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# Increased cortisol levels in hair of recent Ecstasy/MDMA users

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**Abstract**

Previous research has revealed an acute 8-fold increase in salivary cortisol following self-administrated Ecstasy/MDMA in dance clubbers. It is currently not known to what extent repeated usage impacts upon activity of the hypothalamic-pituitary-adrenal axis over a more prolonged period of time. This study investigated the integrated cortisol levels in 3-month hair samples from recent Ecstasy/MDMA users and non-user controls. One hundred and one unpaid participants (53 males, 48 females; mean age 21.75 years) completed the University of East London recreational drug use questionnaire, modified to cover the past 3-months of usage. They comprised 32 light recent Ecstasy/MDMA users (1-4 times in last 3 months), 23 recent heavy MDMA users (+5 times in last 3 months), and 54 non-user controls. Volunteers provided 3 cm hair samples for cortisol analysis. Hair cortisol levels were observed to be significantly higher in recent heavy MDMA users (mean= 55.0 ± 80.1 pg/mg), compared to recent light MDMA users (19.4 ± 16.0 pg/mg; $p=0.015$), and to non-users (13.8 ± 6.1 pg/mg; $p<0.001$). Hence the regular use of Ecstasy/MDMA was associated with almost 4-fold raised hair cortisol levels, in comparison with non-user controls. The present results are consistent with the bio-energetic stress model for Ecstasy/MDMA, which predicts that repeated stimulant drug use may increase cortisol production acutely, and result in greater deposits of the hormone in hair. These data may also help explain the neurocognitive, psychiatric, and other psychobiological problems of some abstinent users. Future study design and directions for research concerning the psychoneuroendocrinological impact of MDMA are also discussed.

## 1. Introduction

The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) is associated with a wide range of central nervous system effects, particularly through its indirect agonistic actions on the serotonergic and dopaminergic systems (Green et al, 2003). MDMA is most widely known for its euphoriant properties, when it is used as an illicit recreational drug under the street name of 'Ecstasy' (Schifano, 2000; Parrott, 2004a, 2013a). The activating effects of MDMA are not limited to positive moods, since negative moods such as anxiety can also be increased (Kirkpatrick et al, 2012; Parrott et al, 2011). The regular use of Ecstasy/MDMA is associated with a range of neurocognitive and other psychological problems, including deficits in memory, higher executive processing, sleep and psychiatric well-being (Laws and Kokkalis, 2007; McCann et al, 2008; Montgomery et al, 2010; Parrott, 2012a, 2013b; Zakzanis and Campbell, 2006).

MDMA also stimulates the hypothalamic-pituitary-adrenal (HPA) axis, with increased secretion of the glucocorticoid hormone cortisol (Mas et al., 1999; Pacifici et al., 2001; Harris et al., 2002). Following acute doses of MDMA in the laboratory, the increase in cortisol can range from 100% to 150% over baseline, depending on factors such as dosage level (Harris et al., 2002), and repeated administration (Farré et al., 2004). Stronger endocrine changes have been reported in real world studies of recreational drug users. In two independent studies, the acute use of Ecstasy/MDMA in combination with dancing and partying induced an almost 8-fold acute increase in salivary cortisol levels (Parrott et al., 2007 2008). Importantly, no significant increases in cortisol were observed in either study on the control weekends, when participants went partying as usual, but without taking Ecstasy/MDMA. The marked cortisol increase in Ecstasy/MDMA-using party-goers may result from the combination of drug and environmental stimulation, with dance clubs typically involving loud music, crowding, and physical exertion (Parrott, 2004b).

Although the short-term neuroendocrine effects of MDMA are well established, the longer-term effects of repeated drug use on HPA axis activity and cortisol remain unclear. Initial evidence suggests that drug-free Ecstasy user's exhibit altered baseline and stress-responsive cortisol secretion (Gerra et al, 2003). Until recently, the measurement of chronic cortisol levels was limited to blood, saliva, or urine samples,

ideally taken repeatedly at several time points (Evans et al, 2012; Hellhammer et al, 2007). Cortisol in hair provides a methodological advance, since it generates a cumulative index of hormone secretion over several months (Stalder & Kirschbaum, 2012). The underlying theory is that the hormone is incorporated into the growing hair, and that the hair cortisol index may provide a valid (Broderick et al, 2004; Kirschbaum et al., 2009; Thomson et al., 2010; Manenschijn et al., 2011), and reliable (Stalder et al, 2012b) measure of longer-term neurohormonal secretion. In the current study we utilized this novel procedure, to investigate the links between Ecstasy/MDMA use and cortisol secretion over the previous 3 months. Our prediction was that more frequent and repeated Ecstasy/MDMA usage would lead to higher levels of cortisol in the hair.

## 2. Experimental Procedures

### Participant characteristics

One-hundred and one participants (53 males, 48 females, mean ± SD age: 21.75 ± 4.23 years) were 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 (8 male, 15 female, mean ± SD age: 21.48 ± 0.89 years) who had consumed MDMA more than five 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 local ethics committee.

- 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, age, smoking status, medication intake, and oral contraceptive use. In addition, hair-related characteristics, such as hair colour, washes per week, and hair treatments were assessed (Stalder et al, 2012a).

*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).

*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 in 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 (Prof. Clemens Kirschbaum). The intra-assay and inter-assay coefficients of variation of this assay are below 8%.

**Statistical analysis**

The data were analysed by one way Analysis of Variance (ANOVA), followed by Tukey paired comparison tests between drug conditions. Probability levels were two-tailed. Since the cortisol values were not found to be normally distributed and showed unequal group variances, the data were subjected to log-transformation. Statistical analyses were performed using SPSS version 20.

- Figure 1 near here -

## 3. Results

Drug usage for the three groups are reported in Table 1, with the ANOVA group effect for amphetamine usage in the past three months ($F$ (2, 106) = 4.46, $p$ = 0.014), tobacco use per day ($F$ (2, 106) = 4.56, $p$ = 0.013), alcohol per week ($F$ (2, 106) = 3.90, $p$ = 0.023) and cannabis use per month ($F$ (2, 106) = 5.04, $p$ = 0.008) being significant. Tukey paired comparisons revealed that the light recent Ecstasy/MDMA users reported higher amphetamine ($p < 0.05$), tobacco ($p < 0.01$) and cannabis ($p < 0.01$) consumption than the non-user controls who did consume more alcohol weekly ($p = 0.05$) than the light recent Ecstasy/MDMA users.

Cortisol values for all participants in the three subgroups are shown in Figure 1. The group mean hair cortisol values were 13.78 ± 6.09 pg/mg for non-users, 19.37 ± 15.96 pg/mg for light recent Ecstasy/MDMA users, and 55.01 ± 80.13 pg/mg for heavy recent Ecstasy/MDMA users. Due to the unequal variances between groups and outliers within each group, the log transformed data was analysed to satisfy the assumptions, and we also analysed the data using non-parametric ANOVA, and this generated a very similar pattern of findings. The ANOVA group effect for cortisol was significant ($F$ (2, 98) = 11.88, $p < .001$). Tukey paired comparison tests revealed that the cortisol levels of heavy recent Ecstasy/MDMA users, were significantly higher than those of non-user controls ($p < .001$), and higher than light recent users ($p = 0.015$); the difference between the latter two groups was not significant. Given the Ecstasy/MDMA usage data from the past three months was collected as a continuous variable, regression analyses concerning the variation in cortisol values was also conducted with the Ecstasy/MDMA usage data as the predictor. For the recent Ecstsay/MDMA usage data, the regression equation ($F$ [1, 99] = 14.20, $p < .001$; $R^2$ = .13) was observed to predict a significant amount of variation in the hair depositis of cortisol.

## 4. Discussion

Recent Ecstasy/MDMA users who had taken the drug on five or more occasions during the past 3-months, demonstrated an almost 4-fold increase in hair cortisol levels compared to non-user controls, and an almost 3-fold increase compared to light Ecstasy/MDMA users. Lighter recent Ecstasy/MDMA users, who had taken the drug on less than 4 occasions in the past 3-months, showed group mean cortisol levels slightly higher than controls, although the difference was not significant. There was a marked difference in the variances of cortisol values across groups. Non-user controls had the lowest variance, whereas heavy recent Ecstasy/MDMA users showed the highest group variance. This variation in scores is illustrated by the box-plot distributions, where the exponential Y-axis for the cortisol values should be noted (Figure 1). Non-user controls showed a tight clustering of scores at the low end of the scale, with most cortisol values under 15 ug/dl, and the highest single value of 35 ug/dl. In contrast, heavy Ecstasy/MDMA users showed far greater variance, with comparatively few cortisol values under 15ug/dl, and five values over 90 ug/dl. The distribution of scores for the light-recent Ecstasy/MDMA users was in between the other two groups, with the majority of users providing mid-range cortisol values.

Although the current data are cross-sectional and technically no causation can be inferred, it may be assumed that the high chronic cortisol values for the heavier users are a result of the neuroendocrine effects of acute MDMA use. In placebo-controlled laboratory studies, MDMA administration causes a significant cortisol release (Mas et al, 1999; Harris et al, 2002), while repeated dosing leads to further rises in cortisol (Pacifici et al, 2001; Farre et al, 2004; Dumont and Verkes, 2006). In real world studies of recreational users, acute self-dosing with Ecstasy/MDMA can generate an even stronger cortisol release, with peak values around 750-800% higher than baseline (Parrott, et al, 2007, 2008). Gerra et al (2003) reported that baseline cortisol levels were significantly higher in drug-free Ecstasy users than non-user controls, and displayed a heightened cortisol response to a psychosocial stressor. This may suggest that in addition to the increased deposits of cortisol in hair in heavy MDMA users from repeated stimulant drug use may also alter basal and cortisol responses to stressful environments. Interestingly, Scholey et al (2011) have also observed that abstinent Ecstasy/MDMA users reported significantly higher feelings of daily stress

than non-user controls. Wetherell et al (2012) too, observed that drug-free Ecstasy/MDMA users required to perform a laboratory stress task, reported feeling significantly 'less calm' than non-user controls. Whether these subjective mood responses to laboratory induced stressors are causally related to the higher cortisol values found both here (Figure 1), and by Gerra et al (2003) in MDMA users is worthy of future investigation.

Cortisol is important for acute metabolic activation and energy mobilization, especially under conditions of high physical demand (Selye, 1956; Lovallo, 1997). MDMA is a powerful central nervous system stimulant and metabolic activator, and cortisol release may contribute to the stimulatory effects of Ecstasy/MDMA in recreational users (Parrott, 2009). MDMA is commonly consumed at dance clubs and raves (Winstock et al, 2001) where the prolonged periods of dancing and hot-crowded conditions may help to boost the reinforcing effects of MDMA - possibly through greater cortisol release (Harris, et al, 2002; Parrott, 2002, 2009). A potential cause of the neurohormonal variation in our Ecstasy/MDMA users may be individual differences in rates of dancing or sympathetic activation (Parrott et al, 2006). Recreational users also report that the positive effect of MDMA are enhanced or reinstated by heat (Bedi and Redman, 2005; Parrott, 2004, 2012b), and this may be another factor contributing to the variance in neurohormonal response recorded in hair samples. There may also be endogenous differences in HPA or hormonal reactivity to MDMA. All these putative factors need to be empirically studied. In chronic terms, the recreational use of MDMA is associated with many functional problems, including neurocognitive deficits and an increased propensity for psychiatric distress (Brier et al, 2012; Laws and Kokkalis, 2007; McCann et al, 2008; Montgomery et al, 2010; Parrott, 2006, 2012a; Verkes et al, 2001; Zakzanis and Campbell, 2006). The heightened levels of cortisol may contribute to these neuropsychobiological effects (Parrott, 2009); furthermore the variance in neuroendocrine responses may help explain any variation in functional findings with chronic users.

These findings need to be interpreted in light of the various study limitations, some of which are inherent to many observational drug usage studies. Firstly, our study relied upon the self-reporting of recreational drug users to detail their recent usage of MDMA (or not for the non-using drug control group), and their recent usage of

MDMA was not qualified via biological sampling. Concerning the levels of cortisol assessed in the current study, the differences in the levels of hair cortisol are mainly prescribed to the greater consumption of MDMA by the 'heavy usage' group. No assessment of trait or state stress levels was administered within the current study, possibly not accounting for any chronic 'stress induced' effect upon cumulative cortisol levels. Assessment of any differences in levels of intense exercise undertaken by the sample, their body-to-mass ratio, or the relative amount of intense dancing (common for this age group and amongst MDMA users) for example, are other possible contributors to the cumulative cortisol index provided by hair samples that could be considered in future research of this kind. Future research could also consider the stability of group assignment, given 'heavier' or 'lighter' users assignment in groups may be effected by the timeframe or the study and whether the heavier users for example were consuming MDMA closer to the hair sampling within the study.

Another potentially important topic for future research is the 'homeostatic integrity' of abstinent Ecstasy/MDMA users. Herbert et al (2007) noted that cortisol was involved in both predictive and reactive homeostasis. Predictive homeostasis is based around circadian rhythms, with cortisol involved in everyday patterns of sleep, waking and alertness (Halberg, 1963). Yet cortisol is a labile and reactive hormone, and its release is also stimulated by physical and psychological stressors. Gerra et al's (2000, 2003) earlier cortisol findings are consistent with deficits in reactive homeostasis. This could help explain why abstinent Ecstasy/MDMA users reported feeling more stressed and less calm, than non-user controls (Scholey et al, 2011; Wetherell et al, 2012). Future research needs to investigate predictive and reactive homeostasis within a unified design. The core hypothesis for future empirical investigation is that both aspects of homeostasis may be adversely affected in recreational Ecstasy/MDMA users.

**References**

Bedi G, Redman J (2006). Recreational ecstasy use: acute effects potentiated by ambient conditions? Neuropsychobiology 53: 113.

Brière FN, Fallu JS, Janosz M, Pagani LS (2012). Prospective associations between meth/amphetamine (speed) and MDMA (ecstasy) use and depressive symptoms in secondary school students. J Epidemiol Community Health. 66: 990-994.

Broderick, J E, Arnold, D, Kudielka, B M, & Kirschbaum, C (2004). Salivary cortisol sampling compliance: comparison of patients and healthy volunteers. Psychoneuroendocrinology 29: 636-650.

Dumont GJ, Verkes RJ (2006). A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers. J Psychopharmacol 20: 176-187.

Evans, P.D., Hucklebridge, F., Loveday, C., Clow, A., 2012. The cortisol awakening response is related to executive function in older age. Int. J. Psychophysiol 84, 201-204.

Farré M, de la Torre R, Mathúna BO, Roset PN, Peiró AM, Torrens M, Ortuño J, Pujadas M, Camí J. (2004). Repeated doses administration of MDMA in humans: pharmacological effects and pharmacokinetics. Psychopharmacology 173: 364-375.

Fox HC, McLean A, Turner, JJD, Parrott AC, Rogers R, Sahakian BJ (2002) Neuropsychological evidence of a relatively selective profile of temporal dysfunction in drug-free MDMA (ecstasy) polydrug users. Psychopharmacology 162, 203-214.

Gerra G, Zaimovic A, Ferri M, Zambelli U, Timpano M, Neri E, Marzocchi GF, Delsignore R, Brambilla F (2000). Long-lasting effects of (+/-)3,4-methylenedioxymethamphetamine (ecstasy) on serotonin system function in humans. Biol Psychiat 47: 127-136.

Gerra G, Bassignana S, Zaimovic A, Moi G, Bussandri M, Caccavari C, Brambilla F (2003). Hypothalamic–pituitary–adrenal axis responses to stress in subjects with 3,4-methylenedioxy-methamphetamine ('ecstasy') use history: correlation with dopamine receptor sensitivity. Psychiatry Res 120: 115–124.

Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol Revs 55: 463-508.

Halberg F (1963). Circadian (about twenty-four-hour) rhythms in experimental medicine. Proc R Soc Med 56: 253-257.

Harris DS, Baggott M, Mendelson JH, Mendelson JE, Jones RT (2002). Subjective and hormonal effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. Psychopharmacology 162: 396-405.

Hellhammer, J, Fries, E, Schweisthal, O W, Schlotz, W, Stone, A A, & Hagemann, D (2007). Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. Psychoneuroendocrinology 32: 80-86.

Herbert J, Goodyer IM, Grossman AB, Hastings MH, de Kloet ER, Lightman SL, Lupien SJ, Roozendaal B, Seckl JR (2007). Do corticosteroids damage the brain? J Neuroendocrinol 18: 393-411.

Kirkpatrick MG, Gunderson EW, Perez AY, Haney M, Foltin RW, Hart CL (2012). A direct comparison of the behavioral and physiological effects of methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) in humans. Psychopharmacology 219: 109-122.

Kirschbaum, C, Tietze, A, Skoluda, N, & Dettenborn, L (2009). Hair as a retrospective calendar of cortisol production--Increased cortisol incorporation into hair in the third trimester of pregnancy. Psychoneuroendocrinology 34: 32-37.

Laws KR, Kokkalis J (2007) Ecstasy (MDMA) and memory function: a meta-analytic update. Hum Psychopharmacol. 22: 381-388.

Lovallo WR (1997). Stress and health: biological and psychological interactions. Sage, California.

Manenschijn, L, Koper, J W, Lamberts, S W J, & van Rossum, E F C (2011). Evaluation of a method to measure long term cortisol levels. Steroids 76: 1032-1036.

Mas M, Farre M, de la Torre R, Roset PN, Ortuno J, Segura J, Cami J (1999). Cardiovascular and neuroendocrine effects and pharmacokinetics of 3, 4-methylenedioxymethamphetamine in humans. J Pharmacol Exp Ther 290: 136-145.

Mc Cann UD, Szabo Z, Vranesic M, Palermo M, Mathews WB, Ravert HT, Dannals RF, Ricaurte GA (2008). Positron emission tomographic studies of brain dopamine and serotonin transporters in abstinent (+/-)3,4-methylenedioxymethamphetamine ("ecstasy") users: relationship to cognitive performance. Psychopharmacology 200: 439-450.

Montgomery C, Hatton NP, Fisk JE, Ogden RS, Jansari A (2010). Assessing the functional significance of ecstasy-related memory deficits using a virtual reality paradigm. Hum Psychopharmacol 25: 318-325.

Pacifici R, Zuccaro P, Farre M, Pichini S, Di Carlo S, Roset PN, Ortuno J, Pujadas M, Bacosi A, Menoyo E, Segura J, de la Torre R (2001). Effects of repeated doses of MDMA ("ecstasy") on cell-mediated immune response in humans. Life Sci 69: 2931-2941.

Parrott AC (2002). Recreational Ecstasy/MDMA, the serotonin syndrome, and serotonergic neurotoxicity. Pharmacol Biochem & Behav 71: 837-844.

Parrott AC (2004a). Is Ecstasy MDMA ? A review of the proportion of ecstasy tablets containing MDMA, dosage levels, and the changing perceptions of purity. Psychopharmacology 173: 234-241.

Parrott AC (2004b). MDMA (3,4-methylenedioxymethamphetamine) or Ecstasy: the neuropsychobiological implications of taking it at dances and raves. Neuropsychobiology 50: 329-335.

Parrott AC (2006). MDMA in humans: factors which affect the neuropsychobiological profiles of recreational Ecstasy users, the integrative role of bio-energetic stress. J Psychopharmacol 20: 147-163.

Parrott AC (2009). Cortisol and MDMA: neurohormonal aspects of bioenergetic stress in Ecstasy users. Neuropsychobiology 60: 148-158.

Parrott AC (2012a). MDMA and 5-HT neurotoxicity: the empirical evidence for its adverse effects in humans - no need for translation. Brit Jour Pharmacol. 166: 1518-20.

Parrott AC (2012b). MDMA and temperature: a review of the thermal effects of 'Ecstasy' in humans. Drug and Alcohol Dependence 121: 1-9.

Parrott AC (2013a). Human psychobiology of MDMA or 'Ecstasy': an overview of 25 years of empirical research. Human Psychopharmacology 28: 289-307.

Parrott AC (2013b). MDMA, serotonergic neurotoxicity, and the diverse functional deficits of recreational 'Ecstasy' users. Neuroscience and Biobehavioral Reviews 37: 1466-1484.

Parrott AC, Milani R, Parmar R, Turner JJD (2001). Ecstasy polydrug users and other recreational drug users in Britain and Italy: psychiatric symptoms and psychobiological problems. Psychopharmacology 159: 77-82.

Parrott AC, Adnum L, Evans A, Kissling C, Thome J (2007). Heavy Ecstasy/MDMA use at cool house parties: substantial cortisol release and increased body temperature. J Psychopharmacol 21: a35.

Parrott AC, Lock J, Conner AC, Kissling C, Thome J (2008). Dance clubbing on MDMA and during abstinence from Ecstasy/MDMA: prospective neuroendocrine and psychobiological changes. Neuropsychobiology 198:

Parrott AC, Gibbs A, Scholey AB, King R, Owens K, Swann P, Ogden E, Stough C (2011). MDMA and methamphetamine: some paradoxical negative and positive mood changes in an acute dose laboratory study. Psychopharmacology 215: 527-536.

Ricaurte GA, Yuan J, McCann UD (2000). (+-) 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") - induced serotonin neurotoxicity: studies in animals. Neuropsychobiology 42: 5-10.

Schifano F (2000). Potential human neurotoxicity of MDMA ('Ecstasy'): subjective self-reports, evidence form an Italian drug addiction centre and clinical case studies. Neuropsychobiology 42: 25-33.

Scholey AB, Owen L, Gates J, Rodgers J, Buchanan T, Ling J, Heffernan T, Swan P, Stough C, Parrott AC (2011). Hair MDMA samples are consistent with reported Ecstasy use: findings from an internet study investigating effects of Ecstasy on mood and memory. Neuropsychobiology 63: 15-21.

Selye H (1956). The Stress of Life. McGraw Hill, New York.

Stalder, T, Hucklebridge, F, Evans, P, Clow, A (2009) Use of a single case study design to examine state variation in the cortisol awakening response: Relationship with time of awakening. Psychoneuroendocrinology 34: 607-614.

Stalder T, Kirschbaum C (2012): Analysis of cortisol in hair - State of the art and future directions. Brain Behav Immun 26: 1019-1029.

Stalder, T, Steudte, S, Alexander, N, Miller, R, Dettenborn, L, Kirschbaum, C, (2012a). Cortisol in hair, body mass index and stress-related measures. Biol Psychol, 90: 218-223.

Stalder, T, Steudte, S, Miller, R, Skoluda, N, Dettenborn, L, Kirschbaum, C. (2012b). Intraindividual stability in hair cortisol concentrations. Psychoneuroendocrinology 37: 602-610.

Thomson, S, Koren, G, Fraser, L A, Rieder, M, Friedman, T C, & Van Uum, S H, (2010). Hair Analysis Provides a Historical Record of Cortisol Levels in Cushing's Syndrome. Exp Clin Endocrinol Diabetes 118: 133-138.

Verkes RJ, Gigsman HJ, Pieters MSM, Schoemaker RC, de Visser S, Kuijpers M (2001). Cognitive performance and serotonergic function in users of Ecstasy. Psychopharmacology 53: 196-202.

Wennig R (2000): Potential problems with the interpretation of hair analysis results. Forensic Sci Int 107: 5-12.

Wetherell MA, Atherton K, Grainger J, Brosnan R, Scholey AB (2012). The effects of multitasking on psychological stress reactivity in recreational users of cannabis and MDMA. Hum Psychopharmacol 27: 167-76.

Winstock AR, Griffiths P, Stewart D (2001). Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. Drug Alc Depend 64: 9-17.

Zakzanis KK, Campbell Z (2006). Memory impairment in now abstinent MDMA users and continued users: a longitudinal follow-up. Neurology 66: 740-741.

**Figure legend**

**Figure 1.** Cortisol levels from 3-month hair samples (pg/ml), for recent light recent users of Ecstasy/MDMA (1-4 occasions in last 3 months), recent heavy users of Ecstasy/MDMA (+5 occasions in last 3 months), and polydrug controls. Each box-plot shows the 10, 25, 50, 75 and 90 percentiles.

**Conflict of interest:** The authors declare no conflicts of interest.

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**Individual Contributions:** Authors Parrott, Sands, Jones, Clow, Evans designed the study and wrote the protocol. Authors Sands and Downey completed the data analysis. All authors managed the literature searches and prepared the manuscript for publication. All authors contributed to and have approved the final manuscript.

**Table 1. Recent 3-month drug consumption: self-reported by recent light MDMA users, recent heavy MDMA users, and non-user controls (modified University of East London Recreational Drug Usage Questionnaire; Parrott et al, 2001).**

| | Non-user Controls | | Recent Light Users | | Recent Heavy Users | |
|---|---|---|---|---|---|---|
| | Number of Users | Times Used (S.D) | Number of Users | Times Used (S.D.) | Number of Users | Times Used (S.D.) |
| MDMA | 0 | 0 (0) | 32 | 2.7 (1.1) | 23 | 7.5 (2.4) |
| Amphetamine | 1 | 1 (0) | 7 | 5 (4.97) | 1 | 3 (0) |
| Cocaine (Nasal) | 2 | 5.5 (66.3) | 12 | 9.4 (16.5) | 11 | 1.9 (1.2) |
| Cocaine (Crack) | 0 | 0 (0) | 0 | 0 (0) | 0 | 0 (0) |
| LSD | 1 | 1 (0) | 1 | 1 (0) | 1 | 1 (0) |
| Mephedrone | 7 | 8.4 (6.6) | 12 | 11.6 (16.9) | 6 | 1.5 (.8) |
| Opiate | 0 | 0 (0) | 1 | 90 (0) | 1 | 1 (0) |
| Barbiturate | 2 | 1.5 (2.1) | 2 | 1.5 (0.7) | 2 | 3.5 (3.5) |
| 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 | 1 (0) | 2 | 1(0) | 4 | 1(0) |
| Ketamine | 0 | 0 (0) | 2 | 3 (2.8) | 2 | 1.5 (0.7) |
| Tobacco/day | 19 | 7.5 (4.7) | 27 | 6.7 (3.8) | 13 | 6.6 (4.4) |
| Alcohol/week | 54 | 13.4 (11.8) | 32 | 19.7 (12.6) | 23 | 19.7 (9.) |
| Cannabis/month | 20 | 19.6 (11.2) | 27 | 18.5 (10.5) | 18 | 14.1 (12.3) |

**Figure(s)**