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MDMA intoxication and verbal memory performance: a placebo-controlled pharmaco-MRI study

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Abstract

The aim of the present study was to identify the neural substrate underlying memory impairment due to a single dose of MDMA (3,4-methylenedioxymethamphetamine) by means of pharmaco-MRI. Based on previous behavioral results it was hypothesized that this deficit could be attributed to a specific influence of MDMA on encoding. Fourteen Ecstasy users participated in this double-blind, placebo-controlled, within-subject study with two treatment conditions: MDMA (75 mg) and placebo. Memory performance was tested by means of a word learning task including two words lists, one addressing reading processes (control task, CWL) and a second (experimental task, EWL) addressing encoding and reading processes. Behavioral data showed that under the influence of MDMA, EWL performance was worse than placebo. Imaging data showed that Encoding was situated mainly in (pre)frontal, temporal and parietal areas. MDMA by Encoding interaction was situated in three areas: the left middle frontal gyrus (BA10), the right fusiform gyrus (BA19), and the left cuneus (BA18). Behavioral and functional data only correlated in BA10. It appeared that EWL performance caused BOLD signal change in BA10 during placebo treatment but not during MDMA intoxication. It is concluded that MDMA influences middle frontal gyrus processes resulting in impoverished memory encoding.

Keywords

Introduction

Previously, a causal association between pharmacological exposure to MDMA (3,4-methylenedioxymethamphetamine) and memory impairment was established in an acute, placebo-controlled study. It was shown that immediate and delayed recall of words was impaired after a single dose of MDMA. Although subjects under the influence of MDMA showed a normal learning curve, they learnt less than placebo (Kuypers and Ramaekers, 2005).

Besides the knowledge that MDMA impairs memory performance, it is also interesting to know which specific memory process is affected. Based on previous results, mentioned above, it was hypothesized that the memory deficit could be attributed to a specific influence of MDMA on the encoding of words. Support for this hypothesis was found in research of Ward et al. (2006) that showed that abstinent MDMA users were able to perform at the same level as a control group but needed more learning trials to achieve comparable performance levels. They ascribed the memory problems of abstinent MDMA users to deficits in encoding (Ward et al., 2006). However, views differ about the exact memory process that MDMA affects. Another group who conducted comparable research to that of Ward et al. (2006) pointed at another process, namely defective recall (Quednow et al., 2006), and demonstrated defective memory in recently abstinent MDMA users. They showed that although learning curves had the same shape, MDMA users learnt less compared to two control groups, that is, a cannabis group and a drug

naive group. Because they also found impairment of other memory-related processes besides learning – such as consolidation, recall, and recognition – they concluded that the origin of impairment was not known, but they hinted at defective recall as a potential candidate (Quednow et al., 2006).

The aim of the present study was to identify the neural substrate underlying memory impairment due to a single dose of MDMA. Based on the hypothesis that encoding processes are affected by MDMA during the word learning task, and previous neuroimaging studies on the encoding-related areas of verbal material, the following regions were expected to be affected: posterior and more ventral regions of the left frontal cortex, medial temporal lobe structures, and inferior parietal regions (Buckner et al., 1999; Dupont et al., 2002; Gabrieli et al., 1998; Mottaghy et al., 2002; Ojemann et al., 1997; Schacter and Wagner, 1999; Smith et al., 1998). Frontal

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areas are believed to supply information to medial temporal lobe structures, which are implied in the formation of memory (Buckner et al., 1999; Squire and Zola Morgan, 1991). Parietal regions are thought to be part of a network of brain areas that mediate short-term storage of phonologically coded verbal material (Jonides et al., 1998).

The commonly used paradigm to study memory performance and related brain activation is the 'depth of encoding paradigm' where subjects categorize words based on semantic (deep processing) or physical (shallow processing) properties without expecting a memory test (recognition). The process that is measured is incidental encoding. The rationale behind this task is that depth of processing is positively related with the chance that the word is remembered later on (Buckner et al., 1999; Craik and Lockhart, 1972). The memory process of interest in the present study was, in contrast to incidental learning, intentional learning or encoding. To investigate the encoding process, we adjusted the word learning task (Kuypers and Ramaekers, 2005; Rey, 1958) so it was suited for imaging. The word learning task is a validated tool to measure memory processes and performance in behavioral studies and it has commonly been used in MDMA research. In the original version, subjects have to learn a list of nouns and subsequently recall them after list presentation. For the new version, we added a control task. This control task was also a list containing 15 nouns that were, in contrast to the experimental word list, pre-memorized or pre-encoded. The new task thus included two word lists: an experimental word list which was hypothesized to involve encoding and reading processes, and a control word list which was hypothesized to involve only reading. Subtraction of the control images from the experimental task images should reveal brain activity related to encoding. It was hypothesized first that left frontal, parietal and temporal areas, often linked with memory encoding, would be especially active during the word encoding phase of the word learning task and, second, that MDMA would affect these encoding related areas.

Data presented here were part of a larger pharmaco-MRI study addressing the acute effects of a single dose of MDMA on verbal memory and prospective memory performance. Results of the latter task have previously been published. It was shown that MDMA induced an impairment of prospective memory performance which positively correlated with MDMA concentrations in the blood. Functional data pointed out that this memory impairment could be due to the loss of deactivation in the inferior parietal lobule (Ramaekers et al., 2009).

Methods

Subjects, design and treatments

The study was conducted according to a double-blind, placebo controlled, randomized, crossover design with balancing of treatments. The treatments were MDMA 75 mg and placebo. Placebo and MDMA were administered orally, in identically appearing formulations. MDMA was administered as a 25-ml solution in bitter orange peel syrup and mixed with 200 ml of orange juice.

In total, fourteen healthy Ecstasy users were included in the study (11 males, 3 females) aged in the range of 19 years to 29 years (mean age (SD): 23.43 (3.00)). Mean (SD) estimated lifetime use of MDMA was 65.79 (134.50); it varied from light ($\leq$15 times) in 6 subjects, to moderate (between 16 and 40 times) in 6 subjects, to heavy in 2 subjects (200 and 500 times). Lifetime use of alcohol, cannabis, and amphetamines varied in a similar way among subjects. They were all native Dutch speakers.

Subjects were recruited by means of advertisements in local newspapers and by word of mouth. Potential candidates were questioned about their drug use and medical condition and were given information about the study. Thereupon they were sent a detailed brochure with information about the study procedure, two questionnaires for medical history and detailed history of drug use, and an MRI screenings list. Inclusion criteria were experience with MDMA use, free from psychotropic medication, good physical health, absence of major medical endocrine and neurological conditions, normal weight (body mass index: 18–28 kg/m^2). Exclusion criteria were history of drug abuse (other than MDMA) or addiction, pregnancy or lactation, cardiovascular abnormalities (assessed by a standard 12-lead electrocardiogram (ECG)), excessive drinking (>20 alcoholic consumptions per week), hypertension (diastolic >100 mmHg; systolic >170 mmHg), history of psychiatric or neurological disorders, presence of parts with magnetic properties in the body (MRI criterion). A physician checked the completed medical questionnaire, and upon approval, subjects were invited for a medical examination. Blood and urine samples were taken for examination and subjects underwent an ECG measurement. In case of no medical objections, they were contacted and sent an information brochure with study procedure details and rules they had to obey during the period of the study. Subjects signed an informed consent to prove they had read the information and agreed on it. They were paid upon completion of the testing periods for their participation.

The study was performed in accordance with the 1975 declaration of Helsinki, adjusted in Seoul (2008) and was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University, The Netherlands. A permit for obtaining, storing and administering MDMA was obtained from the Dutch drug enforcement administration.

Study procedure. Before study onset, subjects were familiarized with the task in a short training session and they were given a list of 15 monosyllabic words which had to be learnt by heart. This list then served as the control list on both test days.

On a test day, subjects were screened for drugs (delta-9-tetrahydrocannabinol, opiates, amphetamine/Ecstasy, benzodiazepines, cocaine, and methamphetamine/Ecstasy) and alcohol in their urine and breath, respectively, upon arrival at the test facilities. If negative, subjects proceeded with a light breakfast. Subjects filled out a sleep questionnaire and after breakfast it was checked whether they could perfectly recall the 15 words of the control list. All subjects could recall the 15 words of the control list. Half an hour later subjects received a drink containing either placebo or MDMA. Seventy-five minutes after drug-intake, subjects had to fill out a mood questionnaire, and a blood sample was taken to

determine MDMA/MDA concentrations in blood serum afterward. Following this, subjects were accompanied to the MR-scanner, positioned on the scanner bed, provided with earplugs to reduce the noise, and a given a headphone set for communication with the person operating the scanner. Foam pads were applied to the head to restrict head motion. The tasks were projected onto a screen at the end of the scanner bore and viewed via a mirror mounted on the volume head-coil. The word learning task started at 100 minutes post-drug administration, followed by another memory task of 20 minutes (not reported on in this paper) and finally the anatomical scan. In case the anatomical scan displayed visual abnormalities, the standard procedure was to contact a radiologist; but this was not necessary in any of the cases.

Between the test days there was a minimal wash-out period of 7 days.

Assessments

Word learning task.
The word learning task used in the present study was an adjusted version of one used previously (Kuypers and Ramaekers, 2005). The task was customized to suit blocked imaging, which was used to examine brain regions specialized for encoding and/or reading words.

The word learning task began with memorizing the experimental list consisting of 15 Dutch monosyllabic nouns. Each word was shown on the computer display for 1 second and the subject read it silently. When the series ended, subjects had to name as many words as possible within a time frame of 60 seconds. Thereupon the control word list – also containing 15 Dutch monosyllabic nouns, but learned before the session and drug administration – was shown in the same manner as the experimental word list. At the end of the control word list, subjects also had to recall as many words as possible. The two lists were presented in alternation on five successive occasions. A stars count-down (3-2-1) preceded each list to signal the beginning of the list for the subject. During scanning, speech was recorded (Goldwave version 4.26) and processed off-line by means of a scanner noise reduction program (Cusack et al., 2005). After the reduction of the scanner noise, it was possible to hear and list the words subjects recalled during the recall periods. The numbers of words correctly recalled in the five experimental trials were summed to yield the total immediate free recall score (Immediate Recall Total). After a 40-minute delay, subjects were asked (outside the scanner) to recall as many words as possible. The number of words correctly recalled was taken as the delayed recall score.

The difference between the control word list and the experimental word list was that, during the presentation of the experimental word list, subjects had to read and learn/encode these words, while they only had to read the words of the control task because these had been encoded already. To denote this difference, lists will be referred to as 'reading task' and 'encoding task' for the control word list and the experimental word list, respectively.

Questionnaires.
Two questionnaires were taken: the Groninger Sleep Scale (GSS) and the Profile of Mood States (POMS). The GSS assessed sleep quality and quantity (hours of sleep). It consisted of 15 dichotomous questions about sleep complaints and an open question concerning the duration of sleep the previous night (Mulder-Hajonides van der Meulen et al., 1980). The POMS (de Wit et al., 2002) is a self-assessment mood questionnaire with 72 five-point Likert scale items, representing eight mood states: anxiety, depression, anger, vigor, fatigue, confusion, friendliness, and elation. Two extra scales were derived, namely arousal ((anxiety plus vigor) minus (fatigue plus confusion)) and positive mood (elation minus depression). A subject had to indicate to what extent these items were representing his/her mood.

Pharmacokinetic assessments.
Blood samples were taken at 75 minutes post-dosing, centrifuged at $4000 \times g$ for 10 minutes and the corresponding serum samples were subsequently frozen at $-20°C$ until analysis for pharmacokinetic assessment. MDMA and MDA concentrations were determined using an integrated on-line SPE-LC-MS/MS system (Symbiosis Pharma, Spark Holland) with tandem mass spectrometric detection (Quattro Premier, Waters Corportation) using deuterated analogues for internal standardization. Quantification limits (LOQ) were 2.5 ng/ml for MDMA and MDA.

Functional MRI data acquisition.
Imaging was conducted in a 3-tesla Siemens Allegra MR head-only scanner, equipped with a standard head coil and echo planar sequences for ultra-fast MRI (Allegra, Siemens medical systems). Anatomical T1-weighted images were acquired using a 3D modified driven equilibrium Fourier transform (MDEFT) sequence with an isotropic spatial resolution of 1 mm. An anatomical run contained 176 slices, lasted 12 minutes, and was performed for each subject in each condition.

Blood oxygen level dependent (BOLD) fMRI images were acquired with a gradient echo T2*-weighted image sequence with the following parameters: $TR = 2000$ ms; $TE = 30$ ms; voxel size $= 3.5 \times 3.5 \times 3.5$; no gap between slices, interleaved slice sampling; flip angle $= 90°$; matrix size $= 64*64$. Functional time series consisted of 32 slices and 600 volumes, and lasted 20 minutes. During this time, the experimental word list was shown five times in alternation with the control word list. Each list presentation was followed by a recall period.

Statistical analysis

Behavioral and subjective data were analysed using the statistical package SPSS version 15.0. The alpha criterion level of significance was set at $p = 0.05$.

Behavioral data of the word learning task (WLT) entered a GLM repeated measures analysis. Data of the reading task and the encoding task were separately analysed. Factors were MDMA (2 levels: MDMA 75 mg, placebo) for the Immediate Recall Total and the Delayed Recall, and Factor Trial (5 levels, Trials 1–5), added for the analysis of the separate trials of the WLT. For **questionnaires** the MDMA effect was assessed by means of paired samples t-tests.

Table 1. Mean (±SEM), *F* (df1, df2) and *p*-values of the dependent variables of the experimental word list (encoding task) of the word learning task. Significance at $p \leq 0.05$

Experimental word list scores (dependent variables) Immediate Recall (Trial #)	MDMA Mean (±SEM)	Placebo	MDMA vs Placebo *F* (df1, df2)	*p*-value
1	5.85 (±0.501)	6.61 (±0.413)	5.166 (1, 13)	0.041
2	9.31 (±0.437)	10.85 (±0.563)		
3	11.77 (±.526)	12.08 (±0.597)		
4	12.61 (±0.386)	13.31 (±0.369)		
5	13.38 (±0.463)	13.69 (±0.338)		
Total score (Σ trial 1-5)	52.92 (±1.853)	56.47 (±1.960)	5.005 (1, 13)	0.043
Delayed Recall	12.07 (0.650)	12.14 (±0.720)	0.021 (1, 13)	NS

For functional data pre-processing and statistical analysis, the software package Brain Voyager QX version 2.1 was used. (Brain Innovation, Maastricht, The Netherlands, www.brain-voyager.com). Before statistical analysis, functional data were pre-processed (3D motion correction (trilinear interpolation, reduced data), slice scan time corrected (sinc interpolation, ascending and interleaved slice scanning order), and temporal data filtered (linear trend removal, high pass filter of 3 cycles/run)). Dummy variables were created for each subject and each session for MDMA and placebo in order to make random effects GLM possible. The estimates of the six motion parameters from this analysis were included, together with the eight predictors (Experimental Word List MDMA, Control Word List MDMA, Stars MDMA, Recall MDMA, Experimental Word List placebo, Control Word List placebo, Stars placebo, Recall placebo) from the task (4 real and 4 dummy), in the multi-subject GLM analysis.

The model for random effects (RFX) ANCOVA GLM analysis included two within-subject factors, that is, MDMA (two levels: MDMA, placebo) and Encoding (2 levels: experimental word list, control word list). This model served to analyze main effects of MDMA and Encoding and the MDMA by Encoding interaction. In regions showing significant activation, epoch-based, event-related averaging was conducted to visualize differences in BOLD signal response. P-criterion was set at $p < 0.005$ ($F_{1,12} > 11.75$) with a minimum cluster size of 4 voxels to account for multiple comparisons (McAvoy and Buckner, 2001; Poldrack et al., 2008). Anatomical names of areas showing statistically significant activation were determined by means of the software package Talairach Client version 2.4 (Lancaster et al., 1997, 2000).

Results

Behavioral data

Experimental word list/encoding task. Analysis revealed the main effects of MDMA ($F_{1,13} = 5.166$; $p = 0.041$) and Trial ($F_{4,52} = 148.223$; $p < 0.000$) on Immediate Recall (trials). Under influence of MDMA, subjects recalled less words compared with placebo. In total (over the 5 trials; Immediate Recall Total), this difference equalled 3.5 words (Table 1, Figure 1). There was no significant effect of MDMA
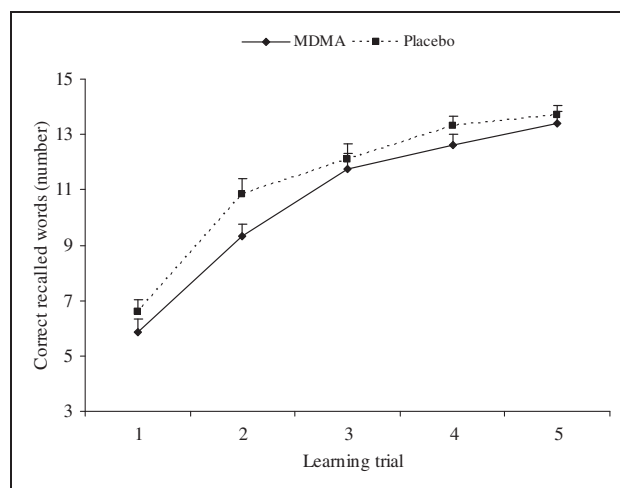

Figure 1. Number of correctly recalled words of the experimental word list per trial during MDMA and placebo treatments.

on Delayed Recall ($F_{1,13} = 0.021$; $p > 0.05$). In the MDMA condition, subjects recalled on average 12.07 words after an interval of 40 minutes relative to the learning trials, in the placebo condition 12.14 words. This was on average one word less compared with the immediate recall of the last trial, in both the MDMA and placebo conditions.

Control word list/reading task. There were no significant effects of MDMA, Trial, or their interaction on the dependent variables of the reading task. Subjects were able to recall all the words of the reading task as intended (mean (±SE): MDMA 15 (±0.00); placebo 14.925 (±0.053)).

Functional imaging data

Functional data from one subject (age 19, male gender, lifetime use 30 times) were not included in the analysis as preprocessing parameters were not normal (too much movement during the MDMA condition, that is, 8 mm translation in the Z-axis direction). For the other 13 subjects, pre-processing parameters were within the normal range.

Encoding effect. RFX analysis revealed a significant main effect of Encoding (encoding vs reading task) in frontal (right: BA46, left and right: BA9), temporal (left: BA37, BA39, hippocampus), parietal (right: BA40), and occipital cortex (right and left: BA18), limbic areas BA23 left and BA32 right, insula (bilateral) and left thalamus $F_{1,12} = (11.75-62.52)$; $p < 0.005$ (see Table 2). BOLD signal response was larger during encoding task performance compared with reading task performance. In all above-mentioned areas but one (posterior cingulate cortex), the encoding task caused an increase in activation compared to the reading task. In the posterior cingulate cortex, the encoding task caused an increase in deactivation (see Figure 2).

MDMA effect. RFX analysis revealed a significant main effect of MDMA (MDMA vs placebo) in the occipital cortex (left: BA30), inferior frontal gyrus (right: BA47) and precentral gyrus (left: BA4) $F_{1,12} = (11.75-33.58)$; $p < 0.005$ (see Table 2). The MDMA-related BOLD signal response was larger than placebo. In both frontal areas, MDMA caused an increase in activation compared with placebo; in the occipital area, it caused an increase in deactivation.

MDMA by Encoding effect. RFX analysis revealed a significant MDMA by Encoding interaction effect in the fusiform gyrus (right: BA19), middle frontal gyrus (left: BA10), and cuneus (left: BA18) $(F_{1,12} = (11.75-36.66)$; $p < 0.005)$ (see Table 2).

Additional correlation analyses showed a significant correlation (i.e. a negative correlation, Pearson $r = -0.30$; $p = 0.024$) between Immediate Recall Total and BOLD signal change in one area: BA 10. Both BA18 and BA19 were not significantly related with word learning list performance.

In the frontal area there was task-related (encoding task > reading task) increase of activation during placebo but not during MDMA (Figure 3). Correlation analysis showed that high word learning performance scores, coming from the reading task, were related with small BOLD signal changes, lower word learning scores, coming form the encoding task were related with greater BOLD signal changes. Further correlation analyses only including the encoding task performance scores revealed no significant correlation between scores and BOLD signal change in the MDMA condition and a positive correlation in the placebo condition ($r = 0.434$; $p = 0.091$; $\alpha = 0.10$, one-tailed; see Figure 4).

Questionnaires

Groninger sleep scale. Sleep Quantity and Quality scores on the evening prior to testing were comparable in both treatment conditions. Subjects slept on average 6.67 h (SD: 1.41) and 6.86 h (SD: 1.79), and had an average score of 2.00 (SD: 2.04) and 2.79 (SD: 2.52) on the sleep complaints questionnaire during MDMA and placebo treatments, respectively.

POMS. Analysis revealed an effect of MDMA on six scales of the POMS, that is, anxiety, vigor, confusion, friendliness, elation, and positive mood. Subjects showed increased scores on these six scales while under the influence of MDMA compared with placebo (Table 3).

Pharmacokinetic assessments

Blood serum concentrations of MDMA were on average ($\pm$SD) 79.3 ng/ml (21.41) 1.25 h post-dosing. Concentrations of MDA were below the limit of quantification in 11 out of 14 subjects. In three subjects MDA concentrations ranged between 2.5 and 3.7 ng/ml (mean (SD): 3 (0.61)) 1.25 h post-dosing.

Concentrations were lower than those in previous studies (e.g. Kuypers and Ramaekers, 2007; Kuypers et al., 2006). This difference can be attributed to the fact that blood samples were collected 75 minutes post-drug administration, that is, 15 minutes before the expected MDMA peak concentrations at 90 minutes post-drug.

Discussion

The present study aimed at revealing neuro-anatomical structures underlying MDMA-induced memory impairment. To that end, a placebo-controlled, within-subject, pharmaco-MRI study was conducted in light-to-moderate Ecstasy users.

Table 2. Summary of the Talairach coordinates and cluster size of anatomical regions showing a significant main effect of Encoding, MDMA, and MDMA by Encoding (Lancaster et al., 1997, 2000)

Brain area Encoding effect	Side	BA	Voxels	X	Y	Z
Frontal cortex						
Inferior frontal gyrus	R	46	185	42	37	5
Middle frontal gyrus	R	9	951	35	29	33
	L		400	−38	19	31
Temporal cortex						
Superior temporal gyrus	L	39	117	−45	−46	7
Fusiform gyrus	L	37	177	−44	−57	−11
Hippocampus	L		229	−29	−41	0
Occipital cortex						
Inferior occipital gyrus	R	18	197	32	−82	−4
Middle occipital gyrus	L	18	408	−28	−81	−1
Parietal cortex						
Supramarginal gyrus	R	40	729	43	−46	36
Limbic lobe						
Posterior cingulate	L	23	556	−2	−44	22
Cingulate gyrus	R	32	574	7	20	38
Insula	R	13	902	35	20	9
	L		821	−31	19	6
Thalamus	L		449	−12	−6	6
(ventral anterior nucleus)						
MDMA Effect						
Frontal cortex						
Inferior frontal gyrus	R	47	178	22	29	−2
Precentral gyrus	L	4	122	−33	−20	57
Occipital cortex	L	30	86	−3	−73	6
MDMA by Encoding Effect						
Middle **frontal** gyrus	L	10	40	−33	38	11
Temporal (fusiform gyrus)	R	19	64	48	−67	−11
Occipital (cuneus)	L	18	61	−2	−91	7

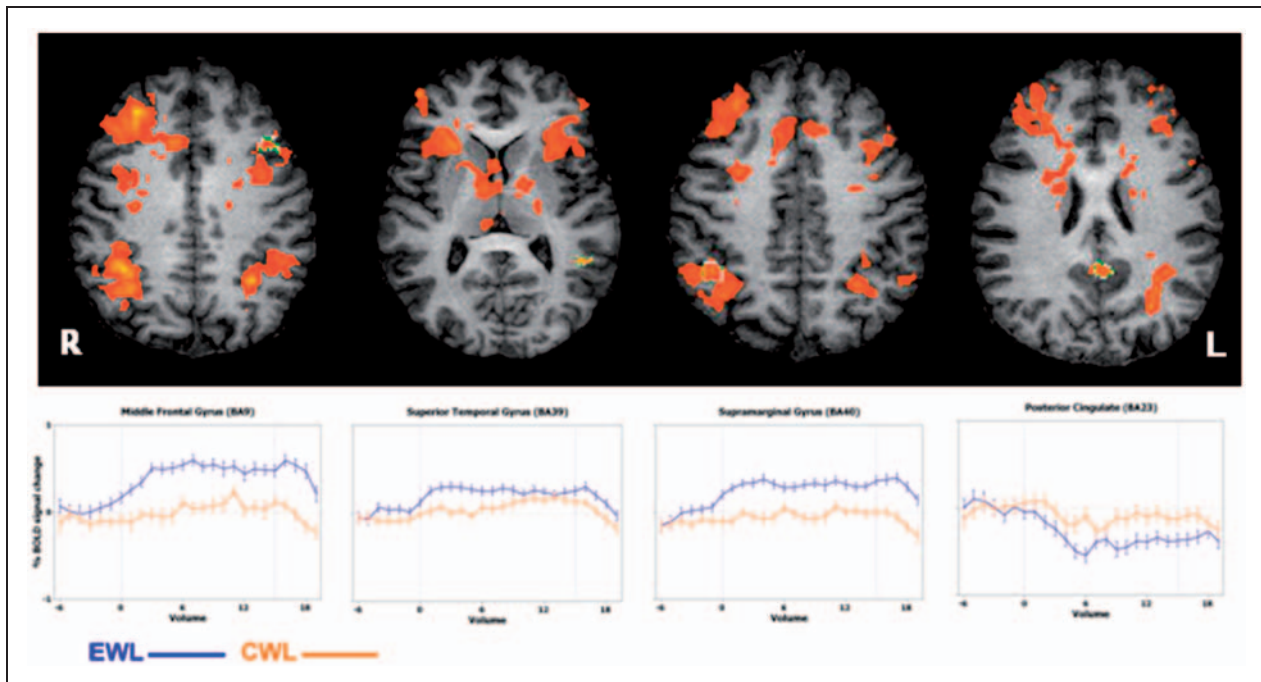BA = Brodmann Area; L = left; R = right.

Figure 2. Statistical *F*-map showing activation (encircled with green line and indicated by green arrow) in (from left to right) middle frontal gyrus, superior temporal gyrus, supramarginal gyrus, and posterior cingulate. In event related plots, corresponding task-related BOLD signal change during the experimental word list (EWL) or encoding task (blue) and control word list (CWL) or reading task (orange) is shown (for coordinates and number of voxels, see Table 2).

Behavioral effects of MDMA

A single dose of MDMA caused an impairment of immediate recall, an indirect measure of word learning (encoding), but not delayed recall in the experimental word list (encoding task). Under the influence of MDMA, subjects recalled on average 3.5 words less in total compared with the number of words recalled during the placebo condition. The finding that there was no effect of MDMA on delayed recall suggests that there was no problem with storage of words. Behavioral data also showed that previously learnt words (control word list/reading task) were perfectly recalled when under the influence of MDMA. In contrast to Quednow et al. (2006), who suggested that defective recall was the prime cause of impaired memory performance, this suggests that the inferior memory performance in MDMA users is, rather, due to defective or slower encoding.

Functional effects of Encoding

To determine the Encoding effect on brain activation patterns, encoding task activation was compared with reading task activation in both treatment conditions. Results showed significant involvement of a number of frontal (BA9, 46), temporal (BA37, 39, hippocampus), parietal (BA40) and occipital (BA18) regions, the insular and limbic cortex, and thalamus during Encoding in the present paradigm. According to previous studies in humans and non-human primates a number of

these above-mentioned regions and structures are included in a network supporting working memory (Buckner et al., 1999; Dupont et al., 2002; Gabrieli et al., 1998; Mottaghy et al., 2002; Ojemann et al., 1997; Schacter and Wagner, 1999; Smith et al., 1998). Of interest is the right-sided activation during Encoding in the dorsolateral prefrontal (BA9, 46) cortex. Previously it has been suggested that higher task complexity in a verbal working memory task is associated with right-sided frontal activation (Mottaghy et al., 2002). In the present study, the encoding task was more complex than the control word list as the former required reading and encoding, whereas the latter only required reading processes.

An odd result, perhaps, was the deactivation in the posterior cingulate gyrus (BA23) during Encoding. This area is part of the so-called default brain network, displaying high resting-state activation as a result of continuously gathering information about the inner and outer world. When the task at hand demands focused attention, activity in the network, and thus BA23, is diminished (Gusnard and Raichle, 2001). In addition it has previously been shown that deactivation in BA23 increases with increasing task complexity (McKiernan et al., 2003). Together, this adequately explains the greater increase in deactivation during the higher demanding encoding task condition.

The paradigm in the present study was previously used by other investigators, though in a slightly different form (Andreasen et al., 1995; Dupont et al., 2002). Dupont et al. (2002) investigated recall and encoding processes and showed activation in five regions during the encoding process,
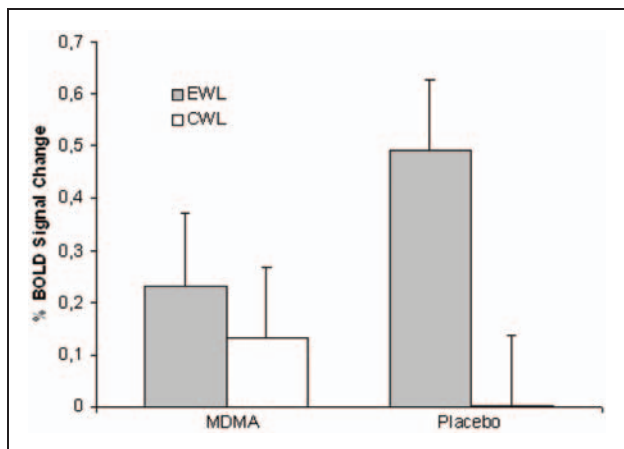
Figure 3. MDMA by Encoding Interaction. Bar chart indicating percentage BOLD signal change in BA10. EWL = Experimental Word List or Encoding task; CWL = Control Word List or Reading task (for coordinates and number of voxels, see Table 2).

Figure 4. Correlation between Encoding (experimental word list performance) and BOLD signal change in BA10 during MDMA and placebo treatments.

namely, occipital, left parietal, left superior temporal cortex, bilaterally ventrolateral frontal cortex, and fusiform gyrus. This activation was observed after subtraction of the experimental task-activation (encoding of 17 words) and the control task-activation (fixation of a single letter). The contrast analysis of Dupont et al. (2002) and the analysis used in the present study disclose consistencies concerning the regions active during the encoding of words. These findings together support the notion that our new paradigm addressed areas that are generally active during encoding of verbal material.

Functional effects of MDMA and limitations. MDMA caused a significant increase in activation and deactivation in frontal and occipital areas, respectively. A general limitation of pharmaco-MRI studies is that drugs may directly affect the BOLD signal response by vasodilation or vasoconstriction. Because MDMA acts generally on the serotonin (5-HT) system, and 5-HT has vasoconstrictor properties, it is possible that MDMA has a general effect on the cerebral blood flow (CBF) in the whole brain (Cohen et al., 1996; Frackowiak, 2004; Meyer et al., 2006). The expected physiological effect of MDMA on CBF is a general decrease in BOLD signal response, independent of task type (Brevard et al., 2006; Meyer et al., 2006). The fact that MDMA caused an increase in activation and deactivation in selective areas in the brain counteracts the notion that brain activations in the present study were secondary to a general physiological effect of MDMA. In support, previous acute MDMA studies in humans have also shown a specific effect of MDMA on task-related activation contrary to a general effect (Bedi et al., 2009; Ramaekers et al., 2009).

Functional effects of MDMA on Encoding: Interaction effect. Functional imaging data showed that a single dose of MDMA caused a selective effect on Encoding-related BOLD
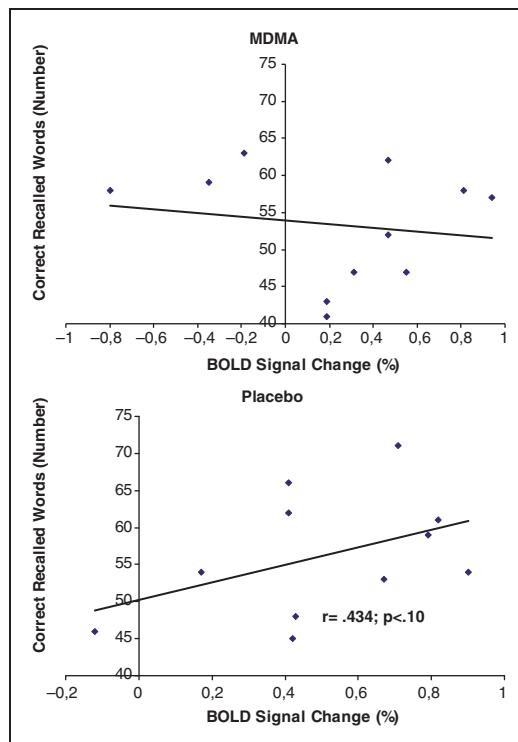
signal response (encoding task minus reading task) in three regions involved, respectively, in memory processes, visual recognition, and basic visual processing: that is, the middle frontal gyrus (BA10), the fusiform gyrus (BA19), and the cuneus (BA18). Functional and behavioral data only correlated in one of these three areas, being the left middle frontal gyrus. BOLD signal response in the left middle frontal gyrus, in the placebo condition, was increased during encoding task performance but not during reading task performance. This was according to expectation, as the reading task contained 'pre-encoded' words opposite to the encoding task which contained new information that had to be encoded.

Subjects under the influence of MDMA showed a small increase in BOLD signal response during encoding and reading tasks. The resemblance of a BOLD signal response during the encoding task and the reading task after taking MDMA is very noteworthy. It suggests that subjects under influence of MDMA invested equal effort during both lists. Behavioral data showed that performance on the reading task was maximal but the presence of BOLD signal response during this list suggested that subjects had to invest effort in these 'pre-encoded' words, while this was not the case during placebo treatment. The small increase in BOLD signal response during the encoding task in the MDMA condition relative to the placebo condition could be an indication for the absence of efficient encoding processes during experimental word learning under the influence of MDMA. Previous memory studies have shown that left frontal areas are a crucial link in the memory formation process, as they initiate the

Table 3. Means (±SEM) of scores on scales of the POMS and *t*- and *p*-values

Scale	Mean (±SE)		*t*-test placebo-MDMA	
	MDMA	placebo	*t*	*p*-values
Anxiety	6.36 (±0.676)	3.57 (±0.626)	2.724	0.017
Depression	0.21 (±0.114)	0.50 (±0.251)	−1.170	0.263
Anger	1.00 (±0.296)	1.14 (±0.345)	−0.342	0.738
Vigor	16.07 (±1.510)	13.07 (±1.659)	2.352	0.035
Fatigue	1.86 (±0.512)	1.86 (±0.783)	0.000	1.000
Confusion	6.36 (±0.843)	3.21 (±0.482)	3.217	0.007
Friendliness	20.00 (±1.726)	16.50 (±1.912)	2.511	0.026
Elation	12.64 (±0.809)	10.14 (±0.994)	3.736	0.002
Arousal	14.21 (±2.349)	11.57 (±2.163)	1.281	0.223
Positive mood	12.43 (±0.789)	9.64 (±1.020)	4.044	0.001

formation of the memory trace. It is to be expected that dysfunctions of these regions can result in defective memory formation (Cabeza and Nyberg, 2000; Kelley et al., 1998; Owen et al., 2005).

Conclusion

A single dose of MDMA impaired memory performance and affected encoding-related areas (BA10, BA19, BA18) as indicated by a change in BOLD signal response during the encoding task compared to the reading task, relative to placebo. Behavioral and functional data correlated only in one of these three mentioned areas, namely BA10. Good performance, as indicated by a high Immediate Recall Total score was related to small BOLD signal change and lower scores to higher BOLD signal change. The highest scores were obtained during the reading task, where performance was maximal, and the lower scores were obtained during the encoding task. When looking at the encoding task scores, it was shown that these only correlated to BOLD signal change in the placebo but not the MDMA condition. These results suggest an absence of effective encoding processing during MDMA influence.

The MDMA effect was not due to a general physiological effect on CBF, as indicated by a selective increase in activation and deactivation in three brain areas. The Encoding effect showed an absence of a BOLD signal response during the reading task, which proved there was no encoding during the reading task. This was expected, as the reading task was pre-memorized or pre-encoded. The behavioral data also showed that the words of the reading task could perfectly be recalled during MDMA treatment. Together these data supported the hypothesis that MDMA-induced memory impairment was due to defective or slower encoding and not due to defective retrieval or storage problems.

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Conflict of interest

The authors declare no conflicts of interest.

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