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ORIGINAL INVESTIGATION

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Effects of a single dose of 3,4-methylenedioxymethamphetamine on circadian patterns, motor activity and sleep in drug-naive rats and rats previously exposed to MDMA

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Abstract *Rationale:* Despite the well documented neurochemical actions of 3,4-methylenedioxymethamphetamine (MDMA), acute effects in rats previously exposed to the drug have not been extensively explored. *Objective:* To examine motor activity and vigilance effects of MDMA in drug-naive rats and in rats exposed to the drug 3 weeks earlier. *Methods:* MDMA (15 mg/kg, IP) was administered to Dark Agouti rats. Motor activity, wakefulness, light slow wave sleep (SWS-1), deep slow wave sleep (SWS-2) and paradoxical sleep (PS), sleep and PS latencies were measured. Acrophases and amplitudes of the 24 h cycles were calculated by cosinor analysis. In parallel groups, local cerebral glucose utilization ($lCMR_{glu}$) and (^3H)-paroxetine binding were measured in motor areas of the brain. *Results:* In drug-naive rats MDMA caused marked increases in motor activity and wakefulness for at least 5–6 h. Circadian patterns of motor activity and sleep/vigilance parameters were altered up to 5 days after treatment. Despite most parameters tending to return to normal, there were still significant effects of MDMA on motor activity, wakefulness, and SWS-2 28 days later. Acute MDMA administration caused significant increases in $lCMR_{glu}$, but after 3 weeks $lCMR_{glu}$ was decreased in the same brain areas. No significant change in [^3H]paroxetine binding was observed in motor areas, although significant reductions were seen elsewhere (neocortex −81%). In rats exposed to MDMA 3 weeks earlier, most acute effects induced by MDMA administration were similar to those in drug-naive rats, but shorter duration of the acute effects were found in motor activity and vigilance. *Conclusions:* Our findings provide evidence that MDMA use can lead to long-term changes in regulation of circadian rhythms, motor activity and sleep generation.

Keywords MDMA · Motor activity · Sleep · Ecstasy · Serotonin transporter · Local cerebral glucose utilization · Circadian rhythm · PS latency · Cosinor analysis · Acrophase

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Introduction

The recreational drug "ecstasy" (3,4-methylenedioxymethamphetamine, MDMA) has become increasingly widely used by young people in Europe and USA (Green et al. 1995; Webb et al. 1996; Schifano et al. 1998; Schuster et al. 1998; Schifano 2000; Doyon 2001; Parrott 2001; Green et al. 2003). MDMA causes a marked increase in serotonin (5-HT) and dopamine (DA) release acutely and, in addition, there is accumulating evidence that in the long term it may damage 5-HT neurons in humans, non-human primates and rodents (Schmidt et al. 1986; Stone et al. 1986; Commins et al. 1987a, 1987b; O'Hearn et al. 1988; Ricaurte et al. 1988a, 1988b; Sprague et al. 1998). While the specific role of 5-HT in the control of behaviour is not known, it has been implicated in the regulation of circadian rhythm, sleep, motor activity and other physiological functions (Meyer-Bernstein and Morin 1996; Meyer-Bernstein et al. 1997; Kantor et al. 2000,

2002; Portas et al. 2000; Colbron et al. 2002). All these functions are likely to be disturbed by MDMA both acutely and in the long term.

A single injection of MDMA to rats produces a biphasic effect, with acute depletion of 5-HT peaking at 3–6 h after administration, and recovery by 24 h, followed by a second phase of 5-HT depletion about a week after drug treatment (Schmidt 1987). The second phase of 5-HT depletion in laboratory animals is accompanied by reductions of 5-hydroxyindoleacetic acid (5-HIAA) concentrations, tryptophan hydroxylase activity and in the density of 5-HT reuptake sites. Additionally, there is histological evidence that MDMA produces morphological changes to 5-HT neurones that arise from the dorsal raphe magnus (for reviews, see McKenna and Peroutka 1990; Green et al. 1995; Morgan 2000). Thus, MDMA might be expected to cause acute and chronic effects in several behaviours regulated by serotonin including circadian rhythm, sleep and motor activity.

The suprachiasmatic nuclei (SCN) of the hypothalamus serve as the primary circadian pacemaker in mammals (Rusak and Zucker 1979; Meijer and Rietveld 1989). Light is the principal cue responsible for the synchronisation of the pacemaker to daily environmental rhythms (Rea et al. 2000). However, non-photic behavioural events, such as exercise and social stimuli also alter the circadian phase in mammals (Mistlberger 1994; Mrosovsky 1996). The origin of serotonergic projections to the SCN is the median raphe, which is in turn innervated by the dorsal raphe (Glass et al. 2003). Depletion of serotonin within the brains of rats produces alterations in circadian parameters (Prosser et al. 1993; Morin 1999).

Sleep/wake cycle is under continuous serotonergic control (Portas et al. 2000; Saper et al. 2001). Recent microdialysis experiments provide evidence that the level of serotonin during wakefulness is elevated in most cortical and subcortical areas receiving serotonergic projections (Portas et al. 2000). In addition, the level of extracellular serotonin is consistent with the pattern of discharge in dorsal raphe nucleus (DRN) serotonergic neurons which show the highest firing rate during wakefulness, followed by a decrease during SWS and by virtual electrical silence during PS sleep (Jacobs and Fornal 1999; Portas et al. 2000; Saper et al. 2001). Serotonergic regulation of the sleep/wake cycle involves a number of 5-HT receptor subtypes, and the role of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3 and 5-HT_7 receptors has been demonstrated by several studies (Borbely et al. 1988; Monti and Jantos 1992; Monti et al. 1995, 1999; Kantor et al. 2000, 2002; Chapin and Andrade 2001a, 2001b; Filakovszky et al. 2001; Monckton and McCormick 2003).

Altered sleep architecture has been documented in two overnight EEG studies in MDMA users. Allen et al. (1993) found reduced total sleep time, mainly due to reduced stage 2 non-REM sleep, in abstinent recreational users. In a later study, the same group documented longer overall sleep times, mainly due to an increase in stage 3 and 4 non-REM sleep (McCann et al. 2000). Methodological problems and uncertainties may provide an explanation for the difference in outcome of these two studies; not least the uncertain nature of the purity and strength of ecstasy tablets, and the parallel use of other drugs, both illicit and licit, but also the inability to randomly assign subjects to drug condition (Parrott 2000).

The aim of our study was to follow circadian patterns of motor activity and sleep parameters in a rat model where the dose of MDMA is firmly within the recreational dosage range for humans (Green et al. 1995, 2003; Colado et al. 1999, 2001; Ricaurte et al. 2000; Esteban et al. 2001; Sanchez et al. 2001). In parallel studies we sought to identify those areas of the brain involved in these behavioural changes using 2-deoxyglucose and radioligand imaging.

Materials and methods

Animals

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health "Principles of laboratory animal care" (NIH Publications No. 85-23, revised 1985), as well as specific national laws (the Hungarian Governmental Regulation about animal studies, December 31, 1998 and the United Kingdom Animals Act, 1986) were followed. Permission was obtained from the local ethical committees.

Male Dark Agouti rats (Harlan, Olac Ltd, Shaw's Farm, Blackthorn, Bicester, Oxon, UK, aged 4–5 weeks and weighing 50–80 g upon arrival), were used in the experiments.

Motor activity and vigilance studies

Rats were kept in single cages (3500 mm×3500 mm×4050 mm) with food and water available ad libitum and their shaving beddings were changed every 2 or 3 days (depending on experiments), drug administrations were minimum 1 day after bedding changes. Animals were maintained on a 12 h light and a 12 h dark cycle (lights: from 0900 to 2100 hours, daylight type fluorescent tubes, 18 W, approximately 300 lux), at an ambient temperature of 21°C and the relative humidity was 40–50%. Animals were chronically equipped with EEG and electromyogram (EMG) electrodes as described previously (Kantor et al. 2002). Briefly, stainless steel screw electrodes were implanted epidurally over the left frontal cortex (L: 2.0 mm and A: 2.0 mm to bregma) and over the left parietal cortex (L: 2.0 mm and A: 2.0 mm to lambda) for fronto-parietal EEG recordings. The ground electrode was placed over the cerebellum. In addition EMG electrodes (stainless steel spring electrodes embedded in silicon rubber, Plastics One Inc., Roanoke, USA, size: 550 mm length, d=1.2 mm with the silicon rubber) were placed in the muscles of the neck. Surgery was performed under halothane (2%) anaesthesia (Fluotec 3) using a Kopf stereotaxic instrument. After surgery, animals were placed into the experimental room (white noise and the same light dark cycle as described above). After an 8-day recovery period, the rats were attached to the polygraph by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movement of the animals. In order to habituate the animals to the recording conditions, the rats were attached to the polygraph and received IP injections of physiological saline for 7 days before the experiments. The animals remained continuously connected to the recording cables throughout the entire study.

EEG, EMG and motor activity were recorded for 24 h periods, starting at light onset. The signals were amplified (amplification

factors approximately 5000 for EEG and motor activity, 20,000 for EMG, respectively), conditioned by analogue filters (filtering: below 0.53 Hz and above 30 Hz at 6 dB/octave) and subjected to analogue to digital conversion with a sampling rate of 64 Hz. The digitised signals were displayed on a PC monitor and stored on computer for further analysis.

Six week old animals (n=66) were randomly divided to four groups (see Table 1). On day −21 of the study (3 weeks before the acute challenge), rats from group 3 (marked as MDMA+Veh on the figures, n=12) and group 4 (marked as MDMA+MDMA on the figures, n=12) received an IP injection of 15 mg/kg (±)3,4-methylenedioxymethamphetamine hydrochloride (MDMA, certified reference compound, purity >99.5%, Sanofi-Synthelabo-Chinoin, Hungary). Rats from group 1 (marked as Veh+Veh on the figures, n=21) and group 2 (marked as Veh+MDMA on the figures, n=21) received an IP injection of 0.9% NaCl (vehicle) in a volume of 1 ml/kg. On day 1 of the study animals of groups 2 and 4 received MDMA (15 mg/kg), and groups 1 (used as control group of group 2) and 3 (used as control group of group 4) received injection of saline. EEG, EMG and motor activity were recorded in animals from group 1 and 2 on days 1, 3, 5, 14, 28 (see Table 1), and in groups 3 and 4 on day 1 (see Table 1). The existence of a solid circadian rhythm was tested on day 0. On all other days, a single vehicle injection was given to all animals at the same time, immediately after light onset. The high number of animals in groups 1 and 2 are explained by the gradual decrease in number of perfect EEG signals during the long-term experiments. EEG, EMG and motor activity were recorded in parallel in four to eight animals at the same time.

To assess motor activity, the potentials generated in electromagnetic transducers activated by the movements of the recording cable were detected and averaged for consecutive 4-s epochs (Svensson et al. 1987; Kantor et al. 2002). From the original motor activity signal, the absolute value or full-wave-rectified signal was derived. The unwanted baseline noise was removed by shifting the rectified signal below zero reference (submerging the band of noise under zero), re-establishing zero at the baseline, and then taking the positive half-wave rectification of the re-zeroed signal. Subsequently, the periods above the zero reference line were quantified, obtaining the time of motor activity. The values obtained by this method yielded very strong correlations with those obtained by visual inspection and scoring (12×2-h intervals, r=0.93, P<0.001).

The vigilance states were classified by SleepSign for Animal sleep analysis software (Kissei Comtec America, Inc., USA) for 4-s periods. Data was obtained by this software was compared to data were obtained by visual analysis: eight animals, 4×30 min each (from 0000 to 0030, from 0900 to 0930, from 1330 to 1400 and from 2230 to 2300 hours). For visual scoring we used the following criteria: wakefulness (Wake): the EEG is characterized by low amplitude activity at beta (14–30 Hz) and alpha (8–13 Hz) frequencies accompanied with high EMG and motor activity; light slow wave sleep (SWS-1): high voltage slow cortical waves (0.5–4 Hz) interrupted by low voltage fast EEG activity (spindles: 6–15 Hz) accompanied with reduced EMG and motor activity; deep slow wave sleep (SWS-2): continuous high amplitude slow cortical waves (0.5–4 Hz) with reduced EMG and motor activity; paradoxical sleep (PS): low amplitude and high frequency EEG activity with regular theta waves (6–9 Hz) accompanied by silent EMG and motor activity with occasional twitching (Gottesmann 1992; Kantor et al. 2000, 2002; Filakovszky et al. 2001). The values obtained by the software yielded strong correlations with those obtained by visual inspection for most parameters (Wake: r=0.968, P<0.001; SWS-1: r=0.736, P<0.001; SWS-2: r=0.920, P<0.001; PS: r=0.837, P<0.001). Sleep latency (time from light onset until the beginning of sleep) and PS latency (time from the beginning of sleep until the first PS) was established visually.

Mean±SEM values of animals are given in the figures. Values of motor activity and sleep were evaluated by multivariate analysis of variance (MANOVA) for measures with two main factors: treatment (non-repeated, vehicle and MDMA or first MDMA and second MDMA) and time (repeated, 1–23 h). Tukey honest significant difference test was used for post-hoc comparisons. Treatment×time interactions (F, df and P-values) are marked on each figure. Amplitude and acrophase values (±confidence limits) were calculated by cosinor analysis (Nelson et al. 1979) using the program ELTemps, 1998 (Cambras et al. 2000).

Measurement of local cerebral glucose utilization

On the day of the experiment, the rats were anaesthetized with halothane (1.5% in a gas mixture of 70% nitrous oxide, 30% oxygen) to allow the insertion of polythene cannulae into both femoral arteries and veins, and into the intraperitoneal space. The incision sites were infiltrated with local anaesthetic and the wounds sutured closed. The rats were lightly restrained and allowed to recover for at least 2 h before further experimental procedures.

Measurements of local cerebral glucose utilization ($lCMR_{glu}$) were performed using the fully quantitative [^{14}C]-2-deoxyglucose (Sokoloff et al. 1977) autoradiographic technique. The measurement protocols were in complete accordance with the methodology as originally published and as previously detailed from this laboratory (Kelly et al. 1995). Animals were exposed to MDMA (3-week MDMA group and 3-week+acute MDMA group) or saline 21 days earlier. On the final experimental day, rats were injected with MDMA (15 mg/kg, acute MDMA group and 3-week+acute MDMA group, see Table 2) or saline as control (Saline group and 3-week MDMA group, see Table 2) via the in-dwelling intraperitoneal cannula. Fifteen minutes later, the measurement of $lCMR_{glu}$ was initiated with a 30-s intravenous injection of tracer (30 μCi in 0.75 ml saline). Over the subsequent 45 min, a total of 14 timed arterial blood samples were collected at predetermined intervals for the determination of plasma [^{14}C] concentrations (20 μl) and glucose levels (10 μl) by liquid scintillation analysis and semi-automated glucose oxidase assay (Beckman) respectively. At the end of the measurement period, the rats were killed with a rapid intravenous injection of barbiturate (Euthatal), the brains dissected intact, frozen, and sectioned in a cryostat for the preparation of autoradiograms.

Sections adjacent to those used for autoradiographic measurement of $lCMR_{glu}$ were thaw-mounted onto gelatin covered glass slides and stored at −70°C for subsequent [^{3}H]-paroxetine autoradiographic binding analysis of 5-HT uptake sites according to the protocol described by De Souza and Kuyatt (1987), with the addition of preliminary wash procedures to remove [^{14}C]-tracers from the tissue (Sharkey et al. 1991).

Analysis of [^{14}C]-2-deoxyglucose autoradiograms was performed using a computer-based image analysis system (MCID/M5+). Local tissue isotope concentrations were derived from the optical density of autoradiographic images of brain tissue, relative to appropriate [^{14}C]-containing standards (Amersham Microscales), and $lCMR_{glu}$ calculated using the operational equations for the techniques (Sokoloff et al. 1977). Data were analysed using Student's t-test for grouped data with acceptable levels of significance set at P<0.05.

Table 1 Study design and name of groups in motor activity and vigilance studies. *MDMA* 15 mg/kg MDMA IP, *Veh* 1 ml/kg 0.9% NaCl (vehicle) IP

Group	Name	Day −21	Day 1	Day 3	Day 5	Day 14	Day 28
Group 1	Veh+Veh	Veh	Veh	Veh	Veh	Veh	Veh
Group 2	Veh+MDMA	Veh	MDMA	Veh	Veh	Veh	Veh
Group 3	MDMA+Veh	MDMA	Veh	–	–	–	–
Group 4	MDMA+MDMA	MDMA	MDMA	–	–	–	–

Table 2 Local cerebral metabolic rate of glucose utilisation ($lCMR_{glu}$) in saline and MDMA (15 mg/kg, IP) treated animals. Data presented as mean±SEM. Percentages are differences from saline control, except for last column, which represents percentage difference from 3-week MDMA group; 3-week animals exposed to MDMA 3 weeks previously

Brain area	$lCMR_{glu}$ (μmol/100 g/min)							
	Saline (*n*=6)	Acute MDMA (*n*=5)		3-week MDMA (*n*=5)		3-week+acute MDMA (*n*=6)		
Cerebellar vermis	81±4	135±6[a]	66%	65±3[a]	−20%	114±10[a,b]	40%	75%
Cerebellum PM	50±2	65±2[a]	30%	43±3	−14%	67±5[a,b]	36%	58%
SNR	51±3	89±10[a]	75%	42±1[a]	−17%	72±6[a,b]	41%	70%
SNC	63±2	91±5[a]	44%	58±5	−8%	108±6[a,b]	71%	86%
Lateral striatum	101±4	126±7[a]	25%	88±3	−13%	133±9[a,b]	32%	51%
Medial striatum	91±3	152±9[a]	67%	72±1[a]	−20%	139±9[a,b]	53%	92%
Ventrolateral thalamus	89±5	104±4[a]	18%	74±4	−17%	110±8[a,b]	24%	49%
Entopeduncular nucleus	58±9	75±3	31%	35±2	−39%	66±6[b]	15%	88%
Globus pallidus	47±2	75±5[a]	60%	39±1[a]	−16%	69±5[a,b]	49%	77%

[a]Significantly different from saline control group

[b]Significantly different from 3 week MDMA group

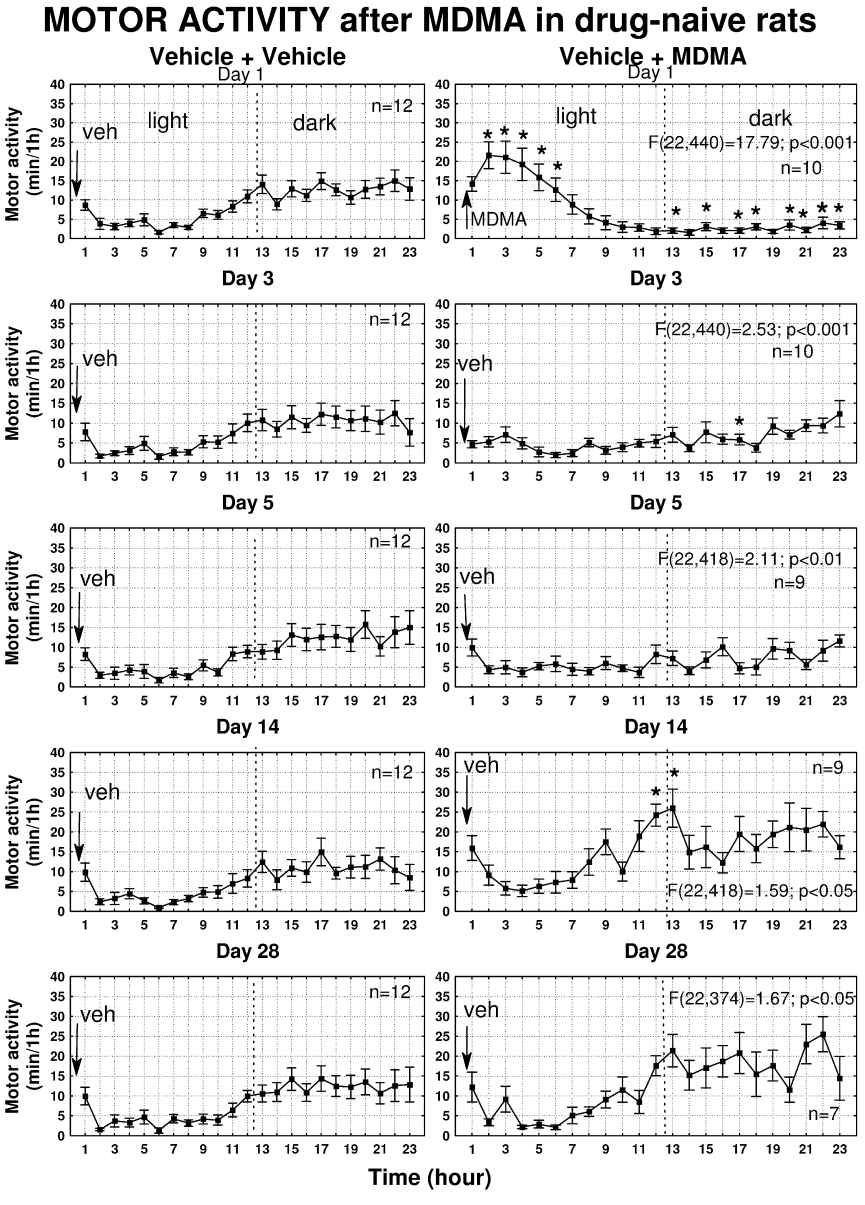

Fig. 1 Effects of MDMA (15 mg/kg) in drug-naïve rats on motor activity in 24-h recordings on days 1, 3, 5, 14 and 28. MDMA (*right column*) or vehicle (*veh*, *left column*) was administered at light onset on day 1. Treatment×time interactions of ANOVA are given for each day. *Significant difference compared with the same time, same day of Vehicle+Vehicle group, Tukey honest post-hoc test, $P<0.05$

Results

Acute effects of MDMA in drug-naive rats

In drug-naive rats, MDMA caused a massive increase in motor activity and inhibited sleep (Figs 1, 2, 3, 4, 5). Motor activity was significantly elevated for 6 h compared to control. From 2 to 4 h, motor activity exceeded the values observed during the normal active phase of the animals. After the cessation of this effect, a significant decrease in locomotor activity compared to control was observed. Wakefulness increased, and SWS-1, SWS-2 and PS decreased during the first 5–10 h.

These effects also caused significant changes in acrophases of day 1. Acrophases for motor activity were 16.9 h (15.7–18.1) and 2.3 h (0.9–3.8), for wakefulness 17.1 h (16.0–18.1) and 2.4 h (1.2–3.6), for SWS-1 1.7 h (0.8–2.5) and 16.0 h (14.2–17.9), for SWS-2 5.4 h (3.1–7.7) and 13.4 h (12.2–14.5) and for PS 2.6 h (1.9–3.2) and 17.3 h (16.4–18.2) in vehicle and MDMA-treated rats on day 1, respectively. Significant increases in amplitude were found for wakefulness: 9.4 min/h (7.0–11.9) and 18.4 min/h (12.8–23.9) and SWS-2:5.4 min/h (3.1–7.7) and 13.1 min/h (9.1–17.1) in vehicle and MDMA-treated rats on day 1, respectively. Regular increase in motor activity and wakefulness in the dark, active phase was abolished on the day of the treatment. These effects were demonstrated by significant shift in acrophases that were 14.6 h, 14.7 h, 14.3 h, 8.0 h, 14.7 h in motor activity, wakefulness, SWS-1, SWS-2 and PS, respectively.

Sleep latency increased from 7±3 to 392±28 min [treatment interaction: $F(1,20)=187.031$, $P<0.001$], PS latency from 53±5 to 230±35 min [treatment interaction: $F(1,20)=25.378$, $P<0.001$] on day 1 compared to control (Fig. 7a,c). Interestingly, sleep latency was much lower

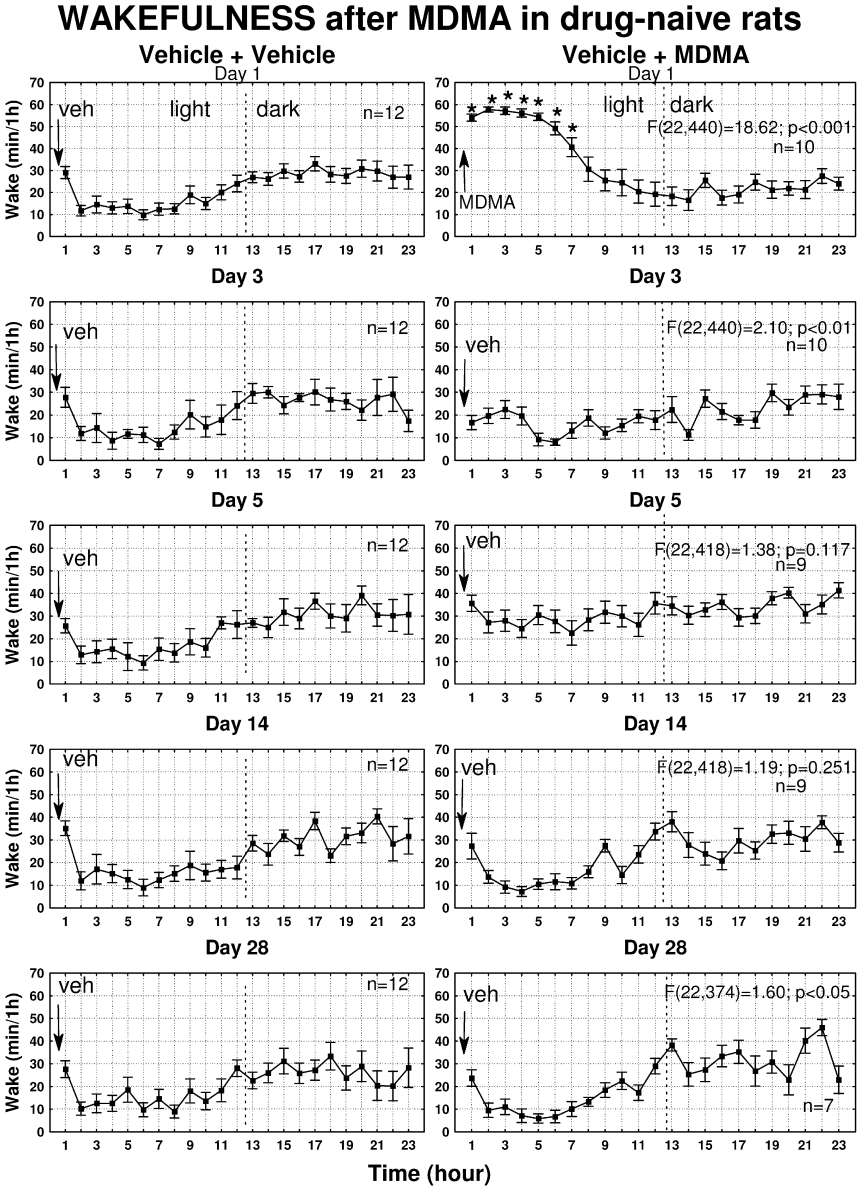

Fig. 2 Effects of MDMA (15 mg/kg) in drug-naïve rats on wakefulness in 24-h recordings on days 1, 3, 5, 14 and 28. MDMA (*right column*) or vehicle (*veh*, *left column*) was administered at light onset on day 1. Treatment×time interactions of ANOVA are given for each day. *Significant difference compared with the same time, same day of Vehicle+Vehicle group, Tukey honest post-hoc test, $P<0.05$

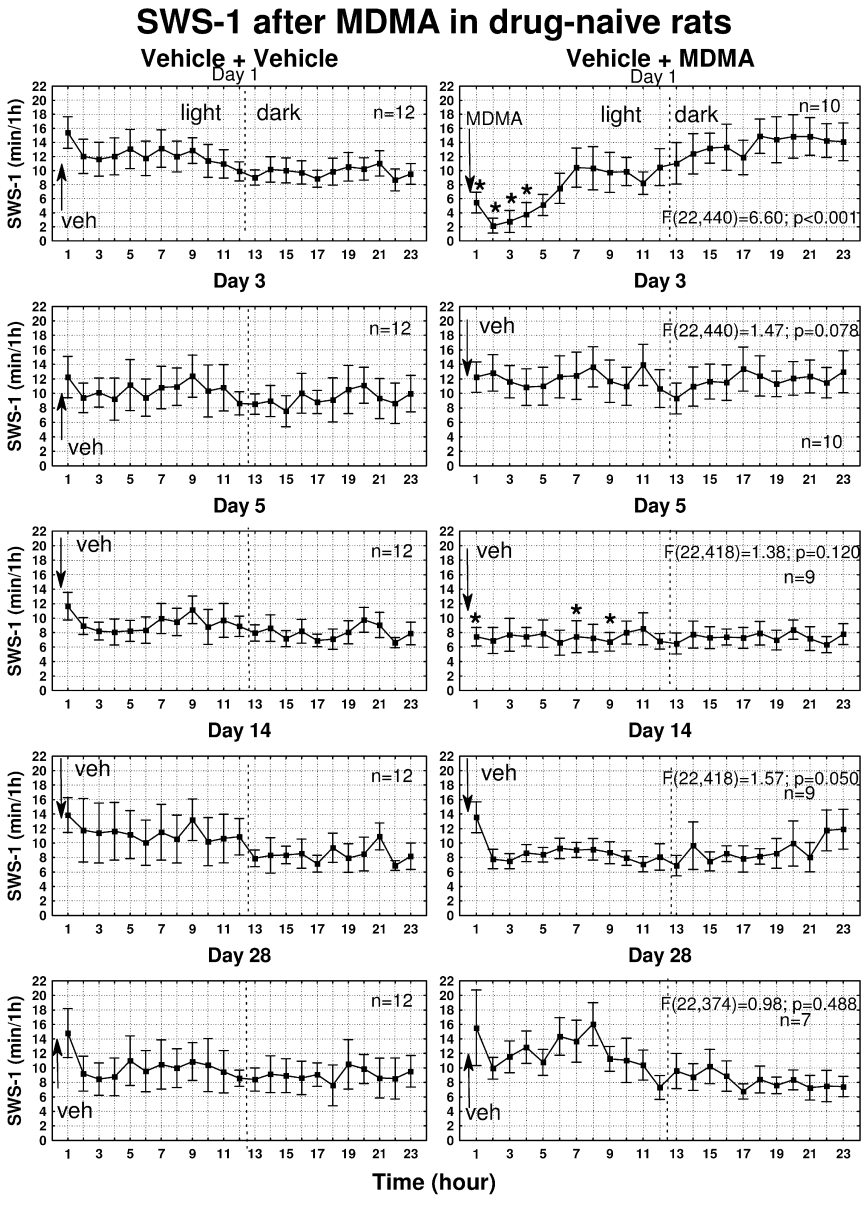

Fig. 3 Effects of MDMA (15 mg/kg) in drug-naïve rats on light slow wave sleep (*SWS-1*) in 24-h recordings on days 1, 3, 5, 14 and 28. MDMA (*right column*) or vehicle (*veh*, *left colum*n) was administered at light onset on day 1. Treatment×time interactions of ANOVA are given for each day. *Significant difference compared with the same time, same day of Vehicle+Vehicle group, Tukey honest post-hoc test, $P<0.05$

than PS latency in control rats, but it exceeded PS latency after MDMA.

Acute effects of MDMA in rats previously exposed to MDMA

In rats previously exposed to MDMA (15 mg/kg, 3 weeks earlier), an acute MDMA injection (15 mg/kg) caused significant increases in motor activity and wakefulness, and a decrease in sleep stages (Fig. 6). Cosinor analysis provided evidence for significant changes in acrophases. Acrophases for motor activity were 16.1 h (14.3–17.9) and 0.9 h (22.5–3.4), for wakefulness 16.6 h (14.8–18.4) and 1.9 h (0.1–3.7), for SWS-1 23.7 h and 14.1 h (12.0–16.2), for SWS-2 4.6 h (2.4–6.8) and 13.0 h (10.8–15.3) and for PS 5.7 h (3.7–7.7) and 16.8 h (15.0–18.7) in vehicle and MDMA-treated rats on day 1, respectively. No significant effects of acute MDMA treatment in amplitudes were found in animals previously exposed to MDMA. Interestingly, there were statistically significant differences in the acute response to MDMA between drug-naive rats and rats previously exposed to the drug; namely, shorter duration of the effects on motor activity, SWS-1, SWS-2 and PS (Figs 1, 3, 4, 5, 6). Significant treatment-time interactions were observed in motor activity [$F(22,330)=1.924$, $P<0.01$] and PS [$F(22,330)=1.665$, $P<0.05$] compared with the effect of MDMA in drug-naive rats. At the same time, we did not find significant treatment-time interactions in wakefulness [$F(22,330)=0.675$, $P=0.863$], SWS-1 [$F(22,330)=0.779$, $P=0.751$] and SWS-2 [$F(22,330)=0.818$, $P=0.704$] compared to drug-naive rats.

Sleep latency increased from 8±3 to 301±19 min [treatment interaction: $F(1,16)=326.761$, $P<0.001$] and PS latency from 39±3 to 258±46 min [treatment interaction: $F(1,16)=34.221$, $P<0.001$] on day 1 compared to

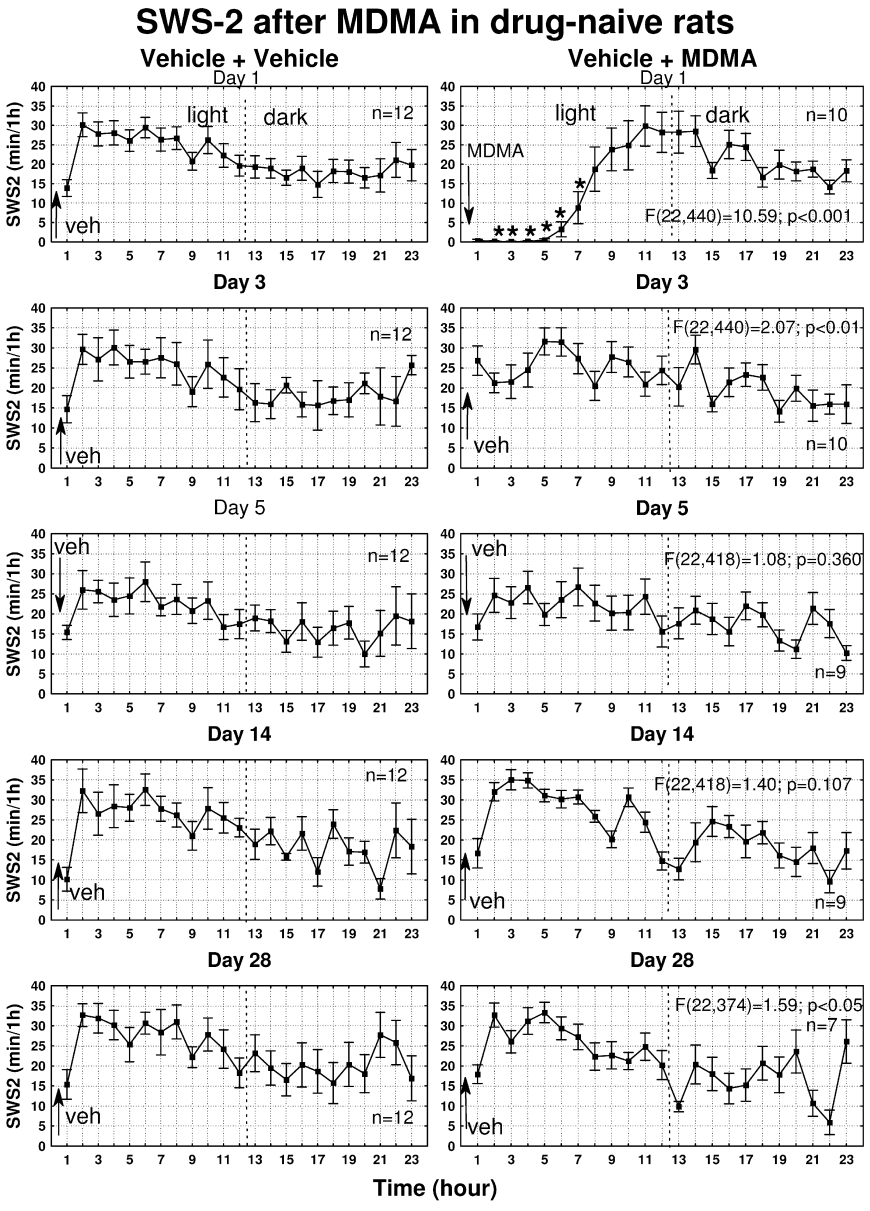

Fig. 4 Effects of MDMA (15 mg/kg) in drug-naïve rats on deep slow wave sleep (*SWS-2*) in 24-h recordings on days 1, 3, 5, 14 and 28. MDMA (*right column*) or vehicle (*veh*, *left column*) was administered at light onset on day 1. Treatment×time interactions of ANOVA are given for each day. *Significant difference compared with the same time, same day of Vehicle+Vehicle group, Tukey honest post-hoc test, $P<0.05$

control (Fig. 7b,d). Sleep latency was significantly decreased compared to drug-naive rats [treatment interaction: $F(1,15)=4.992$, $P<0.05$].

Short-term and long-term effects of MDMA

Effects of MDMA in drug-naive rats on days 3, 5, 14 and 28 are shown in Figs 1, 2, 3, 4 and 5. Significant treatment-time interactions were found for all parameters at most days as indicated on each figure. Significant interactions were caused by the general decreases in circadian activity and/or by higher ultradian fluctuation of parameters in MDMA-treated animals. On day 5, a diminished circadian activity was evident, but on days 14 and 28 fluctuations in parameters over the day were more prominent. For example, marked, but transient increase in motor activity preceded dark onset by 4–5 h on day 14 (Fig. 1). This was accompanied by dramatic decrease in SWS-2 (Fig. 4) and PS (Fig. 5). On day 28, fluctuation was shifted to a later time, close to the end of active phase. On day 3 (Fig. 7c) significant decrease in PS latency was observed compared to vehicle treatment [$F(1,20)=20.589$, $P<0.001$]. This was normalized later and on day 5 and 14 it was not different from control. At day 28, a strong trend towards decrease was observed [$F(1,17)=4.317$, $P=0.053$, control versus treated, Fig. 7c]. Significant treatment effect indicated general increase in motor activity after MDMA compared to controls on day 14 [$F(1,19)=7.065$, $P<0.05$]. Post hoc tests showed significant increase in motor activity only at dark onset, at the beginning of the active phase (hours 12 and 13). Similar trends were observed on day 28.

Despite significant treatment-time interactions, amplitudes and acrophases of motor activity, sleep and vigilance parameters showed less frequent changes between days 3

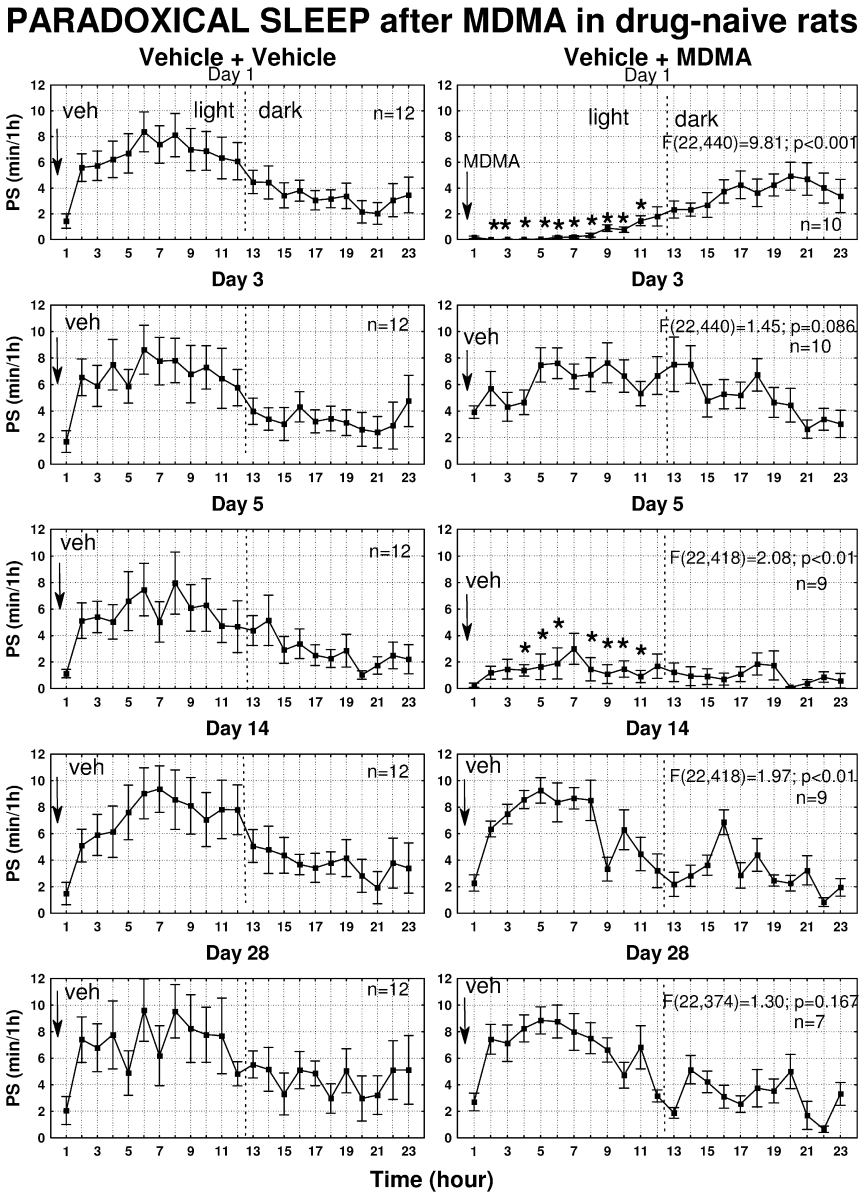

Fig. 5 Effects of MDMA (15 mg/kg) in drug-naïve rats on paradoxical sleep in 24-h recordings on days 1, 3, 5, 14 and 28. MDMA (*right column*) or vehicle (*veh*, *left column*) injection was administered at light onset on day 1. Treatment×time interactions of ANOVA are given for each day. *Significant difference compared with the same time, same day of Vehicle +Vehicle group, Tukey honest post-hoc test, $P<0.05$

and 28. The only exception was day 5. Significant decreases in amplitude were found for motor activity: 5.5 min/h (4.1–7.0) and 1.9 min/h (0.2–3.5), wakefulness: 10.5 min/h (7.7–13.3) and 4.1 min/h (1.1–7.2) and PS: 2.4 min/h (1.7–3.2) and 0.5 min/h (0.0–0.9) in vehicle and MDMA-treated rats on day 5, respectively. Furthermore, PS showed a dramatic decrease on this day resulting significant treatment effect [$F(1,19)=12.002$, $P<0.01$], while wakefulness showed a significant increase [$F(1,19)=12.558$, $P<0.01$].

Effects of MDMA on local cerebral glucose utilization and (^{3}H)-paroxetine binding

Effects of acute MDMA on local cerebral glucose utilization in brain areas involved in the control of movement and motor activity were measured in drug-naive rats, in rats treated with MDMA 3 weeks earlier, and in control animals (Table 2). Significant increases in glucose utilization (18–75%) were found in almost all brain areas studied including cerebellum, substantia nigra, striatum, ventrolateral thalamus and globus pallidus. In contrast, 3 weeks after a single injection, there was a tendency towards decreased $lCMR_{glu}$ in most areas (8–39%), but significant changes were observed only in cerebellar vermis, medial striatum and globus pallidus. MDMA also caused marked increases in glucose utilization in rats previously exposed to MDMA. Generally these increases (15–71% compared to saline treated drug-naive rats) were quantitatively similar to those following the first MDMA treatment. However, previous exposure to MDMA resulted in potentiation of acute effects in pars compacta of the substantia nigra and attenuation in pars reticulata. (^{3}H)-Paroxetine binding was measured in animals exposed to saline or MDMA 3 weeks previously

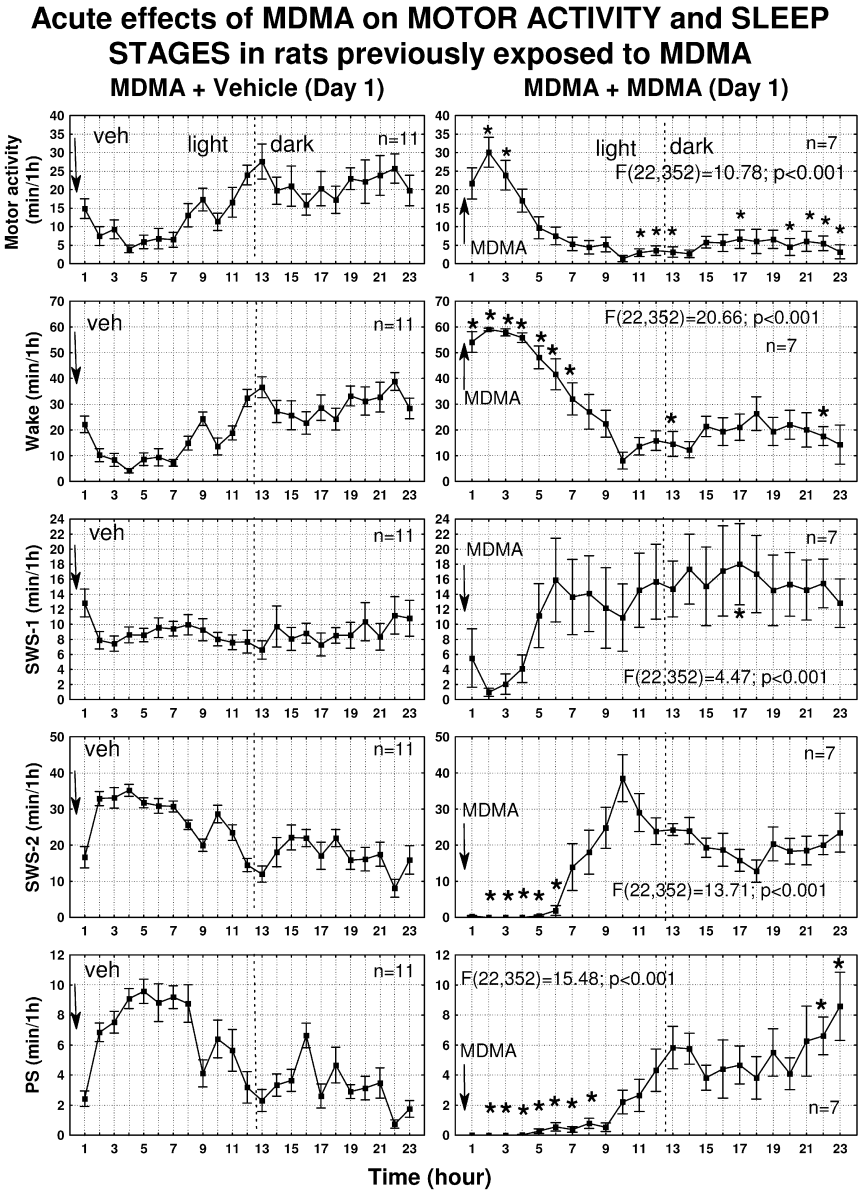

Fig. 6 Effects of MDMA (15 mg/kg) on motor activity, wakefulness, light slow wave sleep (*SWS-1*), deep slow wave sleep (*SWS-2*) and paradoxical sleep (*PS*) in 24-h recordings in rats exposed to MDMA 21 days earlier. MDMA (*right column*) or vehicle (*veh*, *left column*) injection was administered to previously exposed rats at light onset on day 1. Treatment×time interactions of ANOVA are given for each parameter. *Significant difference compared with day 1 of the MDMA +Vehicle group, $P<0.05$

(Table 3). No significant effects of MDMA were observed in substantia nigra pars compacta (−13%), pars reticulata (−2%) and striatum (−15%), although significant reductions were observed elsewhere in the brain (neocortex −81%).

Table 3 [^{3}H]-paroxetine binding in brains exposed to either saline or MDMA 3 weeks previously. Data are presented as mean binding density (fmol/mg tissue)±SEM

Brain Area	Saline (n=6)	MDMA (n=5)	Lesion
SNR	229±40	225±20	−2%
SNC	274±33	237±30	−13%
Striatum	57±11	49±6	−15%
Occipital cortex	77±20	14±7*	−81%

* Significantly different from saline control group

Discussion

MDMA caused marked increases in motor activity and wakefulness, and decreased SWS-1, SWS-2 and PS acutely. Circadian rhythms of these parameters were shifted by 8–15 h on the day of the treatment, and decrease in amplitude were found on day 5. Circadian rhythm of most parameters returned to normal by day 14, although there were still significant effects of MDMA on motor activity, wakefulness, and SWS-2 28 days later. In brain areas involved in motor control MDMA caused significant increases in $lCMR_{glu}$ acutely, but 3 weeks later $lCMR_{glu}$ was decreased. No significant change in [^{3}H] paroxetine binding was observed in subcortical motor areas, although significant reduction was seen in neocortex (−81%).In general, MDMA caused, similar acute effects in rats previously exposed to the drug compared to drug-

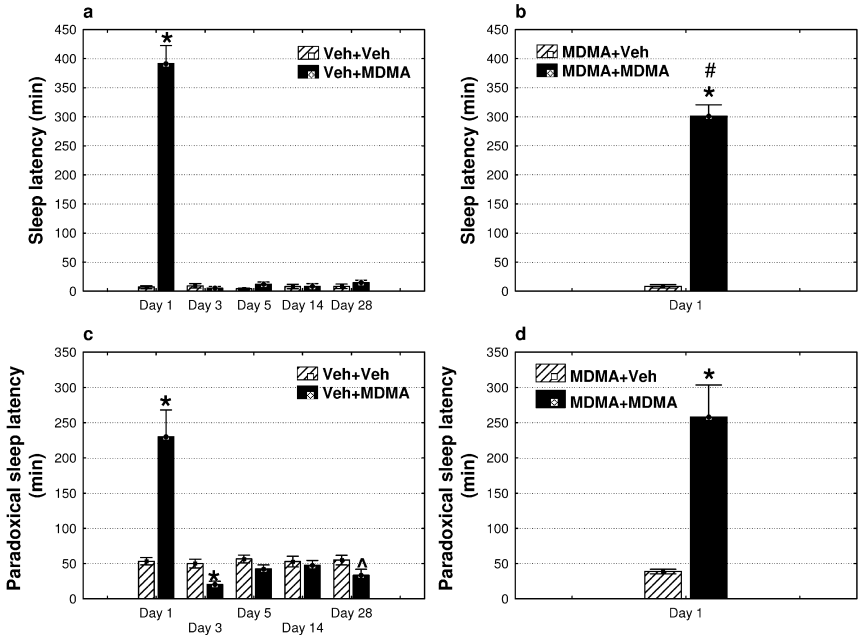

Fig. 7 Sleep latencies and paradoxical sleep (*PS*) latencies in drug-naive rats (**a**, **c**) and rats exposed to MDMA 21 days earlier (**b**, **d**). Day 1: MDMA or vehicle (*veh*) treatment at light onset. *Significant difference compared with the same day of vehicle control (*Veh*+*Veh*, **a**, **c**; *MDMA*+*Veh*, **b**, **d**), $P<0.05$. #Significant compared to day 1 of Veh+MDMA group, $P<0.05$. ^Strong trend compared with the same day of Veh+Veh group, $P=0.053$

naive rats, but significantly shorter duration of the acute effects were found in motor activity and vigilance.

In accordance with our findings, MDMA has been shown to produce acute hyperlocomotion in rats (Callaway et al. 1992; Callaway and Geyer 1993; Rempel et al. 1993; Marston et al. 1999; McCreary et al. 1999; Morgan 2000). Findings of several studies by using agonists and antagonists suggest that the hyperactivity produced by the release of endogenous 5-HT is due to the activation of 5-HT_{1B} receptors (Callaway et al. 1992; Callaway and Geyer 1993; Rempel et al. 1993; McCreary et al. 1999). However, it is possible that DA release also contributes to the hyperlocomotion effect of MDMA, particularly at high doses (Scearce et al. 1997). Enhanced DA efflux induced by the high dose of MDMA may be the result of increased extracellular 5-HT. The enhanced extracelullar 5-HT can, by acting on 5-HT_2 receptors, produce an increase in DA release by eliminating (directly or indirectly) the GABA-ergic inhibition of DA neurons (Yamamoto et al. 1995). There is evidence that 5-HT_{2C} receptors play an important role in the regulation of motor activity, but activation of this receptor subtype by receptor agonists or increase in extracellular 5-HT concentration suppresses motor activity (Bagdy et al. 2001). Furthermore, stimulation of 5-$HT_{1B/1D}$receptors has been shown to depress evoked γ-aminobutyric acid-mediated inhibitory synaptic input to DA neurons of the substantia nigra in vitro (Johnson et al. 1992), an effect that could result in disinhibition of DA neurons. Certainly, the pattern of extrapyramidal activation that we observe in our 2-deoxyglucose studies, would be consistent with such a disinhibitory mechanism.

Acute effects

Consistent with earlier studies, we observed that administration of 15 mg/kg MDMA increased the locomotor activity of rats 2–6 h after the first treatment. Increased wakefulness and decrease in all sleep parameters were observed at this time. In keeping with increases in motility observed acutely following MDMA treatment, in a parallel group of animals, glucose utilisation was found to increase in those areas of the brain known to be involved in motor control. These effects parallel human studies in which restlessness and feeling of heightened energy were described (for reviews see Steele et al. 1994; Green et al. 1995). A reversal of these changes, possibly a rebound effect that started 10–12 h later, followed these effects. A general increase in serotonergic tone by the selective serotonin reuptake inhibitor zimeldine also gave a biphasic effect on sleep and waking. An increase in wakefulness was followed by an increase in deep slow wave sleep (Bjorvatn and Ursin 1990).

Short-term effects

There were marked decreases in circadian amplitudes for almost all parameters on day 5. In addition, significantly increased wakefulness and decreased PS were observed. These changes may be explained by the possible leakage of 5-HT from the degenerating axons.

In humans the acute increase in neurotransmitter activity can generate profound feeling of euphoria and well-being (Parrott et al. 2001), although depression and anhedonia generally follow in the days afterwards, due to monoaminergic depletion (Parrott and Lasky 1998).

Long-term effects

In MDMA-treated rats transient increase in motor activity was observed on day 14 after dark onset compared to saline-treated rats. In addition, we found fluctuations in motor activity on days 14 and 28 especially at around dark onset. Wakefulness of MDMA-treated rats was disrupted during the active phase, but no general decrease was observed. In contrast, Wallace et al. (2001) demonstrated that rats treated with four injections of 10 mg/kg MDMA exhibited significant reductions in both diurnal and nocturnal locomotor activity of rats between days 7 and 14 after treatment. Marston et al. (1999) did not find differences between vehicle and MDMA treated rats in locomotor activity over the following 16 days. The latter group used ascending doses of MDMA (10, 15, 20 mg/kg) twice daily over 3 days, and the different doses used in these studies may explain these discrepancies. In addition, animals in the present study were housed in their home cage and room for at least 2 weeks before the administration of MDMA, and remained there for the whole experiment, while animals in the other studies were removed from their home cages on the day before or even during the studies. Three weeks after a single MDMA treatment, we observed a global decrease in glucose utilization, although significant changes (16–20% decrease) were observed in only three brain areas; cerebellar vermis, medial striatum and globus pallidus. While perturbations in the basal ganglia can be responsible for fluctuations in motor activity, damage to cerebellar regions can cause decline in the quality of movement. Presumably disorders in hypothalamic function, especially SCN, which is involved in development of diurnal rhythm (Saper et al. 2001), along with changes in the basal ganglion and the cerebellar regions, regulators of movement, result in the altered patterns of motor activity described here after injection of MDMA.

Neurotoxic lesions of the 5-HT projection from the median raphe to the SCN modulate activity phase onset (Meyer-Bernstein and Morin 1996; Meyer-Bernstein et al. 1997). MDMA also alters 5-HT innervation from the dorsal raphe to the intergeniculate leaflet (Mintz et al. 1997; Antle et al. 1998), which indirectly alters the response of other neurotransmitters necessary for resetting the circadian clock in Syrian hamsters (Colbron et al. 2002). In the present study, the function of SCN could be partly restored a few days after MDMA treatment; however amplitude in circadian rhythm was attenuated on day 5, and fluctuations in circadian rhythm were still present on days 14 and 28. Consistently, Colbron et al. (2002) demonstrated that MDMA treated hamsters phase shifted significantly less to the 5-$HT_{1A/7}$agonist 8-OH-DPAT and also to the 15 min of light than the controls. Furthermore, phase advances to 8-OH-DPAT in coronal hypothalamic slices of rats were attenuated by pretreatment with MDMA and this response had still not recovered at 20 weeks (Biello and Dafters 2001). It is possible that MDMA alters the response of the circadian pacemaker to the non-photic stimulus 8-OH-DPAT by altering the number and/or the distribution of 5-HT_{1A} and/or the 5-HT_7 receptors at the level of the SCN (Biello and Dafters 2001). The MDMA-induced loss of serotonin content could result in the dysregulation of the sleep-wake cycle, and a subsequent alteration in locomotor activity (Wallace et al. 2001).

Altered sleep architecture has been documented in two overnight EEG studies of ecstasy users (Allen et al. 1993; McCann et al. 2000). Our studies provide evidence for the perturbation of circadian and sleep parameters up to 28 days after MDMA. This fact, in addition to possible regrowth of axons and a pruning effect later, may account for the differences in their studies (Allen et al. 1993; McCann et al. 2000). There are several lines of evidence indicating that MDMA has a pruning effect both in rats and other rodents that may cause changes months and years after administration of MDMA (Ricaurte et al. 1992; Scanzello et al. 1993; Fischer et al. 1995).

In our study, a decrease, which almost achieved significance (P=0.053), was found in PS latency on day 28. Decrease in REM latency has been described in depressed patients (Kupfer et al. 1982; Reynolds et al. 1983). This may reflect a change in CNS function related to late effects of 5-HT depletion related possibly to the circadian cycle. In studies examining longer-term consequences of human ecstasy use, it was established that disruption of the circadian system is linked with a variety of disorders such as sleep, mood and concentration difficulties and depression, as well as decrements in mental and physical performance (Duncan 1996; Richardson and Malin 1996; Manfredini et al. 1998; Reneman et al. 2000).

Acute effects in rats previously exposed to MDMA

The second MDMA treatment, in animals previously exposed to the drug, caused a similar increase in motor activity, wakefulness and decrease in sleep, although the duration of this effect was shorter. Sleep latency, but not PS latency was also significantly decreased compared to the first treatment. In parallel with these effects, an increased glucose utilization was found which was somewhat attenuated in cerebellar vermis, SNR, medial striatum, entopeduncular nucleus and globus pallidus, when compared with the acute response to MDMA in drug-naive rats. Interestingly, we observed a potentiated effect of the second treatment in the substantia nigra pars compacta, the source of ascending dopaminergic neurones. However, no significant change in (^{3}H)-paroxetine binding was found in extrapyramidal areas 3 weeks after treatment, although there was evidence of marked serotonergic terminal depletion in neocortex. Taken together, these data suggest that brain regions other than primary motor areas are responsible for the change in motor and vigilance effects of MDMA after the second treatment. Significant differences compared to the first MDMA injection, were found in other parameters in recent studies. Attenuated temperature response to MDMA was found in animals

pretreated with MDMA 1 week before (Shankaran et al. 2001). Marked decrease in hypothalamic 5-HT concentration was also described in rats previously exposed to MDMA (Mechan et al. 2001). In contrast, we did not find significant decrease in (^{3}H)-paroxetine binding and increase in glucose utilization in areas involved in motor control, although local differences were evident among these areas.

In conclusion, the administration of a single dose MDMA to rats results in long-term alterations in locomotor activity and sleep-wake cycle that consequently outlines the potential dangers of human ecstasy-use.

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