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ORIGINAL INVESTIGATION

Una D. McCann · Victoria Eligulashvili
Melissa Mertl · Dennis L. Murphy
George A. Ricaurte

Altered neuroendocrine and behavioral responses to *m*-chlorophenylpiperazine in 3,4-methylenedioxymethamphetamine (MDMA) users

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Abstract *Rationale:* (±) 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") is a popular drug of abuse and a brain serotonin neurotoxin in animals. Growing evidence indicates that humans are also susceptible to MDMA's neurotoxic effects, although few functional consequences of MDMA-induced 5-HT damage have been identified. *Objective:* The present study sought to determine whether possible differences between MDMA users and control subjects could be unmasked by utilizing a pharmacological challenge with the mixed 5-HT agonist, meta-chlorophenylpiperazine (m-CPP). It was postulated that 5-HT neurotoxicity in MDMA users would be associated with altered 5-HT responsivity, exemplified by altered physiological and behavioral responses to m-CPP. *Methods:* Twenty-five MDMA users who had not taken MDMA for at least 3 weeks and 25 controls received intravenous placebo (normal saline) and m-CPP (0.08 mg/kg) in a fixed order, single blind design. Repeated measures of mood, physical symptoms, and blood samples for neuroendocrine analyses were collected during the 90 min after each infusion. *Results:* MDMA users reported more positive and fewer negative emotions and physical symptoms following m-CPP than controls, and were significantly less likely to report an m-CPP-induced panic attack. Male MDMA users had diminished cortisol and prolactin responses to m-CPP. *Conclusions:* The present data indicate that MDMA users have alterations in 5-HT neuronal function, possibly as a consequence of MDMA-induced brain serotonin neural injury.

Key words Serotonin · Neurotoxicity · Mood · Anxiety · Cortisol · Prolactin

U.D. McCann (✉) · V. Eligulashvili · M. Mertl
Biological Psychiatry Branch, National Institute of Mental Health, Bethesda, MD 20892-1272, USA
e-mail: umccann@helix.nih.gov

U.D. McCann
Department of Psychiatry, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

D.L. Murphy
Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland, USA

G.A. Ricaurte
Department of Neurology, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

Introduction

It is widely accepted that the popular recreational drug, (±) 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy), is a serotonin (5-HT) neurotoxin in animals (Schmidt et al. 1986; Stone et al. 1986; Commins et al. 1987; O'Hearn et al. 1988; Ricaurte et al. 1988a, 1988b; Sprague et al. 1998). Animals treated with MDMA develop persistent decreases in brain concentrations of 5-HT, 5-hydroxy indoleacetic acid (5-HIAA) and tryptophan hydroxylase, as well as a loss of brain serotonin transporters (Stone et al. 1986; Commins et al. 1987; Schmidt and Taylor 1987; Battaglia et al. 1988). Anatomical studies in MDMA-treated animals indicate that these neurochemical changes are secondary to a distal axotomy of 5-HT neurons (O'Hearn et al. 1988; Wilson et al. 1989; Molliver et al. 1990).

Data collected in MDMA users using the two validated methods for detecting MDMA-induced 5-HT injury strongly suggest that some MDMA users incur brain 5-HT damage. In two controlled studies (McCann et al. 1994, 1999), MDMA users have been found to have selective decrements in cerebrospinal fluid (CSF) concentrations of 5-HIAA, with no alterations in CSF homovanillic acid (HVA) or 3-methoxy-4-hydroxyphenylglycol (MHPG), the major metabolites of dopamine and norepinephrine, respectively. Notably, CSF measures of 5-HIAA are also selectively decreased in non-human primates with known MDMA-induced serotonergic injury (Ricaurte et al. 1988c). Further, positron emission tomography (PET) studies in MDMA users (McCann et al. 1998) reveal reductions in the 5-HT transporter, similar to those seen in MDMA-treated baboons with documented MDMA-induced neurotoxicity (Scheffel et al.

1998). Taken together, these results provide strong support for the notion that some human MDMA users incur brain 5-HT neurotoxicity.

The functional consequences of MDMA-induced neurotoxicity remain largely unknown. A handful of controlled studies have attempted to identify behavioral sequelae of MDMA use in humans (Price et al. 1989; Krystal et al. 1992; McCann et al. 1994; Bolla et al. 1998; Gerra et al. 1998; Morgan 1998; Parrott and Lasky 1998; Parrott et al. 1998; McCann et al. 1999). Three of these studies (Price et al. 1989; McCann et al. 1994; Gerra et al. 1998) involved neuroendocrine challenges in an effort to determine whether the homoeostatic regulation of 5-HT-mediated hormonal responses is altered in MDMA users. One of these studies (Price et al. 1989) was suggestive of altered neuroendocrine responses to the serotonin precursor, L-tryptophan, while the other (McCann et al. 1994) was not. In a third neuroendocrine study, Gerra and colleagues (1998) found that male MDMA users had diminished prolactin and cortisol responses to the serotonin releaser, fenfluramine, and that prolactin responses were inversely correlated with measures of aggression and impulsivity. Studies evaluating the potential detrimental cognitive effects of MDMA have been more consistent, with several research groups using a variety of cognitive assessment methods documenting cognitive deficits in MDMA users compared to controls (Krystal et al. 1992; Bolla et al. 1998; Parrott and Lasky 1998; Parrott et al. 1998; McCann et al. 1999). Direct attribution of cognitive changes to MDMA-induced 5-HT neurotoxicity, however, remains problematic.

The purpose of the present study was to determine whether differences between MDMA users and control subjects could be unmasked by utilizing a pharmacological challenge with the mixed 5-HT agonist and 5-HT releaser, meta-chlorophenylpiperazine (m-CPP). Pharmacologic challenges have been shown, in animal studies, to be the most effective method for detecting subclinical neurotoxic injury secondary to neurotoxic amphetamines (Ricaurte et al. 1994). m-CPP is the most extensively utilized probe of serotonin function in psychiatry (Kahn and Wetzler 1991; Murphy et al. 1991), and pretreatment with serotonin neurotoxins has been shown to alter the neuroendocrine and behavioral responses to m-CPP in animals (Quattrone et al. 1981; Lucki et al. 1989; Berendson et al. 1990). In humans, m-CPP reliably induces increases in plasma cortisol and prolactin, effects that have been primarily attributed to actions at post-synaptic 5-HT_{2C} receptors (Conn and Sanders-Bush 1987; Kennett et al. 1989, 1994; Murphy et al. 1991; Aulakh et al. 1992; Calogero et al. 1993; Kennett 1993; Callahan and Cunningham 1994; Mazzola-Pomietto et al. 1996). m-CPP has also been found differentially to affect mood and anxiety in distinct psychiatric populations. In particular, in healthy controls, m-CPP leads to increased anxiety (Charney et al. 1987; Murphy et al. 1989), while in patients with anxiety disorders, m-CPP induces syndrome-specific symptoms, such as obsessive and compulsive symptoms in patients with obsessive compulsive disorder (Zohar et al. 1987; Hollander et al. 1988) and panic attacks in patients with panic disorder (Kahn et al. 1988). The present study postulated that MDMA users, if they had sustained MDMA-induced 5-HT injury, would exhibit altered neuroendocrine and behavioral responses to m-CPP.

Materials and methods

Subjects

Twenty five MDMA users [17 males (age 27±2 years; mean±SE) and eight females (age 28±4 years; mean±SE)] and 25 controls [17 males (age 28±2 years; mean±SE) and eight females (age 34±4 years; mean±SE)] underwent pharmacological challenges with intravenous m-CPP as part of a 5-day inpatient protocol designed to determine the neurotoxic potential of MDMA in humans and its functional consequences. MDMA subjects reported having used MDMA on at least 25 separate occasions, and were self-referred. Control subjects were recruited by advertisements and had never used MDMA. Prior use of recreational drugs other than MDMA was allowed for both subject groups. Exclusionary criteria for both groups included past or current history of major medical illness (e.g., neurologic, renal, endocrine, or hematologic), current axis I psychiatric disorder as determined by SCID-I/P version 2.0 (First et al. 1996), a positive drug screen for illicit or prescribed psychoactive drugs, or current alcohol dependence. Subjects in both groups agreed to abstain from all recreational drugs for a duration of at least 3 weeks prior to testing, and their drug-free status was confirmed by urine and blood drug screens obtained on the first day of the study. Participants were compensated for participating in the study, and their travel expenses were reimbursed. Informed consent was obtained from all subjects and the research protocol was approved by the two Institutional Review Boards where the study was conducted.

Procedure

Subjects were admitted for a 5-day inpatient stay in a clinical research setting. All participants were assessed using physical examinations, structured diagnostic psychiatric interviews using the SCID-I/P version 2.0, electrocardiograms and comprehensive blood and urine laboratory testing to rule out medical illness. During the 5-day study, subjects were evaluated using a battery of biological and behavioral tests designed to probe for evidence of serotonin injury. These included measurement of cerebrospinal fluid (CSF) monoamines, polysomnographic studies, pain testing, cognitive testing, personality assessment, and pharmacological challenges with m-CPP. Results from cognitive testing indicated that MDMA users have cognitive deficits compared to controls, and selective deficits in CSF 5-HIAA were found in MDMA users, but not controls. These data have recently been reported elsewhere (McCann et al. 1999). Only results from neuroendocrine and behavioral effects of m-CPP will be discussed here.

Drug use

Detailed information about MDMA and other drug use was obtained from an initial telephone interview, the drug history section of the Addiction Severity Index, a structured interview that ascertained the number of MDMA experiences and the amount and frequency of MDMA use, and the drug use section of the SCID-IV. Blood and urine samples collected on the day of admission were screened for psychoactive drugs by immunoassay.

Pharmacological challenge paradigm

During the entire 5-day study, subjects were maintained on a low monoamine, no caffeine diet. Pharmacological challenges took place on the fourth day of the study after an overnight fast. At approximately 7:45 a.m., two intravenous catheters were inserted in forearm veins (one in each arm) for drug administration and blood sampling. At approximately 9:00 a.m., 20 cc of a normal saline solution (NS) was infused over a 90-s period. At approximately 11:00 a.m., m-CPP (0.08 mg/kg in 20 cc NS) was infused over a 90-s period. These were administered in a single-blind, fixed order fashion, although subjects were under the impression that the order of drug administration was randomized. Given the possibility that lingering effects of m-CPP might influence neuroendocrine and behavioral ratings obtained hours later, the fixed order paradigm was necessary. Of note, a number of subjects had traveled from other states or countries to participate in the study, and thus performing placebo and m-CPP challenges during two separate admissions was prohibited by cost.

Blood samples for neuroendocrine analyses, m-CPP concentration measurement and vital signs measurements were obtained 30 and 15 min prior to the saline infusion (i.e., –30 and –15 min), and every half hour thereafter until 90 min after the m-CPP infusion. The +90 min time point, which occurred 30 min prior to m-CPP infusion, was used as the baseline measure for the m-CPP neuroendocrine measurements. Blood samples were immediately placed on ice, and subsequently spun at 3600 rpm for 10 min. Serum samples were placed in a –70 C freezer until neuroendocrine assays were performed. Plasma cortisol measures were determined using ImmuChem Coated Tubes, ^{125}[I]RIA Kit 9ICN (Biomedicals, Inc. Costa Mesa, Calif., USA). Plasma prolactin measures were determined using Nichols Prolactin 100 T or 500 T kits (Nichols Institute, San Juan Capistrano, Calif., USA).

In addition to neuroendocrine measurements, subjects also underwent repeated behavioral assessments. In particular, subjects provided a self-assessment of mood and physical symptoms at baseline, +15, +30, +60, and +90 min following placebo, and +15, +30, +60 and +90 min following m-CPP. Rating scales utilized included Lader's Mood Scale (Bond and Lader 1986), eight 100 mm visual analogue scales intended to assess changes in a variety of mood and anxiety states, the NIMH Self-Rating Symptom Scale (van Kammen and Murphy 1975; Murphy et al. 1989), a 24-item questionnaire that assesses a broad array of emotional and physical symptoms, and a 21-item physical symptom checklist, designed to evaluate side effects of drugs. In addition, the NIMH Panic Symptom Scale was self administered at baseline and at 90 min following both infusions. Subjects were asked to rate the severity of their symptoms at their worst as "absent," "mild," "moderate," or "severe."

Statistical analyses

Data were analyzed by repeated measures ANCOVA with two repeated measures (drug and time), one between group factor (group) and one covariant (baseline concentrations of cortisol or prolactin). Greenhouse-Geisser corrections were used. When a significant main effect of Group, or significant Group×Drug or Group×Drug×Time interactions were observed, Bonferroni post-hoc tests were performed at individual time points to determine if significant differences occurred at any individual time points. In addition, comparisons of maximum change from baseline within each drug condition were carried out using Student's *t*-test (independent samples).

For neuroendocrine analyses, only data from male subjects were analyzed since no effort had been made to control for menstrual phase in female subjects, and since prolactin levels are known to fluctuate significantly during the menstrual cycle (Genazzani et al. 1997; Subramanian et al. 1997). For all data analyses, significance was set at $P<0.05$. All statistics were carried out using SPSS for Windows, release 6.1.2, standard version.

Subjects received a positive rating on the panic attack symptom scale if they reported four or more symptoms as "moderate" or "severe." Results from the two subject groups were compared using Fisher's exact test (two-tail).

Results

Drug use patterns

MDMA and other drug use characteristics, past psychiatric illnesses, ethnic backgrounds and educational levels of the two groups are illustrated in Table 1.

Table 1 Demographics and drug use characteristics

	MDMA (*n*=25)	Control (*n*=25)
Age (years±SE)	26.92±1.95	30.36±1.84
Education levels (years±SE)	13.36±0.59	15.28±0.42
Ethnic background[a]		
Caucasian	23	15
African American	1	6
Other	1	4
Past psychiatric history[b]		
Dysthymia	1	0
PTSD	1	3
MDMA exposure		
Number of exposures (times)	196±24 (range: 30–400)	NA
Duration of use (years)	5±3 (range: 1–14)	NA
Frequency of use (per month)	5±1 (range: 0.6–11)	NA
Usual dose (mg)	319±280 (range: 100–1250)	NA
Time since last dose (weeks)	14±29(range: 3–139)	NA
Other recreational drug exposure[c]		
Cocaine	23	10
Sedative hypnotics	19	7
Hallucinogens	24	11
Non-MDMA amphetamines	24	10
Cannabis	25	20
Organic solvents/inhalants	18	7
Opiates	14	10
PCP and related drugs	5	4

[a] Number of MDMA users and controls falling in a particular ethnic category
[b] Number of MDMA users and controls that met criteria in the past for a particular psychiatric disorder
[c] Number of MDMA users and controls that had used a particular drug at least once

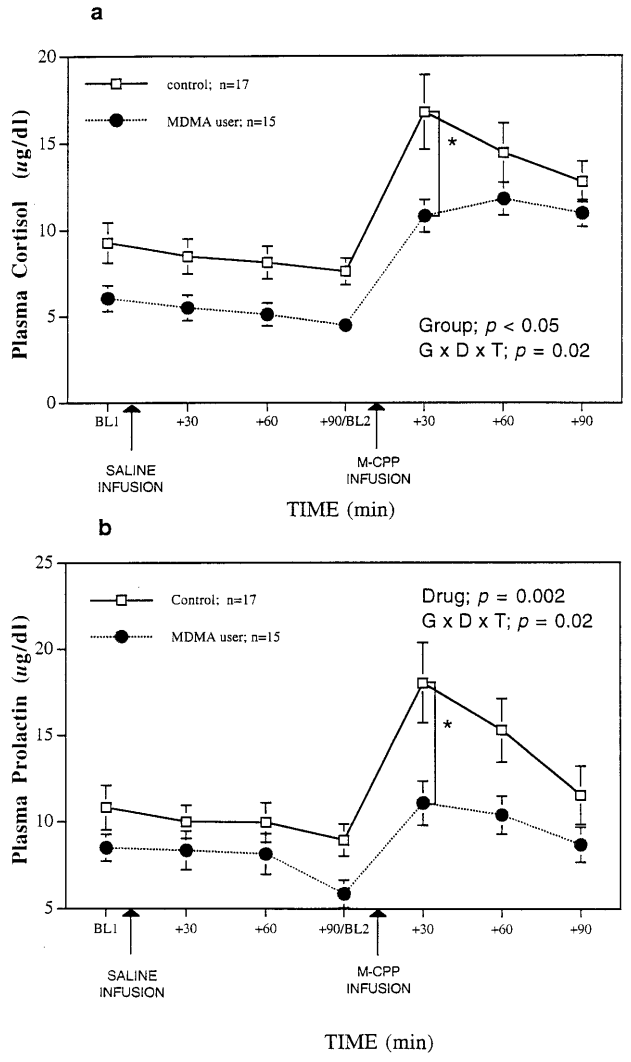

Fig. 1a, b Cortisol and prolactin responses following infusion of normal saline and m-CPP (0.08 mg/kg) in male MDMA users and controls. Data were compared by repeated measures ANCOVA with baseline neuroendocrine values as covariates and Bonferroni post hoc comparisons at individual time points, when indicated. *Significant difference between groups, $P<0.01$. □ Control ($n=17$), ● MDMA ($n=15$)

Neuroendocrine

Cortisol

The analysis revealed a significant main effect of Group [$F(1,29)=4.73$, $P=0.038$] and a significant Group×Drug×Time interaction [$F(2,58)=4.71$, $P=0.015$], reflecting diminished m-CPP-induced increases of plasma cortisol concentrations in MDMA users compared to controls (Fig. 1). "Peak differences from baseline" analyses reflected significantly blunted cortisol responses in MDMA users compared to controls (10.15±1.59 versus 6.32±0.80; mean±SE, $P<0.05$). There were no significant differences between the two experimental groups following placebo.

Prolactin

The analysis revealed a main effect of Drug [$F(1,29)=12.15$, $P=0.002$] and a significant Group× Drug×Time interaction [$F(2,58)=4.30$, $P=0.024$], reflecting diminished m-CPP-induced increases in plasma prolactin concentrations in MDMA users compared to controls. "Peak differences from baseline" analyses reflected no significant difference between MDMA users compared to controls following m-CPP (8.65±0.96 versus 10.09±1.50; mean±SE).

Plasma M-CPP concentrations

A repeated measures ANOVA revealed no differences in plasma concentrations of m-CPP in MDMA users versus control subjects [$F(2,64)=1.50$, $P=0.23$].

Correlation between MDMA use and m-CPP-induced neuroendocrine changes

Pearson correlation analysis revealed no significant relationship between peak changes in cortisol and prolactin concentrations and extent of previous MDMA use.

Behavioral

Lader's mood scales

Repeated measures ANCOVA with baseline values as covariates revealed a significant main effect of Group on the "attentive" scale " [$F(1,47)=4.65$, $P=0.04$], with MDMA users rating themselves higher than controls (Fig. 2). Significant Group×Drug effects were found on four of 16 Lader's scales, including "content" [$F(1,47)=10.07$; $P=0.003$], "energetic" [$F(1,47)=5.11$; $P=0.03$] "happy" [$F(1,47)=15.94$, $P<0.000$], and "quick-witted" [$F(1,47)=4.34$, $P=0.04$. In all cases, MDMA users rated themselves more positively than controls. Significant Group×Drug×Time interactions were observed on nine of 16 Lader's scales, and in every instance, were reflective of more positive mood responses to m-CPP in MDMA users compared to controls. Significant Group×Drug×Time interactions included, "alert" [$F(3,141)=3.48$, $P=0.02$], "amicable" [$F(3,141)=4.72$, $P=0.01$], "content" [$F(3,141)=6.32$, $P=0.002$], "gregarious" [$F(3,141)=2.97$, $P=0.04$], "happy" [$F(3,141)=8.82$, $P=0.000$], "quickwitted" [$F(3,141)=7.29$, $P<0.001$], "strong" [$F(3,141)=3.55$, $P=0.02$], "tranquil" [$F(3,141)=3.15$, $P=0.05$] and "well coordinated" [$F(3,141)=3.19$, $P=0.03$].

Differences between the two groups were also seen on Lader's Mood scales when "peak differences from baseline" were compared (Table 2). In particular, following placebo, MDMA users and controls differed on only one Lader's Scale ("interested"). In contrast, following m-CPP,

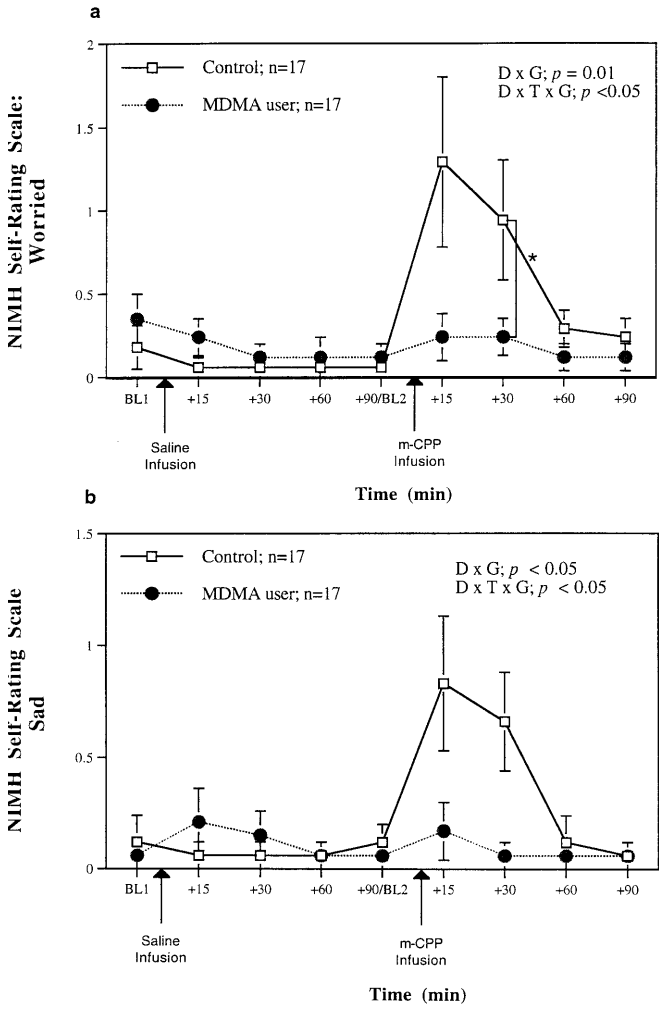

Fig. 2a, b Two examples of altered behavioral responses in male MDMA users following infusion of m-CPP (0.08 mg/kg), compared to controls. Only males are depicted in the figure so that data can be considered with the neuroendocrine data, in Fig. 1. Results including both genders appear in the text. Data were compared by repeated measures ANCOVA with baseline neuroendocrine values as covariates and Bonferroni post hoc comparisons at individual time points, when indicated. Items represented are from the NIMH Self-Rating Scale. *Significant difference between groups, $P<0.05$. □ Control ($n=17$), ● MDMA ($n=17$)

differences were found on 11 of the 16 Lader's Scales. In every instance, MDMA users rated the post-m-CPP experience as being more positive than control subjects.

Visual analog scales

As with the Lader's Mood Scales, repeated measures ANCOVA revealed significant differences between MDMA users and control subjects in their Visual Analog Ratings following m-CPP. In particular, significant Group×Drug interactions were seen on two of eight VAS scales, including "sad" [$F(1,47)=4.73$, $P=0.04$] and "tired" [$F(1,47)=8.54$, $P=0.05$]. Differences on both scales reflected more negative ratings by controls than MDMA users (i.e., MDMA users were less sad and tired than controls subjects following m-CPP infusion). Significant Group×Time×Drug interactions were found on the same two VAS scales and followed the same pattern, with MDMA users reporting lower scores on "sad" [$F(3,141)=5.97$, $P<0.002$], and "tired" [$F(3,141)=5.02$, $P=0.04$] scales.

"Peak differences from baseline" analyses reflected the same differences as those observed by ANOVA (Table 3). In particular, no differences between the two groups were observed following placebo, but following m-CPP, the two groups differed on two of eight scales. In both instances, MDMA users reported a less negative experience following m-CPP than control subjects.

NIMH self-rating scale

Repeated measures ANCOVA revealed significant main effects of Group on the "elated" [$F(1,47)=5.33$, $P=0.03$] and "worried" [$F(1,47)=5.33$, $P=0.03$] items of the NIMH Self-Rating Scale, with MDMA users rating themselves more elated and less worried than controls. Significant Group×Drug interactions were seen on five of 24 NIMH Self-Rating Scales, including "elated" [$F(1,47)=4.72$, $P=0.04$] "sad" [$F(1,47)=5.51$, $P=0.03$], "slowed down, "[$F(1,47)=7.42$, $P=0.01$] "uncomfortable mentally" [$F(1,47)=6.76$, $P=0.01$] and "worried" [$F(1,47)=6.9$, $P=0.01$], again reflecting lower levels of unpleasant symptoms and higher levels of pleasant symptoms in MDMA users compared to controls. Significant Group×Time×Drug interactions were found on five of 24 NIMH Self-Rating Scale items, mirroring the same pattern of more positive and less negative symptoms in MDMA users compared to control subjects. Significant Group×Drug×Time effects were observed on "depressed" [$F(3,141)=4.41$, $P=0.01$], "sad", [$F(3,141)=3.81$, $P=0.03$], "slowed down", [$F(3,141)=3.02$, $P=0.04$], "uncomfortable mentally" [$F(3,141)=7.12$, $P=0.002$], and "worried" [$F(3,141)=5.86$, $P=0.01$]. In all cases, MDMA users reported more positive and less negative experiences following m-CPP.

Differences between the two groups, paralleling those observed using the Lader's Mood Scale and VAS measures, were also found when "peak differences from baseline" were compared between the two groups (Table 4). In particular, following placebo, differences between the two groups were seen on only one of the 24 individual NIMH Self-Rating scale items ("feel mistrustful or suspicious') and none of the six subscales. In contrast, following m-CPP, differences in the peak response were observed on seven of the 24 individual items, and three of six subscales (Anxiety, Dysphoria, and Functional Deficit).

Panic symptom scale

Following m-CPP, controls were significantly more likely to meet criteria for panic attacks than MDMA

Table 2 Lader's mood scale: baseline and peak differences scores. Items on the Lader's Mood Scale that differed significantly between MDMA users and controls following infusion of m-CPP (0.08 mg/kg, IV). Baseline values were obtained prior to infusion of placebo and m-CPP, respectively. Peak values represent the maximum change from baseline. Numbers represent the group mean±SE

Lader's mood scale item	Placebo				mCPP			
	Control (*n*=17)		MDMA (*n*=15)		Control (*n*=17)		MDMA (*n*=15)	
	Baseline	Peak	Baseline	Peak	Baseline	Peak	Baseline	Peak
Alert	62.8 (6.5)	16.5 (5.7)	58.2 (5.4)	10.1 (5.7)	78.4 (4.5)	–23.0 (8.5)	64.0 (4.2)	–0.4 (6.0)[a]
Clearheaded	80.8 (4.2)	–2.3 (3.7)	57.8 (4.5)	6.1 (5.8)	79.9 (4.1)	–40.0 (6.2)	61.6 (4.5)	–16.8 (5.6)[a]
Content	77.0 (4.4)	3.2 (4.9)	56.6 (4.3)	4.3 (5.9)	80.1 (4.1)	–27.3 (7.1)	63.2 (4.1)	7.6 (6.0)[a]
Energetic	60.0 (5.4)	6.3 (6.0)	49.8 (3.4)	2.9 (4.0)	65.6 (4.7)	–23.4 (7.8)	49.9 (3.9)	10.2 (6.4)[a]
Gregarious	68.2 (4.1)	4.2 (2.7)	56.8 (2.8)	2.6 (4.6)	73.2 (3.8)	–16.7 (6.9)	60.2 (3.4)	3.2 (4.3)[a]
Happy	73.0 (4.0)	4.0 (4.0)	62.9 (3.6)	2.4 (2.4)	77.5 (4.0)	–21.9 (5.8)	65.8 (3.6)	10.3 (3.4)[a]
Interested	59.4 (5.9)	14.4 (6.3)	60.0 (4.8)	–4.9 (5.3)[1]	68.4 (4.6)	–5.3 (6.3)	55.5 (5.1)	10.8 (6.2)[a]
Proficient	77.0 (4.1)	–0.5 (3.3)	64.4 (3.8)	–5.2 (4.5)	77.4 (3.9)	–27.0 (6.4)	60.9 (3.3)	–5.9 (4.4)[a]
Relaxed	77.0 (4.4)	2.1 (4.2)	59.1 (3.4)	1.5 (4.7)	79.7 (4.5)	–29.8 (8.2)	62.5 (4.0)	–1.2 (6.4)[a]
Strong	74.7 (4.5)	0.4 (3.1)	60.4 (3.6)	4.7 (2.9)	76.8 (4.1)	–27.1 (6.5)	63.5 (3.2)	–7.7 (3.6)[a]
Tranquil	76.0 (4.3)	5.1 (4.1)	60.6 (3.5)	4.6 (3.9)	82.2 (4.0)	–28.6 (7.3)	65.5 (3.7)	–4.8 (5.8)[a]

[a] Significantly different from controls values (P<0.05)

Table 3 Visual analogue scale for mood: baseline and peak differences scores. Visual analog scale items that differed significantly between MDMA users and controls following infusion of m-CPP (0.08 mg/kg, IV). Baseline values were obtained prior to infusion of placebo and m-CPP, respectively. Peak values represent the maximum change from baseline. Values indicate mean±SE

Visual analogue scale	Placebo				mCPP			
	Control (*n*=17)		MDMA (*n*=15)		Control (*n*=17)		MDMA (*n*=15)	
	Baseline	Peak	Baseline	Peak	Baseline	Peak	Baseline	Peak
Sad	7.3 (3.0)	–1.9 (1.5)	9.0 (2.5)	1.2 (3.5)	5.9 (2.6)	15.0 (5.7)	6.3 (2.7)	3.1 (15.5)[a]
Tired	19.6 (4.4)	–9.4 (3.4)	25.7 (4.7)	–2.3 (4.3)	14.3 (4.7)	22.1 (7.4)	22.7 (4.5)	–7.0 (5.9)[a]

[a]Significantly different from control values (P<0.05)

Table 4 NIMH self-rating symptom scale baseline and peak differences scores. Items on the NIMH Self-Rating Scale that differed significantly between MDMA users and controls following infusion of m-CPP (0.08 mg/kg, IV). Baseline values were obtained prior to infusion of placebo and m-CPP, respectively. Peak values represent the maximum change from baseline. Values are mean±SE

NIMH self-rating scale item	Placebo				mCPP			
	Control (*n*=17)		MDMA (*n*=15)		Control (*n*=17)		MDMA (*n*=15)	
	Baseline	Peak	Baseline	Peak	Baseline	Peak	Baseline	Peak
Difficulty functioning	0.1 (0.09)	–0.08 (0.06)	0.2 (0.1)	0.1 (0.1)	0.04 (0.04)	10.5 (0.4)	0.2 (0.1)	0.8 (0.2)[a]
Elated	0.04 (0.04)	0.3 (0.2)	0.08 (0.06)	0.3 (0.2)	0.08 (0.05)	0.6 (0.3)	0.1 (0.09)	10.6 (0.3)[a]
Energetic	0.04 (0.04)	0.3 (0.2)	0.1 (0.09)	0.4 (0.1)	0.08 (0.08)	0.5 (0.2)	0.08 (0.06)	0.2 (10.1)[a]
Irritable	0.04 (0.04)	–0.2 (0.1)	0.3 (0.1)	0.0 (0.20)	0.2 (0.1)	10.1 (0.4)	0.3 (0.1)	0.2 (0.2)[a]
Slowed down	0.3 (0.1)	–0.08 (0.2)	0.4 (0.1)	0.2 (0.2)	0.1 (0.07)	20.2 (0.4)	0.5 (0.2)	0.4 (0.4)[a]
Uncomfortable mentally	0.08 (0.06)	–0.04 (0.04)	0.2 (0.1)	0.06 (0.08)	0.04 (0.04)	20.1 (0.4)	0.1 (0.07)	0.4 (0.2)[a]
Worried	0.1 (0.09)	–0.08 (0.06)	0.3 (0.1)	–0.2 (0.1)	0.04 (0.04)	10.2 (0.4)	0.1 (0.07)	0.08 (0.1)[a]

[a]Significantly different from control values (P<0.05)

subjects (P=0.023). No subjects had panic attacks after receiving placebo. In contrast, following *m*-CPP infusions, eight controls and one MDMA user reported panic attacks.

Physical symptoms

Repeated measures ANCOVA of physical symptoms revealed a significant main effect of Group on three of 26 physical symptoms, including "dry mouth" [F(1,47)=

Table 5 Physical symptom scale: baseline and peak differences scores. Items on a physical symptom scale that differed significantly between MDMA users and controls following infusion of m-CPP (0.08 mg/kg, IV). Baseline values were obtained prior to infusion of placebo and m-CPP, respectively. Peak values represent the maximum change from baseline. Values indicate mean±SE

Physical symptom scale	Placebo				mCPP			
	Control (*n*=17)		MDMA (*n*=15)		Control (*n*=17)		MDMA (*n*=15)	
	Baseline	Peak	Baseline	Peak	Baseline	Peak	Baseline	Peak
Drowsiness	0.4 (0.1)	–0.3 (0.2)	0.2 (0.1)	0.2 (0.1)	0.1 (0.07)	0.8 (0.2)	0.3 (0.1)	0.2 (0.1)[a]
Dry mouth	0.2 (0.1)	–0.2 (0.1)	0.4 (0.1)	0.2 (0.1)	0.04 (0.04)	10.2 (0.1)	0.6 (0.1)	0.6 (0.2)[a]
Nausea	0.04 (0.04)	–0.04 (0.04)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.0 (0.2)	0.00 (0.00)	0.2 (0.07)[a]
Stomach ache	0.00 (0.00)	0.00 (0.00)	0.08 (0.06)	0.00 (0.06)	0.00 (0.00)	0.3 (0.1)	0.1 (0.07)	–0.08 (0.08)[a]
Sweating	0.00 (0.00)	0.00 (0.00)	0.2 (0.1)	0.04 (0.1)	0.00 (0.00)	10.2 (0.2)	0.2 (0.1)	0.6 (0.1)[a]
Tiredness	0.3 (0.1)	0.08 (0.2)	0.6 (0.2)	0.00 (0.2)	0.2 (0.07)	0.9 (0.2)	0.6 (0.1)	–0.2 (0.2)[a]
Weakness	0.1 (0.07)	–0.08 (0.06)	0.2 (0.08)	0.04 (0.11)	0.04 (0.04)	10.0 (0.2)	0.3 (0.1)	0.04 (0.2)[a]

[a]Significantly different from control values ($P<0.05$)

7.67, $P=0.01$] and "nausea" [$F(1,47)=12.75$, $P=0.001$], and "poor appetite" [$F(1,48)=4.77$, $P=0.03$]. Significant Group×Drug interactions were seen on six of 26 physical symptoms, including "drowsy" [$F(1,47)=12.36$, $P=0.001$], "increased appetite" [$F(1,47)=6.77$, $P=0.01$], "increased sexual thoughts" [$F(1,4) =10.69$, $P=0.02$], "nausea" [$F(1,47)=12.75$, $P=0.001$], "stiffness" [$F(1,47)=4.06$, $P=0.05$], "tiredness" [$F(1,47)=6.91$, $P=0.012$] and "weakness" [$F(1,47)=8.14$, $P=0.01$], and significant Group×Drug×Time interactions were seen on two of 26 scales. These included "nausea" [$F(3,141)=3.59$, $P<0.03$] and "poor appetite" [$F(3,141)=2.50$, $P<0.03$]. MDMA users reported a lesser degree of all unpleasant symptoms (e.g., nausea, poor appetite, drowsiness, weakness and tiredness) following m-CPP than controls, and a greater degree of sexual interest.

Discussion

The principal finding of this study is that MDMA users have altered neuroendocrine and behavioral responses to the mixed serotonin agonist, m-CPP. In particular, following m-CPP infusion, male MDMA users were found to have diminished cortisol and prolactin responses, suggesting altered brain serotonin neurotransmission. Both male and female MDMA users reported fewer negative and more positive emotional and physical experiences following m-CPP than control subjects. Since there is growing evidence that humans, like experimental animals, are susceptible to MDMA-induced neurotoxicity (McCann et al. 1994, 1998, 1999), altered responses to m-CPP in MDMA users may represent persistent functional sequealae of MDMA-induced 5-HT neural injury.

Previous studies using L-tryptophan as a neuroendocrine probe to assess brain serotonergic function found no significant differences between MDMA users and controls (Price et al. 1992; McCann et al. 1994), although the study by Price and colleagues suggested blunted prolactin responses in MDMA users. The present findings are consistent with the observations of Gerra et al. (1998), who found diminished responses to the serotonin releaser, fenfluramine. Taken together, these findings suggest that differences among the various neuroendocrine studies are related to the nature of the neuroendocrine probe utilized. In particular, L-tryptophan is the immediate precursor to 5-HT, and in theory, induces increases in prolactin by increasing 5-HT synthesis and availability. Studies in animals lesioned with other 5-HT neurotoxins, such as 5,7-DHT have shown that following neurotoxic injury, spared 5-HT neurons have enhanced synthesis of 5-HT (Stachowiak et al. 1986; Tsuiki et al. 1995), accompanied by increased tryptophan hydroxylase activity (Bendotti et al. 1990). Thus, the negative findings from studies using L-tryptophan as a neuroendocrine probe could be due to compensatory mechanisms in surviving axons.

Notably, following acute injury with selective serotonin neurotoxins, animals develop denervation supersensitivity and exaggerated neuroendocrine and behavioral responses to m-CPP (Quattrone et al. 1981; Lucki et al. 1989; Berendson et al. 1990). In contrast, MDMA users in the present study had diminished neuroendocrine responses and differential behavioral responses to M-CPP. While our findings seem contrary to those that would be predicted from animal data, it is important to note that in animals, m-CPP studies were conducted shortly after 5-HT lesioning, whereas, on average, MDMA users in this study had used MDMA for nearly 5 years. Thus, if our subjects indeed sustained 5-HT injury, compensatory neuroadaptive processes or even hyperinnervation of hypothalamic 5-HT neurons could potentially have occurred, as has been seen in MDMA-treated non-human primates. Further, although our findings are contrary to what has been reported in animals with serotonin neurotoxicity, a study in healthy controls who received m-CPP following tryptophan depletion found decreased neuroendocrine responses (Coccaro 1998). These clinical data are consistent with the view that lower brain 5-HT levels can be associated with diminished m-CPP-induced neuroendocrine responses.

While the present data are consistent with the hypothesis that MDMA-induced 5-HT injury leads to altered re-

sponses to m-CPP, there are several other plausible explanations for our findings. Most importantly, it is possible that personality features, such as sensation seeking or impulsivity underlie the differential responses to m-CPP. Indeed, we (unpublished data) and others (Morgan 1998) have found recreational MDMA users who attend raves to be more sensation seeking than control subjects. Alternatively, it is possible that increased levels of emotional and physical discomfort experienced by control subjects was associated with increased neuroendocrine responses. Finally, it is possible that MDMA users tend to view altered states of consciousness as favorable, while control subjects tend to view them as unpleasant, thus accounting for the behavioral (and associated neuroendocrine) differences noted. Although the potential neurotoxic effects of MDMA make prospective studies of MDMA-naive individuals unethical, studies in other groups of impulsive drug abusers could help to determine whether altered neuroendocrine and behavioral responses to m-CPP in MDMA users are related to MDMA use or pre-existing constitutional factors. Regardless of the mechanism for the altered response to m-CPP in MDMA users, differences in the neuroendocrine and behavioral responses to m-CPP are indicative of altered brain serotonin function.

The behavioral effects of m-CPP in MDMA users stand in sharp contrast to those that have been observed in patients with a variety of anxiety disorders (Zohar et al. 1987; Charney et al. 1988; Hollander et al. 1988; Kahn et al. 1988). In particular, MDMA users are less sensitive, rather than more sensitive, to the anxiogenic effects of m-CPP than control subjects. This behavioral difference is suggestive of down-regulation of post-synaptic 5-HT_{2C} receptors, because the anxiogenic effects of m-CPP are thought to be secondary to actions at the 5-HT_{2C} receptor. Notably, the general pattern of dysphoria, anxiety and cognitive slowing found in controls in this study is consistent with those from previous studies (Charney et al. 1987; Kahn and Wetzler 1991; Murphy et al. 1991), suggesting that differences between controls and MDMA users are secondary to changes in MDMA users rather than to idiosyncracies of our infusion paradigm. The relatively high rate of panic attacks in control subjects (eight of 25 subjects) is difficult to explain, but could possibly be related to the rate of m-CPP infusion.

As noted earlier, neuroendocrine data were only collected in male subjects, and therefore, it is not known whether female MDMA users develop similar neuroendocrine changes following exposure to MDMA. Given the significant neuroendocrine differences observed in male subjects, it will be important to conduct additional neuroendocrine studies in women MDMA users, controlling for their menstrual phase.

Ideally, each subject would have undergone pharmacological challenge on 2 separate days. Time constraints of the 5-day inpatient study in combination with the prohibitive costs of transporting subjects twice from other states or countries necessitated the current pharmacological challenge paradigm. Fortunately, the sub-optimal design did not appear to compromise the data collected. As there were no systematic differences between the two groups following placebo infusion, it is unlikely that either group had a bias towards responding positively or negatively following drug administration per se. In contrast, following m-CPP, it was the control group, rather than the MDMA group that reported the most dramatic symptom changes, most notably in the form of panic attacks.

In addition to considering alternative explanations for our data, it is important to acknowledge limitations inherent to retrospective studies involving individuals who have used illicit drugs. Namely, drug histories in both subjects groups could only be ascertained by retrospective report. These drug histories could potentially be inaccurate, either because of difficulty in recalling details about illicit drug use or because individuals may have used drugs that they believed to be pure but which were, in fact, tainted. Further, although both subject groups were allowed to have used a variety of recreational drugs, the MDMA subject group had used more drugs to a greater extent. It is possible that drugs other than MDMA (or structurally related amphetamine analogs) could have played a role in neuroendocrine and behavioral alterations following m-CPP. However, this possibility is remote, since none of the other drugs used by MDMA users is a potent serotonin neurotoxin and they are unlikely to alter responses to a selective serotonergic agent.

Subjects in both experimental groups agreed to refrain from drug use for 3 weeks prior to study participation, and were told that they would not be compensated for study participation if drug screens were found to be positive for psychoactive drugs. No subject had a positive drug screen, suggesting that they complied with instructions to refrain from drug use. However, since some of the drugs screened for only remain in the blood and urine for 24–48 h, some subjects could potentially have used recreational drugs more recently. Since subjects underwent m-CPP challenges on day 4 of the study, we are certain that they had not taken drugs for at least 5–6 days prior to study participation. Thus, differences between MDMA users and controls were not secondary to pharmacological interactions between illicit drugs and m-CPP.

In conclusion, the present study demonstrates that MDMA users have altered neuroendocrine and behavioral responses to the mixed serotonin agonist and releaser, m-CPP. When considered with data indicating that MDMA users have persistent deficits in CSF 5-HIAA and loss of brain 5-HT transporters as viewed by PET, altered neuroendocrine and behavioral responses to m-CPP in MDMA users could represent functional manifestations of brain 5-HT neurotoxic injury.

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