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Pharmacogenetic studies of change in cortisol on ecstasy (MDMA) consumption

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Abstract

In this study we investigate the association of cytochrome P450 enzyme CYP2D6, catechol-*O*-methyl transferase (COMT, Val158Met) and serotonin transporter promoter (*5-HTTLPR*) genotypes on change in cortisol concentration following 3, 4-methylenedioxy-methamphetamine (MDMA, 'ecstasy') consumption. Forty-eight subjects (30 males, mean age 23 years), self-nominating regular clubbers provided 'in the field' pre- and post-clubbing biological samples and associated information. Of the 39 subjects who provided a post-clubbing urine sample, 21 were positive for MDMA. Plasma cortisol concentrations increased in subjects ($n = 48$) tested for cortisol, with changes being significantly greater in the MDMA-positive group (736.9 ± 83.2 vs. 350.9 ± 34.5 mmol/l, $p = 0.001$). We found a positive association between the low activity *COMT* genotype (Met/Met) and MDMA-induced change in cortisol and also between this and change in cortisol in the whole sample ($p = 0.039$, Bonferroni corrected). For CYP2D6, there was an association between genotype and change in cortisol, confined to subjects with MDMA-positive urine post-clubbing ($p = 0.003$, Bonferroni corrected). There was no association with *5-HTTLPR* genotype. These associations suggest that chronic use of MDMA may lead to HPA axis dysregulation and that the magnitude of this may be moderated by genetic polymorphism, and warrant further investigation in a larger sample of those who consume the drug on a regular basis.

Keywords

Catechol *O*-methyltransferase (COMT), cortisol, CYP2D6, ecstasy, genetic, glucocorticoid, N-methyl-3, 4-methylenedioxyamphetamine (MDMA), polymorphism, recreational drugs, serotonin transporter

Introduction

'Ecstasy' is the common name for 3,4-methylenedioxymethamphetamine (MDMA), a synthetic amphetamine analogue. Generally available in tablet form, with differing appearance (or 'logos'), there is no guarantee of content, although the majority of tablets sold as 'ecstasy' are thought to contain moderate doses (75–125 mg) of MDMA (Wolff et al., 1995). Use is widespread amongst clubbers whose numbers are high (approximately 14.8 million reported to have visited at least one nightclub) in the UK (Mintel Market Research, 2004). According to the British Crime Survey 2004/2005, over half a million individuals had taken ecstasy in the previous year (Roe, 2005), typically consuming about 1.9 mg/kg orally (Forsling et al., 2001) within a clubbing environment. Ecstasy consumption aside from dance events has also been reported (Lora-Tamayo et al., 1997; Martin, 2001; Libiseller et al., 2005).

The metabolic consequences of MDMA consumption in a clubbing environment have begun to be delineated (Henry et al., 1998; Wolff et al., 2006), and may result in a unique and potentially pathological combination of forces (Cole et al., 2005). However, there are many areas that remain only partially described, such as the neuroendocrine response profile of MDMA: MDMA elicits large increases in the synaptic concentrations of serotonin (Kahn and Wetzler, 1991) and dopamine, both of which are involved in the control of pituitary hormones (Galfi et al., 2005).

There is growing evidence that MDMA has an influence on several key elements of the hypothalamo–pituitary–adrenal (HPA) axis, which mediates many interactions between hormonal secretions and the area of the midbrain that mediates

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the general adaption syndrome (GAS). For instance, vasopressin (AVP, or antidiuretic hormone, ADH), secreted from the paraventricular nucleus of the hypothalamus, has been shown by this group (Wolff et al., 2006) to be significantly elevated following ingestion of MDMA, with concomitant risk of induction of the syndrome of inappropriate antidiuretic hormone (SIADH). In addition, ingestion of MDMA has been shown to result in the release of prolactin and adrenocorticotropic hormone (ACTH) (Dafters, 1995; Henry, 2000). The secretion of the latter being stimulated by the presence of AVP and corticotrophin-releasing hormone (CRH), also released from the paraventricular nucleus. This suggests that both the hypothalamo–lactotroph and the HPA axes are affected during MDMA use. Increased hormone release may also involve active metabolites of MDMA, including 3,4-methylenedioxyamphetamine (MDA) (Forsling et al., 1999).

It is well known that release of stored ACTH acts on the adrenal cortices which stimulates the biosynthesis of glucocorticoids such as cortisol. In the brain, cortisol acts at both mineralocorticoid and glucocorticoid receptors. Cortisol indirectly regulates the activity of ACTH by influencing the secretion of CRH and AVP. Release of CRH itself is also regulated by the sleep/wake cycle and is influenced by stress. This feedback loop is important to the function of the HPA axis. Using plasma cortisol concentration as an indirect biomarker of HPA functionality, we postulate that HPA dysfunction may arise as a consequence of MDMA consumption.

In healthy subjects, cortisol normally follows a diurnal rhythm. Plasma cortisol concentrations have been observed to be significantly raised following ingestion of a typically used dose (1.5 mg/kg) of MDMA by clubbers in experimental conditions (Mas et al., 1999; Harris et al., 2002), following a repeated dose study (FarrÕ et al., 2004), in volunteers with moderate MDMA usage (Tancer and Johanson, 2003), in MDMA users compared to controls (Gerra et al., 2003) and following administration of the analogue entactogen *N*-ethyl-3,4-methylenedioxy-amphetamine (MDEA) (Gouzoulis et al., 1993).

We hypothesize that genetically determined poor metabolism of MDMA may be a predisposing factor for dysfunctional secretion of cortisol. This may be important since elevated secretion of cortisol mediates responses to stress, facilitating an adaptive phase of a GAS in which responses are suppressed, allowing the body to attempt countermeasures. Glucocorticoids such as cortisol have many important functions, including modulation of stress reactions, although in excess there are deleterious effects.

Previously this group (Tsapakis et al., 2006) and others (Gilhooly and Daly, 2002; Schwab et al., 1999; Tucker et al., 1994) have shown that MDMA toxicity may be determined by poor metabolism, with CYP2D6 being responsible for demethylenation, and cathechol-*O*-methyl transferase (COMT) for *O*-methylation (de la Torre et al., 2004). Both *CYP2D6* and *COMT* are highly polymorphic genes with known functional polymorphisms. de la Torre et al. (2005) demonstrated that the pharmacokinetics of MDMA and its metabolites differed according to *CYP2D6* genotype. The multiple allelic variants of CYP2D6 (see http://www.cypalleles.ki.se) result in four well-described phenotypes with respect to *CYP2D6* metabolizer status: poor (PMs), intermediate (IMs), extensive (EMs) and ultrarapid (UMs) metabolizers (Heydari et al., 2004), whereas COMT enzyme activity has a trimodal distribution, with low, intermediate and high levels of activity (Weinshilboum and Raymond, 1977; Spielman and Weinshilboum, 1981). Activity of this polymorphic enzyme has been shown to be associated with a single nucleotide polymorphism in exon 4 (rs4680), a G→A substitution in amino acid codon 158, which results in a valine (Val) to methionine (Met) substitution. Met homozygosity is associated with low enzyme activity, Val/Met heterozygosity with intermediate activity level, and Val/Val homozygosity with a high enzyme activity level (Lachman et al., 1996).

In addition, we took into consideration the possibility that alteration of human serotonin transporter (5-HTT) expression might occur in response to glucocorticoids such as cortisol. For example, clubbers who use ecstasy (MDMA) have also been reported to have persistent deficits in cerebrospinal fluid (CSF) 5-hydroxyindol acetic acid (5-HIAA) (Ricaurte et al., 1988) and loss of brain 5-HTT sites, as viewed by positron emission tomography (PET) (McCann et al., 2005). The above lines of evidence suggest that the excessive secretion of cortisol may interact with serotonergic effects in potential induction of neurotoxicity on MDMA consumption.

The 5-HTT is one of the targets of the serotonin released by MDMA. Alteration in human 5-HTT expression in response to glucocorticoids is thought to be mediated by the serotonin transporter linked polymorphic region (*5-HTTLPR*) (Glatz et al., 2003). The *5-HTTLPR* insertion/deletion polymorphism affects transporter expression and function *in vitro* and *in vivo* (Heils et al., 1996; Collier et al., 1996; Parsey et al., 2006). A long (*l*, 528 bp) and a short (*s*, 484 bp) form affect the expression and function of the serotonin transporter. Those with the *s* variant have reduced transcription of the *5-HTT* gene promoter, resulting in decreased 5-HTT expression and an approximate 50% reduction in serotonin uptake (Heils et al., 1996; Collier et al., 1996). We therefore also hypothesized that *5-HTTLPR* genotype might be associated with cortisol response in those consuming the drug MDMA.

In this paper we report data on a particular phenotype (change in plasma cortisol) in the sample collected 'in the field' (pre- and post-clubbing) and reported by Wolff et al. (2006). Here we investigate (1) change in plasma cortisol concentration associated with MDMA consumption, and (2) association between *CYP2D6*, *COMT* rs4680 and *5-HTTLPR* genotypes with the MDMA-associated change in cortisol.

Methods

Recruitment

Subjects recruited were experienced clubbers who had been clubbing an average of three occasions in the preceding month, were at least 18 years old and able to provide informed consent. A medical assessment was undertaken to exclude those with a past medical or psychiatric history, which were exclusion criteria for the study (Wolff et al., 2006). Subjects were also excluded if they were on medication known to alter the functionality of cytochrome P450 CYP2D6 or COMT enzymes. Ethical approval was granted by the Joint Institute of Psychiatry and South London and Maudsley NHS Foundation Trust Research Ethics Committee (Ref No. 272/

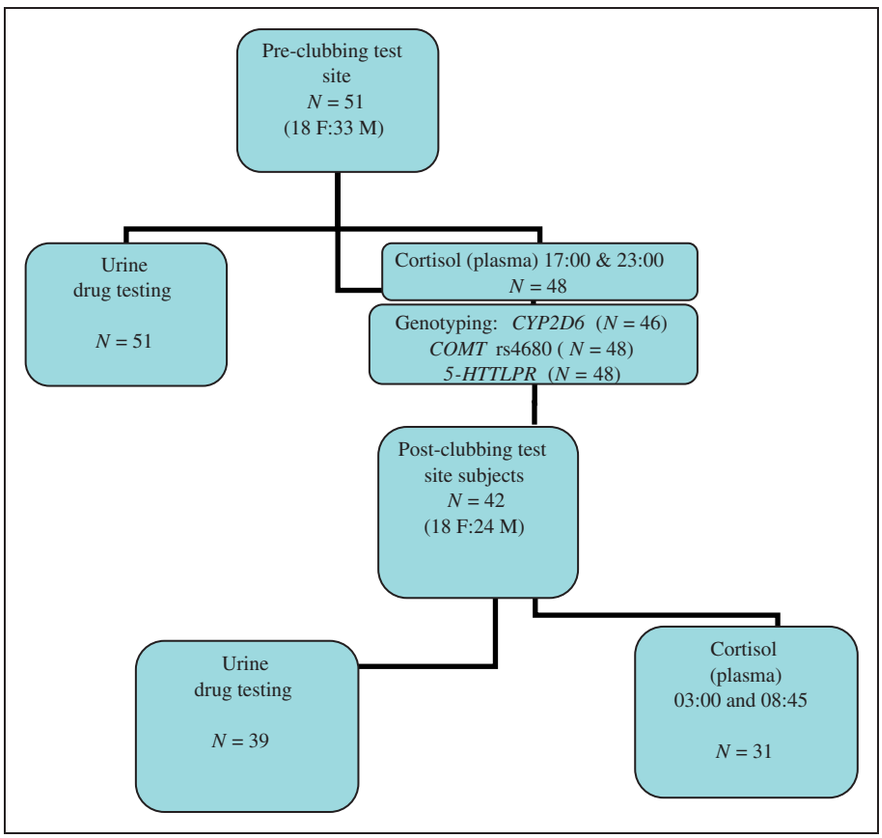

Figure 1. Flowchart of the recruitment process and provisional of samples for analysis by subjects.

00), with all samples and data being anonymized on collection. Of the 51 subjects recruited, 48 were sampled for cortisol concentrations pre-clubbing and each provided a urine sample to screen for drugs of abuse. Alcohol breathalyser testing was also undertaken. All were genotyped for *CYP2D6*, *COMT* rs4680 and the *5-HTTLPR*. Physiological measures including blood pressure were taken, as well as details of the sleeping habits of participants. Thirty-nine of the clubbers (81%) returned to the test station post-clubbing for the same provision of samples (Figure 1).

Procedures

Blood (5 ml) and urine (20 ml) samples were collected; pre-clubbing between 17:00 and 23:00, and post-clubbing between 03:00 and 08:45. Plasma cortisol (nmol/l) was measured. This glucocorticoid has a well-described diurnal variation (Young et al., 2004), rising rapidly and reaching a peak about 45 minutes after waking (normal range at 09:00, 150–650 nmol/l), and declining throughout the day to reach a nadir in the late evening (normal range 00:00, 30–250 nmol/l) (Aardal and Holm, 1995). A qualitative urinary drug screen was performed to identify common drugs of abuse (amphetamines, benzodiazepines, cannabis, cocaine, amyl nitrate, methadone and opiates), MDMA and its main active metabolite (MDA) and most common analogue (MDEA). Breath-alcohol (Br AC) measurements were performed using a handheld device (Lion Alcometer SD-400, L.E. West Ltd, UK) to indicate Br AC (mg/dl).

Genotyping

For genotyping methodology, see the companion paper on this sample also in this special issue. The AmpliChip CYP450 Test® (Roche Molecular Diagnostics, Alameda, CA, USA) was used to detect genetic variants in *CYP2D6* (Heller et al, 2006). Genotype calls were achieved in 46 out of 48 samples genotyped (95.8%): two calls were not achieved due to insufficient DNA of adequate quality being available. *CYP2D6* genotype was coded in order of expected increasing enzyme activity: 1 = PM/PM, 2 = PM/IM, 3 = IM/IM, 4 = PM/EM, 5 = IM/EM, 6 = EM/EM.

Genotyping for the rs4680 single nucleotide polymorphism (SNP; CCAGCGGATGGTGGATTTCGCTGGC[A/G]TG AAGGACAAGGTGTGCATGCCTGA) in the *COMT* gene was initially undertaken by KBiosciences (Hoddesdon, UK), who genotyped 46 out of 48 samples (95.8% genotype call rate). We employed the TaqMan® method independently and were able to call the *COMT* genotypic category of the two remaining samples and confirm other genotypes with 100% concordance.

Genotyping for the *5-HTTLPR* was undertaken using oligonucleotide primers to flank the *5-HTTLPR* and generate a 484/528-bp fragment according to the method of Heils et al

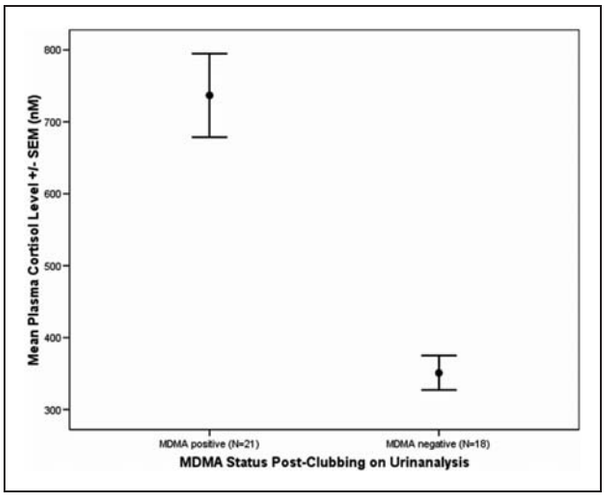

Figure 2. Change in mean plasma cortisol concentration (±SEM, nmol/l) post-clubbing, grouped according to the presence of MDMA by post-clubbing urinalysis (two independent samples *t*-test, $p < 0.001$).

(1996). The cycling conditions included a denaturation step of 95°C for 15 min, 35 cycles of 95°C for 30 s, 66°C for 30 s and 72°C for 40 s and an elongation step at 72°C for 15 min. The genotype was identified by agarose gel electrophoresis on a 3% agarose gel, separating alleles by size, classifying the alleles simply as long (*l*, 16 repeat) or short (*s*, 14 repeat). Genotypes were successfully called in all 48 subjects.

Statistical analysis

Demographic and clinical data on the sub-sample with phenotypes of interest ($N = 39$) versus the whole sample ($N = 48$) were compared using χ^2 analysis. SPSS for Windows (version 15.0, SPSS Inc., Chicago, IL, USA) was used to conduct all phenotypic and genotypic analyses. Data that were not normally distributed were compared by non-parametric analysis; otherwise comparisons were made by using parametric analysis. Linear regression and repeated measures analysis using a general linear model was conducted to investigate the interaction between pre- and post-clubbing plasma cortisol and *CYP2D6*, *COMT* rs4680 and *5-HTTLPR* genotypes. Genotypes were grouped into to the following categories based on the known functionality of the identified alleles: EM/EM, EM/IM, IM/IM, EM/PM, IM/PM, and PM/PM (*CYP2D6*) and Val/Val, Val/Met, and Met/Met (*COMT*) L/L, L/S and S/S (*HTTLPR*), respectively. Graphs were plotted as cluster error bars or repeated measures outputs as appropriate. A *p*-value of less than 0.05 was considered significant after correction for multiple testing. Power analysis was performed using Solo Power Analysis 2.0 (BMDP Statistical Software, Los Angeles, CA, USA). Hardy–Weinberg equilibrium analysis was conducted on the genotypic distributions according to Ott (1991).

Results

The subjects were initially analysed as a total pre- versus post-clubbing cohort, and then were further grouped as either having consumed MDMA (ecstasy) that night on the basis of positive post-clubbing detection of MDMA by urinalysis, or those who had not used the drug. In the pre-clubbing sample, 37.5% (18/48) had positive urine drug tests, which detected cannabis (72.2%, 13/18), MDMA (38.9%, 7/18), cocaine (22.26%, 3/18) and amphetamine (11.8%, 2/18). A total of 11 (22.9%, 11/48) clubbers had consumed alcohol pre-clubbing. In the post-clubbing sample for which plasma cortisol concentrations were available, 61.5% (24/39) had positive urine drug tests for psychoactive substances excluding alcohol, two clubbers being unable to void urine. Only six of the clubbers in the study reported ever using benzodiazepines and pre- and post-clubbing urine samples were negative for benzodiazepine drugs. Exogenous steroid use was not reported by the subjects in the study.

Post-clubbing urinalysis identified MDMA in 21 of 39 clubbers (5 of whom had consumed MDMA before clubbing). Just under a half (9/21, 42.9%) used MDMA alone, others used cannabis (11/21, 52.4%), cocaine (4/21, 19.0%) and/or alcohol (1/21, 4.8%) concurrently. Post-clubbing MDA was detected in the urine of eight subjects in addition to MDMA: in the 5 subjects who had MDMA detected in pre- and post-clubbing, and a further two subjects who also had both MDMA and MDEA detected; only one subject positive for MDA post-clubbing was positive for MDMA pre- but not post-clubbing; HMMA was not analysed. Seven of the 39 clubbers with post-clubbing cortisol measurements had blood alcohol concentrations detectable post-clubbing ranging from 23 to 106 mg/dl, two of which were above the legal UK driving limit (80 mg/dl).

Sleep before entering the study was normal for most of the sample, with only 15.5% reporting interrupted sleep (waking more than twice during the previous night). Nine subjects returning to the test site, who had used MDMA, had a systolic sitting blood pressure >140 mmHg.

Comparing the pre- and post-clubbing samples, the mean plasma cortisol measurements increased; the concentration in those with MDMA-positive urine post-clubbing ($N = 21/39$) was significantly greater (736.9 ± 83.2 nmol/l) than in those who had not consumed the drug (350.9 ± 34.5 nmol/l, two independent samples *t*-test $p < 0.001$; $N = 18/39$, Figure 2). In the pre-clubbing (early evening) samples, approximately two-thirds of clubbers (31/48, 64.6%) had elevated plasma cortisol concentrations (287–724 nmol/l), 287–618 nmol/l (mean 408.0 ± 182.6 nmol/l) in males (19/31) and 551–724 nmol/l (mean $637.5.0 \pm 122.3$ nmol/l) in females (12/31), respectively, as defined by a cortisol concentration greater than 250 nmol/l (Aardal and Holm, 1995). This value was chosen because the nadir in the late evening (normally measured at 00:00) ranges from 30 to 250 nmol/l. The elevated ranges that we observed are similar to concentrations usually observed in 'normal' adults in the early morning.

Linear regression analysis of cortisol measurements pre-clubbing versus detection of MDMA pre-clubbing revealed no significant association, whereas linear regression of cortisol measurements post-clubbing versus detection of MDMA in post-clubbing urinalysis showed a significant association with a positive MDMA post-clubbing ($N = 21/39$) urine test (two-tailed $p < 0.001$). There was no significant correlation between arrival at testing bay pre-clubbing and plasma

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Table 1. Genotypic distributions by status of urinalysis drug test result for MDMA post-clubbing

	COMT rs4680			*CYP2D6*						*5-HTTLPR*		
MDMA urine status post-clubbing	A/A	A/G	G/G	PM/PM	PM/IM	IM/IM	PM/EM	IM/EM	EM/EM	*l/l*	*l/s*	*s/s*
Positive detection of MDMA in urine	6	10	5	0	2	3	4	5	6	5	13	3
Negative drug test result for MDMA in urine	8	8	2	1	2	0	7	4	4	6	8	4
Total	14	18	7	1	4	3	11	9	10	11	21	7

All genotypic distributions were in Hardy–Weinberg equilibrium: for COMT, $\chi^2 = 0.083$, $df = 1$, $p = 0.36$; for CYP2D6, $\chi^2 = 1.21$, $df = 3$, $p = 0.37$; for *5*-HTTLPR, $\chi^2 = 0.310$, $df = 1$, $p = 0.29$ (Ott, 1991). *CYP2D6* genotype was coded in order of expected increasing enzyme activity: 1 = PM/PM, 2 = PM/IM, 3 = IM/IM, 4 = PM/EM, 5 = IM/EM, 6 = EM/EM.

cortisol concentrations pre-clubbing (Pearson's), neither was there a significant correlation between arrival at the test bay post-clubbing with plasma cortisol concentrations post-clubbing (Pearson's). This lack of correlation with time in our sample of regular clubbers is not surprising, given that subjects remained awake all night.

The *COMT* rs4680, *CYP2D6* and *5-HTTLPR* genotypic distributions in the MDMA-positive and MDMA-negative subjects post-clubbing are shown in Table 1. In the case of *CYP2D6*, there were no UMs identified in the sample. Power analysis for repeated measures analysis for change in plasma cortisol, with between subjects factor such as *5-HTTLPR* genotypic category and within subjects factor such as detection of ecstasy (MDMA) in urine status post-clubbing, gave a power of 0.79 for the detection of an interaction between time and genotype, for an effect size of 0.20 ($\alpha = 0.05$). Hence, we may well have not detected an association between *5-HTTLPR* genotype and MDMA-induced cortisol change with an effect size of 0.20 or less, i.e. the non-significant *p*-value may represent a type II error. Further studies in a larger sample of subjects with similar recreational drug use characteristics are therefore indicated.

On repeated measures analysis in subjects positive for MDMA by post-clubbing urinalysis ($N = 21/39$), a nominally significant interaction between *COMT* rs4680 genotype (A/A vs. A/G vs. G/G in an additive model) and change in plasma cortisol concentration was found (two-tailed $p = 0.039$, Figure 3). As the slopes (i.e. change in means in the repeated measures analysis) were more similar for the A/G and G/G groups, these two were grouped together for further analyses. Using this grouping of *COMT* rs4680 genotype (A/A homozygotes vs. A/G or G/G), a significant interaction between low *COMT* activity (A/A genotype, corresponding to Met/Met homozygotes) and change in plasma cortisol concentration was found (two-tailed $p = 0.013$, Figure 4), indicating that carriers of the genotype corresponding to low *COMT* activity showed a greater degree of increase in cortisol. This *p*-value remained significant on applying a Bonferroni correction ($p = 0.039$) for testing three genes versus one phenotype.

Similar repeated measures analysis for *CYP2D6* also revealed a significant association between genotype code and change in plasma cortisol concentration; this time the association was confined to subjects with MDMA-positive urines post-clubbing (two-tailed $p = 0.027$, $N = 21/39$, Figure 5). As the slopes (i.e. change in means in the repeated measures analysis) were very similar for the PM/IM and IM/IM genotypes,

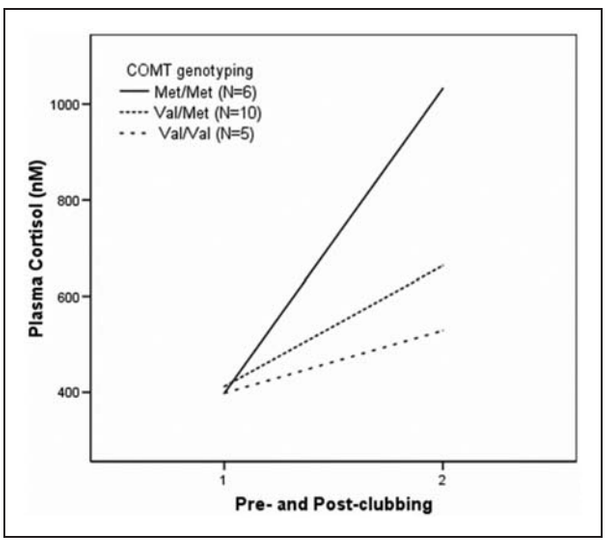

Figure 3. Change in plasma cortisol concentration (nmol/l) pre- and post-clubbing, grouped by COMT rs4680 genotype in subjects positive for MDMA in post-clubbing urinalysis (two-tailed, $p = 0.039$).

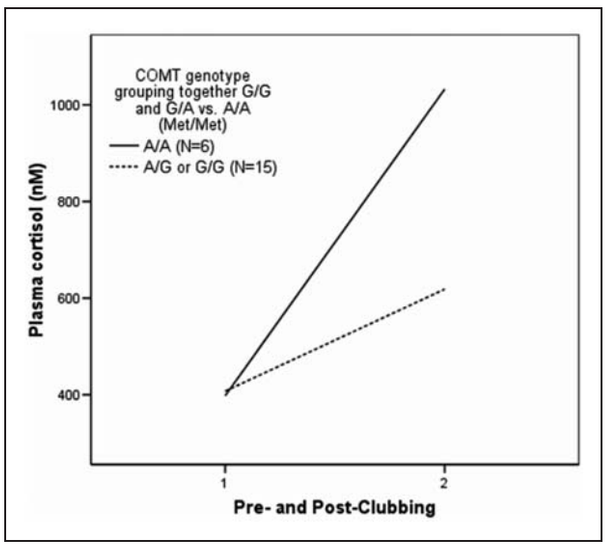

Figure 4. Change in plasma cortisol concentration (nmol/l) grouped by low activity (A/A homozygotes) COMT rs4680 genotype versus combined intermediate or high activity (A/G or G/G) COMT rs4680 genotypes in subjects positive for MDMA in post-clubbing urinalysis (two-tailed Bonferroni, $p = 0.039$).

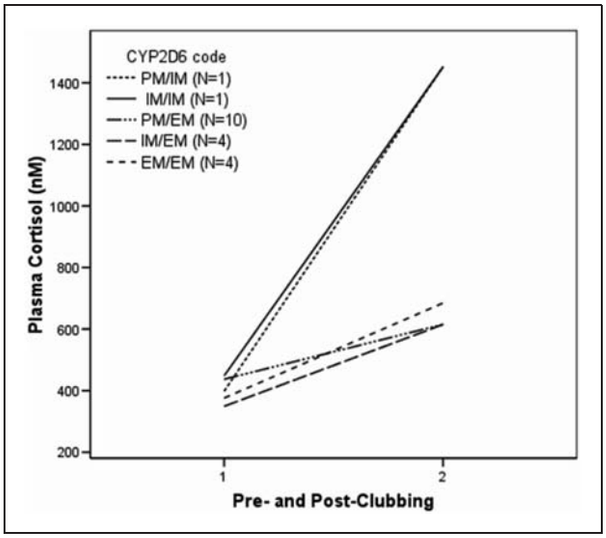

Figure 5. Change in plasma cortisol concentration (nmol/l) pre- and post-clubbing, grouped by CYP2D6 genotype in subjects positive for MDMA in post-clubbing urinalysis (two-tailed, $p = 0.027$).

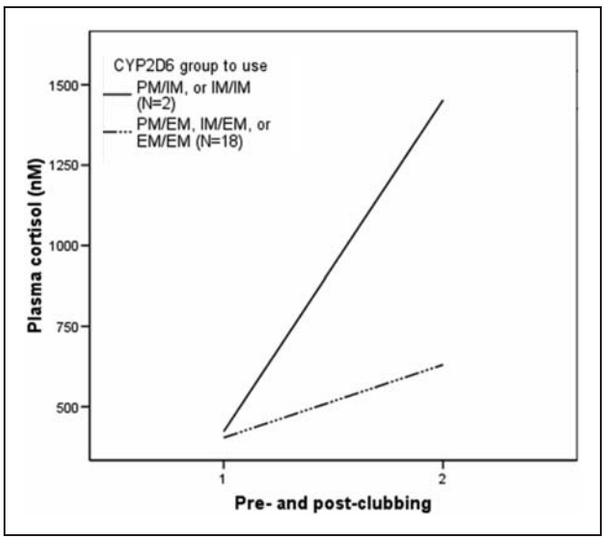

Figure 6. Change in plasma cortisol concentration (nmol/l) pre- and post-clubbing, grouped by low activity (PM/IM or IM/IM) versus the other CYP2D6 genotypes in subjects positive for MDMA in post-clubbing urinalysis (two-tailed Bonferroni, $p = 0.003$).

these two were grouped together for further analyses. Using this grouping of *CYP2D6* genotype, a significant interaction between low *CYP2D6* activity (PM/IM or IM/IM; there were no PM/PM individuals with MDMA-positive urine post-clubbing) and change in plasma cortisol concentration was found (two-tailed $p = 0.001$, Figure 6), indicating that carriers of the genotypes corresponding to low *CYP2D6* activity showed a greater degree of elevation in plasma cortisol. This *p*-value would clearly remain significant on applying a Bonferroni correction ($p = 0.003$). In subjects that screened

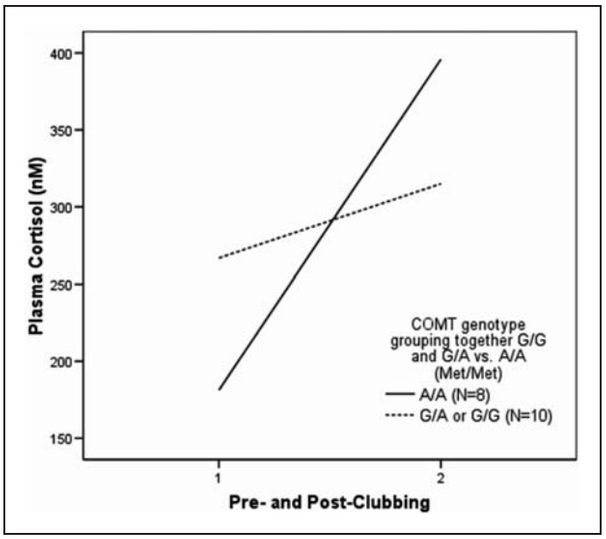

Figure 7. Change in plasma cortisol concentration (nmol/l) grouped by low activity (A/A homozygotes) COMT rs4680 genotype vs. combined intermediate or high activity (A/G or G/G) COMT rs4680 genotypes in subjects negative for MDMA in post-clubbing urinalysis (two-tailed, Bonferroni $p = 0.045$).

negative for MDMA by post-clubbing urinalysis, there was no separation out in terms of slope of change for the PM/PM and IM/IM genotypes versus the rest of the genotypes, or a significant association when the *CYP2D6* low activity groups were grouped together.

Subjects that screened negative for MDMA by post-clubbing urinalysis ($N = 18/39$) in the main also showed an increase in plasma cortisol, and, interestingly, in this subgroup, there was also a significant effect of rs4680 genotype when grouped as above (two-tailed $p = 0.015$, Figure 7). Given the significant findings in both MDMA-positive and MDMA-negative by post-clubbing urinalysis for our clubbers, we also investigated the association between rs4680 genotypic group and overall change in plasma cortisol ($N = 39$). This was also found to be significant (two-tailed $p = 0.013$ using the grouping as above, or $p = 0.048$ using all three genotypes in an additive model, Figure 8).

Despite there being a positive association between both *COMT* and *CYP2D6* genotypes and MDMA-induced post-clubbing increase in plasma cortisol concentration, there was no statistical interaction on repeated measures analysis of these two genes on this phenotype. However, the sub-sample on which this analysis was performed comprised only 20 subjects, and hence this may well be a type II error.

Similar analysis examining the association between *5-HTTLPR* genotype and change in plasma cortisol in subjects positive for MDMA post-clubbing failed to reach significance, and this was the case on using an additive model (treating *l*/*l*, *l*/*s* and *s*/*s* in an additive fashion or when *l*/*l* homozygotes were grouped versus *l*/*s* and *s*/*s* in an *s* allele dominant model, which is in accordance with original *in vitro* functional data (Heils et al., 1996); however, the degree of change in cortisol by *5-HTTLPR* genotypic group was in the order $l/l > l/s > s/s$). The failure to find a statistically significant association may

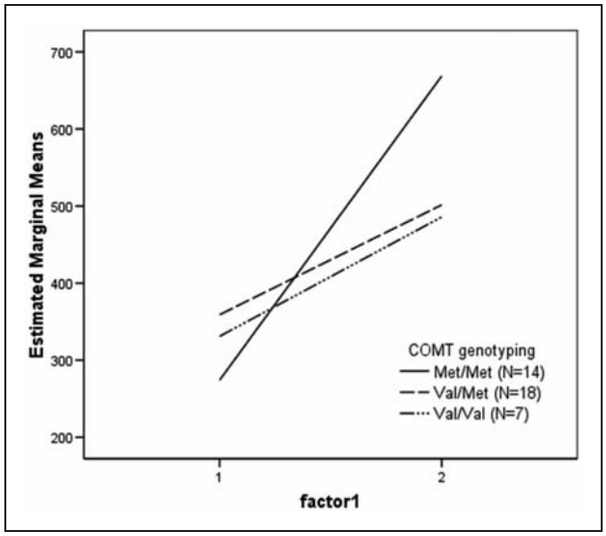

Figure 8. Change in plasma cortisol concentration (nmol/l) in MDMA-positive and MDMA-negative subjects by post-clubbing urinalysis, grouped by COMT rs4680 genotype (two-tailed, $p = 0.048$, using all three genotypes in an additive model).

therefore also be a type II error owing to the sample size. There was also no association between *5-HTTLPR* genotype and overall change in cortisol (i.e. including subjects both MDMA-positive and MDMA-negative by post-clubbing urine).

Discussion

Drug users who use 'ecstasy' are difficult to identify and engage in biological research studies and hence obtaining access to a large sample of this type is very problematic, particularly if biological sampling is to be undertaken. There has, therefore, been increased use of non-random sampling methods in studying drug use within the dance or 'rave' scene. In this paper we report on the genetic association of functional polymorphisms in three relevant candidate genes versus change in plasma cortisol concentration in response to MDMA consumption in a clubbing context.

Our finding of a positive association between plasma cortisol concentration post-clubbing and detection of MDMA by post-clubbing urinalysis ($N = 21/39$, $p < 0.001$) is the first 'in-the-field' replication of previously identified MDMA-induced cortisol increase under experimental (laboratory) conditions in MDMA users (Mas et al., 1999; Harris et al., 2002; Gerra et al., 2003; Tancer and Johanson, 2003; FarrÕ et al., 2004). This report also represents the first genetic association analysis of this phenotype. We found a positive association with low *CYP2D6* and low *COMT* activity genotypes, and no statistical association with low activity serotonin transporter genotypes.

In terms of *CYP2D6,* the PM/IM or IM/IM genotypes were associated with a greater degree of increase in plasma cortisol concentration than the other *CYP2D6* genotypes. The two-tailed *p*-value when all genotypes were analysed separately was 0.027, which as the finding was in the same direction as our hypothesis could be halved to 0.0135. Moreover, on grouping together PM/IM and IM/IM and analysing these versus the rest of the genotypes, the association was strengthened ($p = 0.001$ two-tailed, or 0.0005 one-tailed). The one-tailed *p*-values correspond to 0.0405 and 0.0015 on Bonferroni correcting for testing three genes versus this phenotype, i.e. one-tailed *p*-values remain significant after correction for multiple testing.

The significant finding for CYP2D6 only in subjects MDMA positive by post-clubbing urinalysis may relate to the fact that MDMA inhibits CYP2D6 (Heydari et al., 2004). Genotypes PM/IM and IM/IM would be expected to be particularly susceptible to inhibition (Tandon et al., 2005). It has previously been shown that individuals who have a normal CYP2D6 activity may be rendered equivalent to genetic CYP2D6 poor metabolizers in terms of their CYP2D6 activity by the administration of a CYP2D6 inhibitor (Spina et al., 1991), in this case the consumption of MDMA. Thus, for individuals in the PM/IM and IM/IM genotypic categories, MDMA consumption would render the CYP2D6 activity even lower than it would be in a drug-naÿve state with resulting greatly reduced or absent ability to metabolise MDMA or MDA by this isoenzyme, leading to the accumulation of these substances and likely metabolism by other, less efficient, pathways. As both MDMA and MDA are pharmacologically active, their accumulation would be expected to be associated with a greater rise in plasma cortisol concentration than for individuals with other *CYP2D6* genotypes.

The excessive secretion of cortisol may interact with serotonergic effects in the potential induction of neurotoxicity on MDMA consumption (Poland, 1990; Van Cauter, 1990). It is noteworthy that post-clubbing cortisol concentrations for the PM/IM and IM/IM subjects were the highest out of the whole sample (1452–1453 nmol/l). Chronic, excessive use of MDMA in a clubbing environment may contribute to exaggerated dysfunction of the HPA axis for such individuals. Further work is clearly required in this area.

We found that the genotype predicting low *COMT* activity (Met158Met) was also associated with a greater degree of increase in plasma cortisol concentration than the other *COMT* genotypes ($p = 0.013$, two-tailed). However, this association was also observed in individuals with MDMA-negative urine post-clubbing ($N = 18/39$, $p = 0.015$, two-tailed). This finding of an association between *COMT* genotype and increase in plasma cortisol concentration post-clubbing, whether or not individuals had consumed MDMA is interesting and deserves further comment.

The heritability of total cortisol and the unbound fraction of this glucocorticoid in plasma have been reported as 51% and 58%, respectively (Meikle et al., 1998). Thus, environmental factors are as important as genetic factors in contributing to the overall variance in plasma cortisol concentration. Indeed, it is thought that the HPA axis responsiveness to a given stressor is due to the combination of genetic factors and early environmental influences and this relationship determines the 'set point' of the HPA axis, which predetermines an individual's ability to adapt to chronic mild stress (Moss et al., 1999; Weaver et al., 2004). Release of CRH itself is in turn stimulated by the sleep/wake cycle, blood concentrations of cortisol and is influenced by stress.

The increase in plasma cortisol concentrations in the whole sample of clubbers over the duration of the study may of course, at least partly, reflect the diurnal pattern of cortisol secretion, with a normal peak shortly before rising. However, our subjects' pre-clubbing cortisol concentrations were abnormal, that is in the range usually observed in individuals pre-rising: over two-thirds of the subjects sampled in the early evening (31/48, 64.6%) had abnormal plasma cortisol concentrations (elevated above normal, 287–724 nmol/l), in fact, resembling those usually observed in the early morning in non-clubbing individuals (09:00 range being 150–650 nmol/l). This may be explained in part by the fact that the majority (82.1%) were regular 'clubbers': nearly a third (30.8%) admitted to 'clubbing' at a frequency of at least once per week and a further 51.3% (20/39) to 'clubbing' two or three times per month. In our sample, although duration of sleep before entering the study was reported as normal the timing of sleep when clubbing was clearly altered from their habitual sleep/wake cycle.

HPA axis function is known to be influenced by sleep (irrespective of the time of day when it occurs), which modulates the amplitude of secretory pulses in the circadian pattern of cortisol excretion in normal individuals (Van Cauter, 1990). For instance, shift work patterns alter the normal circadian pattern of secretion, e.g. by advancing the nadir of the cortisol profile forward (Caufriez et al., 2002), and blunting the cortisol peak after sleep deprivation conditions (Mougin et al., 2001). Both sleep/wake and dark/light transitions have been consistently associated with cortisol secretory pulses (Caufriez et al., 2002).

Our study shows concordance with the few studies that have examined the impact on hormones, metabolism and immune function of a common, real-life situation: chronic partial sleep deprivation (Spiegel et al., 1999, 2004; Vgontzas et al., 2004). The first effect of partial sleep loss on circulating levels of pituitary-dependent hormones is an increase in the early evening levels of cortisol (Leproult et al., 1997). Normally at that time of day, cortisol concentrations are rapidly decreasing to attain minimal levels shortly before habitual bedtime. There are risks associated with elevations of early evening cortisol concentration. It has been reported that this occurrence may promote the development of insulin resistance, a risk factor for obesity and diabetes (Spiegel et al., 2004). Regular use of MDMA and chronic partial sleep loss may therefore have unforeseen adverse consequences for the clubber.

It is, therefore, feasible that both chronic use of ecstasy (MDMA) and regular clubbing activity would lead to disturbed, desynchronized or malsynchronized cortisol rhythms (Kripke et al., 2005). This is concerning in the light of previous reports of chronic heavy use of MDMA, with consumption of between 10 and 25 tablets at any one time (Parrott, 2005) and evidence from our own study that some individuals go 'clubbing' as frequently as several times a week (Wolff et al., 2006).

During the course of the study, the cortisol concentrations in our clubbers were further elevated. This may imply that 'clubbing' activities (environmental factors), including dancing, are associated with a rise in cortisol. In addition, four of our clubbers had cocaine detected in their post-clubbing urine. Like MDMA, cocaine inhibits monoaminergic reuptake transporters and also induces catecholamine and monamine release from presynaptic neurones, which may also have contributed to the increase in cortisol concentrations during 'clubbing'.

Ingestion of ecstasy tablets containing MDMA has previously been described to result in the release of anterior pituitary hormones including not only ACTH but also others such as prolactin (Dafters, 1995; Henry, 2000), and a typically used dose of MDMA has been shown to produce a statistically significant increase in plasma cortisol concentrations in man (Harris et al., 2002; Mas et al., 1999). Our observation of elevated plasma cortisol concentrations post-clubbing is consistent with consumption of MDMA leading to a chronically elevated ACTH. For such individuals, the functionality of the HPA axis may well be altered. Further work should include ACTH measurement as well as cortisol in regular clubbers.

In addition to its role in MDMA metabolism, it should be noted that COMT is involved in endogenous neurotransmitter metabolism, specifically, in the degradation of dopamine and noradrenaline, playing a particularly important role in the prefrontal cortex (Egan et al., 2001). The control of the release of CRH from the hypothalamus is complex, including noradrenergic as well as serotonergic mechanisms. Moreover, dopamine has been postulated to play a role in MDMA-induced 5-HT depletion (Nash, 1990). It could, therefore, be hypothesized that COMT plays an indirect role in the regulation of the HPA axis, and hence that COMT genotype could be associated with HPA axis reactivity. Our finding of an association between *COMT* genotype and increase in plasma cortisol concentration during the course of the study may, therefore, reflect this and warrants further investigation.

With regards to the *5-HTTLPR* genotype, we did not find a statistically significant association with MDMA-induced increase in plasma cortisol concentration or with increase in cortisol in the whole sample. However, the degree of change in cortisol by genotypic group was for both of these phenotypes consistent with previous pharmacogenetic studies, with the greatest effect being seen in the *l*/*l* group (Smeraldi et al., 1998; Hu et al., 2006; Huezo-Diaz et al., 2009). The lack of a statistically significant finding may have been due to the fact that our study was not adequately powered to detect such an association, owing to the small sample size of individuals who tested positive for MDMA on post-clubbing urinalysis.

In summary, we have found an association between low *COMT* activity (Met158Met) genotype and increase in plasma cortisol concentration post-clubbing and also between low *CYP2D6* activity (PM/IM or IM/IM) genotypes and MDMA-induced increase in cortisol post-clubbing. Although there was no apparent interaction between these in terms of effect on MDMA-induced cortisol increase, the sample was underpowered to test for interactions.

Further pharmacogenetic analyses of these phenotypes in a larger 'in the field' sample of 'clubbers' are indicated. It would also be interesting to explore the association between change in plasma cortisol concentration on MDMA consumption and mood ratings and relevant candidate genes. Should our findings be replicated, then individuals who intend to go 'clubbing' might want to know their *CYP2D6* and *COMT* genotypes, since low activity genotypes are more vulnerable to increased

production of cortisol, which mediates response to stress, facilitating in the first instance an adaptive phase of a GAS in which alarm reactions including the immune response are suppressed, allowing the body to attempt countermeasures. However, there is evidence that in excess, or in disrupted rhythm, glucocorticoids such as cortisol may well have undesirable effects.

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Conflict of interest

Dr Aitchison has received the AmpliChip CYP450 Test® and associated research support and consultancy fees from Roche Molecular Systems (USA) and consultancy fees from Roche Diagnostics (UK).

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