

Amphetamine-Induced Dopamine Release and Neurocognitive Function in Treatment-Naive Adults with ADHD

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Converging evidence from clinical, preclinical, neuroimaging, and genetic research implicates dopamine neurotransmission in the pathophysiology of attention deficit hyperactivity disorder (ADHD). The *in vivo* neuroreceptor imaging evidence also suggests alterations in the dopamine system in ADHD; however, the nature and behavioral significance of those have not yet been established. Here, we investigated striatal dopaminergic function in ADHD using [¹¹C]raclopride PET with a *d*-amphetamine challenge. We also examined the relationship of striatal dopamine responses to ADHD symptoms and neurocognitive function. A total of 15 treatment-free, noncomorbid adult males with ADHD (age: 29.87 ± 8.65) and 18 healthy male controls (age: 25.44 ± 6.77) underwent two PET scans: one following a lactose placebo and the other following *d*-amphetamine (0.3 mg/kg, p.o.), administered double blind and in random order counterbalanced across groups. In a separate session without a drug, participants performed a battery of neurocognitive tests. Relative to the healthy controls, the ADHD patients, as a group, showed greater *d*-amphetamine-induced decreases in striatal [¹¹C]raclopride binding and performed more poorly on measures of response inhibition. Across groups, a greater magnitude of *d*-amphetamine-induced change in [¹¹C]raclopride binding potential was associated with poorer performance on measures of response inhibition and ADHD symptoms. Our findings suggest an augmented striatal dopaminergic response in treatment-naive ADHD. Though in contrast to results of a previous study, this finding appears consistent with a model proposing exaggerated phasic dopamine release in ADHD. A susceptibility to increased phasic dopamine responsivity may contribute to such characteristics of ADHD as poor inhibition and impulsivity. *Neuropsychopharmacology* (2014) **39**, 1498–1507; doi:10.1038/npp.2013.349; published online 29 January 2014

Keywords: ADHD; dopamine; PET; [¹¹C]raclopride; *d*-amphetamine; executive functions

INTRODUCTION

Converging indirect evidence from clinical, preclinical, neuroimaging, and genetic research implicates catecholamine, particularly dopamine (DA), neurotransmission in the pathophysiology of attention deficit hyperactivity disorder (ADHD) (Faraone and Mick, 2010; Sontag *et al*, 2010; Spencer *et al*, 1996). The nature of this putative DA dysfunction remains to be elucidated. It could involve one or more presynaptic (eg, synthesis, release, reuptake) or postsynaptic (eg, receptor density, receptor affinity, metabolism) system alterations. Based on the clinical efficacy of

stimulants that augment extracellular catecholamine levels, some have hypothesized that ADHD symptoms result from insufficient extracellular DA levels, that is, a hypoactive DA system (Volkow *et al*, 2005). Another model proposes that ADHD symptoms reflect low tonic striatal DA activity coupled with elevated stimulus-evoked phasic DA release (Grace, 2001). According to this model, stimulants confer their therapeutic effects by elevating DA tone that helps attenuate phasic DA release.

More direct measures of DA transmission in ADHD using molecular neuroimaging have produced inconsistent findings. For example, both increases and decreases have been reported for D₂/D₃ receptor availability and for dopamine transporter (DAT) binding in ADHD patients vs controls (Lou *et al*, 2004; Spencer *et al*, 2005; Spencer *et al*, 2013; Volkow *et al*, 2007a; Volkow *et al*, 2009). Only one study has examined DA release in untreated ADHD (Volkow *et al*, 2007b). Using a methylphenidate challenge, this study found lower DA release in the caudate of adult patients than controls. Together, the findings suggest widespread DA system dysfunctions in ADHD, but the direction of these effects remains unclear.

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These data were presented in part at the 47th and 49th (2010) Annual Meetings of the American College of Neuropsychopharmacology at Scottsdale AZ (December 2008) and Miami FL (December 2010), respectively.

Received 26 July 2013; revised 4 December 2013; accepted 5 December 2013; accepted article preview online 30 December 2013

Neurocognitive concomitants of putative striatal DA alterations in ADHD would presumably include executive dysfunction—a key aspect of the cognitive and behavioral profile of ADHD. Executive functions rely on frontal-striatal circuitry (Alexander *et al*, 1990), and functional imaging studies have linked reduced activation in frontal-striatal circuitry in ADHD to performance deficits on executive function tasks (Dickstein *et al*, 2006). However, few studies to date have linked molecular imaging measures of striatal DA function to neurocognitive performance in ADHD (Lou *et al*, 2004; Rosa-Neto *et al*, 2005).

In this study, we measured striatal DA responses in treatment-naïve men with ADHD and healthy controls to a challenge dose of *d*-amphetamine (*d*-amph) using PET with [¹¹C]raclopride. This well-established technique yields an index of tracer binding potential (BP_{ND}) that is proportional to the number of available D₂/D₃ receptors (Laruelle, 2000). The signal is attenuated by drug-induced increases in extracellular DA concentrations, providing an index of DA release. We predicted that *d*-amph-evoked DA release and baseline receptor availability in the striatum would differ significantly between ADHD subjects and healthy controls. As molecular imaging findings in ADHD have been inconsistent, and preclinical models of hyperactivity, impulsivity, and poorly regulated executive function have been linked to both increases and decreases in DA (Sontag *et al*, 2010), group differences and behavior associations in either direction were considered of interest.

PATIENTS AND METHODS

Participants

A total of 15 adult men with ADHD (5 combined subtype; 10 predominantly inattentive subtype) and 18 adult male healthy controls completed the study and were included in the analyses. Four participants completed the study but could not be included: three for poor PET data quality (two ADHD), and one (a control) because of a structural anomaly in striatum on MR. Two additional participants began but did not complete the study because of equipment failure.

The diagnosis of ADHD (DSM-IV-TR) required the presence of at least 6/9 inattention symptoms (with or without 6/9 hyperactivity/impulsivity symptoms) since childhood; diagnosis was ascertained by one of the research psychiatrists (CB, LH, and RJ). Symptoms of ADHD (Table 1) were measured as continuous variables in both groups using the Conners' Adult ADHD Rating Scale (CAARS) (Conners *et al*, 1999).

Participants underwent a Structured Clinical Interview for DSM-IV Axis I disorders (SCID I (First *et al*, 1996)) and were excluded for any current or past history of Axis I disorder, other than ADHD. However, 2/15 ADHD participants reported a single mild depressive episode occurring for ≥ 2 years in the past. Other exclusion criteria were: a first-degree relative with a history of substance dependence; current use of psychotropic medications; a Beck Depression Inventory (BDI) (Beck and Steer, 1987) score > 13 , an estimated IQ < 80 ; a neurological history; a reported history of a head injury with loss of consciousness for > 5 min; a history of any physical disorder (eg, cardiovascular) contradictory to participation as per comprehensive physi-

cal exam; and a positive toxicology screen (cocaine, opiates, phencyclidine, barbiturates, benzodiazepines, Δ^9 -tetrahydrocannabinol, amphetamines) as per the Triage Drugs of Abuse urine test (Biosite, San Diego, CA). Controls were excluded for a reported ADHD diagnosis in a first-degree relative.

All ADHD participants were stimulant treatment naïve except one who, 2 years before his participation, underwent a 6-month methylphenidate trial. Excluding his data did not change the results. Lifetime stimulant exposure did not exceed two uses for any other participants.

The study was carried out in accordance with the Declaration of Helsinki and was approved by the Research Ethics Board of the Montreal Neurological Institute. All participants gave written informed consent.

Procedure

Participants underwent two [¹¹C]raclopride PET scans, one following a lactose placebo and the other following 0.3 mg/kg p.o. of *d*-amph; capsule administration occurred 60 min before tracer injection and was double blind, randomized, and counterbalanced. PET scans occurred at least 3 days apart. Before each scan, participants were asked to abstain from food, caffeine, and smoking for 4 h and from alcohol for 24 h. A structural magnetic resonance image (MRI) was obtained on a separate day. Participants completed the neurocognitive battery a minimum of 24 h from the time of either PET scan to avoid any drug carryover effects. Toxicology screening occurred on the initial screening interview and before both PET scans and neurocognitive testing.

Neuroimaging

Participants were scanned on a Siemens ECAT HR + PET scanner (CTI/Siemens, Knoxville, TN) with lead septa removed (63-slice coverage), with a maximum resolution 4.2-mm, full width at half maximum (FWHM) in the center of the field of view. Attenuation correction was performed using a 12-min ⁶⁸Ga transmission scan immediately before tracer injection. The emission scan started simultaneously with the injection of [¹¹C]raclopride, as an i.v. bolus, and data were acquired for 60 min in 26 time frames of progressively longer duration. Tracer doses did not differ significantly between scans for either group (controls: placebo = 7.25 ± 1.74 , *d*-amph = 7.41 ± 1.55 , $p = 0.46$; ADHD: placebo = 6.44 ± 0.74 , *d*-amph = $6.59 \pm .29$, $p = 0.37$), but were marginally higher across scans for controls (controls = 7.33 ± 1.63 ; ADHD = 6.51 ± 0.56 , $p = 0.06$). The [¹¹C]raclopride-specific activity, sampled for 10/66 scans, ranged from 335 to 925 Ci/mmol, with the injected dose ranging from 2.34 to 6.46 μ g (3.77 ± 1.55). Vital signs were monitored and blood samples for plasma amphetamine collected just before capsule administration, at the time of tracer administration, mid-scan, and at the end of scan. Ratings of subjective drug (or placebo) effects were also obtained (Supplementary Table S4).

High-resolution (1 mm) T1-weighted MRIs were obtained on a 1.5-Tesla Siemens scanner, using gradient echo pulse sequence (TR = 22 ms, TE = 9.2 ms, flip angle = 30°,

Table 1 Sample Characteristics

	Controls (n = 18)	ADHD (n = 15)	Test statistic (d.f.)	p
Age (SD)	25.44 (6.77)	29.87 (8.65)	$U_{(33)} = 99.50$	0.20
Estimated full scale IQ (SD)	116.83 (16.07)	107.13 (12.78)	$U_{(27)} = 51.00$	0.06
Abbreviated WAIS-III ^a	124.25 (14.70)	109.44 (15.16)	$U_{(17)} = 17.00$	0.07
Abbreviated WAIS-R ^b	102.00 (1.63)	103.67 (8.12)	$U_{(10)} = 11.50$	0.91
Years of education (SD)	17.11 (3.32)	16.20 (3.63)	$t_{(31)} = 0.75$	0.46
CAARS t-scores (SD)				
Inattention/memory problems	43.77 (7.41)	74.00 (10.49)	$t_{(26)} = 8.67$	<0.0005*
Hyperactivity/restlessness	43.76 (6.08)	62.27 (12.93)	$t_{(21.79)} = 4.82$	<0.0005*
Impulsivity/emotional lability	42.92 (9.42)	58.53 (11.28)	$t_{(26)} = 3.94$	0.001*
Problems with self-concept	43.08 (5.89)	63.07 (7.64)	$t_{(26)} = 7.66$	<0.0005*
DSM-IV inattention	48.73 (12.49)	84.4 (8.73)	$t_{(26)} = 8.85$	<0.0005*
DSM-IV hyperactivity	44.69 (8.54)	68.13 (14.48)	$t_{(26)} = 5.11$	<0.0005*
DSM-IV total	46.69 (11.64)	81.47 (10.30)	$t_{(26)} = 8.39$	<0.0005*
ADHD index	42.00 (8.45)	66.86 (8.74)	$t_{(26)} = 7.63$	<0.0005*
BDI at intake (SD)	1.53 (2.00)	6.04 (3.86)	$U_{(31)} = 23.50$	<0.0005*
Recreational drug use history				
Stimulants: no. of lifetime uses (SD)	0.06 (0.24)	0.36 (0.74)	$U_{(32)} = 105.00$	0.17
Marijuana: no. of lifetime uses (SD)	18.00 (33.08)	49.27 (90.07)	$U_{(32)} = 119.50$	0.22
Nicotine: no. of lifetime uses (SD)	1359.06 (3677.02)	1614.13 (5171.99)	$U_{(33)} = 128.00$	0.81
No. of smokers	1	1		

Group differences: * $p \leq 0.05$.

^aWechsler Adult Intelligence Scale-Revised (WAIS-R) ($n = 9$) (Reynolds *et al.*, 1983).

^bWechsler Adult Intelligence Scale-III (WAIS-III) ($n = 9$) (Pilgrim *et al.*, 1999).

FOV = 256 mm, and matrix 256 × 256) for co-registration to the PET images.

Neurocognitive Battery

A subset of participants (14 ADHD and 12 controls) completed a battery of neurocognitive tests, tapping functions associated with medium to large effect sizes for ADHD in meta-analyses (Nigg, 2005), and suggested to be in part mediated by catecholamine systems (Allman *et al.*, 2010; Aron *et al.*, 2003; Leyton *et al.*, 2007). The battery included two response inhibition tasks: the Stop Signal Paradigm (Logan *et al.*, 1984) and the antisaccade task (Hallett, 1978). The Stop Signal Paradigm assesses inhibition by measuring the time required to stop a planned response, that is, stop signal reaction time (SSRT). An auditory stop signal instructs participants to withhold responses on 25% of trials in a choice reaction time task. SSRT measures how early the stop signal must occur relative to a participant's mean response time in order for responses to be successfully withheld.

The antisaccade task measures inhibitory oculomotor control (Hallett, 1978). It requires withholding a reflexive saccade to a suddenly appearing peripheral target and, instead, generating a saccade to its mirror location (ie, an antisaccade). Two measures of inhibitory function were

derived from this task: % reflexive saccades toward the target (error rate) and % anticipatory saccades (latency ≤ 80 ms) that reflect impulsive responding when waiting for the peripheral target to appear.

The battery also included tasks of working memory, planning, motor speed and cognitive flexibility, and responsivity to reward and punishment. We will subsequently focus only on those tasks that revealed significant differences between the groups; a detailed description of the battery and the results appears in Supplementary Materials and Methods and Supplementary Table S1.

Analyses

MR volumes were corrected for image intensity nonuniformity (Sled *et al.*, 1998) and transformed into the Montreal Neurological Institute (MNI) space using automated feature matching to the MNI305 template (Collins *et al.*, 1994).

The PET images were reconstructed using a 6-mm FWHM Hanning filter and corrected for motion (Costes *et al.*, 2009). Parametric images were generated by calculating [¹¹C]raclopride binding potential values (BP_{ND}) (Innis *et al.*, 2007) at each voxel using a simplified reference tissue compartmental model (SRTM) with cerebellum as the reference tissue with a very low density of D₂/D₃ receptors (Gunn *et al.*, 1997; Lammertsma and Hume, 1996). BP_{ND} is a function of the estimated concentration of available D₂/D₃ receptors (B_{Avail}),

the dissociation constant of the radiotracer from D₂/D₃ receptors (K_D), and the free fraction of the nonspecifically bound tracer in the brain (F_{ND}): $BP_{ND} = F_{ND} \times (B_{avail}/K_D)$.

As previously described (Boileau *et al*, 2006), mean BP_{ND} values from each individual parametric image were extracted from regions of interest (ROIs) delineated on each individual MRI. The ROIs were based on the functional organization of the striatum (Martinez *et al*, 2003): limbic (LST, includes ventral striatum), associative (AST, includes precommissural dorsal caudate, precommissural dorsal putamen, postcommissural caudate), and sensorimotor (SMST, includes postcommissural putamen). Mean BP_{ND} values were corrected for partial volume effects (Aston *et al*, 2002). ΔBP_{ND} values were calculated as $(BP_{ND \text{ placebo}} - BP_{ND \text{ d-amph}})/BP_{ND \text{ placebo}} \times 100$ and used for intergroup statistical analysis in SPSS 17.

To examine specific loci of *d*-amph-induced change in BP in each group, a voxel-wise statistical mapping method was utilized to determine the *t*-statistic associated with the change in BP_{ND} between the *d*-amph and the placebo conditions (Aston *et al*, 2000). Voxels of statistically significant change had *t*-values of ≥ 3.8 that corresponded to $p < 0.05$ based on the random field theory, considering the search volume of the striatum, the reconstructed image resolution of 8 mm at FWHM, and correction for multiple comparisons (Aston *et al*, 2000; Worsley *et al*, 1996).

Plasma amphetamine concentrations were analyzed using combined gas chromatography-mass spectrometry (GC-MS) (Asghar *et al*, 2002). Area under the curve (AUC) was calculated for each participant to reflect plasma amphetamine concentrations over all measurement points.

Behavioral data were analyzed statistically using SPSS 17. Extreme values outside 3 SDs of the mean for a given variable were Winsorized (replaced by the value of their nearest neighbor) (Dixon and Yuen, 1974). Appropriate transformations were used to normalize the distribution for some measures, and analyses were carried out on the transformed data.

RESULTS

Participants

ADHD participants did not differ significantly from controls on demographic variables. Estimated IQ was marginally higher in controls ($p = 0.06$; Table 1). Although no participant was clinically depressed, the mean BDI score at intake was significantly higher in the ADHD group than controls ($p < 0.0005$).

d-Amph-Induced Change in D₂/D₃ Binding

In the ROI analysis, ΔBP_{ND} values across three ROIs were significantly correlated with intake BDI scores in the ADHD group ($r_s = -0.68$; $p = 0.007$), but not in controls ($r_s = 0.23$; $p = 0.37$). Intake BDI was therefore entered as a covariate in the two-way group \times ROI mixed ANCOVA on the ΔBP_{ND} s. One ADHD and one control participant were missing intake BDI scores; their missing values were replaced by the mean of their respective groups. Excluding those participants produced the same results. The ANCOVA revealed a significant group \times ROI interaction ($F_{(1,32,39.70)} = 4.07$;

$p = 0.04$). Bonferroni adjusted pairwise comparisons showed greater magnitude ΔBP_{ND} in the ADHD group than controls in AST (controls: -0.57% ; ADHD: 6.08% ; $F_{(1,30)} = 4.24$, $p = 0.05$) and SMST (controls: 3.25% ; ADHD: 9.68% ; $F_{(1,30)} = 4.73$, $p = 0.04$), but not in LST (controls: 12.34% ; ADHD: 8.62% ; $F_{(1,30)} = 0.02$, $p = 0.82$) (Figure 1b). There were no other main effects or interactions.

To explore the relationship between ΔBP_{ND} and ADHD symptoms, we performed semipartial correlations between ΔBP_{ND} s and scores on two subscales of the CAARS, the DSM-IV Symptoms of Inattention and the DSM-IV Symptoms of Hyperactivity-Impulsivity subscales. Analyses were conducted across groups and co-varied intake BDI scores. Significance threshold was 0.008 (Bonferroni-corrected to keep 'family-wise' error rate at 0.05). More pronounced ΔBP_{ND} decreases in AST and SMST were associated with higher inattention scores (AST: $r_{(33)} = 0.47$, $p < 0.0005$; SMST: $r_{(33)} = 0.43$; $p = 0.001$) (Figure 2a). Although regression diagnostics suggested a possible 'lever' (leverage values: ≥ 0.47 ; standardized DF β s: ≥ 0.40), nonparametric correlations between inattention scores and residualized ΔBP_{ND} s with intake BDI 'removed' yielded the same results (AST: $Rho = 0.43$, $p = 0.02$; SMST: $Rho = 0.40$, $p = 0.03$). ΔBP_{ND} s were not related to hyperactivity scores (all $r_{s(33)} < 0.27$; $p_s > 0.17$). LST ΔBP_{ND} s were not significantly correlated with ADHD symptoms ($r_{s(33)} < 0.16$; $p_s > 0.29$).

We also examined BP_{ND} on the placebo day. A group \times ROI mixed ANOVA revealed a significant group \times ROI interaction ($F_{(1,70,52.80)} = 4.67$, $p = 0.02$), indicating a different pattern of D₂/D₃ binding across ROIs as a function of group (Table 2).

The loci of significant DA release for each group are presented using voxel-wise *t*-maps in Figure 1 and Supplementary Table S2.

Neurocognitive Performance

In the Stop Signal Paradigm, a two-way mixed ANOVA revealed a significant group \times reaction time (RT) type (Go RT *vs* Stop Signal RT) interaction ($F_{(1,24)} = 5.83$, $p = 0.02$), reflecting longer SSRTs in the ADHD participants than controls ($t_{(24)} = 3.0$; $p = 0.008$), but no group difference on Go RTs (Figure 3).

Independent sample *t*-tests revealed that relative to controls, ADHD participants made significantly more anti-saccade (AS) errors ($t_{(19)} = 2.36$; $p = 0.03$). ADHD participants also made significantly more anticipatory responses during the fixation period on both prosaccade (the control condition, where the participant follows the target) and antisaccade trial blocks ($t_{(19)} = 2.67$; $p = 0.02$) (Figure 3). For saccade latency, a two-way group \times saccade (anti-saccade *vs* prosaccade) ANOVA revealed a trend for a group \times saccade interaction ($F_{(1,17)} = 4.34$; $p = 0.053$), indicating a larger difference between prosaccade and anti-saccade latencies in ADHD participants (70 ms) than controls (48 ms).

d-Amph-Induced ΔBP_{ND} and Response Inhibition

We evaluated associations between ΔBP_{ND} and response inhibition measures that distinguished between the groups

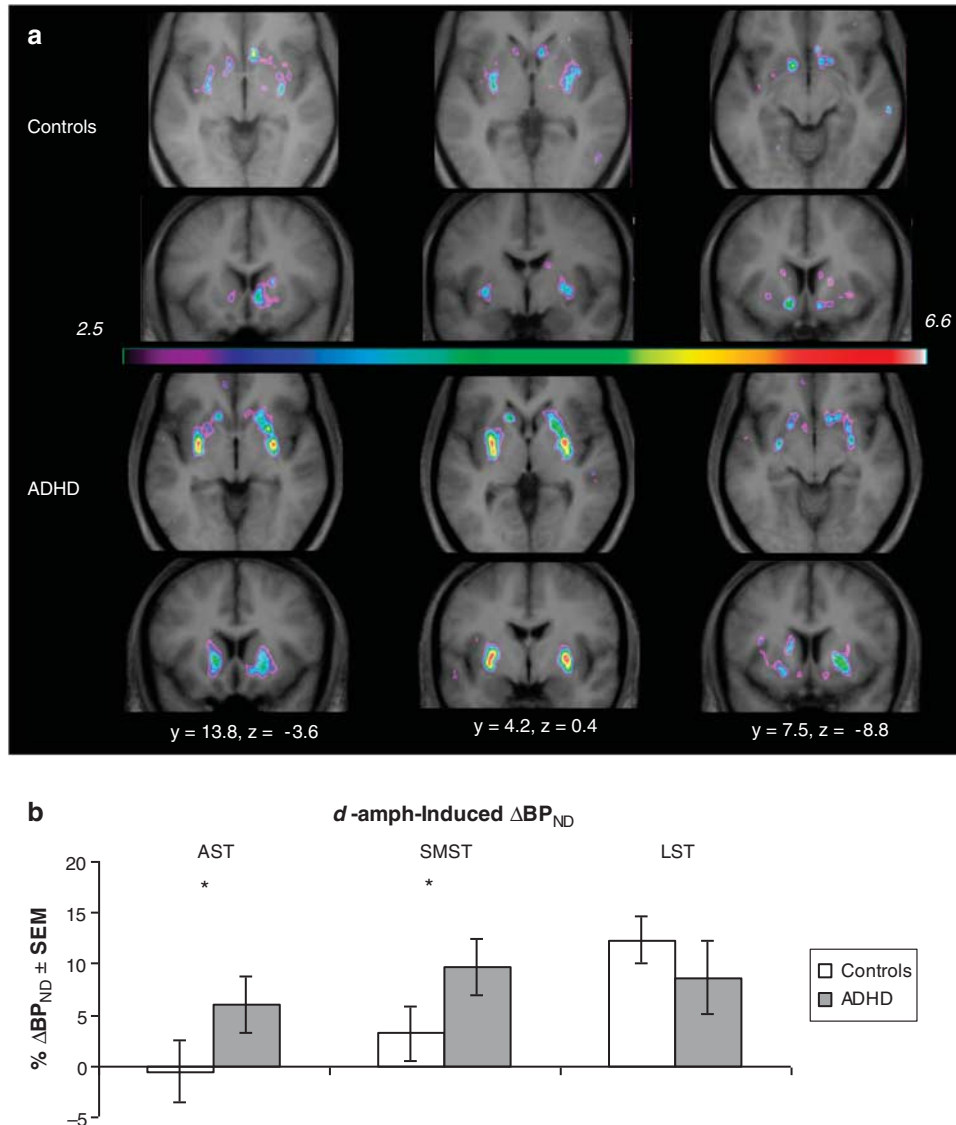


Figure 1 The d -amph-induced change in [^{11}C]raclopride binding. (a) Parametric t -maps showing d -amph-induced decreases in [^{11}C]raclopride BP_{ND} for the control (top) and ADHD (bottom) groups. $t > 3.8$ equivalent to $p \leq 0.05$ based on random field theory. (b) Mean d -amph-induced decreases in [^{11}C]raclopride BP_{ND} extracted from three predetermined ROIs: associative (AST), sensorimotor (SMST), and limbic (LST). The p -values indicate the significance of the between-group differences in % change in BP_{ND} in each region: * $p \leq 0.05$. More positive values indicate larger d -amph-induced BP_{ND} decreases (greater DA release).

using semipartial correlations that controlled for the effect of intake BDI on ΔBP_{ND} . Significance threshold was set at 0.0055 to keep family-wise error rate at 0.05 (3 regions, 3 inhibition measures). Larger ΔBP_{ND} s in AST and SMST were associated with higher proportions of antisaccade errors (AST: $r_{(18)} = 0.62$; $p = 0.003$; SMST: $r_{(18)} = 0.63$; $p = 0.002$) and anticipatory saccades (AST: $r_{(18)} = 0.66$; $p_{(18)} = 0.001$; SMST: $r_{(18)} = 0.65$; $p = 0.001$). Correlations for ΔBP_{ND} in LST were at trend level (AS errors: $r_{(18)} = 0.44$; $p = 0.05$; anticipatory saccades: $r_{(18)} = 0.39$; $p = 0.08$). Longer SSRTs showed trend-level associations with larger ΔBP_{ND} s in AST and SMST (AST: $r_{(23)} = 0.44$; $p = 0.02$; SMST: $r_{(23)} = 0.43$; $p = 0.03$) (Figure 2b). Because regression diagnostics suggested a possible ‘lever’ (leverage values: ≥ 0.47 ; Standardized DF β s: ≥ 0.36), confirmatory nonparametric correlations were conducted using residua-

lized ΔBP_{ND} values with intake BDI ‘removed’. Significant associations remained between both antisaccade variables and ΔBP_{ND} in AST (AS errors: $\text{Rho}_{(19)} = 0.60$; $p = 0.004$; anticipatory saccades: $\text{Rho}_{(19)} = 0.58$; $p = 0.005$) and SMST (AS errors: $\text{Rho}_{(19)} = 0.59$; $p = 0.005$; anticipatory saccades: $\text{Rho}_{(19)} = 0.58$; $p = 0.006$). The trend associations between SSRT and ΔBP_{ND} in these regions also persisted (AST $_{(24)}$: $\text{Rho} = -0.45$; $p = 0.02$; SMST: $\text{Rho}_{(24)} = 0.39$; $p = 0.05$). For LST, the trends were not significant with nonparametric analyses (AS errors: $\text{Rho}_{(19)} = 0.32$; $p = 0.16$; anticipatory saccades: $\text{Rho}_{(19)} = 0.31$; $p = 0.18$).

Plasma Amphetamine

The peak plasma concentrations following d -amph administration were 14.66 ± 12.35 ng/ml for controls and $15.16 \pm$

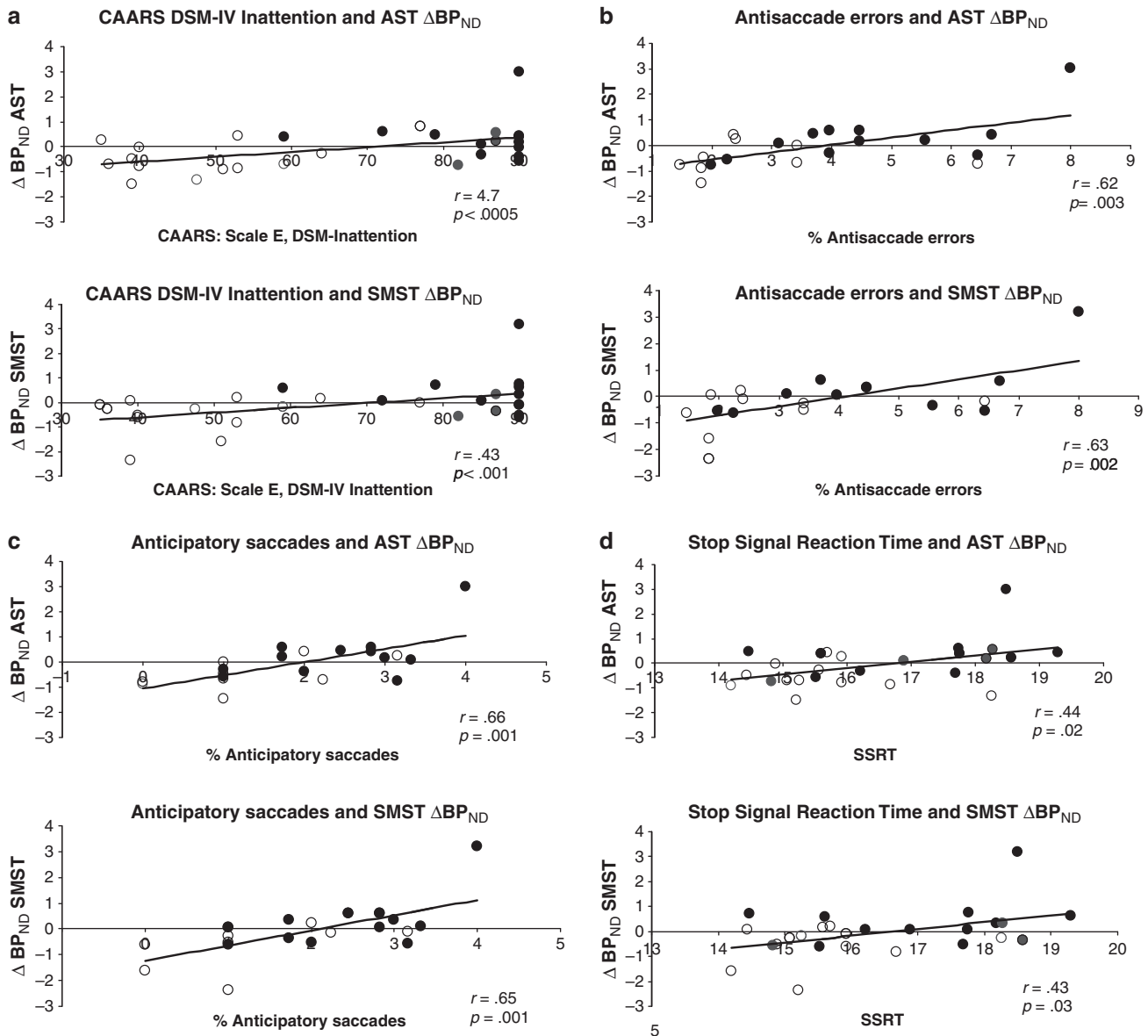


Figure 2 Associations of ΔBP_{ND} with ADHD symptoms on the CAARS and response inhibition. Associations between ΔBP_{ND} and (a) CAARS DSM-IV Inattention symptoms; (b) % antisaccade errors; (c) % anticipatory saccades; and (d) stop signal reaction times. Open symbols represent controls and filled symbols represent ADHD participants. CAARS DSM-IV Inattention t-scores and square root transformed values for neurocognitive performance appear on the x axis; residualized ΔBP_{ND} values, removing the effect of intake BDI, appear on the y axis. More positive values represent greater *d*-amph-induced BP_{ND} decreases (greater DA release). Note that the correlations are not driven by the ADHD participant in the upper right of the graphs and remain significant with nonparametric analyses.

13.72 ng/ml for ADHD participants. AUC values did not differ between groups (ADHD: 27.50 ± 30.4 ; controls: 23.02 ± 25.30) ($t_{(27)} = 0.44$, $p = 0.66$) or correlate with ΔBP_{ND} in any ROI ($ps > 0.36$). No amphetamine was detected in plasma on the placebo day or before drug administration on the *d*-amph day.

DISCUSSION

This study provides evidence of an augmented DA response to an amphetamine challenge in the associative (controls: -0.57% ; ADHD: 6.08%) and sensorimotor (controls:

3.25% ; ADHD: 9.68%) striatal regions in treatment-naive adults with ADHD. Across groups, more pronounced *d*-amph-induced DA responses in these regions were associated with higher self-reported levels of inattention and poorer performance on tests of response inhibition. The two groups did not differ on plasma *d*-amph levels or baseline DA receptor availability.

Although the physiological relevance of an increased *d*-amph-induced ΔBP_{ND} in ADHD participants is uncertain, it could signal an augmented phasic DA release. In nonanesthetized, nonrestrained, behaving animals, phasic DA release contributes most to the overall increases in extracellular DA in response to amphetamine (Daberkow

Table 2 D₂/D₃ Binding Potential (BP_{ND}) in Striatal ROIs

	Controls			ADHD		
	Placebo	<i>d</i> -Amph	Placebo vs <i>d</i> -amph	Placebo	<i>d</i> -Amph	Placebo vs <i>d</i> -amph
AST (SD)	3.00 (0.41)	3.00 (0.49)	$t_{(17)} = -0.04; p = 0.97$	3.25 (0.35)	3.04 (0.43)	$t_{(14)} = 2.15; p < 0.05$
SMST (SD)	3.53 (0.47)	3.41 (0.54)	$t_{(17)} = 1.37; p = 0.19$	3.76 (0.49)	3.37 (0.48)	$t_{(14)} = 3.24; p < 0.006$
LST (SD)	3.26 (0.39)	2.86 (0.47)	$t_{(17)} = 5.32; p < 0.0005$	3.16 (0.53)	2.85 (0.43)	$t_{(14)} = 2.36; p < 0.03$
AST vs LST ^a	$t_{(17)} = 3.52; p = 0.003$	$t_{(17)} = 1.34; p = 0.12$		$t_{(14)} = 0.81; p = 0.43$	$t_{(14)} = 2.31; p = 0.04$	
SMST vs LST ^a	$t_{(17)} = 2.86; p = 0.01$	$t_{(17)} = 5.87; p < 0.005$		$t_{(14)} = 5.21; p = 0.005$	$t_{(14)} = 5.16; p < 0.005$	
AST vs SMST	$t_{(17)} = 9.11; p < 0.0005$	$t_{(17)} = 6.14; p < 0.005$		$t_{(14)} = 5.95; p < 0.0005$	$t_{(14)} = 4.87; p < 0.005$	

Abbreviations: AST, associative ROI; LST, limbic ROI; SMST, sensorimotor ROI.

BP_{ND} values for control and ADHD groups on placebo and *d*-amph. Regional pattern of D₂/D₃ binding on placebo differed as a function of Group, as described by statistics provided in the last three rows.

^aThe group × ROI interaction was driven by group differences in binding discrepancies between AST vs LST ($F_{(1,31)} = 7.20; p = 0.01$) and between SMST vs LST ($F_{(1,31)} = 4.86; p = 0.04$); pairwise comparisons among regions are Bonferroni corrected. Regional BP_{ND} on *d*-amph did not differ significantly as a function of group ($p > 0.45$).

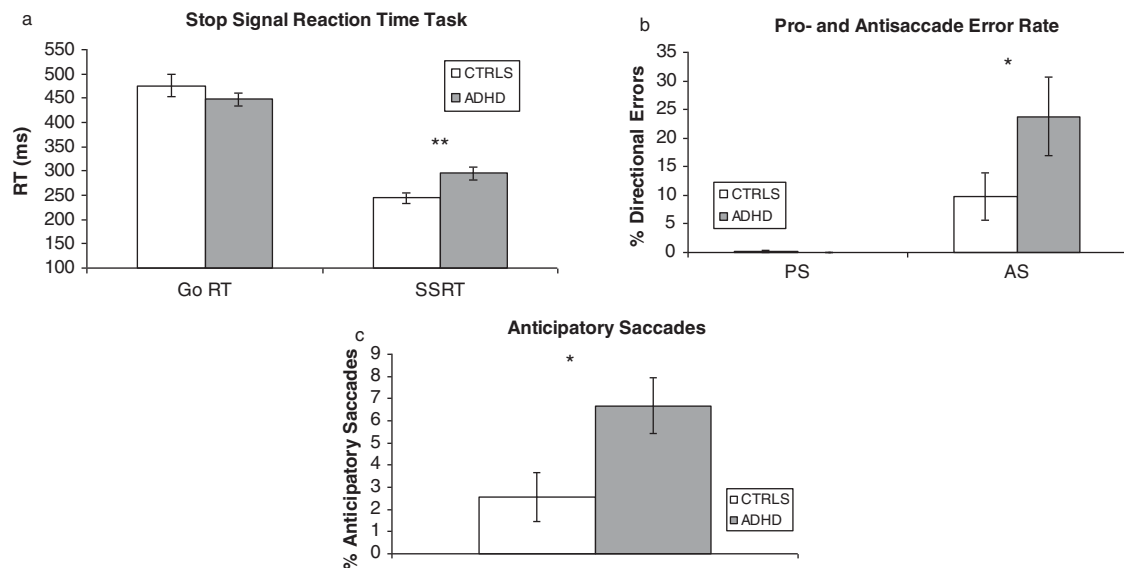


Figure 3 Neurocognitive performance. (a) Stop Signal Paradigm performance. Go RT, Go Reaction Time; SSRT, Stop Signal Reaction Time. (b) Antisaccade and prosaccade error rate. PS, prosaccade; AS, antisaccade. (c) Anticipatory saccades across the pro- and antisaccade blocks. * $p < 0.05$; ** $p < 0.01$.

et al, 2013). Our findings would then appear to be in line with one model of DA function in ADHD that postulates augmented phasic DA release ensuing from abnormally low striatal DA tone (Grace, 2001). Exocytotic DA release is not the sole mechanism contributing to Δ BP_{ND}; DA efflux through reverse transport might also affect BP_{ND}. To the extent that it does, augmented Δ BP_{ND} in the ADHD group could signal altered DAT function, higher DAT density, higher number of DA neurons, or greater presynaptic DA stores. The associations between the magnitude of the DA response and levels of inattention across groups suggest that levels of synaptic DA may modulate attentional function in healthy and ADHD individuals. Notably, the augmented Δ BP_{ND} in ADHD participants is unlikely to have resulted from stimulant sensitization (Boileau *et al*, 2006): the ADHD participants were stimulant treatment naive (except one subject) and the majority had no prior stimulant exposure.

We observed significant differences in DA response between ADHD participants and controls in associative and sensorimotor ROIs but not in limbic striatum. These ROIs are based on the topography of functionally distinct cortico-striatal-thalamo-cortical circuits in non-human primates (Haber, 2010). According to a recent delineation of striatal subregions based on corticostriatal connectivity in humans (Tziortzi *et al*, 2013), our peak voxels of DA release in the AST map onto loci connected with prefrontal executive cortical areas; peak voxels in SMST map onto loci connected with executive and premotor regions. Thus, it is plausible that the group differences in DA release in these striatal regions are linked with executive and motor control, but not with motivation and reward.

Across both groups, greater *d*-amph-induced DA release in associative and sensorimotor ROIs was associated with poorer performance on tasks of response inhibition; the ADHD group had impaired performance of these tasks. This

finding is consistent with previous research in animals and humans that has implicated striatal DA neurotransmission in response inhibition and impulsivity (Buckholtz *et al*, 2010; Dalley *et al*, 2007; Rosa-Neto *et al*, 2005). Some of these findings have suggested that overstimulation of striatal DA receptors by DA is associated with disinhibition: trait impulsivity has been positively associated with amphetamine-induced striatal DA release in healthy controls (Buckholtz *et al*, 2010); in adolescents with ADHD, poorer performance off drug on a behavioral measure of impulsivity and inattention has been associated with greater methylphenidate-induced DA responses (Rosa-Neto *et al*, 2005). The association of an amplified DA release to response inhibition could be mediated by activation of striatal D1 receptors in the *direct pathway* of the basal ganglia circuits that could decrease discharge of the globus pallidus internal and substantia nigra pars reticulata, in turn disinhibiting thalamic nuclei and the superior colliculus (Alexander *et al*, 1990; Hikosaka *et al*, 2000), thus causing weaker inhibition of skeletomotor and oculomotor circuits. Alternatively, the association could be mediated via striatal D2 receptors in the indirect pathway, as D2 stimulation is believed to prevent inhibitory learning (Dagher, 2012).

Our results are at variance with one previous report of a blunted methylphenidate-induced DA response and lower placebo BP_{ND} levels in the caudate of adults with ADHD relative to healthy controls (Volkow *et al*, 2007b). Although placebo BP_{ND} values in the two studies were very similar for controls, they were $\approx 11\%$ lower for the ADHD participants in the study of Volkow *et al* (2007b) than here. Differences in methodology could have contributed to the discrepancy in findings. All of our participants were male; the previous study included females with a trend for a higher proportion of females in the ADHD than control group. Sex differences in striatal DA receptor binding, DA release, and ADHD-relevant personality traits have been reported (Munro *et al*, 2006; Pohjalainen *et al*, 1998; Riccardi *et al*, 2006). Another possible difference is previous substance use. Although the study of Volkow *et al* (2007b) excluded individuals who met criteria for substance abuse or dependence, the paper did not report on the amount of subclinical recreational substance use. Even 'casual' drug use is associated with a blunted DA response to amphetamine (Casey *et al*, 2013); group differences on this variable could affect ΔBP_{ND} . Finally, probes for the dopamine system differed between studies: methylphenidate and *d*-amphetamine augment extracellular DA levels through different mechanisms, and the route of administration (oral *vs* intravenous) and the dose also differed.

Interpretation of the present data should be considered in light of the following. (1) We interpret the association between disinhibition and striatal ΔBP_{ND} as reflecting a meaningful relationship of stable traits. However, these variables were estimated at different times and only once in each participant. Nonetheless, high test-retest reliability of our inhibition measures (Ettinger *et al*, 2003; Logan *et al*, 1984) as well as of ΔBP_{ND} to *d*-amphetamine (Kegeles *et al*, 1999) supports the interpretation that dopaminergic dysfunction is related to response inhibition deficits in ADHD. (2) Our sample size had power to detect only associations of a medium to large effect size, given the Bonferroni correction for multiple comparisons. Larger studies may find associa-

tions of ΔBP_{ND} with other aspects of neurocognition that were not significant here. In addition, there is some inherent instability in correlations found in smaller samples. (3) The magnitude of *d*-amphetamine-induced decreases in [^{11}C]raclopride binding in the control group is at the lower end of the range of ≈ 10 –20% observed in previous studies using a similar dose of oral *d*-amphetamine. However, the difference between groups in ΔBP_{ND} that we observed here cannot be attributed to group differences in dose or to a lack of responsiveness to the drug on the part of the controls. Plasma amphetamine levels were nearly identical between the groups, and the subjective and cardiovascular responses of controls were significant and similar in magnitude to those observed in ADHD participants (Supplementary Tables S3 and S4). (4) Because the sample here was exclusively male, findings cannot be generalized to females.

In conclusion, this study provides evidence of an augmented striatal DA response to an amphetamine challenge in treatment-naïve men with ADHD. Whether an enhanced striatal DA responsivity underlies response inhibition deficits and impulsivity in ADHD remains a matter of conjecture, but the indirect evidence presented here supports this possibility.

FUNDING AND DISCLOSURE

RJ sits on the advisory boards and speakers' bureaus of Pfizer Canada and Janssen Ortho Canada; he has received grant funding from them and from AstraZeneca. He has received honoraria from Janssen Ortho Canada for CME presentations and royalties for Henry Stewart talks. LH has received research support, served on advisory boards, and has been a speaker for Eli Lilly, Glaxo Smith/Kline, Ortho Janssen, Purdue, and Shire; GBB has received grant funding from Pfizer and SinoVeda Canada. The other authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank P Lajeix for ascertaining diagnosis on an ADHD subject; Y Goto for critical discussion and reading of the findings; A Perna for contribution to participant evaluation; K Auclair and F Durand for nursing support; R Fukasawa, G Sauchuck, and S Mattei for technical assistance at the PET unit; D Jolly and M Kovacevic for preparation of radiotracers; G Raw for technical assistance in plasma amphetamine determinations; and K Larcher, SM Cox, and K Welfeld for assistance in PET image analysis. This study was funded by Canadian Institutes of Health Research grant number 77728 to CB and ML.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)