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ORIGINAL INVESTIGATION

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Altered response to tryptophan supplementation after long-term abstention from MDMA (ecstasy) is highly correlated with human memory function

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Abstract *Rationale:* MDMA (ecstasy; +3,4-methylenedioxymethamphetamine) damages brain serotonin (5-HT) neurons and, in non-human primates, a loss of various 5-HT axonal markers persists for several years. This raises the question of whether long lasting effects occur in human beings that persist even after they have stopped using MDMA. *Objectives:* We therefore assessed the effects of an indirect 5-HT manipulation on functions thought to be affected by MDMA use in people who had stopped using MDMA (ex-users) compared with continuing users and non-users. *Methods:* Ninety-six participants were recruited: 32 ex-users who had stopped using MDMA for >1 year (mean, 2.4 years); 32 current users and 32 polydrug controls who had never used MDMA but were matched with ex-users and controls on cannabis use and pre-morbid IQ. Participants were given an amino acid mixture that contained either no tryptophan (T−) or augmented tryptophan (T+) and assessed before and 5 h after the drink on measures of cognitive function and mood. *Results:* T+ and T− produced plasma tryptophan augmentation and depletion, respectively, in all three groups. Ex-users' plasma tryptophan levels in response to T+ were significantly higher than other groups. Ex-users' performance on a delayed prose recall task improved after T+ and lessened after T−. Changes in ex-users' free plasma tryptophan levels correlated highly (r=−0.9) with their baseline performance on immediate and delayed prose recall; change in total plasma tryptophan correlated (r=−0.81) with delayed recall. Further, total baseline plasma tryptophan correlated with number of years they had used MDMA before quitting. Baseline differences between groups were found on learning, working memory, aggression and impulsivity. T− did not produce differential effects in the three groups. *Conclusions:* Our results suggest that prolonged abstinence from MDMA might be associated with altered tryptophan metabolism. Ex-users showing the poorest memory function at baseline were also those who metabolised least tryptophan. These findings may reflect pre-morbid differences in 5-HT function of those who stop using this drug or consequences of MDMA use that emerge after abstention. Aggression is also associated with MDMA use and subsequent abstinence.

Keywords MDMA · Ecstasy · Serotonin · 5-HT · Tryptophan · Memory · Cognition · Ex-users · Abstention

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Introduction

Despite the current widespread use of MDMA (±3,4-methylenedioxymethamphetamine; ecstasy) and the fact that large numbers of people in some parts of the world have been taking MDMA regularly for over a decade, we know little of the long-term consequences of using this drug or whether the damage potentially caused by MDMA in humans is reversible after a period of abstinence. Studies with animals indicate that MDMA damages brain serotonin (5-HT) neurons. Repeated injected doses of 5 mg/kg or single doses of 20 mg/kg have been shown to cause changes in 5-HT markers in both rodents and non-human primates (e.g. Battaglia et al. 1988; Green et al. 1995; O'Shea et al. 1998; Ricaurte et al. 2000). These changes are seen in long-term attenuation of brain 5-HT and 5HIAA as well as attenuated tryptophan hydroxylase activity and reductions in the density of 5-HT uptake sites. Further, there is evidence to suggest that recovery of 5-HT axons in non-human primates remains incomplete 7 years after administration (Hatzidimitriou et al. 1999) and the loss of axonal markers may be more persistent in some brain regions than others (Scheffel et al. 1998). However, to date, little in the way of any functional consequences of these 5-HT changes in animals has been demonstrated so their significance remains unknown.

There is much debate as to whether MDMA may also be neurotoxic in humans, who typically take much lower, oral doses of around 1.7 mg/kg (e.g. Curran 2000; Grob 2000; Ricaurte et al. 2000). Humans who have recently used MDMA show decreased global and regional brain 5-HT transporter binding compared with non-using controls, and this decrement correlates to some degree with extent of use of the drug (McCann et al. 1998). In terms of functional consequences of MDMA use in humans, the most widely reported effect to date has been a subtle but significant impairment (relative to non-using controls) in performance on tasks tapping working and/or episodic memory (e.g. Morgan 1999; Gouzoulis-Mayfrank et al. 2000; Bhattachary and Powell 2001; Zakzanis and Young 2001). The relative consistency of this effect is surprising given the marked procedural differences between studies and general methodological problems in research with such polydrug users. In terms of causation, the degree to which impairments can be attributed to MDMA use rather than pre-existing differences between those who do and do not use the drug or to the conjoint use of several other drugs remain central questions.

Little is known in humans about whether differences between MDMA users and non-users in 5-HT markers or memory performance persist when people stop using MDMA. Given the long lasting damage to axonal terminals in monkey brains, it is important to determine whether similar effects occur in humans. Recently, some evidence of partial recovery of neuroendocrine response to the 5-HT agonist *d*-fenfluramine was found by Gerra et al (1998). Fifteen males who reported having abstained from regularly using MDMA for 12 months showed a normal cortisol response to *d*-fenfluramine but their prolactin response remained as blunted as it was after only 3 weeks abstention from MDMA.

One approach to examining 5-HT function in MDMA users would be to experimentally manipulate 5-HT and examine subsequent effects on cognition and mood. 5-HT cannot cross the blood-brain barrier and so all neuronal 5-HT is synthesised locally in the neurone from its precursor, tryptophan, in a two-stage process. Tryptophan is hydroxylated to 5-HT by the enzyme tryptophan hydroxylase followed by subsequent decarboxylation involving L-aromatic acid decarboxylase. Since tryptophan hydroxylase in humans is only 50% saturated (Young and Gautier 1981), acute alterations in the amount of tryptophan available should cause a corresponding change in the production of 5-HT. In animals, tryptophan depletion results in substantial reduction of plasma tryptophan that is highly correlated with a corresponding reduction in brain 5-HT synthesis and release (Moja et al. 1989; Gartside et al. 1992). In humans, a study using continuous CSF sampling during tryptophan depletion showed that decreased plasma tryptophan levels correlated with a fall in CSF 5HIAA, the metabolite of 5-HT (Williams et al. 1999). On the other hand, tryptophan augmentation in humans has been found to increase 5-HIAA levels in CSF and presumably therefore central 5-HT synthesis (Young and Gautier 1981). Such findings suggest that tryptophan challenge is a valid means of manipulating central 5-HT function in humans (Moja et al. 1989; Anderson et al. 1990).

Tryptophan challenge has already been established as a marker of 5-HT function in people who have recovered from clinical depression (e.g. Delgado et al. 1990; Smith et al 1997), in non-depressed people with a positive family history of depression (Benkelfat et al. 1994) and in people with high trait aggression (e.g. Cleare and Bond 1995). If MDMA users have altered 5-HT function, then manipulation of dietary tryptophan offers a logical challenge in MDMA users. If 5-HT function is impaired in people with a history of MDMA use, there may be little response to tryptophan depletion (cf. Delgado et al. 1994). At the same time, we reasoned that the response to tryptophan augmentation may be altered by changes to tryptophan metabolism perhaps linked to altered 5-HT transporter mechanisms.

We therefore investigated whether humans show altered 5-HT and cognitive function after they stop using MDMA by comparing the effects of tryptophan depletion and augmentation in three groups of participants: ex-users of MDMA, current users of MDMA and non-using controls. Only male MDMA users were recruited because 5-HT variation over the menstrual cycle in females presented practical recruitment difficulties.

If MDMA induces damage that is reversible then we would predict that ex-users would show a similar reaction to the challenge as controls. If MDMA induces damage that is not reversible and ex-users still have compromised 5-HT function, then their response to the tryptophan challenge would be similar to that of current users. Alternately, ex-users may show a different response from either other group if damage emerges when 5-HT is no longer affected by regular MDMA use or if there is some pre-existing difference or vulnerability that led ex-users to quit using MDMA. In terms of cognitive function, the aim was to determine whether long-term abstention from MDMA produced recovery or any persisting deficits and whether these were associated with biological markers of response to tryptophan challenge.

Materials and methods

Participants and design

We recruited three groups of volunteers: (i) regular users who had taken MDMA on more than 20 occasions in the past year; (ii) ex-users who used to take MDMA on a regular basis (>20 occasions per year) but had not used MDMA for at least one year; (iii) controls who had never taken MDMA but who used cannabis and alcohol on a regular basis. Volunteers were recruited through advertisements and then screened in a detailed interview in which standard assessments of mood were administered. A substance use questionnaire was used to obtain detailed information about participants' drug use patterns. Amount, frequency and length of use of all recreational drugs were recorded.

Apart from drug use, other inclusion criteria were that participants were male, aged 18–40 years, within the normal range of bodily mass index (BMI), not taking prescribed psychotropic medication or receiving psychological treatment, score <32 errors

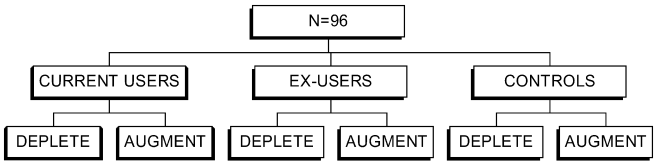

Fig. 1 Study design

on the estimate of pre-morbid IQ (NART—National Adult Reading Test; Nelson 1982), <30 on the Beck Depression Inventory (BDI; Beck 1978) and <60 on trait anxiety (Spielberger 1970). People abusing opiates were also excluded. Current users had to agree to abstain from taking MDMA for 3 weeks prior to testing (to counteract the effects of a temporary reduction in tryptophan hydroxylase activity following an acute dose of MDMA) and not to use LSD for this same period. They also had to agree to give urine and blood samples on the test day and to abstain from using any psychoactive drugs including alcohol for >24 h before the test day. Participants were informed that participation in the study was conditional on a negative urine screen for MDMA. The study was approved by the institutional ethics committee, carried out in accordance with the Declaration of Helsinki, and all participants gave written, informed consent.

The design of the study is depicted in Fig. 1. Participants in each of the three groups were randomly allocated to treatment, half to tryptophan augmentation (T+) and half to tryptophan depletion (T−). Double-blind procedures were followed throughout. Test versions were counter balanced across participants and design. Participants presented at 0900 hours following an overnight fast. Measures were obtained before and beginning 5 h after the amino acid drink. Participants were tested individually and remained in the laboratory throughout the test day, during which they had free access to caffeine-free drinks.

Tryptophan manipulation

The tryptophan-free (T−) mix comprised 15 amino acids in the exact quantities used by Young et al. (1985) and the tryptophan augmented (T+) mix was identical with the addition of 10.3 g tryptophan.. The exact mixtures were as follows: L-alanine, 5.5 g; L-arginine 4.9 g; L-cysteine, 2.7 g; glycine, 3.2 g; L-histidine, 3.2 g; L-isoleucine, 8.0 g; L-leucine, 13.5 g; L-lysine, 8.9 g; L-methionine, 3.0 g; L-phenylalanine, 5.7 g; L-proline, 12.2 g; L-serine, 6.9 g; L-threonine, 6.5 g; L-tyrosine, 6.9 g; and L-valine, 8.9 g; ±10.3 g L-tryptophan. Drinks were made up with 200 ml of water and peppermint essence was added to enhance palatability.

Assessments

Prior to the main test day, participants completed the Beck Depression Inventory (BDI; Beck 1978), the trait anxiety inventory (Spielberger et al. 1970), the Barratt Impulsiveness Scale (BIS; Barratt and Patton 1978) and the Aggression Questionnaire (Buss and Perry 1992).

Tryptophan measures

Blood samples were taken before and 5 h after participants ingested the amino acid drink. Samples were taken into heparinized tubes, immediately centrifuged and plasma was frozen at −20°C until analysis. Plasma was assessed for levels of both free and total tryptophan using high performance liquid chromatography (HPLC).

Memory and cognition

Prose recall

Versions from the Rivermead behavioral memory test (Wilson et al. 1985) were used. A taped prose passage was played to participants who then wrote down all they could recall immediately and then again after 30 min delay filled with other assessments. Scoring was standard.

Buschke selective reminding task (Buschke and Fuld 1974)

A list of 16 words was read aloud to participants at the rate of one word per second. Participants repeated the list back to the experimenter who then "selectively" reminded them only of the words that they had not recalled on the previous trial. Number of words correctly recalled on each of three trials and on a delayed test 30 min later were recorded and learning (trial 3 minus trial 1 recall) was calculated (cf. Park et al. 1994).

Serial sevens

This test tapping working memory and concentration required participants to subtract 7 from a given 3-digit number and then continue to count backwards in 7s. The number of correct subtractions and errors made in 90s were recorded.

Verbal fluency

Semantic and phonemic tests were used to assess verbal fluency. Participants generated as many words starting with a given letter (H and L) or belonging to a particular category (fruit/vegetables) as they could in 90s. Letters and categories were each chosen so that they had similar instances (Battig and Montague 1969).

Cancellation tasks

These were used as measures of focussed attention. In single digit cancellation, one target (4) and in double-digit cancellation two targets (2, 6) were crossed out, each in a random sequence of 400 digits. Time to complete and errors were recorded.

Rapid visual information processing (RVIP) (Wesnes and Warburton 1983)

This was used to index sustained attention and working memory. In the first (10 min) part of this task, single digits were presented at the rate of 100 digits/min and participants were instructed to press a response key to either 3 consecutive odd or 3 consecutive even digits. The second (5 min) part was a dual task in which the participant preformed the same RVIP whilst they were also presented with high and low tones through headphones. They were required to count the number of low tones heard throughout the task and report the total at the end of the task.

Mood

Participants self-rated their current mood pre- and post-drink using a 16-item visual analogue scale which yields three mood factors: alertness, contentedness and calmness (Bond and Lader 1974). Mood was also assessed using a modified state version of the BDI (Beck 1978). The original BDI questions were retained, but the instructions were changed so that participants answered according to how they were feeling at that moment in time. Anxiety was assessed with the State Anxiety Inventory (STAI) (Spielberger et al. 1970). A visual analogue scale was used to assess physical

symptoms (e.g. visual sensitivity, loss of appetite, muscular tension, headache, shaking).

Statistical analyses

Main variables were analysed using repeated measures ANOVA with group and treatment as between-subjects factors and time (pre versus post-drink) as a within subjects factor. Post-hoc comparisons used Bonferroni corrections. For self-paced tasks errors were covaried from time to complete tasks. Drug use patterns were analysed with χ^2. Pearson correlations were used to explore relationships between tryptophan levels, cognition, mood and MDMA use. The α level was raised to 0.01 for these correlations to minimise type I error. All data was analysed using SPSS for Windows version 9.0.

Results

Demographics

A total of 141 screening interviews were conducted. Six ex-users were excluded because they scored in the range for severe depression. Two participants vomited after consuming the amino acid drink and were replaced and 35 people had met inclusion criteria but did not attend the main test day (mostly current users who had not abstained from MDMA for 3 weeks). The remaining 96 completed the study. Overall, 61% had university degree level education (58%, 52%, 72% CU, Ex-U, Con, respectively); 16% "A" levels (23%, 12%, 12%) and the remainder GCSE. Overall, 49% were employed (50%, 70%; 31%); 38% were undergraduate or graduate students (34%, 15%, 59%) and 13% were unemployed (16%, 15%, 10%).

There were no group differences in pre-morbid IQ (estimated from the NART) or in depression scores (BDI) (Table 1). Age differences between groups just reached significance [$F(2,90)=3.27$, $P<0.05$] with ex-users being older than controls ($P<0.05$) but not different from current users. Trait anxiety (STAI) differed across groups [$F(2,92=3.50$, $P<0.05$] with ex-users tending to score higher than current users ($P=0.06$) and controls ($P=0.07$). Both ex- and current users had higher impulsivity scores than controls [$F(2,92)=3.15$, $P<0.05$]. Ex-users scored higher on trait aggression (AQ) than current users and controls respectively [$F(2,91)=3.72$, $P<0.05$].

Patterns of drug use

Ex-users had stopped using MDMA an average of 2.4 (range 1–7) years prior to taking part in the study (Table 2). Ex and current users did not differ in years of MDMA use or in the number of pills taken in a typical session. Ex-users reported having taken MDMA more often in a typical month than current users [$F(1,60=17.63$, $P<0.001$]. The three groups did not differ in years of use of cannabis, alcohol or tobacco (Table 3). Alcohol use was matched in terms of units drank per session although groups differed in frequency of use [$F(2,93=5.52$, $P<0.01$] with current users drinking more often than ex-users ($P<0.005$) although neither group differed from controls. Controls did not currently (within the last 6 months) use any drugs other than alcohol, tobacco and cannabis although 20–30% had previously taken cocaine, amphetamine and/or LSD. Some current and ex-MDMA users took cocaine, amphetamines and other drugs but the frequency of use of such drugs was low compared with their use of alcohol, cannabis, tobacco or MDMA. Current and ex-users did not differ in lifetime use of amphetamine or cocaine.

Tryptophan levels

Total plasma tryptophan

Groups did not differ in tryptophan levels pre-drink and there was no difference in those allocated to T+ or T−. A three-way interaction of group×treatment×time

Table 1 Group means (SD) for age, pre-morbid IQ and scores on BDI, BIS and STAI

	Current users (CU)	Ex-users (ExU)	Controls (CON)
Age (years)	25.22 (5.91)	27.83 (5.92)[b]	23.97 (6.10)
IQ (NART)	111.38 (7.82)	111.27 (8.93)	113.54 (9.80)
Depression (BDI)	6.06 (5.05)	8.48 (5.91)	5.59 (5.77)
Impulsivity (BIS)	74.72 (8.81)[a]	73.77 (9.13)[b]	69.35 (9.67)
Trait anxiety (STAI)	37.09 (8.91)	42.87 (11.22)	37.25 (9.28)
Aggression (AQ total)	72.63 (15.25)	79.67 (17.11)[a,c]	69.38 (12.86)

[a] CU versus CON,$P<0.05$
[b] ExU versus CON,$P<0.05$
[c] ExU versus CU, $P<0.05$

Table 2 MDMA use of current users and previous use by ex-users: means (SD)

	Years of use	Frequency (days/month)	No. tablets per session	Days since last use
Current users	4.33 (2.89)	3.48 (1.83)	2.58 (0.91)	39.0 (22.63)
Ex-users	3.49 (2.63)	6.95 (4.27)[a]	2.43 (1.38)	841.3 (637.4)

[a] ExU versus CU,$P<0.001$

Table 3 Means (SD) of use of drugs (apart from MDMA) in current users (*CU*), ex-users (*ExU*) and controls

Drug	Years of use			Frequency (days per month)			Dose[a]		
	CU	ExU	CON	CU	ExU	CON	CU	ExU	CON
Cannabis	6.7 (4.2)	7.2 (5.1)	7.4 (6.7)	17.5 (12.0)	14.1 (12.2)	17.1 (11.2)	0.6 (0.5)	0.6 (0.9)	0.7 (0.7)
Alcohol	9.3 (5.6)	9.5 (5.4)	7.7 (6.5)	18.2 (9.3)[b]	10.6 (9.3)	14.1 (8.4)	6.7 (3.6)	5.6 (4.4)	7.2 (4.8)
Cigarettes	6.8 (6.0)	8.4 (8.0)	6.0 (8.1)	18.3 (14.5)	18.7 (14.7)	17.7 (14.3)	7.4 (7.2)	7.8 (7.8)	7.1 (7.0)
Cocaine	2.0 (2.1)	3.1 (3.5)	0	0.9 (1.7)	2.7 (6.3)	0	0.2 (0.3)	0.2 (0.6)	0
Amphetamine	3.6 (3.2)	3.4 (2.8)	0	0.6 (1.1)	2.3 (3.5)	0	0.2 (0.5)	0.4 (0.7)	0
LSD	4.1 (4.7)	1.9 (2.0)	0	0.6 (1.7)	1.9 (3.6)	0	0	0	0
Ketamine	0.4 (0.7)	0	0	0.9 (2.6)	0	0	0	0	0
Benzodiazepine	0	0	0	0.4 (2.1)	0	0	0	0	0
GHB	0	0	0	0	0.5 (2.2)	0	0	0	0

[a] Alcohol/units per drinking session; cannabis/ounce per month; cigarettes/day; cocaine and amphetamines: g per session
[b] CU versus ExU, $P<0.05$

Table 4 Group means (SD) for free and total plasma tryptophan levels (μg per ml) in augmented (*T+*) and depleted (*T−*) conditions

	T+		T−	
	Pre	Post	Pre	Post
Free tryptophan				
Current users	1.93 (1.88)	16.85 (5.08)	1.88 (1.43)	0.53 (0.44)
Ex-users	1.49 (1.11)	20.62 (4.11)[a]	1.38 (0.56)	0.47 (0.34)
Controls	1.56 (1.01)	14.89 (9.10)[b]	1.56 (0.65)	0.58 (0.49)
Total tryptophan				
Current users	15.08 (3.95)	108.31(35.99)	12.98 (5.14)	2.93 (1.52)
Ex-users	12.64 (2.66)	142.66(46.99)[a]	13.46 (3.98)	3.80 (2.79)
Controls	13.04 (3.35)	89.56 (52.09)[b]	13.92 (1.46)	3.99 (3.72)

[a] ExU versus CON,$P<0.01$(total),$P<0.05$ (free)
[b] ExU versus CU, (total and free) $P<0.05$. All comparisons are post-pre data

[$F(2,82)=5.23$, $P=0.007$] reflected a post-treatment increase in total plasma tryptophan levels in ex-users more than other groups following T+ (Table 4, Fig. 2). In controls and current users, total plasma tryptophan increased about 8-fold; in ex-users the increase was nearly 12-fold. Post-hoc analysis showed ex-users had a greater increase in total plasma tryptophan than controls ($P<0.01$) and current users ($P<0.05$). Following tryptophan depletion, mean plasma levels dropped to about a quarter of pre-drink levels but there were no significant group differences.

Several two-way interactions were also significant: group×time [$F(2,82)=5.35$, $P<0.01$] and group×treatment [$F(2,82)=4.94$, $P<0.01$], again reflecting ex-users' greater increase than other groups in plasma tryptophan following T+. A treatment×time interaction [$F(1,82)=255.83$, $P<0.001$] showed the tryptophan manipulation had been effective with an increase in total plasma tryptophan after the T+ drink and a decrease after the T− drink. There were also main effects of group [$F(2,82)=4.81$, $P=0.01$], treatment [$F(1,82)=252.36$, $P<0.001$] and time [$F(1,82)=172.04$, $P<0.001$].

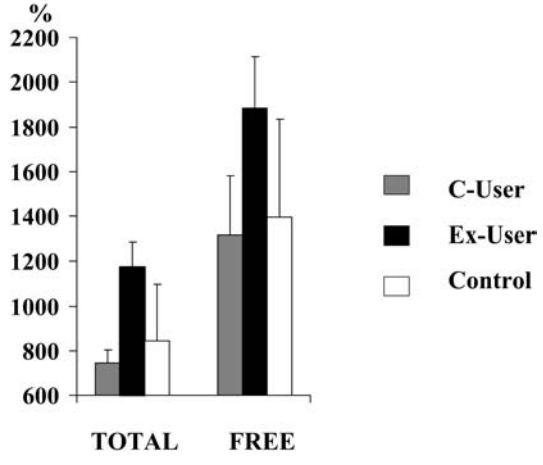

Fig. 2 Mean percentage change (post/pre) in total and free tryptophan levels following tryptophan augmentation (T+) in current users, ex-users and controls

Free plasma tryptophan

There were no pre-treatment group differences. The pattern of results was similar to that for total plasma tryptophan although the three-way interaction of group×treatment×time did not reach significance [$F(2,82)=2.78$, $P=0.07$]. A group×treatment interaction [$F(2,82)=3.08$, $P=0.05$] reflected group differences in free plasma tryptophan in T+ but not T−. Post hoc comparisons showed that ex-users had a greater increase in free plasma tryptophan following T+ than controls ($P=0.036$) and current users ($P=0.026$). In ex-users there was nearly a 19-fold increase in free plasma tryptophan compared

Table 5 Mean scores (SD) of current users (*CU*), ex-users (*ExU*) and controls on memory tasks and double digit cancellation

		T+		T−	
		Pre	Post	Pre	Post
Prose recall (immediate)	CU	7.34 (3.06)	7.19 (3.43)	9.78 (3.86)	10.47 (4.40)
	ExU	6.44 (2.63)	7.50 (3.14)	7.22 (3.28)	7.25 (2.08)
	Control	7.44 (4.39)	9.34 (4.78)	8.84 (4.52)	9.34 (3.16)
Prose recall (delayed)	CU	7.00 (3.15)	6.66 (3.18)	8.13 (3.87)	8.97 (4.19)
	ExU	5.34 (2.51)	6.59 (2.80)	6.31 (2.94)	5.63 (2.43)
	Control	7.06 (4.33)	8.91 (4.60)	7.97 (4.33)	8.19 (3.39)
Bushke trial 1	CU	6.31 (2.06)	6.38 (1.75)	6.00 (1.79)	6.00 (1.83)
	ExU	6.13 (1.82)	6.50 (2.16)	5.38 (1.36)	4.75 (1.29)
	Control	6.63 (2.25)	5.56 (2.78)	6.94 (2.24)	6.38 (1.78)
Bushke trial 3-trial 1	CU	3.25 (2.64)	1.75 (3.13)	3.69 (2.82)	3.37 (2.19)
	ExU	3.31 (2.75)	1.87 (2.06)	3.06 (2.29)	3.37 (2.28)
	Control	3.87 (1.92)	5.31 (2.41)	3.75 (2.35)	4.19 (2.46)
Bushke delayed	CU	7.75 (3.04)	4.56 (3.35)	6.88 (3.05)	4.19 (2.90)
	ExU	5.81 (2.95)	3.81 (2.59)	5.56 (2.39)	3.81 (4.02)
	Control	8.06 (3.51)	6.19 (3.37)	7.75 (2.65)	4.25 (2.21)
Phonemic fluency	CU	15.44 (5.92)	16.19 (6.83)	14.81 (4.64)	18.19 (5.67)
	ExU	17.31 (6.17)	18.50 (5.90)	15.69 (5.35)	16.13 (6.87)
	Control	14.94 (6.90)	17.25 (5.39)	19.38 (7.68)	21.69 (6.78)
Double digit cancellation	U	75.93 (13.53)	78.25 (17.75)	86.74 (20.76)	88.13 (24.20)
	Ex-U	86.44 (16.89)	89.64 (19.12)	100.79 (31.18)	98.30 (28.78)
	Control	85.59 (30.12)	79.20 (21.16)	86.91 (25.34)	81.33 (17.79)

with approximately 14-fold in the other two groups An interaction of group×time [$F(2,82)=3.19$, $P<0.05$] again reflected ex-users' greater increase in free plasma tryptophan levels compared with other groups. A treatment×time interaction [$F(2,82)=281.06$, $P<0.001$] verified the effectiveness of the tryptophan manipulation with an increase and decrease in free plasma tryptophan following T+ and T−, respectively. There were also main effects of treatment [$F(1,82)=292.96$, $P<0.001$] and time [$F(1,82)=213.48$, $P<0.001$] with a marginal effect of group [$F(2,82)=2.43$, $P=0.09$]. Following T−, mean levels dropped to about a third of pre-drink levels and there was no significant difference between groups.

Covariance of frequency of MDMA use (which differed between ex- and current users) did not affect the pattern or level of significance of results for either free or total tryptophan.

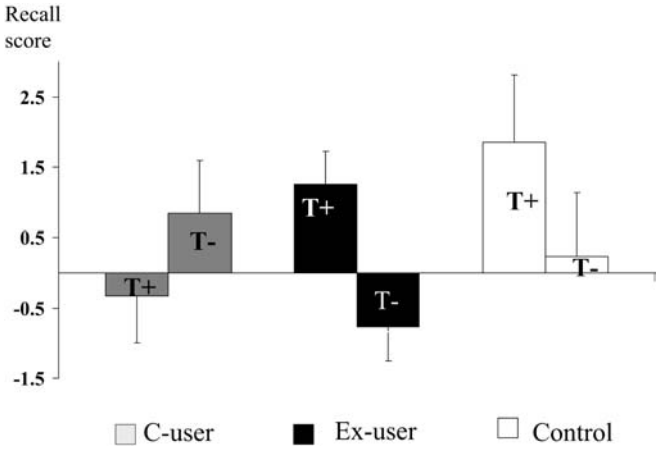

Fig. 3 Change (post minus pre) in prose recall following tryptophan augmentation and depletion in current MDMA users, ex-users and controls

Cognitive assessments

Prose recall

Immediate recall showed only a main effect of time [$F(1,90)=4.23$ $P<0.05$] with higher recall levels post-drink than pre-drink. There was a marginal effect of treatment [$F(1,90)=3.63$, $P=0.06$] reflecting lower scores in T− than T+ [cf. Table 5]. Similarly, group approached significance [$F(2,90)=2.60$, $P=0.08$], with ex-users tending to score lower (mean 7.10) than current users (8.70) and controls (8.74). On delayed recall, there was a trend towards group differences pre-drink [$F(2,93)=2.45$, $P=0.09$) with ex-users recalling less than other groups. Pre-drink scores were therefore used as covariates in the analysis. A significant group×treatment interaction [$F(2,89)=3.65$, $P=0.03$] emerged, with ex-users showing reduced recall following T− and increased recall following T+ (Fig. 3). Post-hoc comparisons of change scores (post minus pre-drink) showed a significant difference between T+ and T− only in the ex-users [$t(30)=-2.86$, $P<0.01$].

Bushke selective reminding task (BSRT)

There were no group differences in initial recall levels (trial 1) (Table 5). Learning across the three trials over trials showed a significant group×trial×time interaction

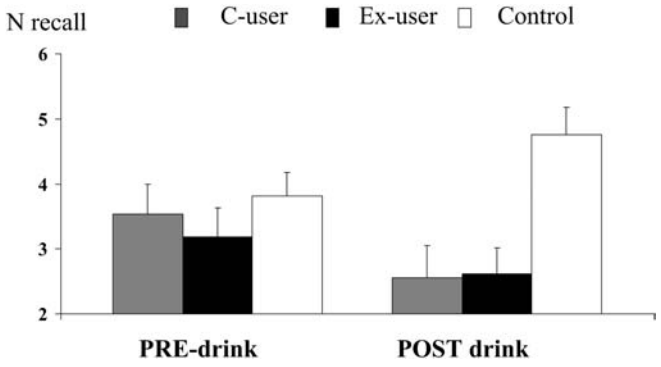

Fig. 4 Learning (trial 3–trial 1) in Bushke selective reminding task (N words)

[$F(4,180)=2.80$, $P<0.05$]. After the drink both current and ex-users showed a more shallow learning curve across trials than controls (see Fig. 4 which depicts the increase from trial 1 to trial 3 in number of words recalled). There was also a group×trial interaction [$F(4,180)=3.37$, $P<0.05$] and a main effect of trial [$F(2,180)=204.94$, $P<0.001$]. In delayed recall, ex-users recalled less than current users or controls before treatment [$F(2,93)=4.96$, $P<0.01$] and so pre-drink scores were entered as covariates. There were no significant group or treatment differences.

Verbal fluency

There were no pre-drink group differences. Phonemic fluency showed only a main effect of time [$F(1,90)=11.53$, $P<0.001$] with more words produced post- than pre-drink (Table 5). Semantic fluency showed no significant group or treatment differences.

Serial sevens

There were pre-drink group differences [$F(2,93)=4.32$, $P=0.016$] whereby ex-users carried out fewer correct subtractions (22.8+5.4) than current users (28.5+13.9) or controls (27.7+11.7). With pre-drink scores covaried, there were no significant group or treatment effects on post-drink scores.

Single and double digit cancellation

There were no group or treatment effects on single digit cancellation. On double digit cancellation, groups did not differ pre-treatment but there was a group×time interaction [$F(2,90)=4.02$, $P<0.05$]. Controls showed a practice effect (were faster post- than pre-drink), whereas current users were slower post- than pre-drink and ex-users were slower than other groups and took about the same time pre and post-drink (Table 5).

RVIP (10-min task)

Ex-users made fewer correct responses (26.4±9.0) than current users (32.4±7.8) or controls (30.5±8.1) pre-drink [$F(2,84)=3.88$, $P<0.025$]. When pre-drink scores were covaried from post-drink scores there was only a main effect of treatment [$F(1,75)=6.47$, $P=0.013$] with more hits after T− than T+. There were no pre-drink differences in reaction times but there was a main effect of group [$F(2,74)=4.70$, $P<0.05$] with ex-users being slower (565±112 ms) than current users (502±73 ms) and controls (525±84 ms). There was also a main effect of time [$F(1,74)=4.16$, $P<0.05$], reflecting faster reaction times post- than pre-drink, regardless of treatment.

RVIP+tone (5 min)

There was a trend for pre-drink group differences ($P=0.06$) so pre-drink scores were covaried from post-drink scores. There was a main effect of group [$F(2,78)=6.24$, $P<0.005$], whereby ex-users made fewer correct responses (13.7±5.8) than current users (19.9±6.7) and controls (19.7±6.4). No differences were found in reaction times or the number of errors made in counting tones whilst performing this task.

Mood

There were no significant effects on the modified BDI or the STAI. The mood rating scale showed only that participants were generally less alert [$F(1,90)=6.29$, $P<0.05$] and more calm [$F(1,90)=5.50$, $P<0.05$] after the drink compared with before (Table 6).

Physical symptoms

There was a group×treatment×time interaction on ratings of visual sensitivity [$F(2,90)=3.36$, $P<0.05$]. Post-hoc tests showed a difference in change scores between T+ and T− only in current users [$t(30)=-2.03$, $P<0.05$] with increased and reduced visual sensitivity following T+ and T−, respectively. There was a main effect of treatment [$F(1,90)=10.23$ $P<0.005$] on ratings of nausea (mean of 23 in T+ and 13 in T−) A main effect of time [$F(1,90)=30.13$, $P<0.001$] showed, as expected, that all groups had higher nausea ratings post- than pre-drink.

Correlations

Plasma tryptophan levels and cognitive function

Ex-users given T+ showed very strong negative correlations between changes in free plasma tryptophan levels and pre-drink prose recall, both immediate ($r=-0.897$, $P<0.001$) and delayed ($r=-0.898$, $P<0.001$) (Fig. 5).

Table 6 Mean (SD) modified BDI scores, STAI (state anxiety) scores and mood factor scores for current users (*CU*), ex-users (*ExU*) and controls

		T+		T−	
		Pre	Post	Pre	Post
BDI	CU	3.56 (3.42)	2.94 (2.41)	2.94 (3.49)	2.56 (3.29)
	Ex-users	5.13 (6.67)	4.88 (5.11)	4.69 (3.79)	3.81 (3.31)
	Controls	2.69 (3.30)	2.44 (3.05)	3.44 (4.00)	3.88 (3.84)
STAI	CU	36.00 (9.54)	33.69 (8.15)	32.00 (8.19)	32.00 (8.44)
	Ex-U	33.50 (8.85)	33.44 (8.55)	35.13 (9.79)	32.31 (8.69)
	Control	32.38 (8.36)	33.88 (8.64)	33.94 (8.96)	35.13 (7.97)
Alertness	CU	38.22 (20.17)	40.15 (14.00)	40.48 (19.64)	42.71 (19.00)
	Ex-U	34.56 (18.98)	44.29 (15.66)	36.22 (15.92)	41.90 (15.94)
	Control	40.94 (13.52)	42.57 (16.40)	41.65 (17.47)	46.48 (16.40)
Contentedness	CU	33.69 (18.33)	31.39 (14.56)	33.10 (14.46)	30.40 (11.88)
	Ex-U	29.23 (14.22)	33.08 (14.08)	30.06 (17.42)	28.44 (14.27)
	Control	29.20 (11.84)	32.59 (13.98)	30.85 (12.71)	30.49 (11.70)
Calmness	CU	28.38 (13.60)	26.28 (16.60)	32.28 (13.85)	28.41 (16.29)
	Ex-U	37.19 (19.14)	30.53 (16.78)	29.13 (11.18)	22.69 (14.51)
	Control	29.38 (16.50)	26.44 (15.36)	28.63 (14.98)	25.50 (10.61)

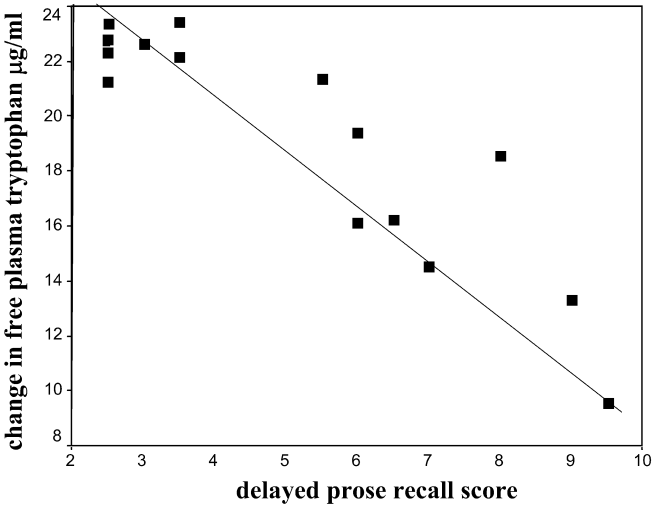

Fig. 5 Correlation between change in free plasma tryptophan and delayed prose recall in ex-users given T+

Similar negative correlations were also found between changes in total plasma tryptophan levels and pre-drink prose recall [immediate: r=–0.65, P<0.01; delayed: r=–0.81, P<0.001]. Prose recall after T+ also correlated with change in free plasma tryptophan (immediate recall: r=–0.66, P<0.01; delayed: r=–0.77, P<0.001) and change in total plasma tryptophan (immediate recall: r=–0.66, P<0.01; delayed: r=–0.74, P<0.001).

Baseline levels of free plasma tryptophan in ex-users correlated positively with total recall in BSRT post-drink (r=0.47, P<0.01).

Current users given T+ showed a negative correlation between change in free plasma tryptophan levels and change in verbal fluency scores post-pre drink (r=–0.65, P<0.01). Thus larger increases in free plasma tryptophan post-pre drink were associated with smaller improvements in scores. In controls, no significant correlations emerged.

Plasma tryptophan levels and MDMA use

Ex-users

Baseline total tryptophan levels were positively correlated with (i) years of use of MDMA (r=0.55, P=0.002) and (ii) frequency of MDMA use (days used in typical month) (r=0.493, P=0.008). No significant associations were found in current users.

MDMA use and cognitive function

Ex-users

Years of MDMA use correlated negatively with total recall in BSRT post-drink (r=–0.51, P<0.005). There was a correlation between dose of MDMA typically taken and baseline phonemic verbal fluency (r=–0.59, P<0.005). Ex-users who used to take more MDMA per session generated fewer words on the phonemic fluency task. Frequency of use was negatively correlated with baseline number of correct hits on the RVIP+tone task (r=–0.48, P<0.01) such that those who took MDMA more frequently when they were using this drug scored fewer correct hits on the RVIP task. There was a tendency for frequency of use to be correlated with baseline scores on serial sevens (r=–0.44, P=0.015).

Current users

There was a negative correlation between length of MDMA use and pre-drink performance on immediate (r=–0.48, P<0.005) and delayed prose recall (r=–0.52, P<0.005) and phonemic fluency (r=–0.54, P<0.005). Thus, current users who had been taking MDMA for a longer period of time recalled less and generated fewer words on the phonemic fluency task. There was a trend

for years of MDMA use to correlate negatively with total recall in BSRT post-drink (r=–0.42, P<0.02).

Mood and MDMA use

Ex-users

Hostility scores on the AQ correlated negatively with years since last use of MDMA (r=–0.55, P<0.005), suggesting that stopping use of the drug leads to reduced hostility. There were also trends reflecting a positive association between years of MDMA use/cumulative dose of MDMA and higher total (AQ) aggression scores (r=0.45, P=0.015/r=0.415, P=0.025).

Current users

Days since last use of MDMA correlated with the physical aggression sub-scale of the AQ (r=+0.5, P=0.005).

No significant correlations emerged between mood and tryptophan levels for any group. There were no significant correlations between indices of cannabis or alcohol use and task performance.

Discussion

The ex-users in our study had all stopped using MDMA for at least a year and on average, 2.4 years. The tryptophan manipulation was successful with all groups showing significant increases in free and total plasma tryptophan following the augmented drink and decreases following the depleted drink. A major finding was differences in how the three groups responded to the tryptophan challenge. Ex-users showed significantly higher levels of total and free plasma tryptophan following T+ than the other two groups. Further, this increase in free and total plasma tryptophan correlated very highly with ex-users baseline performance on prose recall. Bigger changes in free plasma tryptophan were highly associated with poorer immediate and delayed prose recall, with the size of these correlations (both 0.9) indicating that 80% of the variance in recall is shared with the variance in free tryptophan levels. Changes in total plasma tryptophan also correlated highly (0.81) with delayed prose recall.

Although plasma tryptophan levels can be considered only indirect markers of central 5-HT function, the very strong association between these and a behavioural measure of CNS function (a memory task) suggests central mediation. Elevated levels of plasma tryptophan may imply that there is a disruption to tryptophan metabolism in ex-users of MDMA. If tryptophan is not metabolised into 5-HT then the concentration of free tryptophan in the brain will increase, thereby reducing the transport gradient between the brain and plasma, resulting in elevated levels of free plasma tryptophan (Tiihonen et al. 2001). This decreased metabolism could therefore reflect alterations in 5-HT function in ex-users. In conjunction with findings from non-human primates (Hatzidimitriou et al. 1999), it is possible that this relates to degeneration of 5-HT axonal terminals.

To what extent could this putative altered metabolism of tryptophan be attributed to use of MDMA? In terms of drawing any causative links, there is the issue of whether such changes pre-date MDMA use or are a consequence of such use or emerge when people stop using MDMA.

If a direct consequence of MDMA use, then we would expect current users of the drug to show a similar pattern of effects, which they did not. There were no differences between current and ex-MDMA users in the length of MDMA use or the amount used per session. However, when they were using the drug, ex-users took MDMA more frequently than current users. Covarying frequency of use did not affect group differences in changes in tryptophan levels, nor were there any significant correlations between frequency of MDMA use and changes in plasma tryptophan levels. There were positive correlations between the extent that ex-users had previously taken MDMA (both years and frequency of use) and their base levels of total plasma tryptophan (i.e. pre-drink). Thus longer use was associated with higher levels of plasma tryptophan. Again, this may reflect metabolic changes with increasing use of the drug.

That the pattern of effects was different in ex-users from current users could suggest that altered metabolism of tryptophan is an effect which emerges when the drug is stopped. The ex-users had taken MDMA on average about seven times a month and when this frequent use is stopped there are neuronal adaptations to the absence of the drug. It is conceivable, although perhaps unlikely, that such adaptations take place over several years. However, it is also possible that the ex-users had stopped using the drug for some reason that may reflect their increased vulnerability to its effects. Pre-existing differences in 5-HT function may render some users more vulnerable than others to the harmful effects of MDMA. This in turn may influence ex-users decision to stop using MDMA. For some, circumstantial changes in lifestyle may mean that MDMA use is abandoned; others, however, stop taking the drug because they experience negative effects of use on their mental functioning (Verheyden et al. 2003). The main point is that genetic and other factors may mean that some users of this drug are more vulnerable to its toxic effects and therefore are more likely to stop using MDMA. Altered metabolism of tryptophan in ex-users may thus reflect pre-morbid differences in their 5-HT function.

The three groups of participants were matched on pre-morbid IQ and trait depression. All three groups reported similar use of cannabis and alcohol and this is especially important given that these drugs can affect cognitive function and memory (Curran and Hildebrandt 1999; Croft et al. 2001; Curran et al. 2002). Further, current and ex-MDMA users were also well matched for length of

MDMA use and the amount used per session, although when they were using, ex-users took MDMA more frequently than current users. Although the three groups were well matched on pre-morbid IQ, the ex-users differed at baseline on several cognitive tasks (serial 7s, Bushke delayed recall). If we collapse the results across treatments and time, then ex-users performed worse than the other two groups on delayed prose recall, Buschke learning and delayed recall, serial sevens and both RVIP tasks. That deficits were apparent on a number of tasks tapping working and episodic memory is in accord with three studies showing impairments in fairly small numbers of people who had stopped using MDMA for 6 months or more (Wareing et al. 2000; Reneman et al. 2001; Morgan et al. 2002). Our results indicate that subtle impairments in memory may be evident for more than a year after quitting MDMA. Impairments in working memory will influence performance on verbal learning and recall tasks. Ex-users showed negative associations between amount and frequency of use and performance on two working memory tasks (dual RVIP task; serial 7s). Further, the more years they had used MDMA, the worse their total recall levels on the BSRT. It would be interesting to experimentally tease apart the contribution of working memory impairments to performance on tasks that also have an episodic memory component.

Although current users were similar to ex-users in showing impaired learning over trials in the Bushke at the second testing, they generally performed as well as controls on most other tasks despite using MDMA as extensively, if not more than, participants in most published studies. Differences between this and other studies may relate to four factors. First, many previous studies have not matched MDMA and control groups on pre-morbid IQ, and IQ is known to correlate with recall levels. Second, all our participants were male whereas studies reporting memory impairment in current users have generally used mixed gender groups (e.g. Parrott et al. 1998; McCann et al. 1999; Morgan 1999; Gouzoulis-Mayfrank et al. 2000; Rodgers 2000; Wareing et al. 2000). Third, we matched groups for cannabis use (Fletcher et al. 1996) which can combine with MDMA in influencing memory (Gouzoulis-Mayfrank et al. 2000; Croft et al. 2001) but which some studies have not matched in control groups (e.g. Parrott et al. 1998; Klugman et al. 1999). We also matched fairly well for alcohol consumption. Fourth, we used a 3-week abstention period from MDMA prior to testing which again has not always been done in studies showing memory impairment in current users. As far as we are aware, no study reporting memory impairments in current users has controlled for all these potentially confounding factors and used only males. Further, the apparent consistency of studies reporting subtle memory impairment in current users may partially reflect a bias against publishing studies that find no impairment in MDMA users.

Although we did not find differences between current users and controls on several tasks tapping memory, there were associations in current users between dose and frequency of MDMA and deficits in memory and cognitive function. Length of use of MDMA was negatively associated with baseline scores on immediate and delayed prose recall and phonemic fluency. Thus those who had been taking the drug for longer recalled less prose and generated fewer words on the fluency task. These findings support previous studies of current users suggesting an association between length of MDMA use and memory impairment (Bolla et al. 1998; Gouzoulis-Mayfrank et al. 2000).

In terms of changes to cognitive function after the amino acid drink, only the ex-users showed differential changes in prose recall following T+ and T−. Apart from this one effect, groups showed no differential performance effects of the tryptophan challenge. All groups showed faster reaction times on the 10-min RVIP following tryptophan depletion, replicating the findings of Park et al (1994). Those authors suggested that this increase in speed resulted from increased dopaminergic transmission due to decreased inhibitory influence of 5-HT. Some studies have reported that tryptophan depletion in healthy adults impairs learning (Park et al. 1994; Schmitt et al. 2000) or memory performance (Riedel et al. 1999) although other studies do not find such impairments (Shansis et al. 2000). We did not find a memory or learning impairment in controls 5–6 h following depletion. As Reilly et al (1997) summarise in their review, results from tryptophan depletion studies have been inconsistent, partly because of considerable methodological variations. For example, Klaassen et al (1999) found no word recall impairment 6 h after depletion but impairment 24 h after. In general, results are more robust with psychiatric patients than with healthy volunteers. The role of 5-HT in cognitive processes such as memory, learning and information processing is not fully understood. 5-HT probably modulates cognition through its interactions with cholinergic, glutamatergic, dopaminergic and GABAergic systems (Buhot 1997). 5-HT receptors are found in brain areas that are implicated in learning and memory such as the hippocampus and cerebral cortex. Contradictory evidence has led to differing interpretations of exactly how 5-HT modulates cognition. However, more recent studies have suggested that 5-HT has a more complex modulatory role in cognitive processes that is still little understood (Meneses 1999).

In terms of subjective measures, both current and ex-users were significantly more impulsive than controls. Increased impulsivity is a characteristic of drug using populations. Interestingly, ex-users had higher levels of aggression than current users and controls (respectively). The longer they had used MDMA, the higher their trait aggression scores. The link between aggression and 5-HT has been documented by Bond and colleagues using tryptophan augmentation and depletion in aggressive males and in pre-menstrual women (e.g. Cleare and Bond 1996; Bond et al. 2001). Our results also support a link between 5-HT and aggression. However, there was a negative correlation between the hostility sub-scale of the

aggression questionnaire and number of years since stopping MDMA use, whereby those who had stopped for longer were less hostile.

Current users showed an association between days since last use of MDMA and scores on the physical aggression sub-scale: the longer the period since they had last used the drug, the higher their aggression. Recently, we have shown that a significant increase in self-rated aggression occurs in both males and females a few days after an acute dose of MDMA is taken (Verheyden et al. 2002). Our present findings suggest that aggression is not only a residual effect of acute MDMA ingestion but is also a feature associated with long-term use of this drug. Thus the empathy induced acutely by MDMA appears reversed with aggressive mood emerging both days after acute intake and following long-term abstention.

The tryptophan challenge did not affect mood. Mood changes in tryptophan studies are generally only found with vulnerable people such as women who have recovered from clinical depression. The lack of effect on mood we observed may mean that MDMA users are not vulnerable in the same way. In terms of physical reactions, all groups reported increased nausea post-drink and this is likely to have been the combined result of the drink itself and fasting. Current users given T− reported a decrease in visual sensitivity, whereas those given the augmented drink reported increased visual sensitivity. An increase in 5-HT after the augmented drink may have caused current users to experience visual sensitivity similar to that following use of MDMA (Liester et al. 1992; Davison and Parrot 1997).

There are methodological limitations to the present study, many of which are endemic to research in this field (Curran 2000). We had aimed to carry out hair analysis to verify abstinence from use of MDMA but at the time of data collection the fashion among young men was for a hair cut known as a "number one"—almost a shaven head. Only four MDMA participants could provide a reasonable amount of hair and these few samples were not worth analysing. We therefore relied on users' reports of drug use and of abstention from use. Even given their honesty, variation in purity of MDMA tablets (Wolff et al. 1995) means that it is difficult to estimate amount of use. However, participants were informed that participation in the study was conditional on a negative urine screen for MDMA.

This study was restricted to men, so our findings cannot be generalised to women, especially given some reports that women are more susceptible to both the effects of tryptophan depletion (Anderson et al. 1990) and the effects of MDMA (McCann et al. 1994; Liechti et al. 2001; Verheyden et al. 2002). Other limitations concern concern the biochemical markers employed. The amount of tryptophan that can cross the blood-brain barrier for synthesis is determined by the ratio of tryptophan to other large neutral amino acids (LNAAs). Although both treatments used in the present study contained the same mix of other amino acids, it would be important to replicate the present findings in future studies which measure the ratio of tryptophan to LNAAs in plasma which provides an estimation of tryptophan uptake in the central nervous system (Fernstrom and Wurtman 1972).

Research with non-human primates has shown long lasting selective serotonergic damage following high doses (5 or 10 mg/kg) twice daily for 4 consecutive days. This dose regimen does not produce long-term effects on brain dopamine neurons. There is recent animal evidence of dopaminergic neurotoxicity a few weeks after three sequential doses of MDMA (2 mg/kg) were injected in just 6 h (Ricaurte et al. 2002). This lower dose is more like that taken recreationally (but orally) by humans and may mean that some users who "stack" doses over an evening might be vulnerable to dopaminergic (as well as serotonergic) neuronal injury. This could be investigated in such heavy drug users by comparing the effects of tyrosine challenge with those of tryptophan challenge.

In summary, the major finding of this study was that men who had not taken MDMA for an average of 2.5 years showed elevated levels of plasma tryptophan following T+ which were strongly correlated with their performance on a prose recall task. Ex-users showing the poorest memory function at baseline were also those who metabolized least tryptophan. Ex-users showed enhanced sensitivity to tryptophan challenge and performed worse on several cognitive tests than current users or controls. There may be pre-existing differences in tryptophan metabolism in those who quit using this drug (compared with those who continue using it) and these may make them more vulnerable to the adverse effects of MDMA. At the same time, MDMA may have effects on 5-HT function and working and episodic memory that emerge after people stop using the drug and are evident for more than a year later.

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