Basal functioning of the hypothalamic-pituitary-adrenal (HPA) axis and psychological distress in recreational ecstasy polydrug users.

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RUNNING HEAD: BASAL HPA FUNCTION IN ECSTASY USERS

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Rationale: Ecstasy (MDMA) is a psychostimulant drug which is increasingly associated with psychobiological dysfunction. While some recent studies suggest acute changes in neuroendocrine function, less is known about long term changes in HPA functionality in recreational users.
Objectives: The current study is the first to explore the effects of ecstasy-polydrug use on psychological distress and basal functioning of the HPA axis through assessing the secretion of cortisol across the diurnal period.
Method: Seventy-six participants (21 nonusers, 29 light ecstasy-polydrug users 26 heavy ecstasy-polydrug users) completed a substance use inventory and measures of psychological distress at baseline, then two consecutive days of cortisol sampling (on awakening, 30 minutes post awakening, between 1400-1600hrs and pre bed time). On day two, participants also attended the laboratory to complete a 20-minute multitasking stressor.
Results: Both user groups exhibited significantly greater levels of anxiety and depression than nonusers. On day one, all participants exhibited a typical cortisol profile, though light users had significantly elevated levels pre-bed. On day two, heavy users demonstrated elevated levels upon awakening and all ecstasy-polydrug users demonstrated elevated pre-bed levels compared to non-users. Significant between group differences were also observed in afternoon cortisol levels and in overall cortisol secretion across the day.
Conclusions: The increases in anxiety and depression are in line with previous observations in recreational ecstasy-polydrug users. Dysregulated diurnal cortisol may be indicative of inappropriate

anticipation of forthcoming demands and hypersecretion may lead to the increased psychological and physical morbidity associated with heavy recreational use of ecstasy.

KEYWORDS:

MDMA, ecstasy, cortisol, HPA axis, Cortisol Awakening Response

INTRODUCTION

Ecstasy, the common street name for 3,4- Methylenedioxymethamphetamine (MDMA), is an illicit recreational drug. It is estimated (Department of Health, 2010) that 8.7% of the adult population (aged 16 to 59 years) of the United Kingdom have used ecstasy in their lifetime, however, incidence increases to 14.1% in 16 to 34-year-olds. These figures are broadly in line with the USA where lifetime prevalence is estimated at 16% in 18-22 year olds (NIH National Institute on Drug Abuse, 2012). Although acute ecstasy use has been associated with negative mood effects in laboratory conditions (e.g., Parrott et al., 2011), when taken in more representative settings, e.g., house parties and clubs, ecstasy use is typically associated with increases in positive mood, and feelings of intimacy and euphoria (Solowij et al., 1992, Verheyden et al., 2003). Beyond the euphoric acute effects, however, recreational use of ecstasy is associated with a range of deleterious effects on neuropsychological and physical wellbeing. The effects on cognition are well documented and detailed in several comprehensive reviews (*cf* Murphy *et al*., 2009; Solowij & Battisti, 2008; Zakzanis *et al*., 2007); however, in brief, although deficits are observed across a range of cognitive domains, the most consistent effects are observed in learning and memory tasks that involve high levels of executive functioning (e.g., Reay et al. 2006). Chronic use is also associated with increases in psychological morbidity. Ecstasy use is predictive of lower levels of self-reported happiness and increases in perceived stress (Scholey et al., 2011) and survey data suggest that approximately one third of ecstasy users report experiencing adverse psychological symptoms including increased levels of aggression, irritability and impatience, greater levels of sadness and depression and reduced alertness (Fisk et al., 2010). Ecstasy users also report greater levels of frustration, mental demand and time pressure when faced with cognitively demanding tasks representative of real world multitasking situations (Wetherell et al., 2012). Finally, ecstasy users demonstrate increased levels of psychological distress (perceived stress, anxiety and depression) immediately upon awakening compared to non-ecstasy using polydrug users (Wetherell et al. 2012).

Ecstasy use is associated with self-reported and objective sleep problems. Approximately 20% of ecstasy users report difficulty with sleeping beyond the period of acute effect (Verheyden et al.,

2003) and laboratory studies have, through polysomnography, demonstrated increased incidence of obstructive sleep disorders in users who had been abstinent for 2 weeks (McCann et al., 2009). Recreational use of ecstasy can also lead to suppression of innate and adaptive immune responses in animals and humans. For example, users demonstrate significant reductions in numbers of natural killer and T-helper cells and reduced lymphocyte proliferation to antigen challenge (Pacifici et al., 2001, 2007) as well as reduced activity of pro-inflammatory cytokines (Connor et al., 2005). Sustained impairments in immunocompetence can lead to increased susceptibility to infection and more rapid progression of existing disease states (Kiecolt-Glaser et al., 2002), thus these ecstasy induced challenges to the immune system can lead to increased health risks in users (Boyle & Connor, 2010). In support, long term ecstasy users report greater incidences of ill-health (Parrott et al., 2002) and are more susceptible to common ailments such as colds (Pacifici et al., 2007). Furthermore, users report being concerned about the effects of use on their physical health (Verheyden et al., 2003).

Many of the ecstasy-related deficits in neuropsychological and physical functioning are also observed in relation to increased neurohormonal activation, in particular, elevated levels of the stress hormone cortisol. In response to a perceived stressor two physiological mechanisms are activated. The first mechanism operates through sympathetic nervous activation and terminates with the release of catecholamines from the adrenal medulla. This represents the fight-flight response to stress and is responsible for enabling resources to deal with the immediate threat. Simultaneously, the release of corticotropin releasing factor (CRF) from the hypothalamus stimulates the release of adrencocorticotropic hormone (ACTH) from the anterior pituitary gland which triggers the release of the glucocorticoid cortisol from the adrenal cortex. This hormonal cascade represents the action of the hypothalamic-pituitary-adrenal (HPA) axis and has both permissive effects that maintain the fight-flight response and a vast array of direct effects that maintain allostasis through the regulation of metabolic, immune and circadian processes (*cf* Lovell & Wetherell, 2011). To these ends, cortisol typically displays a distinctive circadian profile: values peak approximately 30–45 minutes post awakening (the Cortisol Awakening Response, CAR) and, in the absence of significant external stimulation, gradually decline throughout the day (diurnal decline) to reach a trough at around midnight (Saxbe, 2008). Deviations from this typical pattern of diurnal cortisol have been previously associated with a range of psychosocial variables. For example, chronic on-going stress (Scholtz et al., 2004), work overload (Schultz et al 1998) uncontrollable distal stressors (Miller et al., 2007) and informal caregiving stress (Lovell et al., 2011, 2012) have been associated with atypical levels of cortisol during the CAR period. Further, high levels of perceived and accumulated

psychosocial stress are associated with a flattening of the diurnal decline characterised by relatively higher levels of evening cortisol (Abercrombie et al., 2004, Bower et al., 2005). Such aberrations are indicative of allostatic load, that is, the cumulative wear and tear that can occur following over activation of stress mechanisms in response to chronic or repeated stress (McEwen, 2004).

Dysregulation of the HPA axis has, therefore, been implicated as one physiological mechanism through which psychological factors (e.g., chronic stress) can 'get inside the body' and lead to the initiation or exacerbation of disease processes through the process of allostatic load. In support, dysregulated diurnal cortisol profiles have been associated with a range of deleterious health outcomes including increased upper-respiratory infections (Edwards et al., 2003), increased frequencies of minor health complaints (Lovell et al., 2011, 2012) and earlier mortality rates following breast cancer (Bower et al., 2000; Sephton et al., 2000,). Hypersecretion of cortisol across the day has been linked to the metabolic syndrome (Rosmond, 2005), immunologic decline (Elenkov, 2004), the development of mood disorders (Gold et al., 1998) and cognitive dysfunction (Lupien et al., 1998). In contrast, diurnal hyposecretion has been associated with increased risk of the development of autoimmune disorders such as rheumatoid arthritis (Masi & Chrousos, 1996), Sjögren's syndrome (Johnson et al., 1998) and dermatitis (Buske-Kirschbaum et al., 2002).

The bioenergetic stress model of ecstasy (Parrott 2009) posits that extremely high levels of HPA activation occur following consumption of ecstasy, leading to increased levels of cortisol. Indeed, several laboratory studies have demonstrated significant increases in cortisol following administration of MDMA (e.g., Harris et al., 2002; Mas, 1999). A review of laboratory studies (Dumont & Verkes, 2006) indicates that acute administration of MDMA in the laboratory leads to increases of 100-150%, with peak effects occurring approximately 2 hours following administration. However, outside of the laboratory, more dramatic increases in cortisol are observed. In more ecologically valid studies, recreational users demonstrated an 800% increase in levels of cortisol in house parties and clubs, (Parrott et al., 2007; Parrott et al., 2008). Although these levels were not evaluated in the context of a circadian profile, the observed increases represent significant elevation compared to baseline (pre-drug) levels and in comparison to levels during ecstasy abstinence. Peak levels were observed approximately 2.5 to 4 hours post self-administration of drug; however, levels remained elevated by approximately 130% 24 hours post drug (Parrott et al., 2007) and by 70% 48 hours post drug (Parrott et al., 2008). Increases in cortisol have also been observed following ecstasy use at a club with consideration of circadian variations where cortisol was sampled between 17:00 and 23:00 (pre-clubbing) and again between 03:00 and 08:45 (post-clubbing). The authors

suggest that that the diurnal secretion of cortisol may account for this increase, that is, the period of clubbing coincided with the increases in cortisol that occur during this stage of the circadian rhythm. However, the greatest increases in cortisol were observed in those clubbers who were subsequently identified as MDMA-positive through post-clubbing urine samples, providing support for the notion that MDMA leads to hypersecretion of cortisol over and above the activation induced by other environmental stressors such as dancing and heat stress (Parrott, 2006).

Acute ecstasy use could therefore increase HPA activation as evidenced by increases in levels of cortisol; such increases have been observed in laboratory and field conditions. The greater increases in the latter can be attributed to the combination of a stimulant drug, physical activity and other environmental stimuli that are typical in clubs (Parrott, 2006) and are likely to exert a significant challenge to allostasis. Chronic and frequent use of ecstasy is therefore likely to increase allostatic load, leading to dysregulation of the typical cortisol profile and cumulative damage through wear and tear to those physiological systems reliant on regulation of cortisol (Parrott, 2009). In support, Wolff and Aitchison (2013) note that pre-clubbing levels of cortisol in their own study and that of Parrott et al., (2008) were abnormally high and were in fact more typical of levels observed during the post awakening peak. These pre-drug elevations could be attributed to the frequency of clubbing and ecstasy consumption, for example the majority of the sample described themselves as regular clubbers (Wolff et al., 2012) and have taken ecstasy up to 150 times (Parrott et al., 2008), and subsequent ecstasy-induced elevations of cortisol. Further evidence for a potential alteration in basal HPA activity is offered by Gerra et al. (2003) who assessed levels of cortisol immediately before and after an acute laboratory stressor in recreational users of ecstasy and non-drug using controls. The stressor, which comprised aspects of motivated cognitive performance in front of a socially evaluative audience, elicited significant cortisol increases in control participants. However, ecstasy users demonstrated elevated levels of cortisol immediately before the stressor and a subsequent blunted response to stress. Their 'basal' measure is more accurately defined as a pre-stress measure, and owing to an absence of diurnal timings it cannot be classified in terms of the basal profile of cortisol; however, it does provide preliminary evidence of an alteration in HPA functioning in ecstasy users.

The current study is the first to explore the effects of ecstasy-polydrug use on psychological distress and basal functioning of the HPA axis through assessing the secretion of cortisol across the diurnal period. As indices of the diurnal profile are influenced by state factors (Hellhammer et al., 2007), a two day sampling protocol is adopted to provide markers of diurnal secretion across two

consecutive days, the second of which is characterised by an anticipated acute laboratory stressor. In support of previous studies (Wetherell et al., 2012) it is hypothesised that ecstasy-polydrug users will report greater levels of psychological distress and, in line with the bioenergetic stress model for recreational ecstasy-polydrug use, will demonstrate dysregulated HPA function evidenced by hypersecretion of cortisol.

METHOD

Participants

A total of 76 participants were recruited from an undergraduate student population in Liverpool UK via direct contact with undergraduate students and subsequently using the snowball technique (Solowij et al., 1992). The total sample comprised 48 males and 28 females and had a mean age of 21.6 year (s.d. 2.25). Participant information for the derived groups is presented in Table 1. Participants were requested to abstain from ecstasy use in the 7 days prior to baseline assessment and from other drug use for at least 24 h. Abstinence was verbally confirmed prior to the giving of informed consent.

Measures

Demographic and health data, including contraceptive use and menstrual cycle stage were collected using self-report questionnaires. Drug and alcohol use was assessed via a self-report questionnaire (Montgomery et al., 2005). Participants are asked about the frequency and intensity of ecstasy, cannabis, alcohol, cocaine, amphetamine and other drug use, and their responses are used to calculate scores for frequency of use, total lifetime amount used, average weekly amount used, abstinence, length of use and recent use. Psychological distress was assessed using the Hospital Anxiety and Depression Scale (HADS, Zigmond and Snaith, 1983), which comprises 14 items scored along a 4 point scale (0, never, to 3, considerable). Item scores are summed (from 0 to 21) to create total scores for the depression and anxiety subscales where higher scores indicate more frequent depressive symptoms and feelings of anxiety. Information pertaining to the provision of saliva samples, including time of waking and precise timing of samples, as well as self-reports of prior nights' sleep was recorded using paper diaries (Lovell et al., 2011).

Procedure

All procedures were approved by institutional ethics review boards. At baseline participants attended the laboratory, provided written informed consent and completed questionnaires assessing demographic and health factors, drug use and psychological distress. Participants were

then informed that the study would involve testing over two consecutive days: day one would involve the provision of saliva samples in their own homes and day two would involve a testing session in the laboratory. Details of this testing session were provided, specifically that participants were required to attend in the afternoon to complete a battery of tasks designed to be cognitively demanding and stressful and that their performance would be recorded. Details of this protocol are detailed elsewhere. All participants were then given training regarding the appropriate collection and storage of saliva samples including a demonstration of how to provide saliva using salivettes. In addition, the importance of adherence to the collection protocol was emphasised, specifically, the exact timing and recording of samples and abstinence from behaviours known to affect the integrity of cortisol in saliva. That is, in line with previous research (Kudielka et al., 2003), for 1 hour prior to the provision of each saliva sample participants were asked to refrain from consumption of food, caffeinated or alcoholic beverages, nicotine, brushing of teeth, the use of mouthwashes or antacids and exercise. Full written instructions, detailing the collection protocol were also provided and collection and testing days were agreed between participant and researcher.

On two consecutive typical days, participants collected saliva by chewing on a salivette for 1-2 min at four time points: immediately upon awakening, 30 minutes post awakening, between 1400 and 1600 and immediately before bed. On day one all samples were provided in participants' homes. On day two, participants provided their awakening, 30 minutes post awakening and pre-bed samples at home and their afternoon (1400-1600) sample was provided during a testing session in the laboratory. Samples collected in homes were refrigerated by participants until they were returned to the researcher. All samples were then frozen (-20 °c) and subsequently assayed in house using the enzyme-linked immunosorbent assay method (Salimetrics-Europe, Cambridge UK, intra and inter assay coefficients < 10%). To maximise adherence to the saliva collection protocol and as a means of assessing the timing of samples, participants were instructed to record the precise time at which they provided each of their saliva samples using a paper diary. Following the provision of the 30 minutes post awakening sample on both days, participants completed the HADS and paper diaries. The paper diaries and questionnaires were returned to the researcher during the laboratory testing session.

Treatment of data

Drug use

Ecstasy using participants were classified as either light ecstasy-polydrug users (between 1 and 41 tablets) or heavy ecstasy-polydrug users (between 41.01 and 1351 tablets) using a median split of

their estimated total life time amount used. Light (N = 29) and heavy (N = 26) ecstasy-polydrug users were then compared with non-users of ecstasy (N = 21) in all analyses.

Cortisol sampling and adherence

Diurnal cortisol levels were analysed in two ways to provide differing indices of HPA activity. First the four individual sampling points (awakening, awakening + 30 min, afternoon and pre-bed) across both sampling days were compared between the groups. To normalise distributions, raw cortisol values were log10 transformed (raw data are shown in descriptive statistics, tables and figures). Second, total cortisol, secretion was assessed using area under the curve with respect to ground (AUC_G). AUC_G was calculated for each participant on each sampling day using the cortisol level (nmol/l) at each sampling point and the time (minutes) between each sample (Pruessner et al., 2003). As poor adherence with saliva sampling protocols can affect the accuracy of derived HPA indices, non-adherent participants were excluded from analyses. Specifically, individuals reporting delays of greater than 10 min following the scheduled sampling time of the 30 min post awakening sample were excluded from analyses on that day.

Analyses

One way ANOVAs and independent t-tests were utilised to assess potential between group differences in demographic variables and indices of other drug use. Mixed ANOVAs with group (non-user, light user, heavy user) as the between groups factor and day (1, 2, 3) as the within groups factor were used to analyse distress upon awakening. To maximise the number of participants included in analyses, indices of diurnal cortisol secretion were analysed using a series of one way (non-user, light user, heavy user) ANOVAs with casewise exclusion (individual samples sizes are shown in Tables 2 and 3). Violated assumptions were corrected as required and Bonferroni corrected post-hoc analyses were conducted as appropriate. An alpha of 5% was used for all statistical analyses and F values, degrees of freedom (adjusted for violation of sphericity as necessary) and *p* values are reported for all analyses.

RESULTS

Demographics and other Drug use

The groups were of a similar mean age, ($F_{(2,73)} = 1.84$, $p > 0.05$), contained similar numbers of males and females ($\chi^2_{(2)} = 1.53$, $p > 0.05$) and did not differ significantly in their self-reported health ($F_{(2,73)} = 2.06$, $p > 0.05$) or number of years spent in education ($F_{(2,73)} = 2.14$, $p > .05$). Alcohol consumption

in the 10 days prior to testing and lifetime use of cannabis were greater in ecstasy-polydrug users compared with non-users; however, these differences were non significant ($F_{(2,59)} = 1.73$, $p > .05$, $F_{(2,52)} = 0.10$, $p > 0.05$). Cocaine use was reported in ecstasy-polydrug users only; however, levels of use did not differ significantly between light and heavy ecstasy-polydrug users ($t_{(16)} = 0.74$, $p > 0.05$). In line with the classification of lifetime ecstasy use, heavy ecstasy-polydrug users had a significantly greater lifetime use ($F_{(1,53)} = 15.85$, $p < 0.001$), had used ecstasy for a longer period of time ($F_{(1,53)} = 11.89$, $p < .001$) and had a greater average nightly use than the light users ($F_{(1,53)} = 25.81$, $p < .001$); however, light and heavy users did not differ significantly in terms of frequency of use ($F_{(1,33)} = 0.80$, $p > 0.05$). Demographics and other drug use indices are presented in Table 1.

Distress upon awakening

Levels of anxiety and depression did not differ across testing days (Anxiety: $F_{(2,146)} = 0.10$, $p > 0.05$, Depression: $F_{(1.47, 106.13)} = 1.95$, $p > 0.05$) and there were no significant day x group interactions (Anxiety: $F_{(4,146)} = 0.42$, $p > 0.05$, Depression: $F_{(2.95, 106.13)} = 0.28$, $p > 0.05$). There were, however, significant between group differences in anxiety ($F_{(2,73)} = 5.37$, $p = 0.007$) and depression ($F_{(2,72)} = 4.48$, $p = 0.015$). Post Hoc analyses revealed that light and heavy ecstasy-polydrug users had significantly greater levels of anxiety and depression compared with non-users ($p < 0.05$ in both cases), but there were no significant differences between user groups. Levels of anxiety and depression upon awakening in non-users, light and heavy users across sampling days are presented in Table 2.

Diurnal cortisol

On day one, all groups showed typical patterns of diurnal secretion, characterised by an increase in the 30 minutes following awakening and a decline throughout the day. Significant between group differences were, however, observed in the pre-bed sample ($F_{(2,55)} = 5.14$, $p = 0.009$) characterised by significantly greater levels of cortisol in light users compared to non-users ($p < 0.05$). In terms of total cortisol secretion, there were no significant differences between the groups ($F_{(2,51)} = 0.36$, $p > 0.05$). On day two ecstasy-polydrug users demonstrated atypical patterns of cortisol secretion compared to non-users. Heavy users demonstrated elevated levels upon awakening and a decline in the 30 minutes following awakening and all ecstasy-polydrug users demonstrated elevated pre-bed compared to non-users. Significant between group differences were observed in the afternoon sample ($F_{(2,58)} = 4.45$, $p = 0.02$) characterised by significantly greater levels of cortisol in heavy users compared to light users ($p = 0.02$) and a trend towards elevated levels compared to non-users ($p = 0.09$). The atypical profile in heavy users also contributed to a significant between group difference

in terms of total secretion of cortisol across the day ($F_{(2,49)} = 3.54$, $p = 0.04$) characterised by greater secretion in heavy versus light users ($p = 0.04$). Table 3 presents the cortisol indices for non-users, light and heavy users and Figure 1 demonstrates diurnal cortisol profiles in all groups across the two sampling days and the increases in the total secretion of cortisol (AUC_G) in heavy users.

DISCUSSION

This study assessed psychological distress and indices of basal HPA function in recreational heavy and light ecstasy-polydrug users compared with non-ecstasy polydrug users. This represents the first attempt at measuring the diurnal secretion of cortisol in current, but not on-drug users of ecstasy. In line with predictions, ecstasy-polydrug users reported significantly greater levels of distress upon awakening and demonstrated elevated levels of cortisol compared to non-ecstasy polydrug user controls.

The observed increases in psychological distress support previous studies reporting increased anxiety, depression and stress in ecstasy users (McCardle et al., 2004; Rodgers et al., 2006; Scholey et al., 2011). More specifically, these findings replicate earlier reports of elevated levels of anxiety and depression in the period following awakening (Wetherell et al., 2012). Furthermore, at all assessment points, the levels of anxiety and depression observed in ecstasy-polydrug users exceed the criteria for probable presence of mood disorder (Snaith, 2003). There were, however, no significant differences between light and heavy users with regard to either anxiety or depression and there were no changes across the three days of assessment. The absence of change across time is not surprising and likely reflects the fact that the scale assesses generalised anxiety and depression, not state like changes in mood. The consistency in scores across the assessment period provides further evidence that ecstasy-polydrug users experience increased psychiatric symptomatology (Fisk et al., 2009). The similarity in levels of anxiety and depression between heavy and light ecstasy-polydrug users may be somewhat surprising given the significant differences between the groups in terms of estimated lifetime ecstasy use. Heavy users had also used ecstasy for a longer period of time and had greater average nightly use compared with light users; however, the two groups were indistinguishable on the basis of frequency of use. The frequency of ecstasy use, rather than length or quantity of use per night may, therefore, be the more important contributor to psychological morbidity.

In line with predictions, ecstasy-polydrug use was also associated with dysregulated patterns of cortisol secretion. Although all groups demonstrated typical diurnal profiles on day one, characterised by an increase following waking and a decline throughout the day, light users demonstrated elevated levels of cortisol before bed. On day two, typical profiles were evident in light and non-users; however, heavy users demonstrated diurnal hypersecretion and a dysregulated profile, most notably characterised by elevated levels of cortisol upon waking and an absence of the cortisol awakening response. HPA dysregulation was most notable, however, on day two of the protocol. Given that day two is characterised by a visit to the laboratory to participate in a cognitively demanding stress protocol, this elevation could be attributed to anticipation of forthcoming demands. It has been suggested that the cortisol awakening response plays an important role in anticipating the day ahead and the inability to mount an appropriate response following waking reduces the ability to deal sufficiently with forthcoming demands (Fries et al., 2009). In support, heavy users reported the greatest levels of anxiety on the morning of the anticipated stressor demonstrating an inappropriate level of anxiety in relation to forthcoming events. Furthermore, cortisol was elevated in the afternoon sample which was obtained in the laboratory immediately prior to an acutely stressful cognitive challenge. This finding is analogous to previous observations of elevated cortisol immediately prior to a laboratory stressor (Gerra et al., 2003) and suggests that users may have a greater negative perception of the forthcoming challenge (Gerra et al., 1998). Although activation of cortisol in anticipation of and in response to stress is adaptive, allowing the host to mobilise the resources necessary to deal with threat, excessive or inappropriate responding increases allostatic load (McEwen 1998) and the risk of deleterious consequences for ill-health.

The current study also incorporated additional measures of cortisol and is, therefore, able to assess the impact of recreational use of ecstasy upon indices of basal HPA axis function, for example the CAR and diurnal output. Dysregulation of the CAR is associated with both chronic on-going (Scholtz et al., 2004) and uncontrollable distal (Miller et al., 2007) stressors which would manifest in the increased levels of psychological distress observed in the current sample of recreational users. Elevated levels of cortisol upon awakening have also been observed in a range of clinical populations, for example, in adolescents with an anxious attachment style (Oskis et al., 2009); patients undergoing psychotherapy for depression (Huber et al., 2006); and men experiencing their first episode of psychosis (Pruessner et al., 2013). Similar elevation is also associated with neuropsychological and cognitive deficits; for example, in patients with mild cognitive impairment and global amnesia (Arsenault-Lapierre et al., 2010, Wolf et al., 2005), in individuals with

hippocampal damage (Buchanan et al., 2004) and is a feature of individuals characterised as being in ill-health (Kudielka & Kirschbaum, 2003). Similarly, daytime hypersecretion of cortisol is observed in individuals experiencing high levels of subjective distress and traumatic and / or uncontrollable chronic stressors (e.g., Miller et al., 2007) and is associated with a range of deleterious consequences for health and wellbeing (e.g., Rosmond, 2005; Elenkov, 2004; Gold et al., 1998; Lupien et al., 1998). The consequences of HPA dysregulation, in particular awakening and diurnal hypersecretion are analogous to the range of issues observed in chronic recreational use of ecstasy, suggesting that it may be the effects of ecstasy on the HPA axis and the subsequent effects of excessive cortisol exposure, rather than the direct effects of ecstasy that are responsible for the deleterious health consequences observed in users.

The current findings should, however, be evaluated in the context of a number of limitations. In order to reduce participant burden and increase the opportunity for adherence to the saliva sampling protocol, a minimal number of samples were obtained to provide meaningful indices of the CAR and diurnal secretion. Although this protocol is in line with recommendations (e.g., Hellhammer et al., 2007), a greater number of samples during the post-awakening period (e.g., additional samples at 15, 45 and 60 minutes) and throughout the day would, therefore, provide more robust indices of the CAR (Stalder et al., 2009) and diurnal secretion (Pruessner et al., 2003). Future studies may, therefore, attempt to utilise more intensive sampling protocols; however, this should not be at the expense of recruitment or retention of adherent participants. Steps were taken to minimise the impact of non-adherence to the saliva sampling protocol upon the measurement of HPA indices, and individuals who reported non-adherence to the protocol were excluded from analyses. Furthermore, there were no differences in waking times between the user and non-user groups. Additional techniques may have provided more objective indicators of sleep-wake periods, e.g., polysomnography or actigraphy or sample timing, e.g., medication event monitoring (MEM) caps (Kudielka et al., 2003). Research has, however, demonstrated self-reported adherence to be as reliable as electronic measures (Okun et al., 2010). Furthermore, in line with recommendations (Okun et al., 2010; Saxbe 2008), participants were given training in the provision of saliva, were provided with clear saliva collection instructions and the importance of sample timing was emphasised, all of which encourage protocol adherence (Kraemer et al., 2006). As with many studies in drug users the design was quasi-experimental and consequently it is possible that the groups differed on some variable other than ecstasy use. We have, therefore, excluded some of these possible confounds (e.g. alcohol use, years of education, health). There were differences in the mean levels of cannabis and cocaine used in the ecstasy using groups; however, these differences

were non-significant. Nonetheless, any observed differences could still be attributed to the use of these other drugs, or indeed a synergistic effect of concomitant use of other drugs. It has also been suggested that possible differences in lifestyle, particularly sleep quality, may mediate any ecstasy related differences in function (Cole et al., 2002). Although deficits in cognitive function have been observed in studies that have controlled for differences in sleep (Fisk & Montgomery, 2009; Montgomery et al., 2007; Montgomery et al., 2010), objective and subjective markers of sleep quality have been associated with HPA function; however, these effects differ across sleep (e.g., objective vs. subjective) and HPA indices (*cf* Elder, Wetherell, Barclay & Ellis, 2013). The effects of ecstasy use on sleep may provide one mechanism through which ecstasy use alters cortisol secretion. Future research should, therefore, seek to investigate the effects of sleep disruption and other lifestyle factors on ecstasy related changes in HPA axis function and mood. In summary, while we have attempted to control for a number of extraneous variables, we cannot rule out premorbid conditions that predate drug use (Verheul, 2001). In addition, we cannot guarantee the purity of ecstasy tablets used in the present study. However, in a recent review of the literature, Parrott (2004) reports that purity of tablets was approaching 100% MDMA in tablets that were seized from amnesty bins in UK nightclubs. Due to limited resources we were unable to provide an objective measure of recent drug use (e.g. from hair or urine samples). This is not unusual in studies of this type and many published ecstasy use studies rely on self-report and do not use objective measures (e.g. Fox et al, 2002; Montgomery et al. 2005; Rodgers, 2000). In support, a recent study has demonstrated that self-reported use of ecstasy is consistent with the levels of MDMA obtained from hair samples (Scholey et al., 2011). All participants reported being drug free for at least 7 days (median abstinence period for ecstasy, cannabis and cocaine was 28, 4, and 8 weeks respectively for light users and 4, 2, and 10 weeks for heavy users), and we have no reason to believe this information to be false (participants were not informed that they would be excluded prior to testing). In addition, recent research (Roberts et al., 2013a; Roberts et al., 2013b) has found very low levels of metabolites in the urine of ecstasy-polydrug and non-ecstasy-polydrug users suggesting adherence to abstinence requests. In addition, exclusion of those with low level metabolites did not change the significant findings so we have no reason to believe sub-acute intoxication would affect the results. Despite these possible limitations, the use of a naturalistic sample is more representative of the target population (Carson et al., 2012) and, therefore, these findings are applicable to recreational drug users in the community.

Notwithstanding these limitations, in line with predictions recreational ecstasy-polydrug users demonstrated disturbed psychological and biological functioning in relation to non-ecstasy using

polydrug users. Specifically, ecstasy-polydrug users demonstrated increased psychological distress upon waking and elevated levels of cortisol, particularly on the day of an anticipated stressor. This is the first study to demonstrate diurnal hypersecretion of cortisol in recreational ecstasy-polydrug users and supports evidence that the deleterious effects of frequent and heavy recreational ecstasy-polydrug use may be related to dysregulation of the HPA axis. Moreover, the current findings suggest a more complex link between recreational use of ecstasy-polydrug and HPA function incorporating anticipation of and recovery from perceived stressful events.

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