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Memory and mood during the night and in the morning after repeated evening doses of MDMA

KPC Kuypers *Experimental Psychopharmacology Unit, Department of Neuropsychology and Psychopharmacology, Faculty of Psychology, Maastricht University, Maastricht, The Netherlands.*

M Wingen *Experimental Psychopharmacology Unit, Department of Neuropsychology and Psychopharmacology, Faculty of Psychology, Maastricht University, Maastricht, The Netherlands.*

JG Ramaekers *Experimental Psychopharmacology Unit, Department of Neuropsychology and Psychopharmacology, Faculty of Psychology, Maastricht University, Maastricht, The Netherlands.*

Abstract

Previously it has been shown that MDMA causes memory impairment during daytime testing. However, MDMA is usually taken in the evening or during the night. In addition, it is known that sleep deprivation also causes memory impairment. The present study aimed to assess whether evening doses of MDMA added to, or interacted with the memory impairment due to sleep deprivation. Fourteen healthy subjects participated in a double-blind, placebo-controlled, two-way cross-over study. Treatments consisted of MDMA 75 and 50 mg divided over the evening or double placebo. Memory tests and subjective measures of mood were conducted at baseline and three times post dosing that is at 6.30 pm, 9.30 pm, 1.30 am and 7 am, respectively −1.5, 1.5, 5.5 and 11 h relative to drug intake (first dose). Memory performance detoriated progessively over time as a function of sleep loss, independent of treatment. MDMA added to this impairment as indicated by a significant main effect of MDMA on verbal and spatial memory performance. Mood ratings and response speed revealed an MDMA by Time interaction. After administration of MDMA response speed improved and feelings of vigor, friendliness, elation, anxiety, confusion, arousal and positive mood increased in magnitude during the night, while all these parameters returned to placebo-like levels on the final morning session. It is concluded that evening doses of MDMA selectively impair memory performance, and that this impairment is additional to the effect of sleep deprivation on memory performance.

Keywords

MDMA, verbal, spatial, memory, sleep deprivation, repeated dose

Introduction

Ecstasy has been frequently associated with cognitive impairment. Several studies have shown that abstinent ecstasy users display cognitive deficits, such as verbal, spatial and working memory deficits (reviews (Parrott, 2001; Cole and Sumnall, 2003)). A recent longitudinal study showed that impairment of verbal memory persisted for longer than 2.5 years after quitting the use of ecstasy (Thomasius *et al.*, 2006). However, the possibility exists that other factors contributed to these memory deficits such as the existence of pre-morbid group differences and polydrug use. In addition, even when groups of ecstasy users were compared with polydrug users groups, the pattern of drug use, apart from ecstasy, might differ (Gouzoulis Mayfrank and Daumann, 2006).

Recently, a direct and selective pharmacological link between memory impairment and MDMA intoxication has been established in two acute, placebo-controlled within-subject studies (Kuypers and Ramaekers, 2005, 2007). Verbal memory was impaired in subjects after administration of MDMA compared with placebo as demonstrated by a decrement in immediate and delayed recall in a word learning task. While showing a normal learning curve over successive trials when under influence of MDMA, subjects learned less compared with placebo. Spatial memory was also impaired after an acute dose of MDMA, as shown by a larger localization error during target localization in a simple spatial memory task. Other skills like syntactic reasoning remained unimpaired under the influence of MDMA. Characteristic of these studies was that subjects were tested during daytime after administration of a single dose of MDMA. However, ecstasy users typically take one or more tablets during the evening or night (Farre *et al.*, 2004) and this is is often accompanied by insufficient rest. Parrott and Lasky (1998) who studied the acute and sub-acute effects of ecstasy in combination

Corresponding author: KPC Kuypers, Experimental Psychopharmacology Unit, Department of Neuropsychology and Psychopharmacology, Faculty of Psychology, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands. *Email*: k.kuypers@psychology.unimaas.nl

with other drugs on memory in a naturalistic setting, showed that verbal memory was impaired 2–8 or 8–16 h after drug intake, compared with two control groups (Parrott and Lasky, 1998). This is in line with results of our previous study, showing impairing effects of a single dose of MDMA on memory (Kuypers and Ramaekers, 2005). However, Parrott and Lasky (1998) showed that the impairing effects lasted up to seven days whereas the memory impairment in Kuypers and Ramaekers' study was 25.5 h after MDMA administration not present. This prolonged presence of memory deficits in Parrott and Lasky's study could be due to additional factors like the combined use of several drugs and/or disturbed sleep pattern. It is therefore possible that supplementary stressors like sleep deprivation and repeated dosing magnify memory deficits caused by MDMA.

The aim of the present study was to assess whether evening doses of MDMA added to or interacted with the memory impairment due to sleep deprivation. The design was double-blind, placebo-controlled and two-way cross-over. Subjects received two doses of MDMA, separated by an interval of 4 h, and were tested four times (75 min) throughout the evening and night that is at 6.30 pm, 9.30 pm, 1.30 am and 7 am. Three cognitive tests assessing working memory (e.g., verbal, spatial) and a subjective measure of mood state were included. This mood questionnaire was included to provide additional information about the subjective feelings the subject experienced during the experimental sessions next to the objective measures of impairment.

Data presented here were part of a larger study on the acute effects of nocturnal doses of MDMA on (1) memory and mood and (2) impulsivity measures and psychomotor performance throughout the night. Fourteen subjects completed a testbattery containing measures from the four before-mentioned categories, four times per evening/night, on two separate days. The results from the psychomotor and impulsivity measures showed that nocturnal doses of MDMA may produce impairments of tracking performance and divided attention throughout the night that are additive to performance impairment produced by sleep loss (Kuypers *et al.*, 2007).

Methods

Design and treatments

The study was conducted according to a double-blind, placebo-controlled, two-way within subject design. The treatments consisted of two subsequent doses of MDMA (75 and 50 mg) and double placebo. According to the annual report (2005) of the 'Drugs information and monitoring system' (DIMS) of the Netherlands, average recreational doses of MDMA may vary between 0 and 200 mg, taken as 1 or 2 pills per evening (DIMS, 2005). Doses up to 150 mg have previously been studied in acute studies; It was shown that side effects were limited with doses up to 125 mg but appeared to be more frequent with 150 mg doses (Cami *et al.*, 2000; de la Torre *et al.*, 2000). Dose administration was separated in time by 4 h, at, respectively, 8 pm and 12 pm. A 4-h interval was chosen because subjects tend to take an additional dose when the subjective effects have worn off and that is approximately after 4 h (Hammersley, 1999; Cami *et al.*, 2000). Placebo and MDMA were administered orally in identically appearing formulations. MDMA was administered as a 25 mL solution in bitter orange peel syrup; placebo was given as 25 mL bitter orange peel syrup, both were mixed with 200 mL orange or apple juice.

Participants

Twenty volunteers underwent medical examination. Two volunteers were excluded from participation as they did not meet inclusion or exclusion criteria. The remaining subjects passed the medical screening and entered the training. Four subjects dropped-out prematurely either because of private matters (3x) or because of not feeling well on the first test day after placebo treatment (1x).

In total, fourteen subjects (seven male and seven female) aged in the range of 19–33 years (mean (SD) 22.93 (3.75)) completed all study treatments. Subjects were all polydrug MDMA users and their lifetime use varied from light to moderate (nine subjects: <25 times; five subjects: 30–35 times).

Subjects were recruited by means of advertisements in local newspapers and the snowball technique. 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 and two questionnaires for medical history and detailed history of drug use. 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. A medical doctor checked the completed list, and upon approval, subjects were invited for a medical examination. Blood and urine samples were taken for examination and they underwent an ECG-measurement. When medically fit, they were contacted and received a brochure with information about the study and a number of rules they had to obey during the period of the study. Subjects signed an informed consent to prove they 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 Edinburgh (2000) and was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and the University of Maastricht. A permit for obtaining, storing and administering MDMA was obtained from the Dutch drug enforcement administration.

Study procedure

Before testing days, participants were familiarized with the tests on a training day. Participants were requested to abstain from any drug use

one week before the medical examination until 14 days after the last testing day. They were asked not to use any caffeinated or alcoholic beverages 24 h before testing and to get a normal night's sleep. Participants were screened for alcohol use in breath and for recent drug use in urine (THC/opiates/cocaine/amphetamines/methamphetamines) upon arrival at the testing facilities at 6 pm. Women were given a pregnancy test. When tests were negative, the subjects received a light meal and were given a sleeping questionnaire to assess sleep complaints and a mood questionnaire Profile of Mood States (POMS) to assess their mood state. Cognitive tests and POMS were assessed four times during the evening and night at 6.30 pm (baseline measure), 9.30 pm, 1.30 am and 7 am; respectively 1.5 h pre-dosing and, 1.5, 5.5 and 11 h post dosing (first dose). A test day ended at 8.15 am. A blood sample was collected before each test session, except at baseline, to determine MDMA and MDA concentrations in blood serum. Participants were kept awake the whole night (e.g., they played party games, watched TV and/or listened to music). Testing days were minimally separated by seven days. The ambient temperature in the laboratory was relatively constant and never extreme that is it stayed within a range of 18–22°C.

Assessments

Cognitive assessments

Spatial memory task In the spatial memory task (Vermeeren *et al.*, 1995) subjects were briefly shown a fixation point in the center of the computer screen. Shortly thereafter, a target appeared at a random location for 500 ms. Subjects had to memorize the location of the target and, using a computer mouse, relocate the cursor as accurately as possible over that position. The cursor appeared either immediately upon target offset or after a delay of 2 or 4 s. Subjects depressed the left mouse button to indicate that the cursor was at the recalled position of the target. The task consisted of 75 trials, divided equally among the three response delays, and the sequence of delays was random. Short-term memory for spatial information was assessed. Dependent variables were localization error and reaction time.

Sternberg memory scanning task In the memory scanning test (Sternberg, 1966) subjects were briefly shown a set of unrelated consonants they had to memorise. This was called the 'memory set'. Thereafter a series of 90 letters was displayed on a computer screen. Subjects had to respond to each letter as rapidly as possible by pressing either a 'yes' or 'no' button to indicate whether or not the letter belonged to the memory set. This task was performed with memory sets consisting of 1, 2 and 4 letters, respectively. Half of the presented letters were part of the memory set. The task assessed speed of memory search or retrieval of verbal stimuli from memory. The dependent variable was the mean of the response time for correct responses on targets and non-targets.

Star counting task The star counting task (de Jong and Das Smaal, 1995) consisted of nine trials. Each trial consisted of a matrix of stars with plus and minus signs in between. Subjects had to start adding and subtracting stars from a given number, which was shown in the upper left corner. The plus and the minus signs indicated whether stars should be added to, or subtracted from the total score. This task required the control of a very simple process, namely counting (forward and backward), known to involve working memory. The dependent variables were the number of correct calculations and the time needed to perform the calculation (ms).

Subjective assessments

Two subjective measurements were taken: that is the Groninger Sleep Scale (GSS) and the POMS. The GSS (Mulder-Hajonides van der Meulen *et al.*, 1980) assessed sleep quality and quantity (hours of sleep). It consists of 15 dichotomous questions about sleep complaints and an open question concerning the duration of sleep. The POMS (de Wit *et al.*, 2002) is a self-assessment mood questionnaire with 72 five point-Likert scale items, representing eight mood states; that is Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness and Elation. Two extra scales were derived, that is Arousal ((Anxiety + Vigor) – (Fatigue + Confusion)) and Positive mood (Elation – Depression). The subject had to indicate in how far these items were representing his/her mood.

Pharmacokinetic assessments

Blood samples were taken at 1.5, 5.5 and 11 h post dosing (first dose), centrifuged immediately at 4000x*g* for 10 min 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 system (Symbiosis Pharma, Spark Holland) with tandem mass spectrometric detection (Quattro Premier, Waters Corportation) using deuterated analogues for internal standardization. Quantification limits were 5.0 ng/mL for MDMA and 2.5 ng/mL for MDA.

Statistical analyses

All statistical analyses were conducted by means of SPSS 11.5 for Windows. T-tests were applied to baseline data to check for baseline differences between treatments. All cognitive variables entered GLM univariate repeated measures procedures with MDMA (two levels) and Time (four levels) as main within subject factors. Additional factors were response delay (three levels) in the spatial memory task and memory load (three levels) in the Sternberg memory-scanning task. Separate contrast (drug versus placebo) tests were conducted in case of a significant overall effect of MDMA or MDMA by Session. Subjective parameters of the POMS and Groninger sleep questionnaire were analysed by means of nonparametric Wilcoxon rank tests. The alpha criterion level of significance was set at $P = 0.05$. In case of significant main effects of MDMA or Time, post-hoc analyses were conducted and alpha was then corrected for multiple comparisons.

Results

There were no statistically significant differences between baseline scores of the cognitive tasks. In the subjective tasks there was a baseline difference on the fatigue scale and therefore difference scores for the fatigue scale were calculated.

Cognitive assessments

Spatial memory task Analysis revealed significant main effects of MDMA ($F_{1,13} = 6.152$; $P = 0.028$), Time ($F_{3,39} = 13.207$; $P < 0.001$), Response delay ($F_{2,26} = 130.99$; $P < 0.001$) and MDMA by Response delay ($F_{2,26} = 3.41$; $P = 0.048$) on Localization error. Localization errors were on average higher when subjects were under influence of MDMA (10.38) compared with placebo (8.91). Further analysis of the Time effect revealed that localization error gradually increased as a function of time ($F_{1,13} = 20.87$; $P = 0.001$) (Figure 1). This elevation of localization error over time was independent of MDMA as demonstrated by the absence of a significant MDMA by Time interaction.

Reaction time showed significant effects of MDMA ($F_{1,13} = 9.62$; $P = 0.008$), Time ($F_{3,39} = 9.43$; $P = 0.002$) and MDMA by Time ($F_{3,39} = 4.48$; $P = 0.023$). Subjects responded faster, particularly on session 2 and 3, under influence of MDMA compared with placebo (quadratic contrast, $F_{1,13} = 9.38$; $P = 0.009$) (Figure 2).

An additional multiple regression analysis was conducted to determine whether other factors independently correlated with localization error in the spatial memory task. The analysis was conducted on individual trial data rather than the subjects' means in order to achieve maximal statistical power. The seven predictors that initially entered the model were subject number (1–14), MDMA (placebo, MDMA), Time (2, 3), Trial number (1–75), response delay (0, 2, 4), distance from fixation point (circle) (1–5) and reaction time. The stepwise regression procedure removed eventually two predictors (subject number and reaction time). The resulting model yielded five predictors that is delay, circle, MDMA, session and trial number. Together, these predictors explained about 14% ($R = 0.378$; $R^2 = 0.143$) of the variance in localization error ($F_{5,4194} = 139.76$, $P < 0.001$) Reaction time was not correlated with localization error.

Sternberg memory scanning task Analysis revealed significant effects of MDMA ($F_{1,13} = 5.75$; $P = 0.032$), Time ($F_{3,39} = 10.35$; $P < 0.001$), and Memory Load ($F_{2,26} = 245.19$; $P < 0.001$) on reaction time. Under influence of MDMA, subjects responded 21.85 ms on average slower compared with placebo (Figure 3). Reaction time increased as function of time after drug administration (linear contrast; $F_{1,13} = 16.10$; $P = 0.001$). There was no significant MDMA by Time interaction.

Star counting task Analysis revealed significant effects of MDMA ($F_{1,13} = 6.45$; $P = 0.025$) and MDMA by Time ($F_{3,39} = 2.85$; $P = 0.050$) on number of correct responses. Subjects had a lower score in the MDMA condition compared with placebo. Decrements were particularly noteworthy during the second and third session, but not in the final morning session. There were no significant effects of MDMA, Time or MDMA by Time on reaction time (Figure 4 and Table 1).

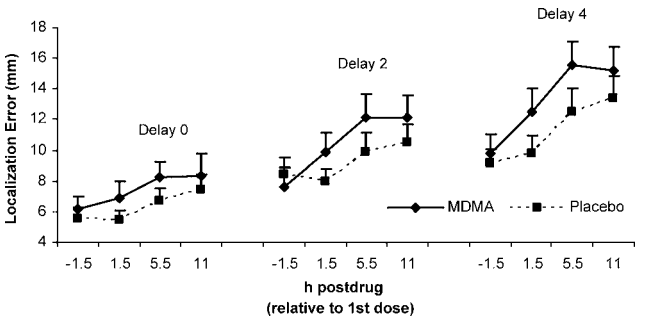

Figure 1 Localization error (mm) in the spatial memory task, per session and response delay (−1.5, 1.5, 5.5 and 11 h post drug correspond respectively with 6.30 pm, 9.30 pm, 1.30 am and 7 am).

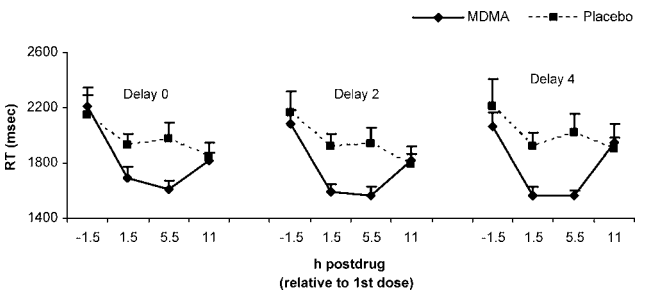

Figure 2 Reaction time (msec) in the spatial memory task, per session and response delay. (−1.5, 1.5, 5.5 and 11 h post drug corresponds respectively with 6.30 pm, 9.30 pm, 1.30 am and 7 am).

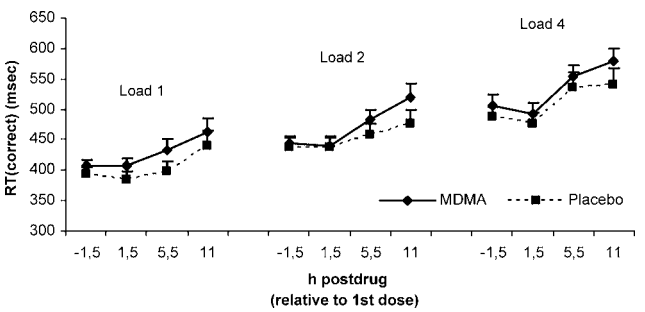

Figure 3 Reaction time (msec) in the Sternberg memory-scanning task, per session and memory load. (−1.5, 1.5, 5.5 and 11 h post drug corresponds respectively with 6.30 pm, 9.30 pm, 1.30 am and 7 am).

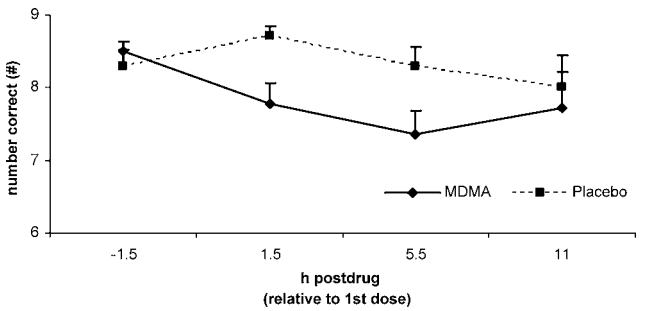

Figure 4 Number of correct sums in the star counting task, per session. (−1.5, 1.5, 5.5, and 11 h post drug corresponds respectively with 6.30 pm, 9.30 pm, 1.30 am and 7 am).

Table 1 Mean scores (SE) of cognitive parameters in the three memory tasks and p-values of the main and interaction effects on these parameters

Spatial memory task	Session	Delay	Treatments		ANOVA		
			MDMA	Placebo	MDMA	Session	MDMA*Session
Localization error					0.028	0.000	—
	1	0	6.17(.80)	5.55(.71)			
		2	7.62(1.22)	8.44(1.08)			
		4	9.75(1.26)	9.17(.92)			
	2	0	6.90(1.06)	5.45(.67)			
		2	9.90(1.20)	8.00(.80)			
		4	12.53(1.51)	9.82(1.11)			
	3	0	8.23(.99)	6.75(.78)			
		2	12.16(1.48)	9.88(1.25)			
		4	15.52(1.60)	12.47(1.57)			
	4	0	8.36(1.46)	7.42(.99)			
		2	12.16(1.46)	10.53(1.11)			
		4	15.23(1.48)	13.41(1.42)			
Reaction time					0.008	0.002	0.023
	1	0	2207.50(138.12)	2143.29(151.41)			
		2	2078.14(99.78)	2160.50(155.11)			
		4	2063.50(103.25)	2204.64(201.65)			
	2	0	1691.86(78.70)	1926.71(81.01)			
		2	1590.07(56.87)	1917.57(93.92)			
		4	1565.79(59.50)	1914.21(102.76)			
	3	0	1613.07(59.14)	1972.93(117.66)			
		2	1567.00(57.06)	1936.14(117.08)			
		4	1559.57(43.54)	2020.71(131.43)			
	4	0	1814.29(56.50)	1845.50(100.68)			
		2	1819.29(95.13)	1786.79(75.22)			
		4	1941.86(142.94)	1895.71(82.08)			
Sternberg task	Load						
RT(correct)					0.032	0.001	—
	1	1	407.53(8.34)	392.98(13.32)			
		2	443.62(11.08)	436.40(16.19)			
		4	505.43(18.40)	487.21(16.91)			
	2	1	406.63(13.02)	385.13(14.28)			
		2	440.25(13.95)	437.22(18.72)			
		4	492.89(18.46)	476.66(17.19)			
	3	1	432.13(19.01)	398.29(15.61)			
		2	483.48(15.96)	457.35(19.14)			
		4	554.04(17.53)	535.92(25.32)			
	4	1	462.08(24.34)	439.79(23.91)			
		2	519.07(24.09)	477.20(21.82)			
		4	578.53(20.63)	539.28(27.81)			
Star counting task							
RT(correct)					—	—	—
	1		30826.30(2045.38)	32966.74(2473.89)			
	2		30992.82(2118.03)	30839.04(1663.36)			
	3		30644.16(1488.10)	31318.41(2128.66)			
	4		32575.10(2269.06)	30915.42(1561.21)			
Correct					0.025	—	0.050
	1		8.50(0.14)	8.29(0.24)			
	2		7.79(0.28)	8.71(0.12)			
	3		7.36(0.32)	8.29(0.27)			
	4		7.71(0.50)	8.00(0.44)			

Subjective assessments

Groninger sleep scale There were no significant main effects of MDMA on Sleep Quantity or Quality scores on the evening prior to testing. Subjects slept on average 8.03 h (SE 0.30) and had an average score of 2.64 (SE 0.74) on the sleep complaints questionnaire.

POMS Analysis revealed effects of MDMA on 7 out of 10 scales of the POMS. Subjects showed increased scores during session 2 and 3 on Anxiety, Vigor, Confusion (only session 2), Friendliness, Elation, Arousal and Positive mood while under the influence of MDMA compared with placebo. There were no significant MDMA effects on measures of Depression, Fatigue and Anger (Figure 5 and Table 2).

Pharmacokinetic assessments

Blood serum concentrations of MDMA were on average (SD) 171.79 (54.63), 298.25 (65.38) and 199.27 (51.90), respectively 1.5, 5.5 and 11 h post dosing (first dose). The higher concentrations in the second and third measurement are in agreement with the expected levels after administration of a second dose, 4 h after the first one. Concentrations of MDA were on average (SD) 6.35 (3.04), 17.84 (7.48) and 21.94 (9.17), respectively 1.5, 5.5 and 11 h post dosing (first dose).

Discussion

Performance on different types of memory tasks (verbal, spatial) deteriorated independent of treatment, as a function of sleep deprivation. MDMA treatment produced a stable memory impairment that was consistent during the night and added to the memory impairment due to sleep deprivation. The fact that memory was impaired after administration of MDMA was in line with previous studies conducted during daytime. These acute studies showed impairment of verbal and spatial memory after administration of 75 mg of MDMA (Kuypers and Ramaekers, 2005, 2007).

In the spatial memory task subjects produced larger localization errors and reacted faster after MDMA as compared with placebo. To investigate the possibility that increments in localization error were caused by increments in speed of responding, a regression analysis was conducted. This analysis showed that localization error was uncorrelated with reaction time and that other factors, for example, response delay and MDMA explained about 14% of the variance in localization error. This was in line with previous results that showed an increase in localization error when under the influence of MDMA, independent of reaction time (Kuypers and Ramaekers, 2007). There was no interaction between MDMA and Time (level of sleep deprivation) on localization error. Localization error increased over successive sessions when hours of sleep loss increased in both MDMA and placebo condition. Reaction time however showed a U-curve-shaped progression over sessions. Reaction time decreased during the evening and night, in session 2 and 3, after administration of the drug, whereas during the morning session it increased to placebo-like levels. Apparently, there is a discrepancy between the stimulant-like effects of MDMA on motor behavior, which seem to have worn off during the morning, and the impairing effects it has on memory performance, which were still present in the morning. The presence of the memory impairment in the morning was in line with the blood plasma concentrations, which at that point were comparable with those measured 1.5 h after administration of 75 mg of MDMA. This discrepancy between effects of MDMA on memory performance and psychomotor

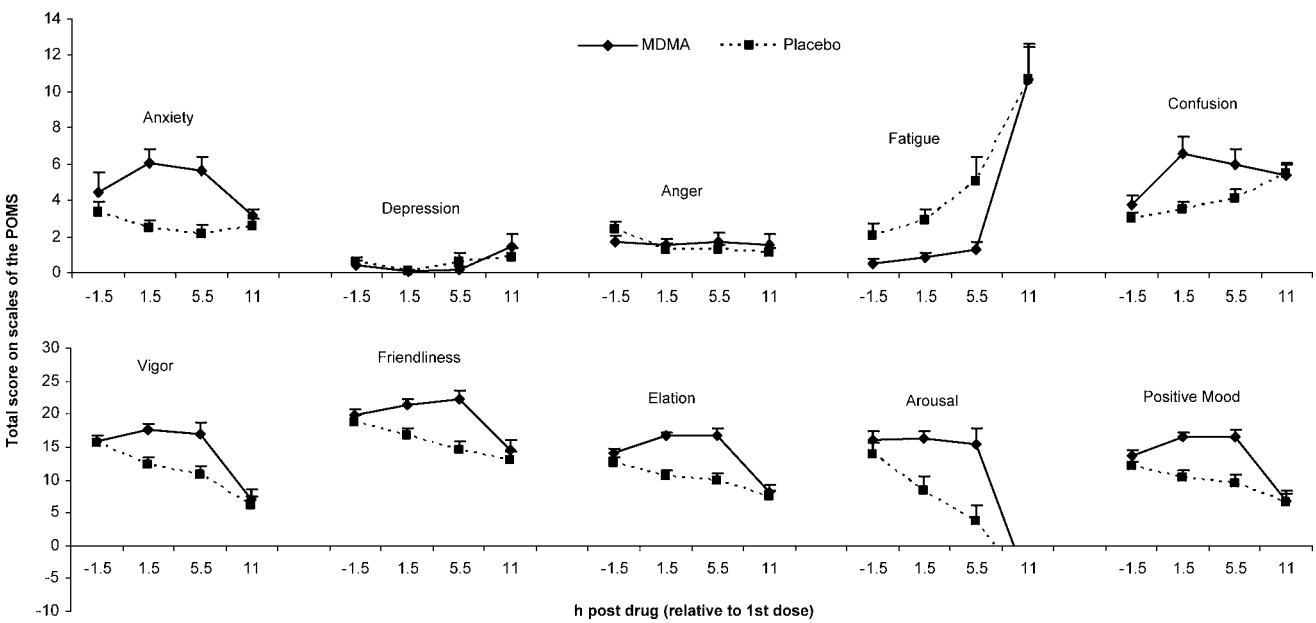

Figure 5 Total score on scales of the POMS (−1.5, 1.5, 5.5 and 11 h post drug corresponds respectively with 6.30 pm, 9.30 pm, 1.30 am and 7 am).

Table 2 Mean scores (SE) of 10 scales of the POMS and Z- and p-values of the statistical tests

Mood scales	Session	Mean (SE)		Wilcoxon signed rank MDMA-placebo	
		MDMA	Placebo	Z	p
Anxiety	1	4.43(1.12)	3.29(0.64)	−0.915	–
	2	6.07(0.77)	2.50(0.36)	−3.190	0.001
	3	5.64(0.72)	2.14(0.48)	−3.084	0.002
	4	3.14(0.39)	2.57(0.43)	−1.705	–
Depression	1	0.43(0.23)	0.57(0.29)	−0.423	–
	2	0.07(0.07)	0.07(0.07)	0.000	–
	3	0.21(0.15)	0.64(0.51)	−0.816	–
	4	1.43(0.69)	0.86(0.53)	−1.480	–
Anger	1	1.71(0.35)	2.43(0.40)	−1.778	–
	2	1.50(0.36)	1.29(0.29)	−0.734	–
	3	1.71(0.50)	1.29(0.27)	−1.100	–
	4	1.57(0.56)	1.07(0.27)	−0.777	–
Vigor	1	15.86(0.91)	15.64(1.06)	−0.362	–
	2	17.57(0.96)	12.29(1.24)	−2.766	0.006
	3	17.07(1.71)	10.71(1.29)	−2.447	0.014
	4	7.00(1.50)	6.21(1.25)	−0.567	–
Fatigue	1	0.50(0.29)	2.07(0.62)	−2.342	–
	2	0.86(0.29)	2.86(0.64)	−2.286	–
	3	1.29(0.44)	5.07(1.32)	−2.449	–
	4	10.71(1.74)	10.71(1.94)	−0.178	–
Confusion	1	3.79(0.51)	3.00(0.31)	−1.848	–
	2	6.57(0.98)	3.50(0.42)	−2.941	0.003
	3	6.00(0.84)	4.14(0.48)	−1.695	–
	4	5.36(0.64)	5.50(0.54)	−0.425	–
Friendliness	1	19.86(0.91)	18.64(1.15)	−1.073	–
	2	21.36(0.99)	16.64(1.11)	−2.556	0.011
	3	22.21(1.29)	14.50(1.43)	−2.923	0.003
	4	14.43(1.58)	13.00(1.35)	−0.668	–
Elation	1	14.07(0.72)	12.57(0.77)	−1.740	–
	2	16.64(0.59)	10.50(0.94)	−3.113	0.002
	3	16.64(1.20)	10.00(0.99)	−2.959	0.003
	4	8.14(1.9)	7.43(1.00)	−0.758	–
Arousal	1	16.00(1.47)	13.86(1.84)	−0.865	–
	2	16.21(1.24)	8.43(2.11)	−2.990	0.003
	3	15.43(2.43)	3.64(2.42)	−2.701	0.007
	4	−5.93(3.45)	−7.43(2.95)	−0.754	–
Positive mood	1	13.64(0.84)	12.00(0.76)	−1.742	–
	2	16.57(0.56)	10.43(0.98)	−3.113	0.002
	3	16.43(1.27)	9.36(1.41)	−2.864	0.004
	4	6.71(1.72)	6.57(1.41)	−0.039	–

performance was, besides the regression analysis, additional support for the view that the impairment of memory after MDMA administration was not due to increments in response speed.

Performance on the verbal memory task, that is the Sternberg memory-scanning task, deteriorated over time and independent of treatment. The speed of retrieval of verbal stimuli from short-term memory slowed down during the night and in the morning. MDMA added to this impairment and caused a larger speed reduction of retrieval speed compared to placebo. There was no interaction between MDMA and Time on the speed of retrieval. This indicated that the rates of decline in speed of retrieval as a function of hours of sleep loss were similar after MDMA and placebo. Subjects' performance on the working memory task, that is the star counting task, was compromised by MDMA especially during the night-time. However, in the morning when the sleep deprivation was maximal, performance was comparable to placebo. Performance in the placebo condition showed a clear decrement over time, with the worst performance in the morning, when the level of sleep deprivation was maximal. This demonstrates that the impairing effect of MDMA on working memory performance was no longer present during the morning session, when subjects' performance after repeated MDMA doses was comparable to that after placebo.

There was a clear interaction between the MDMA effect on mood and hours of sleep loss. After administration of MDMA, feelings of vigor, friendliness, elation, anxiety, confusion, arousal and positive mood seemed to increase in magnitude during the night, while they returned to placebo-like levels on the final morning session. MDMA did not affect measures of depression, fatigue and anger. This latter finding was in line with previous studies conducted during daytime, which showed an absence of MDMA effects on depression and anger scales during MDMA intoxication (Kuypers and Ramaekers, 2005, 2007; Kuypers, 2007; Lamers *et al.*, 2003). The interaction between MDMA's effects on mood and hours of sleep loss seemed to be in line with the stimulant effects MDMA had on reaction time performance in the spatial memory task. This stimulant effect on reaction time also disappeared during the morning session when sleep loss was maximal even though MDMA concentrations in blood were in the morning as high as after the first dose given in the evening. This discrepancy between kinetics and dynamics is also known as hysteresis, which means that the pharmacological effect is dependent on the time since dose as well as on the concentration in the blood (Logan and Isenschmid, 2003). The time since dose in the morning, in the present study, was 11 and 7 h for the first and second dose respectively. This means that subjects were at that moment in the elimination phase, as the half-life of MDMA is approximately 8 h (de la Torre *et al.*, 2004). Its stimulating effects disappeared or were not high enough to compensate for the sleep loss as demonstrated by the interaction effect on reaction time in the spatial memory task and several scales of the POMS.

Previous acute studies have shown that a single dose of MDMA impaired verbal and spatial memory and improved response speed. Results showed that after a single and repeated dose of MDMA, memory was impaired and that this added to the memory impairment due to sleep deprivation. During the night-time sessions, MDMA exerted a stimulant-like effect on response speed, which was also previously observed during day-time testing. This stimulant effect disappeared in the morning session, after a night of sleep deprivation. The same pattern was also reflected in ratings of mood, which were elevated after acute dosing of MDMA but decreased in the morning, when sleep deprivation was maximal.

The present study showed that memory performance decreases when the hours of sleep loss increase. The acute effects of MDMA were additional to these effects that is memory was even more impaired after MDMA during night-time testing compared with placebo performance during the night. It is known that sleep loss is not beneficial for memory and memory structures (Durmer and Dinges, 2005). It is possible that irregular sleep patterns in clubbers can contribute to long-term MDMA-induced impairments of cognitive and physiological functions; although this can not be concluded from the data of the present study and this certainly needs further investigation.

The present study tried to mimic MDMA use in a natural setting by adding two factors that is sleep deprivation and repeated night-time MDMA dosing. Still, some 'real life' factors were not taken into account that is prolonged excersise and high ambient temperatures (Parrott, 2004, 2006). It cannot be excluded that such factors may contribute to MDMA-induced memory deficits as often reported in abstinent MDMA users.

It is concluded that evening doses of MDMA selectively impair memory performance, and that this impairment is additional to the effect of sleep deprivation on memory performance.

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References

Cami J, Farre M, Mas M, Roset P N, Poudevida S, Mas A, San L, de la Torre R (2000) Human pharmacology of 3,4-methylenedioxymethamphetamine ('ecstasy'): psychomotor performance and subjective effects. J Clin Psychopharmacol *20*: 455–466

Cole J C, Sumnall H R (2003) Altered states: the clinical effects of Ecstasy. Pharmacol Ther *98*: 35–58

de Jong P F, Das Smaal E A (1995) Attention and intelligence: The validity of the Star Counting Test. J Edu Psychol *87*: 80–92

de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R, Roset P N, Segura J, Cami J (2000) Non-linear pharmacokinetics of MDMA ('ecstasy') in humans. Br J Clin Pharmacol *49*: 104–109

de la Torre R, Farre M, Roset P N, Pizarro N, Abanades S, Segura M, Segura J, Cami J (2004) Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition. Ther Drug Monit *26*: 137–144

de Wit H, Enggasser J L, Richards J B (2002) Acute administration of d-amphetamine decreases impulsivity in healthy volunteers. Neuropsychopharmacology *27*: 813–825

DIMS (2005) DIMS Jaarbericht 2005: Trimbos institute Netherlands Institute of Mental health and Addiction, pp. 1–4

Durmer J S, Dinges D F (2005) Neurocognitive consequences of sleep deprivation. Semin Neurol *25*: 117–129

Farre M, De La Torre R O, Mathuna B, Roset P N, Peiro A M, Torrens M, Ortuno J, Pujadas M, Cami J (2004) Repeated doses administration of MDMA in humans: pharmacological effects and pharmacokinetics. Psychopharmacology (Berl) *173*: 364–375

Gouzoulis Mayfrank E, Daumann J (2006) The confounding problem of polydrug use in recreational ecstasy/MDMA users: A brief overview

Hammersley R, Ditton J, Smith I, Short E (1999) Patterns of ecstasy use by drug users. Brit J criminol *39*: 625–647

Kuypers K P, Ramaekers J G (2007) Acute dose of MDMA (75 mg) impairs spatial memory for location but leaves contextual processing of visuospatial information unaffected. Psychopharmacology (Berl) *189*: 557–563

Kuypers K P C (2007) Psychedelic bliss: memory and risk taking during MDMA intoxication. In Kuypers K P C, Wingen M, Formisano E, Ramaekers J G (eds), Chapter 5. Imaging the acute effects of a single dose of MDMA on word learning – preliminary results. University of Maastricht, Maastricht, pp. 200

Kuypers K P C, Ramaekers J G (2005) Transient memory impairment after acute dose of 75 mg 3.4-Methylene-dioxymethamphetamine. J Psychopharmacol *19*: 633–639

Kuypers K P C, Wingen M, Limbert N, Samyn N, Ramaekers J G (2007) Acute effects of nocturnal doses of MDMA on measures of impulsivity and psychomotor performance throughout the night. Psychopharmacology (Berl) *192*: 111–119

Lamers C T, Ramaekers J G, Muntjewerff N D, Sikkema K L, Samyn N, Read N L, Brookhuis K A, Riedel W J (2003) Dissociable effects of a single dose of ecstasy (MDMA) on psychomotor skills and attentional performance. J Psychopharmacol *17*: 379–387

Logan B K, Isenschmid D S (2003) Cocaine, methamphetamine and driving impairment. In Burns M (ed.), Medical-legal aspects of drugs, Lawyers and Judges publishing company, Tucson, US

Mulder-Hajonides van der Meulen W R E H, Wijnberg J R, Hollanders J J, DeDiana I, Hoofdakker R (1980) Measurement of subjective sleep quality, Fifth European Congress on Sleep Research. Amsterdam

Parrott A C (2001) Human psychopharmacology of Ecstasy (MDMA): A review of 15 years of empirical research. Human Psychopharmacol Clin Experimental *16*: 557–577

Parrott A C (2004) MDMA (3,4-Methylenedioxymethamphetamine) or ecstasy: the neuropsychobiological implications of taking it at dances and raves. Neuropsychobiology *50*: 329–335

Parrott A C (2006) MDMA in humans: Factors which affect the neuropsychobiological profiles of recreational ecstasy users, the integrative role of bioenergetic stress. J Psychopharmacol *20*: 147–163

Parrott A C, Lasky J (1998) Ecstasy (MDMA) effects upon mood and cognition: before, during and after a Saturday night dance. Psychopharmacology (Berl) *139*: 261–268

Sternberg S (1966) High-speed scanning in human memory. Science *153*: 652–654

Thomasius R, Zapletalova P, Petersen K, Buchert R, Andresen B, Wartberg L, Nebeling B, Schmoldt A (2006) Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users: The longitudinal perspective. J Psychopharmacol *20*: 211–225

Vermeeren A, Jackson J L, Muntjewerff N D, Quint P J, Harrison E M, O'Hanlon J F (1995) Comparison of acute alprazolam (0.25, 0.50 and 1.0 mg) effects versus those of lorazepam 2 mg and placebo on memory in healthy volunteers using laboratory and telephone tests. Psychopharmacology (Berl) *118*: 1–9