Dissociable rate dependent effects of oral methylphenidate on impulsivity and $D_{2/3}$ receptor availability in the striatum

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Conflict of Interest

The authors report no conflicts of interest.

Abstract

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 in 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 5-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 days. Rats were then re-assessed for impulsivity and underwent a second [^{18}F]fallypride PET scan. Prior to MPH treatment we found that $D_{2/3}$ receptor availability was significantly decreased in the left but not right ventral striatum of high-impulsive (HI) rats compared with low-impulsive (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 sub-regions 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.

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 extra-synaptic dopamine (DA) and norepinephrine (NE) levels by blocking their reuptake (Zetterstrom 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 are hypothesized to follow an inverted U-shaped function, which depend on optimising catecholamine levels in the brain (Clatworthy et al., 2009; van der Schaaf et al., 2013).**

Recently, we reported a similar baseline dependent effect of cocaine in a preclinical animal model of impulsivity (Caprioli et al., 2013). 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 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 observed by Volkow and colleagues. Indeed, a set of well-powered case-control positron emission tomography (PET) studies in adult medication-naïve ADHD patients, found ADHD to be associated with reduced $D_{2/3}$ receptor availability in the nucleus accumbens and caudate (Volkow et al., 2007b; Volkow et al., 2007a; Volkow et al., 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 signalling 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 rats on the 5-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 prior to, and following chronic exposure of rats to MPH. In parallel, we investigated the relationship between behavioral impulsivity in selected low (LI)- *vs* high (HI)-impulsive rats and MPH-evoked changes in $D_{2/3}$ receptor availability in the ventral and dorsal striatum.

Material and Methods

Subjects

Ninety-six adult male Lister-hooded rats (Charles River, Margate, UK), 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; n=7 LI), **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 07:00 h). 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 UK 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 elsewhere (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 approximately 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 approximately 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 fewer than 20% of trials. Trials were initiated by subjects entering the magazine. After a fixed inter-trial 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 signalled 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 prior to the onset of the light stimulus (a 'premature' response). Behavioral performance was assessed by *choice accuracy* (% correct responses/ (correct + incorrect trials); *premature responding:* (% premature responses/ (correct + incorrect + omission trials); *omissions* (% omission trials/ (correct + incorrect + omission trials); *latency to collect food* (time from nose-poke response to entering the magazine, ms); *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 five consecutive days of testing with days 1, 2, 4 and 5 comprising sessions each of 100 discrete trials and an ITI of 5 s (short ITI). During day 3, the ITI was increased to 7s to increase the frequency of premature responses (long ITI). HI animals were defined as those exhibiting a level of premature responding greater than 50 on all three L-ITI sessions. LI rats were selected from the remaining rats and responded prematurely on fewer than 30% of trials during the L-ITI sessions.**

Chronic methylphenidate treatment

Methylphenidate hydrochloride (Sigma, Cambridge, UK) was dissolved in Ribena (GlaxoSmithKline, UK) and administered orally (6 mg/ml/kg) twice a day (10:00 and 17:00). 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 prior to 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 days a week for 4 consecutive weeks (see Figure 1). During this period, rats were assessed for performance on the 5-CSRTT at 08:00 when rats were in the drug-free state (i.e., 15 hours after the last MPH administration). During the last 7 days of MPH treatment, rats were challenged with three L-ITI sessions, each spaced 3 days apart.

Analysis of methylphenidate and ritalinic acid

Four non-impulsive 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 anaesthesia 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 minutes 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 prior to the determination of MPH and ritalinic acid using HPLC-MS/MS. Calibration standards (range 0.01-5 mg/L) were prepared with MPH and ritalinic 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 d^3-MPH). The solutions were then placed in an ultrasonic bath for 10 minutes before extracting the drugs by adding to a pre-conditioned Strata Screen-C GF 200mg/6 mL SPE 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^{o}C and reconstituted in 100 µL of methanol/water prior to injection into an HPLC-MS/MS for analysis. HPLC separation was achieved using a FORTIS 3µm C18 150 x 3 mm column with detection using API 3200 MS/MS 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 litre. The gradient run was 20%-100% mobile phase B over 10 minutes. Positive MRM was used to monitor the chromatography column eluent [MPH (MRM 234-84), ritalinic acid (MRM 220-84) and d3-MPH (MRM 237-84)]. The LLOD was 0.008 mg/L (S/N ratio 10) and the LLOQ 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 employed.

Positron emission tomography

HI and LI rats were scanned using [^{18}F]fallypride PET on two occasions; prior to the first oral MPH dose and 2 days after the last MPH dose, which occurred within 48 h of the last L-ITI session on the 5-CSRTT (see Figure 1). The scanning procedure has been described in detail elsewhere (Caprioli et al., 2013). In brief, prior to the injection of tracer, singles-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 labelled and

unlabelled 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, 29 × 300 s. Corrections were applied for randoms, dead time, normalization, attenuation and decay. Fourier re-binning (Defrise et al., 1997) was used to compress the 4D sinograms to 3D prior to reconstruction with 2D filtered back projection with a Hann window cut-off at the Nyquist frequency. The image voxel size was 0.95 x 0.95 x 0.80 mm, with an array size of 128 × 128 × 95. The reconstructed images were converted to kBq/ml 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 normalisation parameters from the above template creation process to late [^{18}F]fallypride images (average image 120-180 min after injection) that had been manually co-registered 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 re-slice 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)(see Figure 2A) was spatially normalised 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 non-displaceable 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 in order to avoid partial volume error from uptake in the Purkinje cell layer. Regional and voxel-wise BP_{ND} 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 post-injection.

Statistical analysis

Behavioral data were subjected to analysis of variance (SPSS, version 17.0, Chicago, USA) 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 corrected to

more conservative values using the Huynh-Feldt epsilon 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 main effects and post-hoc tests. **Pearson product moment correlations were used to assess the strength of the association between: (i) the change in BP_{ND} ((post-MPH−pre-MPH)/(pre-MPH) × 100) and baseline BP_{ND} (pre-MPH scan); (ii) the change in premature responses ((post-MPH−pre-MPH)/(pre-MPH) × 100) and baseline BP_{ND} (pre-MPH scan). All figures show group means ± 1SEM.**

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. Percentage premature responses for HI (n=8) and LI (n=7) rats, averaged across the three L-ITI sessions prior to the commencement of MPH dosing, were 69.6 ± 7.5% (mean ± SEM) and 21.5 ± 2.2%, respectively. HI rats were more impulsive than LI rats regardless of the ITI being set to 5 s ('b1' to 'b10', '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 re-assessed for impulsivity and attentional performance on the 5-CSRTT. It can be seen in Figure 1B that MPH produced divergent effects on impulsivity in the two impulsivity sub-groups 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 1C 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 to increase 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 x 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 (Figure 1D and 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-naïve HI rats compared with LI rats (pre-/post-MPH x hemisphere x group interaction: $F_{(1,13)}$=5.75, p=0.05; group × hemisphere interaction: $F_{(1,13)}$=4.047, p=0.066, Figure 2C). Following 28 days 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 group ($t_{(6)}$ = 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 high impulsive 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 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 (Figure 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 (r_{left} = -0.73, p <0.01) and right (r_{right}= -0.58, p <0.01) hemisphere. Baseline-dependent effects of MPH on $D_{2/3}$ receptor availability were also observed in the anterior and posterior dorsal striatum 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 (Figure 4). We found no significant relationship between these parameters in any of the striatal sub-regions examined for either LI rats 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 chronic oral MPH. We found that repeated oral MPH was sufficient to produce bi-directional 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 suggestive of a non-unitary 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 effects of MPH on impulsivity. T**hese data are consistent with findings showing that MPH modulates performance in humans in a baseline-dependent manner both in healthy controls and in subjects diagnosed with ADHD (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 sub-regions 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} (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 striatum may be due to differences in the route of administration (intravenous *vs* oral), response-contingent cocaine *vs* response non-contingent 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 sub-group compared with MPH in the present study (Caprioli et al., 2013).

The mechanism underling 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} 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 down-regulation 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 chronic 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 co-varied 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 re-uptake 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 GABA-ergic 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 signalling 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 ADHD (del Campo et al., 2013; Volkow et al., 2007a; Volkow et al., 2009).

An analogous PET study in rats found that treating rats with oral MPH for 8 months, initiated during the peri-adolescent 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 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 non-human primates, demonstrates that chronic treatment with extended-release MPH for 1 year has no effect on the DA transporter or $D_{2/3}$ receptors in the striatum (Gill et al., 2012; **Soto 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 Gill study were not pre-selected 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. Firstly, although we dosed MPH orally and assessed serum MPH levels 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 to 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 minutes. 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 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 acute, 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). **Thirdly, our conclusions are based on relatively small group sizes**

(n=7-8). Nevertheless, we have now reported in three independent studies reduced striatal $_{D2/3}$ receptor availability in the ventral striatum of HI rats. In addition, we observed qualitatively similar changes from baseline following 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; Volkow et al., 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).**

392 **Figure legends**

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

408 **Figure 2. MPH-induced effects on $D_{2/3}$ receptor availability in the left ventral striatum of HI and**
409 **LI rats.** (**A**) 3D depiction of regions of interest showing the ventral striatum (blue), anterior dorsal
410 striatum (green), and posterior dorsal striatum (red). (**B**) Horizontal section through [^{18}F]fallypride BP_{ND}
411 maps for HI and LI rats overlaid on the co-registered MR template (L = left; R = right). The images are
412 7 mm below the dorsal brain surface and have a BP_{ND} threshold = 8. (**C**) [^{18}F]fallypride BP_{ND} in the left
413 and right ventral striatum of LI (circle symbols, n=7) and HI (square symbols, n=8) rats before ('pre-
414 MPH') and after ('post-MPH') oral-administration. It can be seen that [^{18}F]fallypride BP_{ND} is significantly
415 reduced in the left ventral striatum of HI rats compared with LI rats prior to MPH exposure (* $p < 0.05$)
416 and that MPH reduces the contrast in $D_{2/3}$ receptor availability between LI and HI rats in the left ventral
417 striatum.

418 **Figure 3. Relationship between the percentage change in [^{18}F]fallypride BP_{ND} in the ventral and**
419 **dorsal striatum before and after the exposure of LI (panel A) and HI (panel B) rats to MPH as a**
420 **function of baseline (i.e. pre-MPH) [^{18}F]fallypride BP_{ND}.** The results show that the effects of MPH on

421 $D_{2/3}$ receptor availability depend inversely on baseline [^{18}F]fallypride BP_{ND} in the anterior and posterior
422 regions of the dorsal striatum, as well as in the ventral striatum of HI but not LI rats. The horizontal
423 dotted line depicts no net effect of MPH on [^{18}F]fallypride BP_{ND}. Pearson product moment correlation
424 coefficients and p-values are given in each panel.

425 **Figure 4. Correlation plots showing the relationship between relative changes in impulsivity**
426 **and [^{18}F]fallypride BP_{ND} in the ventral and dorsal striatum of LI (panel A) and HI (panel B) rats**
427 **produced by MPH.** The results indicate that the effects of MPH on impulsivity and $D_{2/3}$ receptor
428 availability are independent. The vertical dotted line depicts no net effect of MPH on [^{18}F]fallypride
429 BP_{ND}. Pearson product moment correlation coefficients and p-values are given in each panel.
430

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Tables

Table 1

Summary of the effects of oral MPH administration on the behavioral performance of LI and HI rats on the 5-CSRTT. Shown are (mean ± SEM): % premature responses, % accuracy of responding, % omissions, magazine latencies (ms), and correct response latencies (ms) before ('Pre') and after ('Post') drug exposure. * p<0.05; ** p<0.01; *** p<0.001 (LI *vs* HI).

	Pre-MPH		Post-MPH	
Short-ITI sessions	**LI (n=7)**	**HI (n=8)**	**LI (n=7)**	**HI (n=8)**
% 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 lat. (ms)	1169.4±91.9	1156.4±58.4	1213.8±85.5	1159.5±51.2
correct lat. (ms)	751.6±38.0	626.0±41.8	842.4±50.9	688.1±40.0
Long-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 lat. (ms)	1103.9±54.0	1281.9±122.7	1186.6±96.7	1140.1±49.0
correct lat. (ms)	671.7±38.6	601.6±32.5	721.8±63.2	603.0±26.0

Table 2

Summary of the serum concentration (ng/ml) of oral methylphenidate (MPH) and ritalinic acid (RA) obtained from 4 non-impulsive rats after a single oral dose of MPH (6 mg/kg).

Collection time	Mean		SD		Min		Max	
	MPH	**RA**	**MPH**	**RA**	**MPH**	**RA**	**MPH**	**RA**
5	112.5	107.5	20.6	42.7	90.0	60.0	130.0	160.0
15	102.5	142.5	22.2	20.6	90.0	120.0	130.0	170.0
30	87.5	145.0	22.2	20.8	70.0	120.0	120.0	170.0
60	9.5	67.5	7.50	15.0	4.0	50.0	20.0	80.0
90	2.2	35.0	0.91	5.78	1.0	30.0	3.0	40.0
120	1.0	13.0	0.11	8.71	1.0	2.0	1.0	20.0