminergic–cholinergic balance hypothesis of bipolar disorder revisited. Eur. J. Pharmacol. 753, 114–126. doi:10.1016/j.ejphar.2014.05.063 [PubMed: 25107282]

Westfall TC, Grant H, Perry H, 1983. Release of dopamine and 5-hydroxytryptamine from rat striatal slices following activation of nicotinic cholinergic receptors. Gen. Pharmacol. 14, 321–325. doi:10.1016/0306-3623(83)90037-X [PubMed: 6135645]

Yildiz A, Vieta E, Leucht S, Baldessarini RJ, 2011. Efficacy of Antimanic Treatments: Meta-analysis of Randomized, Controlled Trials. Neuropsychopharmacology 36, 375–389. doi:10.1038/npp.2010.192 [PubMed: 20980991]

Young JW, Dulcis D, 2015. Investigating the mechanism(s) underlying switching between states in bipolar disorder. Eur. J. Pharmacol. 759, 151–62. doi:10.1016/j.ejphar.2015.03.019 [PubMed: 25814263]

Young JW, Geyer MA, Halberstadt AL, Enkhuizen J, Minassian A, Khan A, Perry W, Eyler LT, 2019. Convergent neural substrates of inattention in bipolar disorder patients and dopamine transporter-deficient mice using the 5- choice CPT. Bipolar Disord. doi:10.1111/bdi.12786

Young JW, Goey AKL, Minassian A, Perry W, Paulus MP, Geyer MA, 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. doi:10.1016/j.pbb.2010.03.014 [PubMed: 20363246]

Young JW, Meves JM, Geyer MA, 2013. Nicotinic agonist-induced improvement of vigilance in mice in the 5-choice continuous performance test. Behav. Brain Res. 240, 119–133. doi:10.1016/j.bbr.2012.11.028 [PubMed: 23201359]

Young JW, Minassian A, Geyer MA, 2016. Locomotor profiling from rodents to the clinic and back again, in: Current Topics in Behavioral Neurosciences. Springer Verlag, pp. 287–303. doi:10.1007/7854_2015_5015

Young JW, Minassian A, Paulus MP, Geyer MA, Perry W, 2007. A reverse-translational approach to bipolar disorder: rodent and human studies in the Behavioral Pattern Monitor. Neurosci. Biobehav. Rev. 31, 882–96. doi:10.1016/j.neubiorev.2007.05.009 [PubMed: 17706782]

Young JW, van Enkhuizen J, Winstanley CA, Geyer MA, 2011. Increased risk-taking behavior in dopamine transporter knockdown mice: further support for a mouse model of mania. J. Psychopharmacol. 25, 934–43. doi:10.1177/0269881111400646 [PubMed: 21421642]

Zarate CA, Manji HK, 2008. Bipolar disorder: candidate drug targets. Mt. Sinai J. Med. A J. Transl. Pers. Med. 75, 226–247. doi:10.1002/msj.20042

Zhuang X, Oosting RS, Jones SR, Gainetdinov RR, Miller GW, Caron MG, Hen R, 2001. Hyperactivity and impaired response habituation in hyperdopaminergic mice. Proc. Natl. Acad. Sci. 98, 1982–1987. doi:10.1073/pnas.98.4.1982 [PubMed: 11172062]

Highlights

- Novel therapeutics are needed for the symptoms of bipolar mania
- Dopamine transporter knockdown (DAT KD) mice recreate hyperdopaminergia of mania
- DAT KD mice exhibit a mania-like profile in the behavioral pattern monitor (BPM)
- Chronic nicotine partially remediates hyperexploratory BPM profile of DAT KD mice
- Subchronic suramin or resveratrol do not remediate the DAT KD BPM profile

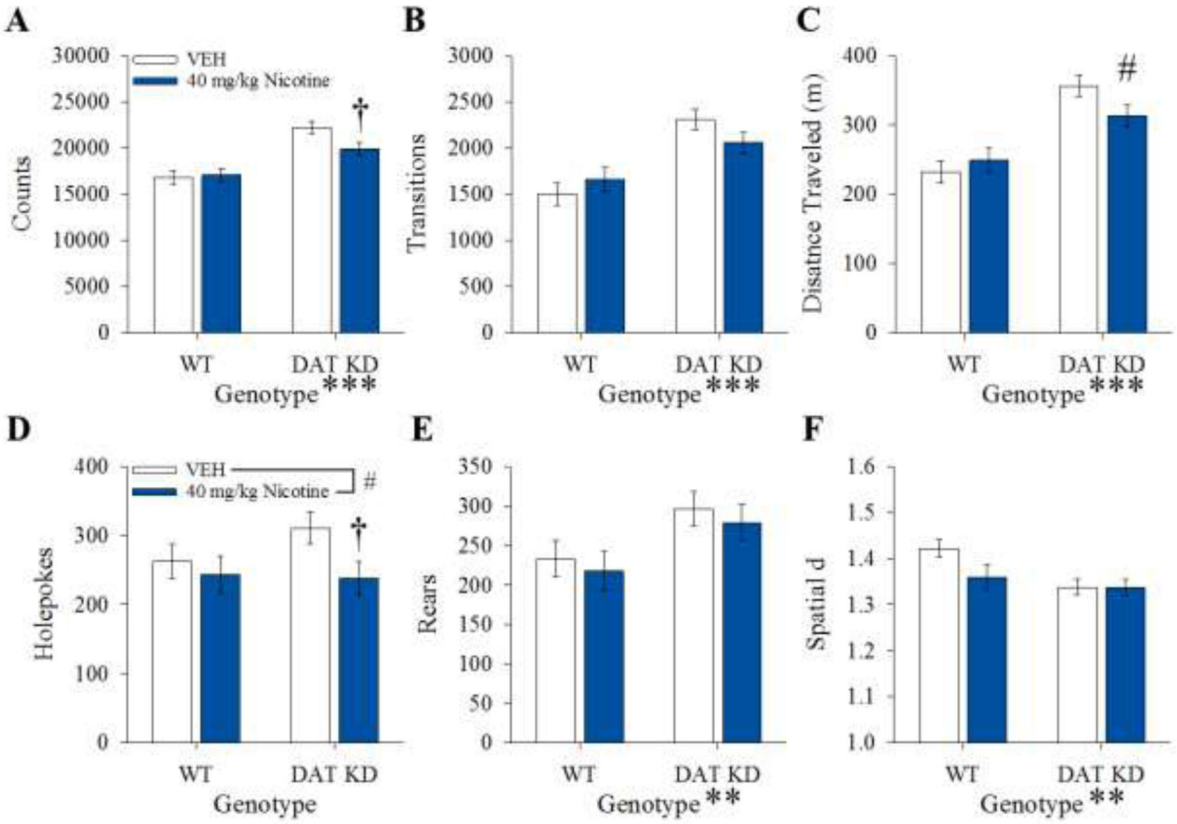

Figure 1. Chronic nicotine partially remediated the hyperactivity phenotype of DAT KD mice.

DAT KD mice displayed increased levels of overall activity and locomotion relative to WT controls. Chronic nicotine decreased activity (counts and distance traveled) in DAT KD mice only, partially normalizing their characteristic hyperactivity (**A-C**). Genotype did not affect exploratory holepoking, though chronic nicotine tended to reduce this behavior across groups. Planned analyses revealed that nicotine significantly reduced holepoking in DAT KD mice only (**D**). DAT KD mice exhibited increased rearing behavior compared to WT mice, with no effect of nicotine (**E**). DAT KD mice demonstrated significantly straighter path trajectories than WT controls, as measured by spatial d, with no main or interactive effects of nicotine (**F**). Data presented as mean ±S.E.M. †=p<0.05 vs VEH; #=p<0.10 vs VEH; **=p<0.01; ***=p<0.001.

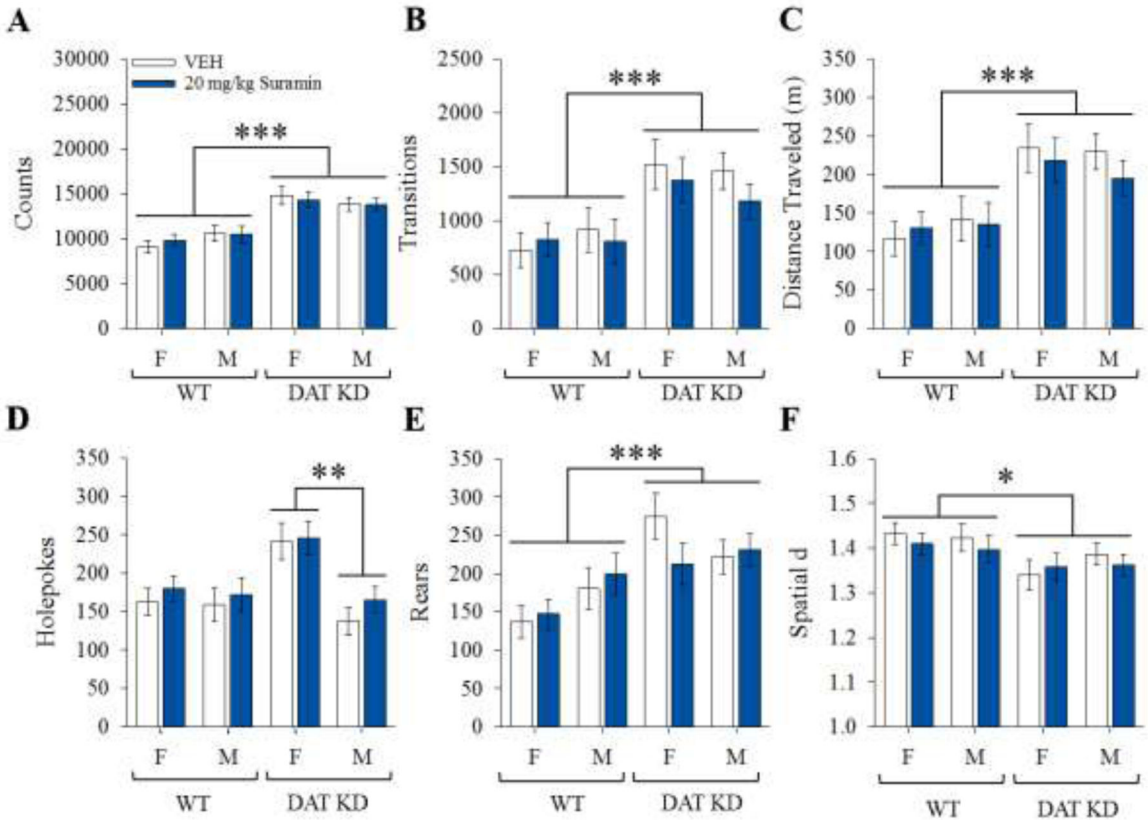

Figure 2. Subchronic suramin did not affect locomotor or exploratory behavior in DAT KD or WT mice.

Regardless of sex or suramin treatment, DAT KD mice exhibited higher activity and locomotion than WT controls (**A-C**). Regardless of suramin treatment, male DAT KD mice exhibited lower levels of holepoking behavior relative to all other groups (**D**). DAT KD mice displayed more rearing behavior than WT littermates, but no effects of sex or suramin treatment were observed (**E**). DAT KD mice exhibited lower spatial d values than WT mice regardless of sex or suramin treatment. Data presented as mean ±S.E.M. *=p<0.05; **=p<0.01; ***=p<0.001.

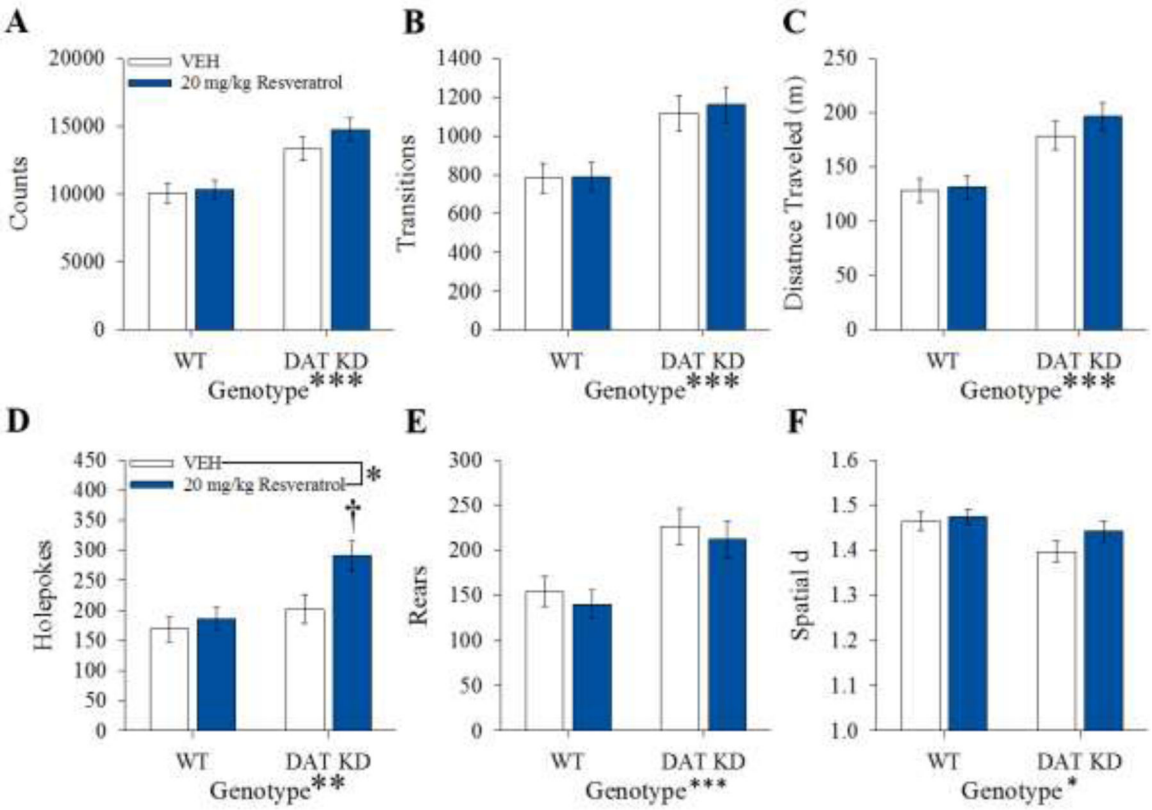

Figure 3. Subchronic resveratrol did not affect the hyperactive profile of DAT KD mice, but non-specifically increased exploration.

DAT KD mice displayed a hyperactive profile that was resistant to the effects of subchronic resveratrol (**A-C**). DAT KD- and resveratrol-treated mice demonstrated increased holepoking behavior relative to WT- and vehicle-treated controls, both of which effects were seemingly driven by the resveratrol-treated DAT KD group (**D**). DAT KD mice displayed increased rearing behavior compared to WT mice, but no effect of resveratrol treatment was observed (**E**). DAT KD mice displayed lower spatial d values than WT mice, regardless of resveratrol treatment. Data presented as mean ±S.E.M. †=p<0.05 vs VEH; *=p<0.05; **=p<0.01; ***=p<0.001.

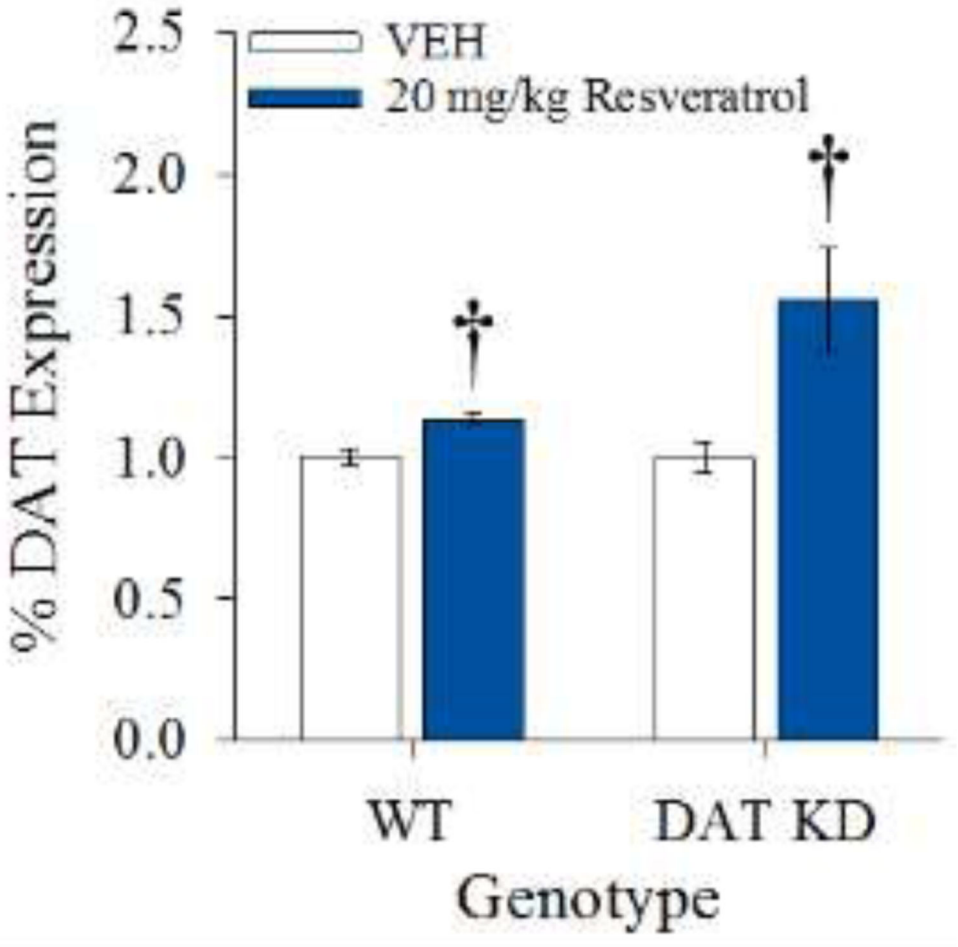

Figure 4. Subchronic resveratrol administration increased striatal DAT expression in DAT KD and WT mice.

DAT expression was normalized to vehicle treatment (1.0). Thus, genotype-specific analyses revealed that, relative to vehicle, resveratrol administration induced a 1.13 fold-change in striatal DAT expression in WT mice, and a 1.55 fold-change in DAT KD mic. Vehicle-treated DAT KD mice expressed ~10% of vehicle-treated WT DAT levels, and resveratrol-treated DAT KD mice expressed ~15% of vehicle-treated WT DAT levels. Data presented as mean ±S.E.M. †=$p<0.05$ vs VEH