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Neuroendocrine and subjective responses to pharmacological challenge with citalopram: a controlled study in male and female ecstasy/MDMA users

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Abstract

Despite evidence that ±3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy') causes persistent alterations to the serotonergic system of animals, evidence for long-term neurological effects of ecstasy/MDMA in humans remains equivocal. The current study assessed serotonin functioning of nine male and 11 female recreational ecstasy polydrug users by measuring neuroendocrine (prolactin, cortisol) responses to pharmacological challenge with the selective serotonin reuptake inhibitor citalopram, compared with nine male and five female cannabis polydrug users and 11 male and 11 female non-drug using controls. A single-blind, randomised, placebo-controlled design was used. Subjective responses, other substance use, mood, personality traits and demographic variables were measured to control for potentially confounding variables. There were no significant differences between ecstasy polydrug users, cannabis polydrug users and non-drug using controls in neuroendocrine or subjective responses to serotonergic challenge, and there were no sex by drug group interactions. There was no relationship between extent of ecstasy use and neuroendocrine functioning, alone or in combination with potential confounding variables. Subjective responses to the pharmacological challenge (nausea, tremor, dry mouth), novelty seeking and lifetime dose of alcohol were the only variables that contributed to one or more of the neuroendocrine outcome variables. These data do not support the premise that recreational ecstasy/MDMA use results in measurable impairment of serotonergic control of endocrine activity.

Key words

citalopram; cortisol; ecstasy; MDMA; neuroendocrine; prolactin; serotonin; sex differences; subjective responses

Introduction

The administration of ±3,4-methylenedioxymethamphetamine (MDMA) to rats and non-human primates results in a number of prolonged changes in the serotonergic [5-hydroxytryptamine (5-HT)] system. These include decreased levels of brain 5-HT and 5-hydroxyindolacetic acid (5-HIAA); decreased tryptophan hydroxylase activity; decreased density of 5-HT transporters; and swelling, distortion and fragmentation of 5-HT axons and nerve terminals (Green, *et al.*, 2003; Ricaurte, *et al.*, 2000). These markers of altered 5-HT function are believed to be indicative of MDMA-induced 'neurotoxicity' (Baumann, *et al.*, 2007). Yet, the relevance of the results of animal studies to humans is unclear, complicated by factors including dosing routes and regimens, varying experimental conditions and a range of interspecies differences (Baumann, *et al.*, 2007; Cole, *et al.*, 2002a; de la Torre and Farre, 2004; Easton and Marsden, 2006).

Based on the evidence from human studies, the long-term consequences of ecstasy/MDMA use on neurological, particularly 5-HT, functioning remain far from clear, although

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measurement of various endpoints has provided indirect evidence of serotonergic alterations. Abnormalities have been reported in 5-HT metabolite levels in cerebrospinal fluid (Bolla, *et al*., 1998), electrophysiological measurements (Croft, *et al*., 2001), structural and functional neuroimaging data (Jacobsen, *et al*., 2004; Reneman, *et al*., 2001) and hormone release following pharmacological challenge of the serotonergic system (Verkes, *et al*., 2001).

Pharmacological challenge studies in particular, are a useful way of investigating central 5-HT functioning in humans, because they provide a dynamic *in vivo* measure of 5-HT system responsivity when it is 'challenged', as opposed to obtaining a 'snapshot' of a single component of the system (Price, *et al*., 1990; Yatham and Steiner, 1993). In addition, this method has the potential to sensitively detect more subtle or subclinical changes in central 5-HT function (Coccaro and Kavoussi, 1994; Price, *et al*., 1989). Because it is possible that the brain may engage compensatory mechanisms in response to 5-HT injury, baseline indicators of serotonergic functioning might still reflect relatively intact functioning; it may be only under conditions of acute perturbation or stress that dysfunctions are revealed (Baumann, *et al*., 1998; Gerra, *et al*., 2003). Furthermore, pharmacological challenge data may be directly comparable between preclinical and human studies, as this methodology has been applied to rats and monkeys (Baumann, *et al*., 2007; Hatzidimitriou, *et al*., 2002; Poland, *et al*., 1997). Pharmacological challenge tests of central 5-HT functioning using endocrine endpoints are based on the assumption that enhanced release of 5-HT stimulates the secretion of hormones, such as prolactin (PRL) and cortisol (CORT), via its innervation of the hypothalamus (Dinan, 1996; Van de Kar, 1991). Hence, they offer practical advantages for examining the potential long-term consequences of ecstasy/MDMA use on central 5-HT function and may provide a functional marker of MDMA-induced 5-HT injury.

Several research groups have examined neuroendocrine functioning of ecstasy/MDMA users with conflicting results. Some of these studies have provided evidence for long-term damage to 5-HT functioning in the form of blunted neuroendocrine responses in ecstasy/MDMA users relative to controls (Gerra, *et al*., 2000; McCann, *et al*., 1999; Verkes, *et al*., 2001), while others have demonstrated no consequential effects of ecstasy use on neuroendocrine function (McCann, *et al*., 1994), or a stronger relationship between neuroendocrine functioning and cannabis, rather than ecstasy, use (Gouzoulis-Mayfrank, *et al*., 2002).

Methodological differences, in addition to a range of limitations that are frequently problematic in research involving human ecstasy/MDMA users, are likely to contribute to mixed findings across studies (see Cole and Sumnall, 2003; Curran, 2000, for review). Some factors are more amenable to experimental control/manipulation than others. One example is the length of the drug-free period required prior to measurement of variables of interest, which varies widely between studies, from 1–3 days (Buchert, *et al*., 2003; Gouzoulis-Mayfrank, *et al*., 2002) to 3 weeks or more (Gerra, *et al*., 1998; Reneman, *et al*., 2001). Findings arising from studies using shorter abstinence periods may reflect (sub)acute, rather than long-term, drug effects.

Some design limitations are difficult to rectify, such as the potential unreliability of participants retrospectively self-reporting their history of ecstasy/MDMA use (Bedi and Redman, 2006), as well as controlling the purity and composition of ecstasy tablets, which are often contaminated with substances other than MDMA (Cole, *et al*., 2002b; Spruit, 2001; Teng, *et al*., 2006). Another factor is the difficulty in accounting for potentially confounding variables that may have a greater impact on serotonergic integrity than ecstasy/MDMA use. For example, concurrent cannabis or other drug use, personality traits (such as novelty seeking, aggressiveness, impulsivity) and mood states have all been shown to correlate with measures of 5-HT functioning in ecstasy users (Gerra, *et al*., 2000; Gouzoulis-Mayfrank, *et al*., 2002; Thomasius, *et al*., 2006). In addition, many studies that have reported deleterious effects of ecstasy/MDMA use on 5-HT function have been unable to demonstrate direct correlations between the extent of ecstasy use and outcome variables (McCann, *et al*., 1999; Tuchtenhagen, *et al*., 2000). This suggests that there are unknown or unmeasured factors contributing to the variance in their findings.

The outcomes of earlier studies may be influenced by inter-individual factors that mediate vulnerability to ecstasy/MDMA-associated harm. Growing evidence suggests that the acute and long-term effects of ecstasy/MDMA may be associated with the sex of the individual (Allott and Redman, 2007). For instance, compared with males, current female ecstasy/MDMA users have been shown to have lower 5-HT transporter binding as measured by positron emission tomography (Buchert, *et al*., 2004; Reneman, *et al*., 2001). Therefore, the potential impact of inter-individual variables, such as sex, which might mediate the effects of ecstasy/MDMA, warrants further investigation.

The current study was designed to address some of the methodological issues that may have contributed to the conflicting and inconclusive findings in human ecstasy/MDMA users. The aim was to assess serotonergic function of current regular ecstasy polydrug (EP) users compared with cannabis polydrug (CP) users and non-drug using controls (NC), via pharmacological challenge of the 5-HT system with the selective serotonin reuptake inhibitor (SSRI) citalopram (CIT). CIT (Lundbeck, 2002) was chosen as the pharmacological challenge agent because of all SSRIs it binds with the highest specificity to central presynaptic 5-HT transporters and has low affinity for other neurotransmitter systems (Popik, 1999; Stahl, 1998). Its use as a 5-HT neuroendocrine probe has been demonstrated in healthy volunteers (Flory, *et al*., 2004; Hawken, *et al*., 2006) and substance users (Gotjen, *et al*., 2002; Netter, *et al*., 2002). It is reported to be well tolerated by participants (Hawken, *et al*., 2006) and a dose-dependent relationship has been demonstrated between CIT (intravenous and oral) treatment and PRL and CORT secretion (Attenburrow, *et al*., 2001; Hawken, *et al*., 2006; Lotrich, *et al*., 2005). The 40 mg dose administered

in the current study is considered a mid-range therapeutic dose and was selected to maximise the chances of stimulating the hypothalamic–pituitary–adrenal (HPA) axis and to ensure that between-group differences could be optimally demonstrated without 'floor' or 'ceiling' effects (Coccaro and Kavoussi, 1994; Gelfin, *et al*., 1995). The inter-individual variability in steady-state plasma concentrations of CIT has been found to be independent of sex in adults (Bezchlibnyk-Butler, *et al*., 2000; de Mendonca Lima, *et al*., 2005; Fredricson Overo, 1982). The present study recruited both males and females to assess the contribution of sex to the neuroendocrine responses of EP users. Furthermore, the present study measured mood and personality variables to investigate their potential contribution to neuroendocrine functioning. An exploration of potential group differences in the subjective effects of the pharmacological challenge and their relationship to neuroendocrine functioning was also undertaken.

Three hypotheses were tested. First, it was hypothesised that if EP users had indeed sustained ecstasy/MDMA-associated injury to the 5-HT system, they would show blunted neuroendocrine responses to serotonergic challenge compared with CP and NC participants. Secondly, if males and females differ in their vulnerability to the effects of ecstasy/MDMA on serotonin functioning, then they would show differences in their neuroendocrine responses to 5-HT challenge that cannot be accounted for by any sex differences observed in the NC participants. Finally, if ecstasy/MDMA use is associated with altered 5-HT functioning, then there should be a significant relationship between extent of ecstasy use and neuroendocrine functioning, that is independent of the effects of other drug use, demographic, mood and personality variables.

Materials and methods

Participants

Participants were recruited using a range of strategies, such as researchers' existing networks (including those who previously participated in ecstasy studies at Monash University), 'snowballing' (Biernacki and Waldorf, 1981) and project advertisements placed in several locations (i.e. websites or e-newsletters pertaining to drug use or the dance party scene, Melbourne street press and university noticeboards and websites). Fifty-nine English-speaking adults (18+ years) who were residents of Melbourne, Australia were recruited into the study between May 2005 and October 2006. Participants belonged to one of three groups: 22 (11 male, 11 female) current regular EP users, who reported to have used ecstasy/MDMA on at least 50 occasions; 15 (10 male, five female) current regular CP users who reported regular cannabis and/or other drug use (e.g. hallucinogens, cocaine), but had never used ecstasy/MDMA and 22 (11 males, 11 females) NC individuals who had never used any illicit substances including ecstasy/MDMA, or reported only past experimental (≤5 occasions) cannabis use. All participants provided voluntary written informed consent and ethical approval was obtained from the Monash University Standing Committee on Ethics for Research Involving Humans, Melbourne, Australia. Each participant received a honorarium of $AU100.

Participation involved four phases: (i) telephone screening interview; (ii) face-to-face screening interview and (iii) two separate pharmacological challenge sessions. Exclusion criteria included: body mass index <14 or >35 (range 17.4–32.2; $M = 23.3$; $SD = 3.4$); current prescription of any medication [apart from oral contraceptives (OC)], or regular use of psychoactive over-the-counter medicines; past or current major medical illness; current or past Axis I psychiatric disorder as defined by DSM-IV criteria (American Psychiatric Association, 1994), assessed via the Mini International Neuropsychiatric Interview, version 5.0.0 [MINI; Sheehan and Lecrubier, 2002; participants were not excluded if they reported a past history (>1 year ago) of anxiety or depressive disorder, but this was examined as a potential covariate in the analyses]; previous or current physical substance dependence, with the exception of ecstasy, nicotine or cannabis, as defined by DSM-IV criteria using the MINI; shift work during the past 3 months; extreme morningness or eveningness assessed using the Morningness-Eveningness questionnaire (MEQ; Horne and Ostberg, 1976); pregnancy or breastfeeding and females not taking a monophasic OC or contraceptive implant/patch. To control for menstrual cycle phase, the female participants were required to be taking a monophasic OC for at least the past 3 months and to have menstruated in the previous two cycles. Controlling for menstrual cycle phase is essential in research assessing sex differences (Becker, *et al*., 2005), as women have been found to respond differently to a wide variety of stimuli, including pharmacological challenge, depending on menstrual cycle phase (Genazzani, *et al*., 1997; O'Keane, *et al*., 1991). The decision to include only females using a monophasic OC was based on uncertain reliability of self-report of natural menstrual cycle phase and large inter-individual variability in hormone concentrations across phases of the natural menstrual cycle (Becker, *et al*., 2005).

Pharmacological challenge procedure

An average of 16.6 ± 18.0 days elapsed between face-to-face screening and the first pharmacological challenge session. The study utilised a cross-sectional, single-blind, randomised, cross-over design, with CIT and placebo (PCB) as the two challenge treatment conditions. Participant groups were compared on the magnitude of neuroendocrine responses – CORT and PRL secretion – to pharmacological challenge with 40 mg CIT (2 × 20 mg tablets orally) and PCB (two inactive tablets orally). Neuroendocrine responses were measured in blood (serum) samples obtained at half-hourly intervals over 5 h. The two challenge sessions were conducted at least 2 weeks apart (mean 23.8 ± 14.9 days) to allow for an adequate washout period and prevent carry-over effects.

Participants adhered to a number of requirements prior to each challenge session: (1) no use of ecstasy/MDMA, other

illegal substances and prescription medications for at least 1 week – to verify this, a serum sample from each participant at both challenge sessions was screened for cannabis, amphetamines, MDMA, opiates and cocaine (Victorian Institute of Forensic Medicine, Melbourne, Australia); (2) no use of alcohol for the previous 24 h, which was verified using a breathalyser (Lion Alcometer SD-2 Pacific Data Systems Pty Ltd, Salisbury, QLD, Australia) screening; (3) no consumption of caffeine-containing products on the day of each procedure; (4) bedtime prior to midnight the night before or at least 6 h sleep and (5) report feeling healthy.

Participants arrived at the laboratory at approximately 12 noon, were seated in a temperature-controlled, quiet and comfortable environment and instructed to remain relaxed, while they completed questionnaires, watched television, read, etc. They were not permitted to sleep, eat or drink (except for water) during the sessions. Smokers were allowed to smoke as they normally would. All female participants were tested during a time when they were not menstruating (i.e. during the 21-day active hormone-based phase of their monophasic OC). An intravenous cannula was inserted into an antecubital vein and kept patent by regularly flushing with ~2.5 ml of sterile saline (0.9% sodium chloride). Participants rested for ~20 min before the first blood sample was taken. Blood samples (~5 ml) were collected through the catheter and drawn into 4 ml Vacuette® serum-separation tubes at 30-min intervals over the 5-h period (total of 10 samples). Two baseline samples (-30 and 0) were collected before the challenge medication was administered. Either CIT (40 mg) or PCB was administered orally as two tablets with water at approximately 1 p.m. Excluding baseline blood sampling, the duration of the challenge procedure was 4 h because previous research had shown peak PRL and CORT responses to occur within this timeframe following 40 mg oral CIT (Gotjen, *et al.*, 2002). To monitor subjective effects, following each blood sample, participants completed a Visual Analogue Scale (VAS; Aitken, 1969; Bond and Lader, 1974) along 100 mm continuums that assessed 14 different mood and physical states commonly associated with CIT and blood-taking. Participants were observed at all times by research staff and were phoned on the day after each challenge session to check recovery.

Following each challenge session, blood samples were centrifuged for 15 min at 3000 rpm, 1500 g at 4 °C and serum was removed and stored at −80 °C for later analysis. After completion of all challenge sessions, serum samples were assayed for CORT and PRL using commercial immunoradiometric assay kits (Coat-A-Count® Prolactin IRMA and Coat-A-Count® IRMA Cortisol; Diagnostic Products Corporation, Los Angeles, California, USA). Data were reported as ng/ml (PRL) and μg/dl (CORT). The limit of sensitivity of the PRL assay was 0.1 ng/ml and the intra- and inter-assay coefficients of variation were 3.6% ($n = 24$ replicates of a single sample with a mean concentration of 9.2 ng/ml) and 8.4% ($n = 24$ replicates of a single sample with a mean concentration of 9.2 ng/ml) respectively. The limit of sensitivity of the CORT assay was 0.2 μg/dl and the intra- and inter-assay coefficients of variation were 11.2% ($n = 24$ replicates of a single sample with a mean concentration of 21.5 μg/dl) and 7.3% ($n = 12$ replicates of a single sample with a mean concentration of 21.5 μg/dl) respectively. All samples were assayed in duplicate and the mean value was used. To reduce the effect of inter-assay variation, all samples from any one participant were measured in the same assay.

Drug use and psychological measures

Each participant completed a detailed demographic, ecstasy and other substance use interview. To maximise reliability of retrospective self-report, lifetime use of ecstasy/MDMA, cannabis and other substances was assessed using a detailed 'timeline' method, employing contextual recall cues (Bedi and Redman, 2006; Ward, *et al.*, 2006). Participants also completed questionnaires examining current mood state and personality traits that are often associated with substance use and altered serotonergic functioning. Measures were chosen based on prior evidence that their scores correlate with indices of central serotonergic function (Cloninger, 1986; Coccaro, 1992; Dougherty, *et al.*, 1999; Manuck, *et al.*, 1998; Moller, *et al.*, 1996), or they had previously been used in ecstasy-related research (de Win, *et al.*, 2006; Gerra, *et al.*, 1998; Moeller, *et al.*, 2002). The measures were: the Center for Epidemiologic Studies Depression Scale – Revised (CESD-R; Eaton, *et al.*, 2004); the Tridimensional Personality Questionnaire (TPQ), version 4 (Cloninger, 1987), which yields three subscale scores of Novelty Seeking (NS), Harm Avoidance (HA) and Reward Dependence (RD); the Barratt Impulsiveness Scale – Version 11 (BIS-11; Patton, *et al.*, 1995) and the Aggression Questionnaire (AQ; Buss and Perry, 1992). The Morningness-Eveningness Questionnaire (Horne and Ostberg, 1976) was also included as a measure of sleep-wake lifestyle. Questionnaires and drug-use interviews were completed during the pharmacological challenge sessions.

Statistical analyses

Data were scrutinised to ensure that exclusion criteria were not violated and there were no anomalies. *Post hoc* serum toxicology screening revealed that of the original 59 participants, three participants had serum samples that contained either amphetamine (one EP male) or Δ^9-tetrahydrocannabinol (THC; one EP male, one CP male); their data were excluded from all statistical analyses, giving a total final sample of 56 participants. Two participants (one EP female, one NC male) were found to have abnormally elevated PRL levels and their PRL data were not included in the analyses. The CORT data of these two participants were included in all analyses, as they did not represent significant outliers (Field, 2005). Finally, four participants who completed the study (two NC females, one CP female, one CP male) vomited during the CIT challenge session. Because none of their PRL or CORT data met criteria for significant outliers, they were retained in the analyses.

Unweighted means independent groups analyses of variance (ANOVA), *t*-tests and Pearson chi-square tests were used to examine group differences in demographic (age, weight, country

of birth, marital status), mood (CESD-R, past history of depression/anxiety), personality trait (MEQ, BIS-11, AQ and TPI subscales RD, HA, NS) and drug use variables. The drug use variables were: other drug use (lifetime dose of alcohol, nicotine, amphetamines, cocaine, gamma hydroxybutyrate, ketamine, hallucinogens, sedatives, amyl nitrate, nitrous oxide, number of different substances ever used); cannabis use (age of first use, last use, lifetime dose, frequency of use per month); and ecstasy use (age of first use, last use, lifetime occasions of use, frequency of use per month, years of use, typical dose used, highest dose used). When ANOVA revealed significant main effects for drug group, Gabriel's *post hoc* comparisons were employed due to unequal sample sizes (Field, 2005). Simple main effects analysis was used when significant interactions were observed. Data were not transformed because *t*-tests and ANOVAs are relatively robust to violations of normality (Gravetter and Wallnau, 1996). No adjustment for possible inflation of experiment-wise error rate was made in these preliminary analyses as it was considered important to ensure that differences between experimental groups were identified as potential confounds to possible group effects. ANOVAs were used to examine between-group differences in the nine peak change VAS ratings found by repeated-measures ANOVA to be significantly greater during CIT vs. PCB administration (i.e. nausea, appetite, dry mouth, wakefulness, hand tremor, drowsiness, lethargy, restlessness, dizziness). A Bonferroni-adjusted Type I error rate of $\alpha = 0.05/9 = 0.006$ (two-tailed) was used.

For neuroendocrine analyses, the 0-min time-point was used as the baseline because initial data screening showed that most participants had elevated PRL and/or CORT responses at the −30 time-point (probably indicating venipuncture-related stress). Furthermore, the 30-min serum samples were not included in the analyses, because previous research had shown that increases in hormone levels following oral CIT did not occur until at least 1 h following administration (Hawken, *et al.*, 2006; Netter, *et al.*, 2002). Hence, including baseline, there were eight time-points (0, 60, 90, 120, 150, 180, 210 and 240 min). Two mixed-model ANOVAs were first conducted to test for group differences in baseline hormone levels. The baseline hormone levels were within the normal range (Diagnostic Products Corporation, 2005a,b). However, females were found to have significantly higher baseline CORT levels than males (21.32 ± 1.39 μg/dl vs. 10.95 ± 1.26 μg/dl respectively), regardless of drug group or treatment condition ($P < 0.001$). While the sex effect for PRL was not significant under the Bonferroni-adjusted Type I error rate, the *P*-value of 0.032 was small, and the differences between females and males (7.75 ± 0.54 ng/ml vs. 6.13 ± 0.49 ng/ml respectively) were in the same direction as was found with CORT. It was considered important to control for the baseline sex differences in subsequent analyses. Therefore, to establish the degree of change from baseline, raw PRL and CORT scores were converted to 'percentage change from baseline', rather than treating baseline values as covariates. Each participant served as his/her own control, with data normalised from individual zero time-point hormone levels. Percentage change from baseline (i.e. 0 min) was calculated with the formula: $(T2 - T1)/T1 \times 100$. Two (PRL, CORT) mixed-model ANOVAs with repeated-measures for challenge agent (CIT, PCB) and seven time-points, were conducted to examine between-group (drug group × sex) differences in neuroendocrine responses to pharmacological challenge. Greenhouse–Geisser corrections were used. A Bonferroni-adjusted Type I error rate of $\alpha = 0.05/2 = 0.025$ (two-tailed) was used. Separate mixed-model ANCOVAs were subsequently performed if potential covariates were identified by preliminary analyses of group differences in demographic, mood, personality or drug use variables.

Relationships between ecstasy use and neuroendocrine outcome variables were examined using the whole sample. For this part of the analysis, peak change in PRL and CORT and Area Under the Curve (AUC) of PRL and CORT were used as the neuroendocrine outcome measures. Peak changes in PRL and CORT were calculated by subtracting the baseline values from the largest change (positive or negative) in PRL and CORT, respectively, after administration of PCB or CIT. Net peak change was determined by subtracting peak change under PCB from peak change under CIT. PRL and CORT AUC from 0 to 240 min was calculated using the trapezoidal method and net hormone responses to CIT were determined by subtracting AUC under PCB from AUC under CIT.

Separate multiple regression analyses were conducted to examine whether ecstasy use variables (lifetime occasions, years of use, frequency use/month, typical dose, highest dose, use in past month) were predictive of the four neuroendocrine outcome variables. Because any relationships between ecstasy use and neuroendocrine variables may be moderated by factors such as other drug use, demographic, mood, personality trait and VAS peak change responses, hierarchical multiple regression analyses were conducted to examine the relationship between ecstasy use and neuroendocrine variables, while controlling for these potentially confounding variables. The regression analysis was compartmentalised because of the large number of predictor variables and the relatively small sample size. Backward multiple regression analyses were conducted first, so as to isolate the confounding variables most likely to contribute to the prediction of the four neuroendocrine variables. Those variables that were retained by these preliminary backward regressions were entered into the final hierarchical regression analyses, with mood variables entered first, personality variables second, demographic variables third, other drug use variables fourth, VAS peak change variables fifth and ecstasy use variables entered last. Type I error rate was set at $\alpha = 0.05$ (two-tailed) for each hierarchical regression. All data analyses were performed using SPSS for Windows, version 15.0 SPSS Australasia Pty Ltd, Chatswood, NSW, Australia.

Results

Demographics, mood and personality

The mean (and SD) values and category proportions for the demographic, mood and personality variables are presented in Table 1. There were four variables in which groups were not

Table 1 Demographic, mood and personality characteristics of participants

	Ecstasy polydrug users (EP)		Cannabis polydrug users (CP)		Non-drug using controls (NC)	
	Male (n = 9)	Female (n = 11)	Male (n = 9)	Female (n = 5)	Male (n = 11)	Female (n = 11)
Demographics						
Age[a]*	26.7 (5.2)	20.3 (2.5)	25.2 (6.3)	20.4 (1.1)	25.1 (3.5)	23.6 (3.6)
Weight (kg)[a]*	74.8 (14.6)	60.3 (13.1)	81.2 (16.7)	53.4 (5.8)	84.6 (9.4)	69.0 (9.7)
% Australian born/Not Australian born	67/33	64/36	78/22	40/60	64/36	73/27
% Married/not married	22/78	18/82	22/78	20/80	18/82	27/73
% Complete/incomplete high school	78/22	82/18	100/0	100/0	82/18	100/0
% Unemployed/working/studying	12/44/44	0/27/73	11/22/67	0/20/80	9/27/64	0/36/64
Mood						
Past history of depression or anxiety[b]	0	3	1	1	0	0
CESD-R total score[a]	8.8 (9.3)	11.1 (11.6)	11.8 (10.4)	12.6 (9.4)	6.9 (4.6)	6.1 (3.0)
Personality						
MEQ total score[a]*	46.2 (4.3)	44.7 (6.9)	49.9 (8.4)	42.4 (6.6)	53.4 (6.8)	58.1 (6.4)
BIS-11 total score[a]	65.6 (9.5)	66.4 (9.6)	69.4 (12.9)	62.0 (7.4)	63.0 (8.0)	56.6 (6.9)
The AQ total score[a]	64.6 (21.6)	70.8 (16.8)	65.7 (16.2)	75.0 (11.5)	66.5 (12.0)	55.3 (7.6)
TPQ–HA[a]	10.9 (6.7)	11.6 (4.9)	8.4 (7.5)	6.8 (4.6)	7.6 (4.9)	12.6 (5.3)
TPQ–RD[a]*	18.3 (5.4)	22.8 (2.9)	16.6 (5.6)	18.0 (4.8)	22.6 (4.0)	19.9 (4.8)
TPQ–NS[a]	18.9 (5.0)	20.4 (4.5)	18.2 (5.5)	18.2 (2.8)	18.4 (4.6)	16.3 (4.9)

MEQ, Morningness-Eveningness Questionnaire; CESD-R, Center for Epidemiological Studies of Depression Scale – Revised; BIS-11, Barratt Impulsiveness Scale – version 11; AQ, Aggression Questionnaire; TPQ, Tridimensional Personality Questionnaire; HA, Harm Avoidance; RD, Reward Dependence; NS, Novelty Seeking.

[a]Mean (SD); [b]n.

*$P < 0.05$, see text for details.

well matched. The first was age, with males being significantly older than females [$F(1,50) = 1.95$, $P = 0.001$]. The second was weight, with males being significantly heavier than females [$F(1,50) = 31.79$, $P < 0.001$]; there was also a main effect for drug group [$F(2,50) = 3.80$, $P = 0.029$], with the EP group weighing significantly less than the NC group ($P = 0.033$). The third variable was MEQ score, with the NC group scoring significantly higher (indicating a greater degree of 'morningness') than the EP ($P < 0.001$) and CP ($P = 0.001$) groups. There was also a significant sex × drug group interaction [$F(2,50) = 3.52$, $P = 0.037$], with simple main effects analysis revealing that the female NC group scored significantly higher on the MEQ than the female EP and CP groups [$F(2,51) = 14.63$, $P < 0.001$], but there were no differences between groups among the males [$F(2,51) = 2.99$, $P = 0.059$]. Despite this, all group mean values still fell within the 'Neither Type' range on the MEQ, indicating that there were no groups with extreme levels of 'morningness' or 'eveningness'. Finally, for reward dependence there was a significant main effect for drug group [$F(2,50) = 3.20$, $P = 0.049$], with the CP group scoring significantly lower than the NC group ($P = 0.031$).

Ecstasy, cannabis and other drug use

The data for ecstasy, cannabis and other drug use are presented in Table 2. There were group differences in the number of different substances ever used [$F(2,50) = 78.33$, $P < 0.001$], with the EP group having used a greater number of different substances than the CP and the NC groups ($P < 0.001$), and the CP group having used a greater number of different substances than the NC group ($P = 0.009$). This finding is not surprising given the group inclusion criteria. There were significant group differences for four other drug-use variables: a significantly higher lifetime dose of alcohol in males vs. females [$F(1,50) = 8.00$, $P = 0.007$]; and a higher lifetime dose of amphetamines [$F(1,30) = 7.82$, $P = 0.009$], ketamine [$F(1,30) = 4.81$, $P = 0.036$], and amyl nitrate [$F(1,30) = 5.14$, $P = 0.031$] in the EP group compared with the CP group.

The EP and CP groups were well matched on all cannabis use variables as analyses revealed no significant group differences. Furthermore, male and female EP users were generally well matched on ecstasy use variables. The only exception was age of first use, wherein females had first used ecstasy at a significantly younger age than males [$t(18) = -3.22$, $P = 0.005$].

Neuroendocrine functioning – group analyses

Figure 1 shows the percentage changes in PRL and CORT following oral administration of PCB and CIT (40 mg) according to drug group (sexes combined). Figures 2 and 3 show the percentage changes in PRL and CORT secretion following PCB and CIT administration in males and females, respectively,

Table 2 Other drug, cannabis and ecstasy use

	Ecstasy polydrug users		Cannabis polydrug users		Non-drug using controls	
	Male (n = 9)	Female (n = 11)	Male (n = 9)	Female (n = 5)	Male (n = 11)	Female (n = 11)
Other drug use lifetime dose						
Alcohol (standard drinks)*	8045.3 (5815.6)	1692.0 (2943.1)	4336.0 (4902.1)	2443.2 (1704.9)	3655.7 (3600.0)	2427.8 (3412.4)
Nicotine (cigarettes)	8321.7 (16,167.8)	4939.5 (5375.5)	12,035.6 (33,333.1)	7460.0 (9366.3)	0.8 (1.2)	0.9 (1.3)
Amphetamines (g)*	10.3 (13.6)	21.4 (24.3)	0.1 (0.4)	0.2 (0.4)	0.0 (0.0)	0.0 (0.0)
Cocaine (g)	1.7 (1.8)	1.1 (2.6)	0.0 (0.1)	4.5 (10.1)	0.0 (0.0)	0.0 (0.0)
Ketamine (g)*	0.9 (1.2)	0.6 (1.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Hallucinogens (occasions)	39.4 (62.3)	5.8 (14.7)	3.0 (7.2)	0.6 (1.3)	0.0 (0.0)	0.0 (0.0)
Nitrous oxide (bulbs)	208.9 (387.9)	219.1 (723.3)	1.4 (4.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
GHB (ml)	83.2 (148.5)	383.5 (895.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Sedatives (occasions)	4.7 (7.6)	24.4 (70.1)	0.0 (0.0)	1.2 (2.7)	0.1 (0.3)	0.0 (0.0)
Amyl nitrate (occasions)*	2.1 (2.5)	1.9 (3.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Cannabis use						
Lifetime use of cannabis[a]	9 (100)	10 (91)	8 (89)	5 (100)	2 (18)	3 (27)
Age at first use	16.8 (2.6)	15.4 (2.0)	15.5 (1.7)	16.0 (3.5)	19.0 (0.0)	20.0 (1.7)
Lifetime dose (g)	148.1 (171.8)	902.4 (2624.2)	587.4 (1304.9)	108.6 (218.8)	0.1 (0.3)	0.0 (0.1)
Frequency use/month	5.2 (9.6)	5.4 (11.2)	5.9 (9.3)	3.4 (6.0)	0.0 (0.0)	0.0 (0.0)
Time since last use (days)	66.8 (133.8)	142.7 (180.9)	14.5 (12.0)	43.4 (52.7)	1551.0 (903.7)	1223.7 (1271.9)
Number of substances used in lifetime*	9.3 (2.7)	8.0 (2.6)	3.9 (1.5)	3.6 (1.3)	2.0 (0.8)	1.8 (0.4)

	Male EP (n = 9)		Female EP (n = 11)	
	Mean (SD)	Range	Mean (SD)	Range
Ecstasy use				
Age of first use**	20.6 (3.5)	18–29	16.8 (1.4)	14–19
Years of use	6.3 (4.1)	2–13	3.6 (2.0)	1.3–7
Frequency use/month	1.2 (0.7)	0.3–2	1.8 (1.4)	0.5–4
Typical dose (tablets)	1.8 (0.8)	1–3	2.0 (0.8)	1–3.5
Highest dose (tablets)	4.5 (1.6)	3–8	6.0 (2.8)	2–11
Lifetime occasions	99.2 (85.3)	43–315	91.6 (59.3)	43–234
Time since last use (days)	24.1 (19.4)	9–70	21.0 (21.7)	6–78

Numbers refer to mean (SD); GHB, gamma hydroxybutyrate.

[a]n (%)

*$P < 0.05$, see text for details.

**$P < 0.05$, male EP compared with female EP.

according to drug group. There was a significant main effect of treatment on PRL secretion, with CIT eliciting a greater percentage change in PRL than PCB [$F(1,48) = 15.75$, $P < 0.001$, $\varepsilon = 1.0$]. Under the Bonferroni correction, there was no significant main effect of time or treatment × time interaction for PRL secretion, but the P-values were small ($P = 0.032$ and $P = 0.040$ respectively), indicating possible trends in the data. There were no significant group effects or interactions involving groups with regard to percentage change in PRL. There were no second- or third-order interactions involving treatment, time, drug group or sex.

With respect to CORT secretion, there was a significant main effect of treatment, with CIT eliciting a greater percentage change in CORT than PCB [$F(1,50) = 21.88$, $P < 0.001$, $\varepsilon = 1.0$]. There was a nonsignificant trend for a main effect of time on CORT secretion ($P = 0.059$). There was a significant treatment × time interaction [$F(2.46,123.01) = 10.56$, $P < 0.001$, $\varepsilon = 0.41$], with CIT producing increased percentage changes in CORT relative to baseline at 90, 120 and 150 min compared with PCB. There were no significant group main effects or interactions with regard to percentage changes in CORT, nor were there second- or third-order interactions involving treatment, time, drug group or sex.

The previously described group differences in age, weight, MEQ score, reward dependence, and lifetime dose of alcohol, amphetamines, ketamine and amyl nitrate, flag the possibility that any underlying effect of drug group and/or sex may have been compromised by these variables. Separate mixed-model

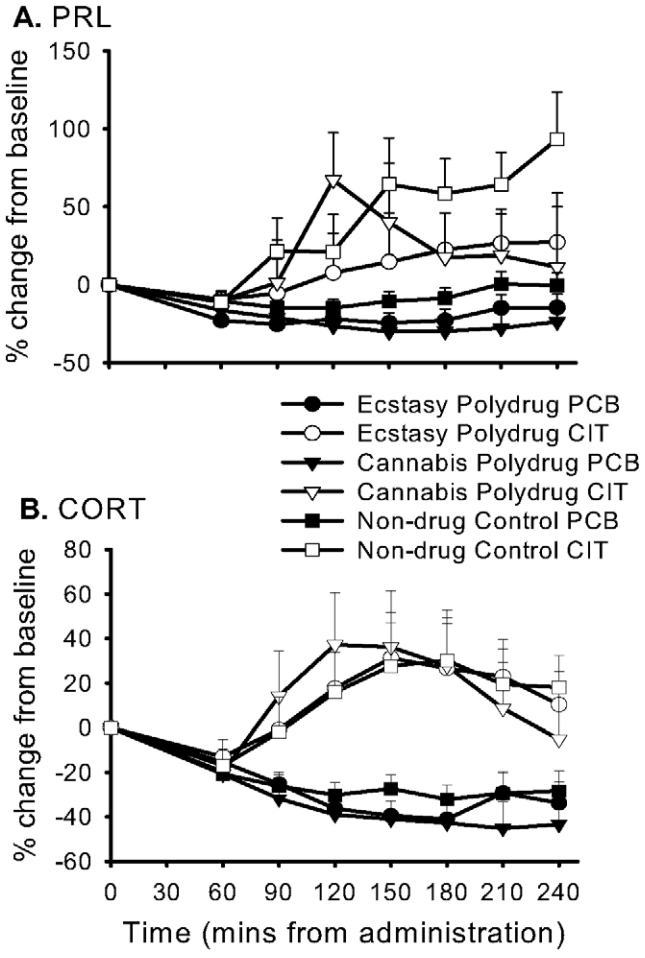

Figure 1 Percentage change in (A) PRL and (B) CORT following oral administration of PCB or CIT (40 mg) according to drug group (sexes combined). When all six groups were compared in two mixed-model ANOVAs, there was a significant main effect of treatment on PRL and CORT secretion, with CIT eliciting a greater percentage change than PCB in PRL and CORT secretion (both $P < 0.001$)

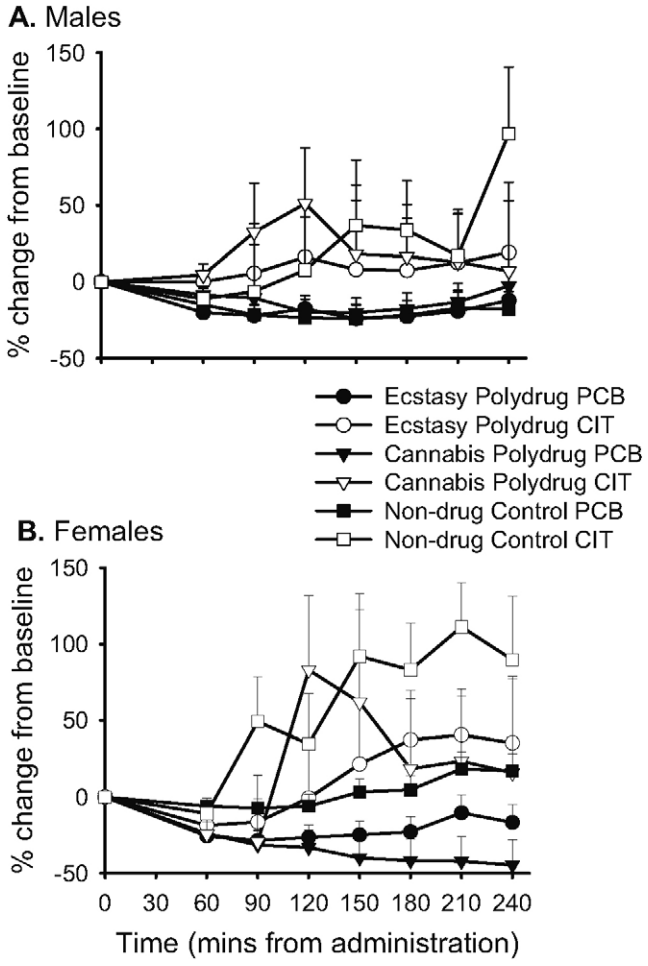

Figure 2 Males (A) and females (B) percentage change in PRL following oral administration of PCB or CIT (40 mg) according to drug group. Note, when all six groups were compared in mixed-model ANOVA, there was a significant main effect of treatment on PRL secretion, with CIT eliciting a greater percentage change in PRL than PCB ($P < 0.001$)

ANCOVAs were repeated for percentage change in PRL and CORT with these potentially confounding variables entered as single covariates. For percentage change in both PRL and CORT, the addition of age, MEQ score, reward dependence and lifetime dose of alcohol, amphetamines, ketamine and amyl nitrate did not alter the between-group effects, which remained nonsignificant. In the case of PRL, weight also did not affect the findings for percentage change relative to baseline. However, for percentage change in CORT, with weight entered as a covariate, there were significant interactions between treatment and sex [$F(1,49) = 5.62$, $P = 0.022$, $\varepsilon = 1.0$], time and sex [$F(2.52,123.28) = 4.20$, $P = 0.011$, $\varepsilon = 0.42$] and treatment × time × sex [$F(2.41,117.99) = 3.58$, $P = 0.024$, $\varepsilon = 0.40$], with males demonstrating an earlier and much greater percentage change in CORT than females following treatment with CIT (data not shown). The CORT responses did not differ between sexes following treatment with PCB.

Subjective responses – group analyses

Table 3 gives the mean (and SD) values for the peak change in VAS variables. Under the Bonferroni correction, none of the mean VAS peak change scores significantly differed between drug groups and sex, alone or in interaction with the other independent variable. However, the *P*-values were small for a main effect of sex ($P = 0.049$) regarding peak change in dry mouth, and for a drug group × sex interaction ($P = 0.031$) regarding peak change in dizziness, indicating possible trends in the data.

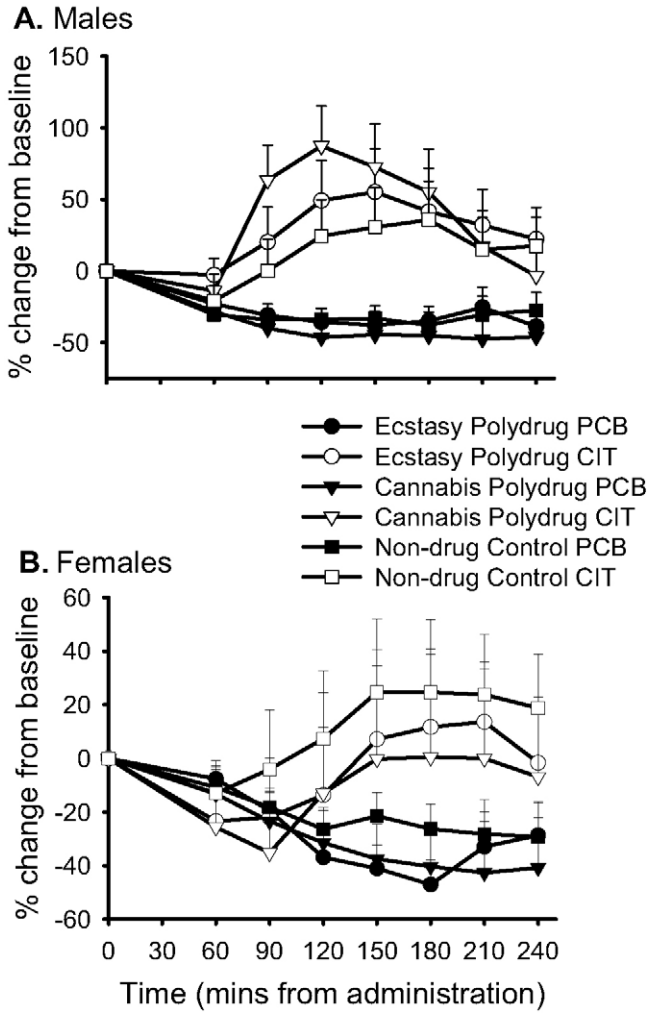

Figure 3 Males (A) and females (B) percentage change in CORT following oral administration of PCB or CIT (40 mg) according to drug group. Note, when all six groups were compared in mixed-model ANOVA there was a significant main effect of treatment on CORT secretion, with CIT eliciting a greater percentage change in PRL than PCB ($P < 0.001$)

Relationship between ecstasy use and neuroendocrine functioning

Table 3 gives the mean (and SD) values for the net PRL and CORT AUCs and the net peak changes in PRL and CORT. There were no significant main effects for drug group or sex, or drug group × sex interactions for any of these neuroendocrine variables.

Preliminary backward multiple regressions revealed that the significant predictors of PRL AUC were VAS peak change scores of nausea, dry mouth and hand tremor. The significant predictors of CORT AUC were novelty seeking and peak change in dry mouth and hand tremor. The only predictor of peak change in PRL was peak change in nausea. The significant predictors of peak change in CORT were lifetime dose of alcohol and peak changes in nausea and dry mouth. None of the demographic, mood or cannabis use variables were retained subsequent to the preliminary backward regression analyses.

For the hierarchical multiple regression analyses, the contributions of the various predictor variables to the neuroendocrine outcome variables (with ecstasy use variables entered last) are shown in Table 4. The VAS variables of peak change in hand tremor, dry mouth and nausea were the significant contributors to the prediction of PRL AUC (explaining in total 28% of the variance). Peak change in nausea (explaining in total 17% of the variance) was the only significant contributor to the prediction of peak change in PRL. Novelty seeking, peak change in hand tremor and dry mouth contributed significantly to the prediction of CORT AUC (explaining in total 29% of the variance). Lifetime dose of alcohol, peak change in nausea and dry mouth formed the best combination of predictors of peak change in CORT (explaining in total 30% of the variance).

The hierarchical multiple regression analyses did not indicate the ecstasy use variables were significant contributors to the prediction of any of the neuroendocrine outcome variables. Therefore, any reported effect of ecstasy *per se* may be due to other confounding variables. Indeed, four backward multiple regressions (one for each neuroendocrine outcome) with only ecstasy use variables entered as predictors, showed that none of the ecstasy use variables, singly or in combination with the remaining variables, significantly predicted any of the four neuroendocrine outcome variables.

Discussion

This is the first challenge study of ecstasy users to utilise CIT, a drug which has highly specific actions at the serotonin transporter, as the neuroendocrine probe. It is only the second study to examine potential sex differences in neuroendocrine functioning of ecstasy users and female hormone status was controlled by recruiting female OC users, whereas hormone status was not controlled in the McCann, *et al*. (1994) study. The experimental groups were generally well matched with respect to most demographic, mood and personality variables and the EP and CP groups were well matched for cannabis use, a common confound in ecstasy-related research. The contribution of potentially confounding variables was carefully measured, including variables that differed between groups. We were able to rule out acute drug effects by employing serum drug screens and EP participants reported themselves not to have used ecstasy for a mean of 3 weeks.

The hypothesis that EP users would show blunted PRL and CORT responses to serotonergic challenge, relative to CP and non-drug (NC) users, was not supported. Furthermore, there was no significant difference between the neuroendocrine responses of female and male EP, CP or NC groups following CIT or PCB treatment, after controlling for baseline differences. However, an effect of sex (regardless of drug group)

Table 3 Descriptives of VAS and neuroendocrine variables

	Ecstasy polydrug users		Cannabis polydrug users		Non-drug using controls	
	Male (n = 9)	Female (n = 11)	Male (n = 9)	Female (n = 5)	Male (n = 11)	Female (n = 11)
VAS variables						
Peak change in nausea	18.4 (22.5)	41.6 (34.1)	19.1 (21.9)	20.8 (39.4)	20.1 (30.3)	20.6 (30.9)
Peak change in appetite	−1.22 (25.1)	−24.3 (24.0)	−2.4 (12.6)	−12.4 (31.4)	−17.6 (38.6)	−0.2 (28.6)
Peak change in dry mouth	2.8 (15.7)	9.6 (19.5)	−8.0 (15.6)	−4.4 (44.2)	−8.5 (20.9)	22.3 (31.7)
Peak change in sleepiness	7.0 (21.3)	3.4 (32.2)	14.0 (27.3)	23.6 (43.6)	13.8 (20.6)	1.9 (26.9)
Peak change in hand tremor	3.9 (5.7)	12.8 (31.2)	0.3 (15.3)	1.6 (29.1)	16.9 (21.7)	7.9 (15.3)
Peak change in drowsiness	5.9 (25.4)	11.3 (30.4)	15.7 (28.0)	1.8 (25.8)	5.4 (13.8)	7.6 (11.5)
Peak change in lethargy	8.8 (27.2)	−2.1 (38.9)	25.4 (27.0)	11.2 (23.5)	17.6 (34.3)	8.2 (21.2)
Peak change in restlessness	9.2 (30.9)	21.3 (30.5)	11.0 (21.2)	−1.4 (11.1)	16.3 (29.2)	2.6 (7.1)
Peak change in dizziness	4.2 (19.3)	30.3 (22.6)	7.6 (16.9)	−7.4 (41.5)	8.6 (27.7)	1.8 (17.9)
Neuroendocrine variables						
PRL AUC	159.4 (232.8)	226.9 (511.9)[a]	247.6 (340.9)	702.6 (1461.5)	215.1 (362.2)[a]	567.6 (1256.2)
CORT AUC	474.9 (731.5)	359.8 (629.0)	666.8 (841.4)	406.8 (867.2)	424.9 (832.5)	791.3 (979.3)
Peak change in PRL	2.2 (3.0)	5.2 (7.9)[a]	4.6 (6.6)	11.9 (23.4)	5.7 (11.4)[a]	9.0 (16.1)
Peak change in CORT	9.0 (13.1)	9.8 (11.8)	11.6 (13.1)	4.5 (13.2)	6.8 (11.4)	13.4 (15.7)

Values represent mean (SD); all peak change and AUC variables are PCB-corrected.
VAS, Visual Analogue Scale; AUC, area under the curve; peak change means peak change from baseline value.
[a]n = 10.

was observed when weight was controlled, with males showing greater CORT responses following CIT treatment. There were no group differences in subjective responses to the pharmacological challenge with CIT. Finally, there was no significant relationship between extent of ecstasy use and neuroendocrine functioning, alone or in combination with other contributing variables. Regression analyses indicated that the best predictors of neuroendocrine functioning were: peak change in hand tremor, dry mouth and nausea for PRL AUC; peak change in nausea for peak change in PRL; novelty seeking, peak change in hand tremor and dry mouth for CORT AUC; and lifetime dose of alcohol, peak change in nausea and dry mouth for peak change in CORT. These results highlight the importance of taking into account non-ecstasy variables that may mediate neuroendocrine functioning, putatively reflecting serotonergic functioning.

The results of the seven previous 5-HT neuroendocrine challenge studies of ecstasy users have been mixed. McCann, *et al.* (1994) found no difference in the neuroendocrine (PRL) responses of 30 ecstasy users and 28 controls, although females showed greater AUC and peak increases in PRL than males regardless of drug group. Price, *et al.* (1989) reported significant increases in PRL from baseline in nine male controls, but not in nine male ecstasy users following L-tryptophan challenge, although there were no significant differences between the groups. McCann, *et al.* (1999) found significantly blunted PRL and CORT responses in 15 male ecstasy users vs. 17 male controls and Verkes, *et al.* (2001) reported significantly reduced CORT, but not PRL responses in 21 'moderate' and 21 'heavy' male ecstasy users vs. 20 male controls. Relative to 15 male controls, Gerra, *et al.* (1998) found blunted release of PRL and CORT in 15 male ecstasy users following 3 weeks of abstinence, and found that the PRL, but not CORT, responses remained blunted in 15 male ecstasy users who were abstinent from ecstasy for 1 year (Gerra, *et al.*, 2000). However, the participants in the latter longitudinal study were not required to remain abstinent from cannabis during the 1-year follow-up period. A stronger relationship between neuroendocrine functioning and cannabis use was reported in a later neuroendocrine study (Gouzoulis-Mayfrank, *et al.*, 2002). However, results of this latter study may have reflected sub(acute) effects, as participants were only required to remain abstinent from cannabis on the day of the study. In the current study, participants were abstinent from cannabis for at least 7 days and no relationship was found between cannabis use and neuroendocrine functioning.

Some of the mixed findings may be associated with the specific challenge agent used. Serotonergic probes that were used by the previous studies included the 5-HT precursor L-tryptophan (McCann, *et al.*, 1994; Price, *et al.*, 1989), the 5-HT releaser D-fenfluramine (Gerra, *et al.*, 1998, 2000; Gouzoulis-Mayfrank, *et al.*, 2002; Verkes, *et al.*, 2001), and the mixed 5-HT receptor agonist *meta*-chlorophenylpiperazine (*m*-CPP; McCann, *et al.*, 1999). L-tryptophan and D-fenfluramine have been criticised for their use as probes of serotonergic functioning because there is evidence that they also affect neurotransmission of dopamine and norepinephrine (Rothman, *et al.*, 2003; van Praag, *et al.*, 1987; Yatham and Steiner, 1993).

An important methodological limitation of several of the previous neuroendocrine studies of ecstasy users was their failure to include a PCB control condition, and therefore, a failure

Table 4 Ecstasy use and other potentially confounding variables as predictors of neuroendocrine variables

Model	*R*	R^2	Adj. R^2	*P*	Change R^2	*P*
PRL AUC ($n = 54$)						
1[a]	0.53	0.28	0.24	0.001	0.28	0.001
2[b]	0.58	0.34	0.20	0.021	0.05	0.727
Peak change in PRL ($N = 54$)						
1[c]	0.42	0.17	0.16	0.002	0.17	0.002
2[d]	0.53	0.28	0.17	0.029	0.10	0.392
CORT AUC ($N = 56$)						
1[e]	0.29	0.09	0.07	0.029	0.09	0.029
2[f]	0.53	0.29	0.24	0.001	0.20	0.002
3[g]	0.57	0.32	0.19	0.026	0.03	0.891
Peak change in CORT ($N = 56$)						
1[h]	0.28	0.08	0.06	0.400	0.08	0.040
2[i]	0.55	0.30	0.26	0.000	0.23	0.001
3[j]	0.63	0.39	0.28	0.003	0.09	0.352

[a]Predictors: Peak change in hand tremor, dry mouth and nausea.

[b]Predictors: Peak change in hand tremor, dry mouth and nausea, years of ecstasy use, frequency ecstasy use/month, typical ecstasy dose, highest ecstasy dose, total occasions of ecstasy use, ecstasy use in last month.

[c]Predictors: Peak change in nausea.

[d]Predictors: Peak change in nausea, years of ecstasy use, frequency ecstasy use/month, typical ecstasy dose, highest ecstasy dose, total occasions of ecstasy use, ecstasy use in last month.

[e]Predictor: Novelty seeking.

[f]Predictors: Novelty seeking, peak change in hand tremor and dry mouth.

[g]Predictors: Novelty seeking, peak change in hand tremor, and dry mouth, years of ecstasy use, frequency ecstasy use/month, typical ecstasy dose, highest ecstasy dose, total occasions of ecstasy use, ecstasy use in last month.

[h]Predictor: Lifetime dose of alcohol.

[i]Predictors: Lifetime dose of alcohol, peak change in nausea, peak change in dry mouth.

[j]Predictors: Lifetime dose of alcohol, peak change in nausea, and dry mouth, years of ecstasy use, frequency ecstasy use/month, typical ecstasy dose, highest ecstasy dose, total occasions of ecstasy use, ecstasy use in last month.

to take into consideration hormone secretion under normal conditions (Gerra, *et al*., 1998, 2000; Gouzoulis-Mayfrank, *et al*., 2002; McCann, *et al*., 1994; Price, *et al*., 1989). Apart from the current study, Verkes, *et al*. (2001) authored the only other study to have utilised a randomised crossover design with a PCB condition. A lack of PCB control is problematic, because there may be group differences in resting diurnal hormone secretion (Kuepper, *et al*., 2006; McBride, *et al*., 1990; Monteleone, *et al*., 1997), as well as inter-individual variations in nonspecific factors, such as responses to cannulation, blood taking and other environmental factors (Thompson, *et al*., 1994).

Assuming that ecstasy/MDMA causes damage to central 5-HT neurons in humans, there are a number of possible explanations for the absence of group differences found in neuroendocrine functioning in the current study. The first is that the participants in the present study were not heavy enough users of ecstasy/MDMA to show neuroendocrine dysfunction. For example, participants' average frequency of ecstasy use per month was low (1–2 times/month) relative to the 2–5 times/month in other studies that have shown altered neuroendocrine functioning in ecstasy/MDMA users (Gerra, *et al*., 1998; Gouzoulis-Mayfrank, *et al*., 2002; McCann, *et al*., 1999). It has been suggested that frequency of ecstasy/MDMA use may be more relevant to 5-HT injury than total cumulative use (Fantegrossi, *et al*., 2004; Hegadoren, *et al*., 1999; O'Shea, *et al*., 1998). It is important to note, however, that the history of ecstasy use reported by the current sample is broadly consistent with the patterns of use reported by other Australian samples (Allott and Redman, 2006; Johnston and Jenkinson, 2006; Topp, *et al*., 1999) and may, therefore, be representative of individuals from this country. At present, it is still not known how much ecstasy/MDMA (i.e. dose, frequency of use, cumulative amount of use) needs to be consumed before potentially permanent changes in the human brain occur. However, it is just as important to understand the levels of use that do not lead to significant harm to the brain (Parrott, 2003).

The second possible explanation for the lack of group differences relates to the finding in monkeys that the hypothalamus appears somewhat more resistant to prolonged 5-HT axon injury (as measured by concentrations of 5-HT and 5-HIAA in brain tissue and ^{3}H-paroxetine-labeled serotonin uptake sites/transporters), relative to other brain regions such as the cerebral cortex, striatum and hippocampus (Ali, *et al*., 1993; Insel, *et al*., 1989), and 'recovery' of serotonergic markers is greatest in the hypothalamus in monkeys in the months following MDMA treatment (Ricaurte, *et al*., 1992; Scheffel, *et al*., 1998). This may in part be due to the fact that the hypothalamus is close to regions containing 5-HT cell bodies, which are known to be spared relative to (more distal) axon terminals from MDMA-induced injury (Ali, *et al*., 1993). These findings may be somewhat less robust in rats (McGregor, *et al*., 2003), although findings in non-human primates are more able to be generalised to humans. Nevertheless, the results in animals are unlikely to be entirely representative of the effects of MDMA in humans. It is notable that despite finding alterations in 5-HT markers in cortical, hippocampal, thalamic and other brain regions, few neuroimaging studies of human ecstasy users have investigated or reported effects in the hypothalamus (see McCann, *et al*., 1998, for exception). Therefore, it may be speculated that the use of neuroendocrine responses that are innervated by 5-HT projections to the hypothalamus, may be a less sensitive measure of central 5-HT changes in ecstasy/MDMA users.

A third possibility is that the ecstasy tablets reportedly consumed by the EP participants contained no or low doses of MDMA. Although it is unlikely that the purity of ecstasy tablets in Australia would be lower than in other countries, especially since Europe is the primary source of ecstasy tablets in Australia (Australian Crime Commission, 2006), the current

study was not able to analyse a sample of any of the ecstasy tablets consumed by the participants, nor was hair analyses conducted to confirm consumption of MDMA (Kish, 2002).

The finding of no significant relationship between ecstasy use variables and neuroendocrine responses is consistent with several other pharmacological challenge studies (Gerra, *et al*., 1998; McCann, *et al*., 1999; Price, *et al*., 1989). Even when there were significant group differences, as reported by some studies (Gerra, *et al*., 1998; McCann, *et al*., 1999), an absence of an association between neuroendocrine release and ecstasy use suggests that neuroendocrine responses may reflect a pre-existing biological milieu that may or may not be associated with initiation and maintenance of ecstasy use or its long-term effects. In support of this hypothesis, the personality trait of novelty seeking significantly contributed to the prediction of CORT AUC in the current study. Gerra, *et al*. (2000) also found a significant association between neuroendocrine release and novelty seeking, although with PRL rather than CORT. Unfortunately, the most convincing and well-controlled study to show impaired neuroendocrine functioning in ecstasy users relative to controls, did not report whether there was a significant correlation between neuroendocrine functioning and the extent of ecstasy use (Verkes, *et al*., 2001).

With the exception of Gouzoulis-Mayfrank, *et al*. (2002), who investigated the effects of cannabis, none of the previous studies examined the impact of substance use other than ecstasy on neuroendocrine release. This is surprising given that polydrug use is the norm among ecstasy users and is frequently cited as a confounding factor. The current study finding that lifetime dose of alcohol contributed significantly to the prediction of peak change in CORT is consistent with previous research showing that heavy alcohol use chronically affects 5-HT-mediated neuroendocrine release (Berggren, *et al*., 2002; George, *et al*., 1997; Krystal, *et al*., 1996; Lee and Meltzer, 1991). This result underscores the importance of considering the impact of substances other than ecstasy and cannabis in studies attempting to disentangle the contribution of ecstasy/MDMA to any outcome.

An interesting finding from the current study was that, although there were no group differences in the subjective responses to pharmacological challenge, the subjective responses of nausea, hand tremor and dry mouth were highly predictive of neuroendocrine responses, indicating that subjective responses to the challenge drug may modulate neuroendocrine release (although the inverse relationship is also possible). Given that PRL and CORT are stress-sensitive hormones, it is possible that nonspecific stress effects of CIT were measured, rather than 5-HT selective effects. Some studies have found subjective effects, such as nausea, to be significantly associated with neuroendocrine responses following challenge of the 5-HT system (Ghaziuddin, *et al*., 2003; Muldoon, *et al*., 1996), while others have not (Kuepper, *et al*., 2006; Mueller, *et al*., 1985). McCann, *et al*. (1999), who found blunted neuroendocrine responses to *m*-CPP in ecstasy users compared with controls, measured subjective responses to the *m*-CPP challenge and found that compared with the ecstasy users, the controls experienced significantly greater negative emotions and physical symptoms, including panic attacks. However, they did not report whether these subjective reactions were associated with neuroendocrine functioning and may have contributed to their findings of group differences in neuroendocrine release. These results emphasise the need to consider the effects of subjective responses in future pharmacological challenge studies.

The finding that females had higher baseline levels of CORT and PRL than males is consistent with previous reports, including those in which females were taking OCs (Alvarez-Tutor, *et al*., 1999; Genazzani, *et al*., 1997; Ghaziuddin, *et al*., 2003; McCann, *et al*., 1994; Meltzer and Maes, 1994). However, the finding of a sex difference, with males showing greater CORT responses to CIT when baseline was controlled and weight was held constant, is novel. Males showed greater CORT responses than females in a study that used 5-hydroxytryptophan to probe 5-HT functioning (Maes, *et al*., 1989), whereas studies that used D-fenfluramine as the neuroendocrine probe have reported greater CORT responses in females (Bond, *et al*., 1995; Monteleone, *et al*., 1997). However, all of these earlier studies failed to account for weight as a covariate. In their neuroendocrine study of ecstasy users and controls, McCann, *et al*. (1994) found a greater PRL response in females regardless of drug group, but they also did not account for weight in their analyses. The finding of significant interactions between sex and treatment/time when weight is held constant indicates that there may be pharmacodynamic factors involved in male and female CORT responses to CIT challenge (Golden, *et al*., 1991). The mechanism underlying these potential sex differences in response to CIT remains unclear and requires further investigation, but underscores the importance of carefully controlling for sex and weight in neuroendocrine challenge studies.

Although there is a lack of research regarding whether OCs interact with CIT to alter CIT's plasma concentration, this is unlikely because CIT is metabolised by multiple cytochrome P450 enzymes, meaning that there is a reduced chance of other drugs altering its biotransformation (Brosen and Naranjo, 2001). Nevertheless, exogenous intake of sex steroids, such as OCs, is intended to mimic endogenous physiology. Therefore, in order to gain a complete understanding of how an individual's sex and possibly sex steroids determine their physiological responses to drugs, conducting additional studies in naturally cycling women and comparing them across various menstrual cycle phases, will enhance our understanding of any sex differences associated with the effects of ecstasy/MDMA (Broadbear, *et al*., 2004).

There are some potential caveats that may limit the conclusions drawn from the current study. First, the influence of pharmacokinetic factors on neuroendocrine responses to CIT must be considered. A fixed oral dose was used and there was no adjustment of dose according to body weight, although it has been recently argued that using weight-adjusted CIT doses may only provide a slight improvement in power (Lotrich, *et al*., 2005). Furthermore, plasma levels of CIT and its major metabolite were not measured. There may have been

individual differences in absorption of the orally administered CIT, which might underlie any variability in responses to it. However, weight was not inversely correlated with (data not shown), or found to be predictive of, AUC and peak change measures of CORT and PRL, therefore arguing against the hypothesis of pharmacokinetic effects. Furthermore, there is only a low to modest relationship between plasma levels of CIT and hormonal responses (Lotrich, *et al*., 2005; Nadeem, *et al*., 2004) and body size does not appear to significantly affect plasma CIT levels (Dr. V. Struwig, Lundbeck, personal communication, July 17, 2006). The use of CIT as a neuroendocrine probe of 5-HT function is still relatively new and the validity and reliability of its use in this way has not been firmly established (Hawken, *et al*., 2006). It is possible that the 40 mg dose and oral route of administration used in the current study was inadequate for producing reliable changes in endocrine release, which may have limited the ability to detect group differences.

Second, as with any cross-sectional study, the reliability of retrospective self-reported use of ecstasy/MDMA is uncertain. However, the reliability of recall was maximised using a detailed 'timeline' interview method, employing contextual recall cues, to obtain histories of drug use. This method has been noted to produce higher estimations of lifetime ecstasy use than other methods (i.e. frequency multiplied by duration of use) and is likely to better capture variability in usage patterns over time (Bedi and Redman, 2006).

Third, the representativeness of the sample to ecstasy users in the general population is unknown, but may be reduced due to potential bias in participant self-selection into the study, and/or due to the stringent inclusion/exclusion criteria. It is possible that we may have recruited 'healthier' ecstasy-using individuals who are less likely to experience serotonergic dysfunction.

Fourth, the length of the neuroendocrine challenge sessions may have been too short, which may have prevented detection of possible group differences. The PRL and CORT levels did not come back to baseline within the 240 min period. Furthermore, Figure 1 shows that relative to the EP and CP groups, the PRL levels still appeared to be increasing in the NC group. Longer sessions were not employed because it was felt that this would have limited recruitment and retention of participants. Furthermore, previous challenge studies of ecstasy users employing similar (oral) challenge periods to the current study showed significant group differences (Gerra, *et al*., 1998, 2000).

Finally, due to some difficulty recruiting suitable participants (especially female CP users), resulting in lower numbers than anticipated, the study may have been underpowered to detect significant group differences, particularly for small effect sizes. Although, the three drug groups were similar in size to the drug groups recruited in previous challenge studies in which significant group differences were reported (Gerra, *et al*., 1998; McCann, *et al*., 1999; Verkes, *et al*., 2001), the statistical power to detect sex × drug group interactions was limited. Based on a mean sample size of approximately nine per group (i.e. 56/6 = 9.3), this study had 57% power to detect a large effect size at $\alpha = 0.05$ (Cohen, 1992; Faul and Erdfelder, 1992), resulting in an increased probability of committing a type II error. It is, therefore, suggested that any future studies incorporate larger sample sizes (e.g. ~15 per group for 80% power).

In conclusion, the results of this study did not demonstrate significant differences between EP users, CP users and non-drug users in neuroendocrine functioning, nor did they demonstrate a significant relationship between ecstasy use and neuroendocrine functioning. The results did, however, highlight the importance of exposing and controlling for potentially confounding variables (such as sex, weight, subjective responses, personality and other drug use) and including a PCB control condition, to show whether any findings of group differences in neurotransmitter (i.e. serotonergic) activity genuinely reflect group differences in the effects of ecstasy/MDMA use.

Based on the current results and results of previous research measuring neuroendocrine 5-HT activity, reliable advice as to the amount and frequency of ecstasy/MDMA consumption that is required before the 5-HT system is significantly compromised cannot be given to users. It may be that the use of other 5-HT endpoints may be more useful markers of 5-HT injury than hypothalamic 5-HT function in human ecstasy/MDMA users. Prospective studies conducted over many years are needed to delineate the threshold for which recreational ecstasy/MDMA use produces persisting brain changes. It is also not currently possible to predict which individuals are more likely to be at increased risk of ecstasy-associated harm; however, this should be an ongoing focus of future studies.

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