

# Monoaminergic dysfunction in recreational users of dexamphetamine



M.L.J. Schouw<sup>a,b,\*</sup>, M.W.A. Caan<sup>a,b</sup>, H.M. Geurts<sup>c</sup>, B. Schmand<sup>c,d</sup>,  
J. Booij<sup>b,e</sup>, A.J. Nederveen<sup>a,b</sup>, L. Reneman<sup>a,b</sup>

<sup>a</sup>Department of Radiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

<sup>b</sup>Brain Imaging Center at the Academic Medical Centre, Amsterdam, The Netherlands

<sup>c</sup>Faculty of Social and Behavioral studies, Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands

<sup>d</sup>Department of Neurology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

<sup>e</sup>Department of Nuclear Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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## Abstract

Preclinical studies suggest that dexamphetamine (dAMPH) can lead to monoaminergic neurotoxicity. This exploratory study aimed to investigate effects of recreational dAMPH use on the dopamine (DA) and noradrenaline (NA) systems in humans. To that purpose, eight male abstinent dAMPH ( $26.0 \pm 4.0$  years) users and 10 age- and IQ-matched male healthy control subjects ( $23.0 \pm 3.8$ ) underwent neuropsychological testing sensitive to DAergic function and single photon emission computed tomography (SPECT) scanning with [ $^{123}$ I]FP-CIT to determine striatal DA transporter (DAT) binding. In addition, changes in cerebral blood flow (CBF) induced by the DA/NA reuptake inhibitor methylphenidate (MPH) were measured using pharmacological magnetic resonance imaging (phMRI). Performance of dAMPH users was significantly worse on executive function and verbal memory tasks. Striatal DAT binding ratios were on average lower in dAMPH users (near-significant,  $p=0.05$ ). In addition, CBF in control subjects decreased significantly in response to MPH in gray matter and basal ganglia, among which the striatum, thalamus and hippocampus by 10% to 29%. However, in dAMPH users the CBF response was blunted in most brain areas studied, only decreasing in the hippocampus and orbitofrontal cortex. When comparing groups, CBF response was found to be significantly different in the thalamus with a decrease for healthy controls and a blunted response in dAMPH users. Collectively, our findings of a blunted hemodynamic response in monoaminergic regions, in combination with indications for lower striatal DAT binding and poorer behavioral measures are likely to represent DAergic dysfunction in dAMPH users, although NAergic dysfunction may also play a role.

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\*Corresponding author at: Department of Radiology, Room G1-241, PO Box 22660, 1100 DD Amsterdam, The Netherlands. Tel.: +31 20 5668323.

E-mail addresses: m.l.schouw@amc.uva.nl, mljschouw@gmail.com (M.L.J. Schouw).

## 1. Introduction

The dopaminergic (DAergic) system plays a pivotal role in many different neurological and neuropsychiatric disorders, such as Parkinson's disease, schizophrenia and attention deficit hyperactivity disorder (ADHD) (for review (Missale et al., 1998)). Consequently, many drugs have been developed, that act on the dopamine transporter (DAT) or dopamine receptors. Of these drugs, dexamphetamine (dAMPH) is prescribed for the treatment of ADHD, but is also frequently used in recreational settings. Preclinical studies in non-human primates however, have indicated that even clinically relevant doses of dAMPH can lead to damage of nerve terminals of DAergic neurons: the concentration of striatal DA, the DAT density and vesicular monoamine transporter (VMAT-2) sites were significantly reduced after dAMPH administration (Ricaurte et al., 2005). Although some positron emission tomography (PET) studies reported loss of striatal DAT in methamphetamine (METH) users (McCann et al., 1998, 2008), little is known about the effects of recreational dAMPH use on the DA system in human users. To our knowledge, only one study has previously investigated the effects of dAMPH in the human brain. In this study reductions in striatal DAT binding were observed using single photon emission computed tomography (SPECT) (Reneman et al., 2002) in combined ecstasy and dAMPH users versus sole ecstasy users. Because DA is involved in many important neurobehavioral functions, such as executive and motor function, attention and inhibition, it is important to study the potential consequences of dAMPH-induced neurotoxicity (Van den Heuvel and Pasterkamp, 2008).

The purpose of the current study was to investigate the effects of recreational dAMPH use on the DAergic system using SPECT and a relatively novel MRI imaging technique, called pharmacological MRI (phMRI), to assess DA (dys)function. phMRI indirectly determines monoaminergic function by looking at the hemodynamic response to a pharmacological challenge (Ogawa et al., 1990). Neurotransmitter-specific drug challenges can evoke changes in synaptic activity, and resultant alterations in metabolic demand, requiring changes in cerebral blood flow (CBF) and/or cerebral blood volume (CBV) as a result of neurovascular coupling (Jueptner and Weiller, 1995). For instance, Jenkins and co-workers (Jenkins et al., 2004) have shown that phMRI adequately assesses DA dysfunction, as dAMPH-induced hemodynamic changes correlated well with loss of DAT densities measured in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned parkinsonian primates, using PET. Interestingly, a recent imaging study showed a reduced amphetamine-induced DA release in cocaine users (Martinez et al., 2007). In addition several behavioral studies have linked DAergic dysfunction to deficits in attention, memory and executive function (for review (Robbins and Arnsten, 2009)).

In this explorative study, we assessed brain monoamine function in dAMPH users and comparison subjects, by examining the hemodynamic response to an acute oral challenge with methylphenidate (MPH). MPH is a DAT blocker causing increased levels of synaptic DA, although it also seems to have effects on norepinephrine (NE) levels, by blocking also the NE transporter (NET) (Hannestad et al., 2010; Volkow et al., 1998). We hypothesized that dAMPH users would have lower striatal DAT binding levels than

controls and a blunted hemodynamic response to MPH. We also predicted that dAMPH users would show an altered performance on tests of attention, memory and executive function.

## 2. Experimental procedures

### 2.1. Study procedure

Participants underwent phMRI with an oral MPH challenge, followed by [<sup>123</sup>I]FP-CIT SPECT scanning (to measure DATs) 1-3 weeks later. In addition, neuropsychological tests and questionnaires were administered on the day of the SPECT scan.

#### 2.1.1. Subjects and study design

The medical ethics committee of the Academic Medical Center in Amsterdam approved the study procedures. Participants were recruited by advertising on the medical campus, websites and in newspapers. They received a small financial compensation of 50 euros per assessment day.

Eight male, recreational users of dAMPH and 10 male, healthy control subjects (matched for age and IQ) participated. Written informed consent was obtained from all subjects. The eligibility criterion for the dAMPH group was previous use of dAMPH on more than 40 occasions. The cut-off point was based on previous studies from our group, where lower striatal DAT binding was found in ecstasy users with combined dAMPH use (Reneman et al., 2002). The control subjects were healthy subjects with no self-reported prior use of dAMPH.

Subjects were asked to refrain from using caffeinated products on assessment days. In addition, both controls and dAMPH users agreed to abstain from all psychoactive drugs for at least 2 weeks before scanning sessions. They underwent urine drug screening on assessment days (with an enzyme-multiplied immunoassay for amphetamines, cocaine, cannabis, alcohol, opiates and benzodiazepines). Exclusion criteria were: self-report of neuropsychiatric diagnosis, history of brain disease or injury, use of medication influencing the DAergic system (e.g., methylphenidate), a positive urine-screen for amphetamines or any contra-indication to MRI.

#### 2.1.2. Behavioral tests

The Dutch Adult Reading Task (DART) was used to estimate IQ (Schmand et al., 1998). The DART is the Dutch counterpart of the National Adult Reading Test (Nelson, 1982). It is relatively insensitive to cognitive deterioration due to neurologic disorders and was used to evaluate the premorbid IQ.

We selected five domains sensitive to DAergic function: executive function, attention, memory, mood and impulsivity. Tests were administered in a mixed balanced order, with questionnaires presented during the recall part of the RAVLT, to ascertain that no other tasks of a verbal nature (e.g. DART) would interfere.

Executive function was measured using the trail making test. Part A of this test (TMT-A), in which the subject draws lines connecting consecutive numbers spread randomly on the page, tests both visual scanning and motor speed (Reitan, 1956). Part B of this test (TMT-B) alternates numbers with letters, focusing more on executive function and visuomotor tracking. The ratio of times to complete both parts of the test (TMT-B/TMT-A) was calculated to assess executive function taking individual differences in visual scanning and motor speed into account.

The sustained attention to response task (SART) displays numbers one through nine randomly, followed by a mask and cue. The subject needs to respond to the cue, except when the number three appears (Bellgrove et al., 2005). The mask insures that speed-accuracy trade-offs are of no major influence on the outcome. Response variability was calculated (standard deviation go

RT/ mean go RT) as well as the number of correct and incorrect responses.

Verbal memory was assessed using the Rey Auditory Verbal Learning Task (RAVLT (Rey, 1964)), in which subjects memorize 15 words in five learning trials. Following a 20-min delay, the word list has to be recalled. Total scores were used for both direct and delayed recall.

Mood and impulsivity were determined using validated questionnaires: the Beck Depression Inventory (BDI, (Beck, 1961)), Behavioral Approach Scale (BAS, (Carver, 1994)) and Barratt Impulsivity Scale (BIS (Patton et al., 1995)). The BDI measures depression severity, with higher scores indicating more severe depressive symptoms. The BAS evaluates the behavioral approach system, regulating an individual's tendency to act on positive impulses. High scores on the BAS tend to correlate with impulsive personalities (Carver, 1994). The BIS is aimed at motor impulsivity, cognitive impulsivity and non-planning (Patton et al., 1995).

Finally, subjective emotional experience of MPH were measured using simulated visual analog rating scales (VAS) 5 min before each MRI scanning session. Subjects rated anxious feelings, tension, feeling comfortable or uncomfortable, lightheadedness, wakefulness, drowsiness and nausea on a scale of 1-10.

### 2.1.3. SPECT

Striatal DAT binding was measured using SPECT with the radioligand [ $^{123}\text{I}$ ]N- $\omega$ -fluoropropyl-2 $\beta$ -carbomethoxy-3 $\beta$ -(4-iodophenyl)nortropane ([ $^{123}\text{I}$ ]FP-CIT). [ $^{123}\text{I}$ ]FP-CIT is a well-validated radiotracer for investigation of DAT availability, and was prepared as previously described (Booij et al., 1998). Potassium iodide was used to block thyroid uptake of free radioactive iodide. SPECT images were acquired with a brain-dedicated SPECT system (Neurofocus, an update of the Strichmann Medical Equipment 810X, Strichmann Medical Equipment Inc., Medfield, Mass., USA). This is a 12-detector single-slice scanner with a full width at half maximum resolution of 6-7 mm. SPECT images were acquired as previously described (de Win et al., 2005). Acquisition was commenced 3 h after i.v. injection of approximately 112 MBq (3.02 mCi) [ $^{123}\text{I}$ ]FP-CIT, since at this time peak specific binding to DATs in the striatum has been reached (Booij et al., 1998).

### 2.1.4. SPECT processing

Reconstruction and attenuation correction of all images were performed as previously described (Booij et al., 1997b). A striatum ROI and separate ROIs for putamen and caudate nucleus were used, which were positioned on the three consecutive SPECT slices corresponding with the highest striatal activity. To correct for background radioactivity (non-specific binding) a predefined region of interest (ROI) was placed over the occipital cortex. Finally, specific to non-specific binding ratios were calculated (ROI counts—occipital cortex counts/occipital cortex counts).

### 2.1.5. PhMRI

MR imaging was performed using a 3.0T Philips MR scanner equipped with an SENSE 8-channel head coil and body coil transmission (Philips Medical Systems, Best, The Netherlands). The protocol consisted of a high resolution 3DT1-weighted anatomical scan for registration and segmentation purposes and a phMRI sequence.

For the phMRI acquisition, we used a pulsed arterial spin labeling (ASL) sequence, based on the PULSAR sequence (Golay et al., 2005). ASL is a non-invasive perfusion imaging modality, using magnetically labeled blood water protons as an endogenous tracer of CBF. Contrary to the more traditionally used BOLD signal, the ASL signal does not suffer from signal drifts in time, making it very suitable to investigate pharmacologically-induced CBF changes (Wang et al., 2002).

phMRI imaging parameters were: TR/TE 3000/14 ms; FOV 240  $\times$  240 mm $^2$ ; matrix size 80  $\times$  79; 17 slices; thickness 7 mm; no

gap; gradient echo single shot EPI; SENSE 2.0; post-labeling delay 1.2 to 2 s; the labeling plane was positioned parallel to the imaging volume with a labeling gap between the imaging volume and the labeling volume of 25 mm. We acquired 100 dynamics (approximately 10 min of scanning time) at baseline and 100 dynamics 1.5 h after administering 35 mg MPH, when it is assumed to be at its peak concentration in the brain (Volkow et al., 1998). Methylphenidate was obtained from Sandoz BV (Weesp, the Netherlands).

### 2.1.6. MR processing

FSL 4.1 (FMRIB-Software-Library, Functional Magnetic Resonance Imaging of the Brain Centre, University of Oxford, UK), the SPM8 toolbox and Matlab (The MathWorks Inc., Natick, USA) were used for offline data processing.

Subtraction of labeled and control ASL images yielded whole brain perfusion weighted images. The mean equilibrium magnetization ( $M_0$ ) of arterial blood per subject was calculated, from which absolute CBF was computed following methods previously described (Chalela et al., 2000). The images were averaged over 100 volumes (10 min) to increase the signal-to-noise ratio (SNR), creating average CBF maps from the baseline and post-challenge scans. One scan from the dAMPH group was excluded due to poor image quality. Average CBF maps were transformed into anatomical space by affine registration to the segmented gray matter masks of corresponding anatomical scans.

3DT1 anatomical scans were segmented into gray and white matter using SPM8. The structural images were non-rigidly normalized to a population-based average using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) (Ashburner, 2007), to allow for a voxel-based analysis. The non-rigid transformations were applied to the average CBF maps, such that all dynamics of all subjects resided in one common frame of reference. An isotropic resampling of 3.0 mm was chosen. Finally, a Gaussian smoothing with FWHM=6 mm was applied to all volumes. Mean perfusion was calculated for the following ROIs taken from the Harvard-Oxford brain atlas provided with FSL 4.1: striatum, prefrontal cortex (PFC), anterior cingulate cortex (ACC), hippocampus, thalamus and cerebellum. The voxel-based analysis was restricted to gray matter, where the CBF can be sufficiently precisely measured, by means of a mask.

## 2.2. Statistical analysis

Continuous variables of group characteristics were analyzed using unpaired two-tailed Student's *t*-tests (log transformed if necessary) and Mann-Whitney tests for drug history variables. Differences in behavioral measures between the two groups on the five cognitive domains were analyzed using multivariate analysis of variance (MANOVA). To limit the number of statistical comparisons per group, TMT and RAVLT were grouped and SART was analyzed in a separate MANOVA as well as results for mood and impulsivity questionnaires. Striatal DAT binding ratios measured with SPECT for whole striatum and putamen and caudate nucleus separately were analyzed using the Mann-Whitney test.

PhMRI data was analyzed by calculating the effect of challenge through paired *t*-testing between baseline and post-challenge scans for both groups separately. Both a ROI-based and a voxel-based analysis were performed. Thus, in the ROI-based analysis we first compared group baseline differences in CBF, followed by comparing baseline with post-MPH scans using paired *t*-test for both groups separately, and finally unpaired *t*-tests to compare the percentage CBF change between the two groups. In the voxel-based analysis, a minimal cluster size of 100 voxels was maintained, besides a significance threshold of  $p=0.01$ . Also in the voxel based analysis, baseline differences and response to the challenge were examined, as well as an interaction analysis to determine any difference in response to MPH between groups.

Finally, correlations were calculated between DAT binding ratios and percentage CBF change in the striatum, and between significantly differing behavioral measurements and percentage CBF change.

All data were analyzed in SPSS version 18.0 (SPSS Inc, Chicago, Ill) and are presented as mean  $\pm$  SD unless otherwise indicated.

### 3. Results

#### 3.1. Characteristics of the sample and behavioral measures

Group characteristics are presented in Table 1. Age, pre-morbid IQ and years of education were similar in both groups. Mean cumulative lifetime exposure to dAMPH was 353 g ( $\pm$ 465, range of use 0.5-3 g per occasion) and time since last dose on average 1.1 ( $\pm$ 1.3) months. dAMPH users had on average used more other recreational drugs of abuse and tobacco than the control subjects.

The behavioral scores on the five DAergic domains (attention, executive control, memory, mood and impulsivity) are shown in Table 2. A significant main effect was revealed on MANOVA of executive function and memory. dAMPH users were significantly slower on TMT-B/TMT-A when compared to controls. dAMPH users were also slower on TMT-A, but this did not reach statistical significance ( $p=0.06$ ). Both on the immediate and delayed verbal learning task, dAMPH users recalled less words than the control subjects, reaching statistical significance only for the delayed recall. Mean group scores for attention, mood and impulsivity (SART, BDI, BAS and BIS scales) did not differ between groups.

Before the MPH challenge, no differences were noted on the VAS scores between dAMPH users and healthy controls. However, after the challenge, control subjects

felt significantly less sleepy ( $-0.9 \pm 1.0$ ;  $p=0.02$ ) and more lightheaded ( $1.7 \pm 1.7$ ;  $p=0.01$ ), in contrast to dAMPH users who did not show such an effect. When comparing the change in VAS scores, there was no significant difference on any of the subjective measures between the groups.

No significant correlations were observed between the TMT-B/TMT-A ratio and percentage CBF change in the PFC (linked mainly to executive functions), nor between the delayed recall and the percentage CBF change in the hippocampus (linked mainly to memory).

#### 3.2. SPECT

No differences in binding between left and right hemispheres were detected, therefore we present grouped data. Binding ratios did not differ significantly between dAMPH users and healthy controls. However, all ROIs studied (whole striatum, caudate nucleus and putamen) showed 8.4-10.4% lower binding ratios (Fig. 1) in dAMPH users and a trend was seen for the whole striatum and putamen ( $p=0.06$  and  $p=0.05$ , respectively). When comparing only non-smoking subjects (healthy controls  $n=8$ ; dAMPH  $n=3$ ) no significant differences in striatal DAT binding ratios were observed between the groups. No significant correlations were observed between DAT binding ratios and the percentage CBF change in the striatum.

#### 3.3. 3 pHMRI

##### 3.3.1. Pre MPH CBF measures: ROI and voxel based analysis

ROI analysis did not show any significant differences in baseline CBF measurements between dAMPH users and healthy controls. Voxel-based analysis, however, showed

**Table 1** Demographics characteristics of drug exposure for dAMPH users and controls with ( $\pm$ SD) and  $p$ -values for  $t$ -test (Age, IQ and Years of education) or Mann-Whitney U test. Significant  $p$ -values ( $<0.05$ ) marked with\*.

	dAMPH users $n=8$	Controls $n=10^{**}$	$p$ -value
Age	26.0 ( $\pm$ 4.0)	23.0 ( $\pm$ 3.8)	0.1
DART-IQ	104.5 ( $\pm$ 3.0)	108.3 ( $\pm$ 6.4)	0.1
Years of education	15.1 ( $\pm$ 3.6)	16.9 ( $\pm$ 2.9)	0.3
<i>dAMPH</i>			
Average dAMPH use (occasions/year)	27.8 ( $\pm$ 17.1)	0	0.00*
Duration of dAMPH use (years)	13.9 ( $\pm$ 8.7)	NA	NA
Usual dose (grams/occasion)	0.8 ( $\pm$ 1.2)	NA	NA
Total exposure (grams)	352.6 ( $\pm$ 465.3)	0	0.00*
Time since last exposure (months)	1.1 ( $\pm$ 1.3)	NA	NA
<i>Other substances</i>			
Average tobacco use (cigarettes/month)	261.0 ( $\pm$ 279.8)	0.4 ( $\pm$ 1.3)	0.02*
Average alcohol use (units/month)	103.5 ( $\pm$ 146.6)	101.8 ( $\pm$ 78.6)	0.4
Average cannabis use (joints/year)	410.3 ( $\pm$ 480.5)	17.2 ( $\pm$ 30.4)	0.02*
Average MDMA use (pills/year)	3.8 ( $\pm$ 10.6)	0	0.3
Average cocaine use (occasions/year)	5.0 ( $\pm$ 5.2)	0.1 ( $\pm$ 0.3)	0.006*

NA=Not applicable.

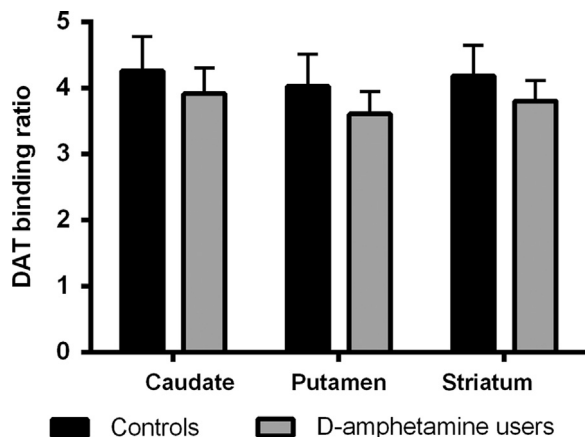
\*\*Drug history questionnaire missing for 1 control subject.

**Table 2** Behavioral measures all measures reflected are averaged means ( $\pm$  standard deviation). *p*-values were obtained using MANOVA. NS= not significant; *p*= *p*-value; *F*= *F*-value; ES= Effect size (Cohen's *d*).

	Controls* <i>n</i> =10	dAMPH <i>n</i> =8	<i>p</i>	<i>F</i>	ES
MANOVA-group			0.02		
<b>Executive function</b>					
TMT-A (seconds)	16.7 ( $\pm$ 2.9)	23.4 ( $\pm$ 9.5)	0.06	4.17	-1.08
Ratio TMT-B/TMT-A	1.91 ( $\pm$ 0.2)	2.65 ( $\pm$ 0.8)	0.04	4.82	-1.48
<b>Memory function</b>					
RAVLT immediate	52.8 ( $\pm$ 8.4)	43.8 ( $\pm$ 8.6)	0.10	3.07	1.06
RAVLT delayed	11.8 ( $\pm$ 2.6)	9.5 ( $\pm$ 2.7)	0.02	6.94	0.87
MANOVA-group		NS			
<b>Attention</b>					
SART correct go RT (milliseconds)**	132.3 ( $\pm$ 48.9)	174.3 ( $\pm$ 35.6)			-0.99
SART response variability	0.50 ( $\pm$ 0.1)	0.39 ( $\pm$ 0.07)			1.29
SART incorrect go (number of trials)	21.6 ( $\pm$ 15.7)	10.0 ( $\pm$ 10.2)			0.90
SART incorrect nogo (number of trials)	2.3 ( $\pm$ 3.3)	2.6 ( $\pm$ 3.0)			-0.10
MANOVA-group		NS			
<b>Mood</b>					
BDI	1.6 ( $\pm$ 2.7)	3.3 ( $\pm$ 1.8)			-0.76
<b>Impulsivity</b>					
BIS	62.4 ( $\pm$ 8.7)	66.3 ( $\pm$ 8.4)			-0.46
BAS	26.8 ( $\pm$ 3.3)	30.0 ( $\pm$ 5.3)			-0.74

\*Neuropsychological data for one control subject was not available.

\*\*Two control and one dAMPH data set from the SART had to be excluded due to poor performance (>25% missed go trials).



**Fig. 1** DAT binding ratios in the putamen, caudate nucleus and whole striatum of dAMPH users and healthy controls. Although dAMPH users showed lower binding ratios, no statistical significance was observed between groups. Error bars reflect standard deviation.

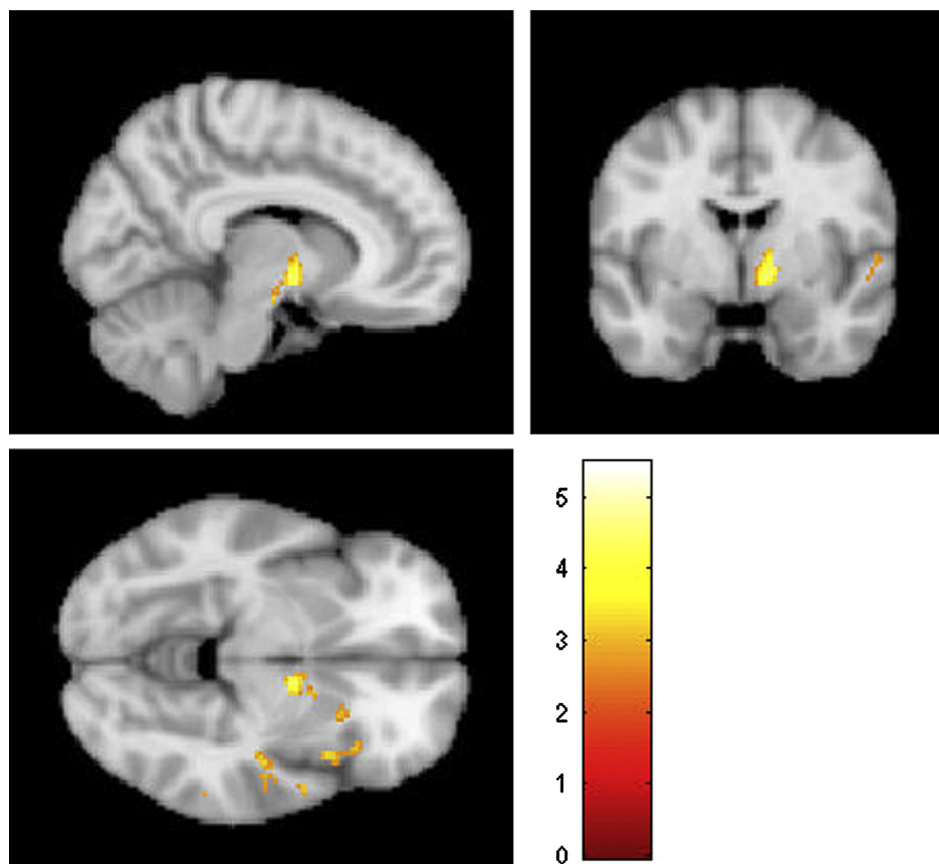
that baseline CBF was higher in the left thalamus (peak level  $Z=3.48$ ,  $p<0.0001$ , uncorrected) and insula (peak level  $Z=3.61$ ,  $p=0.000$ , uncorrected) of dAMPH users compared to healthy controls (Fig. 2).

### 3.3.2. Post MPH CBF measures

**3.3.2.1. ROI-based analysis.** The oral challenge with MPH caused an overall reduction in gray matter CBF of  $-15.8\%$  in control subjects (Fig. 3). In contrast, in dAMPH users MPH did not induce any changes in CBF: a non-significant increase of  $1.0\%$  was observed. In several ROIs in control subjects we observed a significant reduction in CBF: the striatum— $9.6\%$ , hippocampus— $28.1\%$ , thalamus— $28.1\%$  and PFC— $11.6\%$ . In dAMPH users we observed a significant decrease of CBF in the hippocampus of  $-21.0\%$ . The average CBF and *p*-values are represented in Table 3.

We also examined the group difference in response to MPH using unpaired *t*-testing in the percentage CBF change. A significant interaction effect was observed in the thalamus: healthy controls showed a decrease of  $28.1\%$  where dAMPH users showed an increase in CBF of on average  $34.9\%$  ( $p=0.03$ ).

**3.3.2.2. Voxel-based analysis.** When examining the data using a voxel-based approach, several brain areas in healthy controls showed statistically significant decreases in CBF (Fig. 4a). In agreement with the ROI based analysis, the striatum, thalamus, hippocampus and PFC showed a reduction in CBF following an acute challenge with MPH in control subjects with the most significant cluster located in the right striatum (peak level  $Z=4.92$ ,  $p=0.000$ ). The voxel-based analysis identified additional regions to the ROI based analysis in which MPH induced a reduction in CBF: the



**Fig. 2** Statistical parametric map of baseline CBF differences between healthy controls and users of dAMPH. Color bars indicate z-scores of areas where CBF is larger in dAMPH users compared to controls. Lower CBF values were not observed. Statistic images were thresholded to a cluster significance threshold of  $p=0.01$  with a minimum cluster size of 100.

insula, the sensorimotor and visual cortex. In the group of dAMPH users we only observed a significant decrease in CBF in the hippocampus (similar to the ROI based analysis) and in the (orbitofrontal) PFC (Fig. 4b). In the healthy controls, a small area of the orbitofrontal cortex demonstrated an increase in CBF (Fig. 4c). In dAMPH users no increases in CBF were seen in response to the MPH challenge. When examining the interaction effect of group  $\times$  challenge (Fig. 4d), the main difference in response between the groups is located in the (left) thalamus (peak level  $Z=3.29$ ,  $p=0.000$ ).

## 4. Discussion

The results of this study show that several indices of monoaminergic function in recreational dAMPH users differ from those in healthy controls. In addition to a blunted hemodynamic response to a challenge with MPH in DAergic brain regions, we found a trend toward reduced DAT binding in the striatum. Finally, dAMPH users showed impaired performance on executive function and memory.

### 4.1. Baseline behavioral measures

DA function in the PFC in particular is linked to cognitive processes, where DA cells connecting the PFC to several limbic structures, including the amygdala, nucleus accumbens and

hippocampus, have a regulatory role in several cognitive processes (de Almeida et al., 2008). In view of our hypothesis on DA dysfunction in dAMPH it is therefore not surprising that we observed impaired functions in two cognitive domains mediated by the mesocortical pathways (DAergic projections from the ventral tegmental area to the cortex, particularly the PFC) and mesolimbic pathways (projections from the ventral tegmental area to the limbic system and PFC) of dAMPH users, namely: executive functioning and memory consolidation. Our findings of frontal-executive dysfunction in dAMPH users are in agreement with a study by Ornstein et al. (2000). Furthermore, previous studies have shown impaired memory function in users of this drug (Leventhal et al., 2010; Rapeli et al., 2005).

#### 4.1.1. Post MPH behavioral measures

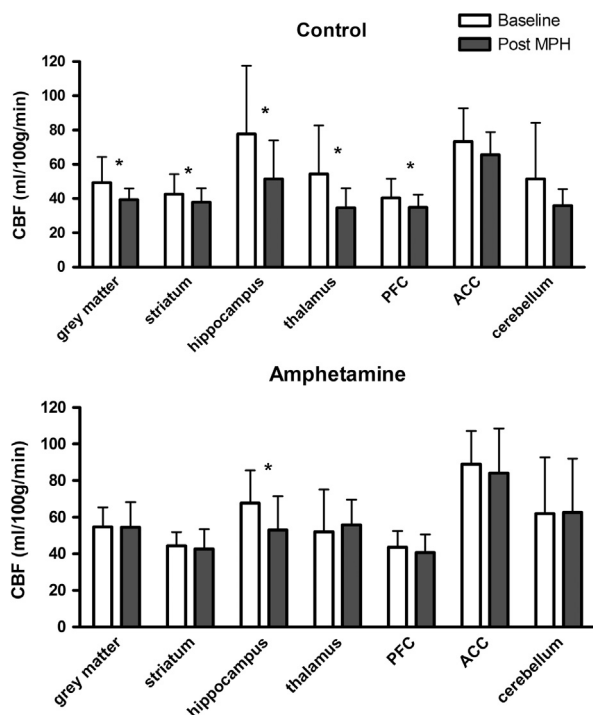
Only control subjects showed a small increase in light-headedness and decreased sleepiness in response to the MPH administration, although there was no interaction effect between the groups. Our findings are in line with other studies that show that even doses as small as 20 mg of MPH can elicit significant changes in measures of subjective drug effect, such as ratings for 'feel an effect' and 'like the drug effect' (Spencer et al., 2006). Interestingly, the absence of an effect of MPH on the VAS score in dAMPH users is in agreement with the decidedly blunted effect of

MPH on CBF. This may reflect monoaminergic dysfunction in dAMPH users.

## 4.2. Baseline imaging data

### 4.2.1. SPECT

Although we found no statistically significant difference in striatal DAT binding when comparing the groups, a trend toward a lower binding (approximately 10%) was observed in the putamen and striatum as a whole. Previous studies showed a relative high inter-individual variation in striatal DAT binding (Booij et al., 2007; Ziebell et al., 2010). Consequently, due to the small sample examined, we can



**Fig. 3** Mean CBF (ml/100 g/min) in the total gray matter and predefined ROIs before MPH (light gray bars on the left) and after ingestion of 35 mg of MPH (dark gray bars on the right) for control subjects (upper panel) and dAMPH users (lower panel) separately. Error bars reflect standard deviation. \*: statistically significant differences between conditions.

not exclude that the trend for a lower striatal DAT binding in our dAMPH users in fact reflects this variation. Also, although the radiotracer [ $^{123}\text{I}$ ]FP-CIT binds with high affinity to the DAT, it binds also with a lower, but still moderate, affinity to serotonin transporters (Booij et al., 1997a; Ziebell et al., 2010). Therefore, we cannot exclude that the lower striatal [ $^{123}\text{I}$ ]FP-CIT binding in dAMPH users reflects changes in serotonin transporter expression. However, in a previous study in healthy controls we showed that oral administration of the selective serotonin transporter inhibitor paroxetine did not induce a significant lower striatal [ $^{123}\text{I}$ ]FP-CIT binding (Booij et al., 1997a). In addition, PET studies in dAMPH-treated vervet monkeys have shown reductions in striatal [ $^{18}\text{F}$ ]DOPA uptake (a marker of the integrity of, and synthesis of DA in, nigrostriatal DAergic neurons) (Melega et al., 1996). Furthermore, studies in rodents, cats and non-human primates have shown that chronic dAMPH exposure results in neurotoxicity characterized by decreases in DA levels and DAT and DA receptor densities, swollen nerve terminals and degenerated axons (Ginovart et al., 2004; Ricaurte et al., 2005). Indeed, we have previously observed 20% lower striatal DAT binding in a group of combined MDMA (ecstasy) and dAMPH users that had used approximately twice the amount of dAMPH lifetime (Reneman et al., 2002). Given the large body of evidence directly documenting the DA neurotoxic potential of dAMPH in rodents and nonhuman primates (reviewed in (Berman et al., 2008)), in this explorative study we provide preliminary evidence that recreational use of dAMPH might lead to loss of DA neurons. Besides neurotoxicity, neuroplastic alterations of monoaminergic neurons related to associative drug conditioning or to non-associative drug sensitization have also been described (Singer et al., 2009). Indeed, neuroplasticity related to the formation of addiction can give rise to both an increase and decrease in dendritic spine formation (reviewed in (Nestler, 2001)). Probably a combination of both neurotoxic and neuroplastic effects has led to the presently observed lower DAT binding ratios in this group of AMPH users.

### 4.3. Pre MPH CBF measures

Our voxel-based analysis showed a higher CBF in the left thalamus of dAMPH users. It has been previously demonstrated that higher DAT availability correlates with lower

**Table 3** CBF measurements average CBF (ml/100 g/min) per ROI per group ( $\pm$ SD). Light gray marking reflects significant paired t-testing between baseline CBF and measurements taken 1½ h after ingestion of 35 mg of oral methylphenidate.

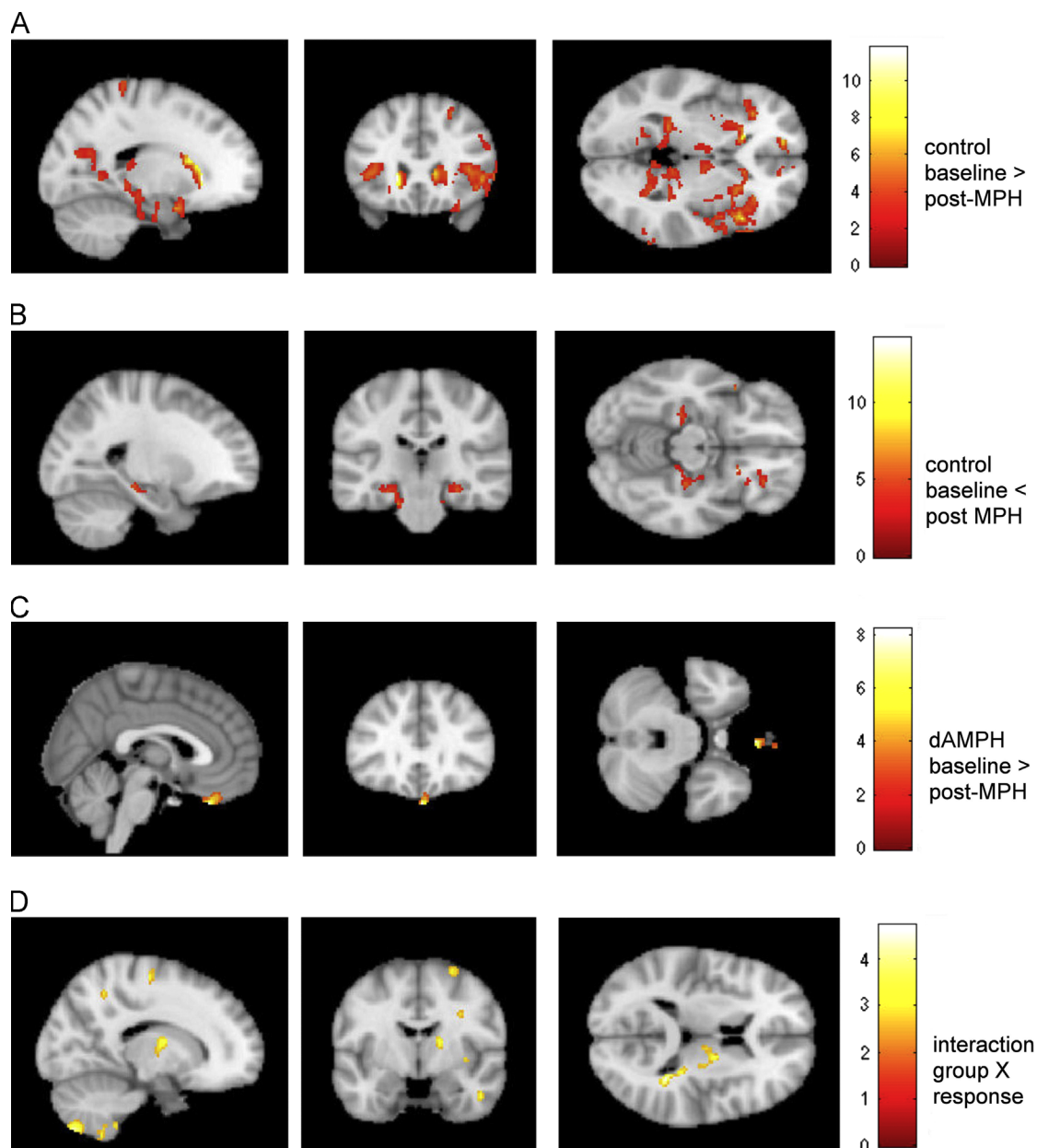
	Control baseline	Control post MPH	p-value	Effect size	dAMPH baseline	dAMPH post MPH	p-value	Effect size
Gray matter	49.2 ( $\pm$ 15.0)	39.4 ( $\pm$ 6.4)	0.022	0.92	54.8 ( $\pm$ 10.6)	54.6 ( $\pm$ 13.7)	0.969	0.02
Striatum	42.6 ( $\pm$ 11.7)	37.8 ( $\pm$ 8.2)	0.049	0.48	44.3 ( $\pm$ 7.6)	42.7 ( $\pm$ 10.8)	0.604	0.17
Hippocampus	77.7 ( $\pm$ 40.0)	51.4 ( $\pm$ 22.6)	0.003	0.84	67.7 ( $\pm$ 17.9)	53.1 ( $\pm$ 18.4)	0.026	0.80
Thalamus	54.4 ( $\pm$ 28.2)	34.6 ( $\pm$ 11.5)	0.022	1.00	52.0 ( $\pm$ 23.2)	55.8 ( $\pm$ 13.9)	0.729	-0.21
PFC	40.5 ( $\pm$ 11.1)	34.8 ( $\pm$ 7.4)	0.035	0.62	43.6 ( $\pm$ 8.8)	40.7 ( $\pm$ 9.9)	0.148	0.31
ACC	73.3 ( $\pm$ 19.3)	65.6 ( $\pm$ 13.2)	0.144	0.47	88.9 ( $\pm$ 18.2)	84.2 ( $\pm$ 24.4)	0.320	0.22
Cerebellum	51.6 ( $\pm$ 32.8)	35.9 ( $\pm$ 9.5)	0.094	0.74	62.0 ( $\pm$ 30.8)	62.6 ( $\pm$ 29.5)	0.966	-0.02

CBF values (da Silva et al., 2011). The higher CBF value we observed in dAMPH users may be linked to the lower DAT availability we observed. Because this increased CBF is a unilateral finding and results were not confirmed in the ROI-based analysis, this data should be interpreted with caution and needs to be replicated in larger groups. Also, the CBF measurements had considerable intra-subject variability. However, ASL has been found to be very consistent across time and scanners and our mean CBF values and variability are in accordance with previous studies (Gevers et al., 2011).

#### 4.4. Post MPH imaging data

##### 4.4.1. In healthy subjects

After administering MPH to healthy subjects, we noted marked changes in CBF in several key DAergic signaling regions: striatum, thalamus, PFC and hippocampus, in addition to the visual and sensorimotor cortex. All these regions, apart from the visual cortex, are rich in DAergic neurons (Missale et al., 1998). In particular, the striatum and PFC are thought to be influenced by MPH's effect on dopaminergic signaling. For example, Volkow and colleagues



**Fig. 4** Statistical parametric map of CBF change induced by 35 mg MPH. (A) Decreased CBF in the striatum, thalamus, hippocampus, prefrontal cortex, insula, motor cortex and visual cortex of healthy controls in response to the challenge. (B) Decreased CBF in several areas of the PFC and a small part of the hippocampus in dAMPH users in response to the challenge. (C) Increased CBF in healthy controls in the orbitofrontal cortex and (D) Interaction effect. Clusters reflect the areas where the response to the MPH challenge differs between healthy controls and dAMPH users. Color bars indicate z-scores. Statistic images were thresholded to a cluster significance threshold of  $p=0.01$  with a minimum cluster size of 100.



demonstrated a decrease in metabolism in the basal ganglia in response to MPH in all subjects and an increase in PFC metabolism, similar to our results, in subjects with high D2 receptor availability (Volkow et al., 1997). Several task related fMRI studies also demonstrated decreased activity in the striatum in response to MPH in healthy subjects, whereas it has been shown to increase brain activation in ADHD patients (Rohde et al., 2003; Vaidya et al., 1998).

Wang et al. found a more generalized decrease in CBF, including the striatum, using [ $^{15}\text{O}$ ]H $_2\text{O}$  PET, and attributed this to the vasoactive properties of MPH (Wang et al., 1994). However, a smaller i.v. dose (0.25 mg/kg) showed regional decreases in CBF, in the ACC, temporal poles and supplementary motor cortex, similar to our results (Udo de Haes et al., 2007). It has previously been demonstrated that the cardiovascular properties of MPH do not alter neuronal hemodynamic coupling. Rao and co-workers observed no effect of oral MPH on a finger tapping task, although heart-rate gradually increased following MPH challenge (Rao et al., 2000). This, taken together with our findings of CBF changes in brain regions commonly linked to DAergic function, strongly suggests that our findings are linked to DA neuronal function rather than reflecting a vascular response.

Besides increasing levels of DA in the striatum, microdialysis studies have shown that MPH increases extracellular NE levels in the hippocampus (Kuczenski and Segal, 1997). Indeed, MPH has been shown to be a very potent inhibitor of DAT and NET ( $K_i=0.06\ \mu\text{M}$  and  $0.10\ \mu\text{M}$ ), whereas it is not a potent inhibitor of SERT ( $K_i=132\ \mu\text{M}$ ) (Han and Gu, 2006). We therefore cannot exclude that increased NE levels contributed to the decrease in CBF we observed in response to MPH in the thalamus and hippocampus, whereas this is unlikely to be the case for 5-HT levels. However, since striatal increases in NE are less pronounced in comparison to that of DA (Koda et al., 2010), the results we observed are likely mainly attributable to an attenuation of DAergic signaling.

#### 4.4.2. In dAMPH users

It has been previously suggested that the hemodynamic response to increased metabolic demand caused by a pharmacological challenge can be an indirect measure of DA release: there is a tight correlation between hemodynamic changes induced by dAMPH and extracellular DA measurements obtained by microdialysis in rats (Chen et al., 1997; Chen et al., 1999). In addition, in non-human primates the extent of the hemodynamic response correlated well with PET markers of DA cell loss (Jenkins et al., 2004). Also, MPH-induced metabolic changes correlate with differences in DA D2 receptor binding and dAMPH has been found to significantly decrease D2 receptor availability in rats (Ginovart et al., 2004). It is therefore likely that the blunted response to the challenge with MPH in dAMPH users reflects DA dysfunction. dAMPH users only showed a decrease in CBF in a small area of the hippocampus and the medial PFC (mPFC). As mentioned before, Volkow and colleagues found a difference in response to MPH in the PFC depending on D2 receptor availability (Volkow et al., 1997). In line with this, Gulley and co-workers demonstrated with single cell recordings in rats that neuronal firing in the

entire PFC increased after the first injection with dAMPH (Gulley and Stanis, 2010). This effect diminished after repeated dAMPH administrations, apart from the mPFC which increased its firing rate. Indeed, Volkow and colleagues (Volkow et al., 2005) saw a similar difference in metabolic rate in response to MPH in the mPFC of cocaine addicts compared to healthy controls. The fact that we observed a blunted response to MPH on a behavioral level (VAS scores), as well as in brain hemodynamics (CBF) in dAMPH users, coupled with the findings in the PFC, leads to the hypothesis that the mPFC may be particularly sensitive to repeated exposure to this drug.

We also found a difference in baseline CBF and response to the challenge in the thalamus using both a ROI based and a voxel-based approach. Oral dAMPH has been shown to lead to increased CBF in the PFC and anterior thalamus in healthy controls (Devous et al., 2001) and subjects detoxified from METH use (a drug very similar to dAMPH), were found to have a lower metabolism located predominantly in the thalamus and to a lesser extent the striatum (Volkow et al., 2001). What remains to be elucidated is whether these thalamic changes are an indirect effect of altered cortico-striato-thalamic signaling as suggested by Honey and colleagues (Honey et al., 2003) or whether repeated dAMPH exposure has a direct influence on thalamic function and integrity.

#### 4.5. Limitations

Here we show a blunted CBF response in dAMPH users to a monoaminergic challenge using several (imaging) techniques. However, there are several limitations to this study that should be mentioned. We designed the study as an explorative study, involving a limited number of subjects. Because interindividual differences may be considerable, this could have affected the reliability of our measurements. Also, although we tried to match groups as much as possible on both age and IQ, the matching was not perfect. Additional analysis of covariance including age and IQ measures, showed that DAT binding ratios were not influenced by these two factors ( $p=0.07$  overall effect of group; a scatterplot of DAT binding and age is provided as supplementary material). Interaction effects in the voxel-based analysis of CBF did not notably change when adding these covariates. DAT binding may be affected by including smoking and cannabis use (Leroy et al., 2012), however again the voxel-based interaction showed similar activation patterns when including the covariates. Nevertheless, the data should be interpreted as such and needs to be confirmed in a larger sample.

Also, the presently observed difference in response could be due to other drugs than dAMPH. However, because other drugs were used only occasionally, whereas dAMPH was used on a regular basis, it is unlikely that the findings of the present study should be attributed to substances other than dAMPH. In addition, the SPECT scan was performed after the first MPH challenge, which may have led to sensitization of the DAergic system. However, to our knowledge alterations in DAT binding ratios have not been described after a single dose of MPH.

Because subjects had to abstain for two weeks from psychoactive drugs, it is unlikely that results are influenced by effects of dAMPH or other drugs (other than MPH that was administered during the study). Other than self-report and urine screening on testing days, we were not able to ensure abstinence from dAMPH. However, a survey in The Netherlands investigated showed that in 93% of the cases ( $n=594$ ) the reported drug use was in agreement with the drug-urine test (Addiction Research Institute 1998). In future studies, hair sample analysis would be a useful way to ascertain previous use of dAMPH.

As with all retrospective studies, there is a possibility that pre-existing differences between dAMPH users and controls underlie differences in DA functioning. Also, we cannot completely exclude the presence of psychiatric disease, such as ADHD, in our dAMPH group although we excluded participants with (a history of) any psychiatric condition. Only a prospective study in users with a high risk of starting to use dAMPH, with an extensive psychiatric assessment, as we previously conducted for MDMA (de Win et al., 2008), can solve this issue.

#### 4.6. Conclusions

The results of this study show that use of dAMPH at recreational doses leads to DA dysfunction, as evidenced by: (a) functional impairments on neuropsychological measures sensitive to DA function (b) a clear trend toward reductions in DATs in the striatum, and (c) a blunted hemodynamic response to a challenge with MPH in several brain regions rich in DA. Future (prospective) studies in larger groups of dAMPH users should be conducted to confirm our preliminary findings. Our results also provide evidence that pHMRI may be a sensitive tool in imaging DA dysfunction in humans non-invasively using an oral challenge.

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#### Contributors

Liesbeth Reneman designed the study and wrote the protocol. Marieke Schouw collected the data, performed the MRI analysis and wrote the first draft of the article. Matthan Caan advised on the MRI analysis. Hilde Geurts and Ben Schmand provided valuable input on analysis of the neuropsychological data. Jan BooiJ helped with study design and analysis of SPECT data. Aart Nederveen helped with study design and implementation of all MRI scanning protocols. All authors contributed to and have approved the final manuscript.

#### Conflict of interest

JB is consultant for GE Healthcare, the manufacturer of the radio-tracer FP-CIT. GE Healthcare, however, has not sponsored this study. All other authors report no conflicts of interest.

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