

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

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42 **Conflict of Interest**

43 The authors report no conflicts of interest.
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47 **Abstract**

48 We have previously shown that impulsivity in rats is linked to decreased dopamine $D_{2/3}$ receptor
49 availability in the ventral striatum. In the present study we investigated, using longitudinal positron
50 emission tomography (PET), the effects of orally-administered methylphenidate (MPH), a first line
51 treatment in attention deficit hyperactivity disorder, on $D_{2/3}$ receptor availability in the dorsal and ventral
52 striatum and related these changes to impulsivity. Rats were screened for impulsive behavior on a 5-
53 choice serial reaction time task. After a baseline PET scan with the $D_{2/3}$ ligand [^{18}F]fallypride, rats
54 received 6 mg/kg MPH, orally, twice each day for 28 days. Rats were then re-assessed for impulsivity
55 and underwent a second [^{18}F]fallypride PET scan. Prior to MPH treatment we found that $D_{2/3}$ receptor
56 availability was significantly decreased in the left but not right ventral striatum of high-impulsive (HI) rats
57 compared with low-impulsive (LI) rats. MPH treatment increased impulsivity in LI rats, and modulated
58 impulsivity and $D_{2/3}$ receptor availability in the dorsal and ventral striatum of HI rats through inverse
59 relationships with baseline levels of impulsivity and $D_{2/3}$ receptor availability, respectively. However, we
60 found no relationship between the effects of MPH on impulsivity and $D_{2/3}$ receptor availability in any of
61 the striatal sub-regions investigated. These findings indicate that trait-like impulsivity is associated with
62 decreased $D_{2/3}$ receptor availability in the left ventral striatum, and that stimulant drugs modulate
63 impulsivity and striatal $D_{2/3}$ receptor availability through independent mechanisms.

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72 **Introduction**

73 Converging evidence from neuroimaging, clinical psychopharmacology and animal models implicates
74 dysregulated dopaminergic and norepinephrinergic neurotransmission in the pathophysiology of
75 attention deficit hyperactivity disorder (ADHD), the prototypical impulse-control disorder (Biederman,
76 2005; Arnsten, 2006; Dalley et al., 2011). Methylphenidate (MPH), which acts by increasing extra-
77 synaptic dopamine (DA) and norepinephrine (NE) levels by blocking their reuptake (Zetterstrom et al.,
78 1988), has been the first-line pharmaceutical therapy for ADHD (Wilens, 2008). Although its
79 pharmacological action has been well characterized, the precise neurobiological mechanisms
80 underlying the therapeutic effects of MPH remain unclear. **Recent findings suggest that only**
81 **specific neurocognitive processes in domains such as impulse control and attention are**
82 **affected by MPH, and that these interact with the drug in a baseline performance-dependent**
83 **manner (Dews and Wenger, 1977; Sahakian and Robbins, 1977; Robbins and Sahakian, 1979;**
84 **Turner et al., 2003; Clatworthy et al., 2009; DeVito et al., 2009). Such effects are hypothesized to**
85 **follow an inverted U-shaped function, which depend on optimising catecholamine levels in the**
86 **brain (Clatworthy et al., 2009; van der Schaaf et al., 2013).**

87 **Recently, we reported a similar baseline dependent effect of cocaine in a preclinical**
88 **animal model of impulsivity (Caprioli et al., 2013).** We reported that impaired response inhibition in
89 a rodent model of impulsivity is associated with a deficiency in DA $D_{2/3}$ receptor availability in the left
90 ventral striatum, and that prior response-contingent exposure to cocaine both restored $D_{2/3}$ receptor
91 availability in this region and improved impulse control. This evidence directly supports the baseline
92 dependency hypothesis at the neurobiological level in the striatum and this may be relevant to recent
93 findings observed by Volkow and colleagues. Indeed, a set of well-powered case-control positron
94 emission tomography (PET) studies in adult medication-naïve ADHD patients, found ADHD to be
95 associated with reduced $D_{2/3}$ receptor availability in the nucleus accumbens and caudate (Volkow et
96 al., 2007b; Volkow et al., 2007a; Volkow et al., 2009), and that treatment response was associated with
97 increased DA transmission in the ventral striatum (Volkow et al., 2012). Thus, the clinical efficacy of
98 stimulant drugs such as MPH in ADHD may depend, in part, on restoring $D_{2/3}$ receptor signalling in the
99 ventral striatum of impulsive individuals.

100 In the present study we therefore investigated the effects of repeated oral administration of MPH
101 on $D_{2/3}$ receptor availability in the ventral striatum of high-impulsive rats on the 5-choice serial reaction
102 time task (5-CSRTT). Impulsivity in this task is measured by the number of anticipatory responses to

103 an imminent visual signal and is analogous to false alarms on the analogous continuous performance
104 test in humans (Robbins, 2002). We used PET and the selective high-affinity $D_{2/3}$ receptor antagonist
105 [^{18}F]fallypride (Mukherjee et al., 1995) to investigate $D_{2/3}$ receptor availability in the ventral and dorsal
106 striatum, both prior to, and following chronic exposure of rats to MPH. In parallel, we investigated the
107 relationship between behavioral impulsivity in selected low (LI)- vs high (HI)-impulsive rats and MPH-
108 evoked changes in $D_{2/3}$ receptor availability in the ventral and dorsal striatum.

109 **Material and Methods**

110 *Subjects*

111 Ninety-six adult male Lister-hooded rats (Charles River, Margate, UK), weighing 250-275 g and 2-3
112 months of age at the beginning of behavioral training, were used. These were housed in groups of four
113 in enclosed ventilation chambers during the initial training and selection of HI and LI rats. Upon
114 completion of the screening and for the remaining period of the study rats were singly housed (n=8 HI;
115 n=7 LI), **similar to our previous study (Caprioli et al., 2013). Rats were singly housed because**
116 **MPH has been shown to disrupt social behavior in adolescent and young adult rats (Beatty et**
117 **al., 1982; Arakawa, 1994; Vanderschuren et al., 2008).** The holding room was humidity- and
118 temperature-controlled (22°C), and rats were maintained under a reversed 12-h light/dark cycle (white
119 lights off/red lights on at 07:00 h). Food was restricted to maintain body weights at 85-90% of free-
120 feeding weights. Water was available *ad libitum*. The present experiment conformed to the UK Animals
121 (Scientific Procedures) Act of 1986 and local ethical guidelines. A timeline of experimental procedures
122 is shown in Figure 1.

123 *Five-choice serial reaction time task*

124 The 5-CSRTT apparatus has been described in detail elsewhere (Bari et al., 2008). The training
125 procedure used in the present study was identical to that previously described (Caprioli et al., 2013). In
126 brief, rats were trained on the 5-CSRTT over approximately 60 daily sessions (6 sessions per week) to
127 detect the location of a brief visual stimulus (0.7 s) presented on a random basis in one of the five
128 recesses. Each session consisted of 100 discrete trials and lasted approximately 30 min. Training was
129 considered complete when rats' responded to the target stimuli of duration 0.7 s with an accuracy of
130 75% and omissions on fewer than 20% of trials. Trials were initiated by subjects entering the
131 magazine. After a fixed inter-trial interval (ITI) of 5 s, a visual stimulus was presented in a single
132 aperture. Rats were rewarded with a single pellet if they correctly located the position of the target

133 stimulus (a 'correct' response). A failure to respond within a limited hold period of 5 s was deemed an
134 'omission' and was signalled by a 5 s time-out period and a loss of food reward on that trial. Similar
135 feedback was given on trials where rats responded in an adjacent aperture (an 'incorrect' response) or
136 prior to the onset of the light stimulus (a 'premature' response). Behavioral performance was assessed
137 by *choice accuracy* (% correct responses/ (correct + incorrect trials); *premature responding*: (%
138 premature responses/ (correct + incorrect + omission trials); *omissions* (% omission trials/ (correct +
139 incorrect + omission trials); *latency to collect food* (time from nose-poke response to entering the
140 magazine, ms); *correct response latency* (time to make a response in the correct aperture after the
141 onset of the light stimulus). **Once rats had acquired the 5-CSRTT they were ranked for impulsivity**
142 **during a 3-week screening period. Each week consisted of five consecutive days of testing with**
143 **days 1, 2, 4 and 5 comprising sessions each of 100 discrete trials and an ITI of 5 s (short ITI).**
144 **During day 3, the ITI was increased to 7s to increase the frequency of premature responses**
145 **(long ITI). HI animals were defined as those exhibiting a level of premature responding greater**
146 **than 50 on all three L-ITI sessions. LI rats were selected from the remaining rats and responded**
147 **prematurely on fewer than 30% of trials during the L-ITI sessions.**

148
149 *Chronic methylphenidate treatment*
150 Methylphenidate hydrochloride (Sigma, Cambridge, UK) was dissolved in Ribena (GlaxoSmithKline,
151 UK) and administered orally (6 mg/ml/kg) twice a day (10:00 and 17:00). The oral route of
152 administration was used to model the normal manner in which this drug is administered clinically
153 (Kuczenski and Segal, 2002; Swanson and Volkow, 2009). Two days prior to the first oral dosing of
154 MPH, rats were trained to consume the Ribena solution from a 1 ml syringe. Chronic MPH exposure
155 was maintained for 7 days a week for 4 consecutive weeks (see Figure 1). During this period, rats
156 were assessed for performance on the 5-CSRTT at 08:00 when rats were in the drug-free state (i.e.,
157 15 hours after the last MPH administration). During the last 7 days of MPH treatment, rats were
158 challenged with three L-ITI sessions, each spaced 3 days apart.

159
160 *Analysis of methylphenidate and ritalinic acid*
161 Four non-impulsive rats were used to quantify plasma levels of MPH and its metabolite ritalinic acid.
162 Rats were orally administered Ribena spiked with 6 mg/kg MPH, as described above, and after 10 min
163 were anesthetized with 5% isoflurane. General anaesthesia was maintained *via* the delivery of 1.5%

164 isoflurane in medical air. Blood samples were taken from a tail vein (0.5 ml) at 5, 15, 30, 60, 90 and
165 120 minutes following MPH administration. Blood was allowed to clot at room temperature (24°C)
166 before being centrifuged at 2000 rpm for 10 min. Plasma was aspirated from the centrifuged sample
167 and stored at -80° C prior to the determination of MPH and ritalinic acid using HPLC-MS/MS.
168 Calibration standards (range 0.01-5 mg/L) were prepared with MPH and ritalinic spiked in blank rat
169 plasma. Quality control samples were similarly prepared at 0.05, 1.00 and 1.50 mg/L. Calibration
170 standards and triplicate controls were carried through with each batch of analysis. Samples were
171 prepared by adding 50 µL of test specimen, calibrators or controls to 2 mL of 100 mM phosphate buffer
172 (pH 6.0) and spiking with the internal standard (10 µL of 20% acetonitrile in 0.1% aqueous formic acid
173 containing 100 pg d³-MPH). The solutions were then placed in an ultrasonic bath for 10 minutes before
174 extracting the drugs by adding to a pre-conditioned Strata Screen-C GF 200mg/6 mL SPE column.
175 Ritalinic acid was eluted first with hexane/ethyl acetate (50:50) followed by MPH using
176 dichloromethane/isopropanol/ammonia (78:20:2). The eluates were evaporated to dryness at 30°C and
177 reconstituted in 100 µL of methanol/water prior to injection into an HPLC-MS/MS for analysis. HPLC
178 separation was achieved using a FORTIS 3µm C18 150 x 3 mm column with detection using API 3200
179 MS/MS with a Turbolon spray interface. The HPLC consisted of a Shimadzu system with mobile phase
180 A consisting of water containing 0.01% ammonia and mobile phase B consisting of methanol
181 containing 0.01% ammonia with 10 mL of isopropanol added per litre. The gradient run was 20%-100%
182 mobile phase B over 10 minutes. Positive MRM was used to monitor the chromatography column
183 eluent [MPH (MRM 234-84), ritalinic acid (MRM 220-84) and d3-MPH (MRM 237-84)]. The LLOD was
184 0.008 mg/L (S/N ratio 10) and the LLOQ was 0.01 mg/L. The overall precision (between batch quality
185 control specimens run on 4 separate days) was 2-11% within the calibration range employed.

186 *Positron emission tomography*

187 HI and LI rats were scanned using [¹⁸F]fallypride PET on two occasions; prior to the first oral MPH
188 dose and 2 days after the last MPH dose, which occurred within 48 h of the last L-ITI session on the 5-
189 CSRTT (see Figure 1). The scanning procedure has been described in detail elsewhere (Caprioli et al.,
190 2013). In brief, prior to the injection of tracer, singles-mode transmission data were acquired using a
191 rotating ⁶⁸Ge/⁶⁸Ga point source (~20 MBq) to provide measured attenuation correction. For all scans,
192 [¹⁸F]fallypride was injected intravenously over 30 s, followed by a 15 s heparin-saline flush. The
193 injected [¹⁸F]fallypride activity (5.3–66.9 MBq) was adjusted so that the total mass of labelled and

194 unlabelled fallypride injected was 0.5 nmol/kg. Dynamic data were acquired in list-mode for 180 min
195 and subsequently binned into sinograms for the following time frames: 6 × 10 s, 3 × 20 s, 6 × 30 s, 10 ×
196 60 s, 10 × 120 s, 29 × 300 s. Corrections were applied for randoms, dead time, normalization,
197 attenuation and decay. Fourier re-binning (Defrise et al., 1997) was used to compress the 4D
198 sinograms to 3D prior to reconstruction with 2D filtered back projection with a Hann window cut-off at
199 the Nyquist frequency. The image voxel size was 0.95 × 0.95 × 0.80 mm, with an array size of 128 ×
200 128 × 95. The reconstructed images were converted to kBq/ml using global and slice factors
201 determined from imaging a uniform phantom filled with a [¹⁸F]fluoride solution.

202 Thirty-two T2-weighted MR brain scans from previous [¹⁸F]fallypride PET studies in Lister-hooded
203 rats were used to create a high resolution MR brain template with SyN (Avants et al., 2008), part of the
204 Advanced Normalization Tools (ANTs) package. A PET template was then created by applying the
205 spatial normalisation parameters from the above template creation process to late [¹⁸F]fallypride
206 images (average image 120-180 min after injection) that had been manually co-registered to their
207 corresponding MR scan. Late [¹⁸F]fallypride images from the 30 PET scans in this study were affine
208 registered to the PET template using ANTs and each transformation was used to re-slice the
209 corresponding dynamic [¹⁸F]fallypride PET image set to template space. Finally, the MR template used
210 in a previous study (Caprioli et al., 2013)(see Figure 2A) was spatially normalised to the new MR
211 template described above, with the resulting transformation applied to the previously defined regions of
212 interest to align them to the new template space.

213 $D_{2/3}$ receptor availability was quantified using non-displaceable binding potential (BP_{ND}) (Innis et
214 al., 2007), determined from reference tissue-based kinetic analysis with the cerebellum acting as the
215 reference region. The borders of the reference region drawn on the MR template excluded the
216 outermost lamina of the cerebellar cortex in order to avoid partial volume error from uptake in the
217 Purkinje cell layer. Regional and voxel-wise BP_{ND} were estimated from the distribution volume ratio
218 (DVR; $BP_{ND} = DVR - 1$) determined using the reference tissue input Logan plot (Logan et al., 1996)
219 with data fitted from 90 to 180 min post-injection.

220 *Statistical analysis*

221 Behavioral data were subjected to analysis of variance (SPSS, version 17.0, Chicago, USA) using a
222 general linear model. Homogeneity of variance was verified using Levene's test. For repeated-
223 measures analyses, Mauchly's test of sphericity was applied and the degrees of freedom corrected to

224 more conservative values using the Huynh-Feldt epsilon for any terms involving factors in which the
225 sphericity assumption was violated. Differences in BP_{ND} between HI and LI rats were evaluated using
226 repeated measures ANOVA. Significantly meaningful interactions ($p < 0.1$) were further analyzed by
227 simple main effects using the pooled sum of square error term (Cochran and Cox, 1957). A
228 significance level of $\alpha = 0.05$ was used to interpret main effects and post-hoc tests. **Pearson product**
229 **moment correlations were used to assess the strength of the association between: (i) the**
230 **change in BP_{ND} ((post-MPH-pre-MPH)/(pre-MPH) \times 100) and baseline BP_{ND} (pre-MPH scan); (ii)**
231 **the change in premature responses ((post-MPH-pre-MPH)/(pre-MPH) \times 100) and baseline BP_{ND}**
232 **(pre-MPH scan). All figures show group means \pm 1SEM.**

233

234 Results

235 *Segregation of high and low impulsivity groups*

236 The behavioral performance of LI and HI rats on the 5-CSRTT is summarized in Table 1. Percentage
237 premature responses for HI ($n=8$) and LI ($n=7$) rats, averaged across the three L-ITI sessions prior to
238 the commencement of MPH dosing, were $69.6 \pm 7.5\%$ (mean \pm SEM) and $21.5 \pm 2.2\%$, respectively.
239 HI rats were more impulsive than LI rats regardless of the ITI being set to 5 s ('b1' to 'b10', 'S-ITI'
240 $p=0.002$) or 7 s ('L-ITI' $p < 0.001$). Among the various behavioral variables recorded only attentional
241 accuracy was significantly impaired in HI rats compared with LI rats during the L-ITI sessions
242 ($p=0.014$). **Although omissions appeared to be increased in HI rats compared with LI rats this**
243 **contrast was not significant ($p=0.054$).**

244 *Interactive effects of methylphenidate on impulsivity in LI and HI rats*

245 Twenty one days after the commencement of daily MPH dosing, rats were re-assessed for impulsivity
246 and attentional performance on the 5-CSRTT. It can be seen in Figure 1B that MPH produced
247 divergent effects on impulsivity in the two impulsivity sub-groups with impulsivity appearing to decrease
248 in HI rats but increase in LI rats (group \times MPH interaction: $F(1,13)=7.59$, $p=0.016$). Although *post-hoc*
249 t-tests failed to reveal a significant decrease in impulsivity, at a group level, following MPH treatment in
250 HI rats ($p=0.095$), the increase in impulsivity observed in LI rats was significant ($p=0.044$). Thus,
251 following exposure to MPH, the initial contrast in impulsivity between LI and HI rats was greatly
252 diminished. Importantly, it can be seen in Figure 1C that MPH increased impulsivity in LI rats in an
253 inverse relationship to the baseline level of impulsivity, while impulsivity decreased in HI rats, with the

254 magnitude of the decrease being positively correlated to the baseline level of impulsivity. Thus, the
255 effect of MPH to increase impulsivity was greatest in LI rats showing the lowest baseline level of
256 impulsivity ($r = -0.80$; $p=0.03$) whereas MPH decreased impulsivity to a greater extent in those HI rats
257 showing the highest baseline level of impulsivity ($r = -0.7$; $p=0.05$). However, MPH did not restore the
258 attentional inaccuracy of HI rats, which remained significantly impaired relative to LI rats (main effect of
259 group $F_{(1,13)} = 3.62$, $p=0.049$; group \times MPH interaction: $F_{(1,13)} = 0.37$, $p=0.55$). Moreover there were no
260 significant effects of MPH on omissions or magazine latencies on the 5-CSRTT (Figure 1D and Table
261 1).

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

263 Consistent with our recent study (Caprioli et al., 2013), the availability of $D_{2/3}$ receptors was significantly
264 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-
265 naïve HI rats compared with LI rats (pre-/post-MPH \times hemisphere \times group interaction: $F_{(1,13)}=5.75$,
266 $p=0.05$; group \times hemisphere interaction: $F_{(1,13)}=4.047$, $p=0.066$, Figure 2C). Following 28 days of
267 exposure to MPH, the difference in $D_{2/3}$ receptor availability between LI and HI rats in the left ventral
268 striatum was no longer evident ($t_{(13)} = 0.09$, $p=0.930$). The normalizing effect of MPH on $D_{2/3}$ receptor
269 availability in the left ventral striatum appeared to be explained by a near-significant reduction in $D_{2/3}$
270 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
271 ($t_{(7)} = -0.495$, $p=0.636$). Exposure of LI and HI rats to MPH had no significant effect on $D_{2/3}$ receptor
272 availability in the right ventral striatum.

273 *Baseline-dependent effects of MPH on striatal $D_{2/3}$ receptors in high impulsive rats*

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

284 <0.01; posterior dorsal striatum HI rats: $r_{\text{left}} = -0.61$, $p < 0.01$, $r_{\text{right}} = -0.72$, $p < 0.01$). In contrast, we did not
285 observe baseline dependent effects on $D_{2/3}$ receptor availability in any of the striatal areas investigated
286 in LI rats.

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

290 Discussion

291 This study investigated striatal $D_{2/3}$ receptor availability in highly impulsive rats and the mechanisms
292 underlying the therapeutic effects of chronic oral MPH. We found that repeated oral MPH was sufficient
293 to produce bi-directional effects on impulsivity that depended on the baseline level of impulsivity. Thus,
294 in LI rats, MPH increased impulsivity whereas in HI rats it reduced impulsivity in animals exhibiting the
295 highest baseline level of impulsivity, consistent with an underlying rate dependent mechanism. Our
296 results indicate that the rate dependency model held for LI and HI rats but with clear differences in the
297 underlying regulatory parameters suggestive of a non-unitary process. Since the baseline dependent
298 effects of MPH on impulsivity and $D_{2/3}$ receptors were dissociable we conclude that $D_{2/3}$ receptors may
299 not play a major contribution to the effects of MPH on impulsivity. **These data are consistent with**
300 **findings showing that MPH modulates performance in humans in a baseline-dependent manner**
301 **both in healthy controls and in subjects diagnosed with ADHD (del Campo et al., 2013).**

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

313 The mechanism underlying the observed rate dependent modulation of impulsivity following MPH
314 treatment is unknown but as discussed above may be distinct for LI and HI rats. Although for LI rats
315 there was no obvious relationship between baseline $D_{2/3}$ BP_{ND} and the change in this parameter
316 following MPH treatment, at a group level, MPH had the trend effect of reducing $D_{2/3}$ BP_{ND} in the left
317 ventral striatum, a deficit associated with increased impulsivity on this task (Dalley et al., 2007) and
318 localized to the nucleus accumbens shell (Besson et al., 2010; Jupp et al., 2013). The reduction in $D_{2/3}$
319 receptor availability in LI rats may reflect a down-regulation of $D_{2/3}$ receptors but since [¹⁸F]fallypride
320 competes with DA in binding to $D_{2/3}$ receptors it could also reflect an increase in synaptic DA release,
321 possibly due to sensitization of the mesolimbic DA systems following repeated MPH treatment
322 (Shuster et al., 1982; Gaytan et al., 1997). However, sensitization of the locomotion response does not
323 appear to develop after chronic oral MPH treatment (McNamara et al., 1993; Kuczenski and Segal,
324 2002). Furthermore, no simple relationship exists between hyperactivity and impulsivity on the 5-
325 CSRTT (Dalley et al., 2007; Molander et al., 2011; Moreno et al., 2013).

326 In HI rats MPH had the dual effect of decreasing impulsivity and modulating striatal $D_{2/3}$ receptor
327 availability according to the principal of rate dependency (Dews and Wenger, 1977). However, neither
328 parameter significantly co-varied after MPH treatment suggesting that the modifying effects of MPH on
329 impulsivity are separable from effects on $D_{2/3}$ receptor regulation. That the measure of impulsivity is not
330 directly related to changes in $D_{2/3}$ receptor availability is possibly due to other actions of MPH,
331 especially for example on NE. Thus, atomoxetine, which reduces impulsivity in HI rats, blocks re-
332 uptake of NE and has no effect on subcortical DA (Bymaster et al., 2002). Moreover, this drug exerts at
333 least some of its anti-impulsive effects within the shell region of the nucleus accumbens (Economidou
334 et al., 2012). Alternatively, the reduction in impulsivity in MPH-treated HI rats may include actions at
335 the level of the nucleus accumbens core. Thus, previously, we have shown that HI rats exhibit a
336 reduced density of markers associated with dendritic spines in this region and GABA synthesis
337 (Caprioli et al., 2014), abnormalities that were mainly restricted to the left hemisphere similar to the
338 locus of deficient $D_{2/3}$ receptor availability in HI rats (Caprioli et al., 2013). Since in the present study
339 MPH had the greatest beneficial effects in the most impulsive animals these effects may be mediated
340 by a restoration of the structural and functional integrity of GABA-ergic medium spiny neurons in the
341 nucleus accumbens core, as previously hypothesized (Caprioli et al., 2014). **The origin of the**
342 **hemispheric imbalance in $D_{2/3}$ receptors in HI rats is unknown but may arise from genetic**
343 **and/or environmental factors affecting trophic signalling during development (Concha et al.,**

344 **2012). Left/right asymmetries in the midbrain DA systems have been reported in rats (Carlson**
345 **and Glick, 1989; Afonso et al., 1993; Rodriguez et al., 1994) and healthy humans (Tomer et al.,**
346 **2008), as well as ADHD (del Campo et al., 2013; Volkow et al., 2007a; Volkow et al., 2009).**

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

361 There are several limitations of the present study that merit discussion. Firstly, although we dosed
362 MPH orally and assessed serum MPH levels and its metabolite ritalinic acid, as endorsed by others
363 (Volkow and Insel, 2003; Gill et al., 2012), peak MPH levels were in excess of the typical therapeutic
364 range of MPH of 8 to 10 ng/ml (Swanson and Volkow, 2002) (Table 2). However, consistent with other
365 research (Patrick et al., 1984; Robb et al., 2014), MPH was rapidly cleared with an elimination half-life
366 of 30-50 minutes. Thus, although serum levels of MPH were initially high these soon declined to
367 clinically-relevant values after the administration of MPH and well before the next dose. Nevertheless,
368 with twice daily dosing and consequent fluctuations in serum MPH our results are difficult to
369 extrapolate to studies in humans that use extended-release oral formulations (Robb et al., 2014). A
370 second consideration is that the primary objective of our research was to investigate the long term
371 effects of MPH on impulse control and $D_{2/3}$ receptors in the striatum. The design of our study thus
372 excluded the analysis of acute, low doses of MPH, which increase NE and DA availability selectively in
373 the prefrontal cortex (Berridge et al., 2006) and facilitate cognitive functions relevant to ADHD
374 (Andrzejewski et al., 2014). **Thirdly, our conclusions are based on relatively small group sizes**

375 **(n=7-8). Nevertheless, we have now reported in three independent studies reduced striatal $D_{2/3}$**
376 **receptor availability in the ventral striatum of HI rats. In addition, we observed qualitatively**
377 **similar changes from baseline following administration of MPH and cocaine (Caprioli et al.,**
378 **2013), using a within-subjects longitudinal design.**

379 In conclusion our results confirm that deficits in impulsive control are associated with reduced $D_{2/3}$
380 receptor availability in the left ventral striatum as previously reported (Dalley et al., 2007; Caprioli et al.,
381 2013) and in three independent studies in ADHD patients (Volkow et al., 2007a; Volkow et al., 2009;
382 del Campo et al., 2013). Although further research is needed to test the full dose-response curve of
383 MPH on impulsivity and $D_{2/3}$ receptor availability, we have now shown how different psychomotor
384 stimulant drugs produce baseline-dependent effects on $D_{2/3}$ receptor availability in the ventral striatum.
385 In addition, we have demonstrated that the therapeutic effects of MPH on impulsivity are unlikely to
386 arise as a direct consequence of changes in the regulation of $D_{2/3}$ receptors in the ventral striatum, a
387 conclusion supported by research in adults with ADHD (Volkow et al., 2012). **Nevertheless, by**
388 **restoring levels of $D_{2/3}$ receptors in the ventral striatum of HI rats, MPH may diminish the risk of**
389 **addiction in addiction-prone highly-impulsive rats (Belin et al., 2008; Economidou et al., 2009).**

390

391

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: $F_{(1,13)}=7.59$, $^{\dagger} p<0.05$) 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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628 **Tables**629 **Table 1**

630 Summary of the effects of oral MPH administration on the behavioral performance of LI and HI rats on
 631 the 5-CSRTT. Shown are (mean \pm SEM): % premature responses, % accuracy of responding, %
 632 omissions, magazine latencies (ms), and correct response latencies (ms) before ('Pre') and after
 633 ('Post') drug exposure. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (LI vs HI).
 634

Short-ITI sessions	Pre-MPH		Post-MPH	
	LI (n=7)	HI (n=8)	LI (n=7)	HI (n=8)
% premature	2.9 \pm 0.5	8.6 \pm 1.2**	2.7 \pm 0.6	4.4 \pm 0.7
% accuracy	83.0 \pm 1.7	80.4 \pm 1.7	83.4 \pm 1.0	79.8 \pm 2.0
% omissions	12.5 \pm 2.2	9.0 \pm 1.4	7.5 \pm 1.1	6.7 \pm 1.2
magazine lat. (ms)	1169.4 \pm 91.9	1156.4 \pm 58.4	1213.8 \pm 85.5	1159.5 \pm 51.2
correct lat. (ms)	751.6 \pm 38.0	626.0 \pm 41.8	842.4 \pm 50.9	688.1 \pm 40.0
Long-ITI sessions				
% premature	21.5 \pm 2.2	69.6 \pm 7.5***	32.1 \pm 2.9	53.3 \pm 4.6**
% accuracy	80.9 \pm 1.0	73.9 \pm 2.1*	80.5 \pm 1.7	74.9 \pm 1.9*
% omissions	12.7 \pm 1.9	18.7 \pm 2.6	8.6 \pm 1.7	11.3 \pm 2.0
magazine lat. (ms)	1103.9 \pm 54.0	1281.9 \pm 122.7	1186.6 \pm 96.7	1140.1 \pm 49.0
correct lat. (ms)	671.7 \pm 38.6	601.6 \pm 32.5	721.8 \pm 63.2	603.0 \pm 26.0

635

636

637 **Table 2**

638 Summary of the serum concentration (ng/ml) of oral methylphenidate (MPH) and ritalinic acid (RA)
 639 obtained from 4 non-impulsive rats after a single oral dose of MPH (6 mg/kg).
 640

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

641