Dopamine Receptor $D_1/D_5$ Gene Expression in the Medial Prefrontal Cortex Predicts Impulsive Choice in Rats

A neuropsychological hallmark of attention deficit/hyperactivity disorder (ADHD) is the reduced ability to tolerate delay of reinforcement, leading to impulsive choice. Genetic association studies have implicated several genes involved in dopaminergic neurotransmission in ADHD. In this study, we investigated whether differences in the expression level of these dopamine-related genes of rats predict the individual level of impulsive choice. Among all frontostriatal brain regions tested, only in the medial prefrontal cortex (mPFC), we observed significant positive correlations between impulsive choice and transcript levels of the dopamine receptor $D_1$, the dopamine receptor $D_5$, and calcyon. Local mPFC infusions of the $D_1/D_5$ receptor antagonist SCH 23390 and agonist SKF 38393 resulted in increased impulsive choice, in agreement with the idea that endogenous receptor $D_1/D_5$ stimulation in the mPFC promotes the choice of large delayed rewards. Together, these data indicate that this class of dopamine receptors in the mPFC plays a pivotal role in impulsive choice, and aberrancies thereof might contribute to ADHD symptomatology.

Keywords: caly, Drd1, Drd5, impulsive decision making, real-time quantitative polymerase chain reaction

Introduction

Attention deficit/hyperactivity disorder (ADHD) is a highly disruptive, disabling disorder clinically characterized by inattention, hyperactivity, and impulsiveness. ADHD patients show deficits on different neuropsychological tasks. Current theories suggest that ADHD is the result of dysfunctions in a spectrum of neuropsychological processes (Sonuga-Barke 2002, 2005; Sergeant et al. 2003; Nigg et al. 2005). One of these dysfunctional processes is the inability to tolerate delay of reinforcement, as ADHD patients show increased preference for immediate small over delayed larger—and therefore more beneficial—rewards (Sonuga-Barke et al. 1992, 2002; Barkley et al. 2001; Solanto et al. 2001). This results in a so-called “impulsive choice” for the small reward. Methylphenidate (Ritalin), currently the most commonly prescribed drug for the treatment of ADHD, reduces impulsive choice in a delayed reward task in humans (Pietras et al. 2003) indicating that this task relates to an important clinical symptom of ADHD.

Manipulation of dopamine, noradrenalin, and serotonin neurotransmission is known to modulate impulsive choice (Winstanley et al. 2005; van Gaalen et al. 2006; Robinson et al. 2008; for review, see Pattij and Vanderschuren 2008). Dopaminergic neurotransmission has received particular attention in this respect because treatment with drugs that specifically (GBR 12909) or less specifically (methylphenidate, amphetamine, and cocaine) inhibit dopamine reuptake decrease impulsive choice as shown in both human (Pietras et al. 2003) and rodent studies (Wade et al. 2000; van Gaalen et al. 2006).

Recently, genetic studies have identified associations between the occurrence of ADHD and genes involved in dopaminergic neurotransmission (for review, see Waldman and Gizer 2006), including genes encoding the dopamine receptor $D_1$ (DRD1; Misener et al. 2004), the dopamine receptor $D_5$ (DRD5; Lowe et al. 2004), the dopamine transporter (SLC6A3 or DAT1; Faraone et al. 2001), and calcyon (CALY; Laurin et al. 2005). Interestingly, the latter gene encodes a protein product that is involved in regulation of the affinity state of $D_1$ receptors for agonists (Lidow et al. 2001). Thus far, polymorphisms within the protein-coding sequence of these dopamine-related genes have not been identified, except for the 7-repeat polymorphism in DAT1 that alters affinity to certain antipsychotics (Van Tol et al. 1992; Faraone et al. 2001). This may suggest that polymorphisms in genes causally related to ADHD reside in regulatory sequences instead and exert their effect by altering the expression levels of these genes. As yet, it is unknown whether differences in gene expression of dopamine-related genes are associated with specific deficits observed in ADHD, such as impulsive choice.

Previously, we have shown that rats vary in their individual level of impulsive choice and that this phenotype is stable over time (Diergaard et al. 2008). In the present study, we investigated whether the individual level of impulsive choice could be predicted by the expression levels of the aforementioned dopamine-related genes in frontocortical and striatal brain regions, areas known to be critically involved in impulsive decision making (for reviews, see Cardinal 2006; Winstanley, Eagle, and Robbins 2006). The gene expression of 2 dopamine receptors ($D_1$ and $D_5$) and calcyon neuron-specific vesicular protein significantly correlated with impulsive choice, notably exclusively in the infra- and prelimbic regions of the medial prefrontal cortex (mPFC). By intracranial infusions of the $D_1/D_5$ receptor antagonist SCH 23390 and agonist SKF 38393 into the mPFC, we examined whether $D_1/D_5$ receptors in the mPFC are functionally involved in impulsive choice. To rule out possible...
agonistic effects of SCH 23390 on 5-HT2c receptors (Millan et al. 2001), SCH 23390 was also coinfused with the 5-HT2a/2c receptor antagonist ketanserin.

Materials and Methods

Subjects
Male Wistar rats (200-250 g, Harlan CPB, Horst, The Netherlands) were housed 2 per cage (lights on from 7 PM to 7 AM) with water available ad libitum during the entire experiment. After a habituation period of 2 weeks, animals were food restricted to 85%-90% of their free feeding weight before training of the operant delayed reward task started. The Animal Care Committee of the VU University Amsterdam approved all experiments.

Delayed Reward Paradigm
Operant test chambers (Med Associates, St Albans, VT) were equipped with 5 nose poke holes with stimulus lights and infrared detectors and a pellet dispenser (45 mg, Noyes Precision Pellets, New Brunswick, NJ) at the opposite wall. Five sessions were scheduled per week, one session each day. More details on the procedure of training the animals to perform the operant delayed reward paradigm have previously been described (van Gaalen et al. 2006). Briefly, after training for ~12 weeks, the task required animals to respond to a 10-s light stimulus in the middle hole at the beginning of each trial (initiation period), which after a response, was followed by 10-s light stimulus in the left and right hole immediately adjacent to the middle hole (choice period). A response in one hole extinguished both stimuli and delivered a small reward (one pellet) into the magazine, whereas a response in the other hole extinguished both stimuli and started the delay period after which a large reward (4 pellets) was delivered into the magazine. For an individual rat, the same hole was always associated with large reward, and left and right associations were counterbalanced across rats. A session consisted of 60 trials in total, with 5 blocks of 12 trials. The first 2 trials of each block were forced trials during which once a small reward and once a large reward could be chosen (randomly presented). In the subsequent 10 trials, a rat was free to choose. After each block of 12 trials, the delay period between a response into the hole associated with the large reward and delivery of the large reward was programmed to increase. No conditioned stimuli were presented during the delay period. The intertrial interval was programmed such that the duration of each trial was 100 s. Failures to respond within the initiation period were registered as errors of omission. Rats that persistently omitted the large reward during all forced and choice trials were excluded. Percentage choice for the large reward was calculated for each block of 10 trials, for each delay [number of large rewards earned/number of large rewards earned + number of small rewards earned]. In this paradigm, an enhanced level of impulsive choice is defined as a reduced percentage of choice for the large reward during all forced and choice trials. For correlation analysis, the average individual level of impulsive choice was calculated across these 17 baseline sessions.

Locomotor Response to Novelty
On the day between the 14th and 15th baseline session, rats were transferred to a dimly lit testing room and introduced in square gray plastic boxes (50 × 50 cm) where their behavior was analyzed using a camera connected to a computer equipped with Ethovision (version 3.1, Noldus, Wageningen, The Netherlands). The total distance moved during 60 min was taken as measure of their locomotor response to novelty.

Gene Expression Measurements
Immediately after the 17th baseline session, rats were decapitated and brains rapidly frozen using -80°C isopentane. In a cryostat, the brains were sliced into 200-μm coronal sections and the following brain regions were isolated according to Paxinos and Watson (1998): orbitofrontal cortex (OFC), agranular insular cortex, prelimbic together with infralimbic cortex (mPFC), anterior cingulated cortex, nucleus accumbens core and nucleus accumbens shell (NAcS), and the medial and lateral caudate putamen (medial to lateral coordinates: ±1.0 to ±2.0 and ±2.0 to ±4.0, respectively). From these tissues, RNA was isolated that was DNase-treated and reverse transcribed to cDNA using random hexanucleotide primers. Gene expression was then analyzed by real-time quantitative polymerase chain reaction (ABI Prism SDS 7900, Applied Biosystems Inc., Foster City, CA) using a SYBR Green (Applied Biosystems Inc., Foster City, CA) approach (300 nM gene-specific primers and the cDNA equivalent of 40 ng RNA in a total volume of 10 μL) and normalized to the geometric mean of 3 housekeeping genes (β-actin, hypoxanthine phosphoribosyltransferase 1, and neuron specific enolase). Normalized gene expression levels (ΔGnorm) were calculated from detection cycle threshold (Ct) values as follows: [ΔGnorm = (Ctgenex - CtHKGenes)]. The fold difference in gene expression level between high impulsive (HI) and low impulsive (LI) groups of rats was calculated as follows: [2ΔCtGnorm HI – ΔCtGnorm LI]. In addition to the aforementioned dopamine-related genes (dopamine receptors D1, D2, D3, D5, D4, Drd1, Dbh, Slc6a3, and Caly) also the expression of 2 known dopamine receptor genes (Drd2 and Drd3) and the catechol-O-methyl-transferase (Cott) gene were measured. Primers were selected for a low probability of folding into loop structures, a low probability of forming primer dimers and were blasted against expressed sequence tags present in the rat database of the National Center for Biotechnology Information to check for specificity. We determined the melting curves for all primer sets used, in order to check for possible primer-dimer formation. All primers yielded a specific product of the correct melting temperature. The amplification efficiency of all primers was checked and found to be between 1.9 and 2.0. Nucleotide sequences of the primers are provided in Supplementary Table 1.

Experiment 2: Infusion of a D1/D5 Receptor Agonist or Antagonist into the mPFC

Surgery
Before surgery, rats were trained to perform the operant delayed reward paradigm at delay periods ranging from 0 to 40 s (0, 5, 10, 20, and 40) for 17 sessions. Five days before surgery operant training was stopped and food was available ad libitum. Rats were anaesthetized using a combination of xylazine (Rompun; Bayer AG, Leverkusen, Germany; 7 mg/kg, intraperitoneal) and ketamine (Alfasan, Woerden, The Netherlands; 100 mg/kg, intramuscularly). A double guide cannula (Plastics One, Roanoke, VA) was placed above the mPFC according to coordinates derived from Paxinos and Watson (1998); anteroposterior +3.2 mm from bregma and lateral ±0.75 mm from midline. After surgery, rats were housed individually and fed ad libitum for 1 week after which they received 12 retraining sessions. Rats were ranked on the average impulsive choice of the last 3 retraining sessions, then divided into pairs, and from each pair one rat was randomly assigned to the group that would receive bilateral infralimbic infusions and the other to the group that would receive bilateral prelimbic infusions.

Drug Infusions
All drugs (SCH 23390 hydrochloride and SKF 38393 hydrochloride [Sigma Aldrich, St. Louis, MO], and ketanserin [Janssen Pharmaceutica, Beerse, Belgium]) were freshly dissolved in sterile saline on the day of infusion. The drug solutions were infused through an injector that was inserted in the guide canula and protruded to the pre- or infralimbic
area (3.7 or 4.9 mm below the surface of the skull). A volume of 0.5 μL was delivered at a flow rate of 0.25 μL/min using 10-μL Hamilton syringes driven by a syringe infusion pump (Harvard Apparatus, South Natick, MA). Injectors were left in place for an additional 2 min to allow diffusion. Rats were placed back in their home cage and were placed in the operant chamber 10 min later. At least 2 training days separated infusion days. For all rats, the order of infusions was the same; sham the operant chamber 10 min later. At least 2 training days separated infusions. Rats were placed back in their home cage and were placed in

Statistical Analysis
Differences between HI and LI rats were analyzed using 1-way analysis of variance (ANOVA). Using the QVALUE package (Storey and Tibshirani 2003) in the statistical software program R (version 2.7.2), the false discovery rate (FDR) was calculated for the list of P values of the dopamine-related genes implicated in ADHD. Pearson correlations coefficients and probabilities were used to analyze correlations between impulsive choice and GEnorm. The effects of local drug infusions were analyzed using repeated-measures ANOVA, with 2 within-subjects factors (dose and delay) and one between-subject factor (area: infusion in either infra- or prelimbic area). If Mauchly’s test for sphericity of data was significant, more conservative Greenhouse-Geisser-corrected probability values were reported. Where appropriate, a post hoc analysis (Fisher least square difference) was used for pairwise comparisons of doses. All statistical analyses, except calculation of the FDR, were performed using Statistical Package for Social Sciences version 14.0 (SPSS Inc, Chicago, IL).

Results

**Experiment 1: Correlations between Impulsive Choice and Expression of Dopamine-Related Genes**

**Stable Individual Differences in Impulsive Choice**
The individual level of impulsive choice was calculated for all 17 baseline sessions, and reliability analysis on all 23 included rats indicated that this individual level was stable across baseline sessions (Cronbach’s α = 0.966). The individual level of impulsive choice between the upper quartile (low percentage choice for large reward; HI) and the lower quartile (high percentage choice for large reward; LI) is shown in Figure 1A. The average impulsive choice across all baseline sessions of HI and LI rats differed significantly ($F_{1,10} = 97.126, P < 0.0001$; Fig. 1B).

![Figure 1](https://academic.oup.com/cercor/article-abstract/20/5/1064/333341)

**Figure 1.** Temporally stable differences in impulsive choice between rats. (A) The level of impulsive choice (100 − % choice large reward) of LI (white circles) and HI rats (black circles) across 17 baseline sessions. (B) LI and HI rats differed in their average percentage choice for large reward calculated from 17 baseline sessions ($\beta$ = baseline). **P < 0.0001.

**Impulsive Choice Does Not Correlate with Errors of Omission or the Locomotor Response to Novelty**
No significant differences between HI and LI rats were observed in distance moved during 1 h in a novel box ($F_{1,10} = 0.415, P = 0.534$) and in errors of omission on trials used to calculate the individual level of impulsivity ($F_{1,10} = 0.530, P = 0.483$). Similarly, when calculated across all rats in the experiment, the individual level of impulsive choice did not correlate with errors of omission (Pearson $r = 0.209, P = 0.338$; not shown) or the distance moved (Pearson $r = -0.04, P = 0.874$; Fig. 2A).

**Brain Region-Specific Correlations of Impulsive Choice with Drd1, Drd5, and Caly**
Significant differences ($P < 0.05$, FDR < 0.33) between HI and LI rats were detected in the mPFC for Drd1, Drd5, and Caly and in the NAcS for Drd5 (Supplementary Table 2). Correlation analysis across all 23 rats in the experiment indicated that impulsive choice correlated significantly ($P < 0.05$) with the expression of Drd1 ($r = 0.52$), Drd5 ($r = 0.56$), and Caly ($r = 0.45$) in the mPFC (Fig. 2B-D) but not with Drd5 in the NAcS ($r = 0.237, P = 0.314$).

**Experiment 2: Inhibition of a D1/D5 Receptor Agonist and Antagonist into the mPFC**
In all results described below, the between-subject factor “area” never had a significant effect on the percentage choice for the large reward, indicating that there was no significant differences between HI and LI rats for A, Drd5, or Caly expression in the mPFC.}

![Figure 2](https://academic.oup.com/cercor/article-abstract/20/5/1064/333341)

**Figure 2.** Scatter plots of correlations between the level of impulsive choice (100 − % choice large reward) and (A) the level of locomotor activity as measured as distance moved and the level of expression in the mPFC of (B) Drd1, (C) Drd5, and (D) Caly. Normalized levels of gene expression are displayed as deviation from the group mean expression level (GEnorm; log2 scale). *P < 0.05. Colors of the circles represent LI (white circles), HI rats (black circles), and intermediate impulsive rats (gray circles).
difference in the response to infusions between the groups receiving infra- and prelimbic mPFC infusions. None of the saline infusions changed the percentage choice for the large reward when compared with the next day (saline1 $F_{1.9} = 0.288, P = 0.604$; saline2 $F_{1.12} = 0.762, P = 0.400$; saline3 $F_{1.8} = 0.009, P = 0.929$). Furthermore, the percentage choice for the large reward after the first, second, and third saline infusion did not differ from each other ($F_{2.14} = 0.036; P = 0.964$). Therefore, all reported drug effects below are compared with the average percentage choice for the large reward calculated over all 3 saline infusions.

**mPFC Infusions of the D$_1$/D$_5$ Receptor Antagonist SCH 23390 Alone and in Combination with an Infusion of Ketanserin**

The effect of mPFC infusions of 2 doses of the D$_1$/D$_5$ receptor antagonist SCH 23390, as well as the effect of SCH 23390 after a preceding infusion of the serotonin 5-HT$_{2a/c}$ receptor antagonist ketanserin were statistically evaluated together (Fig. 3A). These infusions affected the percentage choice for the large reward significantly ($F_{3.55} = 7.020; P < 0.001$), an effect that was not delay dependent (dose $\times$ delay interaction, $F_{2.132} = 1.153; P = 0.324$). Post hoc analyses revealed that the percentage choice for the large reward was significantly increased after an infusion of 1 μg SCH 23390 ($P < 0.001$). When an infusion of 1 μg SCH 23390 was preceded by an infusion of 0.1 μg ketanserin, post hoc analyses indicated that SCH 23390 still significantly increased the percentage choice for the large reward compared with saline ($P < 0.0001$). Moreover, this combination of SCH 23390 and ketanserin did not change the percentage choice for the large reward compared with a single infusion of 1 μg SCH 23390 ($P = 0.566$).

Infusions of SCH 23390 alone and in combination with ketanserin also significantly increased the errors of omission (Supplementary Fig. 1; $F_{3.53} = 3.246; P = 0.034$), an effect that was delay dependent (dose $\times$ delay interaction, $F_{2.132} = 1.880; P = 0.042$). Post hoc analyses revealed that errors of omission increased significantly after 1 μg SCH 23390 when the delivery to the large reward was delayed for 20 s ($P = 0.015$) and 40 s ($P = 0.006$), an effect that was blocked by coinfusion of 0.1 μg ketanserin (Supplementary Fig. 1).

**mPFC Infusions of the D$_1$/D$_5$ Receptor Agonist SKF 38393**

Infusion of SKF 38393 increased the percentage choice for the large reward (Fig. 3B; $F_{2.22} = 4.762; P = 0.019$), an effect that was not delay dependent (dose $\times$ delay interaction, $F_{0.88} = 1.954; P = 0.062$). Post hoc analysis revealed that the percentage choice for the large reward compared with saline increased after infusion of both 0.05 μg ($P = 0.026$) and 0.1 μg ($P = 0.019$) SKF 38393. No effect of SKF 38393 on errors of omission was found ($F_{2.22} = 0.239; P = 0.789$).

**mPFC Infusions of a Single Dose of Ketanserin**

There was no significant effect of infusion of 0.1 μg ketanserin on the percentage choice for the large reward ($F_{1.9} = 1.307; P = 0.282$), its interaction with delay (dose $\times$ delay interaction, $F_{0.72} = 1.559; P = 0.153$) or errors of omission ($F_{1.9} = 2.766; P = 0.131$).

**Discussion**

In this study, we observed stable differences between rats in their inability to tolerate delay of reinforcement, which is a prominent neuropsychological deficit observed in ADHD (Sonuga-Barke et al. 1992, 2002; Barkley et al. 2001; Solanto et al. 2001). We correlated the individual level of impulsive choice in a rodent delayed reward paradigm with the expression level of 6 dopamine-related genes previously associated with ADHD and observed significant positive correlations between the expression level of 3 genes (Drd1, Drd5, and Caly) and impulsive choice only in the mPFC and not in the other corticostriatal brain areas investigated. In line with previous studies (Perry et al. 2005; Wilhelm and Mitchell 2009), we found that the level of locomotor activity did not correlate with individual levels of impulsive choice. This indicates that the observed differences in impulsive choice and gene expression levels do not result from individual differences in activity. Subsequent intracranial infusions of the D$_1$/D$_5$ receptor agonist SKF 38393 and antagonist SCH 23390 into the mPFC confirmed the role of D$_1$/D$_5$ receptor signaling in this brain area in impulsive choice.

With regard to the neuroanatomical circuitry underlying impulsive choice, different regions are thought to promote the choice of immediate small rewards and delayed large rewards (McClure et al. 2004; Cardinal 2006). A human functional magnetic resonance imaging study demonstrated that limbic regions (including the OFC) are activated during a choice for a small immediate reward, whereas regions of the lateral cortex (including the dorsolateral PFC) are activated by choice irrespective of delay (McClure et al. 2004). In line with this, a lesion study in rats showed that the OFC indeed promotes the choice for a small reward (Winstanley et al. 2004). Lesions of the mPFC in rodents, encompassing the prelimbic region that is functionally homologous to the primate dorsolateral PFC.
session-wide temporal discrimination. In support of this, sufficient to bridge the delay times within a trial or the required high to exert beneficial effects.

employed doses of SCH 23390 and SKF 38393 were still too low to be effective. However, the number of rats in this study may have been limited, thereby reducing the power of the study. Two possible reasons may explain why low doses of SKF 38393 and SCH 23390 suggest that endogenous tonically and perhaps phasically activated D1/D5 receptors in the mPFC might promote the choice of large delayed rewards.

D1-like receptors are involved in potentiating and tuning delay-period activity of prefrontal cortical neurons (Goldman-Rakic et al. 2000; Seamans and Yang 2004) and concomitantly prevent behavioral distraction across delays between sample and retention (Robbins 2005). The mechanisms by which mPFC D1/D5 receptor occupancy promotes the choice for delayed large rewards in the current study may possibly be explained by its role in maintaining a correct representation of specific information gathered during the task. Previous studies indicated that blockade or stimulation of mPFC D1/D5 receptors by intracranial infusion of high doses of D1/D5 receptor agonists and antagonists disrupt delay-period activity (Williams and Goldman-Rakic 1995) and impair performance in delayed response tasks (Sawaguchi and Goldman-Rakic 1991; Zahrt et al. 1997; Seamans et al. 1998). In the current study, infusion of the high doses of SKF 38393 and SCH 23390 may have interfered with representation of task-relevant information, such as the length of the delay in a previous trial or the time that has passed with respect to the start of the session. It is conceivable that uncertain or altered representation of this information, for instance, uncertainty regarding the delay duration in the previous trial or altered session-wide timing, contributed to an accelerated discounting of the large reward.

In contrast to high doses of D1/D5 receptor agonists, low doses of D1/D5 receptor agonists have been shown to potentiate the delay-period activity of prefrontal cortical neurons (Goldman-Rakic et al. 2000) and, moreover, to enhance attention in rats with low baseline performance (Granon et al. 2000). Two reasons may explain why low doses of SKF 38393 and SCH 23390 did not decrease impulsive choice in the current study. First, endogenous D1/D5 receptor stimulation may have been sufficient to bridge the delay times within a trial or the required session-wide temporal discrimination. In support of this argumentation, beneficial effects D1/D5 receptor agonists have been observed for delays in the magnitude of 12 h, and not within the range of delays relevant for the current study, that is, in the order of minutes and seconds (Floresco and Phillips 2001). Second, a beneficial effect of low doses of the agonist may only have been visible in rats with a high baseline impulsive choice and presumably low dopamine D1/D5 receptor occupancy. Upon further categorizing rats into high, intermediate, and low impulsive groups, we did not find a significant interaction between baseline impulsivity and the effect of the low doses of SKF 38393 (data not shown). However, the number of rats in this study may have been insufficient to detect small improvements, or alternatively, the employed doses of SCH 23390 and SKF 38393 were still too high to exert beneficial effects.

It should be emphasized that besides affinity for D1/D5 receptors, SCH 23390 may also act as an agonist of the serotonergic 5-HT2c receptor (Millan et al. 2001). In fact, a systemic injection of the serotonin 5-HT2c receptor agonist 2,5-dimethoxy-4-iodoamphetamine has been reported to enhance impulsive choice (Ewen and Ryan 1999). In order to disentangle D1/D5 and 5-HT2c receptor-mediated effects, we coinfused the serotonin 5-HT2c receptor antagonist ketanserin with SCH 23390. Interestingly, the dose of ketanserin used was sufficient to attenuate the increase in omissions by SCH 23390, indicating that effects of SCH 23390 on omissions may be mediated by a nonspecific effect at 5-HT2c receptors. More importantly, ketanserin did not alter the increase in impulsive choice by SCH 23390, indicating that the effect of SCH 23390 on impulsive choice is D1/D5 receptor mediated and not due to its affinity for 5-HT2c receptors.

Taken together, our observation that an endogenous D1/D5 receptor tone in the mPFC is involved in impulsive choice is supported by the observation that dopamine release in the mPFC is enhanced during performance in the delayed reward paradigm (Winstanley, Theobald et al. 2006). The current data extend this observation and suggest that the increase in dopamine during task performance may be related to the optimization of an endogenous dopamine D1/D5 tone to optimize or maintain a representation of task-relevant information.

In addition to the relationship between Drd1 and Drd5 gene expression in the mPFC and impulsive choice, we found a similar relationship between Caly gene expression and impulsive choice. Caly mRNA is abundantly expressed in regions of prefrontal cortex expressing Drd1 or Drd5 (Zelenin et al. 2002), and its protein product calycom is involved in regulation of the affinity state of D1 receptors for agonists (Lidow et al. 2001). Although gene expression differences do not necessarily predict differences in protein abundance or receptor density, collectively these data suggest the existence of a pathway through D1/D5 receptors and calycom in the mPFC that is involved in impulsive choice. In fact, our observation of a positive correlation between Caly gene expression and impulsive choice is in line with a recent study showing that spontaneous hypertensive rats show both enhanced Caly gene expression (Heijtz et al. 2007) and increased impulsive choice (Fox et al. 2008).

The DAT1 has low expression in the frontal lobes (Ciliax et al. 1999) and COMT, which degrades dopamine, has been shown to play an important role in regulating dopamine levels in this brain region (Hong et al. 1998). Although COMT polymorphisms have been associated with prefrontal dopamine levels and executive functioning (for review, see Tunbridge et al. 2006), several studies failed to confirm the initial report (Eisenberg et al. 1999) of an association between COMT and ADHD (for review, see Waldman and Gizer 2006). In the current study, we also measured Comt gene expression in the mPFC but failed to detect a significant difference between HI and LI rats (data not shown).

Previous genetic studies have revealed an association of human DRD1, DRD5, and CALY with ADHD. By and large, in these studies, no variation in the coding region has been identified for DRD1 and CALY (Misserer et al. 2004; Laurin et al. 2005), and in addition, no causal polymorphism in the coding region of DRD5 has been identified thus far (Hawi et al. 2003; Lowe et al. 2004). These observations suggest that
polymorphisms causally related to ADHD reside in regulatory sequences of these genes, thereby altering their expression levels. In this respect, 2 conclusions can be drawn from the current data. First, our findings clearly demonstrate that variation in the expression levels of these 3 aforementioned genes is correlated with impulsive choice in rats, particularly within the mPFC. Second, pharmacological manipulations of D1/D5 receptors within the mPFC increase impulsive choice, further stressing the involvement of prefrontal D1/D5 receptors in impulsive decision making. Together, these data indicate that a pathway involving D1/D5 receptors and possibly calcyon in the mPFC plays a pivotal role in impulsive choice and may constitute a valuable target for further research and possible treatment of ADHD.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Funding
Center for Neurogenomics and Cognitive Research; Neuro-Bsik Mouse Phenomics grant by SenterNovem (BSIK03053).

Notes
Conflict of Interest: None declared.
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