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Downey L.A., Sands H., Jones L., Clow A., Evans P, Stalder T. and Parrott A.C.

Reduced memory skills and increased hair cortisol levels in recent Ecstasy/MDMA users: significant but independent neurocognitive and neurohormonal deficits.

Sands HR[*], Downey LA[*+], Jones L[*], Clow A[**], Evans P[**], Stalder T[++], & Parrott AC[*+]

[*]Department of Psychology, Swansea University, Swansea, UK.

[+] Centre for Human Psychopharmacology (CHP), Swinburne University, Australia

[**] Psychophysiology and Stress Research Group, Westminster University, London, UK.

[++] Department of Psychology, TU Dresden, Dresden, Germany.

Author for correspondence:

Professor AC Parrott,

Department of Psychology

Swansea University,

Swansea SA2 8PP,

Wales, United Kingdom.

Tel: +44 (0) 1792 295271

Email: a.c.parrott@swansea.ac.uk

Abstract

Objectives: to measure the neurocognitive performance of recent users of recreational Ecstasy/MDMA, and investigated whether it was associated with the stress hormone cortisol.

Methods: the 101 participants comprised 27 recent light users of Ecstasy/MDMA (1-4 times in last 3 months), 23 recent heavier Ecstasy/MDMA users (+5 times), and 51 non-user controls. Rivermead paragraph recall provided an objective measure for immediate and delayed recall. The Prospective and Retrospective Memory Questionnaire provided a subjective index of memory deficits. Cortisol levels were taken from near-scalp 3-month hair samples.

Results: Cortisol was significantly raised in recent heavy Ecstasy/MDMA users compared to controls, whereas hair cortisol in recent light Ecstasy/MDMA users was not raised. However *both* Ecstasy/MDMA groups were significantly impaired on the Rivermead delayed word recall, and both groups reported significantly more retrospective and prospective memory problems. Stepwise regression confirmed that lifetime Ecstasy/MDMA predicted the extent of these memory deficits. There were no significant correlations between hair cortisol over the past 3 months, and the memory measures.

Conclusions: recreational Ecstasy/MDMA is associated with increased levels of the bio-energetic stress hormone cortisol, and significant memory impairments. However there was no significant relationship between the neurohormonal levels and the cognitive deficits.

The ring-substituted methamphetamine derivative MDMA or 'Ecstasy' is a powerful central nervous system (CNS) stimulant, that stimulates serotonin (5-hydroxytryptamine, 5-HT) release through indirect agonistic actions on the serotonergic system. MDMA (3.4-methlenedioxymethaphetamine) has been described as neurochemically messy, since it also affects several other neurotransmitters, including dopamine, noradrenaline and histamine (Green et al, 2003; Ricaurte et al, 2000). MDMA is known for its euphoric and entactogenic properties, since it can induce subjective feelings of happiness and closeness to others (Parrott, 2001, 2007; McCann et al, 2007; Taurah et al, 2013). The recreational use of Ecstasy/MDMA is associated with a range of neuropsychobiological problems, in memory and higher cognition (Parrott, 2001, 2013a,b; Rendell et al, 2007; Murphy et al, 2009; Piechatzek et al, 2009), neurohormonal activity (Gerra et al, 2003; Parrott, 2009), psychiatric well-being (Bedi et al, 2010; Karlsen et al, 2008; Potter et al, 2013; Roiser & Sahakian, 2004; Soar et al, 2004; Wallinga et al, 2009; Taurah et al, 2013), mood state (Curran & Travill, 1997; Hoshi et al, 2006; Parrott et al., 2011; Scott et al, 2013), pain sensitivity (McCann et al., 2011), sleep (Blagrove et al., 2011; Carhart-Harris et al, 2009; McCann & Ricaurte, 2007; McCann et al, 2009), and psychomotor ability (Dastrup et al, 2010; Parrott & Lasky, 1998; Rizzo et al, 2005; Verkes et al, 2001; Wilson et al, in press). Furthermore, in many of these psychobiol(log)ial areas the degree of impairment is associated with lifetime Ecstasy/MDMA usage (Parrott et al, 2000; Roderique-Davies & Shearer, 2010; Soar et al, 2006; Sterk et al, 2007).

Over the past 25 years, research into Ecstasy/MDMA has attempted to identify the mechanisms through which it causes these neuropsychobiological changes. Neuroimaging studies on 5-HT markers in recreational ecstasy/MDMA users have identified reduced levels of the 5-HT transporter (SERT) in many brain areas. For instance, serotonergic damage has been identified in the frontal cortex, which is involved with impulsivity and higher cognition, while the hippocampus is closely involved in memory (Benningfield & Cowan, 2013; McCann & Ricaurte, 2003; McCann et al., 2005; Murphy et al, 2009; Kish et al, 2010; Parrott, 2012a; Roberts et al, 2013). Ecstasy/MDMA can also influence neuroendocrine activity, both acutely and chronically (Dumont & Verkes, 2006; Parrott, 2009). An acute dose has stimulatory effects on the hypothalamic-pituitary-adrenal (HPA) axis, leading to

increased secretion of the glucocorticoid hormone cortisol (Harris et al, 2002; see also Dumont & Verkes, 2006; Kuypers et al, 2013). In laboratory studies acute MDMA leads to a cortisol increase of 100-200% in sedentary humans, depending on factors such as dosage (Harris et al, 2002; Kuypers et al, 2013), and single or closely-repeated administration (Farre et al, 2004). Even greater neurohormonal changes have been found in real world studies of recreational users, with self-administered Ecstasy/MDMA by dance clubbers leading to an increase in salivary cortisol of around 800% (Parrott et al, 2013; Parrott et al, 2008). This larger increase is hypothesised to result from the combination of drug and environmental stimulation, since dance clubs involve loud music, dense crowding, and prolonged periods of dancing. These environmental factors have been incorporated into the bio-energetic stress model, attempts to explain the variability in neuropsychological, functional and structural consequences of recreational Ecstasy/MDMA (Parrott, 2001, 2009, 2013a; Parrott et al, 2006).

Corticosteroids are essential for normal brain functioning, and are involved in neuro-adaptive responses to environmental changes; furthermore low or high levels of corticosteroids can be damaging to various psychobiological functions (Herbert et al, 2006). The exposure to repeated stressors, such as Ecstasy/MDMA consumption in biologically stressful situations, may increase cortisol levels, and can be a contributory factor for any associated neurocognitive effects (Parrott et al, 2006, 2008). Wetherell and Montgomery (2014) reported significantly greater levels of anxiety and depression, and elevated diurnal cortisol profiles, in abstinent Ecstasy/MDMA users. As noted earlier, recreational Ecstasy/MDMA use is associated with memory problems and other neurocognitive deficits (Parrott et al, 1998; Gouzoulis-Mayfrank et al., 2000; Rendel et al, 2007; Parrott, 2013a,b; Meyer, 2013). Structural damage to brains areas related to memory function, including the fronto-temporal and hippocampal regions (McEwen, 2005), have been observed to be altered in Ecstasy/MDMA users, and this damage may partially reflect a repeated exposure to high levels of cortisol. Further to this, heavy users of Ecstasy/MDMA can display significant neuroendocrine changes in comparison to controls and lesser users of Ecstasy/MDMA - in the form of reduced cortisol response to serotonin agonists (Gerra et al, 2000; Verkes et al, 2001). As such, the repeated exposure to high levels of cortisol through Ecstasy/MDMA exposure may constitute a potential mechanism for the associated memory impairments detected in Ecstasy/MDMA

users (Parrott, 2009).

Kuypers et al (2012) investigated this hypothesis by giving participants a cortisol synthesis inhibitor (metyrapone), together with a single dose of MDMA. They reported that the cortisol inhibiting effect of metyrapone did not prevent the MDMA-induced verbal memory impairment; this allowed them to conclude that the cortisol response to MDMA was unrelated to the memory deficits. However this was a single does study, and the effects of repeated Ecstasy/MDMA use on cortisol and cognition remain unclear. The bio-energetic stress model predicts that repeated drug usage will lead to chronically increased levels of cortisol (Parrott, 2006, 2009). Furthermore, any basal neurohormonal changes may have adverse practical implications, given the importance of cortisol for homeostasis, neurocognition, and psychobiological integrity (Herbert et al., 2006). Initial evidence suggests that drug-free Ecstasy users exhibit altered baseline cortisol, also stress-responsive cortisol secretion (Gerra et al., 2003). Although some equivocal evidence exists concerning the longer term endocrine changes associated with Ecstasy/MDMA usage. For example, Allott et al (2009) observed no persistent effect of MDMA use on neuroendocrine functioning with respect to a serotonergic challenge study, with Ecstasy/Polydrug, Cannabis/Polydrug and Non-drug using controls not differing in their cortisol responses to citalopram. Whereas, Wolff and colleagues examined cortisol samples 'in the field' in participants pre- and post-clubbing (Wolff et al, 2012). They observed changes in cortisol readings to be significantly greater in MDMA-positive clubbers, and that these changes were related to the low activity catechol-O-methyl transferase genotype. They concluded that chronic use of MDMA may lead to HPA axis dysregulation, although this may be moderated by genetic polymorphism. Frokjaer et al (2013), demonstrated an empirical link between cortisol and prefrontal serotonin, hence supporting the putative links between elevated cortisol, serotonergic neurotoxicity, and disrupted memory (McCann et al, 2008; Kish et al, 2010; Parrott, 2006, 2013b).

Until recently, the measurement of cortisol levels has been limited to biological markers, such as blood, saliva, or urine samples, taken at single time points. Since cortisol is a labile hormone, these measures may be influenced by situational factors and circadian activity (Hellhammer et al, 2007; Stalder et al, 2009). The assessment of cortisol in hair comprises an important methodological advance, since it provides a

single index of neurohormonal level over several months (Stalder & Kirschbaum, 2012). In the current study we utilized this novel procedure to investigate the link between recent Ecstasy/MDMA use, and the amount of cortisol deposited in hair over the previous 3-months. The basic findings of significantly higher cortisol in heavier Ecstasy/MDMA users, have been fully described elsewhere (Parrott et al, 2014). In the current report, we present the memory test findings from that study, and analyse the relationship between the cognitive changes and cortisol levels. We compare the same three groups of light recent MDMA users, heavy recent MDMA users, and non-user controls (Parrott et al, 2014). The first hypothesis was that memory performance would be impaired in the recreational Ecstasy/MDMA users. Secondly, we hypothesized that the extent of memory impairment would be greater in those participants with higher levels of hair cortisol.

Methods

Participant characteristics

One-hundred and one participants (53 males, 48 females, mean ± SD age: 21.75 ± 4.23 years) were recruited via advertisements concerning MDMA usage. Study inclusion was restricted to participants who had hair longer than 3cm at the posterior vertex region of the scalp, and who did not suffer from any chronic medical or psychiatric conditions. Participants were divided into three subgroups depending on their self-reported Ecstasy/MDMA usage over the prior three months. Recent light users comprised 27 participants (18 male, 9 female, mean ± SD age: 21.15 ± 1.09 years) who had consumed MDMA between one and four times in the past 3-months. Recent heavy users comprised 23 participants (7 male (one participant was removed due to multiple outliers across demographic and outcome measures), 15 female, mean ± SD age: 21.48 ± 0.89 years) who had consumed MDMA five or more times in the past 3-months. The control group comprised 51 individuals (27 male, 24 female, mean ± SD age: 21.20 ± 5.85 years) who had not consumed any MDMA in the past 3-months. The drug usage characteristics for the three subgroups are given in Table 1. Written informed consent was provided by all participants. The sign-up form indicated that the University did not condone the use of illicit substances, and provided sources of information for advice on drug related problems. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the

University ethics committee, and the participants were not compensated for their participation.

- Table 1 near here -

Assessment measures

Demographic and hair-related variables. A self-developed questionnaire was used to record socio-demographic and lifestyle variables, such as sex and age, in addition hair-related characteristics, such as hair colour, washes per week, and hair treatments were assessed (as in Stalder et al, 2012).

Recreational Drug Use Questionnaire. This self-rating questionnaire covered recreational drug-usage during the previous 3-months (Parrott et al, 2001). It covered all the main types of drug, both legal (alcohol, tobacco/nicotine), and illegal (cannabis, Ecstasy/MDMA, amphetamine, cocaine, and others; see Table 1). We also covered lifetime usage for the illicit drugs (Table 4).

Hair cortisol. Hair strands (~3 mm diameter) were carefully cut as close as possible to the scalp from the posterior vertex region at the back of the head. The scalp-near 3 cm hair segment was used for analyses. Based on a hair growth rate of ~1cm/month (Wennig, 2000), this segment reflects hair grown over the previous three months. Wash and steroid extraction procedures followed the laboratory protocol described previously (Stalder & et al, 2012b, study II) with 10 mg of whole, non-pulverised hair being used for analyses. After extraction, cortisol concentrations were determined using a commercially available immunoassay with chemiluminescence detection (CLIA, IBL-Hamburg, Germany) in the Biopsychology Laboratory, University of Dresden, Germany (Professor Clemens Kirschbaum). Intra-assay and inter-assay coefficients of variation of this assay are below 8%.

Objective Memory. The Rivermead Behavioural Memory Test (Wilson, Cockburn, Baddeley, & Hiorns, 1989) involved the reading of two paragraphs, containing 60/61 words and 21 key ideas each. One paragraph was to be recalled immediately whereas the other was to be recalled following a 5 minute delay, in which Trail-

making Test B was administered as a distractor task (Mitrushina, 2005). The standardised scoring was for total ideas correctly recalled.

Subjective Memory. The *Prospective and Retrospective Memory Questionnaire (Crawford, Smith, Maylor, Della Sala, & Logie, 2003)* comprised 16 questions - 8 questions covering prospective memory and 8 related to retrospective memory. Reponses were evaluated on the following forced-choice scale: never = 1, rarely = 2, sometimes = 3, quite often = 4, very often = 5.

Design and procedures.

The unpaid volunteer subjects were ….

Results

Cortisol

Group mean cortisol values are presented in Table 2. The between-group ANOVA showed that non-user controls had the lowest group mean cortisol values, and these were marginally higher (non-significantly) in recent light Ecstasy/MDMA users. Recent heavy Ecstasy/MDMA users had cortisol values significantly higher (by around 400%) that non-user controls ($p<0.001$), and significantly higher (by around 300%) than recent light users ($p<0.001$). The cortisol findings are described more fully in Parrott et al (2014).

Objective and Subjective Memory

Means and standard deviations of both objective and subjective memory measures are displayed in Table 2. A higher score on the Objective Memory tasks indicates better recall performance, whereas a higher score on the subjective memory tasks indicates higher self-reported memory problems. Pearson's correlation coefficients between each of these measures and cortisol levels are also displayed; the emergent correlations were all non-significant. The assessment of possible curvilinear relationships between cortisol levels and memory scores also yielded non-significant results. A multivariate analysis of variance (MANOVA) revealed a

significant effect of recent Ecstasy/MDMA use on recall (F (4, 212) = 3.07, p < .05, çp² = .06). Univariate tests of between subject effects demonstrated a non-significant group effect for immediate recall (F (2, 106) = .21, p > .05, *ns*), but a significant group effect with delayed recall (F (2, 106) = 5.51, p < .01, çp² = .10). Comparison tests using Tukey HSD revealed significant differences between the control group and recent Ecstasy/MDMA light users (p < .01), and the control group and recent heavy Ecstasy/MDMA users (p = .01), with no significant differences between the two Ecstasy/MDMA subgroups.

- Table 2 near here -

The Retrospective and Prospective Memory Questionnaire revealed a significant MANOVA effect across the three drug groups (F (4, 212) = 4.71, p < .01, çp² = .08). There were also significant differences between drug groups, for the prospective memory subscale (F (2, 106) = 8.37, p < .01, çp² = .14), and for retrospective memory (F (2, 106) = 9.36, p < .01, çp² = .15). For self-reported prospective memory, Tukey comparison tests revealed significantly more problems in recent light Ecstasy/MDMA users than the control group (p < .01), and significantly more memory problems in recent heavy Ecstasy/MDMA users than controls (p < .01); there were however no significant differences between light and heavy Ecstasy/MDMA user groups (Table 2). A similar pattern emerged with self-reported retrospective memory problems. The recent light Ecstasy/MDMA users reported significantly more retrospective memory problems than the control group (p < .01), while recent heavy Ecstasy/MDMA users also noted significantly more memory problems than controls (p < .01); again there were no significant differences in retrospective memory between the two Ecstasy subgroups.

For the three month drug data, a series of stepwise regressions were undertaken to determine which drug type, or combination of drug types, predicted the memory deficits and cortisol values (Table 3). High collinearity and low usage of certain drug types lead to their exclusion from the regression model. Six variables were entered into the stepwise regression – MDMA, cocaine (nasal), mephedrone, tobacco, alcohol, and cannabis. The regression concerning cortisol levels identified two significant predictors, recent cannabis usage and recent Ecstasy/MDMA usage.

For the objective memory assessments, alcohol consumption was the only significant predictor of immediate recall (4%), and recent Ecstasy/MDMA and mephedrone consumption significantly predicted delayed recall performance (9%). For prospective memory, there were three significant predictors, recent Ecstasy/MDMA, tobacco, and alcohol (Table 3). For self-rated retrospective memory, there were three significant predictors, recent tobacco, recent Ecstasy/MDMA, and recent alcohol (Table 3). Stepwise regression analyses were then repeated with the lifetime usage data - for the same six drugs (Table 5). Cortisol levels were predicted by lifetime cannabis usage, while immediate recall was predicted by lifetime alcohol consumption. Delayed recall was predicted by lifetime Ecstasy/MDMA, while prospective memory was also predicted by lifetime Ecstasy/MDMA. Self-rated retrospective memory was statistically predicted by lifetime Ecstasy/MDMA, and lifetime alcohol (Table 5).

- Tables 3, 4, 5 near here -

Discussion

This study confirmed the adverse effects of recreational Ecstasy/MDMA on objective memory task performance, and self-reported memory deficits. On the Rivermead delayed recall performance test, both groups of recent Ecstasy/MDMA users were significantly impaired, in comparison with non-user controls (Table 2). A similar pattern of deficits was found with the Retrospective and Prospective Memory Questionnaire, where both groups of Ecstasy/MDMA users reported significantly more memory problems, than the control group (Table 2). The contributory role of Ecstasy/MDMA was statistically confirmed in the regression analyses. When recent 3-month drug usage was considered, Ecstasy/MDMA was found to be a significant predictor for delayed word recall, for prospective memory problems, and retrospective memory problems (Table 3). When the lifetime drug usage values were entered into the regression analysis, lifetime Ecstasy/MDMA consumption was a significant predictor for delayed word recall, the prospective memory questionnaire deficits, also the retrospective memory questionnaire deficits (Table 4). The findings

were consistent with the extensive empirical literature on memory deficits in abstinent Ecstasy/MDMA users (Krystal et al, 1992; Bolla et al 1998; Parrott et al, 1998; Parrott and Lasky, 1998; Verkes et al, 2001; Fox et al, 2001, 2002; Laws and Kokkalis, 2007; Rogers et al, 2009; Taurah et al, 2013). However not every study has found deficits (Parrott, 2006, 2013b; Rogers et al, 2009), and this was confirmed here, since there were no group differences in immediate word recall (Table 2). The presence of deficits in delayed recall, but not in immediate recall, suggests that *time* may be important factor – with Ecstasy/MDMA putatively affecting the key process of information storage.

The second aim of the current study was to examine whether hair cortisol levels would be related to neurocognitive performance. As previously reported (Parrott et al, 2014), the only significant group differences in cortisol hair deposits, was found in the recent heavy Ecstasy/MDMA user group. Yet both groups of Ecstasy/MDMA users showed significant memory deficits – recent heavy users, *and recent light* users (Table 2). Hence there was a general dissociation between the neurohormonal and the neurocognitive findings. The independence of the cortisol and cognitive findings, was statistically confirmed in their near zero correlations. Hence for immediate word recall, delayed word recall, retrospective memory, and prospective memory, the 3-month hair cortisol values did not correlate with any memory test score (Table 2). This clearly suggests that the neurocognitive and neurohormonal effects of recreational MDMA are independent. However there may be other potential reasons for the lack of any statistical associations here. Firstly, the cortisol values covered the previous three months, whereas the cognitive deficits would be primarily related to lifetime usage. Furthermore, longer-term Ecstasy/MDMA users tend to use the drug less frequently than novice users (Parrott, 2005), so that the light *current* user subgroup may have contained a number of heavy *lifetime* users. Gerra et al (2003) found that cortisol levels returned to baseline after 12 months of abstinence. Whereas the cognitive performance of ecstasy users tends to remain impaired for a period of time after cessation (Morgan et al, 2002; Taurah et al, 2013).

The regression analyses identified recent cannabis and recent Ecstasy/MDMA usage as significant predictors of cortisol levels ($R^2 = 21\%$), with higher consumption of each drug being related to the increased cortisol deposits

(Table 2). Previous research has shown that acute cannabis consumption (Ranganathan et al., 2009), and acute Ecstasy/MDMA consumption (Kuypers et al., 2013), can increase cortisol secretion. Furthermore the adverse neurocognitive and neuropsychiatric sequelae of chronic cannabis (King et al., 2011), and chronic recreational Ecstasy/MDMA (Parrott, 2009, 2013b), have been suggested to be partially mediated through their effects on cortisol. Heavy recreational use of cannabis is associated with executive function deficits, in attentional impairments and reduced mental flexibility (Lindquist, 2010). In a similar fashion, repeated Ecstasy/MDMA usage may lead to neurocognitive difficulties - in encoding memories for long-term retrieval, verbal learning, and reduced attentional control of higher cognitive processing (Lindquist, 2010). Deficits in this constellation of cognitive components due to MDMA and/or cannabis usage may have negative impacts upon daily functioning, and may be accompanied by alterations in neuroendocrine integrity. Although a significant relationship was not observed between the levels of accumulated cortisol over three months, and Ecstasy/MDMA usage (Table 3. Hence other mechanisms and patterns of licit/illicit drug usage, may be contributing to both the disruptions in memory performance, and the changes in neuroendocrine activity.

In relation to recent drug use, Ecstasy/MDMA consumption was found to be the strongest predictor of variation in delayed recall performance (along with mephedrone consumption), self-rated prospective memory (with tobacco use and alcohol consumption), and the second strongest predictor of self-rated retrospective memory behind tobacco usage (Table 3). Lifetime drug use was also associated with the neurocognitive and neurohormonal changes (Table 5). These findings are consistent with previous findings that have illustrated the metabolic deficiencies in brain areas implicated in working memory and lifetime dosage studies of Ecstasy/MDMA users (Benningfield & Cowan, 2013; Gouzoulis-Mayfrank et al., 2000; Sterk et al., 2007; Verdejo-García, López-Torrecillas, Aguilar De Arcos, & Pérez-García, 2005). Given the participants in this study also consumed alcohol, tobacco, and various illicit drugs in addition to Ecstasy/MDMA, and the drug usage amounts are based upon self-reporting, the results still need to be interpreted with some caution. The regression analyses also only included six of the drug types that were sampled (given the low usage of some drugs, and high collinearity of the usage pattern of the lesser used drugs). Whilst these other drugs may have additive or

synergistic effects upon both the psychological and physiological outcomes utilized in this study, it is beyond the scope of this study to attribute their effects.

This leads into another ubiquitous limitation of these types of cross-sectional design studies, where estimates of retrospective drug use may be considered unreliable. Also we were unable to confirm 'recent' use of the target drug types through biological sampling, therefore again relied upon accurate reporting of drug use, and that the declared drugs were what the participants *believed* they had consumed. With regard to assessment of cortisol, whilst the accumulated deposits of cortisol in the hair over the three month sampling period offer some insight into 'recent' stress, it cannot be causatively linked to on-going memory deficits of the MDMA using groups in this study. Future study designs could consider a more longitudinal approach to assessing changes in memory performance in alongside hair cortisol changes, or consider changes in HPA functionality through daily cortisol profiling, as employed by Wetherell and Montgomery (2014). It is also important to empirically investigate how the different measures of cortisol (saliva, blood, hair) may be interrelated. While future studies should also include subjective indices for self-rated anxiety or stress.

Further research is also needed to examine the additive or synergistic actions of licit and illicit drugs upon multiple mechanisms underlie the cellular adaptations, dysfunction, and neurotoxic or neuroprotective nature of stimulants and other substances of abuse. Use of these substances has been observed to lead to the variable levels of neurocognitive and psychiatric dysfunction (Downey & Loftis, 2014), and it is an important research question to ascertain just how much of certain drugs alone or in combination can be consumed before irreparable damage to the brain is done. It is also likely that a wide range of factors influence synergize with the effects of substances of abuse to impact brain and neuroendocrine function, thus qualifying the molecular changes within the brain and endocrine system with respect to exposure to specifically abused substances like Ecstasy/MDMA, should inform the development of therapeutic approaches to addiction and symptoms of heavy usage.

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Table 1 Recreational drug use over the past 3 months: (group means and standard deviations), from a modified version of the University of East London Recreational Drug Use Questionnaire (Parrott et al, 2001).

	Non-User Controls		Recent Light Users		Recent Heavy Users	
	Number of Users	Mean Times Used (SD)	Number of Users	Mean Times Used (SD)	Number of Users	Mean Times Used (SD)
MDMA	0	0 (0)	31	2.74 (1.13)**	23	7.57 (2.41)**
Amphetamine	1	1 (0)	7	5 (4.97)*	1	3 (0)
Cocaine (Nasal)	2	5.50 (6.36)	12	9.42 (16.57)*	11	1.91 (1.22)
Cocaine (Crack)	0	0 (0)	0	0 (0)	0	0 (0)
LSD	1	1 (0)	1	1 (0)	1	1 (0)
Mephedrone	7	8.43 (6.61)	12	11.58 (16.97)	6	1.5 (0.84)
Opiate	0	0 (0)	0	0 (0)	1	1 (0)
Barbiturate	1	3 (0)	2	1.5 (0.71)	2	3.5 (3.54)
Magic Mushrooms	0	0 (0)	1	1 (0)	1	1 (0)
Steroids	0	0 (0)	0	0 (0)	0	0 (0)
Solvents	0	0 (0)	0	0 (0)	0	0 (0)
Poppers	1	3 (0)	2	1 (0)	4	1 (0)
Ketamine	0	0 (0)	2	3 (2.83)	2	1.5 (0.71)
Tobacco (Cigarettes Daily)	19	7.53 (4.71)	26	6.54 (3.77)*	13	6.62 (4.46)
Alcohol (Units Weekly)	51	14.28 (11.71)	31	20.39 (12.41)*	23	19.70 (9.8)
Cannabis (Uses Monthly)	20	7.30 (10.97)	26	15.19 (11.56)**	18	11.07 (12.11)

** Tukey HSD – Significantly different from control group at $p<0.01$ level
* Tukey HSD – Significantly different from control group at $p<0.05$ level

Table 2 Cortisol levels from three month hair samples, memory recall performance, and self-rated memory problems (means and standard deviations). Correlations between the hair cortisol levels and memory test scores.

	Non User Controls		Recent Light Users		Recent Heavy Users		Cortisol Correlation	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Pearson's** *(r)*	**Sig.** *(p)*
Cortisol (pg/mg)	13.78	6.09	19.37	15.96	55.01	80.13	-	-
Immediate Recall (total correcct)	7.59	3.04	6.03	2.70	7.09	3.63	-0.04	>.05
Delayed Recall (total correct)	6.83	3.49	4.19**	3.28	4.91*	1.98	-0.16	>.05
Prospective Memory (problems)	20.50	5.99	24.32**	4.64	24.30**	3.99	0.01	>.05
Retrospective Memory (problems)	18.26	5.76	21.81**	4.62	22.26**	4.59	0.12	>.05

** Tukey HSD – Significantly different from control group at p<0.01 level
* Tukey HSD – Significantly different from control group at p<0.05 level

Table 3 Stepwise Regression of recent 3-month drug usage upon cortisol levels and objective and subjective memory assessments

	Step	Variable	Beta	t	Adjusted R²
Cortisol	1	Cannabis	0.359	3.81**	0.12
	2	Cannabis	0.306	3.35**	
		MDMA	0.300	3.29**	0.20
Immediate Recall	1	Alcohol	0.223	2.36*	0.04
Delayed Recall	1	MDMA	-0.257	-2.73**	0.06
	2	MDMA	-0.248	-2.69**	
		Mephedrone	-0.213	-2.31*	0.09
Prospective Memory	1	MDMA	0.344	3.78**	0.11
	2	MDMA	0.299	3.34**	
		Tobacco	0.260	2.91**	0.17
	3	MDMA	0.259	2.87**	
		Tobacco	0.255	2.89**	
		Alcohol	0.185	2.08*	0.19
Retrospective Memory	1	Tobacco	0.338	3.69**	0.11
	2	Tobacco	0.291	3.25**	
		MDMA	0.264	2.94**	0.17
	3	Tobacco	0.286	3.24**	
		MDMA	0.223	2.47*	
		Alcohol	0.188	2.11*	0.19

**Significant at the $p<0.01$ level
* Significant at the $p<0.05$ level

Table 4 Lifetime use of illicit drugs (UEL Recreational Drugs Questionnaire, Parrott et al,

	Non-User Controls	Recent Light Ecstasy/MDMA Users	Recent Heavy Ecstasy/MDMA Users
	Mean Times Used (SD)	Mean Times Used (SD)	Mean Times Used (SD)
MDMA	4.41 (12.08)	21.06 (25.19)**	34.74 (26.44)**
Amphetamine	0.37 (1.51)	7.87 (20.19)*	2.87 (5.67)*
Cocaine (Nasal)	7.87 (29.77)	23.55 (44.05)	7.78 (8.82)
Cocaine (Crack)	0.00 (0.00)	0.03 (0.17)	0.00 (0.00)
LSD	0.20 (1.37)	0.26 (0.77)	1.43 (3.68)*
Mephedrone	12.59 (39.29)	28.48 (57.95)	5.22 (5.22)
Opiate	0.09 (0.49)	0.10 (0.40)	0.17 (0.39)
Barbiturate	1.20 (6.92)	1.87 (5.80)	0.65 (2.16)
Magic Mushrooms	0.44 (1.66)	1.26 (1.88)	2.78 (5.44)*
Steroids	0.02 (0.02)	0.00 (0.00	0.00 (0.00)
Solvents	0.46 (2.75)	0.00 (0.00)	0.13 (0.63)
Poppers	2.15 (8.54)	4.87 (18.01)	5.13 (11.03)
Ketamine	2.39 (13.68)	3.00 (7.82)	1.87 (4.57)

2001).

** Tukey HSD – Significantly different from control group at $p<0.01$ level
* Tukey HSD – Significantly different from control group at $p<0.05$ level

Table 5 Stepwise Regression of lifetime drug usage upon cortisol levels and objective and subjective memory assessments

	Step	Variable	Beta	t	Adjusted R²
Cortisol	1	Cannabis	0.292	3.02**	0.12
Immediate Recall	1	Alcohol	0.223	2.36*	0.04
Delayed Recall	1	MDMA	-0.230	-2.44*	0.04
Prospective Memory	1	MDMA	0.398	3.46**	0.15
Retrospective Memory	1	MDMA	0.300	3.24**	0.08
	2	MDMA	0.255	2.72**	
		Alcohol	0.199	2.12*	0.11

**Significant at the p<0.01 level
* Significant at the p<0.05 level