Neural correlates of working memory in pure and polyvalent ecstasy (MDMA) users

Jörg Daumann, Ralph Schnitker,[1] Jürgen Weidemann,[2] Knut Schnell, Armin Thron[3] and Euphrosyne Gouzoulis-Mayfrank[4,CA]

Department of Psychiatry and Psychotherapy; [1]Interdisciplinary Center for Clinical Research–CNS; [2]Department of Neurology and [3]Department of Neuroradiology, Medical Faculty of the University of Technology (RWTH), Aachen, Germany
[4]Present Address: Department of Psychiatry and Psychotherapy, University of Cologne, Joseph-Stelzmann-Strasse 9, 50924 Cologne, Germany

[CA]Corresponding Author: e.gouzoulis@uni-koeln.de

Received 4 July 2003; accepted 14 July 2003

DOI: 10.1097/01.wnr.0000093295.63079.21

Poor cognitive performance in ecstasy (3,4-methylenedioxymethamphetamine; MDMA) users has been related to the well-recognized neurotoxic effects of the drug upon central serotonergic and possibly also dopaminergic systems. However, concomitant use of other drugs has been a critical confound in most investigations. In this study we used an n-back task and fMRI to investigate working memory performance and related cerebral activation in eight, currently abstinent pure MDMA users and two matched groups of polyvalent MDMA users and non-users. Pure MDMA users presented lower activations than controls and/or polyvalent users, most notably in inferior temporal regions, the angular gyrus and the striate cortex, whereas polyvalent users did not differ from controls. Our results suggest that altered brain activation patterns during cognitive processing in ecstasy users may be mainly associated with prior MDMA use. Concomitant use of other drugs may modify this effect. *NeuroReport* 14:1983–1987

Key words: Amphetamine; Cannabis; Ecstasy; fMRI; MDMA; Neuroprotection; Neurotoxicity; Δ^9-Tetrahydrocannabinol; THC; Working memory

INTRODUCTION

During recent years memory problems have been consistently demonstrated in abstinent ecstasy users (MDMA; 3,4-methylenedioxymethamphetamine and similar congeners) [1–4]. Deficits of central executive control, selective attention, working memory as well as elevated impulsivity and poor problem solving have also been reported [3,5,6]. Accumulating evidence suggests that a dose-dependent neurotoxic effect of MDMA upon central serotonergic systems may be responsible for the cognitive problems in ecstasy users [7–9]. According to a recent study in non-human primates neurotoxic effects upon central dopaminergic systems are also conceivable [10].

Despite the striking evidence of neuropsychological impairments and neural abnormalities in MDMA users functional imaging approaches have received little attention in this field of research. An $H_2{}^{15}O$-PET study using a continuous performance test (CPT) reported no performance deficit and no alteration in cerebral activation in ecstasy users compared to controls [11]. In contrast, preliminary evidence from a study by our research group suggests that working memory processing in abstinent MDMA users is associated with subtle alterations in cerebral activation [12]: heavy and moderate users showed stronger activations than controls in right parietal cortex. Furthermore, heavy users had a weaker BOLD response than moderate users and controls in the temporopolar area and to a smaller extent also in frontal regions. However, most participants in that study reported concomitant use of (meth)amphetamine and cannabis [12]. Prolonged abuse of (meth)amphetamine has been associated with significant reduction in dopamine transporter density and long-lasting cognitive impairment in humans [13]. Heavy cannabis users also show neuropsychological deficits which are only reversible after weeks of abstinence [14]. Therefore, non-ambiguous interpretations of the results of our previous study have been difficult [12]. The purpose of the present study was to determine to which extent prolonged MDMA use has an impact on cognitive processing compared to the influence of amphetamine and cannabis use. Differences in fMRI signal changes during a working memory task between pure and polyvalent ecstasy users were taken as a means of separating the effects.

MATERIALS AND METHODS

Participants: Eight pure MDMA users (subjects denying other regular substance use except for ecstasy; mean age 25.30 ± 3.62 years) were compared to eight polyvalent MDMA users (subjects reported regular concomitant

use of amphetamines and cannabis; mean age 26.41 ± 3.70 years) and eight drug-nave healthy controls (mean age 25.55 ± 3.22 years). Regular use was defined as use once per month or more frequently over ≥ 6 months within the last 2 years. Seven MDMA users had participated in our previous study [12]. All subjects were right-handed and matched for age (range 19–35 years), gender (four male, four female in each group) and education. The participants were recruited directly in the dance scene by students who were involved in the study, via word-of-mouth and via the internet. Users were included in the study if they had used MDMA regularly over ≥ 6 months with a minimum frequency of twice a month or if they had used MDMA on > 20 occasions. All ecstasy users agreed to abstain from drug use for at least 7 days prior to the study with the exception of cannabis from which the subjects in the polyvalent group agreed to abstain only on the study day. Given the fact that daily smoking of marijuana is part of the lifestyle of most polyvalent club drug users, it seemed implausible to insist on a longer period of abstinence.

Cognitive task: Subjects performed three n-back tasks consisting of the sequential visual presentation (SOA 3000 ms; duration of presentation 1500 ms) of single letters (B, C, D, G, P, T, F, N, L). Subjects had to press a button, when a target stimulus appeared. Target definition differed with respect to the experimental condition. In the 0-back condition the subjects had to respond when a stimulus within the sequence matched a target stimulus specified at the beginning of the stimulus presentation (letter D). In the 1-back condition, targets were defined as stimuli within the sequence that were identical to the immediately preceding one. In the 2-back condition subjects had to respond if the presented letter matched the one that was presented two trials before. The 0-, 1-, and 2-back tasks differ in their amount of memory load and demands on executive control for the processing of information within working memory.

Experimental procedure: All subjects underwent a structured interview according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV). In addition, we took a medical history and detailed history of drug use. Drug screens were performed on the study day with urine samples for stimulant amphetamines, methylenedioxyamphetamines (ecstasy), cocaine and its metabolite, marijuana, benzodiazepines, barbiturates and opiates. After detailed description of the study to the subjects, written informed consent was obtained. Before entering the scanner, all subjects were carefully instructed and trained in the n-back task by performing it once. In the fMRI study the PC-generated paradigms were presented to the subjects via a rear-projection screen and a mirror attached to the head coil. For each task three off/on cycles (90 s each) with rest as off condition (eyes open, 30 s) were employed (block-design). For each subject performance as measured by reaction times and correct responses for the n-back tasks were recorded. The study was in accordance with the Helsinki Declaration of 1975 and was approved by the local ethics committee at the RWTH Aachen.

Imaging parameters: MRI employing blood oxygenation level-dependent (BOLD) contrast was performed on a clinical 1.5 T Philips ACS NT Gyroscan (Philips, Eindhoven, The Netherlands) using a singleshot multislice T2* weighted gradient echo EPI sequence (imaging parameters: TR 4000 ms, TE 40 ms, FA 90°, matrix 64 × 64, FOV 250 × 250 mm, 15 contiguous slices parallel to the AC-PC line covering the whole brain except for the cerebellum, voxel size 4 × 4 × 7 mm, no interslice gap). For anatomic reference and to exclude subjects with apparent brain pathologies, we additionally obtained a T1-weighted inversion recovery sequence (imaging parameters: TR 2000 ms, TE 22 ms, TI 400 ms, matrix 256 × 256, slice thickness 7 mm). Head movements were minimized in all subjects using foam pads and Velcro straps. Images were acquired using a standard head coil. Every task was introduced by a verbal instruction presented via headphones.

Data analysis: Cognitive performance was analyzed by means of MANOVA with repeated measures for the task-factor (0-back, 1-back, 2-back) using SPSS 10.0 software (SPSS Inc., Chicago, Ill). fMRI data were analyzed using SPM99 software (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 6 (Mathworks Inc., Sherborn, MA). The scans obtained for each subject and each condition were initially realigned to the first image of each scan. All mean images were subsequently normalized with the SPM99 template, which is the current standard Montreal Neurological Institute (MNI) template and smoothed with a Gaussian kernel (triple voxel size). Condition-specific effects in every task were assessed by comparing the on-periods of each task with their respective control conditions (off-periods). Changes in regional cerebral blood flow (rCBF) were determined by applying the general linear model to each voxel. To test hypotheses about significant changes in rCBF in different brain regions, voxel-wise comparisons of rCBF for each subject and condition (0-back, 1-back, 2-back) were performed using *t*-statistics. To correct for the inference due to multi-subject fMRI data a between-subjects second-level analysis (ANOVA) was applied comparing the individual statistical parametric maps of *t*-statistic values for each condition. In this random-effects analysis contrasts were computed with the standard height threshold of $p < 0.001$. Given the fact that the correction for the entire brain volume offered by SPM99 is conservative, *p*-values were not corrected for multiple comparisons. Minimum cluster size was set to five voxels. Because the MNI coordinates do not perfectly match the brain of Talairach and Tournoux [15], we used the Matlab function mni2tal.m by Matthew Brett for the non-linear transformation of MNI to Talairach coordinates (http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html).

RESULTS

Drug histories: The patterns of drug use in the two user samples are presented in Table 1. The two user groups differed significantly in terms of duration of regular use and age at onset of use of ecstasy. In addition, polyvalent users also reported concomitant use of amphetamines and cannabis.

Table 1. Patterns of ecstasy, cannabis and amphetamine use in both user groups ($n = 8$ in each group, mean ± s.d.).

	Pure users		Polyvalent users	
	Ecstasy	Ecstasy	Amphetamine	Cannabis
Lifetime dose	74.50 ± 70.53 tablets	56.25 ± 28.38 tablets	94.25 ± 165.07 g	x
Duration of regular use (months)*	34.29 ± 26.09	18.43 ± 14.12	30.88 ± 25.20	45.00 ± 38.35
Average frequency of use (days per month)	1.44 ± 0.82	3.65 ± 2.32	6.13 ± 3.45	17.50 ± 10.58
Average daily or one night dose	1.66 ± 0.84 tablets	1.44 ± 0.78 tablets	443.75 ± 289.13 mg	499.83 ± 105.30 mg
Age at onset of use*	22.75 ± 3.69	19.00 ± 1.85	18.25 ± 1.28	15.67 ± 1.03
Time since last dose (days)	23.00 ± 18.72	62.38 ± 75.98	156.50 ± 242.21	3.33 ± 3.27

*Significant group differences in terms of ecstasy use between pure and polyvalent users, $p < 0.05$ (unpaired *t*-test, 2-tailed); x: subjects felt that they were unable to give a reliable estimate.

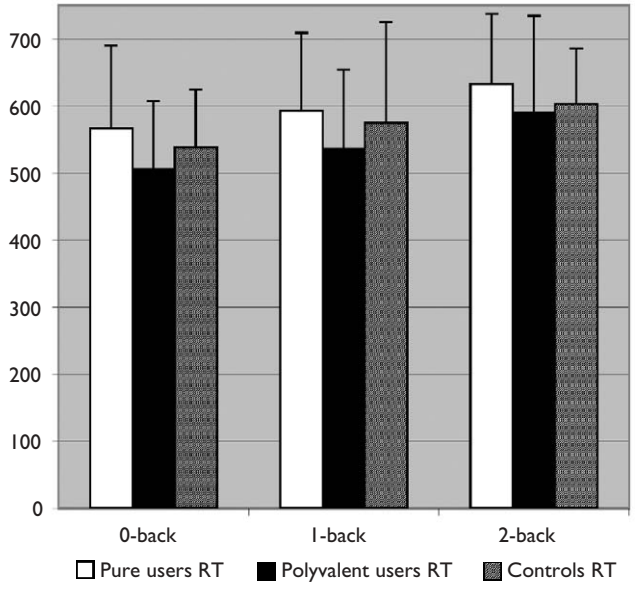

Fig. 1. Cognitive performance in the n-back tasks: reaction time in ms (group means and s.d., $n = 8$ in each group).

Behavioral results: Performance of subjects in the n-back tasks during the fMRI experiment is presented in Fig. 1. The within-subject effect of memory load was significant in respect to reaction times (MANOVA: $F = 7.34$, $df = 2$, $p = 0.003$), but not in respect to correct responses. However, between-subject analysis did not reveal significant performance differences between pure users, polyvalent users and controls in regard to reaction times (MANOVA: $F = 0.89$, $df = 2$, $p = 0.509$) and correct responses (MANOVA: $F = 0.93$, $df = 2$, $p = 0.472$).

fMRI results: All subjects had a normal structural MRI scan without focal brain lesions or anatomical abnormalities. In both groups of MDMA users as well as in the group of controls functional MRI for all three tasks lead to significant and localized hemodynamic changes in prefrontal, parietal, occipital and cingulate brain regions, which are known to be involved in n-back tasks [16]. There were no group differences with regard to cortical activation patterns in the 0-back condition. However, we detected group differences during the 1-back and 2-back task. All significant differences in terms of cortical activation during the 1-back task are presented in Table 2 (upper part). Polyvalent MDMA users did not differ from controls whereas pure MDMA users presented lower activations than controls mainly in the inferior temporal and angular region. In addition, pure users had lower signal changes in the striate cortex and a higher BOLD response in the premotor cortex compared to polyvalent users. Results with regard to the 2-back condition are given in the bottom part of Table 2. Again, pure MDMA users showed lower activations mainly in the angular gyrus compared to both controls and polyvalent users.

DISCUSSION

Eight pure recreational ecstasy users and two equally sized groups of polyvalent ecstasy users and drug-nave controls were examined with fMRI and an n-back task with increasing memory load. Performance in the n-back tasks as measured by reaction times and correct responses revealed no statistically significant group differences. Subjects of all three groups showed typical cortical activation patterns in the n-back tasks. However, despite the unimpaired cognitive performance, pure MDMA users showed significantly lower BOLD responses most strikingly in the inferior temporal gyrus, the angular gyrus and the striate cortex compared to polyvalent users and drug-nave controls. Given the fact that pure MDMA users denied concomitant use of other licit or illicit drugs the differences in fMRI signal changes may be attributable to prior MDMA use, and they may indicate the neurotoxic potential of this drug. Furthermore, it is conceivable that an altered BOLD response might appear before impairments in cognitive performance become significantly apparent. Such an effect was previously reported in HIV patients, who showed altered cortical activation patterns preceding deficits on neuropsychological tests [17]. The authors concluded that BOLD fMRI might be more sensitive for detecting early brain injury than impairments in cognitive performance [17].

Noteworthy, cortical activation patterns of polyvalent users did not differ significantly from drug-nave controls. This finding is contra-intuitive, since we usually expect polyvalent users to exhibit more deviations from controls than users of one drug only. Given the consideration that altered fMRI signal patterns in ecstasy users may reflect drug-related neurotoxicity, the question arises whether concomitant use of amphetamines and/or cannabis might alter (and ameliorate) the long-term effects of MDMA on the

Table 2. Contrasts with significant activation pattern differences between pure and polyvalent ecstasy users and controls during the n-back tasks ($p = 0.001$, uncorrected).

Contrast	Brain region (Brodmann area)	Talairach coordinates			Cluster size (voxels)	Z-score
		x	y	z		
1-back						
Pure > Poly	Premotor cortex (BA6)	33	6	55	10	3.47
Poly > Pure	Striate cortex (BA17)	−21	−81	12	11	3.52
Pure > Control	Superior parietal lobule (BA 7)	24	−38	65	22	4.23
Control > Pure	Posterior cingulate cortex (BA29)	−3	−37	21	21	4.00
	Inferior temporal gyrus (BA20)	−56	−35	−8	25	3.92
	Inferior temporal gyrus (BA20)	50	−33	−11	10	3.64
	Angular gyrus (BA39)	50	−54	28	14	3.83
	Angular gyrus (BA39)	−45	−63	31	22	3.63
2-back						
Pure > Poly	Superior parietal lobule (BA7)	−33	−35	65	10	3.58
Poly > Pure	Striate cortex (BA17)	24	−75	12	20	4.58
	Striate cortex (BA17)	−18	−87	4	27	4.40
	Posterior cingulate cortex (BA31)	24	−51	33	10	4.14
Control > Pure	Striate cortex (BA17)	−15	−84	7	28	3.89

Pure: Subjects who reported no other substance use except for ecstasy; Poly: Subjects who reported use of ecstasy, amphetamines and cannabis; Control: drug-naïve controls.

brain. To our knowledge, interactions between these drugs have not been examined systematically so far. Preliminary evidence suggests that concomitant use of (meth)amphetamine in ecstasy users may be associated with a reduction in dopamine transporter densities [18]. However, it remains unclear how this finding might influence cerebral activation patterns in fMRI studies.

Of course, interactions between the different drugs may also involve indirect mechanisms: for instance, one of the most important physiological effects of MDMA is the induction of hyperthermia, which is a well-known pro-oxidant aggressive condition [19]. Hyperthermia is known to increase the neurotoxic potential of MDMA and (meth)amphetamine, and small decreases in core body temperature were shown to ameliorate MDMA-induced neurotoxic damage in laboratory animals [20,21]. There is evidence that Δ^9-Tetrahydrocannabinol (THC) can lower core body temperature in rats [22] and that co-administration of nicotine can strongly facilitate this effect [23]. Hence, THC might have neuroprotective properties against oxidative stress and might therefore ameliorate long-term neurotoxic consequences of MDMA use. Support for this hypothesis is provided by a recent longitudinal magnetic resonance imaging study [24]. The authors showed that in an *in vivo* animal model of neurodegeneration THC reduced neuronal damage via a CB_1 cannabinoid receptor-mediated mechanism [24]. Finally, head trauma patients treated with dexanabinol, a synthetic nonpsychotropic cannabinoid, achieved neurological recovery more rapidly [25]. However, additional systematic studies are necessary to further clarify the possible neuroprotective properties of THC in ecstasy users, since they commonly report concomitant cannabis use.

Our study has methodological limitations, which are inherent to open trial studies with user populations and simply cannot be solved perfectly. We cannot rule out the possibility that subjects concealed concomitant regular use of other legal and illegal drugs and/or heavier ecstasy use. However, we do not think that this was the case because subjects were not aware of our inclusion and exclusion criteria and there was no motivation for them to falsify their data once they agreed to participate in the study. However, some users reported difficulties recalling the frequency and dosage of their drug use, particularly if they had abstained from drug use for a longer period of time. Finally, the chemical composition and dosage of illegally purchased ecstasy tablets is always uncertain.

CONCLUSION

Ecstasy use appears to be associated with neural alterations in terms of BOLD fMRI during cognitive processing, which may reflect MDMA-induced neurotoxicity. Our results suggest that altered fMRI activation patterns are not related to concomitant use of other drugs. However, further research is needed to definitively establish a causative link between MDMA use and neurotoxic effects in humans. In our view, research strategies should now combine prospective and longitudinal designs with cognitive assessments and modern neuroimaging techniques in order to shed more light onto the course of residual neurocognitive effects of ecstasy use. Furthermore, additional systematic studies are needed to further clarify the reciprocal actions of different licit and illicit drugs in polyvalent ecstasy users. Finally, animal models might help to understand the acute and long-term interactions between MDMA, THC and (meth)amphetamine at different dosages.

REFERENCES

1. Bolla KI, McCann UD and Ricaurte GA. *Neurology* **51**, 1532–1537 (1998).
2. Morgan MJ. *Psychopharmacology* **141**, 30–36 (1999).
3. Gouzoulis-Mayfrank E, Daumann J, Tuchtenhagen F *et al. J Neurol Neurosurg Psychiatry* **68**, 719–725 (2000).
4. Bhattachary S and Powell JH. *Psychol Med* **31**, 647–658 (2001).
5. McCann UD, Mertl M, Eligulashvili V and Ricaurte GA. *Psychopharmacology* **143**, 417–425 (1999).
6. Zakzanis KK, Young DA and Radkhoshnoud NF. *Appl Neuropsychol* **9**, 84–91 (2002).
7. Hatzidimitriou G, McCann UD and Ricaurte GA. *J Neurosci* **19**, 5096–5107 (1999).

8. McCann UD, Eligulashvili V and Ricaurte GA. *Neuropsychobiology* **42**, 11–16 (2000).
9. Reneman L, Lavalaye J, Schmand B *et al. Arch Gen Psychiatry* **58**, 901–906 (2001).
10. Ricaurte GA, Yuan J, Hatzidimitriou G *et al. Science* **297**, 2260–2263 (2002).
11. Gamma A, Buck A, Berthold T and Vollenweider FX. *J Clin Psychopharmacol* **21**, 66–71 (2001).
12. Daumann J, Fimm B, Willmes K *et al. Brain Res Cogn Brain Res* **16**, 479–487 (2003).
13. Volkow ND, Chang L, Wang G-J *et al. Am J Psychiatry* **158**, 377–382 (2001).
14. Pope HG Jr, Gruber AJ, Hudson JI *et al. Arch Gen Psychiatry* **58**, 909–915 (2001).
15. Talairach J and Tournoux P. *Co-planar Stereotaxic Atlas of the Human Brain*. Stuttgart: Thieme; 1988.
16. Cabeza R and Nyberg L. *J Cogn Neurosci* **12**, 1–47 (2000).
17. Ernst T, Chang L, Jovicich J *et al. Neurology* **59**, 1343–1349 (2002).
18. Reneman L, Booij J, Lavalaye J *et al. Psychopharmacology* **159**, 335–340 (2002).
19. Carvalho M, Carvalho F, Remiao F *et al. Arch Toxicol* **76**, 166–172 (2002).
20. Colado MI, Camarero J, Mechan AO *et al. Br J Pharmacol* **134**, 1711–1723 (2001).
21. O'Shea E, Easton N, Fry JR *et al. J Neurochem* **81**, 686–695 (2002).
22. Marsicano G, Moosmann B, Hermann H *et al. J Neurochem* **80**, 448–456 (2002).
23. Valjent E, Mitchell JM, Besson M-J *et al. Br J Pharmacol* **135**, 564–578 (2002).
24. van der Stelt M, Veldhuis WB, Bär PR *et al. J Neurosci* **21**, 6475–6479 (2002).
25. Knoller N, Levi L, Shoshan I *et al. Crit Care Med* **30**, 548–554 (2002).

Acknowledgements: This work was supported by a grant to E.G.-M. from the Deutsche Forschungsgemeinschaft (DFG GO 717/4-1).