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Dopaminergic System Dysfunction in Recreational Dexamphetamine Users

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Dexamphetamine (dAMPH) is a stimulant drug that is widely used recreationally as well as for the treatment of attention-deficit hyperactivity disorder (ADHD). Although animal studies have shown neurotoxic effects of dAMPH on the dopaminergic system, little is known about such effects on the human brain. Here, we studied the dopaminergic system at multiple physiological levels in recreational dAMPH users and age, gender, and IQ-matched dAMPH-naïve healthy controls. We assessed baseline $D_{2/3}$ receptor availability, in addition to changes in dopamine (DA) release using single-photon emission computed tomography and DA functionality using pharmacological magnetic resonance imaging, following a dAMPH challenge. Also, the subjective responses to the challenge were determined. dAMPH users displayed significantly lower striatal DA $D_{2/3}$ receptor binding compared with healthy controls. In dAMPH users, we further observed a blunted DA release and DA functionality to an acute dAMPH challenge, as well as a blunted subjective response. Finally, the lower $D_{2/3}$ availability, the more pleasant the dAMPH administration was experienced by control subjects, but not by dAMPH users. Thus, in agreement with preclinical studies, we show that the recreational use of dAMPH in human subjects is associated with dopaminergic system dysfunction. These findings warrant further (longitudinal) investigations and call for caution when using this drug recreationally and for ADHD.
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INTRODUCTION

Dexamphetamine (dAMPH) is a psychostimulant that is widely used as a recreational drug by up to 12% of youths in the European Union (European Monitoring Centre for Drugs and Drug Addiction, 2012). In addition, dAMPH is frequently used for the treatment of neuropsychiatric disorders, such as attention-deficit hyperactivity disorder (ADHD). It also has a significant abuse potential: 3% of individuals over 11 years of age in the USA reported abuse of prescribed stimulants (Substance Abuse and Mental Health Services Administration, 2012). dAMPH increases extracellular dopamine (DA) levels by the inhibition of the DA transporter (DAT) as well by directly increasing the release of DA from the nerve terminals (Di Chiara and Imperato, 1988).

Over the past decade, a substantial body of preclinical evidence has suggested that dAMPH may be neurotoxic to the central DA system. For instance, repeated administration of dAMPH in rodents and non-human primates induces long-lasting and dose-dependent reductions in several DA brain markers, including striatal levels of DA and its metabolite, the DAT, of the vesicular monoamine transporter type 2 (VMAT-2), striatal DA synthesis capacity, DA release, and in changes in DA receptor densities, particularly the DA D_2-like receptors (Castner *et al*, 2000; McCann and Ricaurte, 2004; Ricaurte *et al*, 2005). However, whether dAMPH also affects the human DA system remains poorly studied, and so far only two studies, both investigating the DAT only, have been conducted (Reneman *et al*, 2002; Schouw *et al*, 2013b). In addition, there have been studies on other drugs of abuse, including other amphetamine derivatives that directly act upon the DA system. For instance, cocaine dependency is associated with lower $D_{2/3}$ receptor availability and reduced DA release (Martinez *et al*, 2007; Volkow *et al*, 1997). Post-mortem studies of methamphetamine (METH) abusers revealed lower levels of DA, DAT, and tyrosine hydroxylase (Wilson *et al*, 1996). In contrast, whereas age-related differences appear to be present in brain function and morphology in rodents (van der Marel *et al*, 2014), long-term treatment with low doses of methylphenidate (MPH) administration do not appear to change DAergic markers in adult non-human

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primates (Gill *et al*, 2012; Soto *et al*, 2012). However, it is unclear whether dAMPH induces changes to $D_{2/3}$ receptor availability and DA release after prolonged exposure as well.

Therefore, the purpose of the current study was to assess the integrity of the DA system in recreational dAMPH users. Traditionally, the central DA system has been studied with positron emission tomography (PET) or single-photon emission computed tomography (SPECT). However, with the development of pharmacological magnetic resonance imaging (phMRI), changes in *in vivo* DA-ergic neurotransmitter function can now be monitored non-invasively and more specifically (Schouw *et al*, 2013a). We used DA system readouts that have consistently shown changes in animals treated with this drug: DA $D_{2/3}$ receptor availability and DA release (using SPECT) and striatal DA neurotransmitter function (using phMRI) in response to an acute challenge with dAMP. On the basis of preclinical literature, we expected lower baseline $D_{2/3}$ receptor availability in the striatum of dAMPH users compared with controls (Ginovart *et al*, 1999). In addition, we expected blunted striatal DA release and DA function in dAMPH users. As DA $D_{2/3}$ receptor binding predicts greater pleasant responses to stimulants (Volkow *et al*, 1999), we further hypothesized that blunted subjective responses to dAMPH would be present in dAMPH users, and that the above-mentioned DA neurobiological readouts would thus only positively relate to behavioral measures in control subjects.

MATERIALS AND METHODS

Participants

Eighteen male, recreational dAMPH users and 20 age, gender, and IQ-matched healthy, drug-naive controls were recruited through online advertisements and flyers at local universities and colleges. After a complete description of the study to the subjects, written informed consent was obtained. The Medical Ethical Committee of the Academic Medical Center in Amsterdam approved the study procedures.

Inclusion criteria for the dAMPH users were at least 30 lifetime exposures of dAMPH and at least 10 exposures in the past year. The 20 controls were healthy subjects with no self-reported prior use of dAMPH or other drugs that affect the DA-ergic system. Exclusion criteria for all participants were a history of a chronic neurological or psychiatric disorder, family history of sudden heart failure, current use of psychostimulant medication, abnormal electrocardiogram (ECG), positive drug screen, and a clinical diagnosis of ADHD, in addition to smoking >15 cigarettes per day, drinking >30 alcoholic beverages per week, and contraindications for undergoing an MRI scan (eg, ferromagnetic fragments) or the SPECT procedure (eg, allergy to iodine).

Participants agreed to abstain from smoking, caffeine, alcohol, and cannabis for 24 h, and from hard drugs (including dAMPH) for 1 week prior to the assessments. This was verified with a multi-drug screen on a urine sample before entry of the scanner with an enzyme-multiplied immunoassay for amphetamines, cocaine metabolite, opiates, and marijuana. Subjects were screened for current axis I psychiatric disorders. The Dutch version of the National Adult Reading Test (DART IQ) was administered as an estimate of verbal intelligence (Schmand *et al*, 1998). In addition, a detailed drug history questionnaire was obtained.

Three participants were excluded from the SPECT data analysis (because of technical issues with the scanner) and two participants were excluded from the ASL data analysis (one because of technical issues with the scanner and one because of excessive movement). Therefore 16 dAMPH users and 19 healthy controls were included in the SPECT analysis, and 17 dAMPH users and 19 healthy controls in the phMRI analysis.

Study Procedures

Participants underwent two SPECT scans and one MRI scan session on two separate occasions at least 1 week apart to ensure full clearance of dAMPH. Scan sessions were counterbalanced within groups to prevent confounding of sensitization or tolerance to dAMPH.

SPECT Acquisition and Processing

Subjects underwent two [^{123}I]IBZM SPECT scans (Figure 1a); the first to assess baseline striatal DA $D_{2/3}$ receptor availability and the second to assess the decrease in binding after the dAMPH challenge, using the sustained equilibrium/constant infusion technique (Videbaek *et al*, 2000). This decrease in binding provides an index of DA release.

The radioligand [^{123}I]IBZM binds with high affinity to DA $D_{2/3}$ receptors (GE Healthcare, Eindhoven, The Netherlands) (Kegeles *et al*, 1999). Participants received potassium iodide tablets prior to the first SPECT scan to block thyroid uptake of free radioactive iodide. Approximately 80 MBq [^{123}I]IBZM was injected via a cannula in the forearm as a bolus, followed by a constant infusion for the duration of the experiment (20 MBq/h for five consecutive hours). The first scan was obtained between 120 and 180 min after the initiation of [^{123}I]IBZM administration. After completion of the first scanning session, dAMPH was injected intravenously at a dose of 0.3 mg/kg over 2 min, and 60 min later the second scanning session commenced. Constant infusion of [^{123}I]IBZM was sustained until the end of the experiment at 300 min.

Images were acquired on a brain-dedicated SPECT system (Neurofocus 810, Medfield, MA) with the following parameters: matrix: 64×64; energy window = 135–190 keV; slice thickness = 5 mm, acquisition time per slice = 300 s, number of slices = 12. SPECT images were corrected for attenuation and reconstructed using iterative algorithms, as earlier described (Booij *et al*, 1997; Boot *et al*, 2008). Non-displaceable binding potential (BP_{ND}) was calculated as follows: (mean striatal binding—mean binding occipital cortex)/mean binding occipital cortex. Standard templates with fixed ROIs were positioned on the striatum and occipital cortex (reflecting non-specific binding), as earlier described (Booij *et al*, 1997; Boot *et al*, 2008). dAMPH-induced decrease in [^{123}I]IBZM BP_{ND} was expressed as a % of the pre-dAMPH BP_{ND} (Booij *et al*, 1997).

phMRI Acquisition and Processing

We probed striatal DA function using phMRI with an acute dAMPH challenge. MRI studies were performed on a 3.0T

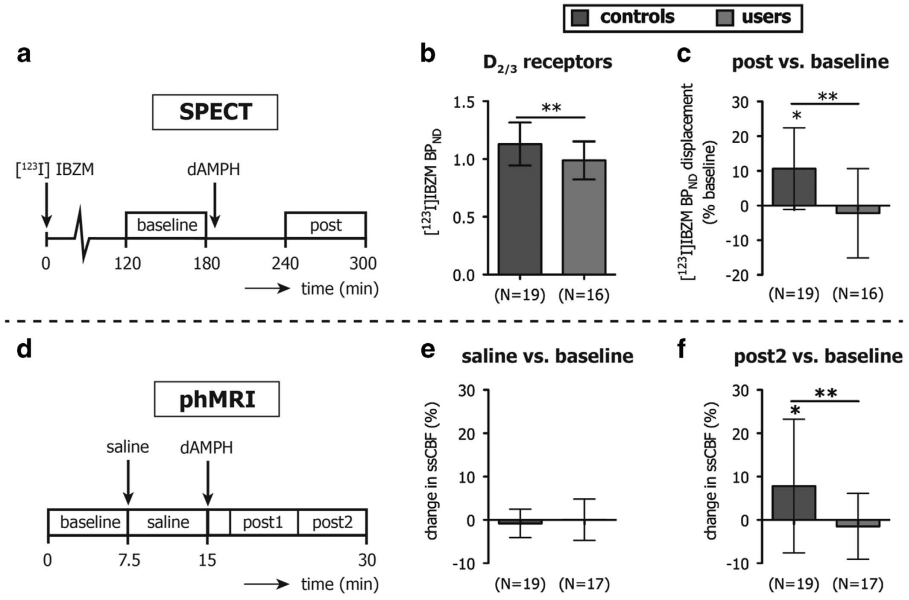

Figure 1 SPECT and phMRI results. (a) Timeline of the SPECT session. (b) **Significant difference in baseline DA $D_{2/3}$ receptor availability (BP_{ND} = non-displaceable binding potential) in the striatum between dAMPH users and controls ($t = 2.363$, df = 33, $p = 0.024$). Means + SD are displayed. (c) *Significant DA release following dAMPH challenge in controls ($t = 3.936$, df = 18, $p < 0.01$), but not in users ($t = -0.704$, df = 15, $p = 0.49$). **The difference between groups was significant ($F = 8.953$, df = 33, $p < 0.01$). Means + SD are displayed. (d) Timeline of the phMRI session. (e) Percentage change of normalized striatal CBF (ssCBF) during saline administration compared with baseline for controls and users. There was no main effect of saline ($F = 0.858$, df = 34, $p = 0.361$) and no interaction effect of time and group ($F = 0.087$,df = 34, $p = 0.769$). Means + SD are displayed. (f) Percentage change of normalized striatal CBF (sCBF) during dAMPH administration ($post_2$) compared with baseline for controls and users. *Significant increased ssCBF in controls ($t = 2.207$, df = 18, $p = 0.04$) but not in users ($t = -0.759$, df = 16, $p = 0.459$). **The difference between groups was significant ($F = 4.956$, df = 34, $p = 0.03$). Means + SD are displayed.

Philips Ingenia scanner (Philips Healthcare, Best, The Netherlands) using a 16-channel receive-only head coil. ASL-phMRI data were acquired using a pCASL sequence, using a GE-EPI readout with parameters: TR/TE = 4000/14 ms, resolution = 3 × 3 × 7 mm, 20 slices, labeling duration = 1650 ms, delay = 1525 ms, GE-EPI read-out, 240 volumes, and scan time 32 min. After 60 baseline volumes (8 min), subjects received a saline challenge (0.9% NaCl) and after 120 image volumes (16 min) a challenge with dAMPH (0.3 mg/kg i.v.) was administered. The saline challenge was given to ensure that changes in cerebral blood flow (CBF) were not induced by the (anticipation of) dAMPH injection.

ASL data were co-registered and motion was corrected before tag-control subtraction. Subsequently, perfusion images were averaged over all volumes, quantified, and normalized using a T1-weighted scan to common image space with DARTEL in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK), with the individual volumes warped accordingly (Ashburner, 2007; Wang *et al*, 2002). Striatal and grey matter (GM) CBF time courses were extracted using masks from the FSL atlas library (FMRIB, Oxford, UK) (Collins *et al*, 1995) and a moving average of 25 volumes. The effect of heart rate was measured in order to correct for potential cardiovascular effects induced by the acute dAMPH challenge. As a result, the post-dAMPH volumes were divided into two bins based on the dynamic time course of changes in CBF and HR: time bin 1 (volumes 150–195) in which heart rate and CBF changed significantly and were also significantly correlated ($r = -0.47$, df = 33, $p < 0.00$), and time bin 2 (volumes 196–240), in which heart rate and CBF stabilized (and did not correlate ($r = -0.19$, df = 33, $p = 0.28$). Both groups did neither differ in heart rate at baseline ($t = -0.68$, df = 33, $p = 0.50$) nor after dAMPH administration in time bin 2 ($t = 0.43$, df = 33, $p = 0.67$).

Subsequently, to obtain CBF changes in the striatum specific to DA neurotransmission, global effects of dAMPH on brain vasculature were corrected by calculating the percentage difference between striatal CBF and GM CBF at that specific time point; specific striatal cerebral blood flow (ssCBF) = (striatal CBF-GM CBF)/GM CBF, as previously described, with minor adaptations (Khalili-Mahani *et al*, 2011) (Figure 2a and b).

Physiological data Acquisition

Heart rate and respiration were monitored during the whole-MRI scan session using a four-lead vector cardiogram signal. For the SPECT, heart rate and blood pressure was measured using a blood pressure cuff at baseline and every 2 min for 20 min, following the administration of dAMPH. In addition, the ECG was monitored during the administration of the drug.

Subjective Effects

As DA $D_{2/3}$ receptor binding may predict the behavioral responses to psychostimulants (Volkow *et al*, 1999), we also investigated the subjective responses to dAMPH. The

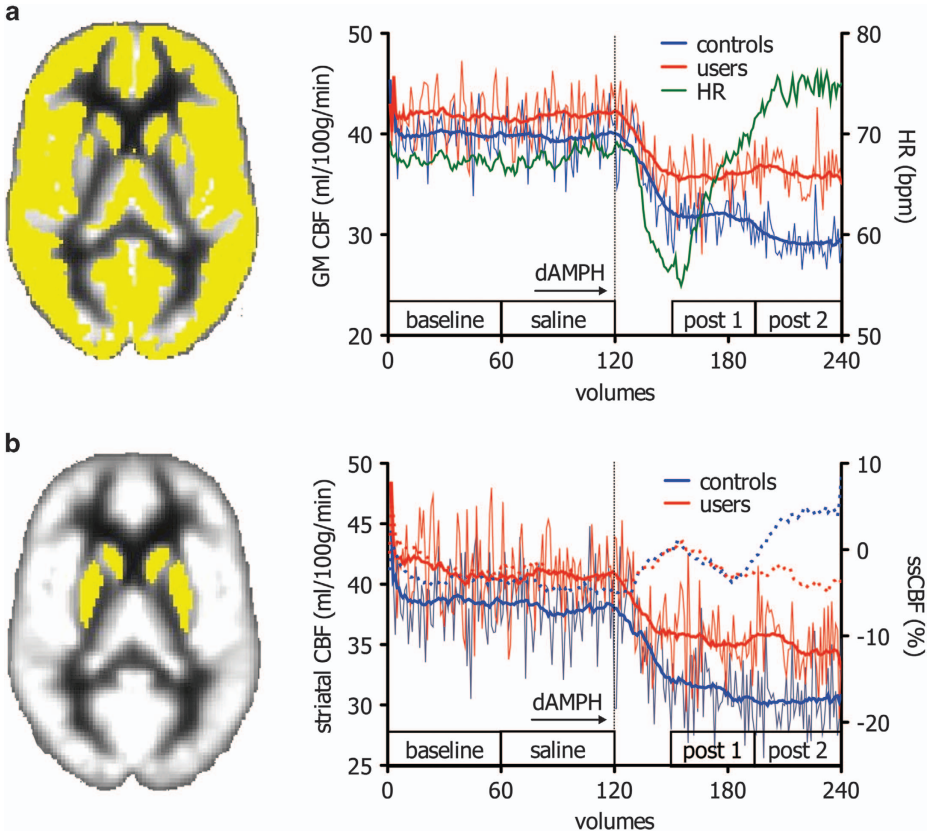

Figure 2 ASL time courses. (a) Grey matter ROI. Mean raw and smoothed grey matter (GM) CBF time courses for controls and users and mean heart rate (HR) for all subjects. (b) Striatal ROI. Mean raw and smoothed striatal time courses in users and controls (solid lines); specific striatal CBF (ssCBF) (dashed lines).

subjective responses were rated by measuring the participants' response to an analog self-rating scale that ranged from 1 (not at all) to 5 (extremely) that assessed 'liking' of the drug infusion (Van Kammen and Murphy, 1975). For the MRI scan, this was assessed directly before the ASL scan commenced, directly after it had finished, and again 20 min later when the participant finished the study day. For the SPECT session, these items were asked at baseline before the dAMPH administration, and at 2, 4, 8, 12, and 20 min subsequent to the dAMPH infusion, as well as at the end of the day. The average time for subjective effects to peak was 12 min for the SPECT and 20 min for the MRI (because this could not be assessed earlier) and the change from baseline to peak, expressed as percentage, was used for statistical analysis.

Statistical Analysis

The SPM8 toolbox was used with MATLAB (The Mathworks, Natick, MA) to process MRI data. IBM SPSS Statistics package Version 20 (SPSS, Chicago, IL) was used to conduct statistical tests on all data. Data were checked for normality and equality of variance. Where appropriate, non-parametric tests were performed. CBF, DA release, heart rate, and blood pressure were compared between groups using repeated measures ANOVA. Differences in baseline DA $D_{2/3}$ receptor binding between groups were calculated using a Student's *t*-test. DA release in each group was assessed using a paired *t*-test. To assess whether SPECT and MRI measures correlated, we used Pearson's *r* correlation coefficient. To examine whether subjective effects differed between groups, we used a logistic regression with a χ^2 significance test. Spearman's *r* correlation coefficient was subsequently used to examine the association between subjective effects and SPECT and phMRI imaging data. All data are presented as means with error bars representing the SD. The statistical significance value was set at $p<0.05$.

RESULTS

Eighteen recreational male dAMPH users (mean age = 21 years, SD = 2) and 20 male healthy controls (mean age = 21 years, SD = 3) were enrolled in this study. No differences between the two groups with regard to age or verbal intelligence were observed. Our dAMPH users used dAMPH typically as a powder, with the main routes of administration being inhalation or by dissolving it in a drink. Apart from anticipated differences between the two groups with respect to inclusion criteria on dAMPH use, they also differed significantly in the extent of tobacco, cannabis, alcohol, XTC, and cocaine use compared with the control group (Table 1).

SPECT Results

Baseline striatal DA $D_{2/3}$ receptor availability was significantly lower in dAMPH users (12%) than in controls ($t=2.363$, df = 33, $p=0.024$; Figure 1b). The challenge with dAMPH induced DA release in the control group by 10.5%

Table 1 Sample Characteristics

	dAMPH users, $N=18$		Controls, $N=20$		p-value
Demographics	**Mean**	**SD**	**Mean**	**SD**	
Age (years)	21.22	1.66	21.10	2.77	0.30
Education (years)[a]	8.72	2.03	8.35	1.87	0.80
IQ (DART)	104.44	5.00	104.16	8.72	0.13
Physiology					
Heart rate (beats/min)[b]	75.47	15.58	68.40	14.6	0.16
Blood pressure (mm Hg)[b]	130/63	126/67	0.53/0.14		
dAMPH use (lifetime)	**Median**	**SD**	**Median**	**SD**	p-value
Frequency[c]	40.00	59.5	—	—	
Usual dose (mg)	250.00	152.59	—	—	
Cumulative dose (g)	12.00	29.52	—	—	
Age of first use	18.00	1.94	—	—	
Duration of use	2.50	2.03	—	—	
Other substance use					
Tobacco (cigarettes/day)	7.50	4.54	0	0.72	<0.00*
Alcohol (units/week)	13.00	9.27	6.75	8.89	0.03*
Cannabis (joints/year)	104.00	157.41	1.2	19.39	<0.00*
MDMA (freq[c]/life time)	6.00	14.22	0	0.48	<0.00*
Cocaine (freq[c]/life time)	2.00	6.71	0	0	<0.00*

[a]Education completed in years from 12 years of age.
[b]Baseline heart rate, measured at the SPECT scanning.
[c]Number of occasions.

compared with baseline BP_{ND} ($t=3.936$, df = 18, $p<0.01$), whereas this effect was absent, or blunted, in dAMPH users (−2%; $t=-0.704$, df = 15, $p=0.49$). This difference between the groups was statistically significant ($t=2.363$, df = 33, $p=0.024$; Figure 1c), which suggests that baseline DA $D_{2/3}$ receptor availability and dAMPH-induced DA release are reduced in dAMPH users.

phMRI Results

Saline administration yielded no significant effects in either group ($F=0.858$, df = 34, $p=0.361$; Figure 1e). However, dAMPH induced a significant increase in ssCBF in controls (8%; $t=2.207$, df = 18, $p=0.04$), but this effect was absent in dAMPH users (1%; $t=-0.759$, df = 16, $p=0.459$; Figure 1f). Also this difference between both groups was statistically significant ($F=4.956$, df = 34, $p=0.03$; Figure 1f), indicative of a blunted DA functionality in dAMPH users. Striatal DA release as measured with SPECT did not correlate with DA functionality ($r=0.29$, df = 33, $p=0.09$), as measured with phMRI (Figure 3). The extent of previous dAMPH exposure was also not related to either measure.

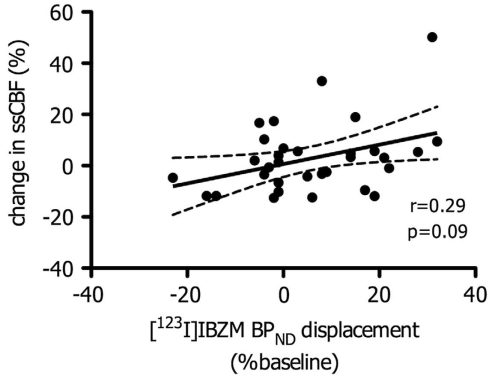

Figure 3 Correlation SPECT and phMRI. The correlation between dAMPH-induced [^{123}I]IBZM displacement and dAMPH-induced changes in ssCBF in the striatum.

Correlation Subjective Effects and Dopaminergic Changes

More control subjects liked the acute administration of dAMPH (ie, experienced a 'pleasant feeling') than dAMPH users. Overall, in subjects in the control group, 'feeling good' increased with an average of 36% (SD = 23.84) following dAMPH administration, compared with 23% (SD = 28.03) in the users. Moreover, the lower the $D_{2/3}$ availability, the more pleasant the dAMPH administration was experienced by controls ($r=-0.45$, df = 19, $p=0.05$), but not by dAMPH users ($r=0.28$, df = 15, $p=0.31$). No

association was present between the subjective response and either DA release ($r = 0.18$, $df = 31$, $p = 0.30$) or DA functionality as assessed with phMRI ($r = 0.20$, $df = 34$, $p = 0.25$).

DISCUSSION

We studied the dopaminergic system in recreational users of dAMPH at multiple physiological levels using SPECT, phMRI, and behavioral responses. We found reduced striatal DA $D_{2/3}$ receptor availability, blunted DA release and DA functionality in chronic dAMPH users relative to controls. We previously reported reduced striatal DAT binding in combined 3,4-methylenedioxymethamphetamine and dAMPH users (Reneman *et al*, 2002). In a subsequent SPECT study with a more selective DAT ligand, we observed reduced striatal DAT binding in the participants who primarily used dAMPH (Schouw *et al*, 2013b).

Our present findings are in line with the extensive preclinical literature, showing that prolonged dAMPH exposure affects the DA system: as reductions in tyrosine hydroxylase activity, long-term DA depletion, loss of the DAT and VMAT-2, reductions in post-synaptic receptors, and even dAMPH-induced apoptosis in certain cell types (Cadet *et al*, 2007; Krasnova *et al*, 2001, 2005, 2013; Ricaurte *et al*, 2005; Soto *et al*, 2012) have been reported. Multiple pathways have been shown to be involved in the neurotoxicity of dAMPH. High levels of DA, eg, induced by administration of amphetamines, can lead to the accumulation of reactive oxygen species, inducing severe oxidative stress. This can cause DA axonal degeneration as well as DNA damage, which can activate mitochondria-mediated cell-death mechanisms (Cadet *et al*, 2007). Taken together, our present findings provide important evidence that recreational use of dAMPH negatively affects the central DA system.

In addition, our findings are concordant with studies of other drugs of abuse that act on the DA system. Lower DA $D_{2/3}$ receptor availability is also found in cocaine and METH-dependent subjects (Volkow *et al*, 2001, 2014a). In addition, cocaine dependency is associated with a blunted DA release and lower levels of endogenous DA (Martinez *et al*, 2007, 2009; Volkow *et al*, 1997).

Interestingly, studies in adult non-human primates did not find any change in DA markers after prolonged administration of low doses of MPH of d-l-AMPH (Gill *et al*, 2012; Soto *et al*, 2012). However, a study in ADHD patients has found DAT upregulation after long-term MPH treatment (Wang *et al*, 2013). Therefore, future research is needed to assess the effect of low-dose long-term exposure of dAMPH on DA function. phMRI is an excellent candidate as this is non-invasive technique without using radiotracers and can therefore be used in longitudinal studies to investigate DA abnormalities.

Previous studies have shown that low DA $D_{2/3}$ receptor binding predicts more pleasant responses to reinforcing substances (Volkow *et al*, 1999). Our current findings in drug-naive subjects are in line with this and suggest that this coupling is lost in dAMPH users, most likely due to the dAMPH exposure. As with all cross-sectional studies, these initial results do not exclude the possibility that pre-existing differences between dAMPH users and controls contribute to our present findings, and low DA $D_{2/3}$ receptor levels may potentially predispose individuals to psychostimulant abuse by favoring their initial pleasant drug responses. Nevertheless, also a longitudinal study in non-human primates has shown that repeated use of dAMPH progressively reduced DA $D_{2/3}$ receptors (Ginovart *et al*, 1999), indicating that our DA $D_{2/3}$ receptor findings are most likely induced by dAMPH use.

PhMRI is thought to provide an index of DA neurotransmitter function, based on the principle that neurotransmitter-specific drug challenges evoke changes in neurovascular coupling and resultant changes in brain hemodynamics, such as CBF. Indeed, a phMRI study in rats has demonstrated a dose–response relationship between dAMPH and increases in CBF (Bruns *et al*, 2009). Also other preclinical studies, including in non-human primates, have shown that time-course changes in the phMRI signal parallel microdialysis measurements of striatal DA release, and also correlated very well with PET and SPECT measurements of DAT availability, as well as behavioral measures of DA dysfunction (Chen *et al*, 1997; Jenkins *et al*, 2004). In line with this, we obtained similar results with both techniques in the current study, ie, a blunted response to a dAMPH challenge in dAMPH users with SPECT imaging and phMRI. Therefore, although physiological variation in an experimental setting may contribute to some discrepancies between the current study and previous preclinical studies, this study also provides further evidence to the existing literature that phMRI is a powerful technique to investigate DA neurotransmission *in vivo*.

Polydrug use was very common in our sample of dAMPH users. It is thus possible that the reduced $D_{2/3}$ receptor availability and blunted DA responses may partly be due to drugs other than dAMPH. Of the drugs used (Table 1), however, only cocaine is known to significantly occupy the DAT and reduced striatal DA release has been reported in detoxified cocaine-dependent subjects (Volkow *et al*, 1997). But cocaine use, in contrast to dAMPH, was very low in our study sample, making it unlikely that the findings of the present study should be attributed to cocaine rather than dAMPH exposure. Other drugs of abuse that were used by our dAMPH group have also been shown to affect the DA system (Martinez *et al*, 2005, 2007; Volkow *et al*, 2014b). However, because none of these have direct neurotoxic effects on the DA system like amphetamine may have, it is thus unlikely that this underlies our results. We particularly acknowledge the difference in alcohol use between our groups as a limitation. We have chosen 30 units of alcohol/week as a cutoff score for exclusion, as alcohol and amphetamines are frequently used together (Hernandez-Lopez, 2002). Indeed, our dAMPH group used relatively large doses of alcohol. However, the absolute threshold levels of alcohol consumption that would constitute a risk for DA neurotoxicity have not yet been determined, and the underlying mechanisms remain to be established (Brodie *et al*, 1999). Although Volkow *et al* (2007) has shown reduced DA release in detoxified alcoholics in the ventral striatum, it has not been firmly established that alcohol is toxic for DAergic neurons, and/or may even protect against amphetamine-mediated DA neurotoxicity (Yu *et al*, 2002). In addition, both heavy and social alcohol users still show clear DA

release, and not a blunted effect, following alcohol administration (Oberlin *et al*, 2014; Urban *et al*, 2010). Thus, although alcohol use disorders have been associated with DAergic abnormalities, changes in the DA system have not been shown to occur in younger heavy drinkers, such as those present in our sample, as these appear to have normal DA release to controls. We further excluded the possibility of acute pharmacological effects of dAMPH on the DA system (apart from the challenge administered on the study day), as participants had to abstain from drug use for at least 1 week before the study, which was also confirmed with a urine drug test.

Another limitation is that the acute dAMPH challenge was administered twice, during the MRI and SPECT scan, which may have led to sensitization effects (Strakowski *et al*, 1996). However, we counterbalanced the visits within groups. In our statistical analyses, we found no session effects, thereby ruling out sensitization effects. To further account for volume effects on physiological parameters (such as heart rate) during phMRI scanning, a saline injection with the same volume preceded the dAMPH challenge. In contrast to the dAMPH challenge, the saline administration did not affect CBF nor heart rate or blood pressure. Furthermore, in this study we used a brain-dedicated SPECT system to analyze DA $D_{2/3}$ receptor binding and striatal DA release. For future studies, it would be of interest to test if our findings could be replicated using PET with DA $D_{2/3}$ radiotracers, which is due to its higher spatial resolution than clinical SPECT systems, would offer the possibility to study the effects of dAMPH in substructures of the striatum. A limitation of our study was that plasma concentrations of dAMPH were not assessed. However, we do not expect large differences between groups, because dAMPH was administered intravenously and was based on body weight.

Finally, we cannot exclude that part of the decrease in striatal DA $D_{2/3}$ binding might be related to differences in striatal volumes between dAMPH users and controls. However, a recent volumetric MR study in amphetamine users showed that the striatal volume may be even higher in users of amphetamine-type stimulants than in controls (Mackey *et al*, 2014). Also, Groman *et al*, (2013) showed a higher putamen gray matter in METH users, and moreover, Koester *et al* (2012) did not find a significant effect of amphetamine use on striatal volume. So, all in all, there is no indication that dAMPH use is associated with a lower striatal volume, which consequently may lead to lower striatal [^{123}I]IBZM binding. However, in future studies, it may be of interest to look into the effects of dAMPH on striatal volumes as well.

Taken together, we found that the recreational use of dAMPH is associated with a dysfunctional DA system, as observed using a multimodal imaging approach. These findings were paralleled by the subjective experience of the drug–responses. Our results are consistent with earlier studies in non-human primates and rodents that demonstrated DA neurotoxicity induced by dAMPH. Whereas our study not only highlights the potential of phMRI to assess changes in DA neurotransmitter function in humans, our main results are also particularly relevant for young users of this drug and call for caution when using dAMPH in a recreational setting. These results warrant further (longitudinal) investigations and bear considerable relevance for ADHD patients for whom these drugs are commonly prescribed.

FUNDING AND DISCLOSURE

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Author contributions

AS and LR were responsible for the study concept and design. In addition, DH, AN, and JB assisted in the setup of the experiments. AS and LV collected the data. AS, DH, and MC carried out ASL data processing and analysis. LV, DH, and AS conducted SPECT data processing and analysis. AS drafted the manuscript. All authors contributed to the interpretation and discussion of the data and edited the manuscript.

REFERENCES

Ashburner J (2007). A fast diffeomorphic image registration algorithm. *Neuroimage* **38**: 95–113.

Booij J, Korn P, Linszen DH, Royen van EA (1997). Assessment of endogenous dopamine release by methylphenidate challenge using iodine-123 iodobenzamide single-photon emission tomography. *Eur J Nucl Med* **24**: 674–677.

Boot E, Booij J, Hasler G, Zinkstok JR, Haan de L, Linszen DH *et al* (2008). AMPT-induced monoamine depletion in humans: evaluation of two alternative [123I]IBZM SPECT procedures. *Eur J Nucl Med Mol Imaging* **35**: 1350–1356.

Brodie MS, Pesold C, Appel SB (1999). Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcohol Clin Exp Res* **23**: 1848–1852.

Bruns A, Künnecke B, Risterucci C, Moreau J-L, Kienlin von M (2009). Validation of cerebral blood perfusion imaging as a modality for quantitative pharmacological MRI in rats. *Magn Reson Med* **61**: 1451–1458.

Cadet JL, Krasnova IN, Jayanthi S, Lyles J (2007). Neurotoxicity of substituted amphetamines: molecular and cellular mechanisms. *Neurotox Res* **11**: 183–202.

Castner S, Al-Tikriti MS, Baldwin RM, Seibyl JP, Innis RB, Goldman-Rakic PS (2000). Behavioral changes and [123I]IBZM equilibrium SPECT measurement of amphetamine-induced dopamine release in rhesus monkeys exposed to subchronic amphetamine. *Neuropsychopharmacology* **22**: 4–13.

Chen Y, Galpern WR, Brownell AL, Matthews RT, Bogdanov M, Isacson O *et al* (1997). Detection of dopaminergic neurotransmitter activity using pharmacologic MRI: correlation with PET, microdialysis, and behavioral data. *Magn Reson Imaging* **38**: 389–398.

Di Chiara G, Imperato A (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* **85**: 5274–5278.

Collins DL, Holmes CJ, Peters TM, Evans AC (1995). Automatic 3-D model-based neuroanatomical segmentation. *Hum Brain Mapp* **3**: 190–208.

European Monitoring Centre for Drugs and Drug Addiction (2012). Statistical Bulletin. Online publication: http://www.emcdda.europa.eu/stats12.

Gill KE, Pierre PJ, Daunais J, Bennett AJ, Martelle S, Gage HD *et al* (2012). Chronic treatment with extended release methylphenidate does not alter dopamine systems or increase vulnerability for cocaine self-administration: a study in nonhuman primates. *Neuropsychopharmacology* **37**: 2555–2565.

Ginovart N, Farde L, Halldin C, Swahn CG (1999). Changes in striatal D2-receptor density following chronic treatment with amphetamine as assessed with PET in nonhuman primates. *Synapse* **31**: 154–162.

Groman SM, Morales AM, Lee B, London ED, Jentsch JD (2013). Methamphetamine-induced increases in putamen gray matter associate with inhibitory control. *Psychopharmacology (Berl)* **229**: 527–538.

Hernandez-Lopez C (2002). 3,4-Methylenedioxymethamphetamine (Ecstasy) and alcohol interactions in humans: psychomotor performance, subjective effects, and pharmacokinetics. *J Pharmacol Exp Ther* **300**: 236–244.

Jenkins BG, Sanchez-Pernaute R, Brownell AL, Chen YCI, Isacson O (2004). Mapping dopamine function in primates using pharmacologic magnetic resonance imaging. *J Neurosci* **24**: 9553–9560.

Van Kammen DP, Murphy DL (1975). Attenuation of the euphoriant and activating effects of d- and l-amphetamine by lithium carbonate treatment. *Psychopharmacologia* **44**: 215–224.

Kegeles LS, Zea-Ponce Y, Abi-Dargham A, Rodenhiser J, Wang T, Weiss R *et al* (1999). Stability of [123I]IBZM SPECT measurement of amphetamine-induced striatal dopamine release in humans. *Synapse* **31**: 302–308.

Khalili-Mahani N, Osch MJP, van, Baerends E, Soeter RP, Kam de M, Zoethout RWM *et al* (2011). Pseudocontinuous arterial spin labeling reveals dissociable effects of morphine and alcohol on regional cerebral blood flow. *J Cereb Blood Flow Metab* **31**: 1321–1333.

Koester P, Tittgemeyer M, Wagner D, Becker B, Gouzoulis-Mayfrank E, Daumann J (2012). Cortical thinning in amphetamine-type stimulant users. *Neuroscience* **221**: 182–192.

Krasnova IN, Chiflikyan M, Justinova Z, McCoy MT, Ladenheim B, Jayanthi S *et al* (2013). CREB phosphorylation regulates striatal transcriptional responses in the self-administration model of methamphetamine addiction in the rat. *Neurobiol Dis* **58**: 132–143.

Krasnova IN, Ladenheim B, Cadet JL (2005). Amphetamine induces apoptosis of medium spiny striatal projection neurons via the mitochondria-dependent pathway. *FASEB J* **19**: 851–853.

Krasnova IN, Ladenheim B, Jayanthi S, Oyler J, Moran TH, Huestis MA *et al* (2001). Amphetamine-induced toxicity in dopamine terminals in CD-1 and C57BL/6J mice: complex roles for oxygen-based species and temperature regulation. *Neuroscience* **107**: 265–274.

Mackey S, Stewart JL, Connolly CG, Tapert SF, Paulus MP (2014). A voxel-based morphometry study of young occasional users of amphetamine-type stimulants and cocaine. *Drug Alcohol Depend* **135**: 104–111.

Marel K, van der, Klomp A, Meerhoff GF, Schipper P, Lucassen PJ, Homberg JR *et al* (2014). Long-term oral methylphenidate treatment in adolescent and adult rats: differential effects on brain morphology and function. *Neuropsychopharmacology* **39**: 263–273.

Martinez D, Gil R, Slifstein M, Hwang D-R, Huang Y, Perez A *et al* (2005). Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. *Biol Psychiatry* **58**: 779–786.

Martinez D, Greene K, Broft A, Kumar D, Liu F, Narendran R *et al* (2009). Lower level of endogenous dopamine in patients with cocaine dependence: findings from PET imaging of D(2)/D(3) receptors following acute dopamine depletion. *Am J Psychiatry* **166**: 1170–1177.

Martinez D, Narendran R, Foltin RW, Slifstein M, Hwang D-R, Broft A *et al* (2007). Amphetamine-induced dopamine release: markedly blunted in cocaine dependence and predictive of the choice to self-administer cocaine. *Am J Psychiatry* **164**: 622–629.

McCann UD, Ricaurte GA (2004). Amphetamine neurotoxicity: accomplishments and remaining challenges. *Neurosci Biobehav Rev* **27**: 821–826.

Oberlin BG, Dzemidzic M, Tran SM, Soeurt CM, O'Connor SJ, Yoder KK *et al* (2014). Beer self-administration provokes lateralized nucleus accumbens dopamine release in male heavy drinkers. *Psychopharmacology (Berl)*; doi:10.1007/s00213-014-3720-1.

Reneman L, Booij J, Lavalaye J, Bruin de K, Reitsma JB, Gunning WB *et al* (2002). Use of amphetamine by recreational users of ecstasy (MDMA) is associated with reduced striatal dopamine transporter densities: a [I-123] beta-CIT SPECT study-preliminary report. *Psychopharmacology (Berl)* **159**: 335–340.

Ricaurte GA, Mechan AO, Yuan J, Hatzidimitriou G, Xie T, Mayne AH *et al* (2005). Amphetamine treatment similar to that used in the treatment of adult attention-deficit/hyperactivity disorder damages dopaminergic nerve endings in the striatum of adult nonhuman primates. *J Pharmacol Exp Ther* **315**: 91–98.

Schmand B, Geerlings MI, Jonker C, Lindeboom J (1998). Reading ability as an estimator of premorbid intelligence: does it remain stable in emergent dementia? *J Clin Exp Neuropsychol* **20**: 42–51.

Schouw M, Kaag A, Caan M (2013a). Mapping the hemodynamic response in human subjects to a dopaminergic challenge with dextroamphetamine using ASL-based pharmacological MRI. *Neuroimage* **72**: 1–9.

Schouw MLJ, Caan MWA, Geurts HM, Schmand B, Booij J, Nederveen AJ *et al* (2013b). Monoaminergic dysfunction in recreational users of dexamphetamine. *Eur Neuropsychopharmacol* **23**: 1491–1502.

Soto PL, Wilcox KM, Zhou Y, Kumar A, Ator NA, Riddle MA *et al* (2012). Long-term exposure to oral methylphenidate or dl-amphetamine mixture in peri-adolescent rhesus monkeys: effects on physiology, behavior, and dopamine system development. *Neuropsychopharmacology* **37**: 2566–2579.

Strakowski SM, Sax KW, Setters MJ, Keck PE (1996). Enhanced response to repeated d-amphetamine challenge: evidence for behavioral sensitization in humans. *Biol Psychiatry* **40**: 872–880.

Substance Abuse and Mental Health Services Administration (2012). National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-46, HHS Publication No. (SMA) 13-4795. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2013.

Urban NBL, Kegeles LS, Slifstein M, Xu X, Martinez D, Sakr E *et al* (2010). Sex differences in striatal dopamine release in young adults after oral alcohol challenge: a positron emission tomography imaging study with [^{11}C]raclopride. *Biol Psychiatry* **68**: 689–696.

Videbaek C, Toska K, Scheideler MA, Paulson OB, Moos Knudsen G (2000). SPECT tracer [(123)I]IBZM has similar affinity to dopamine D2 and D3 receptors. *Synapse* **38**: 338–342.

Volkow ND, Chang L, Wang GJ, Fowler JS, Ding YS, Sedler MJ *et al* (2001). Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *Am J Psychiatry* **158**: 2015–2021.

Volkow ND, Tomasi D, Wang G-J, Logan J, Alexoff DL, Jayne M *et al* (2014a). Stimulant-induced dopamine increases are markedly blunted in active cocaine abusers. *Mol Psychiatry* **19**: 1037–1043.

Volkow ND, Wang G-J, Fowler JS, Logan J, Gatley SJ, Gifford A *et al* (1999). Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. *Am J Psychiatry* **156**: 1440–1443.

Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Hitzemann R *et al* (1997). Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. *Nature* **386**: 830–833.

Volkow ND, Wang G-J, Telang F, Fowler JS, Alexoff D, Logan J *et al* (2014b). Decreased dopamine brain reactivity in marijuana abusers is associated with negative emotionality and addiction severity. *Proc Natl Acad Sci USA* **111**: E3149–E3156.

Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Jayne M *et al* (2007). Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. *J Neurosci* **27**: 12700–12706.

Wang G-J, Volkow ND, Wigal T, Kollins SH, Newcorn JH, Telang F *et al* (2013). Long-term stimulant treatment affects brain dopamine transporter level in patients with attention deficit hyperactive disorder. *PLoS One* **8**: e63023.

Wang J, Alsop DC, Li L, Listerud J, Gonzalez-At JB, Schnall MD *et al* (2002). Comparison of quantitative perfusion imaging using arterial spin labeling at 1.5 and 4.0 Tesla. *Magn Reson Med* **48**: 242–254.

Wilson JM, Kalasinsky KS, Levey AI, Bergeron C, Reiber G, Anthony RM *et al* (1996). Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nat Med* **2**: 699–703.

Yu L, Cherng C-FG, Chen C (2002). Melatonin in concentrated ethanol and ethanol alone attenuate methamphetamine-induced dopamine depletions in C57BL/6J mice. *J Neural Transm* **109**: 1477–1490.