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# Dissociable Rate-Dependent Effects of Oral Methylphenidate on Impulsivity and $D_{2/3}$ Receptor Availability in the Striatum

**Daniele Caprioli,[1] Bianca Jupp,[2,3] Young T. Hong,[4] Stephen J. Sawiak,[2,4] Valentina Ferrari,[4] Laura Wharton,[2,3] David J. Williamson,[4] Carolyn McNabb,[5] David Berry,[6] Franklin I. Aigbirhio,[2,4] Trevor W. Robbins,[2,3] Tim D. Fryer,[2,4] and Jeffrey W. Dalley[2,3,7]**

[1]Behavioral Neuroscience Research Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, Maryland 21224, [2]Behavioural and Clinical Neuroscience Institute, and [3]Department of Psychology, University of Cambridge, Cambridge CB2 3EB, United Kingdom, [4]Wolfson Brain Imaging Centre, Department of Clinical Neurosciences, University of Cambridge, Cambridge Biomedical Campus, Cambridge CB2 0QQ, United Kingdom, [5]School of Pharmacy, University of Auckland, Auckland 1142, New Zealand, [6]Epilepsy Society, Chalfont St Peter SL9 0RJ, United Kingdom, and [7]Department of Psychiatry, University of Cambridge, Cambridge Biomedical Campus, Cambridge CB2 2QQ, United Kingdom

**We have previously shown that impulsivity in rats is linked to decreased dopamine $D_{2/3}$ receptor availability in the ventral striatum. In the present study, we investigated, using longitudinal positron emission tomography (PET), the effects of orally administered methylphenidate (MPH), a first-line treatment for attention deficit hyperactivity disorder, on $D_{2/3}$ receptor availability in the dorsal and ventral striatum and related these changes to impulsivity. Rats were screened for impulsive behavior on a five-choice serial reaction time task. After a baseline PET scan with the $D_{2/3}$ ligand [$^{18}$F]fallypride, rats received 6 mg/kg MPH, orally, twice each day for 28 d. Rats were then reassessed for impulsivity and underwent a second [$^{18}$F]fallypride PET scan. Before MPH treatment, we found that $D_{2/3}$ receptor availability was significantly decreased in the left but not the right ventral striatum of high-impulse (HI) rats compared with low-impulse (LI) rats. MPH treatment increased impulsivity in LI rats, and modulated impulsivity and $D_{2/3}$ receptor availability in the dorsal and ventral striatum of HI rats through inverse relationships with baseline levels of impulsivity and $D_{2/3}$ receptor availability, respectively. However, we found no relationship between the effects of MPH on impulsivity and $D_{2/3}$ receptor availability in any of the striatal subregions investigated. These findings indicate that trait-like impulsivity is associated with decreased $D_{2/3}$ receptor availability in the left ventral striatum, and that stimulant drugs modulate impulsivity and striatal $D_{2/3}$ receptor availability through independent mechanisms.**

*Key words:* **addiction; attention-deficit hyperactivity disorder; dopamine; methylphenidate; nucleus accumbens; positron emission tomography**

## Introduction

Converging evidence from neuroimaging, clinical psychopharmacology, and animal models implicates dysregulated dopaminergic and norepinephrinergic neurotransmission in the pathophysiology of attention deficit hyperactivity disorder (ADHD), the prototypical impulse control disorder (Biederman, 2005; Arnsten, 2006; Dalley et al., 2011). Methylphenidate (MPH), which acts by increasing extrasynaptic dopamine (DA) and norepinephrine (NE) levels by blocking their reuptake (Zetterström et al., 1988), has been the first-line pharmaceutical therapy for ADHD (Wilens, 2008). Although its pharmacological action has been well characterized, the precise neurobiological mechanisms underlying the therapeutic effects of MPH remain unclear. Recent findings suggest that only specific neurocognitive processes in domains such as impulse control and attention are affected by MPH, and that these interact with the drug in a baseline performance-dependent manner (Dews and Wenger, 1977; Sahakian and Robbins, 1977; Robbins and Sahakian, 1979; Turner et al., 2003; Clatworthy et al., 2009; DeVito et al., 2009). Such effects, which depend on optimizing catecholamine levels in the brain, are hypothesized to follow an inverted U-shaped function (Clatworthy et al., 2009; van der Schaaf et al., 2013).

Recently, we reported (Caprioli et al., 2013) a similar baseline-dependent effect of cocaine in a preclinical animal model of impulsivity. We reported that impaired response inhibition in a rodent model of impulsivity is associated with a deficiency in DA $D_{2/3}$ receptor availability in the left ventral striatum, and that

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Correspondence should be addressed to Dr. Jeffrey W. Dalley, Department of Psychology, University of Cambridge, Downing Street, Cambridge CB2 3EB, UK. E-mail: jwd20@cam.ac.uk.

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prior response-contingent exposure to cocaine both restored $D_{2/3}$ receptor availability in this region and improved impulse control. This evidence directly supports the baseline dependency hypothesis at the neurobiological level in the striatum, and this may be relevant to recent findings (Volkow et al., 2007a,b, 2009, 2012). Indeed, a set of well powered case-control positron emission tomography (PET) studies in adult medication-naive ADHD patients, found ADHD to be associated with reduced $D_{2/3}$ receptor availability in the nucleus accumbens and caudate (Volkow et al., 2007a,b, 2009), and that treatment response was associated with increased DA transmission in the ventral striatum (Volkow et al., 2012). Thus, the clinical efficacy of stimulant drugs such as MPH in ADHD may depend, in part, on restoring $D_{2/3}$ receptor signaling in the ventral striatum of impulsive individuals.

In the present study, we therefore investigated the effects of repeated oral administration of MPH on $D_{2/3}$ receptor availability in the ventral striatum of high-impulsive (HI) rats on the five-choice serial reaction time task (5-CSRTT). Impulsivity in this task is measured by the number of anticipatory responses to an imminent visual signal and is analogous to false alarms on the analogous continuous performance test in humans (Robbins, 2002). We used PET and the selective high-affinity $D_{2/3}$ receptor antagonist [$^{18}$F]fallypride (Mukherjee et al., 1995) to investigate $D_{2/3}$ receptor availability in the ventral and dorsal striatum, both before, and following chronic exposure of rats to MPH. In parallel, we investigated the relationship between behavioral impulsivity in selected low-impulsive (LI) versus HI rats and MPH-evoked changes in $D_{2/3}$ receptor availability in the ventral and dorsal striatum.

## Materials and Methods

*Subjects.* Ninety-six adult male Lister-hooded rats (Charles River), weighing 250–275 g and 2–3 months of age at the beginning of behavioral training, were used. These were housed in groups of four in enclosed ventilation chambers during the initial training and selection of HI and LI rats. Upon completion of the screening and for the remaining period of the study, rats were singly housed ($n = 8$ HI rats; $n = 7$ LI rats), similar to our previous study (Caprioli et al., 2013). Rats were singly housed because MPH has been shown to disrupt social behavior in adolescent and young adult rats (Beatty et al., 1982; Arakawa, 1994; Vanderschuren et al., 2008). The holding room was humidity and temperature controlled (22°C), and rats were maintained under a reversed 12 h light/dark cycle (white lights off/red lights on at 7:00 A.M.). Food was restricted to maintain body weights at 85–90% of free-feeding weights. Water was available *ad libitum*. The present experiment conformed to the United Kingdom Animals (Scientific Procedures) Act of 1986 and local ethical guidelines. A timeline of experimental procedures is shown in Figure 1.

*Five-choice serial reaction time task.* The 5-CSRTT apparatus has been described in detail previously (Bari et al., 2008). The training procedure used in the present study was identical to that previously described (Caprioli et al., 2013). In brief, rats were trained on the 5-CSRTT over ~60 daily sessions (6 sessions per week) to detect the location of a brief visual stimulus (0.7 s) presented on a random basis in one of the five recesses. Each session consisted of 100 discrete trials and lasted ~30 min. Training was considered complete when rats responded to the target stimuli of duration 0.7 s with an accuracy of 75% and omissions on <20% of trials. Trials were initiated by subjects entering the magazine. After a fixed intertrial interval (ITI) of 5 s, a visual stimulus was presented in a single aperture. Rats were rewarded with a single pellet if they correctly located the position of the target stimulus (a "correct" response). A failure to respond within a limited hold period of 5 s was deemed an "omission" and was signaled by a 5 s time-out period and a loss of food reward on that trial. Similar feedback was given on trials where rats responded in an adjacent aperture (an "incorrect" response) or before the onset of the light stimulus (a "premature" response). Behavioral performance was assessed by the following: choice accuracy, (percentage correct responses/(correct + incorrect trials); premature responding, (percentage premature responses/(correct + incorrect + omission trials); omissions, (percentage omission trials/(correct + incorrect + omission trials); latency to collect food, (time from nose-poke response to entering the magazine, in milliseconds); and correct response latency, (time to make a response in the correct aperture after the onset of the light stimulus). Once rats had acquired the 5-CSRTT, they were ranked for impulsivity during a 3 week screening period. Each week consisted of 5 consecutive days of testing with days 1, 2, 4, and 5 comprising sessions of 100 discrete trials each and an ITI of 5 s [short ITI (S-ITI)]. During day 3, the ITI was increased to 7 s to increase the frequency of premature responses [long ITI (L-ITI)]. HI animals were defined as those exhibiting a level of premature responding of >50 on all three L-ITI sessions. LI rats were selected from the remaining rats and responded prematurely on <30% of trials during the L-ITI sessions.

*Long-term methylphenidate treatment.* Methylphenidate hydrochloride (Sigma) was dissolved in Ribena (GlaxoSmithKline) and administered orally (6 mg/ml/kg) twice a day (10:00 A.M. and 5:00 P.M.). The oral route of administration was used to model the normal manner in which this drug is administered clinically (Kuczenski and Segal, 2002; Swanson and Volkow, 2009). Two days before the first oral dosing of MPH, rats were trained to consume the Ribena solution from a 1 ml syringe. Chronic MPH exposure was maintained for 7 d a week for 4 consecutive weeks (Fig. 1). During this period, rats were assessed for performance on the 5-CSRTT at 8:00 A.M. when rats were in the drug-free state (i.e., 15 h after the last MPH administration). During the last 7 d of MPH treatment, rats were challenged with three L-ITI sessions, each spaced 3 d apart.

*Analysis of methylphenidate and ritalinic acid.* Four nonimpulsive rats were used to quantify plasma levels of MPH and its metabolite ritalinic acid. Rats were orally administered Ribena spiked with 6 mg/kg MPH, as described above, and after 10 min were anesthetized with 5% isoflurane. General anesthesia was maintained via the delivery of 1.5% isoflurane in medical air. Blood samples were taken from a tail vein (0.5 ml) at 5, 15, 30, 60, 90, and 120 min following MPH administration. Blood was allowed to clot at room temperature (24°C) before being centrifuged at 2000 rpm for 10 min. Plasma was aspirated from the centrifuged sample and stored at −80°C before the determination of MPH and ritalinic acid using HPLC-tandem mass spectrometry (MS/MS). Calibration standards (range, 0.01–5 mg/L) were prepared with MPH and ritalinic acid spiked in blank rat plasma. Quality control samples were similarly prepared at 0.05, 1.00, and 1.50 mg/L. Calibration standards and triplicate controls were carried through with each batch of analysis. Samples were prepared by adding 50 μl of test specimen, calibrators, or controls to 2 ml of 100 mM phosphate buffer, pH 6.0, and spiking with the internal standard (10 μl of 20% acetonitrile in 0.1% aqueous formic acid containing 100 pg trideuterated-MPH). The solutions were then placed in an ultrasonic bath for 10 min before extracting the drugs by adding to a preconditioned Strata Screen-C GF 200 mg/6 ml solid phase extraction column. Ritalinic acid was eluted first with hexane/ethyl acetate (50:50) followed by MPH using dichloromethane/isopropanol/ammonia (78:20:2). The eluates were evaporated to dryness at 30°C and reconstituted in 100 μl of methanol/water before injection into an HPLC-MS/MS spectrometer for analysis. HPLC separation was achieved using a FORTIS 3 μm C18 150 × 3 mm column with detection using an API 3200 MS/MS system with a TurboIon spray interface. The HPLC consisted of a Shimadzu system with mobile phase A consisting of water containing 0.01% ammonia and mobile phase B consisting of methanol containing 0.01% ammonia with 10 ml of isopropanol added per liter. The gradient run was 20–100% mobile phase B over 10 min. Positive multiple reaction monitoring was used to monitor the chromatography column eluent [MPH (MRM 234–84), ritalinic acid (MRM 220–84) and d$^3$-MPH (MRM 237–84)]. The lower limit of detection was 0.008 mg/L (signal-to-noise ratio, 10) and the lower limit of quantification was 0.01 mg/L. The overall precision (between batch quality control specimens run on 4 separate days) was 2–11% within the calibration range used.

*Positron emission tomography.* HI and LI rats were scanned using [$^{18}$F]fallypride PET on the following two occasions: before the first oral MPH dose and 2 d after the last MPH dose, which occurred within 48 h of

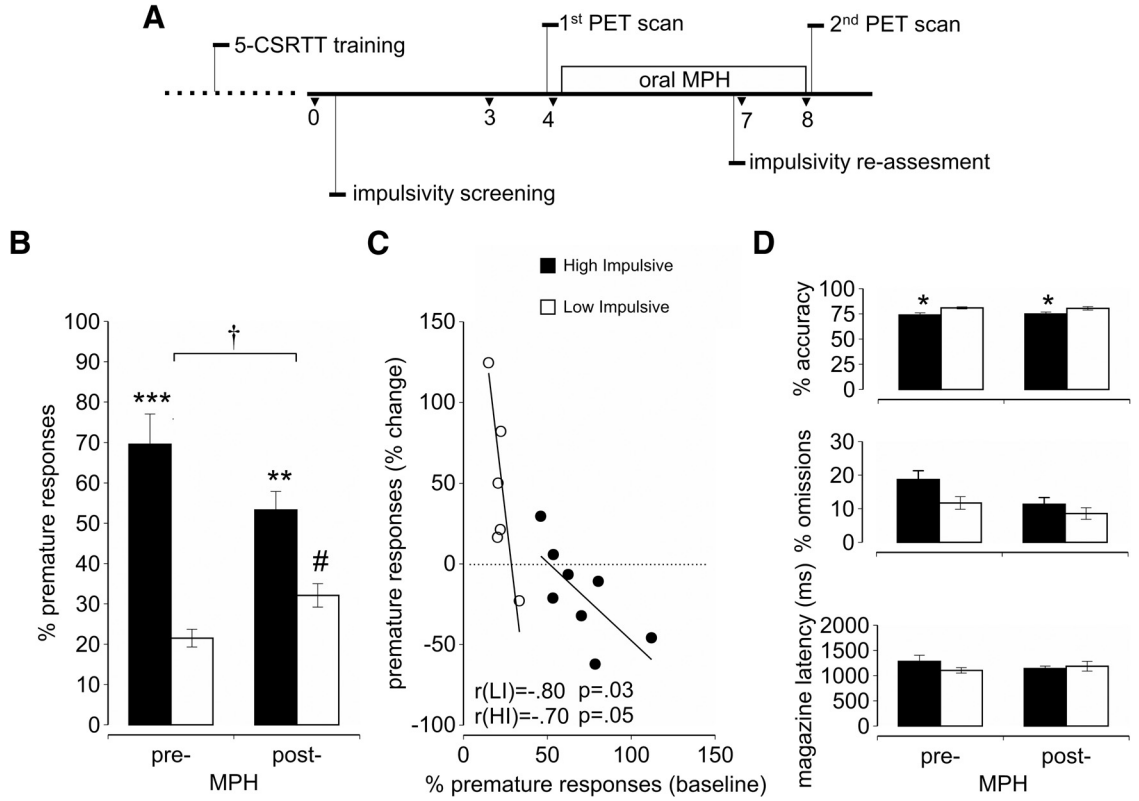


**Figure 1.** Effects of methylphenidate on sustained attention and impulsivity in selected LI versus HI rats. ***A***, Timeline of the experimental procedure in rats expressing differential levels of impulsive behavior on the 5-CSRTT. The dashed line refers to 5-CSRTT training, which took ~3 months. Values shown are weeks. ***B***, Effects of prior oral MPH administration on impulsivity in LI (white bars) and HI (black bars) rats on the 5-CSRTT. Pre-cocaine administration values are averaged across 3 weekly spaced L-ITI sessions. It can be seen in ***B*** that impulsivity was altered both in HI and LI rats (group × MPH interaction: $F_{(1,13)} = 7.59$, † $p < 0.05$) during the challenge sessions. The increase in impulsivity post-MPH was significant in LI rats (#$p = 0.044$). ***C***, Correlation plots showing the relationship between relative changes in impulsivity from baseline produced by MPH. It can be seen in ***C*** that the effect of MPH on increasing impulsivity was greatest in LI rats showing the lowest baseline level of impulsivity ($r = -0.80$; $p = 0.03$), whereas MPH decreased impulsivity to a greater extent in those HI rats showing the highest baseline level of impulsivity ($r = -0.7$; $p = 0.05$). ***D***, Differences in accuracy, omissions, and magazine latency before and after the oral MPH dosing between HI and LI rats (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ HI vs LI).

the last L-ITI session on the 5-CSRTT (Fig. 1). The scanning procedure has been described in detail previously (Caprioli et al., 2013). In brief, before the injection of tracer, single-mode transmission data were acquired using a rotating $^{68}$Ge/$^{68}$Ga point source (~20 MBq) to provide measured attenuation correction. For all scans, [$^{18}$F]fallypride was injected intravenously over 30 s, followed by a 15 s heparin-saline flush. The injected [$^{18}$F]fallypride activity (5.3–66.9 MBq) was adjusted so that the total mass of labeled and unlabeled fallypride injected was 0.5 nmol/kg. Dynamic data were acquired in list mode for 180 min and subsequently binned into sinograms for the following time frames: 6 × 10 s, 3 × 20 s, 6 × 30 s, 10 × 60 s, 10 × 120 s, and 29 × 300 s. Corrections were applied for randoms, dead time, normalization, attenuation, and decay. Fourier rebinning (Defrise et al., 1997) was used to compress the four-dimensional sinograms to three dimensions before reconstruction with two-dimensional filtered back projection with a Hann window cutoff at the Nyquist frequency. The image voxel size was 0.95 × 0.95 × 0.80 mm, with an array size of 128 × 128 × 95. The reconstructed images were converted to kilobecquerels per milliliter using global and slice factors determined from imaging a uniform phantom filled with a [$^{18}$F]fluoride solution.

Thirty-two T2-weighted MR brain scans from previous [$^{18}$F]fallypride PET studies in Lister-hooded rats were used to create a high-resolution MR brain template with SyN (Avants et al., 2008), part of the Advanced Normalization Tools (ANTS) package. A PET template was then created by applying the spatial normalization parameters from the above template creation process to late [$^{18}$F]fallypride images (average image 120–180 min after injection) that had been manually coregistered to their corresponding MR scan. Late [$^{18}$F]fallypride images from the 30 PET scans in this study were affine registered to the PET template using ANTS, and each transformation was used to reslice the corresponding dynamic [$^{18}$F]fallypride PET image set to template space. Finally, the MR template used in a previous study (Caprioli et al., 2013; Fig. 2*A*) was spatially normalized to the new MR template described above, with the resulting transformation applied to the previously defined regions of interest to align them to the new template space.

$D_{2/3}$ receptor availability was quantified using nondisplaceable binding potential ($BP_{ND}$) (Innis et al., 2007), determined from reference tissue-based kinetic analysis, with the cerebellum acting as the reference region. The borders of the reference region drawn on the MR template excluded the outermost lamina of the cerebellar cortex to avoid partial volume error from uptake in the Purkinje cell layer. Regional and voxel-wise $BP_{ND}$ values were estimated from the distribution volume ratio (DVR; $BP_{ND} = DVR - 1$) determined using the reference tissue input Logan plot (Logan et al., 1996) with data fitted from 90 to 180 min postinjection.

*Statistical analysis.* Behavioral data were subjected to ANOVA (SPSS version 17.0) using a general linear model. Homogeneity of variance was verified using Levene's test. For repeated-measures analyses, Mauchly's test of sphericity was applied, and the degrees of freedom were corrected to more conservative values using the Huynh-Feldt $\varepsilon$ for any terms involving factors in which the sphericity assumption was violated. Differences in $BP_{ND}$ between HI and LI rats were evaluated using repeated-measures ANOVA. Significantly meaningful interactions ($p < 0.1$) were further analyzed by simple main effects using the pooled sum of square error term (Cochran and Cox, 1957). A significance level of $\alpha = 0.05$ was used to interpret the main effects and *post hoc* tests. Pearson product moment correlations were used to assess the strength of the association between the following: (1) the change in $BP_{ND}$ ((post-MPH − pre-MPH)/(pre-MPH) × 100) and baseline $BP_{ND}$ (pre-MPH scan); and (2) the change in premature responses ((post-MPH − pre-MPH)/(pre-

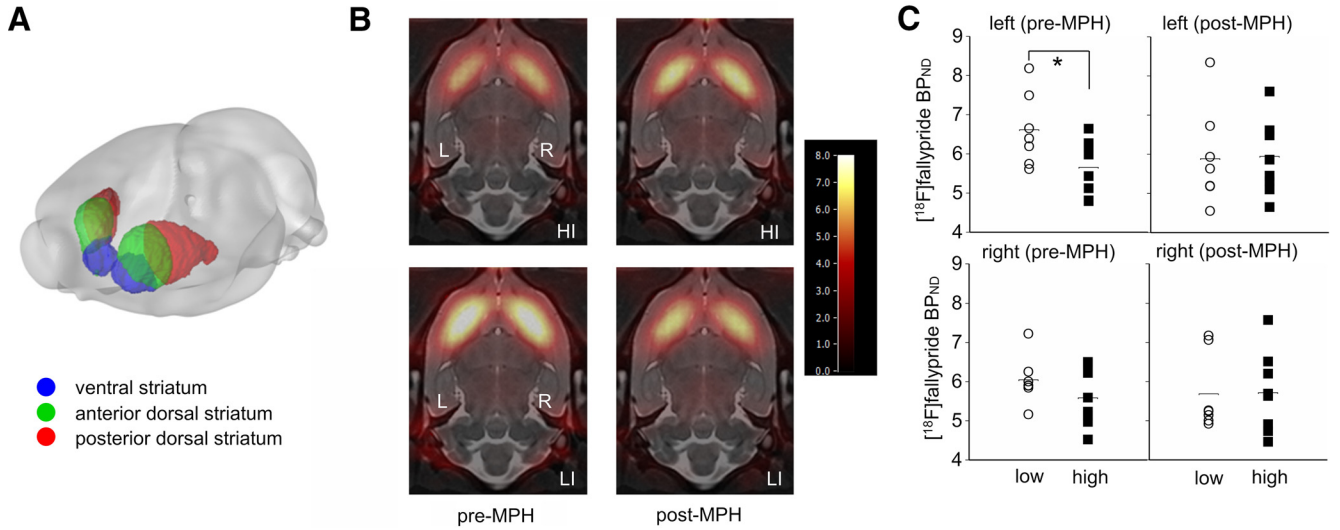


**Figure 2.** MPH-induced effects on $D_{2/3}$ receptor availability in the left ventral striatum of HI and LI rats. ***A***, Three-dimensional depiction of regions of interest showing the ventral striatum (blue), anterior dorsal striatum (green), and posterior dorsal striatum (red). ***B***, Horizontal section through [$^{18}$F]fallypride $BP_{ND}$ maps for HI and LI rats overlaid on the coregistered MR template (L, left; R, right). The images are 7 mm below the dorsal brain surface and have a $BP_{ND}$ threshold = 8. ***C***, [$^{18}$F]fallypride $BP_{ND}$ in the left and right ventral striatum of LI (circle symbols, $n = 7$) and HI (square symbols, $n = 8$) rats before (pre-MPH) and after (post-MPH) oral administration. It can be seen that [$^{18}$F]fallypride $BP_{ND}$ is significantly reduced in the left ventral striatum of HI rats compared with LI rats before MPH exposure (*$p < 0.05$) and that MPH reduces the contrast in $D_{2/3}$ receptor availability between LI and HI rats in the left ventral striatum.

**Table 1. Summary of the effects of oral MPH administration on the behavioral performance of LI and HI rats on the 5-CSRTT**

| | Pre-MPH | | Post-MPH | |
|---|---|---|---|---|
| | LI ($n = 7$) | HI ($n = 8$) | LI ($n = 7$) | HI ($n = 8$) |
| S-ITI sessions | | | | |
| Premature (%) | 2.9 ± 0.5 | 8.6 ± 1.2* | 2.7 ± 0.6 | 4.4 ± 0.7 |
| Accuracy (%) | 83.0 ± 1.7 | 80.4 ± 1.7 | 83.4 ± 1.0 | 79.8 ± 2.0 |
| Omissions (%) | 12.5 ± 2.2 | 9.0 ± 1.4 | 7.5 ± 1.1 | 6.7 ± 1.2 |
| Magazine latency (ms) | 1169.4 ± 91.9 | 1156.4 ± 58.4 | 1213.8 ± 85.5 | 1159.5 ± 51.2 |
| Correct latency (ms) | 751.6 ± 38.0 | 626.0 ± 41.8 | 842.4 ± 50.9 | 688.1 ± 40.0 |
| L-ITI sessions | | | | |
| Premature (%) | 21.5 ± 2.2 | 69.6 ± 7.5** | 32.1 ± 2.9 | 53.3 ± 4.6* |
| Accuracy (%) | 80.9 ± 1.0 | 73.9 ± 2.1*** | 80.5 ± 1.7 | 74.9 ± 1.9*** |
| Omissions (%) | 12.7 ± 1.9 | 18.7 ± 2.6 | 8.6 ± 1.7 | 11.3 ± 2.0 |
| Magazine latency (ms) | 1103.9 ± 54.0 | 1281.9 ± 122.7 | 1186.6 ± 96.7 | 1140.1 ± 49.0 |
| Correct latency (ms) | 671.7 ± 38.6 | 601.6 ± 32.5 | 721.8 ± 63.2 | 603.0 ± 26.0 |

Data are reported as the mean ± SEM. *$p < 0.01$; **$p < 0.001$; ***$p < 0.05$ (LI vs HI).

MPH) × 100) and baseline $BP_{ND}$ (pre-MPH scan). All figures show group means ± SEM.

## Results

### Segregation of high- and low-impulsivity groups

The behavioral performance of LI and HI rats on the 5-CSRTT is summarized in Table 1. The percentages of premature responses for HI ($n = 8$) and LI ($n = 7$) rats, averaged across the three L-ITI sessions before the commencement of MPH dosing, were (mean ± SEM) 69.6 ± 7.5% and 21.5 ± 2.2%, respectively. HI rats were more impulsive than LI rats regardless of the ITI being set to 5 s (baseline 1 to baseline 10; S-ITI; $p = 0.002$) or 7 s (L-ITI; $p < 0.001$). Among the various behavioral variables recorded only attentional accuracy was significantly impaired in HI rats compared with LI rats during the L-ITI sessions ($p = 0.014$). Although omissions appeared to be increased in HI rats compared with LI rats, this contrast was not significant ($p = 0.054$).

### Interactive effects of methylphenidate on impulsivity in LI and HI rats

Twenty-one days after the commencement of daily MPH dosing, rats were reassessed for impulsivity and attentional performance on the 5-CSRTT. It can be seen in Figure 1*B* that MPH produced divergent effects on impulsivity in the two impulsivity subgroups, with impulsivity appearing to decrease in HI rats but increase in LI rats (group × MPH interaction: $F_{(1,13)} = 7.59$, $p = 0.016$). Although *post hoc t* tests failed to reveal a significant decrease in impulsivity, at a group level, following MPH treatment in HI rats ($p = 0.095$), the increase in impulsivity observed in LI rats was significant ($p = 0.044$). Thus, following exposure to MPH, the initial contrast in impulsivity between LI and HI rats was greatly diminished. Importantly, it can be seen in Figure 1*C* that MPH increased impulsivity in LI rats in an inverse relationship to the baseline level of impulsivity, while impulsivity decreased in HI rats, with the magnitude of the decrease being positively correlated to the baseline level of impulsivity. Thus, the effect of MPH on increasing impulsivity was greatest in LI rats showing the lowest baseline level of impulsivity ($r = -0.80$; $p = 0.03$), whereas MPH decreased impulsivity to a greater extent in those HI rats showing the highest baseline level of impulsivity ($r = -0.7$; $p = 0.05$). However, MPH did not restore the attentional inaccuracy of HI rats, which remained significantly impaired relative to LI rats (main effect of group: $F_{(1,13)} = 3.62$, $p = 0.049$; group × MPH interaction: $F_{(1,13)} = 0.37$, $p = 0.55$). Moreover, there were no significant effects of MPH on omissions or magazine latencies on the 5-CSRTT (Fig. 1*D*; Table 1).

### Modulation of $D_{2/3}$ receptor availability in the ventral striatum by MPH

Consistent with our recent study (Caprioli et al., 2013), the availability of $D_{2/3}$ receptors was significantly reduced in the left ($t_{(13)} = 2.25$, $p = 0.043$), but not the right ($t_{(13)} = 1.29$, $p = 0.219$), ventral striatum of drug-naive HI rats compared with LI rats (pre-/post-

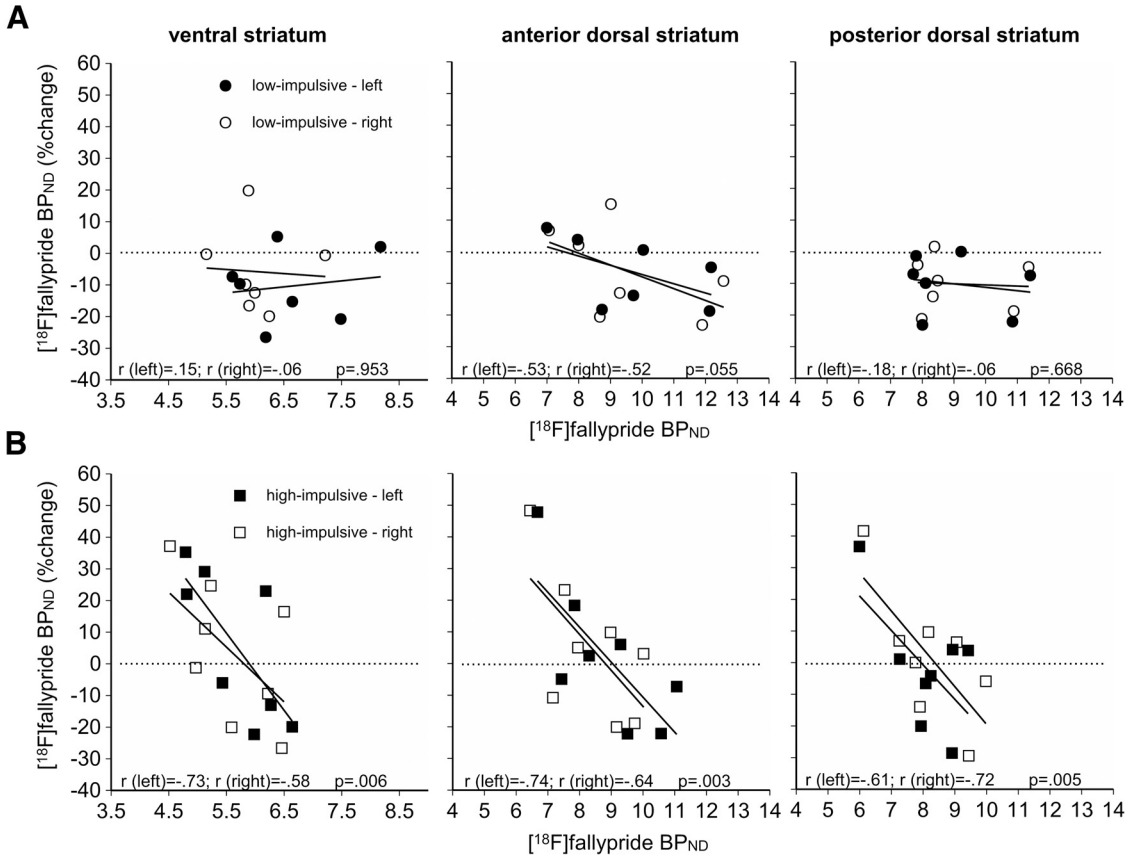


**Figure 3.** ***A***, ***B***, Relationship between the percentage change in [$^{18}$F]fallypride $BP_{ND}$ in the ventral and dorsal striatum before and after the exposure of LI (***A***) and HI (***B***) rats to MPH as a function of baseline (i.e., pre-MPH) [$^{18}$F]fallypride $BP_{ND}$. The results show that the effects of MPH on $D_{2/3}$ receptor availability depend inversely on baseline [$^{18}$F]fallypride $BP_{ND}$ values in the anterior and posterior regions of the dorsal striatum, as well as in the ventral striatum of HI but not LI rats. The horizontal dotted line depicts no net effect of MPH on [$^{18}$F]fallypride $BP_{ND}$ values. Pearson product moment correlation coefficients and *p* values are given in each panel.

MPH × hemisphere × group interaction: $F_{(1,13)} = 5.75, p = 0.05$; group × hemisphere interaction: $F_{(1,13)} = 4.047, p = 0.066$; Fig. 2*C*). Following 28 d of exposure to MPH, the difference in $D_{2/3}$ receptor availability between LI and HI rats in the left ventral striatum was no longer evident ($t_{(13)} = 0.09$, $p = 0.930$). The normalizing effect of MPH on $D_{2/3}$ receptor availability in the left ventral striatum appeared to be explained by a near-significant reduction in $D_{2/3}$ $BP_{ND}$ in the LI rats ($t_{(6)} = 2.292$, $p = 0.062$) rather than an increase in $D_{2/3}$ $BP_{ND}$ in this region of HI rats ($t_{(7)} = -0.495$, $p = 0.636$). Exposure of LI and HI rats to MPH had no significant effect on $D_{2/3}$ receptor availability in the right ventral striatum.

### Baseline-dependent effects of MPH on striatal $D_{2/3}$ receptors in HI rats

We found no significant group differences in $D_{2/3}$ receptor availability between LI and HI rats in the dorsal striatum, either at baseline (pre-MPH) or following MPH treatment (data not shown). However, when we compared the change in [$^{18}$F]fallypride $BP_{ND}$ before and after drug administration, we found that MPH both increased and decreased [$^{18}$F]fallypride $BP_{ND}$ in the ventral and dorsal striatum in HI rats, depending on the baseline availability of $D_{2/3}$ receptors (Fig. 3). In the ventral striatum, we observed a strong inverse relationship between the percentage change in [$^{18}$F]fallypride $BP_{ND}$ and baseline [$^{18}$F]fallypride $BP_{ND}$ in both the left hemisphere ($r_{left} = -0.73$, $p < 0.01$) and right hemisphere ($r_{right} = -0.58$, $p < 0.01$). Baseline-dependent effects of MPH on $D_{2/3}$ receptor availability were also observed in the anterior and posterior dorsal striata of HI rats. For all regions of interest, the relationship was strongly inversely related to baseline $D_{2/3}$ receptor availability (anterior dorsal striatum HI rats: $r_{left} = -0.74$, $<0.01$; $r_{right} = -0.64$, $p < 0.01$; posterior dorsal striatum HI rats: $r_{left} = -0.61$, $p < 0.01$, $r_{right} = -0.72$, $p < 0.01$). In contrast, we did not observe baseline-dependent effects on $D_{2/3}$ receptor availability in any of the striatal areas investigated in LI rats.

We next compared the relative changes in $D_{2/3}$ receptor availability and impulsivity produced by MPH treatment (Fig. 4). We found no significant relationship between these parameters in any of the striatal subregions examined for either LI or HI rats.

## Discussion

This study investigated striatal $D_{2/3}$ receptor availability in highly impulsive rats and the mechanisms underlying the therapeutic effects of long-term oral MPH treatment. We found that repeated oral MPH administration was sufficient to produce bidirectional effects on impulsivity that depended on the baseline level of impulsivity. Thus, in LI rats, MPH increased impulsivity, whereas in HI rats it reduced impulsivity in animals exhibiting the highest baseline level of impulsivity, consistent with an underlying rate-dependent mechanism. Our results indicate that the rate dependency model held for LI and HI rats but with clear differences in the underlying regulatory parameters, which is suggestive of a nonunitary process. Since the baseline-dependent effects of MPH on impulsivity and $D_{2/3}$ receptors were dissociable, we conclude that $D_{2/3}$ receptors may not play a major contribution to the

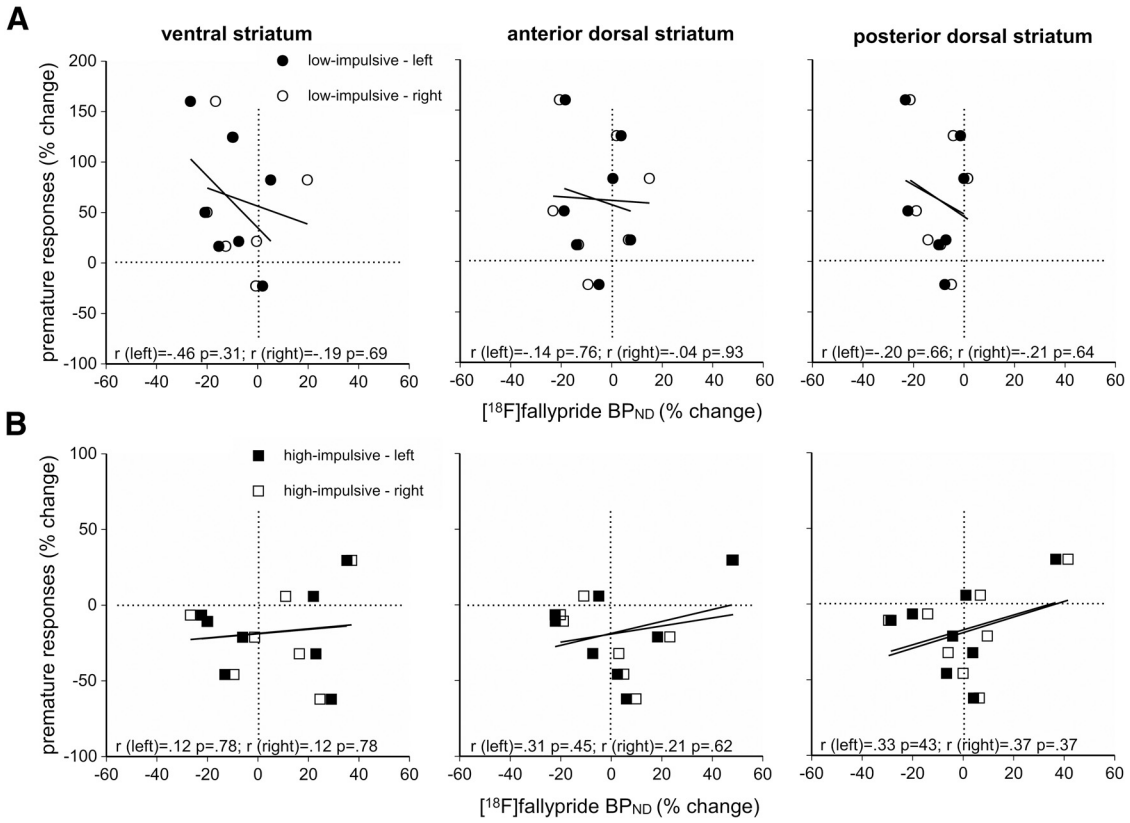


**Figure 4.** ***A***, ***B***, Correlation plots showing the relationship between relative changes in impulsivity and [$^{18}$F]fallypride $BP_{ND}$ in the ventral and dorsal striatum of LI (***A***) and HI (***B***) rats produced by MPH. The results indicate that the effects of MPH on impulsivity and $D_{2/3}$ receptor availability are independent. The vertical dotted line depicts no net effect of MPH on [$^{18}$F]fallypride $BP_{ND}$. Pearson product moment correlation coefficients and *p* values are given in each panel.

**Table 2. Summary of the serum concentration of oral MPH and ritalinic acid obtained from four nonimpulsive rats after a single oral dose of MPH (6 mg/kg)**

| | Mean (ng/ml) | | SD (ng/ml) | | Minimum (ng/ml) | | Maximum (ng/ml) | |
|---|---|---|---|---|---|---|---|---|
| Collection time | MPH | RA | MPH | RA | MPH | RA | MPH | RA |
| 5 min | 112.5 | 107.5 | 20.6 | 42.7 | 90.0 | 60.0 | 130.0 | 160.0 |
| 15 min | 102.5 | 142.5 | 22.2 | 20.6 | 90.0 | 120.0 | 130.0 | 170.0 |
| 30 min | 87.5 | 145.0 | 22.2 | 20.8 | 70.0 | 120.0 | 120.0 | 170.0 |
| 60 min | 9.5 | 67.5 | 7.50 | 15.0 | 4.0 | 50.0 | 20.0 | 80.0 |
| 90 min | 2.2 | 35.0 | 0.91 | 5.78 | 1.0 | 30.0 | 3.0 | 40.0 |
| 120 min | 1.0 | 13.0 | 0.11 | 8.71 | 1.0 | 2.0 | 1.0 | 20.0 |

RA, Ritalinic acid.

effects of MPH on impulsivity. These data are consistent with findings showing that MPH modulates performance in humans in a baseline-dependent manner both in healthy controls and in subjects in whom ADHD has been diagnosed (del Campo et al., 2013).

We found a strong inverse relationship between baseline $D_{2/3}$ $BP_{ND}$ and the change in this parameter after MPH treatment in HI rats. However, no such relationship was found for LI rats in any of the striatal subregions examined. These findings correspond with our earlier findings in rats self-administering cocaine, which had the similar effect of modulating $D_{2/3}$ $BP_{ND}$ in a manner dependent on baseline $D_{2/3}$ $BP_{ND}$ values (Caprioli et al., 2013). However, in our previous study, cocaine also modulated $D_{2/3}$ receptors in the dorsal striatum. The more pervasive effects of cocaine on $D_{2/3}$ receptors throughout the ventral and dorsal striata may be due to differences in the route of administration (intravenous vs oral), response-contingent cocaine vs response noncontingent MPH, differing quantities of cocaine and MPH, and higher relative efficacy of cocaine over MPH. This may explain why cocaine produced a more substantial reduction in impulsivity in the HI subgroup compared with MPH in the present study (Caprioli et al., 2013).

The mechanism underlying the observed rate-dependent modulation of impulsivity following MPH treatment is unknown but, as discussed above, may be distinct for LI and HI rats. Although for LI rats there was no obvious relationship between baseline $D_{2/3}$ $BP_{ND}$ and the change in this parameter following MPH treatment, at a group level, MPH had the trend effect of reducing $D_{2/3}$ $BP_{ND}$ values in the left ventral striatum, a deficit associated with increased impulsivity on this task (Dalley et al., 2007) and localized to the nucleus accumbens shell (Besson et al., 2010; Jupp et al., 2013). The reduction in $D_{2/3}$ receptor availability in LI rats may reflect a downregulation of $D_{2/3}$ receptors, but since [$^{18}$F]fallypride competes with DA in binding to $D_{2/3}$ receptors, it could also reflect an increase in synaptic DA release, possibly due to sensitization of the mesolimbic DA systems following repeated MPH treatment (Shuster et al., 1982; Gaytan et al., 1997). However, sensitization of the locomotion response does not appear to develop after long-term oral MPH treatment (McNamara et al., 1993; Kuczenski and Segal, 2002). Furthermore, no simple relationship exists between hyperactivity and impulsivity on the 5-CSRTT (Dalley et al., 2007; Molander et al., 2011; Moreno et al., 2013).

In HI rats, MPH had the dual effect of decreasing impulsivity and modulating striatal $D_{2/3}$ receptor availability according to the principal of rate dependency (Dews and Wenger, 1977). However, neither parameter significantly covaried after MPH

treatment, suggesting that the modifying effects of MPH on impulsivity are separable from effects on $D_{2/3}$ receptor regulation. That the measure of impulsivity is not directly related to changes in $D_{2/3}$ receptor availability is possibly due to other actions of MPH, especially for example on NE. Thus, atomoxetine, which reduces impulsivity in HI rats, blocks reuptake of NE and has no effect on subcortical DA (Bymaster et al., 2002). Moreover, this drug exerts at least some of its anti-impulsive effects within the shell region of the nucleus accumbens (Economidou et al., 2012). Alternatively, the reduction in impulsivity in MPH-treated HI rats may include actions at the level of the nucleus accumbens core. Thus, previously, we have shown that HI rats exhibit a reduced density of markers associated with dendritic spines in this region and GABA synthesis (Caprioli et al., 2014), abnormalities that were mainly restricted to the left hemisphere, similar to the locus of deficient $D_{2/3}$ receptor availability in HI rats (Caprioli et al., 2013). Since in the present study MPH had the greatest beneficial effects in the most impulsive animals, these effects may be mediated by a restoration of the structural and functional integrity of GABAergic medium spiny neurons in the nucleus accumbens core, as previously hypothesized (Caprioli et al., 2014). The origin of the hemispheric imbalance in $D_{2/3}$ receptors in HI rats is unknown but may arise from genetic and/or environmental factors affecting trophic signaling during development (Concha et al., 2012). Left/right asymmetries in the midbrain DA systems have been reported in rats (Carlson and Glick, 1989; Afonso et al., 1993; Rodriguez et al., 1994) and healthy humans (Tomer et al., 2008), as well as in individuals with ADHD (Volkow et al., 2007a, 2009; del Campo et al., 2013).

An analogous PET study in rats found that treating rats with oral MPH for 8 months, initiated during the periadolescent period, increased $D_{2/3}$ availability in the striatum (Thanos et al., 2007). By contrast, striatal $D_{2/3}$ availability decreased 2 months after starting MPH treatment. These findings demonstrate that the MPH-induced changes in striatal $D_{2/3}$ receptors depend on treatment length and developmental stage (Rodriguez et al., 2010; Gill et al., 2012). The mechanisms underlying these changes in $D_{2/3}$ receptors are unknown but may involve alterations in synaptic DA and/or the pool of receptors available for binding in the striatum. However, research in nonhuman primates demonstrates that long-term treatment with extended-release MPH for 1 year has no effect on the DA transporter or $D_{2/3}$ receptors in the striatum (Soto et al., 2012; Gill et al., 2012). This discrepancy with rodent studies may be species specific or a consequence of differing doses of MPH and/or length of treatment. In the context of the present study, it may also reflect the fact that animals in the study by Gill et al., 2012 were not preselected for impulsivity-related traits. This may be relevant, as it has been shown that treating adults with ADHD for 1 year decreases striatal $D_{2/3}$ receptor availability, as assessed using PET (Volkow et al., 2012).

There are several limitations of the present study that merit discussion. First, although we dosed MPH orally and assessed the levels of serum MPH and its metabolite ritalinic acid, as endorsed by others (Volkow and Insel, 2003; Gill et al., 2012), peak MPH levels were in excess of the typical therapeutic range of MPH of 8–10 ng/ml (Swanson and Volkow, 2002; Table 2). However, consistent with other research (Patrick et al., 1984; Robb et al., 2014), MPH was rapidly cleared with an elimination half-life of 30–50 min. Thus, although serum levels of MPH were initially high, these soon declined to clinically relevant values after the administration of MPH and well before the next dose. Nevertheless, with twice-daily dosing and consequent fluctuations in serum MPH levels, our results are difficult to extrapolate to studies in humans that use extended-release oral formulations (Robb et al., 2014). A second consideration is that the primary objective of our research was to investigate the long-term effects of MPH on impulse control and $D_{2/3}$ receptors in the striatum. The design of our study thus excluded the analysis of short-term treatment with low doses of MPH, which increase NE and DA availability selectively in the prefrontal cortex (Berridge et al., 2006) and facilitate cognitive functions relevant to ADHD (Andrzejewski et al., 2014). Third, our conclusions are based on relatively small group sizes ($n = 7–8$). Nevertheless, we have now reported in three independent studies reduced striatal $D_{2/3}$ receptor availability in the ventral striatum of HI rats. In addition, we observed qualitatively similar changes from baseline following the administration of MPH and cocaine (Caprioli et al., 2013), using a within-subjects longitudinal design.

In conclusion, our results confirm that deficits in impulsive control are associated with reduced $D_{2/3}$ receptor availability in the left ventral striatum, as previously reported (Dalley et al., 2007; Caprioli et al., 2013), and in three independent studies in ADHD patients (Volkow et al., 2007a, 2009; del Campo et al., 2013). Although further research is needed to test the full dose–response curve of MPH on impulsivity and $D_{2/3}$ receptor availability, we have now shown how different psychomotor stimulant drugs produce baseline-dependent effects on $D_{2/3}$ receptor availability in the ventral striatum. In addition, we have demonstrated that the therapeutic effects of MPH on impulsivity are unlikely to arise as a direct consequence of changes in the regulation of $D_{2/3}$ receptors in the ventral striatum, a conclusion supported by research in adults with ADHD (Volkow et al., 2012). Nevertheless, by restoring levels of $D_{2/3}$ receptors in the ventral striatum of HI rats, MPH may diminish the risk of addiction in addiction-prone highly impulsive rats (Belin et al., 2008; Economidou et al., 2009).

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