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The effect of reduced dopamine D4 receptor expression in the 5-choice continuous performance task: Separating response inhibition from premature responding

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Abstract

Impairments in attention/vigilance and response disinhibition are commonly observed in several neuropsychiatric disorders. Validating animal models could help in developing therapeutics for cognitive deficits and improving functional outcomes in such disorders. The 5-choice continuous performance test (5C-CPT) in mice offers the opportunity to assess vigilance and two forms of impulsivity. Since reduced dopamine D4 receptor (DRD4) function is implicated in several disorders, DRD4 is a potential therapeutic target for cognition enhancement.

We trained wildtype (WT), heterozygous (HT), and knockout (KO) mice of the murine *Drd4* to perform the 5C-CPT under baseline and variable stimulus duration conditions. To dissect motor impulsivity (premature responding) from behavioral disinhibition (false alarms), we administered the 5-HT_{2C} antagonist SB242084 during an extended inter-trial-interval session. We also examined the preattentive and exploratory profile of these mice in prepulse inhibition (PPI) and the Behavioral Pattern Monitor (BPM).

Reduced *Drd4* expression in HT mice, as confirmed by quantitative RT-PCR, resulted in response disinhibition and impaired 5C-CPT performance, while premature responding was unaffected. Conversely, SB242084 increased premature responding without affecting response inhibition or attentional measures. No genotypic differences were observed in PPI or BPM behavior.

Thus, reduced *Drd4* expression impairs attentional performance, but not other behaviors associated with neuropsychiatric disorders. Moreover, the use of signal and non-signal stimuli in the 5C-CPT enabled the differentiation of response disinhibition from motor impulsivity in a vigilance task.

Keywords

Attention; Dopamine D4 receptor; Impulsivity; Vigilance; 5-HT_{2C} receptor

1. Introduction

Attentional dysfunction features prominently in numerous neuropsychiatric disorders such as bipolar disorder (BD) and schizophrenia, and is a primary hallmark of attention deficit hyperactivity disorder (ADHD) irrespective of age or subtype [1]. Given that attentional performance can impact multiple cognitive domains [2], improving attention in

neuropsychiatric patients remains paramount to their treatment. Moreover, a better understanding of the neurobiological underpinnings of attentional performance may enable the development of improved therapeutics. Abnormal attentional performance in patient populations is often assessed in terms of vigilance, as assessed by any of a variety of continuous performance tests (CPT [3]).

Irrespective of the type of CPT used, e.g., X-CPT [4], Connor's CPT [5], or CPT IP [6], the subject is required to respond to signal but inhibit from responding to non-signal stimuli in the environment [7]. Since CPT measures have been used extensively in humans, the development of therapeutics is likely to require animal models of disorders tested in a CPT-like task. Moreover, a rodent CPT provides opportunities to ascertain the neurobiological underpinnings of performance. While animal tests of attention, such as the 5-choice serial reaction time task (5CSRTT) or the sustained attention task exist [8–11], they often do not assess vigilance in a manner that is consistent with the human CPT [12]. For example, in the 5CSRTT the rodent is not required to inhibit responding to non-signal stimuli, with the only impulsivity measure being a “premature” response made during a waiting period (inter-trial interval; ITI) prior to a signal stimulus onset [10]. Recently, the 5CSRTT has been elaborated to develop a rodent 5-choice CPT (5C-CPT) that corresponds more closely to the CPT used in humans [12]. In the 5C-CPT, rodents continue to respond to signal stimuli, but now must also inhibit responding to non-signal stimuli. Thus, consistent with the human CPT, it is possible to measure response inhibition in terms of false alarm responding to non-signal stimuli in the 5C-CPT [12]. Measuring response inhibition is important when generating animal models of neuropsychiatric disorders because increased false alarm responding often accompanies or mediates impaired CPT performances, for example in ADHD [13–15] or BD [16].

Abnormal dopaminergic control has been linked to both response disinhibition and the etiology of ADHD and BD [14,15,17]. The dopamine transporter [18–20,17] has been associated genetically to both ADHD and BD, while the dopamine D4 receptor (DRD4) has been linked predominantly with ADHD [20–24], with limited linkage to BD [25]. The DRD4 abnormalities linked to ADHD occur as a variable number of tandem repeat polymorphisms in the DRD4 coding region, which may result in a blunted response to dopamine [26] and/or a reduction of DRD4 expression [27,28]. Given that dopamine D4 receptors are highly expressed in the prefrontal cortex in mice [29] and humans [30], reduced expression and/or function are likely to deleteriously impact cognitive functioning including impulsivity and attention. Impulsivity in the 5CSRTT is measured by premature responses and is mediated predominantly by the serotonergic system, however [10,31–35]. For example the 5-HT_{2C} antagonist SB24284 increases premature responding in rats and mice (Fletcher et al. [32]). These findings are consistent with serotonergic contributions to temporal control and timing [36,37]. As discussed above, impulsivity in the human CPT is measured as false alarms to non-signal stimuli [3] and has been linked to dopaminergic mechanisms [14,38,39].

Other symptoms of neuropsychiatric disorders can be assessed in tests with cross-species validity, such as over-activity in the Behavioral Pattern Monitor [40–44] or sensorimotor gating deficits as measured by prepulse inhibition (PPI) [45–49]. Patients with schizophrenia and BD exhibit altered exploration in the BPM [40,41], and impaired PPI [45,48], when compared with control subjects. Although limited to date, preliminary data suggest that adult patients with ADHD do not exhibit altered exploration in the BPM [50], while several studies support normal PPI in ADHD in the absence of attentional challenges [51–53]. The use of these cross-species translational tests may provide insight regarding the contribution of reduced DRD4 expression to specific symptoms across neuropsychiatric disorders.

In the present studies, we examined the effects of altered *Drd4* receptor expression in mice on behaviors that are abnormal in ADHD and/or BD. We characterized the vigilance and impulsivity of male *Drd4* wildtype (WT), heterozygous (HT), and knockout (KO) mice in the 5C-CPT. To confirm that the 5C-CPT was sensitive to detect changes in premature responding, we also administered the 5-HT_{2C} antagonist SB242084 to these mice in an extended ITI challenge [32]. We then assessed the activity levels of these mice in the BPM, as well as their sensorimotor gating as measured by PPI.

2. Methods

2.1. Animals

Drd4 mutant mice were generated at The Oregon Health and Sciences University (Portland, OR). Male WT ($n = 7$), HT ($n = 7$), and KO ($n = 7$) mice used for the current study were derived from heterozygous breeding pairs in a line that had been back-crossed onto C57BL/6 mice for over 20 generations and genotyped as previously described [54]. Procedures were approved by the UCSD Institutional Animal Care and Use Committee and conformed to NIH Guidelines. Training began at approximately 3 months of age, with mice weighing between 20 and 30 g. Mice were housed in groups of maximum 4/cage. Mice were maintained at 85% of free-feeding weight, with water available *ad libitum*, and housed in a vivarium on a reversed day–night cycle (lights on at 8.00 PM, off at 8.00 AM). Mice were brought to the laboratory 60 min before testing between 9.00 AM and 6.00 PM.

2.2. Apparatus

2.2.1. 5-Choice chambers—Training and testing took place in four 5-hole operant chambers (25 cm × 25 cm × 25 cm, Med Associates Inc., St. Albans, VT). 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 opposite a food delivery magazine (Lafayette Instruments, Lafayette, IN) at floor level and a house-light near the ceiling. The chamber was located in a sound-attenuating box, ventilated by a fan that also provided a low level of background noise. An infra-red camera installed in each chamber enabled the monitoring of performance during training and testing. Mice were trained to respond with a nose-poke to an illuminated LED recessed into the holes. Responses were detected by infrared beams mounted vertically located 3 mm from the opening of the hole. Liquid reinforcement in the form of strawberry milkshake (Nesquik[®] plus non-fat milk, 30 μ l) was delivered by peristaltic pump (Lafayette Instruments, Lafayette, IN) to a well located in the magazine opposite the 5-hole wall. Magazine entries were monitored using an infrared beam mounted horizontally, 5 mm from the floor and recessed 6 mm into the magazine. 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) using custom programming [12].

2.2.1.1. 5-Choice continuous performance test training: Mice were trained in the 5-choice serial reaction-time task daily, 5 days per week as described previously [55]. Each session lasted 30 min or 120 trials, whichever was completed first. Each trial was initiated by the mouse nose-poking, then removing its nose, from the magazine. After a 5 s ITI, a light stimulus appeared in one of the 5 apertures located opposite the magazine. A nose-poke in the lit aperture during the stimulus duration (SD) plus a 2 s limited hold period resulted in a *correct* (Hit) response being registered and a reward being delivered in the magazine. A nose-poke in any other aperture over this period was registered as an *incorrect* response and resulted in a 4 s time-out. Failure to respond in any aperture during the SD + limited hold was registered as an *omission* (omission + incorrect = Miss) and also resulted in a time-out (TO). Response in any aperture during the ITI registered a *premature* response and triggered

a TO. The next trial began when the mouse entered, then exited the magazine. The SD started at 20 s and was reduced to 10, 8, and 4 s after the attainment of each criterion (a mean correct latency less than half the current SD for two consecutive days) across sessions. At this point, mice were transferred to a variable ITI (3–7 s). Once performance stabilized (approximately 1 week), the mice were then transferred to the 5C-CPT. For the 5C-CPT, 100 trials were go (signal) trials, identical to trials described in the 5CSR task where a cue stimulus could appear in any 1 of the 5 apertures, 20 trials were no-go (non-signal) trials, unique to the 5C-CPT in which all 5 apertures were illuminated and the mouse was required to inhibit responding. Training took approximately 4 months. Consistent with human CPTs [3], successful inhibition of a response in a no-go trial resulted in a *correct rejection* (CR) being recorded and reward delivered. Responding in a no-go trial, however, resulted in a *false alarm* (FA) being registered and a TO occurring. These no-go trials were interspersed pseudo-randomly within the 100 go trials (maximum of 3 sequential no-go trials). *False alarm latency* was also recorded.

For all three tasks, the *mean correct latency* (MCL) was calculated along with the following parameters:

$$\text{accuracy} = \frac{\text{Hit}}{\text{Hit} + \text{Incorrect}},$$

$$\% \text{Omissions} = \left(\frac{\text{omissions}}{\text{Total Trials}} \right) \times 100 \quad (\text{measures resulting from signal trials only})$$

$$p(\text{HR}) = \frac{\text{Hit}}{\text{Hit} + \text{Miss}},$$

$$p(\text{FA}) = \frac{\text{FA}}{\text{FA} + \text{CR}} \quad (\text{measures resulting from signal and non-signal trials})$$

Based upon these basic parameters, signal detection indices [56,57] were then calculated to assess both sensitivity index (SI) and responsivity index bias (RI). The SI was calculated using the following formula:

$$\text{SI} = \frac{p(\text{HR}) - p(\text{FA})}{2[p(\text{HR}) + p(\text{FA})] - [p(\text{HR}) + p(\text{FA})]^2}$$

SI provides a non-parametric assessment of sensitivity [58]. Values for SI vary from –1 to +1, with +1 indicating that all signal events were responded to, while all non-signal events were inhibited from responding to, while zero indicates chance levels of distinguishing between signal and non-signal events. SI was also the basis by which Sarter and co-worker [9] developed their vigilance index measure and so would produce comparable results for mice to those seen in rats performing their vigilance paradigm. To mirror the use of SI, the non-parametric response bias measure RI [58] was chosen to provide a measure of the “tendency to respond” [58–60]:

$$\text{RI} = \frac{p(\text{HR}) + p(\text{FA}) - 1}{1 - [p(\text{FA}) - p(\text{HR})]^2}$$

Both SI and RI are based on the same geometric logic and are both appropriate for use with single choice procedures (respond or not) [59].

2.2.1.2. Experimental challenges: Once fully trained, stable baseline performance was recorded (experiment 1). Vigilance performance was then challenged on a single test day

(Wednesday) between normal training sessions (Monday, Tuesday, Thursday, and Friday) where the session was extended to include 250 trials (200 signal and 50 non-signal trials) and the stimulus duration (SD) was varied to be 0.8, 1, or 2 s in length pseudo-randomly across trials (maximum of three consecutive presentations of any given SD). Session duration was also lengthened to 60 min on this challenge day (experiment 2). Extending the session duration has successfully differentiated genotypic performance in the past when no differences were observed during baseline testing [61]. Finally, in a within-subject cross-over design, mice received vehicle or one of two doses of the 5-HT_{2C} antagonist SB242084 while being challenged in an extended session (60 min), with a constant SD, and an extended vITI (7, 8, 9, 10, and 11 s; experiment 3). Doses and extended ITI challenge utilized were based on previous reports [32].

2.2.1.3. Drugs: SB242084 (Sigma, St. Louis, MO) was dissolved in 0.9% saline solution and was administered intraperitoneally 30 min prior to testing in a 5 ml/kg injection volume [32].

2.2.2. Behavioral Pattern Monitor (BPM)—Ten mouse BPM chambers were used to assess spontaneous exploratory behavior as described previously [62,63]. Each chamber is illuminated from a single light source above the arena (30.5 cm × 61 cm × 38 cm area with a Plexiglas holeboard floor equipped with 3 floor holes and 8 wall holes). Holepoking behavior was detected using an infrared photobeam. The location of the mouse was recorded every 0.1 s using a grid of 12 × 24 infrared photobeams located 1 cm above the floor recorded. The position of the mouse was assigned to 9 unequal regions described by a tic-tac-toe pattern [43,64]. Rearing behavior was recorded using an array of 16 infrared photobeams 2.5 cm above the floor aligned with the long axis of the chamber. At the start of each test session, mice were placed in the bottom left hand corner of the chamber, facing the corner and the test session started immediately.

Three main factors were investigated: locomotor activity as measured by transitions (calculated as a movements between the 9 regions); investigatory behavior as measured by holepoking and rearing; locomotor pattern as measured by spatial *d*. Spatial *d* uses analyses based on fractal geometry to quantify the geometrical structure of the locomotor path, where a value of 2 represents highly circumscribed localized movement and a value of 1 represents straight line distance-covering movements [65].

2.2.3. Prepulse inhibition (PPI)—Startle and PPI testing were performed in commercial startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA). Within each chamber there was a Plexiglas cylinder (3.7 cm in diameter) into which the animal was placed. Sudden movements by a mouse were detected by a piezoelectric accelerometer attached below the cylinder. A loudspeaker provided the broadband background noise and acoustic stimuli, and the whole apparatus was housed within the ventilated, sound-attenuating chamber (39 cm × 38 cm × 58 cm). Stimulus presentations and response measures were controlled by a standard computer. The experimental session consisted of a 5 min acclimatization period to a 65 dB background noise (continuous throughout the session). During the session, 12 trial types were presented: a 40 ms, 120 dB startle pulse (*pulse alone*); a no stimulus trial (*nostim*); four 20 ms prepulse + pulse combinations [67, 69, 73, or 81 dB prepulses followed 100 ms later by a P120 stimulus; *prepulses + pulse*]; six 20 ms prepulse + pulse combinations with varying inter-stimulus intervals [73 dB prepulse followed 25, 50, 100, 200, 500, or 1000 ms later by a P120 stimulus; *prepulses(vISI) + pulse*]. Trial types were presented in a varied order (12 presentations of *pulse alone* trial, 8 presentations of each *prepulses + pulse* combination, 8 presentations of each *prepulses(vISI) + pulse* combination, and *nostim* trials occurring between each trial) with an average inter-trial interval (ITI) of 15 s. In addition, 6 of the *pulse alone* trials, which were not included in

the calculation of PPI values, were presented at the beginning of the test session to achieve a relatively stable level of startle reactivity for the remainder of the session (based on the observation that the most rapid habituation of the startle reflex occurs within the first few presentations of the startling stimulus [66]). Another 6 of the *pulse alone* trials, which were also not included in the calculation of PPI values, were presented at the end of the test session to assess startle habituation.

2.2.4. Quantitative real-time PCR—For quantitative RT-PCR studies, individual frontal cortical RNA was isolated from a new cohort of male mice (WT $n = 3$; HT $n = 3$; KO $n = 1$). Five micrograms of total RNA were reverse transcribed into cDNA with random hexamers by using SuperScript III first strand Kit (Invitrogen, Carlsbad, CA). Taqman primers for *Gapdh* and dopamine D4 receptor genes were purchased from Applied Biosystems (Foster City, CA). Each genotype contained three individual RNA samples, and their standard curves during PCR amplification were made. *Gapdh* expression was used as an internal control for normalization, and one wildtype mouse RNA sample was selected arbitrarily as a control for the relative expression of all other samples. The quantitative RT-PCRs were conducted in the Center for Aids Research Genomic Core (UCSD).

2.2.5. Statistics—Acquisition of the 5C-CPT was analyzed by comparing the A50 (time at which they learn to nose-poke in a singly lit hole) as previously described [55]. The A50 of these mice were compared using a one-way ANOVA with genotype as a between subjects factor. Baseline data were analyzed using a two-way repeated measures ANOVA with day as a within subjects factor and genotype as the between subjects factor. The extended session/variable SD challenge data were analyzed using a two-way repeated measures ANOVA with SD as a within subjects factor and genotype as a between subject factor. Within session performance of this challenge was analyzed by combining performance within 5×50 trial-bins then analyzing performance using a two-way repeated measures ANOVA with trial-bin as a within subject factor and genotype as a between subjects factor. These aspects of performance were analyzed separately due to the limited trials available for analysis if data were binned into separate stimulus durations within trial-bins. The effects of drug were analyzed using a two-way repeated measures ANOVA with drug as the within subjects factor and genotype as the between subjects factor. For qRT-PCR, the *Drd4* expression levels in WT and HT were compared using a Student's *t*-test. KO mice were not included in the analysis due to confirmed lack of expression [54]. All 5C-CPT and qRT-PCR data were analyzed using SPSS (Chicago, IL). For BPM, the primary measures were analyzed using one-way ANOVAs, with genotype as the between subject factor. Significant main effects were analyzed using Tukey *post hoc* analyses. For PPI, mean startle magnitude for each trial type presentation, the dependent measure, was determined by averaging 65 1-ms readings taken from the onset of the startle P120 stimulus. Startle reactivity, mean startle magnitudes within the test session, was analyzed using three factor ANOVAs with drug as a within-subjects factor and genotype and sex as between-subjects factors. The BPM and PPI data were analyzed using the Biomedical Data Programs (BMDP) statistical software (Statistical Solutions Inc, Saugus, MA). The alpha level was set to 0.05 within each experiment. The relationships of measures within and between tasks were investigated by comparing 5C-CPT performance measures from experiment 2 to BPM, and PPI measures. Performance was collapsed across SD and subject to a Spearman's rho correlation due to limited sample size. All correlations were analyzed using SPSS (Chicago, IL), with a Bonferroni correction setting the alpha level to 0.002273.

3. Results

3.1. 5C-CPT: acquisition and baseline performance

The acquisition of the 5C-CPT was compared between genotypes in terms of acquisition for nose-poking in a singly lit hole (A50), as well as total trials to criterion. No effect of genotype was observed for A50 acquisition [$F < 1$, ns; WT = 15.6 ± 4.2 ; HT = 18.1 ± 1.2 ; KO = 15.6 ± 5.3]. Once performance had stabilized, there was little variation in performance as demonstrated by a lack of day effect or interaction for any measure. Finally, there was no main effect of genotype on total trials, premature responses, accuracy, % omissions, MCL, $P[HR]$, $P[FA]$, SI, or RI (Table 1).

3.2. 5C-CPT: experiment 2—variable stimulus duration challenge

During training, it was noted that *Drd4* HT mice exhibited increased $P[FA]$ responses as the SD was reduced. Hence, stable performance was challenged using a variable SD and extended session. A genotype X SD interaction was observed for SI [$F(4,32) = 2.7$, $p < 0.05$; Fig. 1A], with Tukey *post hoc* analyses revealing superior performance of WT compared to HT ($p < 0.05$), but not KO ($p > 0.1$) mice, across the three SDs. There was a trend toward a genotype by SD effect observed on $P[FA]$ [$F(4,32) = 2.2$, $p = 0.096$; Fig. 1B], with HT mice exhibiting more false alarms than either WT or KO mice across SDs ($p < 0.05$), although no main effect of genotype on $P[FA]$ was observed [$F(2,16) = 2.2$, $p = 0.138$]. No genotype or genotype $\times$ SD interaction was observed for RI, $P[HR]$, accuracy, premature responding, % omissions, or MCL [$F < 1.8$, ns].

Main effects of SD was observed for RI [$F(2,32) = 6.5$, $p < 0.01$], $P[HR]$ [$F(2,32) = 63$, $p < 0.0001$], % omissions [$F(2,32) = 60.6$, $p < 0.0001$], and MCL [$F(2,32) = 38.3$, $p < 0.0001$], but not for $P[FA]$, accuracy, or premature responding [$F < 1.7$, ns]. *Post hoc* analyses revealed that mice were slower and more responsive at longer SDs.

During this 60 min 250 trial session data was binned into 50 trial increments. Performance deteriorated over trial-bin as measured by SI [$F(4,68) = 3.5$, $p < 0.05$], with *post hoc* analyses revealing poorer performance in bin 5 compared to trial-bins 1–4 ($p < 0.05$). No effect of trial-bin [$F(4,68) = 2.3$, ns] or trial-bin by genotype interaction [$F(8,68) < 1$, ns] was observed for RI.

3.3. 5C-CPT: experiments 3–5—HT2C antagonist effect in an extended ITI challenge

No effects of drug, genotype, or their interaction were observed for SI (Fig. 2A), RI (Fig. 2B), $P[FA]$ (Fig. 2C), $P[HR]$ (Fig. 2D), accuracy (Fig. 2E), % omissions, or the MCL. SB242084 significantly increased premature responding [$F(2,34) = 4.4$, $p < 0.05$; Fig. 2F] at both doses compared to saline ($p < 0.05$). No drug interaction with or effect of genotype was observed for premature responses ($F < 1.7$, ns).

When analyzed within the challenge session (binned into first and second half of session), a significant improvement in performance was observed as measured by increased SI [$F(1,11) = 4.3$, $p < 0.05$; mean $0.1\text{--}0.3$, ± 0.05 s.e.m.], reduced % omissions [$F(1,11) = 17.8$, $p < 0.0001$; mean $33.1\text{--}15.3\%$, ± 4.8 s.e.m.], reduced premature responses [$F(1,11) = 105.7$, $p < 0.0001$; mean $8.0\text{--}4.6$, ± 0.9 s.e.m.], and a trend toward improved accuracy [$F(1,11) = 4.3$, $p = 0.063$; mean $0.89\text{--}0.92$, ± 0.24 s.e.m.] over time. No interactions were observed between time and genotype or drug for any of these measures [$F(2,11) < 1$, ns].

3.4. BPM: exploratory activity

The exploratory behavior of *Drd4* mutant mice was assessed in the mouse BPM and presented in Fig. 3. *Drd4* KO and HT mice did not differ from their WT littermates in

activity levels [transitions; $F(2,16) = 1.4$, ns], specific exploration [holepoking; $F(2,16) = 1.1$, ns; rearing; $F(2,16) < 1$, ns], or locomotor patterns [spatial d ; $F(2,16) < 1$, ns].

3.5. PPI: sensorimotor gating

The sensorimotor gating of *Drd4* mutant mice was assessed across multiple prepulse intensities (2, 4, 8, and 16 dB above background), as well as across multiple inter-stimulus intervals (ISI; 25, 50, 100, 200, 500, and 1000 ms between the prepulse, 8 dB above background, and the pulse). No main effect of genotype or interaction was observed for prepulse intensities or ISI [$F < 1$, ns]. Although there was a trend toward a genotype effect on habituation to the startle pulse [$F(2,27) = 2.9$, $p = 0.073$], there was no genotype by habituation interaction [$F(6,81) = 1.7$, $p = 0.12$], and a strong habituation of startle observed across all genotypes [$F(3,81) = 8.5$, $p < 0.0001$]. PPI increased across all three genotypes with increasing prepulse intensities [$F(3,81) = 48.6$, $p < 0.0001$] and a main effect of ISI was observed [$F(3,81) = 14.7$, $p < 0.0001$].

3.6. Quantitative RT-PCR: *Drd4* RNA expression levels

The qRT-PCR levels of *Drd4* RNA expression from the frontal cortex were normalized to *Gapdh* levels in each sample. It was confirmed that *Drd4* expression in HT was approximately 40% compared to that of WT mice [$t(2) = 6.09$, $p < 0.05$; Fig. 4]. KO mice were not included in the analysis given the confirmation of 0% expression [54].

3.7. Correlations across testing paradigms

The only correlations significant beyond the Bonferroni corrections were all within the same paradigms. For the 5C-CPT, $P[FA]$ negatively correlated with SI [$\rho = -0.822$, $p < 0.001$], $P[HR]$ negatively correlated with % omissions [$\rho = -0.977$, $p < 0.001$], and MCL negatively correlated with accuracy [$\rho = 0.677$, $p < 0.002$]. As expected, the two forms of impulsivity, $P[FA]$ and premature responding, were not significantly correlated. No significant correlations between transitions, holepoking, rearing, or spatial d were observed within the BPM. For PPI, pulse-alone reactivity did not correlate with any PPI conditions. Several PPI conditions exhibited strong correlations with each other.

4. Discussion

The present studies demonstrate that a genetically induced reduction in *Drd4* expression impaired attentional performance as measured by the 5C-CPT. This impaired performance was driven predominantly by increased false alarm responding to non-signal stimuli, indicative of deficient response inhibition. Importantly, these mice did not exhibit an increase in premature responding, the traditional measure of impulsivity in the 5CSRTT. To confirm that this distinction was not due to an insensitivity of the 5C-CPT to detect changes in premature responding, we demonstrated that the 5-HT_{2C} antagonist SB242084 induced the expected increase in premature responding in these mice. Furthermore, SB242084 did not affect response inhibition as measured by false alarm responding, a differentiation uniquely enabled by the 5C-CPT design. In addition, these two forms of impulsivity were not correlated. Thus, the present data provide support that (a) reduced functioning of the *Drd4* receptor in mice produces a deficient attentional profile similar to neuropsychiatric disorders and (b) impulsivity measures in the 5C-CPT can be differentiated into the dissociable independent factors of response inhibition ($P[FA]$) and motor impulsivity (premature responses). Finally, the demonstrations of normal exploratory activity and PPI in these mice confirm and extend previous findings, broaden these findings for HT mice, and shed further light on the specificity of behavioral effects of reducing *Drd4* expression.

Consistent with previous reports, the present data confirm that *Drd4* mutant mice can be trained to perform an operant task for food rewards [67,68]. During training, it was observed that *Drd4* HT mice exhibited increased false alarm responding when the stimulus durations were shortened. Despite these increases, performance normalized within a few training days. Thus, it was not surprising that no effect of genotype was observed when baseline performance was assessed. When challenged using an extended session and variable stimulus duration, however, HT mice once again exhibited increased false alarm ($P[FA]$) responding. Although when all three genotypes were assessed there was only a trend toward a genotype by stimulus duration interaction on $P[FA]$, *post hoc* analyses revealed that HT mice exhibited increased $P[FA]$ when compared with WT mice at every stimulus duration. This increase in $P[FA]$ was not simply due to increased responding because $P[HR]$ was not increased, and there was no difference in the responsivity index, suggesting that there was no change in response bias. Previous reports suggest that motivation for food reward is normal in these mice [69]. Hence, we conclude that *Drd4* HT mice exhibited significantly poorer attentional performance due to a selective increase in $P[FA]$ in response to non-signal stimuli. This pattern of deficient performance in *Drd4* HT mice in the 5C-CPT is consistent with ADHD and BD patients in multiple CPT tasks, although hit rate was unaffected [16,70,71]. The data presented here support the hypothesis that reduced dopamine D4 receptor expression may contribute to the increased false alarm responding, impairing an aspect of attention that contributes to poor CPT performance observed in neuropsychiatric patients. Further support for this hypothesis derives from evidence of DRD4 polymorphisms associated with volumetric abnormalities in the dorsolateral pre-frontal cortex in patients with ADHD [72]. Given evidence that the dorsolateral prefrontal cortex mediates response inhibition in standard CPTs [73,74], as well as in a newly created human 5C-CPT (Eyler, personal communication), reduced DRD4 expression may contribute to dysfunctional attentional performance.

While *Drd4* HT mice exhibited increased impulsivity as measured by false alarms, premature responding was not increased in these mice. Thus, reduced expression of *Drd4* increases impulsivity in response to non-signal stimuli (response inhibition), but not while awaiting a stimulus to be presented (motor impulsivity). In contrast to the heterozygous animals, *Drd4* KO mice did not exhibit abnormal response inhibition, consistent with previous reports of these mice in a go/nogo task [67]. Furthermore, the KO mice did not differ from their WT littermates in other aspects of attentional performance. Thus, it may be that a complete constitutive knockout of the *Drd4* gene results in neuronal adaptations such as increased striatal extracellular glutamate [75–77] that compensate for the complete lack of *Drd4*, not occurring in *Drd4* HT mice [76]. Altered glutamate levels can affect impulsivity as measured by rating scales in humans [78], but more studies utilizing cross-species translational tests would be required to identify whether this mechanism compensates for the complete lack of *Drd4s* in KO mice. If confirmed in future studies, such compensatory mechanisms may explain the lack of gene dose-dependent effects on performance as have been observed in earlier 5CSRTT studies with alpha 7 nicotinic acetylcholine receptor mutant mice [61].

Studies examining *Drd4* HT mice are limited, but KO vs. WT studies have revealed few behavioral abnormalities of KO mice [67,68], with some suggestions of reduced [79], or increased novelty seeking [67] when compared with WT mice. The data presented here also confirm and broaden previous reports of normal PPI in *Drd4* KO mice [80], and extend these findings to HT mice. Thus *Drd4* HT mice appear to exhibit a selective attentional deficit driven by response disinhibition, without concomitant deficits in PPI or abnormal activity levels, behaviors consistent with some aspects of ADHD patients in similar tasks [50,51,53,70,71,81].

The BPM measurement of activity and exploration in humans has successfully differentiated patients with schizophrenia and BD in the past, leading to the generation of animal models that may be specific to BD [42,44,82]. Although hyperactivity is a prominent symptom of ADHD, it is not always observed in patients and has not been observed to date in adult ADHD patients in the BPM [50]. The inattentive subtype category of ADHD was created to encompass patients who exhibit inattentive behavior but without the combined hyperactive behaviors [83]. This subtype is more common in adults compared to children [84] and provides evidence that the hyperactive and inattentive subtypes may have dissociable neurobiological underpinnings. The majority of animal models of ADHD focus on the hyperactivity component of ADHD [85–88], with few attempts to model the inattentive component [89]. These *Drd4* HT mice may therefore provide a model of the inattentive subtype of ADHD with construct validity that is lacking in other models. Moreover, the selectivity of the behavioral deficit in these *Drd4* HT mice supports a role for the DRD4 in the watershed model of mental disorders [90] and the use of attention as a viable endophenotype [91–94].

Previous reports using the 5CSRTT suggest that impulsivity (measured as premature responses) is mediated by the serotonergic system. More specifically, 5-HT_{2A} agonists and 5-HT_{2C} antagonists increase, while 5-HT_{2A} antagonists and 5-HT_{2C} agonists decrease motor impulsivity [10,31–35]. Moreover, it has been demonstrated that elevated serotonin levels correlate with increased motor impulsivity in rats performing the 5CSRTT [95]. This relationship is in contrast to correct responses to target stimuli which correlated to acetylcholine release [96]. In the present studies, we have replicated previous reports that administration of the 5-HT_{2C} antagonist SB242084 increased motor impulsivity as measured by premature responding [32]. The present studies extend these findings by demonstrating that 5-HT_{2C} antagonist administration does not increase response inhibition impulsivity (*P*[FA]), in contrast with genetic reduction of *Drd4* receptors. These data therefore support the view that impulsivity is a multi-faceted concept [97,98] and suggest that the 5C-CPT can differentiate between motor impulsivity and response inhibition. The orthogonal nature of motor impulsivity and response inhibition suggested here is further supported by a lack of correlation between these measures at each stage.

Previous reports in the 5C-CPT demonstrated a vigilance decrement in two strains of mice [12], providing support that the 5C-CPT assesses vigilance in a manner that is consistent with the human CPT. The vigilance decrement observed in *Drd4* mutant mice in the present studies provides further support for the validity of the 5C-CPT. Moreover, the finding that vigilance performance in the task (SI) varied depending upon signal strength (variable stimulus duration), provides further support that the 5C-CPT assays vigilance consistent with human CPTs [2,3]. The present data highlight another important aspect of assaying attentional performance, however. Challenging performance in the 5CSRTT has proven to be a viable means by which to identify neurobiological underpinnings of task performance [10], as well as assay putative therapeutics [99]. The challenge utilized in experiment 3 (extended ITI) altered the protocol sufficiently that mice improved as time went on. The mice therefore demonstrated a within session learning effect, in contrast with the vigilance decrement observed in the standard task [12] and in experiment 2 (although testing for experiment 3 occurred over three distinct test sessions with training days between testing). These data support the possibility that a learning confound may exist in studies employing an extended ITI challenge, whereby drug- or gene-induced effects on performance may interact with learning and not necessarily reflect changes in attentional performance [11].

4.1. Conclusions

The present studies demonstrate that dopamine D4 receptors likely contribute to normal attentional performance by facilitating response inhibition. Reduced *Drd4* expression in

mice, confirmed by qRT-PCR, impaired 5C-CPT performance by reducing response inhibition. These findings provide behavioral support for the hypothesis that the DRD4 polymorphisms associated with ADHD may reduce *Drd4* expression in patients [27,28] and thereby contribute to some symptoms these patients exhibit. The transitory effect of response disinhibition of the HT mice could reflect initial testing as occurs in a laboratory setting with practice effects obscuring this effect upon repeated testing. This transitory effect may also be attributable to low sample sizes, however, with replication of the current findings prudent. Future studies could also examine whether selective antagonists of the DRD4 could produce response disinhibition consistent with that of the *Drd4* HT mice here. Moreover, these studies support the hypothesis that DRD4 agonists may be effective in the treatment of attentional dysfunction in neuropsychiatric patients [100]. Previous reports have demonstrated that agonists to the DRD4 can improve other forms of inhibition such as inhibitory avoidance in the spontaneous hypertensive rat model of ADHD [101]. The present data also highlight the utility of the 5C-CPT, whereby a measurement of response inhibition, consistent with CPTs in humans, was required to delineate impaired attentional performance in the *Drd4* HT mice. Furthermore, the double dissociation in the effects of *Drd4* and 5-HT_{2C} receptor manipulations on response inhibition and motor impulsivity clearly differentiates these separable forms of impulsivity, highlighting the value of assessing response inhibition in vigilance tasks. Finally, the 5C-CPT may prove useful in assessing the validity of animal models and the efficacy of putative therapeutics related to other psychiatric populations such as schizophrenia [11] and BD [42,82].

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Fig. 1.

Performance of *Drd4* mutant mice in the 5C-CPT. The vigilance performance of *Drd4* wildtype (WT), heterozygous (HT), and knockout (KO) mice was assessed under a variable stimulus duration, extended session challenge. HT mice exhibited poorer vigilance as measured by the sensitivity index (A), which was apparently driven by an increased probability of false alarm responding, suggesting poor response inhibition (B). This increased responding to non-signal stimuli was not due to higher responding overall since there were no differences observed in the responsivity index (C) or in the probability of hit rate (D). All mice maintained high levels of accuracy (E), demonstrating that they could discriminate well between lit and unlit holes. Despite response disinhibition in HT mice, their level of premature responding did not differ from WT, nor did KO mice (F). Finally,

these mice also did not differ in terms of % omissions (G) or speed of responding as measured by mean correct latency (H). Data presented as mean $\pm$ s.e.m., *denotes $p < 0.05$ compared to WT littermate mice.

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Fig. 2.

Effects of the 5-HT_{2C} antagonist on performance in the 5C-CPT. To ascertain the effects of a 5-HT_{2C} antagonist on performance of the 5C-CPT, SB242084 was administered to *Drd4* mutant mice and performance was assessed over an extended variable inter-trial interval. Given no drug by genotype interactions, data are presented as means collapsed across all genotypes. SB242084 did not affect vigilance as measured by the sensitivity index (A), nor bias as measured by responsivity index (B). SB242084 also did not affect the probability of false alarms (C), hit rate (D), or accuracy (E). SB242084 did, however, increase premature responding (F). Data presented as mean + s.e.m., *denotes $p < 0.05$ when compared to vehicle performance.

Fig. 3.

Exploratory activity of *Drd4* mutant mice. The exploratory behavior of *Drd4* mutant mice was assessed using the cross-species test of exploration, the Behavioral Pattern Monitor. Neither *Drd4* heterozygous (HT) nor knockout (KO) differed from *Drd4* wildtype (WT) littermates in terms of activity (A), specific exploration as measured by holepoking (B) or rearing (C), and linear movement measure spatial d (D). Data presented as mean + s.e.m.

Fig. 4. Q-PCR analysis of *Drd4* mRNA expression in prefrontal cortex. Heterozygous (HT) *Drd4* mice have about 50% reduction of *Drd4* mRNA expression compared to wildtype (WT) mice, while no detectable expression was found in *Drd4* knockout (KO) mice. Data presented as mean + s.e.m., *denotes $p < 0.05$ compared to WT littermate mice (KO not analyzed due to lack of data).

Table 1

Stable baseline performance of dopamine D4 mutant mice.

Measure	Genotype effect		Mean $\pm$ s.e.m. by genotype
	<i>F</i> (2,16)	<i>p</i> -Value	
Total trials	1.3	0.321	WT = 112 $\pm$ 5.1 HT = 104 $\pm$ 8.2 KO = 107.4 $\pm$ 3.3
Premature responses	2.5	0.134	WT = 10.0 $\pm$ 3.8 HT = 2.9 $\pm$ 1.3 KO = 6.6 $\pm$ 2.1
Accuracy	1.3	0.312	WT = 0.89 $\pm$ 0.01 HT = 0.87 $\pm$ 0.03 KO = 0.93 $\pm$ 0.02
% Omissions	0.9	0.440	WT = 43.5 $\pm$ 3.3 HT = 51.3 $\pm$ 2.1 KO = 47.8 $\pm$ 3.2
Mean correct latency	1.4	0.280	WT = 0.78 $\pm$ 0.03 HT = 0.80 $\pm$ 0.04 KO = 0.70 $\pm$ 0.02
Proportion of hit rate	0.4	0.659	WT = 0.68 $\pm$ 0.04 HT = 0.64 $\pm$ 0.05 KO = 0.69 $\pm$ 0.03
Proportion of false alarms	0.4	0.673	WT = 0.35 $\pm$ 0.04 HT = 0.41 $\pm$ 0.06 KO = 0.35 $\pm$ 0.04
Sensitivity index	0.7	0.536	WT = 0.59 $\pm$ 0.11 HT = 0.50 $\pm$ 0.12 KO = 0.60 $\pm$ 0.10
Responsivity index	0.1	0.952	WT = 0.23 $\pm$ 0.08 HT = 0.25 $\pm$ 0.16 KO = 0.30 $\pm$ 0.11