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Persistent nigrostriatal dopaminergic abnormalities in ex-users of MDMA ('Ecstasy'): an ^{18}F -dopa PET study

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ABSTRACT

Ecstasy (MDMA) is a popular recreational drug with known serotonergic neurotoxicity. Its long-term effects on dopaminergic function are less certain. Studying the long-term effects of ecstasy is often confounded by concomitant polydrug use and the short duration of abstinence. We used ^{18}F -dopa positron emission tomography (PET) to investigate the long-term effects of ecstasy on nigrostriatal dopaminergic function in a group of male ex-recreational users of ecstasy who had been abstinent for a mean 3.22 years. We studied 14 ex-ecstasy users, 14 polydrug-using controls (matched to the ex-users for other recreational drug use) and 12 drug-naïve controls. Each participant underwent one ^{18}F -dopa PET, cognitive assessments, and hair and urinary analysis to corroborate drug use history. The putamen ^{18}F -dopa uptake of ex-ecstasy users was 9% higher than drug-naïve controls ($p=0.021$). The putamen uptake of polydrug-using controls fell between the other two groups, suggesting the hyperdopaminergic state in ex-ecstasy users may be due to the combined effects of ecstasy and polydrug use. There was no relationship between the amount of ecstasy use and striatal ^{18}F -dopa uptake. Increased putamen ^{18}F -dopa uptake in ex-ecstasy users after an abstinence of over three years (mean) suggests that the effects are long-lasting. Our findings suggest potential long-term effects of ecstasy use, in conjunction with other recreational drugs, on nigrostriatal dopaminergic functions. Further longitudinal studies are needed to elucidate the significance of these findings as they may have important public health implications.

INTRODUCTION

±3,4-methylenedioxymethamphetamine (MDMA) is an amphetamine derivative which is the main psychoactive compound in 'ecstasy' tablets. Ecstasy is a popular recreational drug and is frequently taken in multiple doses in nightclubs and parties. In the United Kingdom, the prevalence of ecstasy use in the 15-34 age group is 13.6% ((EMCDDA), 2005). Neurotoxicity of MDMA on the serotonergic system, as defined by a reduction in the neuronal or neurochemical markers, has been demonstrated in both animals and human (Green *et al*, 2003), though this may be partly reversible after a prolonged abstinence [for review, see (Cowan, 2007; Reneman *et al*, 2006)]. The effects of MDMA on dopaminergic neurons are less certain. Acutely, MDMA releases dopamine from the dopaminergic terminals (Colado *et al*, 2004). In animals, with the exception of mice and, under certain conditions, rats (Commins *et al*, 1987; Yuan *et al*, 2002), MDMA has not been found to cause dopamine neurotoxicity (Colado *et al*, 2004; Logan *et al*, 1988) - unlike amphetamine (Melega *et al*, 1997) or methamphetamine (Wilson *et al*, 1996). In humans, some studies have suggested chronic effects of MDMA usage on dopaminergic neurons. One study showed that three weeks after discontinuation of MDMA, the growth hormone response to dopamine agonist bromocriptine was significantly attenuated in ecstasy users compared to controls, possibly reflecting reduced dopamine D2 receptor sensitivity in the hypothalamus (Gerra *et al*, 2002). There have also been several reports suggesting an association between MDMA usage and subsequent development of parkinsonism (Kuniyoshi and Jankovic, 2003; Mintzer *et al*, 1999), but no definite causal link has been established.

The interpretation of many studies involving ecstasy users can be confounded by concomitant polydrug use given that most ecstasy users have also taken other recreational drugs (Gouzoulis-Mayfrank and Daumann, 2006). Previous neuroimaging studies investigating the effects of ecstasy on dopaminergic system have produced heterogeneous results. The relatively short period of abstinence from ecstasy in participants included in these studies also cannot address long-term toxicity. Using ^{123}I -beta-CIT single-photon emission computed tomography, one study reported no difference in striatal dopamine transporter (DAT) uptake between a group of current MDMA users and polydrug-using controls (Semple *et al*, 1999). Another study showed an increase in striatal DAT binding in ecstasy users who had been abstinent for at least three weeks compared with controls and ecstasy users who also used amphetamine (Reneman *et al*, 2002). Finally, one group used ^{11}C -WIN35428 positron emission tomography (PET) and did not find any difference in striatal DAT binding between MDMA users who had been abstinent for a mean of 2.75 months and a group of unmatched polydrug users (McCann *et al*, 2008).

^{18}F -dopa PET provides an *in vivo* marker of presynaptic dopaminergic terminal function (Brooks, 2003). Using ^{18}F -dopa PET, we investigated the long-term effects of ecstasy exposure on nigrostriatal dopaminergic function in ex-ecstasy users who have abstained from this drug for at least a year. Aspects of mood and cognition associated with dopaminergic function or with impairment following ecstasy use were also assessed. We included a group of polydrug-using controls to minimise the possible confounding effects of exposure to other recreational drugs.

MATERIALS AND METHODS

Design and participants

We recruited three groups of healthy participants: (1) ex-ecstasy users (EE) who had taken ecstasy on at least 25 occasions but had not taken any for the past 12 months, though they could continue to use other recreational drugs; (2) polydrug-using controls (PC) who were matched to the EE in their recreational drug use but had never taken ecstasy; and (3) drug-naïve controls (DC) who had never taken any recreational drugs except alcohol. The 25 occasions of use of ecstasy was an arbitrary cut-off to exclude very light ex-users from this study. Participants were recruited through magazine advertisements and word of mouth. Participants were not informed of inclusion criteria beforehand, and they were reimbursed travel expenses and £50 for their time. All three groups were matched for age, pre-morbid IQ, level of education and, for the drug-using groups, history of drug use apart from ecstasy.

Inclusion criteria for all groups were that the participants were male, aged 25-50, not taking prescribed psychotropic medication or receiving psychological treatment, no current or history of drug addiction, not being depressed as determined by the Structured Clinical Interview for DSM-IV (SCID) (First *et al*, 1997), have a score of <18 on the Beck Depression Inventory (BDI), have a score of <55 on the Spielberger Trait Anxiety Scale (STAI), no significant medical illness or serious head injuries in the past, and no history of schizophrenia or parkinsonism among first degree relatives. Participants were

also required not to drink more than three units of alcohol in the 24 hours prior to testing and not to use any recreational drugs for at least three days prior to testing (for the drug using groups). Only male volunteers above the age of 25 were included because the local ethics committee has imposed restrictions on exposing young adults and healthy women of childbearing age to ionising radiation. All participants had a neurological examination to detect any signs of parkinsonism. The participants in this study were part of a larger cohort of volunteers who took part in a related neuropsychological study (Hoshi *et al*, 2007), where a separate subgroup also had ^{11}C -DASB PET to assess serotonin transporter status (Selvaraj *et al*, 2009).

PET scanning and neuropsychological testing were performed on the same day. Each participant also provided urine and hair samples on the day which were analyzed to corroborate their account of recent and longer term history of drug use respectively. The hair and urine samples were tested using enzyme-multiplied immunoassay to detect the presence of amphetamines, cocaine and methamphetamines groups of drugs, while the urine samples were tested additionally for opiates, benzodiazepines, barbiturates and cannabis. If the initial screen was positive for amphetamines, cocaine, methamphetamines or opiates, the sample was analyzed further by gas/liquid chromatography-mass spectrometry to determine the actual substance present. Most recreational drugs persist in the urine for 48 to 72 hours after ingestion apart from cannabis, which could be detected for two to three weeks after use. Every 1cm of hair length approximates to a one-month window of detection. The duration tested is limited by the length of hair available for analysis (Pragst and Balikova, 2006). Hair analysis was performed by TrichoTech Ltd,

Cardiff, UK. If the urine or hair analysis showed that the participants no longer fulfilled the inclusion criteria, then they would be excluded from further analysis.

The study was approved by the local research ethics committee and permission to administer ^{18}F -dopa was granted by the Administration of Radioactive Substances Advisory Committee of the UK. All participants gave written informed consent to take part in this study in accordance with the Declaration of Helsinki.

PET scanning

All participants had one ^{18}F -dopa PET. PET was performed in the morning and participants were asked to have a light breakfast at least four hours before imaging and to avoid caffeine for at least 12 hours. Participants arrived at our PET unit at least two hours before the scan, so that they could settle down in a controlled environment. They were given orally 150mg of carbidopa and 400mg of entacapone one hour before the injection of ^{18}F -dopa to block the activity of peripheral aromatic amino acid decarboxylase and catechol-*O*-methyl transferase respectively. PET was performed using an ECAT EXACT HR++ (Knoxville, TN) camera. The camera has a transaxial spatial resolution of $4.8 \pm 0.2\text{mm}$ and axial resolution of $5.6 \pm 0.5\text{mm}$ after image reconstruction. A 5-minute transmission scan was performed prior to injection of ^{18}F -dopa to correct for tissue attenuation of 511keV γ -radiation. 114.5 ± 4.7 (mean \pm SD) MBq of ^{18}F -dopa was injected intravenously over 30 seconds, and the dynamic emission data was acquired as 26 time-frames over 95 minutes.

Image analysis

The investigator (Y.F.T.) analysing the scans was blinded to the drug use history of the participants.

The ^{18}F -dopa dynamic images were corrected for between-frame head movements during PET scans by applying a frame-by-frame realignment paradigm (Lang *et al*, 2006; Montgomery *et al*, 2006). Briefly, the non-attenuation corrected dynamic images were first denoised using a Battle-Lemarie wavelet (Turkheimer *et al*, 1999). Frames 4 to 26 of dynamic scans were co-registered to frame 3 (the first frame with high signal-to-noise ratio) and the transformation matrices were applied to the corresponding frames of the attenuation corrected dynamic images.

^{18}F -dopa images were analyzed as previously described (Whone *et al*, 2004). Individual parametric images of ^{18}F -dopa influx rate constant (Ki maps) were created using a standard multiple-time graphical approach (Patlak and Blasberg, 1985) with an occipital reference input function. An “ADD” image of integrated ^{18}F -dopa signal from 30 to 90min was also created for each participant. The ADD images were then spatially normalised to an in-house ^{18}F -dopa template in standard stereotactic (MNI) space using statistical parametric mapping (SPM) software (Wellcome Functional Imaging Laboratory, London). The transformation matrices were then applied to the corresponding Ki maps. A standard region-of-interest object map that outlined putamen, heads of caudate nucleus and ventral striatum was defined on the ^{18}F -dopa template with magnetic resonance imaging guidance (Whone *et al*, 2004). This object map was then

applied to each normalised parametric Ki map to measure individual Ki values. For each subject, the left- and right-sided Ki values were averaged.

Cognitive and mood assessments

The participants in this study form part of a larger cognitive study, and the cognitive and mood assessments used have been described elsewhere (Hoshi *et al*, 2007). They included, amongst others, tests which are known to tap dopaminergic and serotonergic functions: immediate and delayed prose recall (Rivermead Behavioural Memory Tests; Wilson *et al*, 1985); the Buschke Selective Reminding Task (BSRT; Buschke and Fuld, 1974); Go/No-go task (Mesulam, 1985); Rapid Visual Information Processing (Wesnes and Warburton, 1983); the Serial Sevens task (Hayman, 1942); semantic and phonemic verbal fluency; the Trail Making Test (Reitan, 1959); CANTAB spatial working memory (Owen *et al*, 1990); CANTAB Stockings of Cambridge (Owen *et al*, 1995); and Gibson's spiral maze (Gibson, 1977). Impulsivity was assessed using the Barratt Impulsiveness Scale (BIS; Barratt and Patton, 1983) and aggression was measured using the Aggression Questionnaire (AQ; Buss and Perry, 1992).

Drug use history

A semi-structured interview was carried out on all participants to ascertain their levels of drug usage. For each drug, a lifetime estimate of amount used was obtained using the formula: (number of days used per month) x 12 x (number of years of regular use) x (dosage per session).

Statistical analysis

Statistical analysis was performed using SPSS v16 (SPSS Software Inc, Chicago, IL). The Kolmogorov-Smirnov test confirmed the normal distribution of drug-use data. The majority of group differences were assessed using one-way ANOVA. The prose recall task was analyzed using repeated measure ANOVA. Post-hoc comparisons were performed with a Bonferroni correction and the significance level was set at $p=0.05$. Chi-squared tests were used to compare non-parametric data. Pearson's correlations were used to explore the relationship between PET data, drug use and cognitive performance. Due to the number of correlations performed, $\alpha=0.01$ was adopted to minimise the probability of type I errors.

RESULTS

Demographic details and drug use history

A total of 46 participants were tested: 17 EE, 16 PC and 13 DC. Several participants were eventually excluded from analysis as they were found not to fulfil the inclusion criteria: three EE (two tested positive for cocaine in urine analysis indicating recent cocaine use, one reported the use of ecstasy four months prior to testing), two PC (one due to scan failure and one tested positive for MDMA in hair analysis) and one DC (tested positive for MDMA in hair analysis). Thus, the final analysis included 14 EE, 14 PC and 12 DC. One PC was diagnosed with 'depressed mood' by his family doctor more than a year prior to his PET study and had a trial of a selective serotonin reuptake inhibitor for two weeks. He decided to discontinue the medication after that as he felt he was much better.

There was no other history of psychiatry disorder or history of use of psychotropic drugs amongst the participants.

The demographic details of the participants are listed in table 1. There were no group differences in age, pre-morbid IQ (Spot the Word), depression, anxiety, aggression and impulsivity (Table 1), or groups in level of education attained and current employment status (not listed). All participants had a normal neurological examination. The EE have on average been using ecstasy for 4.38 years but have abstained from it for 3.22 years (table 2). There was no significant difference in the drug use pattern of EE and PC (table 3). A group difference was found in units of alcohol consumed per week ($F_{2,39}=4.479$, $p=0.018$), with the PC reported drinking more than DC ($p=0.02$, corrected). Three participants tested positive for cocaine on hair analysis: one EE (within last three months based on hair analysis, self-reported last use 5 days ago) and two PC (one PC within last three months, self reported last use 30 days ago; the second PC within last 3.5 months, self reported last use 5 days ago). The remaining participants tested negative on hair analysis. Excluding these three participants, the average length of hair tested was approximately 3cm in all three groups, equivalent to 3 months being free from amphetamines, cocaine and methamphetamines groups of drugs (table 3). Three participants also tested positive for cannabis in urine: one EE (the same participant who also tested positive for cocaine on hair analysis) and two PC.

Striatal ^{18}F -dopa uptake

One-way ANOVA detected a significant group difference in the putamen ^{18}F -dopa uptake [$(F_{2,39}=4.108, p=0.024)$; table 4 & figure 1]. Post-hoc comparison revealed the EE had 9% higher putamen ^{18}F -dopa uptake than the DC ($p=0.021$, corrected). The putamen ^{18}F -dopa uptake of PC fell between that of EE and DC but there were no significant group differences. There were no significant group differences in ^{18}F -dopa uptake in the ventral striatum and caudate nucleus, though these were 6% and 5% higher in the EE than the DC. Exclusion of the five participants who tested positive for cocaine and cannabis on hair and urine analysis had minimal impact on the results [putamen ^{18}F -dopa uptake ($F_{2,34}=3.969, p=0.029$); EE > DC, $p = 0.025$, corrected].

To further ensure that the differences seen in the putamen ^{18}F -dopa uptake were not due to the influences of other recreational drugs which could potentially affect dopaminergic activity, we performed an ANCOVA with putamen ^{18}F -dopa uptake as the dependent variable, participants' drug-use group as fixed factor and lifetime estimated amount of amphetamine, cocaine and cannabis as covariates. We also included amount of alcohol consumed as a covariate since there were group differences in its consumption.

ANCOVA confirmed a significant effect of group on the putamen ^{18}F -dopa uptake ($F_{2,33}=4.116, p=0.025$), with the EE having higher values than the DC ($p=0.024$, corrected). The effects of other recreational drugs on putamen ^{18}F -dopa uptake were not significant.

Neuropsychological data

Data for two participants (one EE and one DC) on the RVIP and Go/No-Go were lost due to computer failure.

A significant group difference was observed on the first trial of the BSRT ($F_{2,39}=5.85$, $p=0.006$). Post-hoc tests revealed that both the EE ($p=0.04$) and PC ($p=0.01$) recalled fewer words than DC. Analysis of the Go/No-Go task (table 5) revealed a significant group difference on the number of hits ($\chi^2=7.57$, $df = 2$, $p=0.023$), with the PC making significantly fewer hits than the EE ($p=0.008$). A group difference was also found in number of false alarms ($F_{2,37}=6.16$, $p=0.005$): PC made more false alarms than both EE ($p=0.01$) and DC ($p=0.02$). A group difference was observed on reaction times to hits ($F_{2,37}=0.395$, $p=0.028$). PC reacted faster to hits than EE ($p=0.03$). There were no significant group differences in other assessments.

Correlations

Ex-ecstasy users group

^{18}F -dopa uptake in the putamen correlated negatively with the total number of false alarms on the Go/No-Go task ($r=-0.815$, $p=0.001$) and correlated positively with reaction time to hits ($r=0.761$, $p=0.003$). There was no correlation between striatal ^{18}F -dopa uptake and the following: lifetime amount of ecstasy or other recreational drugs, time since last use of ecstasy and O-LIFE scores (see below).

Assessment of schizotypy

The striatal hyperdopaminergic state found in EE was not predicted *a priori*. The main disorder in the literature that demonstrates a consistent association with a hyperdopaminergic state is psychosis, usually in the context of schizophrenia (Hietala *et al*, 1995; Howes *et al*, 2007). We therefore performed a post-hoc assessment using the Oxford-Liverpool Inventory of Feelings and Experiences (O-LIFE) short scale (Mason *et al*, 2005) to measure the participants' schizotypy or psychosis-proneness as none of our participants has frank psychotic symptoms. O-LIFE consists of four subscales, Unusual Experiences, Cognitive Disorganisation, Introverted Anhedonia and Impulsive Nonconformity (table 6). It was administered within three months of completion of their PET scans. The rate of response and the O-LIFE scores are listed in table 6. There were no group differences in the response rates or O-LIFE scores. The relatively poor response rate was probably due to the post-hoc nature of the questionnaire with associated drop-outs.

DISCUSSION

This is the first study looking into the long-term effects of ecstasy exposure, in the setting of polydrug use, on nigrostriatal dopaminergic system after a prolonged abstinence. We have detected an increase in the putamen ^{18}F -dopa uptake in a group of male EE, who had abstained for over three years (mean), compared to DC. The putamen ^{18}F -dopa uptake of PC lay between that of EE and DC, suggesting that the difference seen between the latter two groups may be due to the combined effects of ecstasy and polydrug use rather than ecstasy alone. The lack of correlation between striatal ^{18}F -dopa uptake and ecstasy use

also suggests that polydrug use could be an important factor. We have not demonstrated any evidence of ecstasy-induced nigrostriatal dopaminergic neurotoxicity.

As mentioned in the introduction, previous neuroimaging studies investigating the effects of ecstasy on nigrostriatal dopaminergic functions produced rather mixed results. There were significant methodological differences, including periods of abstinence and control groups chosen, with our study which made direct comparisons difficult. None of the studies mentioned used hair analyses to corroborate the drug use history of their participants. Semple and colleagues studied current ecstasy users, not ex-users, and they did not include any drug-naïve controls (Semple *et al*, 1999). McCann and colleagues did not detect any difference in DAT binding in ecstasy users who had been abstinent for 2.75 months (mean) and a group of controls with low-level polydrug use (McCann *et al*, 2008); but they did not include a drug-naïve control group which may partially explain the lack of significant difference in DAT binding. Reneman *et al* reported significantly higher striatal DAT binding in a group of ecstasy users who had never used amphetamine and who had been abstinent from ecstasy for a mean 3.4 months, compared to a group of ecstasy users who had also used amphetamine and a group of controls who had never used these two drugs (Reneman *et al*, 2002). The conclusion of the study focused on the ‘lower’ DAT binding with amphetamine use, which in fact was not different from the controls, rather than the increase in DAT binding with ecstasy use. The authors proposed that amphetamine, a probable dopamine neurotoxin, in conjunction with ecstasy might be responsible for the lower DAT binding compared to ecstasy users naïve to amphetamine. Of the three studies quoted above, the latter study had a more similar set-up with our

study, with a relatively drug-free control groups. Taken together with their findings, our results are compatible with the presence of a striatal hyperdopaminergic state in ex-ecstasy users and its persistence after an abstinence from ecstasy for over 3 years mean suggests that the effects maybe long-lasting.

A post-mortem study found increased homovanillic acid, a dopamine metabolite, in the putamen of the single MDMA chronic user studied compared to drug-naïve controls (Kish *et al*, 2000), indicating possible increased dopamine turnover. Other studies have also shown increased cerebrospinal fluid and plasma homovanillic acid in patients with psychosis (Maas *et al*, 1997; Pickar *et al*, 1986; Ramirez-Bermudez *et al*, 2008), a condition linked to hyperdopamnergic state. An increase in striatal ^{18}F -dopa uptake has been reported in neuropsychiatric conditions particularly schizophrenia and psychosis - including those with prodromal psychotic symptoms (Howes *et al*, 2007; Howes *et al*, 2009); whilst less consistent findings have been seen in Tourette syndrome and attentional deficit hyperactivity disorder (Nikolaus *et al*, 2009), which are mainly disorders of childhood onset. The magnitude of increase in putamen ^{18}F -dopa uptake in the EE compared to DC in our study is similar to that seen in the at-risk mental state subjects who have prodromal symptoms of schizophrenia (Howes *et al*, 2009). The O-LIFE questionnaire in our study did not reveal any significant differences in schizotypy amongst our participants but the results are limited by the variable response rate, and the retrospective and cross-sectional nature of our survey. Chronic ecstasy use may lead to multiple long-term neuropsychiatric consequences including psychosis (McGuire *et al*, 1994; Montoya *et al*, 2002; Winstock, 1991). One study looked at substance abuse in

patients presenting with first-episode psychosis and found that age at first use of cannabis, cocaine, ecstasy and amphetamine was independently associated with age at first psychotic symptom (Barnett *et al*, 2007).

The underlying molecular mechanisms for ecstasy-related hyperdopaminergic state are still unclear but our findings suggest they may be related to synergistic or interactive effects with other recreational drugs. The increased striatal ^{18}F -dopa uptake and DAT binding are consistent with increased dopamine turnover. This may be a reaction to chronic exposure to a dopamine depleting agent or may reflect long-term sensitization of the nigrostriatal dopaminergic system to MDMA, where repeated exposure to a stimulant drug resulted in heightened dopamine release after re-exposure, as demonstrated *in vivo* with amphetamine one year following limited exposure to the substance (Boileau *et al*, 2006). Acutely, MDMA increases DOPA accumulation in the striatum which is blocked by 5-HT₂ antagonist ketanserin, suggesting that MDMA activates nigrostriatal dopaminergic pathways via 5-HT₂ receptors (Nash *et al*, 1990). It is uncertain if this effect persists after repeated dosing or prolonged abstinence but this mechanism could offer a potential explanation for our findings.

An alternative, albeit speculative, explanation for our findings is that there are pre-existing differences in the nigrostriatal dopaminergic system between ecstasy and non-ecstasy users, i.e. the hyperdopaminergic state leads to a desire to use stimulants such as ecstasy. We do not have baseline or pre-drug use data on these participants. However, a longitudinal multi-modality neuroimaging study did not reveal any difference in the

baseline neuroimaging data between subjects who remained ecstasy-naïve versus those who started using ecstasy at follow-up, though dopaminergic function was not measured (de Win *et al*, 2008).

Cognitive assessments revealed minor differences across groups. Both drug-using groups were impaired on the BSRT, indicating they had a shorter immediate memory span. The lack of major cognitive impairment in the EE is in line with other published studies (Hoshi *et al*, 2007; McCann *et al*, 2008). These cognitive tests probably have lower sensitivity than PET in detecting neurochemical abnormalities in the drug users as previous studies have demonstrated reduced serotonin transporters in ecstasy users but without concomitant changes in their cognitive performances (McCann *et al*, 2008). Higher putamen ^{18}F -dopa uptake in EE correlated with fewer false alarms but a slower reaction time to hits on a choice reaction time task. The significance of these correlations is unclear and it needs to be interpreted with caution since there were no differences between EE and DC in these parameters. We excluded participants with significant anxiety or depression, which can often be seen in chronic drug users. This may also explain the lack of group differences in the personality traits and psychological data in table 1.

There are several potential limitations of our study. As with all studies involving recreational drug use, we have to rely on the participants' subjective recall of their drug use history. We attempted to corroborate that as far as possible with urine and hair analysis but inevitably recall biases or errors could occur. The small number of

participants tested positive for cannabis on urine analysis and cocaine on hair analysis indicated that the confounding effects from recent use of other drugs were likely to be small. Excluding these participants from analysis or incorporating other drug use as covariates in the ANCOVA analysis did not alter our study results. The average duration of three months being free from amphetamines, cocaine and methamphetamines groups of drugs as shown on hair analysis also implied that the impact from recent ingestion of these drugs on the dopaminergic system was likely to be small. Nevertheless, we had not been able to test for the longer history of drug use especially with regards to ecstasy to verify that the EE had indeed been abstain from the drug for more than a year. Whilst we tried to minimize the confounding effects of polydrug use by matching EE and PC groups, in reality it was difficult to do so exactly especially when we also had to rely on subjective recalls of the participants. One further limitation is that the purity of recreational drugs taken by the participants in our study cannot be ascertained. Our study included male participants only, and hence the results may not be generalised to female EE. One study has reported that women might be more susceptible than men to MDMA-induced alterations of the serotonergic system (Reneman *et al*, 2001). The EE showed a modest 9% increase in the putamen ^{18}F -dopa uptake compared to DC. Whilst this is significantly greater than the striatal ^{18}F -dopa uptake test-retest variability of about 5% (Egerton *et al*, 2010) and of a similar magnitude seen in at-risk mental state subjects with prodromal symptoms of schizophrenia (Howes *et al*, 2009), it would be prudent to repeat this in a larger scale study. Finally, we studied participants with a history of recreational drug use only. Therefore, our results may not be applicable to those with drug addiction and heavy use.

The long-term sequelae of our findings are as yet uncertain, and follow-up of the ex-ecstasy users would be important to evaluate any development of psychopathology or further changes in dopaminergic function. Nevertheless, our study highlights potential long-term adverse effects from recreational use of a very popular illicit substance, in the setting of polydrug use, with consequent public health implications. Larger scale prospective studies (de Win *et al*, 2008) would be able to confirm and to further delineate the effects of ecstasy on human nigrostriatal dopaminergic system, whilst avoiding difficulties and limitations frequently encountered with cross-sectional drug studies as outlined above.

Conflicts of interest

DJB serves or has served on scientific advisory boards for CeNes, Synosia, Genentech, GlaxoSmithKline, and Orion; receives funding for travel from TEVA; serves or has served as an editorial board member for the *Journal of Neural Transmission*, *Brain*, *Movement Disorders*, the *Journal of Neurology, Neurosurgery and Psychiatry*, and *Synapse*; has received speaker honoraria from GlaxoSmithKline and Orion Pharma; serves as Head of Neurology for GE Healthcare; and receives research support from the Alzheimer's Research Trust and the Medical Research Council Clinical Sciences Centre. PP serves as a member of The Research Advisory Panel for the Parkinson's disease society UK and receives research support from the Parkinson's disease society UK and the Michael J Fox Foundation US. The other authors declare that they have no conflicts of interest.

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Table 1: Group means (standard deviations) for age, pre-morbid IQ (Spot The Word), depression (BDI), anxiety (STAI), aggression (AQ) and impulsivity (BIS). The range of possible scores for each of the rating scale is listed in the first column. There was no significant difference between groups for any of the items

	Ex-ecstasy users	Polydrug controls	Drug-naïve controls
Age (yrs)	31.07 (5.62)	30.50 (7.30)	30.58 (8.15)
Spot The Word (0-60)	51.79 (3.09)	49.79 (4.49)	50.67 (3.98)
BDI (0-63)	8.07 (6.08)	7.71 (4.29)	5.42 (6.36)
STAI (20-80)	38.93 (10.64)	37.50 (6.73)	36.25 (8.47)
AQ (29-145)	72.79 (21.24)	74.36 (13.80)	72.25 (17.75)
BIS (30-120)	53.50 (13.27)	55.29 (17.53)	52.25 (16.13)

Table 2: Pattern of previous ecstasy use in ex-users

	Mean (SD)	Range
Time since last use (years)	3.22 (2.77)	1-10.5
Years of regular use	4.38 (2.88)	1-12
Frequency of use (days per month)	5.75 (3.37)	1-12
Number of tablets used in a typical session	2.32 (0.91)	0.5-4
Lifetime amount (tablets)	754.71 (750.38)	25-2520

Table 3: Group means (standard deviations) for alcohol, cannabis, amphetamine, and cocaine use; and length of hair tested for amphetamines, cocaine and methamphetamines use‡ (excludes 1 EE and 2 PC who tested positive for cocaine). Significant comparison:

*PC > DC (p=0.02, corrected).

	Ex-ecstasy users (EE)	Polydrug controls (PC)	Drug-naïve controls (DC)
Alcohol			
Units per week*	19.35 (16.11)	23.03 (17.32)	6.84 (5.01)
Days since last use	16.57 (47.25)	4.29 (3.63)	64.17 (209.71)
Cannabis			
Weeks since last use	82.95 (129.98)	45.87 (62.26)	
Years of regular use	8.58 (5.76)	7.15 (3.27)	
Amount used per month (oz)	1.09 (1.37)	1.58 (1.65)	
Lifetime amount (oz)	143.36 (314.32)	100.83 (95.78)	
Amphetamine			
Weeks since last use	379.97 (312.59)	362.63 (457.92)	
Years of regular use	4.17 (2.99)	4.67(5.20)	
Dose per session (g)	0.83 (0.26)	1.06 (0.58)	
Lifetime amount (g)	42.65 (82.41)	346.73 (550.54)	
Cocaine			
Weeks since last use	53.03 (120.75)	70.52 (103.68)	
Years of regular use	3.39 (1.80)	5.22 (4.02)	

Dose per session (g)	1.09 (1.04)	1.12 (0.88)	
Lifetime amount (g)	399.26 (645.50)	199.40 (330.89)	
Length of hair tested for amphetamines, cocaine and methamphetamines use (cm)‡	3.00 (1.17)	2.83 (0.39)	2.88 (0.74)

Table 4: Mean (and standard deviations) striatal ^{18}F -dopa influx rate constant (K_i ; min^{-1}) in the three groups of participants. Significant comparison: *EE > DC ($p=0.021$, corrected).

	Ex-ecstasy users	Polydrug controls	Drug-naïve controls
	(EE)	(PC)	(DC)
Caudate	0.01468 (0.00134)	0.01473 (0.00134)	0.01397 (0.00141)
Putamen*	0.01591 (0.00144)	0.01532 (0.00110)	0.01455 (0.00100)
Ventral striatum	0.01390 (0.00114)	0.01339 (0.00104)	0.01310 (0.00121)

Table 5: Means (SD) for total number of hits, false alarms, reaction time to hits and reaction times to false alarms on the Go/No-Go task. Significant comparisons: *EE > PC (p=0.008); ‡PC > EE (p=0.01) and PC > DC (p=0.02); §EE > PC (p=0.03). All p values are corrected.

	Ex-ecstasy users (EE)	Polydrug controls (PC)	Drug-naïve controls (DC)
Total hits*	70.31 (0.84)	66.21 (5.56)	69.36 (2.42)
Total false alarms‡	8.62 (4.19)	13.64 (4.73)	8.73 (3.47)
Reaction time to hits (msec)§	404.05 (48.61)	352.95 (45.05)	384.43 (49.85)
Reaction time to false alarms (msec)	363.51 (42.49)	320.51 (37.51)	354.27 (68.76)

Table 6: The mean (SD) Oxford-Liverpool Inventory of Feelings and Experiences (O-LIFE) scores of the participants. The range of possible scores for each of the subscale is listed in the first column (brackets). Population mean scores are derived from Mason *et al.*, (2005) but unmatched for age of the participants in this study. There was no significant difference between groups for any of the items.

	Ex-ecstasy users	Polydrug controls	Drug-naïve controls	Population mean
Responses (%)	9/14 (64.3)	12/14 (85.7)	7/12 (58.3)	
Unusual experiences (0-12)	2.13 (1.81)	4.18 (2.86)	3.00 (1.26)	3.17 (2.92)
Cognitive Disorganisation (0-11)	5.25 (3.92)	5.09 (2.74)	5.40 (2.65)	4.28 (3.00)
Introvertive Anhedonia (0-10)	2.75 (2.12)	2.91 (2.30)	2.60 (2.33)	2.80 (2.16)
Impulsive Nonconformity (0-10)	2.88 (2.36)	3.91 (2.34)	4.80 (1.60)	2.70 (1.99)

Figure legends

Figure 1: Mean ^{18}F -dopa uptake in the putamen of ex-ecstasy users, polydrug-using controls and drug-naïve controls. Error bars indicate standard deviations.

Significant comparison: *Ex-ecstasy users > drug-naïve controls ($p=0.021$, corrected).

