Equivalent effects of acute tryptophan depletion on REM sleep in ecstasy users and controls

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Abstract

Introduction This study sought to test the association between 3,4-methylenedioxymethamphetamine use, serotonergic function and sleep.

Materials and methods Ambulatory polysomnography was used to measure three nights sleep in 12 ecstasy users and 12 controls after screening (no intervention), a tryptophan-free amino acid mixture (acute tryptophan depletion (ATD)) and a tryptophan-supplemented control mixture.

Results ATD significantly decreased rapid eye movement (REM) sleep onset latency, increased the amount of REM sleep and increased the amount of stage 2 sleep in the first 3 h of sleep. There was no difference between ecstasy users' and controls' sleep on the screening night or after ATD.

Discussion These findings imply that the ecstasy users had not suffered significant serotonergic damage as indexed by sleep.

Keywords Serotonergic · Abuse · Amphetamine · Sleep

Introduction

Ecstasy, 3,4-methylenedioxymethamphetamine (MDMA), is an amphetamine derivative popular for its mood-enhancing and energising effects. MDMA acts on monoamine transporters, especially those for 5-hydroxytryptamine (5-HT), to block reuptake and stimulate release (Rudnick and Wall 1992). Blockade of the 5-HT transporter with 5-HT reuptake

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inhibitors significantly attenuates MDMAs mood-enhancing effects (Liechti et al. 2000a, b; Farré et al. 2007; Tancer and Johanson 2007). MDMA has also been shown to cause serotonergic neurotoxicity in animals (e.g., Ricaurte et al. 1985; Mechan et al. 2006) and it has been argued that it may also damage serotonergic fibres in humans (e.g., McCann et al. 2008). There is, however, still some uncertainty about the precise nature of the long-term effects of MDMA on serotonergic functioning, the threshold of use required to cause changes, and the nature of the recovery (Hatzidimitriou et al. 1999; Thomasius et al. 2006; McCann et al. 2008; Jager et al. 2008; Selvaraj et al. 2009).

In order to assess the long-term effects of MDMA, it is important to find a reliable index of serotonergic function. Previous studies have tended to concentrate on emotional processing (e.g., Reay et al. 2006) and cognition (Kalechstein et al. 2007) since both are known to be modulated by 5-HT (Merens et al. 2007). Sleep is another sensitive index of serotonergic function (Bhatti et al. 1998; Haynes et al. 2004). Antidepressant medications that increase synaptic 5-HT concentrations reliably inhibit REM sleep (Wilson and Argyropoulos 2005); whereas, interventions that decrease 5-HT tend to promote REM sleep (e.g., Bhatti et al. 1998; Jouvet 1999). Preclinical studies have shown that serotonergic projections from the median and dorsal raphe nuclei terminate in tegmental nuclei that are involved in the onset of REM sleep (Cornwall et al. 1990; Luebke et al. 1992). Serotonin has an inhibitory effect on cholinergic neurons involved in the onset of REM sleep (Jouvet 1999; Hobson 1992) and modulates the putative REM "flip flop" switch (Lu et al. 2006). The application of 5-HT and 5-HT1A agonists into the brainstem hyperpolarises these cholinergic tegmental cells, thus inhibiting REM sleep (Luebke et al. 1992; Bjorvatn et al. 1997; Thakkar et al. 1998).

It is speculated that MDMA-induced serotonergic damage will have implications for sleep (McCann and Ricaurte 2007). Previous objective and subjective data have indicated that ecstasy users do have different sleep to controls (Allen et al. 1993; McCann et al. 2007; Parrott 2000, 2006; Dughiero et al. 2001; Morgan et al. 2002; Carhart-Harris et al. 2009). Decreased stage 2 sleep (Allen et al. 1993; McCann et al. 2007), decreased total sleep time (Allen et al. 1993), increased stage 1 sleep and trends toward decreased REM onset latency (ROL) (Allen et al. 1993; McCann et al. 2007) have been recorded in two relatively large samples of ecstasy users. Decreased ROL, increased sleep fragmentation and reductions in 5-HT transporter densities were found in rats 21 days after a single large dose of MDMA (Kirilly et al. 2008) but these changes were no longer significant after 180 days.

Acute tryptophan depletion (ATD) is a dietary manipulation that is used to decrease central 5-HT availability (Moja et al. 1989). It has also been used to probe functional changes related to MDMA (Curran and Verheyden 2003; Taffe et al. 2003). ATD has been shown to reduce concentrations of 5-HT in the cerebrospinal fluid (CSF) of animals (Moja et al. 1989) and humans (Carpenter et al. 1998; Williams et al. 1999), with a maximal decrease in plasma tryptophan 5–7 h after intake of the amino acid mixture (Rubia et al. 2005). ATD has been shown to have clear effects on several sleep parameters, especially REM sleep (Haynes et al. 2004; Moore et al. 1998; Evans et al. 2002; Landolt et al. 2003; Bhatti et al. 1998; Riemann et al. 2002; Voderholzer et al. 1998).

Previous studies have suggested that individuals with serotonergic abnormalities are more sensitive to ATD (e.g., Delgado 2006; Booij et al. 2002; Moore et al. 2001; Riedel et al. 2002; Neumeister 2003; Neumeister et al. 2006; Roiser et al. 2006; Jans et al. 2007). Positron emission tomography studies have found lower radioligand binding to the 5-HT transporter (a putative marker of cell integrity) in ecstasy users relative to controls (McCann et al. 1998, 2005, 2008; Buchert et al. 2004; Thomasius et al. 2006), although a recent study reported no such changes in a well-matched sample of former users (Selvaraj et al. 2009). We hypothesised that if ecstasy users had suffered serotonergic damage, this might lower their threshold for entering REM sleep.

This study sought to test the association between MDMA use, serotonergic function and sleep. We used ambulatory polysomnography to measure three nights sleep in 12 ecstasy users and 12 controls: once after no intervention, once after a tryptophan-free amino acid drink (depletion) and once after a tryptophan-inclusive amino acid drink (sham). Ambulatory recordings have been shown to reduce the disturbance caused by sleeping in unfamiliar surroundings (Iber et al. 2004) and serotonergic manipu-

lations have produced consistent effects with both methods (Wilson et al. 2004, 2005). It was predicted that ATD would decrease ROL and increase the amount of REM sleep in both ecstasy users and controls and that if ecstasy users had suffered significant serotonergic damage, this effect would be more pronounced in them.

Materials and methods

This was a double-blind placebo-controlled crossover study approved by a National Health Service Research Ethics Committee and conducted in accordance with The Declaration of Helsinki and Good Clinical Practice guidelines.

Participants

Twelve ecstasy users and 12 controls participated in this study. Ecstasy users were recruited via web-based message boards concerned with dance music and youth culture. Controls were recruited from previous study participants and acquaintances. Participants were physically and mentally healthy with no history of psychiatric disorder. Due to high rates of nausea after tryptophan depletion in females, we only included males. Participants were matched for age, alcohol use and work status. Shift workers were excluded. Ecstasy users and controls drank less than 17 units of alcohol per week on average and heavy smokers were excluded. All participants were required to abstain from illegal drug use 7 days before screening and for the remainder of the study. Caffeine use was kept to a minimum on the screening night and only two caffeinated drinks (tea or coffee) were allowed on study days. Ecstasy users were required to have used a minimum of 150 ecstasy pills and controls were only included if they had never taken ecstasy. All controls recruited for screening went on to complete the study. Three ecstasy users were excluded for poor compliance with the study requirements; two were excluded for showing poor reliability after screening and one gave a positive urine test for amphetamines.

Procedure

Screening

All subjects underwent screening in a clinical research unit in the Bristol Royal Infirmary. Demographic information was recorded and medical history was taken. A physical examination including electrocardiogram, routine blood tests, urine screen for drugs of abuse and screen for recent alcohol use. A standard psychiatric assessment was conducted and participants gave full disclosure of their

drug-taking histories. Participants completed the following questionnaires: the profile of mood states (POMS), the Spielberger state-trait anxiety inventory (STAI), the Beck depression inventory (BDI), the Bristol sleep profile and the Pittsburgh sleep quality index (PSQI). Once all these procedures had been completed, ambulatory sleep recording equipment was attached to the participants by a trained sleep researcher, the participant was asked to go to bed and get up at their usual time, and they went home to sleep.

Study days

Approximately 1 week after the screening recording, participants came in for the first study day where they received either a tryptophan-free (depletion) or tryptophan-inclusive (sham) amino acid drink in a double-blind randomised manner. Participants were instructed to refrain from using alcohol 48 h before each study day (confirmed by a breathalyser test on day of sleep recording) and to refrain from using illegal drugs 7 days prior to beginning the study (confirmed by urine analyses).

On the evening before each study day, participants began a low protein diet. They were instructed to have their normal breakfast and lunch but to have a low protein meal for dinner. They were asked to refrain from consuming meat or dairy products, and pasta or rice with vegetables was recommended. On the day of the recordings, participants were asked to keep to a strict low protein diet. For breakfast, they could have fruit (but not bananas), orange juice, and two slices of bread or toast with margarine (but not butter) and jam. They were asked not to eat peanut butter or to have any milk—except for a small amount allowed in one cup of tea or coffee. Participants were asked to have a light lunch consisting of salad, celery, carrots and fruit (excluding bananas, apples, pears or grapes as they have been found to contain traces of tryptophan). If no bread was eaten at breakfast, one slice was allowed at lunchtime with a small amount of margarine. One cup of coffee or tea was allowed at lunchtime.

Participants arrived at the research clinic at approximately 4:00 p.m. Most participants reported that they had eaten very little and were quite hungry. The drink was consumed at exactly 5:00 p.m.; this time was chosen so that the period of maximum depletion of CSF tryptophan would roughly coincide with sleep onset and the first REM epoch. Maximum depletion of CSF tryptophan takes place approximately 7 h after ingestion of the drink (Carpenter et al. 1998; Williams et al. 1999).

Flavouring (orange, blackcurrant or cherry) and water was added, and the contents stirred. The amino acid content of the tryptophan-free drink was consistent with Young et al. (1985) with 2.3 g of tryptophan added to the mixture.

This method is consistent with previous ATD sleep studies (e.g., Voderholzer et al. 1998; Riemann et al. 2002). Other studies have used 1.9 g/70 kg tryptophan (Haynes et al. 2004), 4 g tryptophan (Evans et al. 2002) and a 25% strength amino acid drink as their control mixtures (Moore et al. 1998; Bhatti et al. 1998) with generally consistent effects on sleep. All participants tolerated the drinks reasonably well. Some participants expressed dislike of the gritty consistency and unpleasant taste but all of the drinks were completely consumed and no participants vomited. After the drink had been ingested, participants were prepared for the sleep recording as before. Visual analogue rating scales of alertness, dizziness, nausea, anxiety, sadness, sleepiness, irritability and stomach upset were completed at half-hour intervals before participants returned home at about 7:30 p.m. Participants were instructed not to eat anything until the morning and to drink only water. Participants were telephoned at 10:30 p.m. and POMS and visual analogue scales (VAS) were carried out. Participants returned the equipment the following morning, underwent a brief health check and the St Mary's Hospital sleep questionnaire (SMHSQ) and Leeds sleep evaluation questionnaire (LSEQ) were completed.

Sleep recordings

Polysomnography recordings were obtained using an ambulatory digital sleep recorder (Embla), with electroencephalography, electrooculography, and electromyography electrodes attached according to standard methodology (Rechtschaffen and Kales 1968). An actiwatch and sleep diary was issued to record the regularity of the sleep cycles throughout the study. Participants returned home and were instructed to abstain from strenuous activity and to go to bed and get up at their usual time. The equipment was returned to the study centre in the morning and the data was retrieved. All recordings were scored blind to the study drink by two experienced sleep scorers. Each recording was separated by an interval of about 1 week.

Data analysis

Sleep variables scored included sleep onset latency, ROL, total sleep time, minutes of waking, REM, stages 1, 2, 3, and 4, number of awakening, and number of awakenings lasting longer than 60 s. ROL was the primary outcome.

Individual T tests were used to compare the screening sleep recordings of ecstasy users and controls, and analysis of variance was used to test for effects within and between subjects for the study days. In each test, we took the drink (depletion versus sham) as the within-subject variable, and the subject group (ecstasy versus controls) and the order of

Table 1 Demographics (mean values (SD))

	Sample size	Age	Work status	Weekly alcohol (units)	Daily cigarettes	STAI	BDI	PSQI (global)
Controls	12	27.1 (6.4)	1.1 (1)	11.4 (8.4)	0.8 (2.9)	21.8 (5.9)	1 (1.3)	2 (1.7)
Ecstasy	12	27.3 (7.2)	1.3 (0.9)	16.5 (8.3)	0.9 (2.2)	26.3 (7.2)	2.25 (1.6)	*4.1 (1.9)

*Significant group difference (unpaired T test, $T=3.1$, $p=0.006$ (two-tailed))

the drinks (e.g., depletion first night versus sham second night) as the between-subject variables.

Full night sleep recordings were incomplete in five cases due to participants taking the equipment off prematurely, batteries failing and participants having to get up especially early for work commitments. Since complete recordings for the first 3 h of sleep (post-sleep onset) were available in all of the participants, we decided this dataset would provide the most reliable index of sleep after ATD.

Power analysis indicated that a sample size of $n=24$ was sufficient to achieve 80% power to detect an effect size of $f=0.33$ in the primary outcome measure of ROL, equivalent to a difference of 30 min in one cell of the 2×2 design, at an alpha level of 0.05. This would translate as ecstasy users having an ROL of 30 min (±40) and controls having an ROL of 60 min (±40) after ATD, reduced from 90 min (±40) in both groups after the control drink.

Results

Baseline characteristics

There were no significant differences between ecstasy users and controls on age, work status, caffeine, tobacco or alcohol use.

Both groups' anxiety and depression scores on the STAI and BDI were low and clinically non-significant (Table 1). Ecstasy users scored significantly higher on the global PSQI (4.1±1.9) than controls (2±1.7; Table 1). Meta-analyses have shown that patients suffering from sleep disorders score at least 5 on the PSQI and healthy controls average around 2 (Buysse et al. 1989).

All ecstasy users reported having used at least 150 ecstasy pills in their lifetime. Their ecstasy use can be considered relatively high. Ecstasy users' mean lifetime use of ecstasy was 358.3±273 (150–1,000), their mean highest number of pills per session was 8.6±5.4 (4–20) and they had not used ecstasy for a mean of 60 days± 48.9 (17–181). In addition to MDMA, the ecstasy group had used considerably more other drugs than controls (Table 2).

No significant differences were found between the sleep of ecstasy users and controls during the screening night recording (Table 3).

As predicted, ATD significantly shortened ROL ($p=0.006$; Fig. 1), increased the amount of REM sleep ($p=0.03$; Fig. 2) and decreased the amount of stage 2 ($p=0.008$) in the first 3 h of sleep (Table 4). However, contrary to our predictions, there were no differences in ecstasy users' and controls' sleep at screening or after ATD versus sham. There were also no significant differences between ecstasy users' and controls' subjective sleep ratings of sleep (SMHSQ and LSEQ) on the screening night or after depletion versus sham.

There were no significant drink or drink×group effects in participants' POMS ratings at 10:30 p.m. (Table 5). VAS ratings of irritability were higher after ATD than after the sham drink ($p=0.01$), but again, there were no differences

Table 2 Drug use (mean values, SD, and range)

	Controls	Ecstasy
Cannabis (occasions used in last month)	0	1.5±1.5 (0–5)
Amphetamine (lifetime uses)	0.25±0.6 (0–2)	18.1±20.4 (0–100)
Amphetamine (days since last use)	4,375±1,025 (3,650–5,100)	1,534±1,849 (100–5,100)
Methamphetamine (lifetime uses)	0	one person, one occasion
Methamphetamine (days since last use)	NA	900
Cocaine (lifetime uses)	0	18±12 (2–40)
Cocaine (days since last use)	NA	309.5±408.1 (8–1,825)
Opiates (lifetime uses)	0	0.8±1.3 (0–6)
Opiates (days since last use)	NA	75±30.4 (30–120)
Lifetime ecstasy pills	0	358.3±273 (150–1,000)
Highest number of pills in a single session	NA	8.6±5.4 (4–20)
Days since last use of ecstasy	NA	60±48.9 (17–181)

Table 3 Screening sleep architecture (3-h recording following sleep onset; mean values (SD))

	Controls	Ecstasy
Time of sleep onset	12:12 a.m. (39.5 min)	11:43 p.m. (39.8 min)
Time of final waking	08:02 a.m. (43.6 min)	07:34 a.m. (72.2 min)
Sleep onset latency (min)	18.8 (14)	24.8 (22.6)
REM onset latency (min)	70.2 (16.4)	72.3 (21.9)
Wake minutes (3 h)	9.9 min (14.1)	8 min (5.6)
REM minutes (3 h)	23.6 min (14.6)	28.5 min (12.7)
Stage 1 minutes (3 h)	7.4 min (5.8)	8.8 min (5.6)
Stage 2 minutes (3 h)	69.3 min (16.7)	73.8 min (17.9)
Stage 3 minutes (3 h)	13.8 min (6.5)	14.3 min (7.5)
Stage 4 minutes (3 h)	56.6 min (17.1)	47.2 min (14.3)
Number of awakenings (3 h)	5.4 (2.4)	6.8 (4.4)
Number of awakening lasting >60 s (3 h)	1.1 (1.3)	1.5 (1.2)

No significant group differences (unpaired T tests, two-tailed)

between the groups. Although they did not reach significance, POMS ratings for fatigue and VAS ratings for "sleepy" were higher after ATD than sham, which might explain why participants had a significantly earlier sleep onset after ATD than sham ($p=0.008$; see Table 4). VAS ratings for "sad" were also higher after ATD but did not reach significance (Table 6).

Correlational analyses of extent of ecstasy use (and age, time since last use and psychiatric scores) versus sleep and mood measures did not reveal any significant relationships. Regarding order effects, ROL was significantly shorter when the ATD drink was given on the first study night, and POMS scores for fatigue ($p=0.01$) and VAS scores of sleepiness ($p=0.03$) were also significantly higher when ATD was given on the first study night.

Discussion

Summary of findings

ATD significantly decreased ROL, increased the amount of REM sleep and increased the amount of stage 2 sleep in the first 3 h of sleep. However, we did not find differences between ecstasy users' and controls' sleep on the screening night or after ATD. There were also no group differences in mood after ATD.

There are two possible explanations for lack of group effects: either tryptophan depletion is an ineffective probe for revealing MDMA-induced serotonergic damage and/or the ecstasy users had not suffered significant damage. Other studies have suggested that ATD does not reliably reveal MDMA-induced serotonergic damage (Taffe et al. 2003; Curran and Verheyden 2003). Taffe et al. (2003) administered four large doses of MDMA (10 mg/kg intramuscular) to rhesus monkeys over 4 days and carried out tryptophan depletion 1 year later. Although large reductions in hippocampal and neocortical 5-HT (76–93%) were detected

after 12 months post-MDMA treatment, decrements in CSF 5-hydroxyindoleacetic acid concentrations after ATD were similar in both groups (MDMA-treated animals 22%; controls 23%) and there were few behavioural differences. Curran and Verheyden (2003) carried out tryptophan depletion in a large sample of ecstasy users ($n=64$) and controls ($n=32$) which induced similar decreases in plasma tryptophan in both groups (31% for ecstasy users and 37% for controls). There were no significant differences between the groups' performances on a range of cognitive assessments and mood was also not significantly affected.

The ineffectiveness of ATD to reveal behavioural changes related to putative MDMA-induced serotonergic damage might be explained by a hyperresponsivity of mechanisms compensating for subacute deficiencies in synaptic 5-HT. Up-regulation of tryptophan hydroxylase activity (Neckers et al. 1997) or down-regulating the 5-HT transporter (Buchert et al. 2004) might be involved.

A simpler and perhaps more convincing explanation for the absence of group effects is that the ecstasy users had not
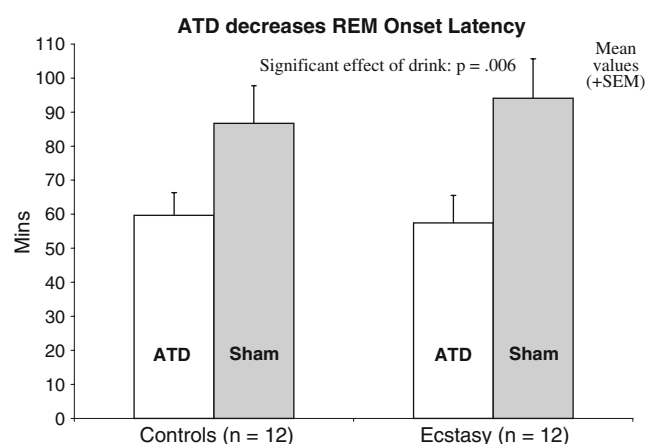
Fig. 1 Acute tryptophan depletion decreases rapid eye movement onset latency
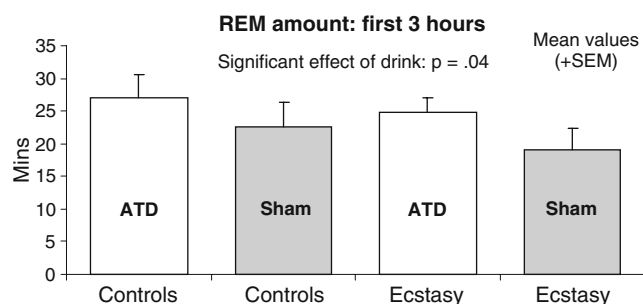
Fig. 2 Rapid eye movement amount: first 3 h

suffered enduring serotonergic damage. A growing number of functional and behavioural studies have failed to find significant differences between ecstasy users and controls (Daumann et al. 2004; Sumnall and Cole 2005; Hoshi et al. 2007; Medina and Shear 2007; Durdle et al. 2008; Bedi and Redman 2008; Bedi et al. 2008; Allott et al. 2008; Selvaraj et al. 2009). In addition, improved preclinical models have suggested that MDMA toxicity may be more moderate than previously supposed (Meyer et al. 2008). Another possibility is that selective regions may have been more resistant to damage. The hypothalamus is important for sleep (Saper et al. 2001) and is relatively resistant to MDMA-induced serotonergic damage (Ali et al. 1993; Insel et al. 1989; Scanzello et al. 1993; Ricaurte and McCann 1992; Sabol et al. 1996; Scheffel et al. 1998; Fischer et al. 1995). Activity in the raphe nuclei is closely associated with REM sleep

(McGinty and Harper 1976) and although serotonergic cell bodies are resistant to damage, their distally projecting axons are especially vulnerable (Hatzidimitriou et al. 1999). Serotonergic innervations of the hippocampus are sensitive to MDMA toxicity (Insel et al. 1989; Ricaurte and McCann 1992; Fischer et al. 1995; Sabol et al. 1996; Meyer et al. 2008) and medial temporal structures are significantly implicated in the regulation of REM sleep (Maquet et al. 1996; Braun et al. 1998; Wehrle et al. 2007; Cano et al. 2008). If MDMA had damaged serotonergic functioning in excitatory limbic structures, we might have expected disinhibition of REM sleep in ecstasy users, but this was not found.

Our findings support the hypothesis that ATD promotes REM sleep and decreases stage 2 sleep (Haynes et al. 2004; Moore et al. 1998; Evans et al. 2002; Landolt et al. 2003; Bhatti et al. 1998; Voderholzer et al. 1998) and they also provide further evidence that ATD is well tolerated by ecstasy users (Curran and Verheyden 2003). However, our finding that ecstasy users sleep similarly to controls conflicts with previous findings (Allen et al. 1993; McCann et al. 2007). Less total sleep time and less stage 2 sleep was found by Allen et al. in a relatively large sample of ecstasy users (23 MDMA and 22 controls). Contrary to the argument that our sample was not large enough to detect these differences, our results for group×drink on ROL gave an effect size $f=0.089$, indicating that a sample of 304

Table 4 Study night sleep architecture (3-h recording following sleep onset; mean values (SD))

	Controls		Ecstasy	
	Depletion	Sham	Depletion	Sham
Time of sleep onset[a]	11:55 p.m. (50 min)	12:14 a.m. (68.5 min)	11:20 p.m. (34.6 min)	12:14 p.m. (65 min)
Time of final waking	07:58 a.m. (45.3 min)	07:54 a.m. (46.9 min)	08:00 a.m. (56.4 min)	07:27 a.m. (48.9 min)
Sleep onset latency (min)	13.2 (6)	16.9 (9)	23.3 (26.5)	20.8 (22.6)
REM onset latency (min)[b,c]	59.7 (23.1)	86.7 (38.5)	57.5 (27.8)	94.1 (40.1)
Wake minutes (3 h)	8.7 (7.4)	6.9 (3.4)	4.6 (2.4)	7.1 (7.2)
REM minutes (3 h)[d]	27 (12.1)	22.7 (12.8)	24.8 (7.9)	19 (11.9)
Stage 1 minutes (3 h)	8 (4.2)	8.3 (5.7)	8.2 (5.6)	6.1 (2.6)
Stage 2 minutes (3 h)[e]	61.3 (22.4)	75.4 (11.5)	62.2 (18.4)	71 (17.9)
Stage 3 minutes (3 h)	14.7 (11.8)	19.7 (11.8)	18.3 (17.8)	18.2 (14.3)
Stage 4 minutes (3 h)	56.8 (26.9)	49.3 (10.7)	62 (23.9)	56.2 (22)
Number of awakenings (3 h)	7.2 (3.1)	5.5 (2.2)	5.2 (2.7)	5.4 (2.3)
Number of awakening lasting >60 s (3 h)	1.7 (1.8)	1.2 (1.2)	0.9 (0.7)	0.9 (1.2)

[a] Significant effect of drink ($F=8.7$, $df=1,19$, $p=0.008$)

[b] Significant effect of drink ($F=9.4$, $df=1,20$, $p=0.006$)

[c] Significant effect of drink×order ($F=5.2$, $df=1,20$, $p=0.03$)

[d] Significant drink effect ($F=4.8$, $df=1,20$, $p=0.04$)

[e] Significant drink effect ($F=5.9$, $df=1,20$, $p=0.03$)

Table 5 Study nights (profile of mood states 5 1/2 h post-drink (10:30 p.m.); mean values (SD))

	Controls		Ecstasy	
	Depletion	Sham	Depletion	Sham
POMS 10:30 p.m. tension	0.3 (0.5)	0	0.8 (1.5)	0.6 (1.1)
POMS 10:30 p.m. depression	0.8 (1.4)	0	0.5 (1.2)	0.1 (0.3)
POMS 10:30 p.m. anger	0.3 (0.5)	0	0.7 (0.8)	0.6 (1.7)
POMS 10:30 p.m. vigour	4.3 (4.6)	4.5 (4)	3.3 (2.4)	3.8 (2.1)
POMS 10:30 p.m. fatigue[a]	4.1 (3.4)	3.4 (2.8)	7.9 (4.4)	6.6 (5.9)
POMS 10:30 p.m. confusion	0.2 (0.4)	0	1.2 (1.9)	0.6 (1)

[a] Significant drink×order effect ($F=8$, $df=1,20$, $p=0.01$)

would be required in order to achieve 80% power at an alpha level of 0.05. Thus, according to our data, the effect, if real, is likely to be too small to be of clinical significance.

Another possible explanation might be that unlike the Allen study, our recordings were ambulatory, allowing participants to sleep at home, going to bed and waking up at their usual times. In the Allen study, participants slept in a sleep centre where they were required to sleep between the hours of 11:00 p.m. to 7:00 a.m. It is possible that the ecstasy users' were less accustomed to these times, particularly since a disproportionate number had travelled across US time zones to participate (21 ecstasy users versus five controls). Previous subjective data has suggested that ecstasy users go to bed and wake up later than controls (Carhart-Harris et al. 2009). It is possible that the sleep differences recorded in the Allen study would have been different had the participants been allowed to go to bed and wake when they were ready. It is not known whether the more recent study by the same team also implemented sleep time restrictions (McCann et al. 2007).

Limitations

The results of this study are only relevant to male ecstasy users since females were not included. Females show higher rates of nausea and vomiting after ATD (Hood et al. 2005) but their inclusion would have made the study more representative. Females are especially sensitive to

ATD (Ellenbogen et al. 1996; Weltzin et al. 1994; Nishizawa et al. 1997; Smith et al. 1997; Menkes et al. 1994) and there are also suggestions that they are more susceptible to MDMA-induced damage (Reneman et al. 2001; Buchert et al. 2004). It would have been interesting to have measured female ecstasy users' responses to ATD.

With regards to self-reported MDMA use, it may have been informative to have asked about consecutive use patterns and average dose. Some studies have suggested that these parameters might correlate better with functional and behavioural changes than total lifetime use (e.g., Meyer et al. 2008).

Our lack of an objective measure of tryptophan depletion is a more serious limitation. Measures of pre and post-drink plasma tryptophan levels would have achieved this. Subjects could have been asked to remain inside the study centre for a longer period of time post-drink allowing us to carry out these measures as well as additional behavioural tests that might also have been sensitive to serotonergic changes. This would also have allowed us greater control over subjects' behaviour, limiting opportunities for breaking the study requirements and strengthening the implications of our findings.

Conclusions

This study sought to test the hypothesis that ecstasy users sleep differently to controls using ATD as a potential means of

Table 6 Study nights (visual analogue scales 5 1/2 h post-drink (10:30 p.m.); mean values (SD))

	Controls		Ecstasy	
	Depletion	Sham	Depletion	Sham
VAS 10:30 p.m. sad	3.3 (6.5)	0	2.5 (6.2)	0.8 (2.9)
VAS 10:30 p.m. nauseous	0	0.8 (2.9)	3.3 (11.5)	1.7 (3.9)
VAS 10:30 p.m. irritable[a]	1.7 (3.9)	0	7.9 (10.3)	2.9 (6.2)
VAS 10:30 p.m. sleepy[b]	34.6 (19)	32.1 (21.6)	62.5 (17.5)	51.7 (24.4)
VAS 10:30 p.m. alert	44.2 (16.1)	37.1 (20.7)	39.2 (14.7)	43.3 (15)
VAS 10:30 p.m. dizzy	0.8 (2.9)	0.8 (2.9)	4.6 (5.8)	1.7 (3.9)
VAS 10:30 p.m. anxious	0	0	2.1 (5.8)	3.3 (6.9)
VAS 10:30 p.m. stomach churn	2.1 (7.2)	10.8 (19.3)	5.4 (8.9)	5.8 (7.6)

[a] Significant drink effect ($F=6.8$, $df=1,22$, $p=0.01$)

[b] Significant drink×order effect ($F=5.7$, $df=1,20$, $p=0.03$)

amplifying any differences between the groups. ATD significantly decreased ROL and increased the amount of REM sleep and stage 2 in the first 3 h of sleep. However, these pronounced effects were equivalent in both groups. The results support the hypothesis that ATD promotes REM sleep but do not support the hypothesis that MDMA is associated with persistent sleep abnormalities. It is possible that ATD is an ineffective probe for revealing long-term serotonergic changes caused by MDMA, but the inference that ecstasy users had not suffered significant serotonergic damage seems more likely. Further research is required to explain discrepancies between our findings and those of previous studies.

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