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Decreased cerebral cortical serotonin transporter binding in ecstasy users: a positron emission tomography/[^{11}C]DASB and structural brain imaging study

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Animal data indicate that the recreational drug ecstasy (3,4-methylenedioxymethamphetamine) can damage brain serotonin neurons. However, human neuroimaging measurements of serotonin transporter binding, a serotonin neuron marker, remain contradictory, especially regarding brain areas affected; and the possibility that structural brain differences might account for serotonin transporter binding changes has not been explored. We measured brain serotonin transporter binding using [^{11}C] *N,N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine in 50 control subjects and in 49 chronic (mean 4 years) ecstasy users (typically one to two tablets bi-monthly) withdrawn from the drug (mean 45 days). A magnetic resonance image for positron emission tomography image co-registration and structural analyses was acquired. Hair toxicology confirmed group allocation but also indicated use of other psychoactive drugs in most users. Serotonin transporter binding in ecstasy users was significantly decreased throughout all cerebral cortices (range –19 to –46%) and hippocampus (–21%) and related to the extent of drug use (years, maximum dose), but was normal in basal ganglia and midbrain. Substantial overlap was observed between control and user values except for insular cortex, in which 51% of ecstasy user values fell below the lower limit of the control range. Voxel-based analyses confirmed a caudorostral gradient of cortical serotonin transporter binding loss with occipital cortex most severely affected. Magnetic resonance image measurement revealed no overall regional volume differences between groups;

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however, a slight left-hemispheric biased cortical thinning was detected in methamphetamine-using ecstasy users. The serotonin transporter binding loss was not related to structural changes or partial volume effect, use of other stimulant drugs, blood testosterone or oestradiol levels, major serotonin transporter gene promoter polymorphisms, gender, psychiatric status, or self-reported hyperthermia or tolerance. The ecstasy group, although 'grossly behaviourally normal', reported subnormal mood and demonstrated generally modest deficits on some tests of attention, executive function and memory, with the latter associated with serotonin transporter decrease. Our findings suggest that the 'typical'/low dose (one to two tablets/session) chronic ecstasy-polydrug user might display a highly selective mild to marked loss of serotonin transporter in cerebral cortex/hippocampus in the range of that observed in Parkinson's disease, which is not gender-specific or completely accounted for by structural brain changes, recent use of other drugs (as assessed by hair analyses) or other potential confounds that we could address. The striking sparing of serotonin transporter-rich striatum (although possibly affected in 'heavier' users) suggests that serotonergic neurons innervating cerebral cortex are more susceptible, for unknown reasons, to ecstasy than those innervating subcortical regions and that behavioural problems in some ecstasy users during abstinence might be related to serotonin transporter changes limited to cortical regions.

Keywords: MDMA; ecstasy; PET; serotonin transporter; methamphetamine; cortical thickness

Abbreviations: BP_{ND} = non-displaceable binding potential; CVLT = California Verbal Learning Test; DASB = *N,N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine; HAM = Hamilton; IDS = Inventory of Depressive Symptomatology; MDA = 3,4-methylenedioxyamphetamine; MDMA = 3,4-methylenedioxymethamphetamine; MNI = Montreal Neurological Institute; MRTM2 = multilinear reference tissue model 2; NART = National Adult Reading Test; PASAT = Paced Auditory Serial Addition Test; SERT = serotonin transporter; SIGHD/SAD = Structured Interview Guide for the Hamilton Depression Rating Scale and Seasonal Affective Disorder scale; SRTM2 = simplified reference tissue model 2; WASI = Wechsler Abbreviated Scale of Intelligence

Introduction

Ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is an analogue of methamphetamine that is widely used recreationally and is also being tested in clinical trials for the treatment of post-traumatic stress disorder (Bouso *et al*., 2008). Ecstasy and methamphetamine produce increased energy and euphoria at higher doses (Dumont and Verkes, 2006); however, recreational interest in ecstasy appears chiefly related to distinct positive social and emotional effects (e.g. increased sociability, closeness and emotional well-being) (Cohen, 1995). In preclinical studies, ecstasy causes release of the neurotransmitter serotonin (Schmidt *et al*., 1986; Stone *et al*., 1986; Gough *et al*., 1991) probably via an action at the serotonin transporter (SERT) (Rudnick and Wall, 1992; Verrico *et al*., 2007). The involvement of SERT is suggested by animal data showing the blockade of ecstasy-induced serotonin release following exposure to selective serotonin reuptake inhibitors (Schmidt *et al*., 1987; Hekmatpanah and Peroutka, 1990) and in SERT-knockout mice (Renoir *et al*., 2008), as well as by reports in humans of antagonism, increased sociability and other ecstasy effects in human users pre-treated with selective serotonin reuptake inhibitors (Liechti *et al*., 2000; Liechti and Vollenweider, 2000).

Animal data also indicate that high-dose ecstasy exposure can cause long-term reduction in brain serotonin markers (Ricaurte and McCann, 2001). Direct measurement of major brain serotonin markers in human ecstasy users has only been accomplished in our single post-mortem brain study of a high-dose user that showed markedly decreased levels of all serotonin neuron markers: serotonin itself, its metabolite, 5-hydroxyindoleacetic acid, and protein concentrations of its rate limiting biosynthetic enzyme, tryptophan hydroxylase and of its transporter (SERT) (Kish *et al*., 2010). To establish whether damage to the brain serotonin system might occur in living human ecstasy users, a variety of radioligand binding brain imaging studies have investigated SERT binding status in subjects withdrawn from the drug. However, findings continue to be inconsistent (even within the same laboratory) with respect to presence, magnitude and regional extent of brain areas affected [e.g. massive global brain (McCann *et al*., 1998*a*) versus regionally selective reduction (McCann *et al*., 2005, 2008); striatal involvement (caudate, putamen; Buchert *et al*., 2007) versus no striatal involvement (McCann *et al*., 2005); cerebral cortical reduction (Reneman *et al*., 2001) versus no cerebral cortical reduction (de Win *et al*., 2008*b*)] and appear in some cases to be dependent on the method of quantification of SERT binding (Buchert *et al*., 2004 versus Buchert *et al*., 2007).

The lack of consistency is due in part to use of radioligands not specific for SERT ([^{123}I]β-CIT; e.g. Reneman *et al*., 2001; de Win *et al*., 2008*a*) or with only modest specific to non-specific binding ratio ([^{11}C]McN5652; e.g. Buchert *et al*., 2003; see Frankle *et al*., 2004 for discussion). Consequently, inadequate measurements in some regions (particularly neo-cortex) were not sufficiently powered or sensitive, or had highly variable data (e.g. McCann *et al*., 1998*a*). Most investigations also failed to prove by drug analysis that subjects used ecstasy on even a single occasion (e.g. McCann *et al*., 1998*b*; Reneman *et al*., 2001; de Win *et al*., 2008*a*; Selvaraj *et al*., 2009). This is a highly relevant concern as ecstasy users are often not aware of the drugs provided in the 'cocktail' and may be untruthful with respect to other drugs used (Kalasinsky *et al*., 2004).

The present imaging investigation, employing SERT binding as the primary outcome measure, used a highly specific PET

radioligand capable of measuring both cerebral cortical and subcortical brain areas, and was designed to address systematically the above and other issues already identified in the literature (Kish, 2002). We utilized procedures including scalp hair analysis in a large sample size (49 users, 50 controls) to provide proof-of-use of ecstasy or other stimulants (especially methamphetamine and cocaine), as these stimulants can also act on the brain serotonin system (Ramamoorthy and Blakely, 1999); a detailed questionnaire providing additional information supporting ecstasy use and identifying behaviour (e.g. hyperthermia during ecstasy exposure) that might influence any drug toxicity (Parrott, 2002); assessment of major SERT promoter gene variants (5-HTTLPR; Lesch *et al*., 1996; Praschak-Rieder *et al*., 2007) and blood levels of two hormones (Rehavi *et al*., 1987; Lu *et al*., 2003) that might affect concentration of brain SERT; a voxel-based procedure to confirm region of interest analysis; and efforts to address possible confound of tissue loss (partial volume adjustment, MRI-based structural imaging assessment). The latter issue is especially relevant given the report of brain tissue loss in ecstasy users (Cowan *et al*., 2003), and the lack of attention to this possible confound in previous SERT imaging investigations. To make our findings generally applicable, we selected for investigation subjects who could be considered 'typical' ecstasy users (i.e. one to two tablets per session; 200 lifetime tablets). By employing a large cohort of confirmed (by hair toxicology) ecstasy users and by addressing comprehensively a variety of potentially important confounds and methodological issues, this investigation may help bring some consensus to the question of ecstasy and brain serotonin marker changes.

Materials and methods

Subject recruitment and screening

This study was approved by the Centre for Addiction and Mental Health Research Ethics Board. Subjects were recruited from the Toronto area by advertisements. Potential subjects underwent a full assessment interview which included (i) psychiatric evaluation using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) Axis I disorders (First *et al*., 2002); (ii) assessment of general health using a self-report questionnaire; (iii) brief neurological evaluation (Unified Parkinson's Disease Rating Scale neurological exam: Fahn and Elton, 1987); (iv) urine pregnancy screen in females (exclusion for PET); (v) height and weight recording for body mass index calculation; and (vi) completion of mood [Structured Interview Guide for the Hamilton Depression Rating Scale and Seasonal Affective Disorder scale (SIGHD/SAD) which includes the 17 and 21 item Hamilton (HAM) and 8 item Seasonal Affective Disorder scale (SAD): Hamilton, 1960; Hedlund and Vieweg, 1979; and Inventory of Depressive Symptomatology (IDS): Rush *et al*., 2000] and sleep quality questionnaires (Toronto Hospital Alertness Test: Shapiro *et al*., 2006; Zogim-A: Shapiro *et al*., 2006; Epworth Sleepiness Scale: Johns, 1991; Fatigue Severity Scale; Krupp *et al*., 1989). Current and past drug-use was assessed by: (i) a comprehensive drug-history questionnaire (structured and open-ended, locally developed) including subjective effects of acute administration and early and extended abstinence following ecstasy; (ii) a broad-spectrum (>900) urine drug screen (gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, immunoassay) for illicit and prescription drugs by our in-house CAMH Laboratory Service; (iii) test-strip urine drug-screens (9-Drug Test Panel, BTNX Inc., Markham, ON) conducted at each visit and just prior to PET scan; and (iv) a drug scalp-hair screen (Center for Human Toxicology, Salt Lake City, UT) using minor modification of the procedure of Kalasinsky *et al*. (2004).

Study inclusion criteria for control subjects included: (i) males and non-pregnant, non-lactating females between 18 and 40 years; (ii) free of psychotropic medication; (iii) without history of illicit drug use per hair/urine toxicology (with exception of cannabis); (iv) without personal or first-degree relative history of DSM-IV Axis I psychiatric disorder (with exception of nicotine dependence); and (v) without medical illness or injuries (brain trauma) that might conceivably affect the brain serotonin system. Study criteria for ecstasy users were the same as for control subjects with the exception of evidence for ecstasy use by positive hair screen and self-report. Ecstasy users meeting criteria for a DSM-IV Axis I condition (e.g. mood disorder) were excluded if the condition occurred before ecstasy use, but those meeting criteria for an Axis I condition after first use of ecstasy were not excluded. Drug hair testing provided information on some drugs (ecstasy, methylenedioxyamphetamine, methamphetamine, amphetamine, paramethoxyamphetamine, paramethoxymethamphetamine, ephedrine, cocaine, norcocaine, benzoylecgonine, codeine, morphine, 6-monacetylmorphine and phencyclidine) over a time period related to the length of hair, which grows ~0.5 inch/month. We relied on self-report for use of drugs prior to the time which could be inferred from drug hair analysis (e.g. ~6 months if hair is 3 inches in length) and for drugs that were not assessed by drug hair analysis [e.g. gamma-hydroxybutyrate (GHB), lysergic acid diethylamide (LSD)].

PET neuroimaging

Synthesis of [^{11}C]DASB (*N,N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine) and the image acquisition protocol has been previously described (Wilson *et al*., 2002; Meyer *et al*., 2004). PET dynamic emission scans were acquired using a high-resolution head-dedicated (CPS-HRRT) PET camera system (Siemens Medical Imaging, Knoxville, TN) in which head movement minimization was achieved with a head immobilization system (Tru Scan Imaging, Annapolis USA). The CPS-HRRT neuro-PET camera system has an intrinsic in plane resolution of ~2.8 mm full width at half maximum and measures radioactivity in 207 brain slices with a thickness of 1.2 mm each. After subjects were placed on the PET bed, transmission scans were obtained using a single-photon ^{137}Cesium ($E\gamma = 662$ keV) point-source, and used to correct the emission scans for the attenuation of 511 keV photons through tissue and head support. The 90 min emission data were acquired in list-mode following a bolus injection in the antecubital vein of [^{11}C]DASB (mean injected dose: 348 MBq; specific activity 1095 mCi/μmol). Raw data were scatter-corrected (Bailey *et al*., 1997). Fourier re-binning was applied to convert the sinograms into 2D data (Defrise *et al*., 1997). The 2D sinograms were reconstructed as described (Ginovart *et al*., 2001).

Subjects were allowed to smoke cigarettes on the day of the scan but were instructed to withhold alcohol consumption at least overnight (12 h before the scan) and all illicit drug-use for a minimum of 14 days.

Region of interest selection and analysis

A structural proton density weighted MRI scan (repetition time = 6000 ms; echo time = 17 ms; slice thickness 2 mm and zero

gap; 85 slices; field of view = 22 cm × 22 cm; 256 × 256, yielding a voxel size of 1.5 mm × 0.86 mm × 0.86 mm; slice thickness of 2 mm, number of excitations = 2) and a T_1-weighted scan (3D spoiled gradient recalled acquisition in the steady state; repetition time = 8.9–12 ms; echo time = 5.3–15 ms; flip angle of 45°; slice thickness 1.5 mm and zero gap; 124 slices; field of view 22 cm × 16 cm; 256 × 256 matrix, yielding a voxel size of 1.5 mm × 0.78 mm × 0.78 mm, number of excitations = 1) performed on a Sigma 1.5 T scanner (GE Medical Systems, Milwaukee, WI, USA) were acquired in every subject for PET image co-registration and segmentation purposes and for structural brain analysis (see below). Segmentation of the images for region of interest analysis was performed semi-automatically using in-house image analysis software following previously published methods (ROMI, Rusjan *et al*., 2006; see Boileau *et al*., 2009 for details). Regions of interest included hippocampus, thalamus, midbrain (including substantia nigra and part of the dorsal raphé), major striatal sub-compartments (Martinez *et al*., 2003), globus pallidus and all cortices including cingulate, insular, temporal, parietal, occipital, frontal and cerebellar. The latter was used as a reference region because of the very low density of SERT in this area (Kish *et al*., 2005). Only the posterior half of the cerebellar cortex excluding vermis and cerebellar white matter served as the reference region. In addition to the automated approach, cortical and sub-cortical regions of interest were manually delineated on the co-registered MRI by a research assistant blinded to group assignment using commercially available image analysis software.

Partial volume effect correction

Time activity curves extracted from regions of interest were adjusted for quantification errors (under and over estimation) resulting from the limited spatial resolution of the scanner (i.e. partial volume effect) by using the geometric transfer matrix (Rousset *et al*., 1998). Data uncorrected for partial volume effect were investigated as the primary PET outcome measure; the partial volume effect adjusted regional time activity curves were estimated to address potential bias resulting from possible volume loss in the ecstasy group.

Modelling/quantification of [^{11}C]DASB binding

[^{11}C]DASB binding was quantified using the non-invasive graphical approach of Logan *et al*. (1990) for estimation of reversible radioligand (implemented under PMOD; PMOD Technologies Ltd, Zurich, Switzerland) using cerebellar cortex as the reference tissue. This analysis method has previously been shown to provide valid and reproducible [^{11}C]DASB PET measurements of SERT non-displaceable binding potential (BP_{ND}, Innis *et al*., 2007) in regions of interest (Ginovart *et al*., 2001; Frankle *et al*., 2004; Meyer *et al*., 2004). For comparison, additional analyses were also carried out using the Modified Simplified-Reference Tissue Method (SRTM2, Wu and Carson, 2002), and Ichise Multilinear Reference Tissue Model (MRTM2, Ichise *et al*., 2003). The rationale for the use of these methods has been described in detail and reliability data have been previously published (Ginovart *et al*., 2001; Ichise *et al*., 2003).

Voxel-based analysis

Parametric images of [^{11}C]DASB BP_{ND} were generated (under ROMI) by estimating Logan at every voxel. The tissue time activity curve of the cerebellar reference region served as input function. The application of the model was performed on the 3D dyadic wavelet transformed dynamical PET image using ROMI. This approach has been shown to overcome noise susceptibility when solving linear models in the real-space and to be reliable across regions of different receptor density in the presence of noise (Turkheimer *et al*., 1999; Cselenyi *et al*., 2002, 2006).

Each parametric map was spatially normalized to an anatomical template [Montreal Neurological Institute (MNI) space] using SPM5 normalization and co-registration tools (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Once in the same space, BP_{ND} maps were statistically investigated to assess significant contrasts between groups using independent sample t-test analysis (SPM5). An implicit mask excluding voxel with BP_{ND} values <0.01 was applied to restrict statistical search to areas of specific binding (i.e. excluding cerebrospinal fluid, background and the reference region). The threshold for significant cluster was set to P (false discovery rate corrected) <0.05. This approach is aimed at detecting between group changes in neuroreceptor ligand binding at the voxel-level with no *a priori* anatomical hypothesis and enables circumvention of some limitations of region of interest placement; i.e. the investigation of regions that are not classified by our automatic segmentation (the pons) or that are problematic to define (e.g. amygdala).

Structural brain imaging

T_1-weighted MRI was used to investigate possible differences in the regional volume (of grey and white matter) or cortical thickness, which could (in principle) affect the partial volume effect and the true activity emitted and recovered from a given region of interest.

Each subject's T_1 image was submitted to the CIVET pipeline (version 1.1.7) (http://wiki.bic.mni.mcgill.ca/index.php/CIVET) developed at the Montreal Neurological Institute (Ad-Dab'bagh *et al*., 2006). The processing steps included registration to the symmetric ICBM 152 template (Mazziotta *et al*., 2001) with a 12-parameter linear transformation (Collins *et al*., 1994), correction for inhomogeneity artefact (Sled *et al*., 1998), skull stripping (Smith, 2002), tissue classification into white and grey matter, cerebrospinal fluid and background (Zijdenbos *et al*., 2002; Tohka *et al*., 2004) and neuroanatomical segmentation using ANiMAL (Collins Dl, 1995). Total volumes for each cortical lobe and sub-cortical structures were estimated for each individual by non-linearly warping each T_1 image towards a segmented atlas (Chakravarty *et al*., 2008). The regions of interest resulting from this segmentation were grossly similar (though not identical) to the ones delineated using ROMI. Volume (ml) was extracted from each of these regions using the *RMINC* package (version 0.4.) for reading and analysing MINC2 output files.

Along with regional measures of volume, cortical thickness was estimated as described (Lerch *et al*., 2005). After the main processing steps (described above) deformable models were used to first fit the white matter surface for each hemisphere separately, followed by an expansion outward to find the grey matter/cerebrospinal fluid intersection (MacDonald *et al*., 2000; Kim *et al*., 2005), resulting in 4 surfaces of 41 962 polygons each. From these surfaces the distance between the white and grey surfaces was used to measure cortical thickness (Lerch and Evans, 2005). The thickness data were subsequently blurred using a 20 mm surface-based diffusion blurring kernel (Chung and Taylor, 2004) and non-linearly aligned using surface based registration techniques (Robbins *et al*., 2004; Lyttelton *et al*., 2007) in preparation for statistical analyses. Un-normalized, native-space thickness values were used in all region of interest analyses owing to the poor correlation between cortical thickness and brain volume (Ad-Dab'bagh *et al*., 2005; Sowell *et al*., 2007).

A search for areas of different cortical thickness between-groups was initially conducted using a general linear model (using *R* environment for statistical computing within Linux; R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org/). Candidate regressors such as age, gender, education level and intelligence quotient (IQ) [National Adult Reading Test (NART) score] were investigated using step-wise regression (backward elimination). The resulting *t* statistical maps were thresholded to *P* (false discovery rate) < 0.05. Cortical thickness values (mm) were extracted from each vertex identified during the whole brain search using the *mni.cortical.statistics* tool and analysed for between group differences.

Neuropsychological testing

Each subject completed a broad battery of neuropsychological assessments which included tests of (i) general verbal and non-verbal intelligence (Wechsler Abbreviated Scale of Intelligence, WASI; Psychological Corporation, Wechsler, 1981) and premorbid IQ (NART/Barona index B, Nelson, 1982); (ii) executive function focusing on working-memory and mental-manipulation [Paced Auditory Serial Addition Test (PASAT), Gronwall, 1977; Auditory Consonant Trigrammes Test, Spreen and Strauss, 1998; Digit Ordering Test, Hoppe *et al*., 2000; Trail Making B, Reitan, 1958]; (iii) attention (Seashore Rhythm Test, Reitan, 1958; Trail Making A, Reitan, 1958; Symbol Digit Modalities, Smith, 1982); and (iv) memory including measures of encoding, delayed-recall, interference, cued-recall and (verbal and non-verbal) recognition [California Verbal Learning Test (CVLT), Delis *et al*., 2000; Warrington Recognition Test, Warrington, 1984; Denman Memory Scale, Denman, 1987].

Hormone levels

Oestradiol, testosterone and follicle-stimulating hormone were measured by high performance liquid chromatography mass-spectrometry and electrochemiluminescent assay (Esoterix Lab Service Inc., Calabasas Hills, CA) from venous blood samples taken on scan day.

DNA genotyping for SERT polymorphism

Peripheral blood samples were collected at time of scan. DNA was extracted from about 8 ml of blood from each sample using a high-salt extraction procedure. Polymerase chain reaction-based methods and high-resolution agarose gel electrophoresis were used for major SERT promoter gene polymorphism genotyping (Cook *et al*., 1997). DNA bands were then assigned allele numbers as described in Praschak-Rieder *et al*. (2007).

Statistical analysis

Outcomes related to SERT BP_{ND}, region of interest volume (ml) and cortical thickness (mm extracted from vertices) were analysed by standard repeated measures ANOVAs or analyses of covariance (ANCOVAs), with region of interest as a repeated factor and group as a cofactor (region of interest × group). Sphericity was assessed with the Mauchly test and, when indicated, corrections were made with Greenhouse–Geisser adjustments. When appropriate, least significant difference *t*-tests, Bonferroni corrected, were applied to determine the significance of regional differences in BP_{ND} between groups. Unpaired Student's *t*-tests and chi-square tests for non-parametric data and univariate ANCOVA's were used to investigate between-group differences in demographics, blood hormone levels and genotyping. Neuropsychological tests scores were investigated using *t*-tests or, for tests having more than one sub-score, sphericity-corrected repeated-measures ANCOVAs taking into account verbal IQ whenever appropriate (for test of verbal memory); Bonferroni correction for multiple comparisons were used. As we were only interested in cognitive performance 'deficits', a one-tailed probability value of $P < 0.05$ was chosen. Relationships between continuous variables were analysed with the Pearson product moment correlation coefficient and Spearman's Rank test for categorical data. Stepwise linear regression analysis was used to examine whether region of interest BP_{ND} in users could be predicted by qualifying variables including demographics, drug-use (e.g. years, max dose, frequency), subjective-effects (e.g. acute drug-induced hyperthermia), psychopathology or depressed mood or neurocognitive deficits.

Results

Demographic characteristics and drug profiles

Table 1 shows the major demographic characteristics of the subjects. Ecstasy users and control subjects were matched on age ($P = 0.9$) and groups were not statistically different in terms of gender composition [$\chi^2(1, n = 99) = 0.51$, $P = 0.48$] and race [$\chi^2(3, n = 99) = 1.8$, $P = 0.6$]. Relative to control subjects, ecstasy users had slightly lower body mass index ($P = 0.04$), education level ($P = 0.002$) and estimated premorbid IQ (NART-S; $P = 0.002$).

Drug history was ascertained by self-report and, for major stimulants and opioid drugs, verified by testing hair samples taken at interview, whereas a comprehensive drug toxicology screen was conducted in urine taken at interviews and just prior to PET scan (see Table 2 for drug use profiles). All ecstasy users reported using the drug by the oral route with the exception of a single subject who used ecstasy (taken from crushed tablets) intra-nasally. Patterns of ecstasy use were variable across the sample with self-reported estimates of cumulative lifetime (4–922 pills), monthly (0.2–60 pills), typical (0.5–10 pills) and maximum (1–20 pills) doses ranging across users. However, the majority was composed of typically low-dose users (one to two pills; 80%), more often consuming less than four pills a month (65%). Subjects used the drug for an average of 4 years, with only 10% of subjects using the drug for less than 1 year and 6% abstinent (based on self-report) for 3 months (or more).

As expected, consumption of other drugs was prevalent. The most commonly self-reported co-used substances (in the 6-months prior to the scan) were alcohol (71% weekly users), tobacco (61% current smokers), cannabis (51% regular users), ketamine (37%) and stimulants methamphetamine (18%) and cocaine (29%); some users also occasionally used other hallucinogens (mushrooms, 18%) and sedatives (GHB, 20%). There were significantly more tobacco [$\chi^2(1, n = 99) = 28.4$, $P < 0.0001$] and regular ($\geq 1\times$/month) cannabis [$\chi^2(1, n = 99) = 30.7$, $P < 0.0001$] smokers in the ecstasy group relative to the control group and significantly more ecstasy users drank on a weekly basis [$\chi^2(1, n = 99) = 3.95$, $P = 0.047$] and had a tendency to consume

Table 1 Demographics of control subjects and ecstasy users

	Controls ($n=50$)	Ecstasy users ($n=49$)	P
Age	26.0 ± 0.9	25.9 ± 0.8	0.90
Gender	25 male; 25 female	28 male; 21 female	(χ^2) 0.51 0.48
Body mass index	24.7 ± 0.4	23.4 ± 0.5	**0.04**
Handedness	5 left handed 43 right handed 2 not recorded	4 left handed 43 right handed 2 not recorded	(χ^2) 0.10 0.75
Education (years)	16.0 ± 0.3 ($n=49$)	14.6 ± 0.4 ($n=47$)	**0.002**
% College/university educated (1 year or more)	96.0%	79.6%	**(χ^2) 6.23** **0.01**
Premorbid IQ[a]	111.6 ± 0.9 ($n=48$)	107.4 ± 0.9 ($n=45$)	**0.002**
Employment status (%)			
student	27 (54)	9 (18)	**(χ^2) 15.37**
employed	21 (42)	31 (63)	**0.0005**
unemployed	2 (4)	9 (18)	
Current cigarette smokers	5	30	**(χ^2) 28.4** **<0.0001**
Alcohol use	1.4 ± 0.3 drinks per week	3.1 ± 0.4 drinks per week	**0.001**
Current Cannabis use (≥1×/month)	1	25	**(χ^2) 30.7** **<0.0001**
SERT promoter gene polymorphism			
L_AL_A	7	13	
L_AL_G	4	5	
L_GL_G	4	0	
L_AS	18	16	
L_GS	5	5	
SS	12	9	
Hormonal contraception in females	10	8	(χ^2) 0.0174 0.895
Race	12 Asian, 7 Black or African American, 27 White, 3 Mixed, 1 unknown/adopted	14 Asian, 3 Black or African American, 28 White, 4 Mixed	(χ^2) 1.8 0.6

Data are mean ± SEM. SERT polymorphism type (see 'Materials and methods' and 'Results' for details).
a Intelligence Quotient (IQ) as per results on the National Adult Reading Test.

slightly more alcohol ($P=0.001$). The average length of hair tested was 2.3 inches in males (representing ~5 months) and 10.5 inches in females (~21 months). As expected, of the 49 ecstasy users, all demonstrated presence of ecstasy (MDMA) in hair and most (82%) also tested positive in hair for methylenedioxyamphetamine (MDA), a de-methylated metabolite of ecstasy (Kalasinsky *et al.*, 2004). Levels of MDA in 39 of these subjects were lower than that of ecstasy (data not shown), suggesting that MDA had derived from metabolism of ecstasy (Kalasinsky *et al.*, 2004). Hair toxicology revealed presence in hair of other stimulants in many ecstasy users (cocaine, 47%; methamphetamine, 65%). Incidence of drug use for the two stimulants as demonstrated by hair analyses was higher than that disclosed by self-report. This difference was significant in the case of self-reported methamphetamine, where only 9 of 32 subjects (18%), with hair positive for methamphetamine, reported recent use [χ^2 (1, $n=49$) = 5.86, $P=0.016$]. Of the 32 users who tested positive for methamphetamine in hair, 16 also demonstrated levels of the methamphetamine metabolite amphetamine, at a lower drug level (with the exception of one subject).

Hormonal measurements and SERT genotyping

All female subjects were pre-menopausal. Female controls (40%) and ecstasy users (38%) were matched in terms of self-reported current use of hormonal contraception (Table 1). Univariate ANOVA with gender as a grouping variable indicated that levels of the hormones testosterone [group × gender $F(1,92)=1.03$; $P=0.31$], oestradiol [group × gender $F(1,91)=0.154$; $P=0.70$], and follicle-stimulating hormone [group × gender $F(1,92)=0.93$; $P=0.34$] were similar in the ecstasy users and controls (Table 3). Preliminary data, still not entirely consistent, suggest that SERT promoter gene polymorphism variants might modestly influence SERT levels in some brain areas, in which SERT levels are higher in the homozygous L_A/L_A genotype and lower in other genoytypes (L_A/L_G, L_GL_G, L_AS, L_GS, SS) (Praschak-Rieder *et al.*, 2007; Reimold *et al.*, 2007; Kalbitzer *et al.*, 2009; but see Parsey *et al.*, 2006). Fourteen percent of the control subjects and 27% of the ecstasy users had the L_A/L_A genotype [χ^2 (1, $n=99$) = 2.58, $P=0.11$] (Table 1).

Table 2 Drug use profiles of control subjects and ecstasy users

Number (%)	Controls ($n=50$)				Ecstasy users ($n=49$)			
	Confirmed[a]	Self-report			Confirmed[a]	Self-report		
		(<6 months)	(>6 months)	≥1×/month		(<6 months)	(>6 months)	≥1×/month
MDMA	0	0	0		49 (100)	49 (100)	48 (98)	40 (82)
MDA	0	0	0		40 (82)	0	0	0
Meth/Amph	0	0	0		32 (65)	9 (18)	21 (43)	5 (10)
Cocaine	0	0	1 (2)		23 (47)	14 (29)	29 (59)	11 (22)
Opioids	0	0	0		1 (2)	0 (0)	7 (14)	2 (4)
LSD	0	0	0		n/e	4 (8)	13 (27)	2 (4)
Mushrooms	0	0	2		n/e	9 (18)	22 (45)	3 (6)
Ketamine	0	0	0		4 (8)	18 (37)	22 (45)	13 (27)
GHB	n/e	0	0		n/e	10 (20)	19 (39)	6 (12)
THC	1 (2)	7 (14)	13 (26)	5 (10)	15 (31)	37 (76)	40 (82)	29 (59)
EtOH	0	35 (70)			0	41 (84)		
Tobacco	n/e	5 (10)	9 (18)		n/e	30 (61)	36 (74)	
PCP	0	0	0		0	0	0	

Pattern of MDMA use	Controls	Ecstasy users
Years of ecstasy use	n/a	4.1 ± 0.4 years; range 0.5–12 years
Age at onset of use	n/a	21.8 ± 0.8; range 14–38
Lifetime pills used	n/a	206 ± 31. pills; median 126; range 4–922
Typical monthly dose	n/a	5.3 ± 1.3 pills; median 2.3; range 0.2–60
Dose taken per use	n/a	2.2 ± 0.3 pills; median 1.5; range 0.5–10
Times used per month	n/a	2.2 ± 0.3 times; median 1.5; range 0.3–10
Maximum dose per use	n/a	4.4 ± 0.5 pills; median 3; range 1–20
Days withdrawn prior to PET	n/a	45.2 ± 4.7 days; median 38; range 11–194

n/e = not examined; Meth = methamphetamine; Amph = amphetamine; LSD = lysergic acid diethylamide; GHB = gamma-hydroxybutyric acid; THC = tetrahydrocannabinol (cannabis); EtOH = alcohol; PCP = phencyclidine.
a Use confirmed by hair/urine testing at interviews.

Table 3 Hormonal measurements in blood serum of control subjects and ecstasy users

	Males				Females			
	Controls ($n=25$)		Users ($n=27$)		Controls ($n=25$)		Users ($n=20$)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Oestradiol (ng/dl)	1.87	0.15	2.27	0.12	5.21	1.22	5.02	0.99
Total testosterone (ng/dl)	465	25	509	31	22	2	23	3
Free testosterone (pg/ml)	83.6	5.7	92.4	4.0	1.7	0.2	1.9	0.5
Follicle stimulating hormone (mIU/ml)	3.65	0.42	4.40	0.72	2.76	0.45	4.61	0.61

Psychiatric, mood and neurological status

Based on information obtained at interview, none of the control subjects had evidence of any current or past psychiatric disorder with the exception of one control subject who had a past history of mild alcohol abuse. None of the ecstasy users reportedly had a primary Axis I psychiatric disorder prior to ecstasy use; however, 12 ecstasy users (25%) met diagnostic criteria for concurrent (with ecstasy use) past and/or present psychiatric illnesses. Three of those 12 cases met criteria for major depressive disorder (partial–full remission), three had major depressive disorder with generalized anxiety disorder, one had generalized anxiety disorder, one had generalized anxiety disorder with obsessive compulsive disorder, one had (mild) alcohol abuse, two had specific phobias and one had trichotillomania. The presence of an Axis I disorder diagnosis did not correlate with ecstasy use but was slightly related to cannabis use ($\rho = 0.27$, $P = 0.03$). Relative to control subjects, ecstasy users as a whole scored significantly higher on all mood rating scales after taking into account previous or current (Axis I) diagnosed psychopathology [univariate ANCOVA: IDS, $F(1,97) = 18.7$; SIGHD/SAD, $F(1,96) = 24.5$; HAM17, $F(1,96) = 14.3$; HAM21, $F(1,97) = 14.6$; SAD, $F(1,97) = 20.6$; $P < 0.001$]. Self-rated depressive symptomatology correlated with ecstasy dosage such that subjects categorized (*post hoc*) as generally taking greater than the 'typical' one to two pill per occasion

reported lower mood (IDS, $\rho = 0.32$, $P = 0.02$; HAM17, $\rho = 0.31$, $P = 0.03$). Co-use of cannabis (SIGHD/SAD, $\rho = 0.28$, $P = 0.03$) and nicotine (SIGHD/SAD, $\rho = 0.28$, $P = 0.03$; HAM17, $\rho = 0.36$, $P = 0.005$) were also related to higher rating on depressive symptom inventories. Brief neurological assessment disclosed no gross abnormalities in any of the subjects and no significant inter-group difference in mean Unified Parkinson's Disease Rating Scale ($P = 0.3$); however, measurement of subclinical motor changes (e.g. using Purdue Pegboard test) was not conducted.

Responses to ecstasy-behavioural questionnaire

A broad battery of specific and open-ended investigator-administered questions was completed at interview (Supplementary Table 1). Items most commonly reported during acute (30 min to 6 h) exposure were: 'changes in appetite', 'pupil-dilation' and 'jaw-clenching' (>90% reported); 'increased sociability/friendliness/talkativeness', 'increased tolerance', 'urge to drink/dehydration' and 'altered sense of time' (>80%); and 'a high/mood lifting' (>75%) and 'overheating/sweating' (>60%). Effects most commonly reported during drug withdrawal (1–4 days) included 'exhaustion/somnolence', 'problems concentrating/focusing on work', 'mood-alterations', 'physical weakness', 'thirsty' and 'loss of appetite' (>55%). Adverse 'long-term' effects of ecstasy (weeks, months off-drug) were rarely reported with exception of mood (16%), memory (10%) and sleep (4%) problems in a minority of users. 'Decreased shyness' (65%) was the most reported long-term off-drug effect of ecstasy, followed by 'increased openness' (27%). Potential relationships between extent of ecstasy use and common (items reported by >50% of the sample) negative (acute and chronic) withdrawal effects were investigated using Spearman's Rank tests. Correlation analysis indicated that amongst items assessed, only 'mood-alterations' survived correction for multiple comparison, suggesting a positive link between severity of use and depressed symptoms during withdrawal (monthly dose, $\rho = 0.43$, P corrected = 0.01; average dose, $\rho = 0.51$, P corrected = 0.007; lifetime dose, $\rho = 0.41$, P corrected =0.02; maximum dose, $\rho = 0.51$, P corrected = 0.007).

Sleep questionnaire

Sleep quality and level of alertness was investigated using standard questionnaires (Fatigue Severity Scale, Epworth Sleepiness Scale, Toronto Hospital Alertness Test, Zogim-A). Unpaired t-tests revealed no statistically significant differences between the ecstasy and control groups on sleep and alertness inventories. However, there was a trend for increased fatigability in the drug-using group as rated with Epworth Sleepiness Scale (mean ± SD score, users 7.7 ± 3.8 versus controls 6.2 ± 3.8; $P = 0.07$).

PET [^{11}C]DASB non-displaceable binding potential

Visual inspection of brain time activity curves revealed that three subjects had probably moved during emission and the scans were corrected using a frame-to-frame realignment method as described by Mawlawi *et al*. (2001). There was no significant difference in mean cerebellar cortical (reference region, see 'Materials and methods' section) areas under the time activity curve for the control and ecstasy groups ($P = 0.49$).

As expected (Kish *et al*., 2005), [^{11}C]DASB BP_{ND} regional distribution was highly heterogeneous [region of interest: $F(12,1152) = 1129.5$, $P < 0.0001$], with low levels in cerebral cortices and higher levels in sub-cortical grey matter. ANOVA entering cerebral cortices (six regions of interests), striatum (caudate, putamen, ventral striatum; three regions of interest), thalamus, globus pallidus, hippocampus and midbrain (repeated-measures) and Group (2) as factors indicated a highly significant region-specific decrease in [^{11}C]DASB BP_{ND} [region of interest × group: $F(12,1152) = 7.24$, P corrected < 0.0001] in ecstasy users relative to controls. Decreased [^{11}C]DASB BP_{ND} was restricted to the entire cerebral cortices and hippocampus (P corrected < 0.001) (Fig. 1), with occipital cortex having the most marked reduction (–46%). No changes were observed in striatum (caudate, putamen and ventral striatum), thalamus, globus pallidus or midbrain; however, there was a significant positive correlation between [^{11}C]DASB BP_{ND} in cerebral cortex and that in subcortical areas ($r = 0.52$; $P < 0.0001$).

Partial volume effect correction increased [^{11}C]DASB BP_{ND} in all regions of interest in the ecstasy and control groups equally such that decreases of similar magnitude were observed after adjustment and only cerebral cortices and hippocampus showed significant SERT loss (occipital, –39%; frontal, –17%; parietal, –19%; temporal, –34%; insular cortex, –27%; hippocampus, –31%; cingulate, –30%; $P < 0.01$). In cortical regions and hippocampus,

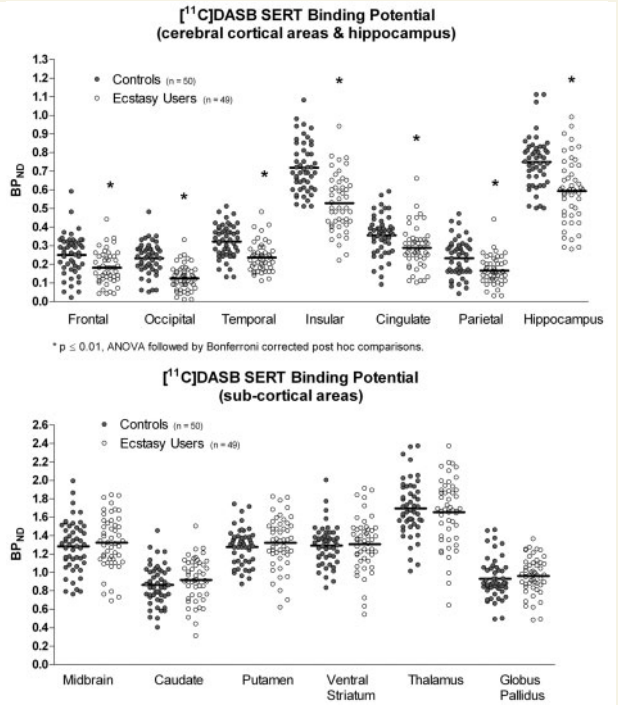

Figure 1 Scattergram of [^{11}C]DASB BP_{ND} in control subjects and ecstasy users. Note group differences in cerebral cortical but not subcortical brain areas.

areas prone to partial volume effect, the adjustment lead to a global recovery of 70% such that occipital and frontal BP_{ND} values, for example, were increased from 0.2 to 0.5 and 0.2 to 0.4, respectively. [^{11}C]DASB BP_{ND} extracted from manually segmented regions of interest tended to have slightly lower BP_{ND} values but yielded similar results (Supplementary Table 2 and Supplementary Fig. 1).

We investigated whether co-abused substances might account for the observed [^{11}C]DASB BP_{ND} loss. Separate ANOVAs indicated that ecstasy use was associated with significantly decreased [^{11}C]DASB BP_{ND} independently of whether or not the subject was using methamphetamine (in hair) [group × region of interest, $F(12,1140) = 4.28$, $P = 0.002$; Supplementary Table 3], cocaine (in hair) [group × region of interest, $F(12,1140) = 5.53$, $P < 0.0001$], cannabis (self-report/urine) [group × region of interest, $F(12,1140) = 6.04$, $P < 0.0001$], ketamine (self-report) [group × region of interest, $F(12,1140) = 4.68$, $P = 0.001$], LSD (self-report) [group × region of interest, $F(12,1140) = 6.61$, $P < 0.0001$], psilocybin mushroom (self-report) [group × region of interest, $F(12,1140) = 6.63$, $P < 0.0001$] or was a current tobacco smoker [group × region of interest, $F(12,1140) = 5.54$, $P < 0.0001$]. Average decreased binding was not confounded by gender, body mass index, presence of an Axis I disorder or hormone levels ($P < 0.0001$). A SERT binding reduction was observed in the ecstasy users (versus genotyped matched controls) having the putatively (see above) 'low' SERT-expressing genotypes (L_A/L_G, L_GL_G, L_AS, L_GS, SS, grouped together) and in the much smaller sub-group having the 'high' SERT-expressing genotypes (L_A/L_A) (data not shown). Seasonal changes in SERT [^{11}C]DASB BP_{ND}, previously reported in the literature (Praschak-Rieder *et al.*, 2008), were unlikely to influence findings as only a small number of subjects (13) were scanned in winter. Visual inspection of cerebral cortical [^{11}C]DASB BP_{ND} scatter indicated that between-group overlap, marked in most regions, appeared less in occipital and especially insular cortices where ecstasy users' values fell mostly in (or below) the lower half of control range (Fig. 1).

A step-wise linear regression analysis entering variables including demographic information, drug history and neuropsychological, mood and subjective drug-effect ratings investigated factors that were associated with (could predict) lower [^{11}C]DASB BP_{ND} in ecstasy users. The only factors which could predict low SERT binding across all cortices were 'years of use' and 'maximum drug dose' [$F(2,44) = 9.57$, $P < 0.0001$]. 'Years of use' also predicted lower SERT BP_{ND} in caudate nucleus [$F(1,41) = 4.91$, $P = 0.032$]. There was no relationship between depressive symptom ratings ($P > 0.05$), presence of Axis I disorder ($P > 0.05$) and [^{11}C]DASB BP_{ND} in all examined brain areas. There was no significant difference in SERT binding in the 59% of subjects reporting a sense of overheating during drug taking than those not reporting hyperthermia (data not shown). [^{11}C]DASB BP_{ND} was also estimated using SRTM2 and MRTM2. A good agreement between regional BP_{ND} estimates from all methods was found and overall variability of BP_{ND} derived by either model was comparable, although slightly lower with values extracted using Logan (Supplementary Table 4). The correlation coefficient between the BP_{ND} estimated from each method and across regions of high and low SERT density was generally good (cortex SRTM2, $r = 0.97$; MRTM2, $r = 0.94$; striatum SRTM2, $r = 0.91$, MRTM2, $r = 0.84$; hippocampus, SRTM2, $r = 0.87$, MRTM2, $r = 0.84$). Correlation between Logan and SRTM2 and MRTM2 parameters in midbrain was weaker (SRTM2, $r = 0.78$; MRTM2 $r = 0.60$). The methods were found to yield between-group changes of similar magnitude and significance ($P < 0.05$); for example, differences reported using Logan, SRTM2 and MRTM2 respectively were –46, –46 and –41% in occipital cortex, –21, –21 and –15% in the hippocampus and 3, 2 and 8% in striatum.

Voxel-wise analysis of [^{11}C]DASB BP_{ND}

Voxel-wise analysis on whole brain volume confirmed region of interest analysis and revealed significant clusters of decreased [^{11}C]DASB BP_{ND} in ecstasy users relative to controls, which did not appear to be confounded by co-use of stimulants (Fig. 2) or cannabis (data not shown). Large peak clusters covered bilateral caudal brain, insular and temporal lobe cortices, hippocampus and frontal and cingulate cortices. The largest, most significant cluster occurred in the occipital cortex [MNI coordinates, 8, –70, 12; $t_{max} = 10.45$; $k = 80892$; P (false discovery rate corrected) < 0.0001] (Fig. 2). The magnitude of decrease at peak maxima (in a 10 mm radius spherical search region) corresponded to –62%. Significant clusters did not occur outside of areas identified by region of interest analysis (e.g. no changes were observed in the SERT-containing amygdala or pons) with the exception of a small cluster of significant voxels (which was not identified by the region of interest analysis) in an area including the edge of the right thalamus (MNI coordinates 10, –8, 0) with a peak in white matter adjacent to the globus pallidus [MNI coordinates 14, −8, −2; $t = 3.6$; P (false discovery rate) = 0.002]. Voxel-wise search did not reveal any clusters of significantly increased [^{11}C]DASB BP_{ND} in ecstasy users relative to controls or in ecstasy users who co-used methamphetamine relative to those who did not.

Structural imaging

Overall, there was no significant difference between ecstasy users and control subjects in whole brain volume ($P = 0.95$), extracerebral cerebrospinal fluid ($P = 0.26$), size of ventricles [region of interest × group, $F(3,291) = 0.036$, $P = 0.99$], white matter volume [region of interest × group, $F(7,679) = 0.190$, $P = 0.80$] or that in any of the cortical [region of interest × group, $F(7,679) = 0.119$, $P = 0.94$] or subcortical [region of interest × group, $F(8,776) = 0.193$, $P = 0.71$] brain structures investigated (Supplementary Table 5a). However, there was a significant difference in subcortical grey matter volumes in ecstasy users who co-used methamphetamine versus those who did not, such that ecstasy users who did not co-use methamphetamine ($n = 17$) had below normal grey matter volumes (versus controls) in all subcortical regions examined [including globus pallidus, caudate, putamen and thalamus; range −3 to −11%; between group, $F(2,96) = 4.43$, $P = 0.014$, controls versus users without methamphetamine $P = 0.007$]. Co-use of methamphetamine did not affect whole brain ($P = 0.26$) or ventricle size [region of interest × group,

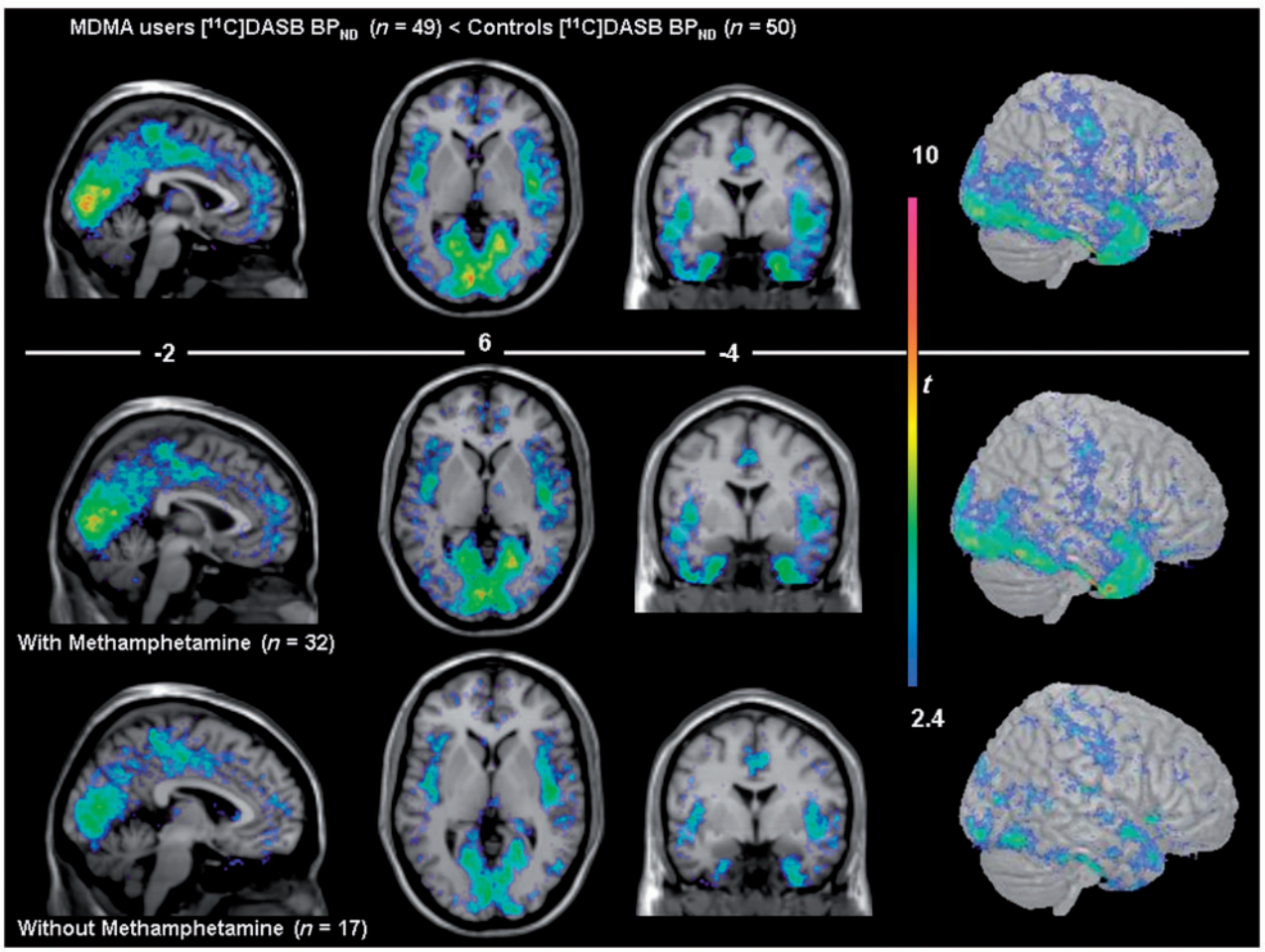

Figure 2 *T*-statistical map overlaid template MRI (ICBM template) illustrating clusters of significant decrease in [^{11}C]DASB BP_{ND} at a *P* (false discovery rate corrected) < 0.05 (height threshold $t = 2.4$) in (top): ecstasy users (whole group; $n = 49$) versus control ($n = 50$) subjects [MNI coordinates global maxima, 8, –70, 12; $t_{max} = 10.45$; $k = 80892$; *P* (false discovery rate corrected) < 0.0001] (middle): ecstasy users positive in hair for methamphetamine ($n = 32$) versus control ($n = 50$) subjects [MNI coordinates global maxima, –8, –84, 4; $t_{max} = 9.98$; $k = 66204$; *P* (false discovery rate corrected) < 0.0001]; (bottom): ecstasy users negative in hair for methamphetamine ($n = 17$) versus control ($n = 50$) subjects (MNI coordinates global maxima, –4, –80, –10; $t_{max} = 6.52$; $k = 42398$; *P* (false discovery rate corrected) < 0.0001). Image coordinates (–2, –4, 6) are in MNI space. The *t*-map shows reduced cerebral cortical and hippocampal [^{11}C]DASB BP_{ND} in ecstasy users as a whole and in ecstasy users irrespective of co-use of methamphetamine.

$F(3,288) = 0.160$, $P = 0.78$], white matter volume [region of interest × group, $F(7,672) = 0.109$, $P = 0.88$], extracerebral cerebrospinal fluid ($P = 0.87$) or volume of cortical grey matter [$F(7,672) = 0.238$, $P = 0.86$] (Supplementary Table 5a). Co-use of other substances including cocaine and cannabis was not associated with volumetric changes ($P > 0.05$).

Clusters of significantly (false discovery rate corrected <0.05) decreased cerebral cortical thickness were identified in the ecstasy group (Fig. 3). Areas of cortical thinning were mostly biased to the left-hemisphere but also included right-hemisphere localized clusters in medial frontal, parietal, parahippocampal and cingulate gyri. The clusters of greatest cortical thinning were in the left medial (MNI coordinates; −38, 56, 5; $t_{max} = -5.0$; $P < 0.0001$; magnitude –5.9%) and inferior frontal gyri (MNI coordinates; −55, 8, 28; $t_{max} = -4.3$; $P < 0.0001$; magnitude −4.2%), parietal lobe (MNI coordinates; –53, –47, 49; $t_{max} = -4.3$; $P < 0.0001$; magnitude –4.8%) and occipital lobe (MNI coordinates; −13, −101, −5; tmax = –3.7; $P = 0.0003$; magnitude −6.6%). The peak decrease in the right hemisphere occurred in the parietal (precuneus) lobe (MNI coordinates; 32, −72, 35; $t_{max} = -3.3$, $P = 0.002$; magnitude –3.9%). Clusters of significantly increased cortical thickness were also found bilaterally in the parietal lobes (post-central gyri; *t* range 2.45–3.84; magnitude 4.1–5.7%) (Supplementary Table 5b). Voxel-wise analyses investigating whether co-used stimulants and other substances (cannabis, nicotine, alcohol) affected cortical thinning revealed that co-use of methamphetamine (Fig. 3) but not cocaine or cannabis (data not shown) largely accounted for decreased thickness. Thus, ecstasy users who did not use methamphetamine had only small clusters of significantly thinner cortex; these were mostly unilateral and restricted to the left middle frontal gyrus. A stepwise linear regression analysis including potential explanatory variables

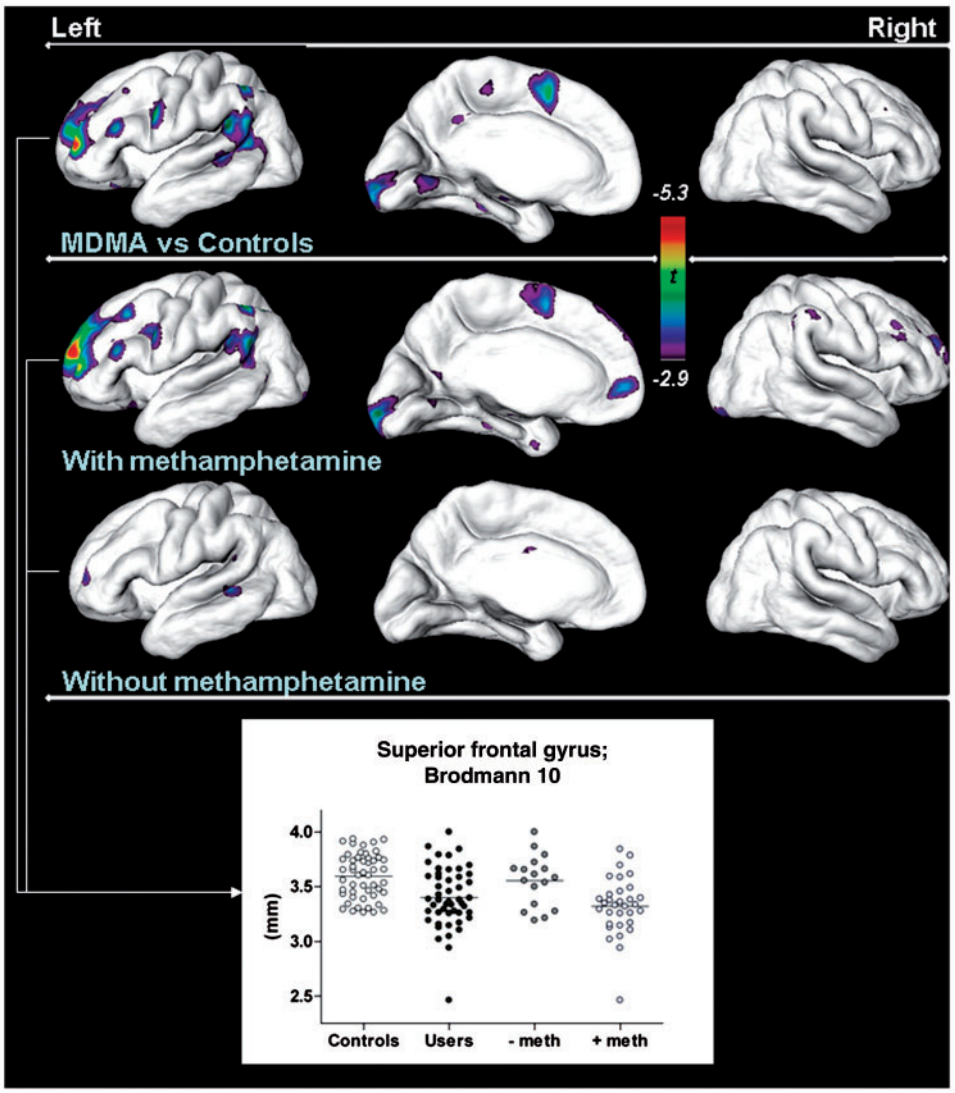

Figure 3 *T*-statistical maps of cortical thickness differences (top) in ecstasy users ($n = 49$) versus control subjects ($n = 50$) [MNI coordinates, −38, 56, 5; $t_{max} = -5.0$; *P* (false discovery rate corrected) < 0.0001]; (middle) ecstasy users positive in hair for methamphetamine ($n = 32$) versus control ($n = 50$) subjects [MNI coordinates, −23, 63, 16; tmax = −5.3; *P* (false discovery rate corrected) < 0.0001]; (bottom) ecstasy users negative in hair for methamphetamine ($n = 17$) versus control ($n = 50$) subjects [MNI coordinates, −57, −36, 1; $t_{max} = -3.7$; *P* (false discovery rate corrected) < 0.0001]. Results are displayed on a standardized brain (ICBM template). Significant differences at *P* (false discovery rate corrected) < 0.05 can be seen in the parietal, temporal, occipital, cingulate and frontal cortices. (Bottom) Scattergram of cortical thickness (mm) in left superior frontal gyrus, the area of peak cortical thinning in ecstasy users. Cerebral cortical thinning is observed in ecstasy users as a whole, but which is primarily restricted to those ecstasy users who also use methamphetamine.

[gender, IQ (NART score), education and age] further revealed that differences in IQ could explain cortical thinning (mostly restricted to the left middle frontal gyrus) in the ecstasy group who did not use methamphetamine.

We found an association between cortical thinning in the left hemisphere and drug use severity such that higher dosage and frequency of use were associated with greater cortical thinning ($\rho = -0.29$, $P = 0.04$); this association also occurred in the right hemisphere ($\rho = -0.31$, $P = 0.02$). The magnitude of the [^{11}C]DASB BP_{ND} reduction in cerebral cortical regions was similar in left (in which cortical thinning was more prominent) and right hemispheres (data not shown). Mean cortical thickness extracted from frontal, parietal, occipital, cingulate and parahippocampal gyri, peak thickness extracted from significant clusters and region of interest volume were unrelated to our outcome measure [^{11}C]DASB BP_{ND} ($P > 0.05$ in all regions) suggesting that morphological differences alone are unlikely to account for decreased SERT in the drug-using population.

Neuropsychological testing

As shown in Table 4, mean scores on current structured intelligence (WASI Verbal and Performance IQ) and premorbid verbal IQ (NART) were in the normal range for both groups

Table 4 Mean neuropsychological test scores of control subjects and ecstasy users

	Controls			Ecstasy users					ANCOVA	
	n	Mean	SD	*n*	Mean	SD	*t*-test	% diff	Education	V.IQ
Age	50	26.0	6.1	49	25.9	5.7	0.90	–0.5		
Education (total years)	49	16.0	1.9	47	14.6	2.4	**0.002**	–8.8		
Intelligence										
WASI										
Full (four subtest) IQ	49	113	11	47	107	9	**0.001**	–6.0	**0.008**	
Full (two subtest) IQ	49	113	11	47	105	9	**0.0001**	–7.4	**0.001**	
Verbal IQ	49	111	10	47	102	11	**0.0001**	–8.0	**0.0005**	
Performance IQ	49	113	12	47	109	10	0.09	–3.5	0.22	
NART										
Standard	48	112	6	45	107	6	**0.002**	–3.7	**0.01**	
Willshire	48	116	7	45	111	7	**0.0004**	–4.5	**0.02**	
Executive functions										
PASAT										
2.4 s delay	49	47.8	10.1	47	41.7	9.6	**0.003**	–12.9	**0.03**	0.15
2.0 s delay	49	43.3	9.2	47	37.6	8.7	**0.002**	–13.2	**0.04**	0.07
1.6. delay	49	37.4	10.2	45	32.2	8.2	**0.008**	–13.9	**0.05**	0.11
Digit Ordering Test										
Max (15)	48	9.9	4.1	46	7.3	3.1	**0.0007**	–26.8	**0.004**	**0.05**
Cooper (105)	48	98.2	7.2	46	93.8	6.0	**0.002**	–4.4	**0.009**	0.12
Trails B time	46	48.2	17.7	44	62.7	24.6	**0.002**	30.2	**0.02**	0.09
Attention										
Trails A time	48	20.4	6.9	47	22.0	5.2	0.21	7.8	0.26	0.73
Seashore	49	26.8	2.2	47	27.1	2.3	0.59	0.9	0.48	0.20
Symbol Digit Modalities										
Oral score	49	76.0	13.0	47	68.0	11.1	**0.002**	–10.5	**0.02**	0.13
Written score	49	61.6	9.8	47	54.5	8.3	**0.0003**	–11.4	**0.005**	**0.008**
Memory										
Consonant Trigrams										
0 s delay	49	15.0	0.2	47	14.9	0.3	0.67	–0.2	0.90	0.95
3 s delay	49	13.1	1.8	47	12.6	2.3	0.30	–3.4	0.97	0.10
9 s delay	49	12.7	2.1	47	11.9	2.4	0.08	–6.4	0.30	0.59
18 s delay	49	12.1	2.4	47	11.5	2.5	0.25	–4.7	0.56	0.20
CVLT										
List A Trials 1–5	49	63.5	8.5	47	56.5	8.7	**0.0001**	–11.0	**0.003**	**0.04**
List A Trial 1	49	9.6	2.2	47	8.3	2.0	**0.003**	–13.6	**0.04**	0.29
List A Trial 5	49	14.3	1.8	47	13.3	2.1	**0.009**	–7.3	0.08	0.48
List B	49	8.9	2.5	47	7.3	1.8	**0.0006**	–17.9	**0.004**	**0.04**
Delay time (min)	49	16.0	3.6	47	15.3	2.0	0.23	–4.5	0.11	0.16
SDFR	49	13.4	2.4	47	11.0	2.6	**0.0000**	–17.8	**0.004**	**0.04**
SDCR	49	13.4	2.4	47	11.1	3.0	**0.0001**	–16.9	**0.003**	0.26
LDFR	49	13.5	2.3	47	11.9	2.6	**0.003**	–11.4	0.08	0.68
LDCR	49	13.4	2.4	47	11.5	3.0	**0.0009**	–14.2	**0.02**	0.49
Perseverations	49	3.9	4.3	47	4.9	3.9	0.23	26.1	0.11	0.14
Free intrusions	49	1.1	2.1	47	2.1	3.8	0.13	84.3	0.18	0.67
Cued intrusions	49	1.0	1.6	47	1.3	1.6	0.51	20.6	0.77	0.53
Recognition hits	49	15.6	1.1	47	15.1	1.2	**0.04**	–3.1	0.22	0.83
Discriminability	49	98.0	4.0	47	95.5	6.1	**0.02**	–2.5	0.15	0.28
False positives	49	0.45	0.96	47	1.00	2.10	0.10	122.7	0.30	0.25
Response bias	49	–0.02	0.16	47	–0.01	0.23	0.85	–47.9	0.95	0.40
Warrington Recognition										
Words score	49	48.6	2.1	46	48.1	2.1	0.37	–0.8	0.55	0.64
Faces score	49	43.8	4.2	46	42.0	4.7	0.06	–4.0	0.19	0.38
Denman										
Story	49	277	6.6	46	25.2	6.0	0.06	–8.9	0.28	0.64
Pairs	49	33.5	6.9	46	31.0	5.4	0.06	–7.4	0.17	0.50
Delayed pairs	49	12.8	1.9	46	12.5	1. 9	0.48	–2.2	0.59	0.89
Delayed story	49	24.2	6.9	46	21.9	6.3	0.09	–9.6	0.56	0.80
Delay time (min)	49	28.3	4.5	46	28.7	4.9	0.67	1.5	0.61	1.0

SDFR = short delay free recall; SDCR = short delay cued recall; LDFR = long delay free recall; LSCR = long delay cued recall. Differences were analysed by *t*-test and by ANCOVA using education or verbal IQ (V.IQ) as covariates.

(range 102–116). An ANCOVA controlling for level of education indicated that ecstasy users had significantly lower overall IQ scores compared to control subjects [WASI × group with education level, $F(3,279) = 3.87$, $P = 0.03$; NART with education level, $P = 0.02$]; however, this difference was attributed to verbal IQ (P corrected = 0.004) and not to performance (non-verbal) IQ ($P = 0.3$).

In general, ecstasy users performed more poorly than control subjects on most cognitive measures. Typically the magnitude of the mean test score differences was modest and the variance and distribution of scores showed near total between-group overlap. Ecstasy users had lower scores on a single test of attention [Symbol Digit Modalities Test, $F(1,93) = 7.34$; $P = 0.008$] but performed in the normal range on other tests of the same domain (Trails A, $P = 0.2$; Seashore, $P = 0.6$). An ANCOVA taking into account differences in verbal IQ across-group revealed significant group differences in memory as revealed by the CVLT as a whole [$F(1,93) = 8.53$; $P = 0.004$], with short free-recall being the most affected component [group × CVLT scores, $F(6,558) = 3.83$; $P = 0.001$; short free-recall P corrected = 0.002]. We did not find group differences in memory using the Denman Memory Scale [$F(1,92) = 0.50$, $P = 0.5$] or the Warrington Recognition Test [$F(1,92) = 1.105$, $P = 0.3$]. Executive function as tested with all tasks used including Trails B ($P = 0.002$), the Digit Ordering Test [$F(1,93) = 11.34$; $P = 0.001$] and the mental arithmetic of the PASAT [$F(1,91) = 4.84$, $P = 0.03$], indicated poorer performance by the ecstasy group. Co-use of cannabis, nicotine, alcohol, methamphetamine or cocaine did not significantly affect results of cognitive testing (non-significant interaction; $P > 0.05$). Co-use of cocaine disclosed a trend for slower performance on the Trails B test ($P = 0.06$) whereas ecstasy users who smoked tobacco on a regular basis had better performance on that task ($P = 0.05$). When compared to ecstasy users who did not also use methamphetamine, co-users had slightly more recall errors (CVLT short-delay free-recall, $P = 0.08$).

Correlation analyses were conducted to investigate whether task deficits were related to decreased [^{11}C]DASB BP_{ND}. Lower performance on tasks of short-term memory (CVLT short-delay free-recall, insular cortex, $\rho = 0.38$, $P = 0.004$; hippocampus, $\rho = 0.38$, $P = 0.004$) and mental flexibility and speed of processing (Trails B, insular, $\rho = -0.38$, $P = 0.005$; hippocampus, $\rho = -0.35$, $P = 0.01$) were associated with SERT binding loss in the insular cortex and hippocampus. Experienced users, consuming higher dosage per occasion (greater than the 'typical' 1–2 pills per occasion) had the greatest impairment in the test of verbal memory (CVLT short-delay free-recall; $\rho = -0.33$, $P = 0.01$).

We investigated whether the presumed cortical thinning and decrease in subcortical regional volume in ecstasy users was associated with deficits in mood and neuropsychological function (at tests where deficits were noted). Poorer performance on the CVLT was related to cortical thinning in bilateral frontal lobes (left: $r = 0.41$, P uncorrected = 0.005; right: $r = 0.35$, P uncorrected = 0.02), anterior cingulate (left: $r = 0.38$, P uncorrected = 0.009; right: $r = 0.33$, P uncorrected = 0.02) and decreased grey matter volume in the caudate ($r = -0.29$, $P = 0.04$); however these correlations did not survive correction for multiple comparison.

Discussion

Our major finding is an overall mild to marked decrease in SERT binding in cerebral cortex and hippocampus, but not in SERT-rich striatum, in a representative number of recreational polydrug-users proven, by hair analysis, to have used ecstasy. Our findings suggest that a brain SERT binding reduction can be highly regionally selective in some ecstasy users and also is unlikely to be explained, at least *in toto*, by a variety of potential confounds including structural brain changes, major hormonal level differences, SERT promoter gene polymorphisms or recent use of other stimulant drugs.

Characteristics of ecstasy users

The ecstasy users of our investigation can be classified (for the most part) as 'low to moderate' (versus heavy) or perhaps 'typical' users of the drug as indicated by the median/average use of 1.5/2.2 tablets per session, 2 uses/month, 206 lifetime tablets used, and four years duration (Parrott and Marsden, 2006). All (but one) used other drugs, knowingly or unknowingly (Kalasinsky *et al*., 2004), as demonstrated by drug testing and/or self-report.

Most ecstasy users reported the typical acute effects of ecstasy, including increased sociability and hyperthermia, and features of a drug discontinuation/withdrawal syndrome (sometimes severe) occurring one or more days after cessation of drug use and that resolved within a week. It was our impression that the ecstasy users, who enjoyed the drug taking experience, did not have a strong compulsion to use the drug, based on their pattern of use which was primarily weekend and often sporadic and by the successful attempts by some to reduce or stop consumption.

Brain SERT binding levels are low in ecstasy users

Preferential SERT changes occurred in cerebral cortex and hippocampus

We did not find a global, massive reduction of brain SERT binding as reported in the first SERT imaging study of ecstasy users (McCann *et al*., 1998*a*). SERT binding changes were regionally heterogeneous and preferentially affected the cerebral, especially occipital cortex and hippocampus (archicortex) with high SERT density striatal subdivisions distinctly normal. SERT binding differences were maintained irrespective of method of estimation (Logan, SRTM2, MRTM2, manual region of interest, voxel-based), unlike some findings reported with [^{11}C]McN5652 (Buchert *et al*., 2007). The cerebral cortical, but not striatal [^{11}C]DASB BP_{ND} decrease was also observed in our pilot study of seven ecstasy users scanned on a lower resolution camera (GEMS 2048-15B PET camera, Scanditronix Medical, General Electric, Uppsala, Sweden) (Kish, unpublished observations). Below normal SERT binding could be explained by decreased concentration of SERT in intact neurons or that of SERT-containing neurons (e.g. loss of axons/nerve terminals in cerebral cortex/hippocampus with sparing of cell bodies), or possibly by a premorbid difference in levels. We found near total overlap between control and ecstasy

user ranges in striatum and thalamus, whereas in the cerebral cortical regions and hippocampus, most ecstasy user values fell within the bottom half of the control range. The brain area showing least overlap was insular cortex, in which 51% of ecstasy user values fell below the lower limit of the control range (i.e. were 'below normal'). It has been suggested that females might be more susceptible to the effects of ecstasy than males (Reneman *et al*., 2001); however, extent of SERT binding reduction in females versus males (versus respective controls) was similar (e.g. occipital cortex: males, –51%; females, –40%; insular cortex: males, –22%, females, –31%; hippocampus: males, –21%, females, –21%). In line with previous studies (Thomasius *et al*., 2003; Buchert *et al*., 2004; McCann *et al*., 2005), we found a relationship between longevity and intensity (years of use and maximum dose) of drug-usage and magnitude of SERT binding decrease affecting all areas of SERT-binding reduction (including caudate) equally. Results of a recent imaging study showing normal brain [^{11}C]DASB binding in (self-reported) ecstasy users following extended abstinence (mean 2.7 years) suggest that any drug-induced SERT reduction might be reversible in some subjects (Selvaraj *et al*., 2009). However, we could not address this question in our study as the typical withdrawal times were only 1–2 months duration and we observed no significant correlation between ecstasy withdrawal time and brain SERT binding.

SERT binding, major SERT gene polymorphisms and hormone levels

We attempted to address, as much as possible, potential confounds in grouping factors that might have influenced SERT levels. Mean ages of control and ecstasy user groups were similar and distribution of variants of a SERT promoter gene polymorphism (5-HTTLPR) that might have influenced SERT expression (Praschak-Rieder *et al*., 2007) were unrelated to SERT binding. Seasonality, which has been reported to influence brain SERT binding in normal individuals (Praschak-Rieder *et al*., 2008), can also probably be ruled out as a confounding factor since the same proportion of users and controls were scanned across seasons (i.e. most subjects in our study were examined during spring and summer months).

Some animal data suggest that androgens and ovarian steroids might be involved in regulation of brain SERT concentration (Rehavi *et al*., 1987; Lu *et al*., 2003), and increased levels of testosterone (in saliva) have been reported in ecstasy users (Parrott *et al*., 2008). However, blood levels of oestradiol, testosterone, and follicle-stimulating hormone (subdivided by gender) taken at time of PET scan, were similar between groups and we observed no statistically significant correlation between blood hormone and SERT binding levels in either control or ecstasy user groups subdivided by gender. This suggests that chronic ecstasy exposure might not alter levels of these hormones when measured several months following last drug use.

Ecstasy, unlike other drugs used, was associated with low SERT binding levels

A highly relevant potential confound was use of other substances (especially methamphetamine, cocaine) that might have influenced SERT binding. We addressed this issue by measuring drugs in scalp hair to establish, to some extent, whether some key drugs were 'recently' used and by performing statistical analyses (ANCOVA; comparison of SERT binding in users versus non-users of other drugs) based on hair data. We are still, however, dependent on self-report information regarding drugs used for the period of time that could not be assessed by drug hair analysis and for those drugs that were not measured in hair (e.g. tetrahydrocannabinol). We were also limited by length of hair from males (average 2.6 inches) and females (average 10.3 inches) representing ~5 and 20 months, respectively, and by generic issues surrounding drug hair analysis (e.g. variable and uncertain rate of drug uptake and retention in different subjects, sensitivity to hair treatments). In this regard, data derived from females (in which the SERT binding decrease was observed) having much longer hair than males, addresses this potential confound better.

Given animal data that methamphetamine can damage brain serotonin neurons (Davidson *et al*., 2001; McCann and Ricaurte, 2004) and our own post-mortem brain findings showing low SERT protein in brain of human methamphetamine users (Kish *et al*., 2009), co-use of methamphetamine by ecstasy users could itself explain reduced SERT binding. However, extent and regional pattern of brain SERT binding changes were similar in ecstasy users who tested positive in hair for methamphetamine (typical ecstasy dose 2.5 tablets) versus those who did not test positive (1.8 tablets), and in the smaller groups of subjects subdivided by gender (data not shown). While we cannot exclude that use of methamphetamine prior to that estimated by hair testing had caused some SERT binding reduction, these observations suggest that at least 'recent' (~5 months in males, 1–2 years in females) methamphetamine use might not have been a significant factor. Similarly, the autopsied human brain report of increased SERT binding in human cocaine users (Mash *et al*., 2000) suggests that co-use of cocaine could have antagonized to some extent any reduction in SERT binding caused by ecstasy. However, SERT binding changes were similar in ecstasy users who tested positive for cocaine (median typical ecstasy dose 2 tablets/session; 2 uses/month) and those who did not (median typical dose 1.5 tablets/session; 1 use/month). Information on use of non-stimulant drugs came primarily from self-report. As with the stimulant drugs, SERT binding changes in ecstasy users who reported using and not using these drugs (cannabis, GHB, 'mushrooms', ketamine, tobacco or alcohol) were similar.

SERT binding levels were not confounded by structural brain changes

We found little evidence of substantial structural changes in subcortical and cerebral cortical brain areas of the ecstasy users as a group. However, we did find two structural brain differences when taking into account co-use of methamphetamine. Thus, in the subcortical regions grey matter volume was globally lower than normal by ~6% in ecstasy users who did not use methamphetamine, whereas the ecstasy users who used methamphetamine had normal volumes. While this may appear paradoxical, these findings could be explained in the context of the literature on methamphetamine users reporting above-normal striatal volume (Chang *et al*., 2005; Jernigan *et al*., 2005). Thus, ecstasy might cause or be related to a reduction of subcortical grey matter

that would not be apparent in those users who also use methamphetamine. Unlike Cowan *et al*. (2003), we did not find significant grey matter loss in cerebral cortex of the ecstasy users, but did observe some cerebral cortical thinning which was, however, largely accounted for by co-use of methamphetamine. We caution however that although the ecstasy users who used methamphetamine were not statistically significantly different from the rest with respect to other drugs used (cocaine, alcohol, cannabis, nicotine), they were slightly 'heavier' users (mean 2.5 versus 1.8 tablets used/session) such that the structural differences in the two groups might be related to an ecstasy dose effect.

Correlational analyses disclosed that cerebral cortical thickness and regional volume were unrelated to [^{11}C]DASB BP_{ND}. Furthermore, the magnitude of SERT binding decrease in cerebral cortical regions and hippocampus was similar in the subgroups subdivided with respect to use of methamphetamine; both subgroups showed lack of significant changes in striatum, and SERT changes or lack of changes were similar following partial volume adjustment. This suggests that brain morphological differences are unlikely to account for the SERT findings.

Are reduced SERT levels or structural brain changes related to behaviour?

Given suspected roles of serotonin in mood (Shopsin *et al*., 1976) and cognition (Mendelsohn *et al*., 2009), a brain serotonergic disturbance might be causally related to some psychiatric and cognitive problems in ecstasy users. It is also possible that aspects of these behaviours could be related to structural changes in the brain (Peterson *et al*., 2009).

SERT and brain structure versus depressive symptomatology

We did not find evidence of a relationship between brain SERT binding and psychiatric status, as SERT binding in the subgroup of ecstasy users having an Axis I psychiatric disorder was not significantly different from that in the rest of the ecstasy users. Heavier users reported, as expected, more marked mood alterations after drug-discontinuation; however, we did not find a relationship between brain SERT binding and individual scores on depressive symptomatology indices. This suggests that the magnitude of any functional change (e.g. serotonergic deficiency) associated with decreased SERT binding might have been too small to have consequence on mood or explain the psychiatric conditions present in some of the drug users. It is also conceivable that structural changes in brain, especially in cerebral cortex, might be related to psychiatric problems observed in some ecstasy users, as cerebral cortical thinning has been reported in some subjects at risk for major depressive disorder (Peterson *et al*., 2009). However, we found no relationship in ecstasy users between cortical thinning and presence of Axis I disorder and depressive symptomatology based on the mood rating scales.

SERT and brain structure versus cognitive status

Some (but not all) evidence points to persistent, probably modest, memory problems (particularly verbal) in some ecstasy users during extended abstinence (see de Sola Llopis *et al*., 2008 for review). Even after taking into account IQ and education (lower in drug users), ecstasy users performed, on average, more poorly than control subjects on many cognitive tasks (working memory, verbal memory, speeded visual code transposition) and the effect size for the tests showing significant differences was 'moderate' to 'moderately large' (Cohen's *d* effect size range 0.43–0.86). [For comparison, the effect size was larger for SERT binding differences in occipital (1.14) and insular (1.10) cortices.] Nevertheless, most ecstasy users had few cognitive complaints after the acute effects and the drug withdrawal phase had passed and user values generally fell within the normal control range. In principle, users might have been poor performers due to impact of their drug use and compromised effort or they may have drifted to a poor performance peer group because of inherent (premorbid) cognitive issues.

The observation of normal or close to normal performance on cognitive testing is consistent with much of the ecstasy literature (Thomasius *et al*., 2003; McCann *et al*., 2008) and might be explained by inclusion in our study of relatively 'low dose' ecstasy users. Co-use of cannabis, nicotine, alcohol, methamphetamine or cocaine did not significantly affect cognitive findings. We did find several modest correlations between performance on some tests of memory (CVLT) and mental flexibility (PASAT, Digit Ordering Test) and SERT binding in hippocampus, an area related to memory processing, and in insular cortex. The findings in hippocampal formation and insular cortex, the latter being a region suggested to be involved in self-awareness and insightful cognition (Goldstein *et al*., 2009), raise the possibility that an uncompensated serotonergic disturbance, related to low SERT, might lead to subtle problems in memory (short-term and working memory), self-awareness, and insight which could, if sufficiently severe, lead to functional difficulty.

We also found that poorer performance on a memory task dependent on integrity of frontal-striatal loops (CVLT) (Hartley and Speer, 2000) was related to cerebral cortical thinning, particularly in frontal areas, and decreased grey matter concentration in the caudate. Although these observations are only preliminary (differences did not survive correction for multiple comparison) they raise the possibility that morphological changes affecting frontal cortical output in brain of ecstasy users might cause cognitive problems unrelated to a disturbance of the brain serotonin system.

SERT and self-reported drug tolerance

Our observations support the view that tolerance to ecstasy is a characteristic of chronic use of the drug (Parrott, 2005) as most ecstasy users reported decreased behavioural effects of the drug after chronic use (which could arguably be explained in part by decreased novelty/expectancy) and two-thirds reported escalation in number of tablets typically used. Since the mechanism of action of ecstasy may be dependent on SERT integrity (Tancer and Johanson, 2007; Trigo *et al*., 2007), cerebral cortical SERT binding reduction, assuming this reflects actual loss of transporter protein *in vivo*, could explain drug tolerance. Although we found no difference in brain SERT binding levels between the majority ($n = 41$) of subjects reporting drug tolerance versus the small subgroup ($n = 8$) that did not, it would be important to establish in

future studies whether individual differences in tolerance to the effects of ecstasy might relate to extent of decreased brain SERT.

Emerging consensus in PET imaging SERT literature on ecstasy: is the cerebral cortex preferentially affected?

Excluding the McCann (1998*a*) study, two main findings emerge in the brain SERT ecstasy literature involving use of radioligands having some selectivity for SERT. First, in the [^{11}C]McN5652 investigations of Buchert *et al*. (2003, 2004, 2006, 2007) SERT binding is decreased in striatum and thalamus (cerebral cortex was not analysed by region of interest measurement and voxel-based analyses in cerebral cortex were inconsistent). Second, in the recent [^{11}C]DASB and [^{11}C]McN5652 studies of McCann and colleagues (2005, 2008), binding is, in contradistinction, normal in striatum but decreased in cerebral cortex. Our findings of cerebral cortical but not striatal changes, with the occipital cortex most severely affected, are almost identical to those of the two recent investigations of McCann *et al*. (2005, 2008) employing the same PET probe ([^{11}C]DASB). The subjects in the two McCann studies (although not confirmed by drug analysis as ever having used ecstasy) were also comparable to ours in terms of general behavioural characteristics ('grossly behaviourally normal', modest reduction on cognitive performance and self-reported mood). This first-time 'replication' of SERT binding data by an independent laboratory may help bring some consistency to the ecstasy SERT literature.

Why, however, do we and McCann not detect a SERT binding reduction in the SERT-rich striatum as does the Buchert group? Previously, McCann explained their 'puzzling' failure to detect striatal [^{11}C]DASB reduction in ecstasy users by high 'variability' of values in this region (McCann *et al*., 2005). However, variability of striatal values was not high in our study or in McCann's follow up study (McCann *et al*., 2008). Indeed, given our sample size and coefficient of variation (16% in striatum) we could have detected a change as small as 8% in striatum with a power of 80%. We suggest that differences in ecstasy user characteristics amongst the studies might account, at least in part, for the 'discrepancy' regarding the striatum. Thus, low/typical doses of ecstasy (one to two tablets as in our study and McCann's work) might somewhat selectively cause reduced cerebral cortical SERT binding, whereas higher typical doses (four tablets in Buchert *et al*., 2003, 2004) could affect striatum in addition to cerebral cortex. The possibility that striatum might be affected in some high-dose ecstasy users is indicated by our single post-mortem case study finding of marked (~50%) reduction of SERT protein concentration in striatum (and in occipital cortex) in a very heavy dose ecstasy user (Kish *et al*., 2010). The suggestion that more distal targets of brainstem raphé serotonergic neurons, including occipital cortex, might be more susceptible to potential toxic damage from ecstasy is supported by some limited non-human primate data showing that the cerebral (especially occipital) cortex is more vulnerable to ecstasy than striatum in terms of persistence of serotonin reduction, perhaps because of different nerve ending characteristics or proximity from cell body (Hatzidimitriou *et al*., 1999). It is also possible that serotonergic neurons innervating subcortical brain areas might initially be 'damaged' by ecstasy use, but during chronic exposure become excessively innervated (e.g. see Scheffel *et al*., 1998). Our human findings suggest that experimental animal studies aimed at addressing these possibilities are warranted.

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Supplementary material

Supplementary material is available at *Brain* online.

References

Ad-Dab'bagh Y, Einarson D, Lyttelton O, Muehlboeck JS, Mok K, Ivanov O, et al. The CIVET image-processing environment: a fully automated comprehensive pipeline for anatomical neuroimaging research. In: NeuroImage, editor. Organization for Human Brain Mapping. Italy: Florence; 2006.

Ad-Dab'bagh Y, Singh V, Robbins S, Lerch J, Lyttelton O, Fombonne E, et al. Native space cortical thickness measurement and the absence of correlation to cerebral volume. In: Zilles K, editor. Organization for human brain mapping. Toronto, Canada: Neuroimage; 2005.

Bailey DL, Young H, Bloomfield PM, Meikle SR, Glass D, Myers MJ, et al. ECAT ART—a continuously rotating PET camera: performance characteristics, initial clinical studies, and installation considerations in a nuclear medicine department. Eur J Nucl Med 1997; 24: 6–15.

Boileau I, Guttman M, Rusjan P, Adams JR, Houle S, Tong J, et al. Decreased binding of the D3 dopamine receptor-preferring ligand [11C]-(+)-PHNO in drug-naive Parkinson's disease. Brain 2009; 132: 1366–75.

Bouso JC, Doblin R, Farre M, Alcazar MA, Gomez-Jarabo G. MDMA-assisted psychotherapy using low doses in a small sample of women with chronic posttraumatic stress disorder. J Psychoactive Drugs 2008; 40: 225–36.

Buchert R, Thiele F, Thomasius R, Wilke F, Petersen K, Brenner W, et al. Ecstasy-induced reduction of the availability of the brain serotonin transporter as revealed by [11C](+)McN5652-PET and the multi-linear reference tissue model: loss of transporters or artifact of tracer kinetic modelling? J Psychopharmacol 2007; 21: 628–34.

Buchert R, Thomasius R, Nebeling B, Petersen K, Obrocki J, Jenicke L, et al. Long-term effects of ''ecstasy'' use on serotonin transporters of the brain investigated by PET. J Nucl Med 2003; 44: 375–84.

Buchert R, Thomasius R, Petersen K, Wilke F, Obrocki J, Nebeling B, et al. Reversibility of ecstasy-induced reduction in serotonin transporter availability in polydrug ecstasy users. Eur J Nucl Med Mol Imaging 2006; 33: 188–99.

Buchert R, Thomasius R, Wilke F, Petersen K, Nebeling B, Obrocki J, et al. A voxel-based PET investigation of the long-term effects of

''Ecstasy'' consumption on brain serotonin transporters. Am J Psychiatry 2004; 161: 1181–9.
Chakravarty MM, Sadikot AF, Germann J, Bertrand G, Collins DL. Towards a validation of atlas warping techniques. Med Image Anal 2008; 12: 713–26.
Chang L, Cloak C, Patterson K, Grob C, Miller EN, Ernst T. Enlarged striatum in abstinent methamphetamine abusers: a possible compensatory response. Biol Psychiatry 2005; 57: 967–74.
Chung MK, Taylor J. Diffusion smoothing on brain surface via finite element method, biomedical imaging: macro to nano. IEEE Int Symp 2004; 1: 432–5.
Cohen RS. Subjective reports on the effects of the MDMA ('ecstasy') experience in humans. Prog Neuropsychopharmacol Biol Psychiatry 1995; 19: 1137–45.
Collins Dl HC. Automatic 3D model-based neuroanatomical segmentation. Hum Brain Mapp 1995; 3: 190.
Collins DL, Neelin P, Peters TM, Evans AC. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr 1994; 18: 192.
Cook EH Jr, Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A, et al. Evidence of linkage between the serotonin transporter and autistic disorder. Mol Psychiatry 1997; 2: 247–50.
Cowan RL, Lyoo IK, Sung SM, Ahn KH, Kim MJ, Hwang J, et al. Reduced cortical gray matter density in human MDMA (Ecstasy) users: a voxel-based morphometry study. Drug Alcohol Depend 2003; 72: 225–35.
Cselenyi Z, Olsson H, Farde L, Gulyas B. Wavelet-aided parametric mapping of cerebral dopamine D2 receptors using the high affinity PET radioligand [11C]FLB 457. Neuroimage 2002; 17: 47–60.
Cselenyi Z, Olsson H, Halldin C, Gulyas B, Farde L. A comparison of recent parametric neuroreceptor mapping approaches based on measurements with the high affinity PET radioligands [11C]FLB 457 and [11C]WAY 100635. Neuroimage 2006; 32: 1690–708.
Davidson C, Gow AJ, Lee TH, Ellinwood EH. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. Brain Res Brain Res Rev 2001; 36: 1–22.
de Sola Llopis S, Miguelez-Pan M, Pena-Casanova J, Poudevida S, Farre M, Pacifici R, et al. Cognitive performance in recreational ecstasy polydrug users: a two-year follow-up study. J Psychopharmacol 2008; 22: 498–510.
de Win MM, Jager G, Booij J, Reneman L, Schilt T, Lavini C, et al. Sustained effects of ecstasy on the human brain: a prospective neuroimaging study in novel users. Brain 2008a; 131: 2936–45.
de Win MM, Jager G, Booij J, Reneman L, Schilt T, Lavini C, et al. Neurotoxic effects of ecstasy on the thalamus. Br J Psychiatry 2008b; 193: 289–96.
Defrise M, Kinahan PE, Townsend DW, Michel C, Sibomana M, Newport DF. Exact and approximate rebinning algorithms for 3-D PET data. IEEE Trans Med Imaging 1997; 16: 145–58.
Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test-II. San Antonio, TX, 2000.
Denman SB. Denman Neuropsychology Memory Scale: Norms, 1987.
Dumont GJ, Verkes RJ. A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers. J Psychopharmacol 2006; 20: 176–87.
Fahn S, Elton RL. Unified Parkinson's Disease Rating Scale, in Recent development in Parkinson's disease. Vol. 2. Macmillan Healthcare Information, 1987.
First MB, Spitzer RL, Gibbon M, Williams JW. Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Non-patient Edition (SCID-I/NP, 11/2002 revision). New York: New York State Psychiatric Institute; 2002.
Frankle WG, Huang Y, Hwang DR, Talbot PS, Slifstein M, Van Heertum R, et al. Comparative evaluation of serotonin transporter radioligands 11C-DASB and 11C-McN 5652 in healthy humans. J Nucl Med 2004; 45: 682–94.
Ginovart N, Wilson AA, Meyer JH, Hussey D, Houle S. Positron emission tomography quantification of [(11)C]-DASB binding to the human serotonin transporter: modeling strategies. J Cereb Blood Flow Metab 2001; 21: 1342–53.
Goldstein RZ, Craig AD, Bechara A, Garavan H, Childress AR, Paulus MP, et al. The neurocircuitry of impaired insight in drug addiction. Trends Cogn Sci 2009; 13: 372–80.
Gough B, Ali SF, Slikker W Jr, Holson RR. Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamines in rat caudate. Pharmacol Biochem Behav 1991; 39: 619–23.
Gronwall DM. Paced auditory serial-addition task: a measure of recovery from concussion. Percept Mot Skills 1977; 44: 367–73.
Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960; 23: 56–62.
Hartley AA, Speer NK. Locating and fractionating working memory using functional neuroimaging: storage, maintenance, and executive functions. Microsc Res Tech 2000; 51: 45–53.
Hatzidimitriou G, McCann UD, Ricaurte GA. Altered serotonin innervation patterns in the forebrain of monkeys treated with (+/−)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. J Neurosci 1999; 19: 5096–107.
Hedlund JL, Vieweg BW. The Hamilton ratting scale for depression. J Operat Psychiatry 1979; 10: 149–65.
Hekmatpanah CR, Peroutka SJ. 5-hydroxytryptamine uptake blockers attenuate the 5-hydroxytryptamine-releasing effect of 3,4-methylenedioxymethamphetamine and related agents. Eur J Pharmacol 1990; 177: 95–8.
Hoppe CD, Muller UD, Werheid KD, Thone AD, von Cramon YD. Digit Ordering Test: clinical, psychometric, and experimental evaluation of a verbal working memory test. Clin Neuropsychol 2000; 14: 38–55.
Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, et al. Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. J Cereb Blood Flow Metab 2003; 23: 1096–112.
Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 2007; 27: 1533–9.
Jernigan TL, Gamst AC, Archibald SL, Fennema-Notestine C, Mindt MR, Marcotte TD, et al. Effects of methamphetamine dependence and HIV infection on cerebral morphology. Am J Psychiatry 2005; 162: 1461–72.
Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991; 14: 540–5.
Kalasinsky KS, Hugel J, Kish SJ. Use of MDA (the ''love drug'') and methamphetamine in Toronto by unsuspecting users of ecstasy (MDMA). J Forensic Sci 2004; 49: 1106–12.
Kalbitzer J, Frokjaer VG, Erritzoe D, Svarer C, Cumming P, Nielsen FA, et al. The personality trait openness is related to cerebral 5-HTT levels. Neuroimage 2009; 45: 280–5.
Kim JS, Singh V, Lee JK, Lerch J, Ad-Dab'bagh Y, MacDonald D, et al. Automated 3-D extraction and evaluation of the inner and outer cortical surfaces using a Laplacian map and partial volume effect classification. Neuroimage 2005; 27: 210–21.
Kish S, Fitzmaurice P, Chang L, Furukawa Y, Tong J. Low striatal serotonin transporter protein in a human polydrug MDMA (ecstasy) user: a case study. J Psychopharmacol 2010; 24: 281–4.
Kish SJ. How strong is the evidence that brain serotonin neurons are damaged in human users of ecstasy? Pharmacol Biochem Behav 2002; 71: 845–55.
Kish SJ, Fitzmaurice PS, Boileau I, Schmunk GA, Ang LC, Furukawa Y, et al. Brain serotonin transporter in human methamphetamine users. Psychopharmacology (Berl) 2009; 202: 649–61.
Kish SJ, Furukawa Y, Chang LJ, Tong J, Ginovart N, Wilson A, et al. Regional distribution of serotonin transporter protein in postmortem human brain: is the cerebellum a SERT-free brain region? Nucl Med Biol 2005; 32: 123–8.
Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989; 46: 1121–3.

Lerch JP, Evans AC. Cortical thickness analysis examined through power analysis and a population simulation. Neuroimage 2005; 24: 163–73.
Lerch JP, Pruessner JC, Zijdenbos A, Hampel H, Teipel SJ, Evans AC. Focal decline of cortical thickness in Alzheimer's disease identified by computational neuroanatomy. Cereb Cortex 2005; 15: 995–1001.
Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996; 274: 1527–31.
Liechti ME, Baumann C, Gamma A, Vollenweider FX. Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, ''Ecstasy'') are attenuated by the serotonin uptake inhibitor citalopram. Neuropsychopharmacology 2000; 22: 513–21.
Liechti ME, Vollenweider FX. The serotonin uptake inhibitor citalopram reduces acute cardiovascular and vegetative effects of 3,4-methylenedioxymethamphetamine ('Ecstasy') in healthy volunteers. J Psychopharmacol 2000; 14: 269–74.
Logan J, Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ, et al. Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl]-(-)-cocaine PET studies in human subjects. J Cereb Blood Flow Metab 1990; 10: 740–7.
Lu NZ, Eshleman AJ, Janowsky A, Bethea CL. Ovarian steroid regulation of serotonin reuptake transporter (SERT) binding, distribution, and function in female macaques. Mol Psychiatry 2003; 8: 353–60.
Lyttelton O, Boucher M, Robbins S, Evans A. An unbiased iterative group registration template for cortical surface analysis. Neuroimage 2007; 34: 1535–44.
MacDonald D, Kabani N, Avis D, Evans AC. Automated 3-D extraction of inner and outer surfaces of cerebral cortex from MRI. Neuroimage 2000; 12: 340–56.
Martinez D, Slifstein M, Broft A, Mawlawi O, Hwang DR, Huang Y, et al. Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. J Cereb Blood Flow Metab 2003; 23: 285.
Mash DC, Staley JK, Izenwasser S, Basile M, Ruttenber AJ. Serotonin transporters upregulate with chronic cocaine use. J Chem Neuroanat 2000; 20: 271–80.
Mawlawi O, Martinez D, Slifstein M, Broft A, Chatterjee R, Hwang DR, et al. Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D(2) receptor parameter measurements in ventral striatum. J Cereb Blood Flow Metab 2001; 21: 1034.
Mazziotta J, Toga A, Evans A, Fox P, Lancaster J, Zilles K, et al. A probabilistic atlas and reference system for the human brain: International Consortium for Brain Mapping (ICBM). Philos Trans R Soc Lond B Biol Sci 2001; 356: 1293–322.
McCann UD, Ricaurte GA. Amphetamine neurotoxicity: accomplishments and remaining challenges. Neurosci Biobehav Rev 2004; 27: 821–6.
McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA (''Ecstasy'') on brain serotonin neurons in human beings. Lancet 1998a; 352: 1433–7.
McCann UD, Szabo Z, Seckin E, Rosenblatt P, Mathews WB, Ravert HT, et al. Quantitative PET studies of the serotonin transporter in MDMA users and controls using [11C]McN5652 and [11C]DASB. Neuropsychopharmacology 2005; 30: 1741–50.
McCann UD, Szabo Z, Vranesic M, Palermo M, Mathews WB, Ravert HT, et al. Positron emission tomographic studies of brain dopamine and serotonin transporters in abstinent (+/−)3,4-methylenedioxymethamphetamine (''ecstasy'') users: relationship to cognitive performance. Psychopharmacology (Berl) 2008; 200: 439–50.
McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA. Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [11C]WIN-35,428. J Neurosci 1998b; 18: 8417–22.
Mendelsohn D, Riedel WJ, Sambeth A. Effects of acute tryptophan depletion on memory, attention and executive functions: a systematic review. Neurosci Biobehav Rev 2009; 33: 926–52.
Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N, et al. Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. Arch Gen Psychiatry 2004; 61: 1271–9.
Nelson HE. National Adult Reading Test (NART). NFER-Nelson: Windsor; 1982.
Parrott AC. Recreational Ecstasy/MDMA, the serotonin syndrome, and serotonergic neurotoxicity. Pharmacol Biochem Behav 2002; 71: 837–44.
Parrott AC. Chronic tolerance to recreational MDMA (3,4-methylenedioxymethamphetamine) or Ecstasy. J Psychopharmacol 2005; 19: 71–83.
Parrott AC, Lock J, Conner AC, Kissling C, Thome J. Dance clubbing on MDMA and during abstinence from Ecstasy/MDMA: prospective neuroendocrine and psychobiological changes. Neuropsychobiology 2008; 57: 165–80.
Parrott AC, Marsden CA. MDMA (3,4-methylenedioxymethamphetamine) or ecstasy: the contemporary human and animal research perspective. J Psychopharmacol 2006; 20: 143–6.
Parsey RV, Hastings RS, Oquendo MA, Hu X, Goldman D, Huang YY, et al. Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. Am J Psychiatry 2006; 163: 48–51.
Peterson BS, Warner V, Bansal R, Zhu H, Hao X, Liu J, et al. Cortical thinning in persons at increased familial risk for major depression. Proc Natl Acad Sci USA 2009; 106: 6273–8.
Praschak-Rieder N, Kennedy J, Wilson AA, Hussey D, Boovariwala A, Willeit M, et al. Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: a [(11)C] DASB positron emission tomography study. Biol Psychiatry 2007; 62: 327–31.
Praschak-Rieder N, Willeit M, Wilson AA, Houle S, Meyer JH. Seasonal variation in human brain serotonin transporter binding. Arch Gen Psychiatry 2008; 65: 1072–8.
Ramamoorthy S, Blakely RD. Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. Science 1999; 285: 763–6.
Rehavi M, Sepcuti H, Weizman A. Upregulation of imipramine binding and serotonin uptake by estradiol in female rat brain. Brain Res 1987; 410: 135–9.
Reimold M, Smolka MN, Schumann G, Zimmer A, Wrase J, Mann K, et al. Midbrain serotonin transporter binding potential measured with [11C]DASB is affected by serotonin transporter genotype. J Neural Transm 2007; 114: 635–9.
Reitan RM. Validity of the trail making test as an indicator organic brain damage. Perceptual and Motor Skills 1958; 8: 271–6.
Reneman L, Booij J, de Bruin K, Reitsma JB, de Wolff FA, Gunning WB, et al. Effects of dose, sex, and long-term abstention from use on toxic effects of MDMA (ecstasy) on brain serotonin neurons. Lancet 2001; 358: 1864–9.
Renoir T, Paizanis E, Yacoubi ME, Saurini F, Hanoun N, Melfort M, et al. Differential long-term effects of MDMA on the serotoninergic system and hippocampal cell proliferation in 5-HTT knock-out vs. wild-type mice. Int J Neuropsychopharmacol 2008; 11: 1149–62.
Ricaurte GA, McCann UD. Experimental studies on 3,4-methylenedioxymethamphetamine (MDA, ''ecstasy'') and its potential to damage brain serotonin neurons. Neurotox Res 2001; 3: 85–99.
Robbins S, Evans AC, Collins DL, Whitesides S. Tuning and comparing spatial normalization methods. Med Image Anal 2004; 8: 311–23.
Rousset OG, Ma Y, Evans AC. Correction for partial volume effects in PET: principle and validation. J Nucl Med 1998; 39: 904.
Rudnick G, Wall SC. The molecular mechanism of ''ecstasy'' [3,4-methylenedioxy-methamphetamine (MDMA)]: serotonin transporters are targets for MDMA-induced serotonin release. Proc Natl Acad Sci USA 1992; 89: 1817–21.

Rush AJ, Carmody T, Reimitz PE. The Inventory of Depressive Symptomatology (IDS): Clinician (IDS-C) and self-report (IDS-SR) ratings of depressive symptoms. International Journal of Methods in Psychiatric Research 2000; 9: 45–59.

Rusjan P, Mamo D, Ginovart N, Hussey D, Vitcu I, Yasuno F, et al. An automated method for the extraction of regional data from PET images. Psychiatry Res 2006; 147: 79–89.

Scheffel U, Szabo Z, Mathews WB, Finley PA, Dannals RF, Ravert HT, et al. In vivo detection of short- and long-term MDMA neurotoxicity–a positron emission tomography study in the living baboon brain. Synapse 1998; 29: 183–92.

Schmidt CJ, Levin JA, Lovenberg W. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem Pharmacol 1987; 36: 747–55.

Schmidt CJ, Wu L, Lovenberg W. Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue. Eur J Pharmacol 1986; 124: 175–8.

Selvaraj S, Hoshi R, Bhagwagar Z, Murthy NV, Hinz R, Cowen P, et al. Brain serotonin transporter binding in former users of MDMA ('ecstasy'). Br J Psychiatry 2009; 194: 355–9.

Shapiro CM, Auch C, Reimer M, Kayumov L, Heslegrave R, Huterer N, et al. A new approach to the construct of alertness. J Psychosom Res 2006; 60: 595–603.

Shopsin B, Friedman E, Gershon S. Parachlorophenylalanine reversal of tranylcypromine effects in depressed patients. Arch Gen Psychiatry 1976; 33: 811–19.

Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. IEEE Trans Med Imaging 1998; 17: 87.

Smith A. Symbol Digits Modalities Test. Western Psychological Services, Los Angeles, 1982.

Smith SM. Fast robust automated brain extraction. Human Brain Mapp 2002; 17: 143–55.

Sowell ER, Peterson BS, Kan E, Woods RP, Yoshii J, Bansal R, et al. Sex differences in cortical thickness mapped in 176 healthy individuals between 7 and 87 years of age. Cereb Cortex 2007; 17: 1550–60.

Spreen O, Strauss E. A compendium of neuropsychological tests. 2nd edn., New York: Oxford University Press; 1998.

Stone DM, Stahl DC, Hanson GR, Gibb JW. The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. Eur J Pharmacol 1986; 128: 41–8.

Tancer M, Johanson CE. The effects of fluoxetine on the subjective and physiological effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. Psychopharmacology (Berl) 2007; 189: 565–73.

Thomasius R, Petersen K, Buchert R, Andresen B, Zapletalova P, Wartberg L, et al. Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users. Psychopharmacology (Berl) 2003; 167: 85–96.

Tohka J, Zijdenbos A, Evans A. Fast and robust parameter estimation for statistical partial volume models in brain MRI. Neuroimage 2004; 23: 84–97.

Trigo JM, Renoir T, Lanfumey L, Hamon M, Lesch KP, Robledo P, et al. 3,4-methylenedioxymethamphetamine self-administration is abolished in serotonin transporter knockout mice. Biol Psychiatry 2007; 62: 669–79.

Turkheimer FE, Brett M, Visvikis D, Cunningham VJ. Multiresolution analysis of emission tomography images in the wavelet domain. J Cereb Blood Flow Metab 1999; 19: 1189–208.

Verrico CD, Miller GM, Madras BK. MDMA (Ecstasy) and human dopamine, norepinephrine, and serotonin transporters: implications for MDMA-induced neurotoxicity and treatment. Psychopharmacology (Berl) 2007; 189: 489–503.

Warrington EK. Manual for recognition memory test. NFER-Nelson: Windsor; 1984.

Wechsler D. Manual for the Wechsler Adult Intelligence Scale-Revised. New York: The Psychological Corporation; 1981.

Wilson AA, Ginovart N, Hussey D, Meyer J, Houle S. In vitro and in vivo characterisation of [^{11}C]-DASB: a probe for in vivo measurements of the serotonin transporter by positron emission tomography. Nucl Med Biol 2002; 29: 509–15.

Wu Y, Carson RE. Noise reduction in the simplified reference tissue model for neuroreceptor functional imaging. J Cereb Blood Flow Metab 2002; 22: 1440–52.

Zijdenbos AP, Forghani R, Evans AC. Automatic ''pipeline'' analysis of 3-D MRI data for clinical trials: application to multiple sclerosis. IEEE Trans Med Imaging 2002; 21: 1280–91.