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Brexpiprazole reduces hyperactivity, impulsivity, and risk-preference behavior in mice with dopamine transporter knockdown – a model of mania

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Abstract

Rationale—Bipolar Disorder (BD) is a unique mood disorder defined by periods of depression and mania. The defining diagnosis of BD is the presence of mania/hypomania, with symptoms including hyperactivity and risk-taking. Since current treatments do not ameliorate cognitive deficits such as risky decision-making, and impulsivity that can negatively affect a patient's quality of life, better treatments are needed.

Objectives—Here, we tested whether acute treatment with brexpiprazole, a serotonin-dopamine activity modulator with partial agonist activity at $D_{2/3}$ and 5-HT_{1A} receptors, would attenuate the BD mania-relevant behaviors of the dopamine transporter (DAT) knockdown mouse model of mania.

Methods—The effects of brexpiprazole on DAT knockdown and wild-type littermate mice were examined in the Behavioral Pattern Monitor (BPM) and Iowa Gambling Task (IGT) to quantify activity/exploration and impulsivity/risk-taking behavior respectively.

Results—DAT knockdown mice exhibited hyper-exploratory behavior in the BPM and made fewer safe choices in the IGT. Brexpiprazole attenuated the mania-like hyper-exploratory phenotype and increased safe choices in risk-preferring DAT knockdown mice. Brexpiprazole also reduced safe choices in safe-preferring mice irrespective of genotype. Finally, brexpiprazole reduced premature (impulsive-like) responses in both groups of mice.

Conclusions—Consistent with earlier reports, DAT knockdown mice exhibited hyper-exploratory, risk-preferring, and impulsive-like profiles consistent with patients with BD mania in these tasks. These behaviors were attenuated after brexpiprazole treatment. These data therefore indicate that brexpiprazole could be a novel treatment for BD mania and/or risk-taking/impulsivity disorders, since it remediates some relevant behavioral abnormalities in this mouse model.

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Keywords

Iowa Gambling Task; Risk taking; D_2 receptor; Cognition; Bipolar disorder

INTRODUCTION

Multiple psychiatric disorders manifest with both abnormal impulsive behavior and increased risk-taking. One such disorder is bipolar disorder (BD), which affects 1–2% of the global population for BD type I (Merikangas et al. 2011). The seriousness of BD is indicated by an increased suicide mortality rate (Osby et al. 2001) where one in three patients attempt suicide (Novick et al. 2010), which has been linked to impulsive action. BD is a unique mood disorder however since it is defined by periods of depression and (hypo-)mania during which the patients' symptoms differ markedly (Belmaker and Bersudsky 2004). In fact, symptoms can largely be opposite from each other with hyperactivity, risk-taking, and impulsive action being hallmark features of mania, whereas lethargy, anhedonia, and increased sleep being characteristic of depression (American Psychiatric Association 2013). The defining diagnosis of BD is the presence of mania/hypomania. Impulsive action and high reward risk-taking can be measured by the Iowa Gambling Task [IGT; (van Enkhuizen et al. 2015b)], providing objective measures of these behaviors. Treatments exist for mania and depression as well as to maintain a patient's state between periods (Malhi et al. 2012), but their efficacy is limited and low acceptability profiles are low (Cipriani et al. 2011). Moreover, no treatments are approved specifically for the treatment of the cognitive deficits, such as risky-choices, which have been linked to patients' ability to live independently (Green 2006). Therefore, better therapeutics for BD are needed, particularly in the domains of risk-taking and impulsive action.

Studies implicate dopaminergic abnormalities in impulsivity and risk-taking, particularly in BD mania. For example, polymorphisms for the gene encoding for the dopamine transporter (DAT) have been associated with BD (Greenwood et al. 2006). Such polymorphisms likely result in reduced functional expression of DAT (Horschitz et al. 2005). Reduced DAT functioning elevates extracellular dopamine in mice (Zhuang et al. 2001). The effects of reduced DAT function can be mediated by dopamine receptors, i.e. D_1-family (D_1 and D_5) and D_2-family (D_2, D_3, and D_4) receptors (Kwek and van den Buuse 2013; Young and Geyer 2010; Young et al. 2011b; Young et al. 2011d). The dopamine D_3 receptor has been associated with reward-related processing. The dopamine D_3 partial agonist BP897 (N-[4-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl]naphthalene-2-carboxamide), inhibits cocaine-seeking behavior (Pilla et al. 1999). Activation of striatal D_2 receptors facilitates avoidance learning in rodents, which suggests that reduced functioning might lead to diminished sensitivity to negative or punishing outcomes. On the other hand, low D_2 receptor availability in the substantia nigra/ventral tegmental area has been positively associated with amphetamine-induced striatal dopamine release in healthy humans and with novelty-seeking in rats (Oswald et al. 2015). Hence, D_2-family receptors have been associated with reward- and punishment-associated learning in rodents.

Brexpiprazole is a serotonin-dopamine activity modulator that acts as a partial agonist at 5-HT_{1A} and dopamine $D_{2/3}$ receptors, and as an antagonist at 5-HT_{2A} and noradrenaline $alpha_{1B/2C}$ receptors, all at similar potencies (Maeda et al. 2014; Oosterhof et al. 2014). Since brexpiprazole improved cognitive flexibility and executive function in an attentional set-shifting test in rats and improved social recognition in the Social Recognition Test in mice (Citrome et al. 2015), we speculate that it may remediate cognitive deficits in psychiatric patients. Brexpiprazole has recently been approved in the USA for the treatment for schizophrenia and as an adjunctive treatment with antidepressants in major depressive disorder patients exhibiting inadequate responses to monotherapy. Considering that some treatments cross diagnostic boundaries (e.g., antipsychotics), brexpiprazole may prove efficacious for treating symptoms of BD, a hypothesis that should first be tested in a viable animal model.

Mice with reduced dopamine transporter (DAT) levels [knockdown (KD) mice] exhibit mania-like behavior when tested in the cross-species behavioral pattern monitor [BPM (Young et al. 2007)]. These behaviors include hyperactivity, increased exploration, and altered locomotor patterns with straighter patterns of movement (reduced spatial *d*) and less predictable patterns of movements (increased entropy h). This pattern replicates that seen in BD patients during an acute manic episode (Perry et al. 2009), and is consistent over time (Minassian et al. 2011). Importantly, valproate reduced hyperactivity in this model (van Enkhuizen et al. 2013). Given that valproate reduced hyperactivity in BD mania patients (Minassian et al. 2011) without affecting specific exploration or spatial *d*, these data provide further pharmacological predictive validity of the model (Young et al. 2011a). Another cross-species behavioral task is the IGT, which utilizes high-yield/high-risk versus low-yield/low-risk options to measure decision making with real-world translational validity in one test-session (Bechara et al. 1994). BD patients exhibit poor performance on the IGT (Adida et al. 2011; Ibanez et al. 2012). Moreover, a diagnosis-specific performance profile can be discerned. While manic BD patients are hypersensitive to rewards (Cassidy et al. 1998), schizophrenia patients exhibit disrupted contingency learning (Brambilla et al. 2013) and depressed patients are more sensitive to punishment (Adida et al. 2011; Must et al. 2013). Consistent with BD mania patients in the human IGT (Adida et al. 2011; van Enkhuizen et al. 2014b), DAT KD mice exhibited preference for high-risk/reward leading to increased risk-taking behavior and also exhibited increased motoric impulsivity as measured by premature responses in the IGT (van Enkhuizen et al. 2014b). These behaviors are important given that risk-taking and impulsivity in patients are linked to suicidal behavior (Jollant et al. 2010). Identifying treatments that ameliorate these risk-taking/impulsivity-related behaviors could provide an excellent new class of therapeutics for BD.

Here, we tested whether acute treatment with brexpiprazole would attenuate the BD mania-relevant behaviors of DAT KD mice in the BPM and IGT. We hypothesized that brexpiprazole treatment would reduce the hyper-exploratory profile of DAT KD mice and normalize the risk-taking behavior seen in these mice, while having no effect on their WT littermates.

METHODS

Animals

Male and female DAT KD and WT littermates were generated from heterozygous pairings. Male mice were used for the IGT study, while female and male mice were used in the BPM study. These mice were congenic on a C57BL/6J background (>10 generations of backcrossing). All animals were group housed (maximum four/cage) unless this was not possible due to concerns about the mice's health and well-being (i.e. due to fighting or if the mice had body weight differences above 10%). Mice were maintained in a temperature-controlled vivarium (21±1 °C) on a reversed day-night cycle (lights on at 7.00 PM, off at 7.00 AM) and had *ad libitum* access to food and water except during training and testing. Training and testing occurred during the dark phase of the day-night cycle between 8.00 AM and 6.00 PM. All behavioral testing 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 and was approved by the American Association for Accreditation of Laboratory Animal Care.

Mice were 2 to 3 months old at the time of training, 5 months old at testing and weighed between 22–29 g for the IGT study (n=53 ♂; 29 WT & 24 KD). The second group of mice that was tested in the BPM was 3 months old at the time of testing and weighed between 18–28 g (n= 78 ♀; n= 81 ♂).

Drug treatment

Brexpiprazole (Brex; MW: 433.566; batch: C09G92M; Lundbeck/Otsuka Pharmaceuticals) was prepared in 10% HPBCD (Hydroxy-propyl-β-cyclodextrin) vehicle solution. Methanesulfonic acid or hydrochloric acid was used to aid dissolution of the compound. Sodium hydroxide was used to increase the pH of the solution to 4 before injection. For the BPM study, solutions of 0.006, 0.02, and 0.06 mg/ml were prepared for p.o. (oral gavage) administration at a 5 ml/kg volume. Mice were treated with 0.03, 0.1, or 0.3 mg/kg or vehicle when tested in the BPM. Doses were selected based on mouse in-vivo binding data (unpublished) as well as previous publications (Yoshimi et al. 2015), in order to have clinically relevant D_2 receptor occupancies and to avoid motor depressant doses. In the IGT study, solutions of 0.002, 0.006, and 0.02 mg/ml were prepared for s.c. (subcutaneous) administration at a volume of 5 ml/kg. Mice were thus treated with doses of 0.01, 0.03, or 0.1 mg/kg or vehicle. The dose adjustment for the s.c. injections reached approximately similar systemic exposure as the p.o. administration of the drug (Christoffer Bundgaard, Lundbeck, personal communication). Acute dosing was first chosen to determine the effects of this drug on this model given its known acute mechanism of action, as well as because treating mania initially relies on the efficacy of acute dosing in the inpatient ward.

Behavioral Pattern Monitor (BPM)

Locomotor activity and exploratory behavior were examined in eight mouse BPM chambers (San Diego Instruments, USA) as described previously (Risbrough et al. 2006a; Tanaka et al. 2012). In brief, each Plexiglas arena consists of a 30.5 × 61 × 38 cm area with three floor and eight wall holes (three in each long wall and one in each short wall; 1.25 cm in diameter,

1.9 cm from the floor), each equipped with an infrared photobeam to detect holepoking. Each chamber is enclosed in an outer box with an internal white house-light above the arena (350 lux in the center and 92 lux in the four corners) that minimizes external light and noise. Activity was obtained -from a grid of 12 × 24 infrared photobeams 1 cm above the floor (2.5 cm apart; 24 × 12 X-Y array), recording the location of the mouse every 0.1 s, with its position defined across nine unequal regions (four corners, four walls, and center (Geyer et al. 1986)). Another set of 16 photobeams, placed 2.5 cm above the floor, was used to detect rearing behavior. At the start of the session, mice were placed in the bottom left-hand corner of the arena and the test session started immediately. The primary endpoint measures included **transitions** across the defined regions and **center entries** (locomotor activity), **holepoking** and **rearing** (exploratory behavior), and **spatial *d*** (dimensionality of locomotor patterns). Spatial *d* measures the degree to which the animal makes more straight-line movements versus more circumscribed paths of movement. It quantifies the geometric dimensionality of the locomotor path, where a value closer to 1 is representative of a one-dimensional straight path, and values closer to 2 indicating highly circumscribed small-scale movements (Geyer et al. 2001; Henry et al. 2014; Powell et al. 2008; Powell et al. 2009; Risbrough et al. 2006b; Young et al. 2010b; Young et al. 2011b; Young et al. 2011c; Young et al. 2007).

Iowa Gambling Task (IGT)

Mice were trained and tested in 15 five-hole operant chambers (25 cm × 25 cm × 25 cm, Med Associates Inc., St. Albans, VT, USA). Each chamber consisted of an array of five square holes (2.5 cm × 2.5 cm × 2.5 cm) arranged horizontally on a curved wall 2.5 cm above the grid floor with, on the opposite panel, a food-delivery magazine (Lafayette Instruments, Lafayette, IN, USA) at floor level and a house light near the ceiling. The chamber was enclosed in a sound-attenuating box, ventilated by a fan that also provided a low level of background noise. An infrared camera installed in each chamber enabled the monitoring of performance during training and testing when necessary. The animals were trained to respond with a nosepoke to an illuminated LED recessed into the holes. Infrared beams, mounted vertically and located 3 mm from the opening of the hole, were used to detect the responses. The food-delivery magazine opposite to the middle hole contained a well in which liquid reinforcement in the form of strawberry milkshake (Nesquik® plus non-fat milk, 30 μL) was delivered by a peristaltic pump (Lafayette Instruments, Lafayette, IN, USA). An infrared beam mounted horizontally, 5 mm from the floor and recessed 6 mm into the magazine, was used to detect the magazine entries. The control of stimuli and recording of responses were managed by a SmartCtrl Package 8-In/16-Out with additional interfacing by MED-PC for Windows (Med Associates Inc., St. Albans, VT, USA) using custom programming (Young et al. 2011d).

Mice were trained to respond to nosepoke for food rewards (strawberry milkshake, ~25 μl) in one of four illuminated holes (Hab2). Once trained and responding consistently (i.e. responding to > 70 stimuli in 30 min for 2 consecutive days), these mice were challenged in the IGT. The test lasted 400 trials or 60 minutes whichever came first. During the IGT, mice were presented with 4 options. Selecting one of these options resulted in either a reward or punishment at the level and probabilities provided in Figure 1. Specifically, selecting the left

two holes (“risky”) could result in 2 rewards (~50 μl), or long time-out punishments (66 or 132 s) at a probability of 50/50 or 25/75 respectively. Selecting the two right holes (“safe”) could result in 1 reward (~25 μl), or short time-out punishments (12 or 6 s) at a probability of 75/25 or 50/50 respectively. The risky and safe holes were on the left or right, with the safe side opposite the side-bias displayed by the mice during training. The degree of safe vs. risk-preference was quantified using % **advantageous choices** [safe/(risky+safe)*100). Consistent with earlier reports (Rivalan et al. 2009; van Enkhuizen et al. 2014c), for final analysis mice were binned into three different subpopulations based on their individual within-session learning. The total trials of mice were split into three trial periods and the three subpopulations quantified by subtracting % advantageous choices of trial period 1 from trial period 3 (**Difference Score**; primary endpoint). Safe, chance, and risk-preferring decision-makers were stratified as 1) >0.5, 2) between 0.5 and −0.5, and 3) <0.5 standard deviations from the mean respectively. This stratification was made for each genotype separately. Mice were treated with each dose on 4 separate days (Tuesday, Friday, Tuesday, and Friday) in a within-subjects design, with a 30 min training day prior to each day of testing by an experimenter not blinded to treatment. With each subsequent test day, the safe and risky hole sides were switched. An overview of additional measures collected during the IGT is presented in table 1.

Experimental design

Effects of acute brexpiprazole on activity, exploration and patterns of movement in the Behavioral Pattern Monitor—DAT KD and WT littermates (n= 78 ♀; n= 81 ♂) were monitored for 60 min in the BPM following brexpiprazole administration at 0.03, 0.1, or 0.3 mg/kg or vehicle via p.o. 2 hours before testing the BPM.

Demonstrating the test-retest reliability of the mouse IGT—The within-session performance of C57BL/6N mice (*n*=44; from van Enkhuizen et al. 2014b) was assessed two times in the IGT twice with 3 weeks days of Hab2 training between test sessions. Thus, the reliability of performance across time in the IGT could be assessed.

Effects of acute brexpiprazole on performance in the Iowa Gambling Task—DAT KD and WT littermates (n= 53 ♂) were treated with brexpiprazole at 0.01, 0.03, and 0.1 mg/kg or vehicle 60 min prior to being tested via s.c. injection due to long-term food-restriction in these mice, which proved intolerable to oral administration. All animals were tested in the IGT during a session of 60 min in a within-subjects design.

Statistical Analyses

Data from the BPM were analyzed using a three-way analysis of variance (ANOVA) with sex, drug, and genotype as between subject factors after confirmation of a normal distribution. Significant main effects and interactions were followed up with more detailed 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). Test-retest reliability data from the IGT were analyzed by conducting a Pearson’s r correlation coefficient analysis on the %advantageous choices in the final trial period of mice with test data separated by 3 weeks. Data from the IGT studies were analyzed using a four-way

analysis of variance (ANOVA) with genotype and learning score as between subject factors, and drug and time-period as within-subjects factors after confirmation of a normal distribution. Each variable was binned according to dose and trial period. Significant main effects and interactions were followed up with more detailed ANOVAs as well as Tukey *post hoc* analyses. The data were analyzed for 60 min testing periods using SPSS (version 20; Chicago, IL). The alpha level was set at 0.05.

RESULTS

Effects of acute brexpiprazole on activity, exploration, and patterns of movement in the BPM

The effects of acute brexpiprazole (0.03, 0.1, or 0.3 mg/kg: p.o.) on exploratory behavior of DAT WT and KD mice were examined using the BPM in a 60 min session.

Activity—No main effect of sex was observed on any of these measures, nor were there interactions between sex, drug, and genotype ($F<1.4$, ns). For each measure of activity, main effects of genotype [counts; $F_{(1,143)}=47.0$, $p<0.01$: transitions; $F_{(1,143)}=42.2$, $p<0.01$: center entries; $F_{(1,143)}=36.6$, $p<0.01$] and drug [counts; $F_{(3,143)}=14.1$, $p<0.01$: transitions; $F_{(3,143)}=9.8$, $p<0.01$: center entries; $F_{(3,143)}=8.5$, $p<0.01$] were observed, with no interaction between the two factors ($F<1$, ns). *Post hoc* analyses revealed that KD mice were more active than WT mice in every measure. Furthermore, brexpiprazole reduced counts, transitions, and distance travelled at 0.1 and 0.3 mg/kg, while lowering center entries at 0.3 mg/kg compared to vehicle in WT mice ($p<0.05$). Brexpiprazole only reduced counts and distance travelled at 0.3 mg/kg compared to vehicle in KD mice ($p<0.05$), however, only tending to lower transitions and center entries at this dose compared with vehicle-treated mice (see Fig. 2A for transitions; other data not shown).

Exploration—A trend effect of sex on center duration was observed ($F_{(1,143)}=3.5$, $p=0.063$), but this factor did not interact with genotype or drug ($F<1.1$, ns). For each measure, a main effect of genotype [holepoking; $F_{(1,143)}=9.1$, $p<0.01$; rearing; $F_{(1,143)}=44.0$, $p<0.01$; center duration; $F_{(1,143)}=1.3$, ns] and drug [holepoking; $F_{(3,143)}=9.7$, $p<0.01$; rearing; $F_{(3,143)}=12.6$, $p<0.01$], with a trend effect on center duration [$F_{(3,143)}=2.4$, $p=0.070$] were observed. The only interaction between genotype and drug was observed for rearing ($F_{(3,143)}=3.1$, $p<0.05$), for every other measure no interaction was observed ($F<1.5$, ns). *Post hoc* analyses revealed that KD mice had higher exploratory levels in every measure compared with WT mice. Moreover, in WT mice, brexpiprazole treatment reduced every measure (except center duration) at 0.1 and 0.3 mg/kg compared with vehicle. In KD mice however, brexpiprazole did not affect holepoking, or center duration at any dose, although 0.3 mg/kg did reduce rearing in these mice compared with vehicle ($p<0.05$). The results for holepokes and rearing are shown in Fig. 2B & C.

No effect of sex or interaction with genotype and drug was observed for holepoking, or rearing ($F<1.8$, ns; data not shown).

Locomotor Patterns—Main effects of sex was observed for spatial d ($F_{(1,143)}=13.9$, $p<0.0005$), but no interaction between sex, genotype, or drug treatment was observed

(F<1.9, ns). Genotype affected spatial *d* ($F_{(1,143)}$=13.0, p<0.0005), as did brexpiprazole treatment ($F_{(3,143)}$=11.6, p<0.0001). No interactions between these factors were observed (F<1, ns). *Post hoc* analyses revealed that KD mice exhibited lower spatial *d* than WT mice (p<0.05). In terms of brexpiprazole effects, in WT mice brexpiprazole increased spatial *d* at 0.3 mg/kg compared to vehicle-treated mice (p<0.05). In KD mice, brexpiprazole also increased spatial *d* at 0.3 mg/kg compared to vehicle-treated mice (see Fig. 2D).

Acute effects of brexpiprazole on DAT KD and WT mice performance in the IGT

The mice were trained on Hab2 and it took WT mice on average 7 days (+/− 4.0) to reach criterion (>70 responses for 2 consecutive days) while KD mice needed on average 4.5 days (+/− 1.5) to reach that same criterion in HAB2. As previously described, mice were grouped into safe- (30%), intermediate- (38%), or risk- (32%) preferring decision makers based on their IGT learning performance (van Enkhuizen et al. 2015b). Mice were treated with vehicle or doses of 0.002, 0.006, and 0.02 mg/ml via s.c. administration. For details see table 2.

% Advantageous choices—Safe-preferring DAT KD mice selected more slowly from the advantageous choices compared to WT mice, consistent with our previous observations (van Enkhuizen et al. 2015a). Indeed there was a genotype by trial-period by learning score interaction on % advantageous choices ($F_{(4,94)}$=2.9, p<0.05). *Post hoc* analyses revealed that the genotype by learning score interaction was also evident for mice treated with vehicle when we examined the difference score of % advantageous choices from trial period 3 minus trial period 1 as a gauge of learning ($F_{(2,47)}$=3.9, p<0.05), supporting the conclusion that DAT KD mice learned more slowly than WT mice.

Difference score: There was a main effect of genotype on difference score in safe-preferring mice ($F_{(1,14)}$=13.8, p<0.005; Fig. 3A). No effect of genotype was observed for intermediate and risk-preferring mice (F<1, ns; Figs. 3B &C). There was a main effect of brexpiprazole treatment in safe-preferring mice on difference score ($F_{(3,42)}$=5.9, p<0.005), irrespective of genotype (F<1, ns). There was a trend towards an effect of brexpiprazole treatment on the difference score for intermediate- or chance-level performing mice ($F_{(3,54)}$=2.6, p=0.063). Finally, there was a main effect of brexpiprazole treatment on difference score in risk-preferring mice ($F_{(3,45)}$=5.0, p<0.005). *Post hoc* analyses revealed that brexpiprazole treatment reduced the difference score of safe-preferring mice at all doses compared to vehicle-treated mice (p<0.05). In intermediate performers, brexpiprazole reduced the performance at the highest dose compared to vehicle treatment (p<0.05) irrespective of genotype (F<1, ns). For risk-preferring mice, every dose of brexpiprazole increased their difference score compared to vehicle (p<0.05), irrespective of genotype (F<1, ns). The planned statistical comparison confirmed this finding: under vehicle treatment safe-, intermediate-, and risk-preferring mice selected the preferred option significantly more often than chance. The choice made by the animals reflects a deliberate preference that was not random. This effect was absent in mice treated with brexpiprazole. Similarly, safe-, intermediate-, and risk-preferring KD mice treated with vehicle performed above chance as was also the case for intermediate-performing mice treated with brexpiprazole 0.1 mg/kg (s.c.). When treated with brexpiprazole, safe- and risk-preferring mice perform at chance

levels. Thus, the mice were selecting as often from advantageous as disadvantageous choices.

Motoric impulsivity—There was no interaction between learning scores and % premature responses ($F<1$, ns). DAT KD mice exhibited higher levels of % premature responses compared to WT mice ($F_{(1,47)}=23.9$, $p<0.0001$; Fig. 4A). Brexpiprazole treatment had a main effect on % premature responses ($F_{(3,141)}=3.8$, $p<0.05$) irrespective of genotype, learning score, or trial period ($F<1$, ns). *Post hoc* analyses revealed that brexpiprazole reduced % premature responses at the highest dose compared to vehicle treatment ($p<0.005$).

Total trials: No interaction between learning score and total number of trials completed was observed ($F<1.7$, ns). Thus when analyzed together, there was a main effect of brexpiprazole treatment ($F_{(3,138)}=8.6$, $p<0.0001$) and a main effect of genotype ($F_{(1,46)}=11.1$, $p<0.005$) on total trials completed (see Fig. 4B). More importantly, there was an interaction between genotype and brexpiprazole treatment ($F_{(3,138)}=4.5$, $p<0.01$). *Post hoc* analyses revealed that brexpiprazole reduced total trials in WT mice [$F_{(3,81)}=12.0$, $p<0.0001$] at every dose compared with vehicle treatment ($p<0.005$). Brexpiprazole only tended to alter total trials in KD mice [$F_{(3,81)}=2.7$, $p=0.054$), driven by differences between the highest and intermediate dose, with no dose significantly affecting total trials compared to vehicle treatment ($p>0.1$).

Win Stay/ Lose Shift strategies—DAT KD mice exhibit reduced safe win-stay choices compared with WT mice at time period 2 [$F_{(1,40)}=4.2$, $p<0.05$], with a similar trend at time period 3 [$F_{(1,40)}=3.5$, $p=0.069$]. There was a genotype by brexpiprazole interaction [$F_{(3,129)}=3.1$, $p<0.05$; Fig. 5A] e on safe-stay choices, as well as a main effect of brexpiprazol [$F_{(3,78)}=2.7$, $p<0.05$]. *Post hoc* analysis revealed that brexpiprazole reduced safe win-stay choices significantly at the highest dose ($p<0.05$) compared with vehicle treatment. In WT mice, brexpiprazole reduced safe-stays [$F_{(3,36)}=5.0$, $p<0.01$] at all doses ($p<0.05$) compared with vehicle treatment. Brexpiprazole did not affect safe-stays in KD mice however ($F<1$, ns). There was no main effect of treatment on risky win-stay ($F<1$, ns) nor was there an interaction with genotype ($F<1.9$, ns; Fig. 5B).

For lose-shift strategies, there was a trend towards an interaction between brexpiprazole treatment and genotype [$F_{(3,51)}=2.6$, $p=0.062$; Fig. 5C], with no main effect of brexpiprazole treatment ($F<1.1$, ns). In terms of risky lose-shifts, there was a main effect of brexpiprazole treatment [$F_{(3,84)}=3.1$, $p<0.05$] while a treatment by genotype interaction was observed [$F_{(3,84)}=3.4$, $p<0.05$; Fig. 5D]. *Post hoc* analysis showed that in WT mice, a main effect of brexpiprazole treatment on risky lose-shifts was observed [$F_{(3,48)}=6.2$, $p<0.005$] driven by brexpiprazole lowering risky lose-shifts at all doses compared with vehicle treatment ($p<0.01$). Interestingly, no main effect of drug was observed in KD mice ($F<1$, ns), likely due to significantly lower risky lose-shifts of KD compared to WT mice during vehicle treatment [$F_{(1,51)}=7.5$, $p<0.01$].

Secondary measures

Latencies: There was a main effect of genotype ($F_{(1,45)}$=33.8, p<0.0001) for mean choice latency, indicating that KD mice chose significantly faster than WT mice. Additionally, there was a main effect of brexpiprazole treatment ($F_{(3,135)}$=6.5, p<0.0001) without interaction with genotype (F<1, ns). *Post hoc* analyses revealed that brexpiprazole slowed choice latency at the highest dose tested compared with vehicle (p<0.001). In terms of reward collection latency, there was no main effect of genotype (F<1, ns) but there was a main effect of brexpiprazole treatment ($F_{(3,135)}$=7.0, p<0.0001). *Post hoc* analyses again revealed that brexpiprazole slowed reward collection latency significantly at the highest dose (p<0.05). See table 3 for details.

Omissions: There was no effect of learning scores on % omission ($F_{(2,46)}$=2.3, p=0.108). There was a main effect of genotype on % omission ($F_{(1,46)}$=19.2, p<0005). There was also a main effect of brexpiprazole treatment on this measure ($F_{(3,138)}$=10.3, p<0.0001). *Post hoc* analyses revealed that DAT KD mice exhibited lower % omission and brexpiprazole treatment increased % omissions at the lowest and highest doses compared with vehicle (p<0.05). In WT mice brexpiprazole treatment increased % omission at all doses (p <0.05) while it only increased this measure in KD mice at the highest dose (p<0.05). See table 3 for details.

Test-retest reliability—The consistency of performance over time and upon repeated testing was evaluated in the same cohort of mice by reassessing performance 21 days after session 1. There was a significant correlation between advantageous choices in the final trial period of the first and second test (r=0.946, p<0.001), indicating consistent performance over time.

DISCUSSION

DAT KD mice exhibited behavioral characteristics that were consistent with patients with BD mania, replicating earlier findings. These characteristics included hyper-exploratory behavior in the BPM (Perry et al. 2009), and increased reward seeking and impulsive behavior in the IGT (van Enkhuizen et al. 2014c). The present studies also examined whether brexpiprazole (a serotonin-dopamine activity modulator with partial agonist activity at $D_{2/3}$ and 5-HT_{1A} receptors and antagonist at 5-HT_{2A} and noradrenaline $alpha_{1B/2C}$ receptors), remediated these behaviors. At the highest dose (0.3 mg/kg; p.o.), brexpiprazole attenuated the mania-like hyper-exploratory phenotype of DAT KD mice. This dose also reduced the WT behavioral profile however, with the lower dose (0.1 mg/kg; p.o.) also reducing activity in WT mice.

Interestingly, in the IGT, brexpiprazole treatment increased safe scores in risk-preferring KD mice more than in WT mice. Brexpiprazole also lowered safe choices in safe-preferring mice irrespective of genotype, likely driven by brexpiprazole-induced reduction in safe-stays and increased safe-shifts at the highest dose. Brexpiprazole also reduced the % premature responses in both groups of mice, and the effect in KD mice was not confounded by the brexpiprazole-induced reduction in trials seen in WT mice. Overall, brexpiprazole treatment

at the highest dose equalized performance of KD mice to match that of vehicle-treated WT mice on these secondary measures, consistent with efficacy as a treatment for BD mania.

Effects of brexpiprazole treatment on the exploration of DAT WT and KD mice

Increased activity is a hallmark of BD (Cheniaux et al. 2014). In fact, in the transition from DSM IV to V, hyperactivity was identified as a core feature of BD mania. This goal-related activity measure has been reliably quantified in patients in the BPM (Henry et al. 2013; Minassian et al. 2011; Perry et al. 2009). As reported previously, mice with reduced functioning of DAT (DAT KD mice) exhibited a behavioral profile irrespective of sex that closely recreates the behavioral profile of mania: hyper-exploration, hyperactivity, and abnormal linear patterns of movement that is also irrespective of gender (Perry et al. 2009; van Enkhuizen et al. 2014a; van Enkhuizen et al. 2013; Young et al. 2010a). Specifically, acute pharmacologic or chronic genetic reductions of DAT recreate a manic profile that is more comparable to the BD mania profile than is the profile induced by the combined DAT/NET inhibitor amphetamine (Minassian et al. 2015). Thus, selectively reducing DAT function recreates altered exploration in mania patients, which can be partially ameliorated by antimanic treatments such as valproate (van Enkhuizen et al. 2013) and lithium (van Enkhuizen et al. 2015b). It should be noted, however, that valproate increased rather than ameliorated exploration of specific stimuli, which could be linked to the deleterious effects it reportedly has on aspects of cognition such as decision-making (Larkin et al. 2015). Clearly, a strong need still exists for therapies that do not negatively impact cognition while specifically treating the behavioral deficits seen in BD mania.

Brexpiprazole was developed to work on both dopamine and serotonin systems. It is approved in the USA for the treatment of schizophrenia and as adjunct treatment to antidepressants in patients with major depressive disorder with inadequate response, but could potentially have beneficial effects for BD patients and other conditions characterized by impulse control issues. Here, brexpiprazole at 0.3 mg/kg (p.o.) attenuated hyperactivity and exploration in DAT KD mice and increased spatial *d*, likely mediated by partial agonist activity at $D_{2/3}$ receptors. It is also possible that synergistic effects due to the combined modulation of dopamine and serotonin results in the reduction of the hyper-exploratory profile of DAT KD mice by brexpiprazole. Indeed, treatments impacting the serotonin system, including the 5-HT_{1B} receptor, reduced locomotion in DAT knockout mice (Dalley and Roiser 2012; Gainetdinov et al. 1999; Hall et al. 2014). Future studies could assess the specific mechanism of action of brexpiprazole on hyper-exploration in DAT KD mice by blocking its target receptor effects with selective ligands. Importantly, since this brexpiprazole effect was also observed in DAT WT mice, it was not selective to DAT KD mice. While DAT KD mice tested in the BPM are considered to model mania, the lack of a specific effect of brexpiprazole on the model alone without affecting the control (WT) mice is inconsistent with our original hypothesis. As we described previously, ideally any treatment would affect the model only and leave the WT unaffected (Young et al. 2011). It is apparent therefore that brexpiprazole did not specifically impact the mechanisms underlying the hyper-exploratory behavior of DAT KD mice. Brexpiprazole did however, reduce the mania-relevant behavior in these mice and hence could still be an effective treatment for BD mania. These findings are consistent with current antipsychotic treatments that exert greater

effects on healthy human participants at doses too low to treat psychotic patients. Hence, these findings are consistent with currently approved therapies.

One finding that has been consistent with our human BD mania observations (Perry et al. 2009; Minassian et al. 2011), is that neither sex effects nor interactions of sex with genotype were observed in our mouse model. These data are also consistent with previous observations in these mice (Young et al. 2011; van Enkhuizen et al. 2013).

Effects of brexpiprazole treatment on feedback-related decision-making, risk-preference, and motoric impulsivity in DAT WT and KD mice

This study, as well as previous studies, investigated the effects of reduced DAT function in male mice only due to the sample sizes required to reliably assess effects in the IGT. In addition, there have been long-held concerns regarding the impact of the estrogen cycle in female mice on their behavioral profile. More recently however, studies have found that the effects of the estrogen cycle on behavior might not be as pronounced as initially presumed, indicating that future studies should include female subjects in order to better represent the population (Kokras and Dalla 2014).

DAT KD mice exhibited a risk preference behavioral profile consistent with that previously described (van Enkhuizen et al. 2014c). This profile consists of a poorer % advantageous choice over time of safe-preferring DAT KD compared with WT mice. Hence, these findings support earlier work suggesting that a dopaminergic imbalance could contribute to poor decision-making in this task (Fitoussi et al. 2015). It should be noted, that the distributions of the WT and KD mice are even across groups (table 2) because group assignments were conducted within each genotype so as not to skew group conditions. In other studies, D_2 receptor activation in the basolateral amygdala attenuated risky choices in risk-prone rats leading to a behavioral profile of decision-making that resembled risk-aversive rats (Larkin et al. 2015). A similar trend can be observed in the present study in which risk-preferring mice switch to an increased preference for the advantageous choices when treated with brexpiprazole. Mice treated with brexpiprazole selected significantly more often from the advantageous choices than mice treated with vehicle. Nevertheless, they did not select the advantageous choices significantly more often than chance.

It remains possible that brexpiprazole simply impaired learning in mice, although earlier reports indicate no effect of brexpiprazole on social- or digging-based learning tasks (Maeda et al. 2014; Yoshimi et al. 2014). This premise requires confirmation however, in a simpler operant-based learning task.

It is unclear in the current study what brain region might have contributed to the effects of brexpiprazole on decision making in the IGT. While DAT is predominantly located in the striatum, it is also located in other brain regions in which DAT KD mice exhibit altered dopaminergic homeostasis (Milienne-Petiot et al. 2016). Certainly, each of these brain regions contribute toward risk-based decision making as reviewed (Orsini et al. 2015). Specifically in the IGT, a prefrontal-subcortical network appears to underlie decision-making (Fitoussi et al. 2015; Rivalan et al. 2011), but the precise mechanism driving poorer

decision-making in DAT KD mice remains unclear. Future studies examining the deficits and mechanistic actions of brexpiprazole are required.

Additionally, DAT KD mice exhibited higher rates of motoric impulsivity (% premature responses), faster reaction-times, and fewer % omissions compared to WT mice (van Enkhuizen et al. 2014c). Brexpiprazole reduced the impulsivity of DAT KD mice without affecting overall total trials, unlike effects in WT mice. These data indicate a more selective effect of brexpiprazole toward lowering waiting impulsivity in KD vs. WT mice. Impulsivity could be a major trait of BD during the manic and euthymic states, although behavioral assessments have not provided sufficient evidence to-date (Newman and Meyer 2014). The mechanism by which brexpiprazole reduced impulsivity more in DAT KD mice than WT mice is unclear.

CONCLUSIONS

Consistent with earlier reports, DAT KD mice exhibited hyper-exploratory and risk-preference profiles consistent with BD mania patients in cross-species tasks that mirror the clinical tasks. The motoric impulsivity of DAT KD mice could also mirror the impulsive actions of BD mania patients. Brexpiprazole represents a putative new treatment option for BD mania and/or other conditions characterized by increased impulsivity, since it remediates some of the behavioral abnormalities exhibited by the DAT KD mice. Although these studies were conducted with acute treatments – as commonly done in mania – future studies will need to assess the effects of chronic treatments, which may exert differential effects in WT vs. KD mice (van Enkhuizen et al. 2015a; Young et al. 2011a). Nevertheless, the effectiveness of acute brexpiprazole in the present studies suggests that it may have efficacy in the treatment of acute mania.

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Abbreviations

IGT	Iowa Gambling Task
DAT	dopamine transporter
BD	bipolar disorder
WT	wildtype
BPM	behavioral pattern monitor

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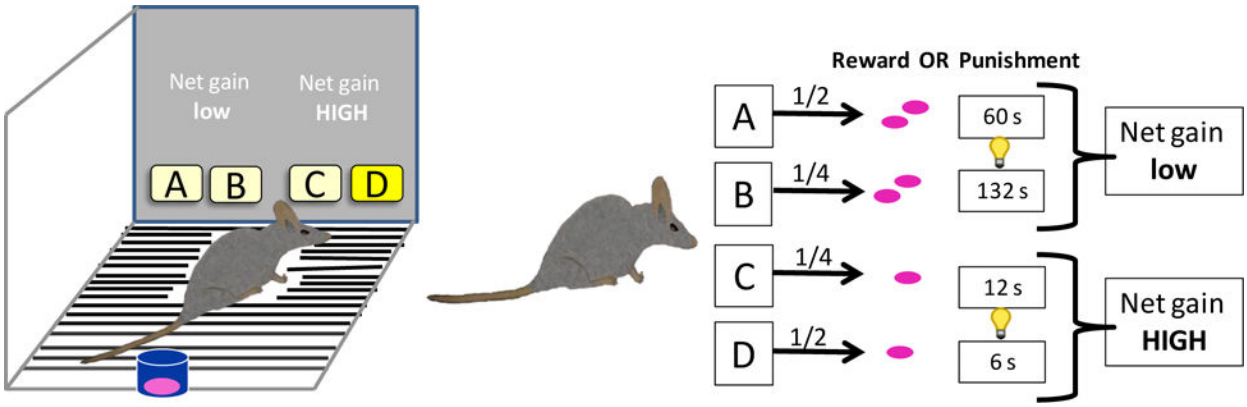

Figure 1. Schematic representation of the mouse Iowa Gambling Task

Mice were trained to holepoke for a single reward. Then in a single test session, mice were provided with 4 options resulting in varying reward (25 or 50 μl strawberry milkshake), and punishment (timeout with the chosen flashing light for varying durations) levels were altered. The probability of each result is presented, with the final gains of milkshake vs. punishment represented. Ultimately, as previously demonstrated (van Enkhuizen et al. 2014c), mice received the lowest level of punishment and the highest reward if they selected from the lower reward options (C and D).

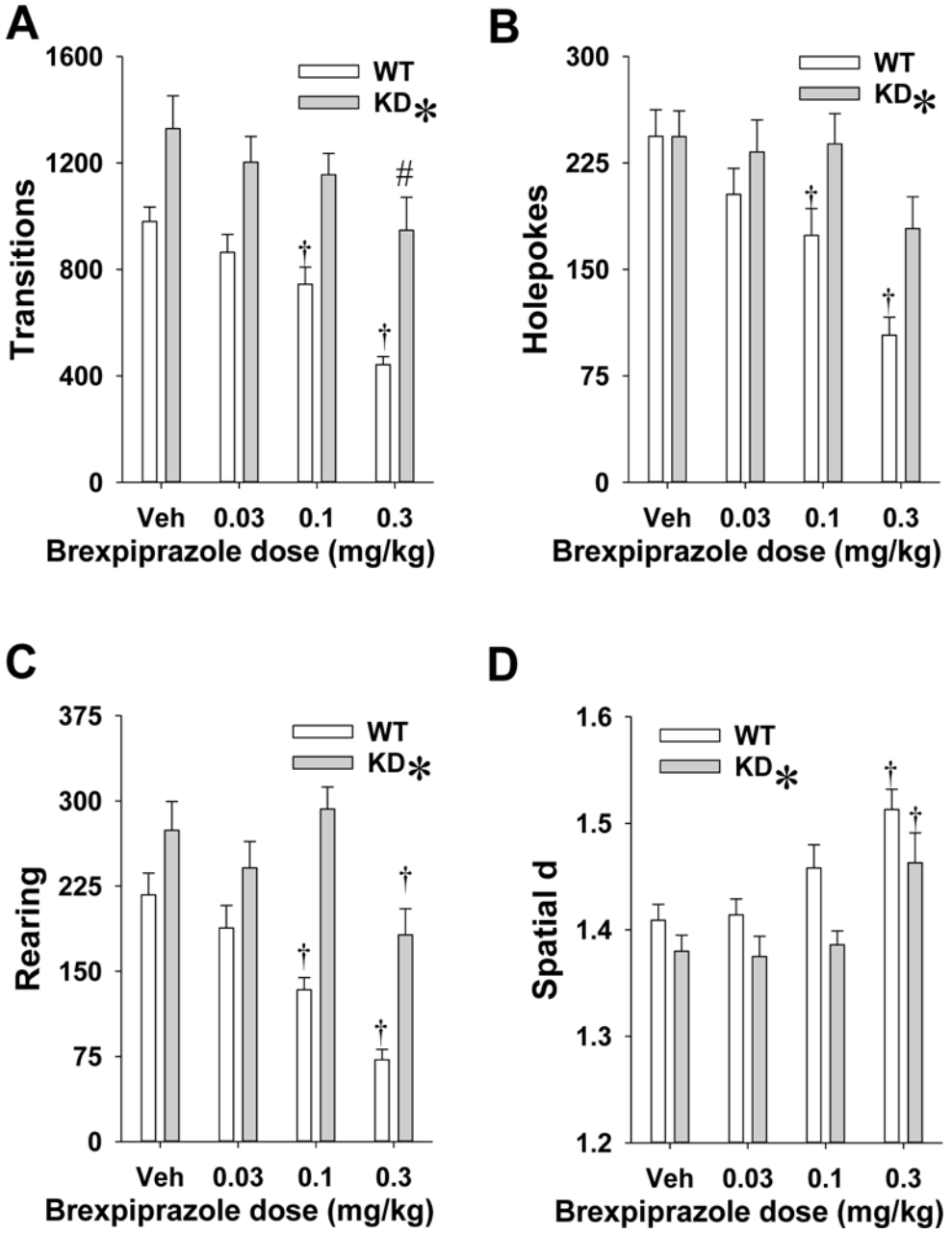

Figure 2. Effects of acute brexpiprazole (p.o. administration) on the activity, specific exploration, and locomotor patterns of DAT WT and KD mice

DAT KD mice were more active than WT mice in every activity measure including transitions (**A**). Brexpiprazole reduced activity in each of these measures, with WT mice more sensitive to its effects. Overall, DAT KD mice displayed greater exploration than WT mice in every exploration measure including holepoking (**B**), and rearing (**C**). Brexpiprazole reduced exploration in each of these measures, particularly in WT mice. Finally, DAT KD mice displayed altered locomotor patterns when compared with WT mice including lower spatial *d* (**D**). Brexpiprazole increased spatial d at 0.3 mg/kg. Data are presented as mean ±

SEM. * denotes p <0.05 cf. WT mice † denotes p <0.05 cf. vehicle (Veh) treated mice, # denotes p <0.1 cf. vehicle-treated mice.

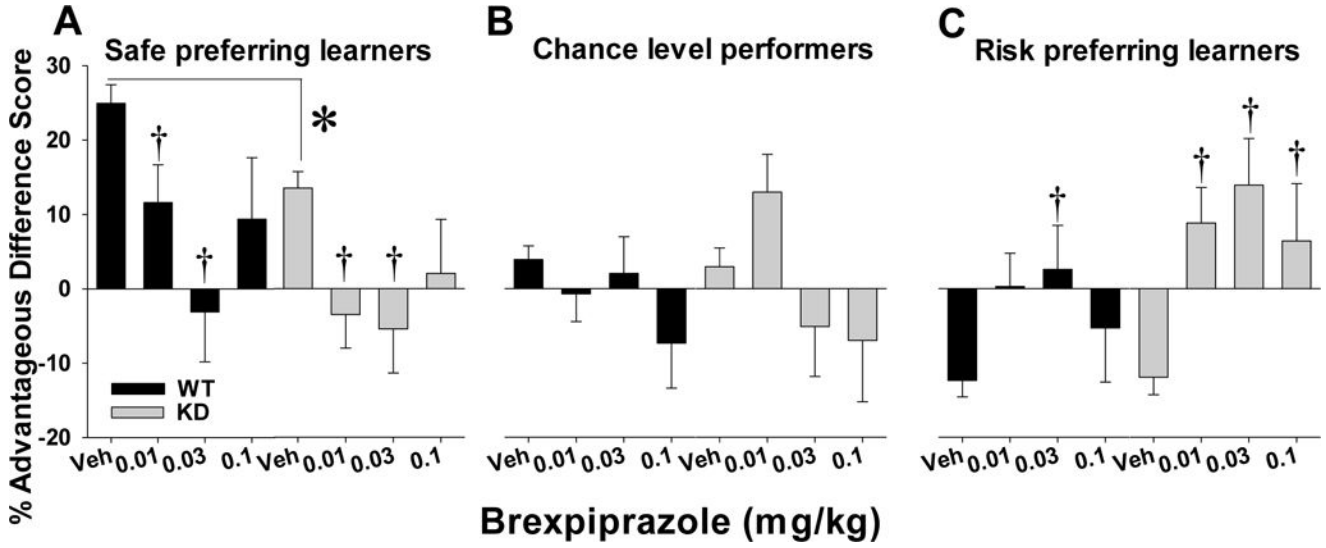

Figure 3. Effects of acute brexpiprazole (s.c. administration) on risk-taking as measured by the % Advantageous Difference Score of DAT WT and KD mice

The performance of mice in the IGT was categorized by their learning score (% advantageous difference score from trial period 3 minus trial period 1). Some mice exhibited a safe-preferring learning score (**A**), some showed no change over time (**B**), while some exhibited a preference for the risky side (**C**). Safe-preferring KD mice exhibited a poorer change compared with safe-preferring WT mice. Brexpiprazole treatment reduced the positive change score of safe-preferring WT mice, while tending to do so in safe-preferring KD mice, without affecting chance level performers. Brexpiprazole increased change scores of risk-preferring mice however, primarily in KD mice. Data are presented as mean ± SEM. * denotes $p < 0.05$ cf. vehicle (Veh)-treated WT mice, † denotes $p < 0.05$ cf. vehicle-treated mice in the same learning category.

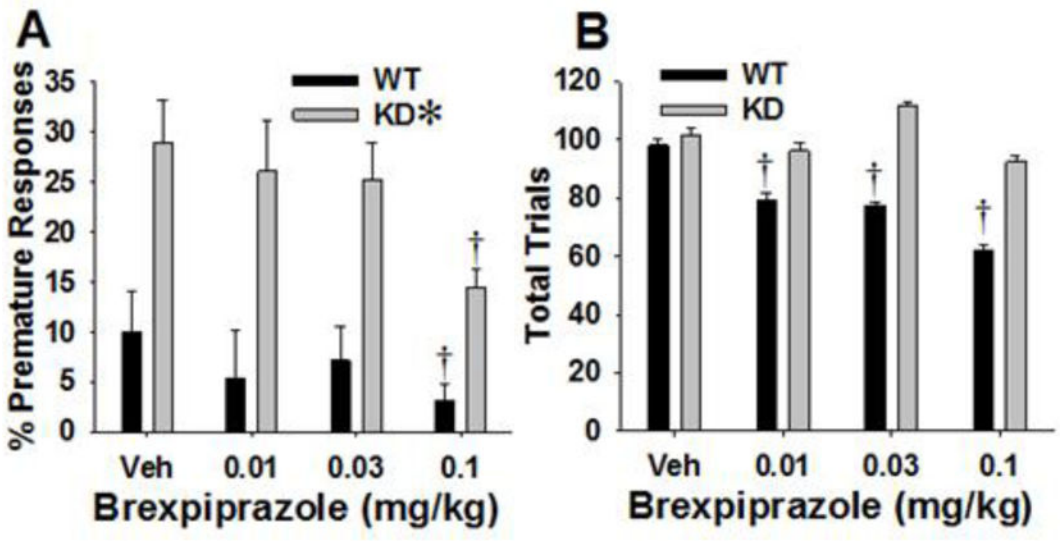

Figure 4. Effects of acute brexpiprazole on secondary outcome measures of DAT WT and KD mice in the IGT

DAT KD mice exhibited behaviors on the secondary outcome measures that were consistent with previous reports (van Enkhuizen et al. 2014c). These behaviors included increased % premature responses (**A**), normal total trials completed (**B**). Brexpiprazole treatment reduced % premature responses in both WT and KD mice, the latter to levels comparable with vehicle-treated WT mice (**A**). Brexpiprazole also reduced total trials in WT but not KD mice (**B**). Data are mean ± SEM. * denotes $p < 0.05$ cf. WT mice † denotes $p < 0.05$ cf. vehicle (Veh) treated mice.

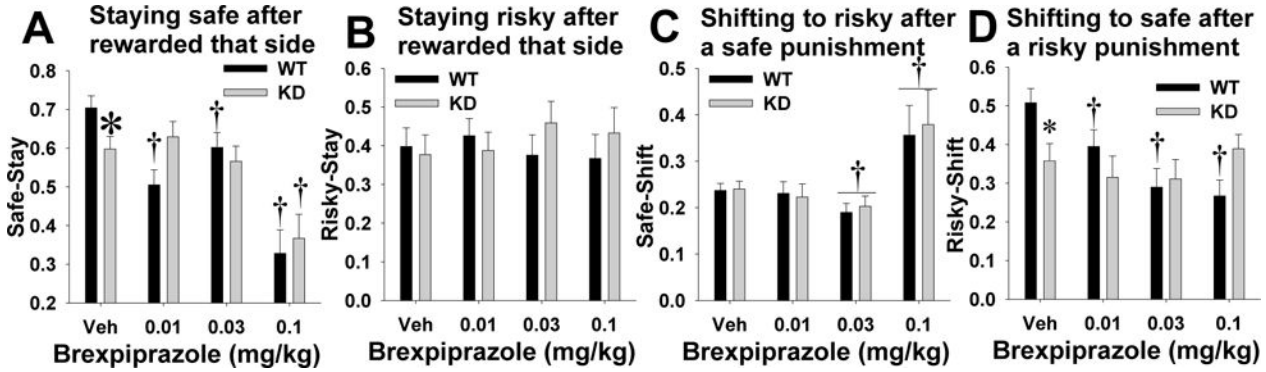

Figure 5. Effects of acute brexpiprazole on strategy measures from the IGT of DAT WT and KD mice

DAT KD mice exhibited behaviors on the secondary outcome measures of IGT that were consistent with previous reports (van Enkhuizen et al, 2014c). This secondary measure was a reduction in the likelihood of repeating a response at the safe side after being rewarded at that side (**A**). Brexpiprazole treatment reduced this safe-stay behavior in both genotypes. Neither drug nor genotype affected staying at the risky side after a reward was received at that side (**B**). No genotype effect was seen on shifting to a risky choice after being punished on the safe side, although brexpiprazole reduced or increased this behavior at different doses (**C**). Finally, little genotype effect was observed on the measure indicating shifting to the safe side after being punished on the risky side, with brexpiprazole lowering this shift at low doses (**D**). Data are mean ± SEM. * denotes $p<0.05$ cf. WT mice † denotes $p<0.05$ cf. vehicle treated mice.

Table 1

Terms and definition from the mouse IGT (van Enkhuizen et al. 2014c)

Measures	Definition
Learning Score	The risk-, chance- or safe-preferring group scores for mice
% Advantageous choices	The preference of safe vs. risk choices in the IGT
Difference Score	Difference in % advantageous choices from time period 3 - time period 1
Premature Responses	Motoric impulsivity: Responding prior to stimulus presentation
% Omissions	Not responding to a stimulus - could reflect motivation, responsiveness, and/or attentional processes
Mean Choice Latency	Latency to make a choice, irrespective of safe or risky preference - could reflect speed of processing or responsiveness
Mean Reward Collection Latency	Latency to collect the reward after its presentation - could reflect motivation or responsiveness
Safe-Stay	Selecting the safe side after being rewarded there
Risky-Stay	Selecting the risky side after being rewarded there
Safe-Shift	Selecting the risky side after being punished at safe side
Risky-Shift	Selecting the safe-side after being punished at risky side

Table 2

Distribution of cohort based on learning score by genotype for the IGT

Genotype	Safe preferring mice	Chance level performers	Risk preferring mice
WT	7	13	9
KD	9	7	8

Table 3

Secondary IGT measures after brexpiprazole (Brex) treatment (mg/kg; s.c.), mean (± SEM).

Measure	WT				KD			
	vehicle	Brex 0.01	Brex 0.03	Brex 0.1	Vehicle	Brex 0.01	Brex 0.03	Brex 0.1
% Omissions	19.5 (2.6)	28.1 (3.3)	24.7 (2.6)	35.3 (3.5)	9.7 (2.8)	9.7 (3.5)	8.9 (2.7)	16.0 (3.7)
Mean Choice Latency (s)	4.9 (0.2)	5.3 (0.2)	5.3 (0.2)	5.7 (0.2)	3.7 (0.3)	3.7 (0.2)	3.7 (0.2)	4.2 (0.2)
Mean Reward Collection Latency (s)	1.6 (0.1)	1.9 (0.4)	1.7 (0.2)	2.6 (0.7)	1.1 (0.1)	1.7 (0.4)	1.2 (0.2)	3.2 (0.7)