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ORIGINAL RESEARCH

Effects of methylphenidate during emotional processing in amphetamine users: preliminary findings

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Abstract D-amphetamine (dAMPH) and methylphenidate (MPH) are stimulants used in the treatment of Attention Deficit Hyperactivity Disorder (ADHD). Preclinical studies have shown that in healthy animals, dAMPH induces dopamine (DA) dysfunction, as evidenced for instance by loss of DA levels and its transporters. It has also been suggested that DA plays an important role in emotional processing, and that altered DA-ergic intervention may modulate amygdala function. To explore the role of the DA system in emotional processing we examined emotional processing using functional magnetic resonance imaging (fMRI) in eight male recreational users of dAMPH and eight male healthy controls. We compared brain activation between both groups during an emotional face-processing task with and without an oral MPH challenge. All subjects were abstinent for at least 2 weeks during the baseline scan. The second scan was performed on the same day 1½hours after receiving an oral dose of 35 mg MPH. A significant Valence*Group interaction (p=.037) indicated amygdala hyperreactivity to fearful facial expressions in dAMPH users that wasrobust against adjustment for age (p=.015). Furthermore, duration of amphetamine use in years was positively correlated with amygdala reactivity in dAMPH users (r=.76; p=.029). These exploratory findings are in line with previous findings suggesting that DA plays a role in emotional processing.

Keywords Dextroamphetamine · fMRI · Face recognition · Emotional lability · Methylphenidate · Neurotoxicity

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Introduction

Although emotional function is typically thought to involve the serotonergic system, several experimental studies support the idea of a dopamine (DA)-ergic contribution to an emotional response, as suggested by biochemical, pharmacological, and lesion experiments (for review see (Bolaños et al. 2003; Carlezon et al. 2003; Delaveau et al. 2005). Although much less studied clinical studies now also support DA disruption in emotional processing. For instance, several studies have found positive effects of drugs that increase DA

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concentrations in the brain, such as the DA reuptake inhibitor methylphenidate (MPH): this drug stabilized mood in patients suffering from major depression (El-Mallakh 2000). Likewise, in healthy controls, the DA releaser and reuptake inhibitor d-amphetamine (dAMPH), potentiated the response of the amygdala during the perceptual processing of angry and fearful facial expressions (Hariri et al. 2002). Also, in adolescents suffering from attention deficit hyperactivity disorder (ADHD), fear processing is associated with amygdalar hyperactivation (Brotman et al. 2010; Posner et al. 2011) and MPH normalized increased activity of the right amygdala (Posner et al. 2011). Hence, there seems to be a role for DA disruption in emotional processing.

Indeed, emotional dysregulation has been described in children with ADHD 6 years, but not 8 years, after stimulant treatment (Molina et al. 2009). In line with this, several preclinical studies have demonstrated that exposing preadolescent rats to a DA-ergic agents like MPH results in profound changes associated with a depression-like state later in life (Bolaños et al. 2003; Carlezon et al. 2003). It has been shown in animals that these emotional deficits can be reversed by antidepressant treatment in adulthood with a serotonine reuptake inhibitor, such as fluoxetine (Bolaños et al. 2008). Bolanos suggested that antidepressants enhance DA transmission resulting in a reverse of depression-like behavior is induced by early (preadolescent) MPH exposure.

The amygdala is a brain structure critical for emotional processing and activates most strongly during the processing of emotional faces (Hariri et al. 2002). Amygdala function measured with functional Magnetic Resonance Imaging (fMRI) is a well-known biomarker of emotional dysregulation, i.e., depression (Tao et al. 2012). For instance, increased amygdala activity assessed with fMRI has been found in patients suffering from major depressive disorder (MDD), which decreased after successful treatment with paroxetine (Ruhé et al. 2011)

To further explore the role of the DA system in emotional processing, we examined emotional function in a group of dAMPH users, using task related fMRI before and after oral administration of MPH. We included dAMPH users, because there is preliminary evidence that users of this drug suffer from a dysfunctional DA system. We set out to answer the following questions: 1) Does amygdala function differ between recreational dAMPH users and healthy control subjects? 2) Does a DA-ergic challenge with MPH modulate amygdala function? 3) If so, does it affect amygdala function differently in recreational dAMPH users when compared to control subjects?

If indeed DA plays a role in emotional processing, as suggested in the literature described above, we hypothesized that in recreational dAMPH users we would observe an increased responsiveness of the amygdala to negative or fearful faces, which would decrease following a challenge with MPH, presumably due to enhanced DA transmission.

Methods

This study was approved by the medical ethics committee of the Academic Medical Centre Amsterdam. Written informed consent was obtained from all subjects.

Subjects

Subjects were recruited by posting advertisements around the medical campus, on websites and in regional newspapers. A total of eight male recreational amphetamine users and eight male healthy control subjects were recruited. The eligibility criterion for the dAMPH group was previous use of dAMPH on more than 40 occasions. This threshold was chosen based on the work of Reneman and co-workers (Reneman et al. 2001) who found lower DAT binding in ecstasy users with an average dAMPH use on more than 45 occasions. The eight control subjects were healthy subjects with no self-reported use of amphetamines.

Subjects were asked to refrain from using caffeinated products on assessment days, and to abstain from all psychoactive drugs for at least two weeks before scanning. Therefore dAMPH dependence was an exclusion criterion. All subjects were asked to comply with urine drug screening on the day they were scanned (with an enzyme-multiplied immunoassay for amphetamines, cocaine, cannabis, alcohol, opiates, and benzodiazepines). Exclusion criteria for all participants were: any neuropsychiatric diagnosis or history of brain disease or injury, use of medication with affinity for DA (e.g., MPH), a positive urine-screen for any DA-ergic drugs or any contra indication to MRI such as metallic implants or claustrophobia. Subjects received a small financial compensation for their participation.

Procedure

The tasks were presented in the same order for every subject; first a go-nogo task, then a reward task, and then the emotional face recognition task. Results of the go-nogo task (in preparation) and the reward task (Schouw et al. 2013) are reported elsewhere. To minimize learning effects, a practice run for each task was presented outside of the scanner. After the first scanning session, subjects received 35 mg MPH immediate release formulation (approximately 0.5 mg per kg body weight) orally. Subjects were then free to relax for 1½hours until peak plasma levels were expected (Demenescu et al. 2011; Hysek et al. 2013) and then re-entered the MRI scanner for the second session that was identical to the first. MPH was obtained from Sandoz B.V. (Weesp, the Netherlands).

Imaging

All MR imaging was performed using a 3.0 Tesla Philips MR scanner equipped with an SENSE 8-channel head coil and body coil transmission (Philips Medical Systems, Best, The Netherlands). The session protocol consisted of a high-resolution 3D T1-weighted anatomical scan for registration and segmentation purposes and a fast single shot echo planar image (EPI) sequence for BOLD analysis. The BOLD acquisition imaging parameters were: TR/TE 2300/30 ms; FOV 220×220 mm^2; 40 slices; voxel size 3×3×3 mm; no gap; 80° flip angle, SENSE 2.0.

Emotion processing task

The implicit emotion processing task was presented by a video projection system onto a white screen using E-prime software (Psychological Software Tools, USA). Subjects saw the screen via a mirror attached to the head coil. Responses were logged via a response box attached to the computer presenting the stimuli.

All subjects performed a modified version of the event-related implicit emotion processing task (Demenescu et al. 2011). Color photos of fearful, happy, and neutral facial expressions were presented. The stimuli were selected from the Karolinska Directed Emotional Faces (KDEF) stimulus set and consisted of standardized facial expressions of emotions expressed by amateur actors. Twenty-four stimuli (12 male and 12 female faces) were presented for each of the three facial expressions. In addition, two control stimuli consisting of an arrow pointing to the left or right, overlaid on a scrambled face, were presented. The control stimuli were presented 80 times (40 with an arrow pointing to the left, 40 with an arrow pointing to the right). Each stimulus type was not presented more than twice in a row. Each stimulus was shown on the screen for 2.5 s with an interstimulus interval (black screen) varying between 0.5 and 1.5 s. Participants were instructed to indicate each face's gender by pressing one of two buttons with the index finger of the left or right hand on two button boxes (left for a male face and right for a female face). For the control stimuli, participants had to push a button according to the direction the arrow was pointing in (left button for left direction and right button for right direction), the direction of the arrows and correct faces were counterbalanced.

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. All demographic and behavioral data were analyzed in SPSS version 18.0 (SPSS Inc, Chicago, Ill) and are presented as mean ± standard deviation unless indicated otherwise. Reaction times were entered into a mixed model ANOVA in SPSS with the factors Group (two levels: healthy controls and dAMPH), Drug challenge (two levels: pre and post) and Stimulus type (four levels: fearful, happy, neutral, and baseline). MRI scans were analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) implemented in Matlab version 7.13. Images were first manually reoriented to the anterior commissure. Subsequently, fMRI images were realigned to the first volume, corrected for differences in slice acquisition time, co-registered to the anatomical scan, segmented into grey matter, white matter, and cerebrospinal fluid, spatially (non-linearly) normalized to the Montreal Neurological Institute (MNI) T1 template, resampled into 2×2×2 mm voxels and spatially smoothed using a 6 mm full width at half maximum Gaussian kernel. Statistical analysis was performed within the framework of the general linear model. To determine BOLD activation in response to different facial expressions, box-car regressors convolved with a canonical hemodynamic response function were used to model responses to each facial expression. Data were high-pass filtered at 128 s and temporal autocorrelation was modeled with an AR(1) process provided within SPM8. To assure that the faces paradigm elicited reliable activations, a whole brain second level fMRI analysis across groups and sessions was performed for each facial expression (random effects analysis). The resulting statistical parametric maps were initially thresholded at $p<0.001$. Clusters significant at the $p<0.05$ level family-wise error (FWE) corrected for multiple comparisons were considered statistically significant. Next, a region of interest (ROI) analysis was performed by extracting mean BOLD activation in the bilateral amygdala with the MarsBaR toolbox (Matthew Brett, Jean-Luc Anton, Romain Valabregue, Jean-Baptiste Poline). Region of interest analysis using an SPM toolbox (presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2–6, 2002, Sendai, Japan. Available on CD-ROM in NeuroImage, Vol 16, No 2.) using a mask of the bilateral amygdala as defined by the Automated Anatomical Labeling atlas. Because no statistically significant differences were found for the left and right amygdala, the mean amygdala response across hemispheres was used. Mean amygdala activations for the left and right amygdala were analyzed with mixed models in SPSS with the factors Group (two levels: healthy controls and dAMPH), Hemisphere (two levels: left and right), Drug challenge (two levels: pre and post) and Affective Valence (three levels: fearful, happy and neutral). Additional analyses were run with age as a covariate. The association between the extent of dAMPH use and amygdala reactivity was studied with Pearson product–moment correlation coefficient provided within SPSS (two-tailed).

Results

Sample characteristics

The dAMPH group used dAMPH on average for 13.9 (±8.7) years with on 27.8 (±17.1) occasions/year and a usual dose of 0.8 (±1.2) grams/occasion. The mean cumulative lifetime exposure to dAMPH was 352.6 (465.3) grams and mean time since the last dose was 1.1 (±1.3) month. Table 1 shows that the dAMPH group was slightly older and had a normal, but slightly lower, pre-morbid IQ than the control group although years of education did not differ significantly. In addition, dAMPH users had used significantly more tobacco, cannabis, and cocaine.

Task performance

Reaction times are shown in Table 2. Subjects were faster after than before MPH challenge (F (1, 14)=11.70, p<.01). Responses to control stimuli (scrambled faces that required a left/right response) were faster than to the emotional stimuli (requiring sex discrimination (F (3, 12)=36.27, p<.001). No statistically significant effects were found involving the factor 'Group'.

Whole brain analyses across groups and challenge

Whole brain fMRI analyses across groups (dAMPH and HC) and challenge (pre and post MPH) were performed to verify whether the facial expressions elicited reliable activation (see Table 3 and Fig. 1). Presentation of fearful faces was associated with activation of right amygdala extending into anterior hippocampus, left and right fusiform gyrus, right prefrontal cortex and right middle temporal cortex (Fig. 2). However, activation of left amygdala did not survive correction for multiple comparisons. Presentation of happy and neutral faces was generally associated with the same pattern of activation, albeit less extensive. Therefore, activation by happy and neutral faces in most of the above mentioned areas did not survive correction for multiple comparisons.

Table 2 Mean reaction time RT (SD)

	dAMPH pre		dMAPH post		Controls pre		Controls post	
Fear	798	(125)	726	(167)	710	(83)	651	(132)
Happy	749	(91)	654	(84)	686	(88)	659	(75)
Neutral	799	(129)	651	(66)	692	(81)	665	(165)

ROI analyses

The omnibus ANOVA at amygdala ROIs did not show significant differences between the left and right amygdala so this factor was dropped from the analyses. Although the Session*Valence*Group interaction was not significant (p=.73), a significant Valence*Group interaction indicated that only for fearful faces, significant group differences were present (p=.037) that were robust against adjustment for age (p=.015) (Table 4, Fig. 3). For fearful faces, a trend significant Group*Session interaction (p=.073, p=.26 after adjustment for age) indicated that before the oral MPH challenge, dAMPH users showed more activation to fearful faces than the control group (Fig. 4). This was confirmed by post-hoc analyses: before the oral MPH challenge, the two groups differed significantly in amygdala activation (p=.027, p=.05 after adjustment for age). The MPH challenge was associated

Table 1 Demographics for dAMPH users and controls with standard deviation (±) and p-values for t-test (Age, IQ and Years of education) or Mann–Whitney test

	dAMPH n=8	Controls n=8	p-value
Age	26.0 (±4.0)	22.0 (±3.0)	0.04
DART-IQ	104.5 (±3.0)	110.4 (±4.2)	0.007
Years of education	15.1 (±3.6)	16.4 (±2.9)	0.46
dAMPH			
Average dAMPH use (occasions/year)	27.8 (±17.1)	0	0.00
Duration of dAMPH use (years)	13.9 (±8.7)	NA	NA
Usual dose (grams/occasion)	0.8 (±1.2)	NA	NA
Total exposure (grams)	352.6 (±465.3)	0	0.00
Time since last exposure (months)	1.1 (±1.3)	NA	NA
Other substances			
Average tobacco use (cigarettes/month)	261.0 (±279.8)	0	0.01
Average alcohol use (units/month)	103.5 (±146.6)	104.5 (±83.5)	0.49
Average cannabis use (joints/year)	410.3 (±480.5)	19.4 (±31.8)	0.02
Average MDMA use (pills/year)	3.8 (±10.6)	0	0.32
Average cocaine use (occasions/year)	5.0 (±5.2)	0.1 (±0.4)	0.009

NA, Not applicable

Table 3 Whole brain fMRI analyses for fearful, happy, and neutral faces averaged across all participants and sessions

	Fearful						Happy						Neutral					
	MNI			Z	k		MNI			Z	k		MNI			Z	k	
	x	y	z				x	y	z				x	y	z			
Amygdala (l)	−20	−12	−16	3.30	10		−18	−20	−16	3.71	16		−22	2	−14	3.21	3	
Amygdala (r)	20	−10	−10	4.46	118	*	18	−12	−10	4.98	49		18	−10	−10	3.21	5	
Fusiform gyrus (l)	−38	−56	−18	4.88	128	*	−38	−58	−20	4.12	45		−34	−62	−16	4.61	95	*
Fusiform gyrus (r)	36	−58	−22	5.80	519	*	38	−58	−20	6.42	434	*	40	−60	−22	6.19	412	*
PFC BA 44/45 (r)	50	34	24	4.67	320	*												
Middle temp. cortex (r)	50	−74	16	4.21	246	*												

Montreal Neurological Institute (MNI) Coordinates, Z=statistical Z value; k=number of voxels in cluster. All activations significant at $p<.001$ uncorrected. * cluster level FWE corrected significant at $p<.05$, *L*=left; *r*=right

with a marginally significant increase in amygdala reactivity in the controls ($p=.075$) whereas the challenge was associated with a nonsignificant decrease in reactivity in the amphetamine users ($p=.51$). No significant Group*Session interactions were present for happy faces ($p=.27$) or neutral faces ($p=.35$).

Correlation analysis

Before the challenge, duration of amphetamine use in years was positively correlated with amygdala reactivity in dAMPH ($r=.76$; $p=.029$; Fig. 5).

Discussion

In this study of amygdala activation before and after MPH challenge in dAMPH users and controls, we found that only presentation of fearful faces was associated with abnormal increased activation of the amygdala. However, the modulating effect of MPH was not significant, although we observed a trend that MPH increased amygdala activity in the control group, without such an effect in the dAMPH group. Interestingly, in the dAMPH users the extent of dAMPH exposure was positively correlated with amygdala reactivity.

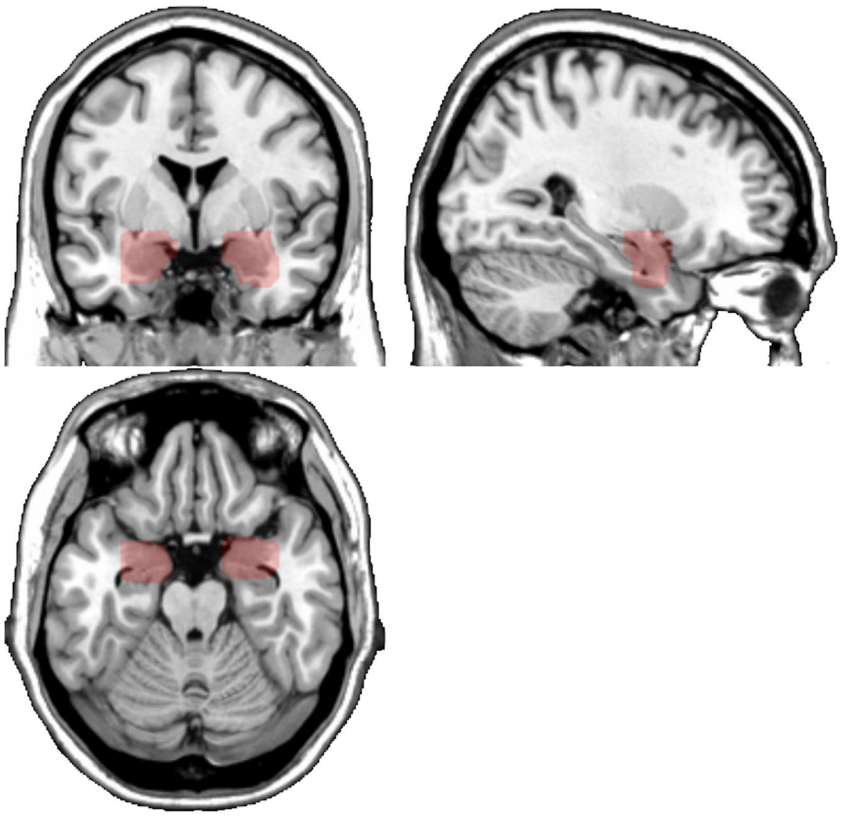

Fig. 1 Mask used to extract mean BOLD activation for region of interest (ROI) analysis

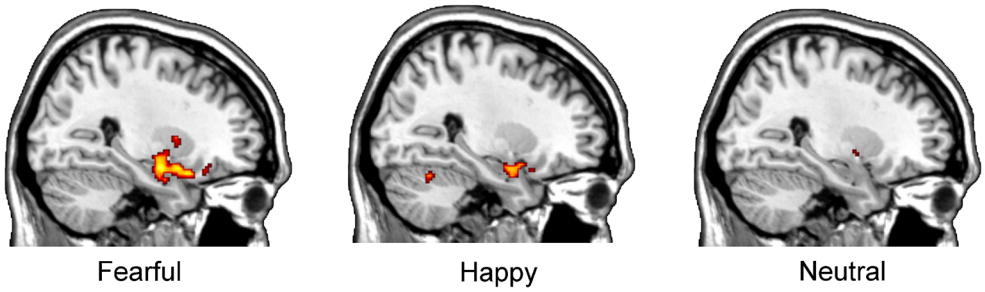

Fig. 2 Main effects of participants during the task and session. Sagittal view (MNI coordinate x=27) of BOLD activation in right amygdala to presentation of fearful, happy, and neutral faces across groups (AMPH and HC) and challenge (pre and post MPH). Data are shown at $p<.005$ for display purposes

Our experimental and control groups were not homogenous; our dAMPH group was slightly older, and we therefore corrected our statistical analysis for age. The dAMPH users also had a normal but lower premorbid IQ than the control group, although the years of education did not differ significantly. In addition, the dAMPH group had used significantly more tobacco, cannabis, and cocaine. Unfortunately, the groups were too small to also correct for IQ and drug use.

Our findings of abnormal baseline amygdala activation in recreational dAMPH users most likely reflect an abnormal DA transmission in this brain region, as dAMPH has been previously found to affect the DA system in animals treated with this drug and the amygdala is under modulatory influence of DA. For instance, preclinical studies have shown that even relatively low doses of dAMPH (equivalent to the doses used in clinical practice) induce DA dysfunction in rodents and non-human primates (Ricaurte 2005) as evidenced by for instance reductions in DA, the DA transporter (DAT) and an increase in D1 receptor density (Bonhomme et al. 1995). In line with this, in humans, Reneman and co-workers have shown that recreational dAMPH use is linked with reduced striatal DA transporter (DAT) availability (Reneman et al. 2001). Because the DAT is a structural component of the DA-axon, loss in DAT likely indicates DA-ergic neurotoxicity. In addition, in recreational users of dAMPH, we previously found a blunted hemodynamic response to a challenge with MPH (Schouw et al. 2013), as well as blunted DA release induced by an i.v. challenge with dAMPH (Schrantee et al. 2014). These (pre)clinical findings in the literature provide additional evidence that our results of abnormal emotional processing in dAMPH users likely underly abnormal DA neurotransmission.

Indeed, DA has been found to modulate the human amygdala response in anxious patients (Bergman et al. 2014), but also in patients suffering from Parkinson's disease (Tessitore et al. 2002) as well as ADHD (Posner et al. 2011). The dose–response correlation between lifetime exposure of dAMPH use and amygdala activity in the present study further supports a relationship between dAMPH use and amygdala hyperactivity and is in line with previous findings (Hariri et al. 2002).

We did not find amygdala (or other brain region) activity with the neutral and happy faces. This might be surprising in relation to our DA hypothesis and the role of DA in rewarding processes. We think this is related due to the overall weak responses to our stimuli. Since aversive stimuli are known to give the strongest activation this might be the reason that other stimuli used did not threshold for brain activity. Furthermore, in healthy controls there may also be a greater top down regulatory activity from the ventrolateral and medial prefrontal cortex when stimuli are negative rather than positive

Table 4 Omnibus ANOVA results. When $p<.05$ the effect is also reported adjusted for age (ANCOVA)

Factor	*p* value	Adjusted *p* value
Session	.553	
Session * Group	.171	
Valence	.175	
Valence * Group	.037	.015
Session * Valence	.683	
Session*Valence*Group	.725	
Group	.474	

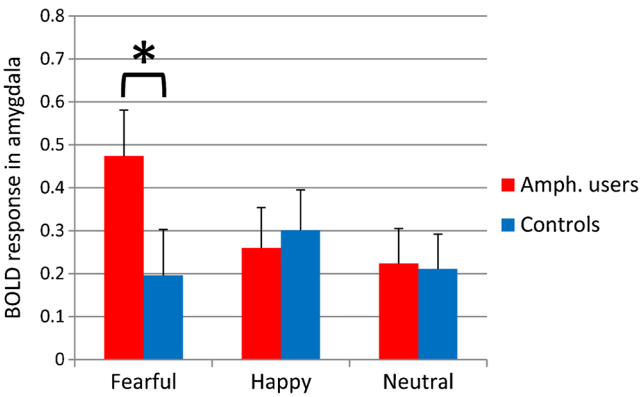

Fig. 3 Amygdala activation to fearful, happy, and neutral facial expressions for amphetamine users and controls. Y axis shows mean BOLD response in bilateral amygdala (anatomical ROI). '*' denotes statistically significant difference ($p<.05$)

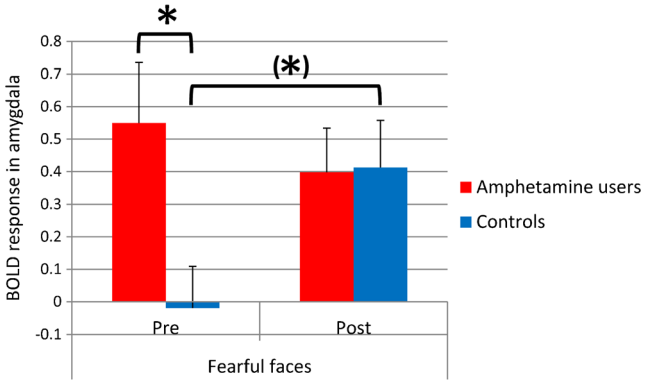

Fig. 4 Amygdala activation before and after MPH challenge in dAMPH users and controls. Y axis shows mean BOLD response in bilateral amygdala (anatomical ROI). '*' denotes statistically significant difference ($p<.05$)

(Musser et al. 2013). This top down regulation of emotional responses might be more disturbed in the dAMPH users, like in patients with DA-ergic dysfunction such as ADHD, than in the control group, giving less strong emotional responses in the control group (Shaw et al. 2014).

We observed a trend that MPH may induce a different (opposite) effect in dAMPH users as in healthy volunteers: MPH seems to increase in amygdala activation in healthy controls, whereas no effect in dAMPH users. Studies with lager sample sizes are needed to replicate these findings. Increased amygdala activation induced by a DA-ergic challenge in healthy volunteers has been reported in other studies using dAMPH as a challenge (Delaveau et al. 2005; Hariri et al. 2002). Interestingly, in patients suffering from ADHD, stimulants had a normalizing effect on the activity of the right amygdala (Posner et al. 2011; Volkow et al. 2007, for a review see Shaw et al. 2014) as well as emotional processing (Conzelmann et al. 2011) and emotional lability, although negative mood persisted after treatment with MPH (Williams et al. 2008).

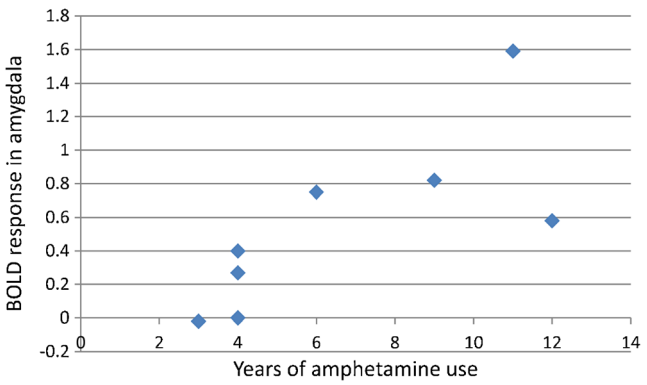

Fig. 5 Correlation of years of amphetamine use with amygdala reactivity in dAMPH users. $r=.76$, $p=.029$. X-axis shows years of amphetamine use, Y axis shows mean BOLD respone in bilateral amygdala (anatomical ROI).). '*' denotes statistically significant difference ($p<.05$).'(*) denotes marginally statistically significant difference ($p<.1$)

Studies with similar results as ours, in subjects with dysfunctional DA systems, lend further support to our hypothesis that DA dysfunction, or DA deficiency, most likely underlies the abnormal fear processing in dAMPH users. Also structural effects have been found after prolonged MPH use, for example in a study on patients with a bipolar I disorder, a decrease in amygdala volume was found in adolescents using MPH (Geller et al. 2009).

In fact, it has been shown that DA in the basolateral amygdala is critical for fear-processing (Fadok et al. 2009). Additionally, some midbrain DA neurons increase their firing rates to aversive stimuli and predictive cues (Guarraci et al. 1999; Horvitz 2000). DA levels in the ventral midbrain increase during aversive events and DA neurons of these brain areas project to limbic brain areas important for fear learning (Abercrombie and Zigmond 1989; Kalivas and Duffy 1995). In these areas, DA facilitates long term potentiation, an important neural correlate of memory (Lemon 2006). Finally, in a recent human study dimensional scores on a fear subscale and not on a depressive subscale were found to be predictive for right amygdala activity in human adolescents when processing fearful, happy, and neutral faces (van den Bulk et al. 2014) and higher amygdala activity is displayed by a common polymorphism in a region of the DAT gene (SLC6A3), during the processing of aversive emotional stimuli in humans (Bergman et al. 2014).

Besides this, not only DA but also norepinephrine (NE) may play a role since NE is known for its relevance in the treatment of depression (Blier 2013). Both MPH and dAMP activate the NE system. Previously, NE has been implicated in the therapeutic action of MPH as it significantly occupied the NE transporter at clinically significant doses (Hannestad et al. 2010). Alternatively, our findings may be explained by an increased sensitivity to corticotropin-releasing factor, as chronic amphetamine treatment has been shown to enhance corticotropin-releasing factor-induced serotonin release in the amygdala of rats (Scholl et al. 2010). The corticotropin-releasing factor increases serotonin release in the central nucleus of the amygdala, and this neurochemical circuitry has been shown to mediate fear processing as well.

Our findings may, when replicated in a larger sample, have clinical implications. What happens if DA-acting agents are administered to healthy subjects (e.g., to improve scholarly achievements), or in ADHD patients that have not been correctly diagnosed as suffering from ADHD? We would like to argue that in these cases, MPH administration has a detrimental effect, as in our study MPH seems to induce an increase in amygdala activity in healthy subjects. Indeed, in animal studies MPH has been shown to induce anxiety-like behavior. For example, Bolanos et al. found that in normal Wistar rats, prolonged treatment with MPH during adolescence induced anxiety-like behavior, in which the animals were significantly more sensitive to stressful situations and had enhanced levels

of corticosterone (Bolaños et al. 2003). Additionally, MPH has been shown to enhance decoding of negative emotions including fear in healthy human subjects (Hysek et al. 2013)

A limitation of the current study is the study design, because it lacks a placebo-controlled condition. Therefore, we cannot exclude that the effects we measured were indeed induced by MPH or due to sensitization or habituation. However, as mentioned previously, the dose–response correlation further supports a relationship between dAMPH use and amygdala hyperactivity. Another limitation of this study is the possibility that pre-existing differences between dAMPH users and healthy controls underlie differences in amygdala function. People with a dysfunctional DA system with low response on an acute DA-ergic challenge, may be predisposed to use dAMPH. Our findings in this relatively small sample size in this explorative study, should thus be replicated in a larger study population.

Conclusion

During fear processing, we observed increased amygdala activity in a group of recreational dAMPH users compared to healthy control subjects. MPH induced a marginally significant increase in amygdala activity in healthy control subjects. These exploratory findings are in line with the literature, underpinning the modulatory influence of DA on amygdala function.

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Conflict of interest M. A. Bottelier, M. L. J. Schouw, M. B. de Ruiter, H. G. Ruhe, R. J. L. Lindauer, and L. Reneman declare that they have no conflicts of interest.

Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all patients for being included in the study.

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