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Sustained effects of ecstasy on the human brain: a prospective neuroimaging study in novel users

Maartje M. L. de Win,[1,2] Gerry Jager,[3] Jan Booij,[4] Liesbeth Reneman,[1] Thelma Schilt,[5] Cristina Lavini,[1] Sílvia D. Olabarriaga,[6] Gerard J. den Heeten[1] and Wim van den Brink[5]

[1]Department of Radiology, University of Amsterdam, Academic Medical Center, Amsterdam, [2]Graduate School Neurosciences Amsterdam, [3]Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, University Medical Center, Utrecht, [4]Department of Nuclear Medicine, University of Amsterdam, Academic Medical Center, Amsterdam, [5]Amsterdam Institute for Addiction Research and Department of Psychiatry, University of Amsterdam, Academic Medical Center, Amsterdam and [6]Informatics Institute, University of Amsterdam, Amsterdam, The Netherlands

Correspondence to: Maartje M. L. de Win, MD, PhD, Department of Radiology, G1-229, University of Amsterdam, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
E-mail: m.m.dewin@amc.uva.nl

Previous studies have suggested toxic effects of recreational ecstasy use on the serotonin system of the brain. However, it cannot be excluded that observed differences between users and non-users are the cause rather than the consequence of ecstasy use. As part of the Netherlands XTC Toxicity (NeXT) study, we prospectively assessed sustained effects of ecstasy use on the brain in novel ecstasy users using repeated measurements with a combination of different neuroimaging parameters of neurotoxicity. At baseline, 188 ecstasy-naive volunteers with high probability of first ecstasy use were examined. After a mean period of 17 months follow-up, neuroimaging was repeated in 59 incident ecstasy users and 56 matched persistent ecstasy-naives and their outcomes were compared. Neuroimaging included [^{123}I]β-carbomethoxy-3β-(4-iodophenyl)tropane (CIT) SPECT to measure serotonin transporter densities as indicators of serotonergic function; ^{1}H-MR spectroscopy (^{1}H-MRS) to measure brain metabolites as indicators of neuronal damage; diffusion tensor imaging (DTI) to measure the apparent diffusion coefficient and fractional anisotropy (FA) of the diffusional motion of water molecules in the brain as indicators of axonal integrity; and perfusion weighted imaging (PWI) to measure regional relative cerebral blood volume (rrCBV) which indicates brain perfusion. With this approach, both structural (^{1}H-MRS and DTI) and functional ([^{123}I]β-CIT SPECT and PWI) aspects of neurotoxicity were combined. Compared to persistent ecstasy-naives, novel low-dose ecstasy users (mean 6.0, median 2.0 tablets) showed decreased rrCBV in the globus pallidus and putamen; decreased FA in thalamus and frontoparietal white matter; increased FA in globus pallidus; and increased apparent diffusion coefficient in the thalamus. No changes in serotonin transporter densities and brain metabolites were observed. These findings suggest sustained effects of ecstasy on brain microvasculature, white matter maturation and possibly axonal damage due to low dosages of ecstasy. Although we do not know yet whether these effects are reversible or not, we cannot exclude that ecstasy even in low doses is neurotoxic to the brain.

Keywords: ecstasy; prospective; neuroimaging; [123I]β-CIT SPECT; MRI

Abbreviations: ADC = apparent diffusion coefficient; CIT = carbomethoxy-3β-(4-iodophenyl)tropane; DART = Dutch version of the National Adult Reading Test; DTI = diffusion tensor imaging; FA = fractional anisotropy; ^{1}H-MRS = ^{1}H-MR spectroscopy; MDMA = 3,4-methylenedioxymethamphetamine; MNI = Montreal Neurological Institute brain template; NAA = N-acetylaspartate; NeXT = Netherlands XTC toxicity; PWI = perfusion weighted imaging; ROI = regions of interest; rrCBV = regional relative cerebral blood volume; SERT = serotonin transporter; SPECT = single photon emission computed tomography

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Introduction

After cannabis, ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is the most commonly used illegal substance in most countries worldwide, especially among adolescents and young adults. Despite its popularity, there are strong indications from animal and human studies that ecstasy is toxic to the axons of serotonin cells (Ricaurte *et al.*, 2000; Reneman *et al.*, 2006). Serotonin is an important neurotransmitter for the regulation of processes such as mood and memory. However, results from animal studies cannot simply be extrapolated to humans, and most human studies are littered with methodological problems, including inadequate subject sampling, retrospective designs, lack of baseline data before first ecstasy use and inclusion of only moderate or heavy ecstasy users (Lyvers, 2006). This leaves the possibility that observed differences between ecstasy users and controls are biased or pre-existent, and explains why the discussion of whether ecstasy is really neurotoxic in humans is ongoing (Turner and Parrott, 2000). Recently, studies have even started to assess potential benefits of MDMA as adjuvant in psychotherapy (Check, 2004). Only a few studies prospectively examined the effects of ecstasy in volunteers without prior ecstasy experience, but these studies were all focused on acute effects of ecstasy (Dumont and Verkes, 2006).

Only a long-term prospective study in ecstasy-naive individuals randomly assigned to MDMA or placebo can determine decisively whether recreational ecstasy use is neurotoxic in humans. Given the existing data on the potential neurotoxicity of ecstasy, such a study is ethically disputable. Therefore, it has recently been advocated to start prospective studies in specific groups with increased risk of future ecstasy use and re-examine these volunteers over time (Gouzoulis-Mayfrank and Daumann, 2006). The current study, part of the Netherlands XTC Toxicity (NeXT) study, succeeded in this approach and is the first prospective study focusing on the sustained effects of ecstasy.

We prospectively assessed the parameters for neurotoxicity of ecstasy in 59 volunteers before and after their first period of drug use and compared these novel ecstasy users with a matched group of 58 persistent ecstasy-naive volunteers. The parameters included [^{123}I]β-carbomethoxy-3β-(4-iodophenyl)tropane (CIT) single photon emission computed tomography (SPECT) to measure serotonin transporter (SERT) densities; proton magnetic resonance spectroscopy (^{1}H-MRS) to measure the brain metabolites *N*-acetylaspartate (NAA; neuronal marker), choline (Cho; reflecting cellular density) and myo-inositol (mI; marker for gliosis) relative to (phospho)creatine (Cr) (Miller *et al.*, 1996; Ross *et al.*, 1997; Pouwels and Frahm, 1998); diffusion tensor imaging (DTI) to measure apparent diffusion coefficient (ADC) and fractional anisotropy (FA) of the diffusional motion of water molecules in the brain to indicate axonal integrity (Le Bihan *et al.*, 1992; Haykin *et al.*, 2006); and perfusion weighted imaging (PWI) to measure regional relative cerebral blood volume (rrCBV) which indicates brain perfusion (Belliveau *et al.*, 1990; Levin *et al.*, 1996). With this approach, we combined imaging techniques that measure both structural (^{1}H-MRS and DTI) and functional ([^{123}I]β-CIT SPECT and PWI) aspects of neurotoxicity. Based on previous findings in ecstasy users (Chang *et al.*, 1999; Chang *et al.*, 2000; Reneman *et al.*, 2000; Reneman *et al.*, 2002b; Daumann *et al.*, 2004; Reneman *et al.*, 2006), we hypothesized that if ecstasy is neurotoxic, novel ecstasy users, and not persistent ecstasy-naives, would show a decrease (after a short period of abstinence from ecstasy) or increase (after longer period abstinence from ecstasy) in rrCBV and ADC; an increase in brain metabolite ratios of choline and myo-inositol relative to creatine (Cho/Cr and mI/Cr); a decrease in [^{123}I]β-CIT binding to SERTs; and a decrease in FA and the ratio of *N*-acetylaspartate to creatine (NAA/Cr).

Methods

Design and subjects

A cohort of 188 ecstasy-naive young adults (77 males, 111 females, 21.7 ± 3.0 year) with a relatively high probability of starting to use ecstasy in the near future was recruited between 2002 and 2004 using a combination of targeted site sampling, advertisement and snowball sampling, as described in a special design paper on the NeXT study (de Win *et al.*, 2005*b*). Main inclusion criteria were high probability of using ecstasy in the near future, indicated by the intention to probably or certainly use ecstasy for the first time in near future and/or having one or more friends who already use ecstasy. Exclusion criteria were: age <18 years or >35 years, past ecstasy use, severe physical or mental illness, use of psychotropic medications (e.g. serotonin reuptake inhibitors), pregnancy and use of intravenous drugs. Subjects had to abstain from psychoactive substances for at least 2 weeks and from alcohol for at least 1 week before examinations. This was checked in urine (enzyme-multiplied immunoassay for amphetamines, MDMA, opioids, cocaine, benzodiazepine, cannabis and alcohol). None of the subjects had to be excluded because of a positive urine drug test.

At baseline examination, all subjects underwent SPECT and MR imaging. Thereafter, subjects were sent four questionnaires by mail regarding their drug use during a follow-up period of 12–24 months. Between 12 and 36 months after baseline assessments, all novel ecstasy users and an individually matched (gender, age, verbal intelligence and cannabis use) control group of persistent ecstasy-naives (subjects from the same baseline population who did not start to use ecstasy during follow-up) were invited for a follow-up session during which brain imaging was repeated.

Subjects were paid for their participation (per session €100–€150). The study was approved by the local medical ethics committee and informed consent of each subject was obtained according to the Declaration of Helsinki. Some of the new ecstasy users took part in an intermediate session with MRI and psychopathology measurements soon after first ecstasy use (de Win *et al.*, 2007). Subjects also completed questionnaires on psychopathology (de Win *et al.*, 2006), underwent functional MRI (Jager *et al.*, 2007) and cognitive testing (Schilt *et al.*, 2007), as reported in separate publications.

Assessment of potential confounders

Potential confounders, such as demographic variables, education and substance use were measured at baseline and follow-up. Aspects of lifetime ecstasy use (frequency and duration of use, cumulative number of tablets), use of alcohol in the previous year (units per week), tobacco (cigarettes per week), cannabis (number of joints in the previous year), amphetamines (number of uses in the previous year) and cocaine (number of uses in the previous year) were assessed using validated questionnaires (Van de Wijngaart *et al.*, 1999). An estimate of verbal intelligence was measured using the Dutch version of the National Adult Reading Test (DART) (Nelson, 1991). Genotyping of the SERT was performed as described elsewhere (Lesch *et al.*, 1996). Genotyping was successful in 93 out of 115 samples, because of poor DNA isolation in the others.

MRI acquisition and post-processing

Acquisition

MRIs were performed on a 1.5 T scanner (Signa Horizon, LX 9.0, General Electric Medical Systems, Milwaukee, WI, USA) using the standard head coil. MRI acquisition, post-processing and quality control were performed with the same methods used in previous NeXT substudies (de Win *et al.*, 2007; de Win *et al.*, 2008). The most relevant aspects are summarized. The protocol included axial proton density- and T_2-weighted imaging; three voxel-based ^{1}H-MRS scans; DTI; PWI; and high-resolution T_1-weigthed 3D imaging. The ^{1}H-MRS voxels were placed in the left frontoparietal white matter (centrum semiovale) and in mid-frontal and mid-occipital grey matter in analogy to previous studies (Reneman *et al.*, 2002*b*). Positioning of subjects, slices and voxels were performed by the same examiner and according to a protocol in order to keep positioning as reproducible as possible.

Post-processing

From the ^{1}H-MRS, ratios of NAA/Cr, Cho/Cr and mI/Cr were analysed using LCModel (Linear Combination of Model spectra) (Provencher, 1993). ADC and FA maps were calculated from the DTI scans (Hunsche *et al.*, 2001) and CBV maps from the PWI scans (Levin *et al.*, 1996). FA, ADC and CBV were spatially normalized by registration to the Montreal Neurological Institute brain template (MNI152) and segmentation was performed into CSF, white and grey matter (Fig. 1). The CBV maps were intensity-scaled to mean individual CBV intensity of white matter derived from the segmentation procedure to generate relative CBV (rCBV) maps.

Regions of interest (ROIs) were drawn on the MNI152 brain template in thalamus, putamen, globus pallidus, head of caudate nucleus, frontoparietal white matter and dorsolateralfrontal, mid-frontal, occipital, superior parietal and temporal cortex. Only grey matter voxels were included for the cortical ROIs; whereas, white and grey matter voxels were included in the ROIs of the basal ganglia (i.e. excluding CSF voxels). Selection of ROIs was based on the findings of previous studies, which indicated that ecstasy-induced abnormalities are most prominent in the thalamus, basal ganglia and certain cortical areas; ecstasy-induced abnormalities in white matter were rarely reported and thus not expected. As cortical grey matter has very low anisotropy, it is difficult to get reliable FA and ADC measurements in cortical areas. For this reason only ROIs in white matter and basal ganglia were taken into account in the measurements of FA and ADC. Within the ROIs, individual mean values of FA, ADC and rrCBV were calculated. Values of FA, ADC and rrCBV from ROIs in left and right hemispheres were averaged.

SPECT acquisition and post-processing

Acquisition

SPECT imaging was performed with the radioligand [^{123}I]β-CIT that binds non-selectively to SERTs. The procedure of radiosynthesis of [^{123}I]β-CIT and acquiring of SPECT images were the same as previously described (de Win *et al.*, 2005a). SPECT images were acquired 4 h after the injection, when stable specific binding uptake to the SERTs is reached (Pirker *et al.*, 2000).

Post-processing

Attenuation correction and post-processing were performed as previously described (de Win *et al.*, 2008). In short, the SPECT images were first registered (rigid body) to the T1-3D MRI scans of the same subject and both were registered to the 152MNI brain (affine transformations; Fig. 1). For quantification, both ROI and voxel-by-voxel analyses were performed. For the ROI analysis, regions were drawn on the 152MNI template in midbrain, thalamus, temporal cortex, frontal cortex and occipital cortex. We did not measure SERT uptake in the putamen, caudate nucleus and globus pallidus, because there is no specific binding to SERT or DAT in these regions 4 h after [^{123}I]β-CIT injection. Activity in the cerebellum was assumed to represent non-specific activity. Specific to non-specific binding ratios were calculated as (activity in ROI − activity in cerebellum)/activity in cerebellum. Image registration steps were visually inspected to check the quality of the registration.

Voxel-by-voxel analysis was performed with Statistical Parametric Mapping software (SPM2, Wellcome Department of Imaging Neuroscience, Functional Imaging Laboratory, London, UK; www.fil.ion.ucl.ac.uk/spm) as previously described (Friston *et al.*, 1995;

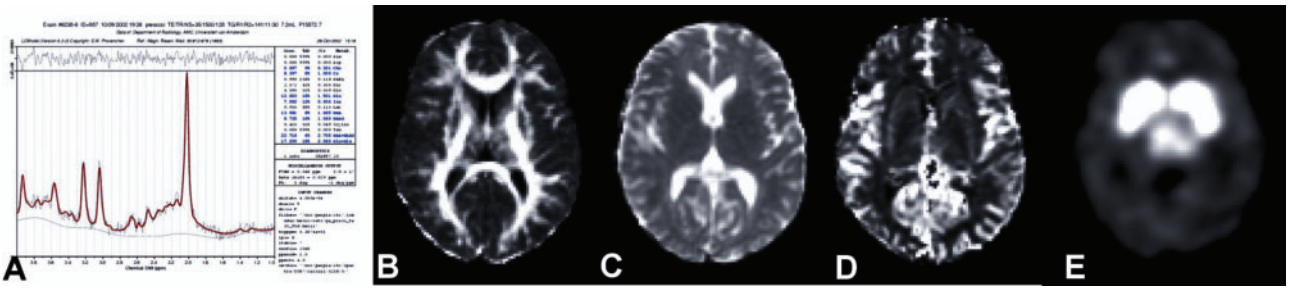

Fig. 1 Representative images of an individual (**A**) ^{1}H-MRS after analysis by LCModel and representative; (**B**) FA; (**C**) ADC; (**D**) rrCBV and (**E**) [^{123}I]β-CIT binding images after transformation to the spatially normalized MNI brain template.

de Win *et al.*, 2008). Difference images were created by subtracting the follow-up images from the baseline images.

Statistical analyses

Substance-use variables were log-transformed because they were not normally distributed. Future ecstasy use, i.e. ecstasy use between baseline and follow-up, was categorized as a binary variable: novel ecstasy user versus persistent ecstasy-naive volunteer. Baseline differences between novel ecstasy users and persistent ecstasy-naives in gender and SERT polymorphism were analysed with chi-square tests and age, DART-IQ, years of education and imaging parameters with the Student *t*-tests (two-sided).

To test whether ecstasy use had an effect on imaging parameters, separate linear regression analyses were performed for the total group with the follow-up outcomes as dependent variables, ecstasy use (yes/no) as the independent variable and baseline imaging data as covariates (model 1). If the effect of novel ecstasy use in model 1 was statistically significant, a second linear regression analysis (enter) was performed in which the observed relationship was adjusted for the effect of potential confounders, i.e. gender, baseline verbal IQ and follow-up measures of age, years of education and the use of alcohol, cannabis, amphetamines, cocaine and tobacco (model 2). In order to test whether changes in imaging parameters were related to the amount of ecstasy use, linear regression analyses were performed in the group of novel ecstasy users with follow-up outcomes as dependent variables, cumulative number of ecstasy tablets (log-transformed) as the independent variable and baseline imaging parameters and potential confounders as covariates (model 3).

ROI analyses were performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Mean values reported in the result and discussion sections are followed by their SDs (mean ± SD). Regression coefficient B is reported with 95% CI in the tables and in the text with their two-tailed significance level (*P*-values).

For the SPECT voxel-by-voxel analysis, difference images from the novel ecstasy users and the persistent ecstasy-naives were compared on a voxel-by-voxel basis by means of the spatial extent statistical theory using SPM2 (Friston *et al.*, 1995). The PET/SPECT model 'compare-populations' was chosen (two-sample *t*-test). An effect was considered statistically significant if a cluster of at least 20 connected voxels reached the one-sided P-value < 0.001 ($T = 3.30$, uncorrected for multiple comparisons).

Results

Characteristics of the sample and substance use

Of the 188 volunteers at baseline, 158 (84%) completed the follow-up drug-use questionnaires. The other 30 volunteers refused to participate in follow-up or were lost to follow-up. Of the 158 subjects, 64 (41%) started to use ecstasy during follow-up, whereas the other 94 subjects (59%) remained ecstasy-naive. Of the 64 novel ecstasy users, 59 (92%) agreed to participate in the follow-up session. From the 94 persistent ecstasy-naives, 56 subjects best matching with the ecstasy users (on gender, verbal IQ and cannabis use) were selected to take part in the follow-up session as controls. This resulted in 115 subjects with a follow-up session. Time between baseline and follow-up measurements was 15.9 ± 4.6 months in the ecstasy group and 18.3 ± 6.5 months in the control group ($P = 0.024$).

During the follow-up period, novel ecstasy users used six tablets on average (range: 0.5–80; median 2 tablets) in a mean period of 20.4 ± 23.8 weeks (Table 1). At baseline, the two groups did not significantly differ in terms of gender, age, verbal IQ, SERT polymorphism, alcohol use, smoking and use of cannabis, amphetamine and cocaine ($P > 0.05$; Table 1). Between baseline and follow-up, novel ecstasy users reported modestly higher levels of consumption of alcohol ($P = 0.036$), cannabis ($P = 0.029$), amphetamines ($P = 0.047$) and cocaine ($P = 0.017$) than persistent ecstasy-naive controls.

Brain imaging

The SPECT scans of one subject had to be excluded because of mis-registration to the standard brain. In addition, due to incidental technical problems or the refusal of a subject for a particular part of the study, we do not have complete baseline and follow-up data sets of each subject for all imaging modalities. The numbers of complete data sets per modality are given in Table 2.

Table 2 also shows results from baseline and follow-up comparisons. The two groups did not significantly differ on any of the neuroimaging parameters at baseline. Neither ROI nor voxel-by-voxel analysis showed any significant effect of ecstasy use on [^{123}I]β-CIT binding. Also no significant effect of ecstasy was observed on brain metabolite ratios. However, ecstasy users, relative to non-users, showed a small but significant decrease of FA in the thalamus (−2.1% in XTC+, +1.8% in XTC−; $P = 0.025$) and fronto-parietal white matter (−1.0% in XTC+, +1.5% in XTC−; $P = 0.009$), and of rrCBV in the globus pallidus (−3.9% in XTC+, +3.9% in XTC−; $P = 0.022$) and putamen (−4.5% in XTC+, +1.5% in XTC−; $P = 0.029$); whereas, they showed a significant increase of FA in the globus pallidus (+2.9% in XTC+, −2.9% in XTC−; $P = 0.020$) and of ADC in the thalamus (+1.6% in XTC+, −4.0% in XTC−; $P = 0.017$) (Fig. 2). After correction for potential confounders such as the use of other substances (model 2), all effects of ecstasy according to model 1 remained significant. Within the group of novel ecstasy users, we found no significant dose-response effects of cumulative doses of ecstasy on follow-up outcomes (model 3).

Discussion

This first prospective imaging study in novel ecstasy users suggests that even a low to moderate ecstasy dose of 1–80 tablets (mean 6, median 2 tablets) has some sustained effects on the brain.

Decreased FA and increased ADC in the thalamus may reflect ecstasy-induced axonal damage, because axonal cell membranes are known to be responsible for most of the restriction of water diffusion (Le Bihan *et al.*, 1992) and

Table 1 Demographics, characteristics of ecstasy use and use of other substances[a]

	Baseline			Follow-up		
	Persistent ecstasy naives ($N = 56$)	Future ecstasy users ($N = 59$)	Mean difference (95% CI)[b]	Persistent ecstasy naives ($N = 56$)	Incident ecstasy users ($N = 59$)	Regression coefficient B (95% CI)[d]
Gender	23M, 33F	25M, 34F	$P = 0.888$[c]			
Age	21.5 ± 2.1	21.7 ± 3.1	0.24 (−0.71; 1.19)	23.1 ± 2.1	23.0 ± 3.2	−0.20 (−0.38; −0.03)*
DART-IQ	105.3 ± 10.2	103.5 ± 9.0	−1.56 (−4.98; 1.87)			
Years of education	14.4 ± 1.8	13.9 ± 2.7	−0.45 (−1.26; 0.37)	15.9 ± 2.0	15.0 ± 2.8	−0.39 (−0.84; 0.05)
SERT polymorfism ($n = 93$)	15 l,15 l/s,16 s	18 l,21 l/s,8 s	$P = 0.140$[c]			
Ecstasy						
Cumulative dose (tablets)	NA	NA		NA	6.0 ± 11.6	
Time since first tablet (weeks)	NA	NA		NA	39.2 ± 23.4	
Time since last tablet (weeks)	NA	NA		NA	18.7 ± 17.5	
Duration of ecstasy use (weeks)	NA	NA		NA	20.4 ± 23.8	
Other substances (last year)						
Alcohol (units/week)	10.5 ± 9.1	8.6 ± 7.7	−0.24 (−0.59; 0.10)	8.7 ± 8.1	9.3 ± 8.6	0.59 (0.06; 1.11)*
Tobacco (cig/week)	25.8 ± 54.4	33.4 ± 47.5	0.85 (−0.01; 1.71)	24.9 ± 46.1	39.6 ± 62.6	0.28 (−0.32; 0.88)
Cannabis (joints in last year)	17.6 ± 25.4	48.0 ± 99.8	0.67 (−0.02; 1.37)	21.1 ± 51.8	48.9 ± 114.2	0.59 (0.06; 1.11)*
Amphetamine (number of times used last year)	0.0 ± 0.0	0.1 ± 0.8	0.04 (−0.04; 0.13)	0.0 ± 0.0	0.6 ± 2.1	0.19 (0.00; 0.37)*
Cocaine (number of times used last year)	0.4 ± 1.6	0.9 ± 2.2	0.21 (−0.09; 0.51)	0.4 ± 1.6	2.5 ± 7.3	0.43 (0.08; 0.79)*

[a]Values expressed as mean ± SD, outcomes as regression coefficients (95% CI).
[b]Future ecstasy users versus persistent ecstasy-naives at baseline (*t*-test, substance use log-transformed).
[c]Future ecstasy users versus persistent ecstasy-naives at baseline (chi-square test).
[d]Incident ecstasy users versus persistent ecstasy-naives at follow-up (linear regression adjusted for baseline scores, substance use log-transformed).
*Significant difference, $P < 0.05$.

axonal damage lead to decreased FA and increased ADC. This finding of ecstasy-induced brain pathology in the thalamus corroborates findings from previous studies showing decreased thalamic SERT densities in (heavy) ecstasy users, most probably reflecting damage to terminals of serotonergic axons (Reneman *et al.*, 2001a; Buchert *et al.*, 2004; Reneman *et al.*, 2006; de Win *et al.*, 2008). A study in rats even showed large numbers of degenerating axons in the thalamus after a single exposure to MDMA (Schmued, 2003). As the thalamus is important for neurocognitive processes, one can speculate that ecstasy-induced thalamic damage is (partly) responsible for decreased verbal memory performance frequently reported in heavy ecstasy users (Verkes *et al.*, 2001; Herrero *et al.*, 2002) and recently also shown in the current prospective cohort of low-dose ecstasy users (Schilt *et al.*, 2007). Decreased FA in the frontoparietal white matter may also reflect axonal injury, although white matter was shown to remain relatively unaffected by MDMA. Therefore, we suspect that the reduced FA in frontoparietal white matter reflects abnormal brain maturation, as the difference in FA values between novel ecstasy users and persistent ecstasy-naive subjects in this study results from a combination of a relative decrease in FA in novel ecstasy users and an increase in FA in persistent ecstasy-naives during the follow-up period (Fig. 2). In line with this, a previous study showed a positive relation between mean FA and age in young adults up to 39.5 years, suggesting that structural changes in the brain continue in normal young adults, especially in areas with low anisotropy (Yoshiura *et al.*, 2005). Therefore, we speculate that this normal maturation did occur in the persistent ecstasy-naives; whereas, this failed to occur in the group of novel ecstasy users.

In contrast, we observed an increase in FA in the globus pallidus of novel ecstasy users relative to persistent ecstasy-naives. Increased anisotropy was previously observed in acute stroke (Yang *et al.*, 1999). However, the increased FA in the globus pallidus may also be related to the decreased rrCBV values observed in the globus pallidus and putamen in the current study. Decreased rrCBV may reflect a prolonged vasoconstriction after ecstasy use, resulting in a decrease in extracellular water content due to ecstasy-induced changes in the serotonergic regulation of the microcirculation (Cohen *et al.*, 1996). A previous SPECT study observed subacute decreases in cerebral blood flow (CBF) after only two doses of MDMA, 2–3 weeks after ecstasy intake (Chang *et al.*, 2000). The mean period of abstinence in our present study was 18.7 weeks, and therefore subacute ecstasy-induced vasoconstriction seems unlikely. Moreover, in previous studies we observed increased rrCBV and ADC in heavy ecstasy users and suggested that this was caused by ecstasy-induced serotonergic depletion (Reneman *et al.*, 2001*b*; de Win *et al.*, 2008). These apparent inconsistencies suggest that there is a complex, yet to be unraveled, relationship between ecstasy

Table 2 Results from [^{123}I]β-CIT SPECT, ^{1}H-MRS, DTI and PWI[a]

Imaging	Parameter	Region of interest	Baseline			Follow-up		
			Persistent ecstasy naives	Future ecstasy users	Mean difference (95% CI)[b]	Persistent ecstasy naives	Incident ecstasy users	Regression coefficient B (95% CI)[c]
SPECT	[^{123}I]β-CIT Binding ratios	Midbrain	1.30 ± 0.31	1.33 ± 0.34	0.032 (−0.090; 0.154)	1.27 ± 0.29	1.32 ± 0.32	0.036 (−0.068; 0.140)
($N_{xtc\text{-}naives}$ = 53)		Thalamus	1.26 ± 0.33	1.24 ± 0.29	−0.016 (−0.132; 0.101)	1.12 ± 0.22	1.11 ± 0.21	−0.001 (−0.077; 0.076)
($N_{(future)xtc}$ = 57)		Frontal grey matter	0.03 ± 0.14	0.07 ± 0.12	0.041 (−0.008; 0.089)	0.00 ± 0.08	0.01 ± 0.10	−0.003 (−0.036; 0.31)
		Occipital grey matter	0.06 ± 0.11	0.05 ± 0.13	−0.006 (−0.051; 0.038)	0.05 ± 0.09	0.03 ± 0.10	−0.018 (−0.051; 0.015)
		Temporal grey matter	0.32 ± 0.14	0.32 ± 0.15	0.000 (−0.055; 0.055)	0.28 ± 0.12	0.27 ± 0.14	−0.009 (−0.053; 0.35)
^{1}H-MRS	NAA/Cr	Mid-frontal grey matter	1.30 ± 0.22	1.25 ± 0.25	−0.053 (−0.143; 0.037)	1.27 ± 0.20	1.29 ± 0.18	0.015 (−0.059; 0.089)
($N_{xtc\text{-}naives}$ = 51)		Mid-occipital grey matter	1.49 ± 0.24	1.50 ± 0.18	0.015 (−0.067; 0.097)	1.52 ± 0.24	1.50 ± 0.19	−0.022 (−0.108; 0.064)
($N_{(future)xtc}$ = 52)		Left frontoparietal white matter	1.49 ± 0.30	1.49 ± 0.21	0.002 (−0.100; 0.103)	1.46 ± 0.17	1.50 ± 0.22	0.043 (−0.032; 0.117)
	Cho/Cr	Mid-frontal grey matter	0.24 ± 0.05	0.24 ± 0.04	−0.001 (−0.019; 0.017)	0.23 ± 0.05	0.25 ± 0.05	0.018 (−0.001; 0.038)
		Mid-occipital grey matter	0.15 ± 0.02	0.15 ± 0.02	−0.001 (−0.011; 0.008)	0.15 ± 0.02	0.15 ± 0.02	−0.002 (−0.010; 0.006)
		Left frontoparietal white matter	0.33 ± 0.05	0.34 ± 0.04	0.011 (−0.008; 0.030)	0.34 ± 0.04	0.35 ± 0.05	0.004 (−0.011; 0.018)
	mI/Cr	Mid-frontal grey matter	0.90 ± 0.23	0.90 ± 0.15	−0.003 (−0.079; 0.073)	0.88 ± 0.18	0.89 ± 0.18	0.006 (−0.063; 0.076)
		Mid-occipital grey matter	0.79 ± 0.12	0.81 ± 0.10	0.015 (−0.028; 0.058)	0.83 ± 0.13	0.81 ± 0.11	−0.023 (−0.071; 0.025)
		Left frontoparietal white matter	0.84 ± 0.16	0.83 ± 0.16	−0.012 (−0.074; 0.050)	0.86 ± 0.17	0.84 ± 0.14	−0.019 (−0.077; 0.038)
DTI	FA (×1000)	Thalamus	276 ± 27	281 ± 19	5.30 (−3.39; 14.00)	281 ± 22	275 ± 20	−8.16 (−15.30; −1.02)*
($N_{xtc\text{-}naives}$ = 55)		Globus pallidus	310 ± 67	314 ± 72	4.44 (−21.79; 30.67)	298 ± 38	323 ± 70	23.19 (3.65; 42.73)*
($N_{(future)xtc}$ = 56)		Putamen	222 ± 36	224 ± 46	1.94 (−13.72; 17.59)	221 ± 29	224 ± 38	2.63 (−7.87; 13.14)
		Caudate nucleus	172 ± 34	176 ± 43	4.48 (−10.15; 19.12)	173 ± 38	173 ± 37	−2.26 (−11.78; 7.26)
		Frontoparietal white matter	413 ± 29	421 ± 27	7.83 (−2.77; 18.42)	419 ± 30	417 ± 26	−8.23 (−14.35; −2.10)*
	ADC 10^{-5} mm^2/sec	Thalamus	88.3 ± 14.2	86.3 ± 9.4	−2.05 (−6.58; 2.48)	84.8 ± 8.7	87.7 ± 9.3	3.68 (0.67; 6.69)*
		Globus pallidus	71.9 ± 2.3	72.1 ± 3.6	0.22 (−0.93; 1.37)	71.8 ± 2.3	72.1 ± 3.5	0.13 (−0.81; 1.07)
		Putamen	70.5 ± 1.7	70.7 ± 1.9	0.23 (−0.45; 0.92)	70.5 ± 1.6	70.5 ± 1.8	−0.12 (−0.71; 0.48)
		Caudate nucleus	92.5 ± 15.2	91.6 ± 15.0	0.86 (−6.54; 4.82)	91.51 ± 15.6	93.3 ± 14.3	2.74 (−1.76; 7.25)
		Frontoparietal white matter	69.9 ± 2.2	70.3 ± 1.9	0.38 (−0.40; 1.15)	69.9 ± 2.1	70.4 ± 1.8	0.22 (−0.26; 0.70)
PWI	rrCBV	Thalamus	1.63 ± 0.18	1.64 ± 0.20	0.005 (−0.068; 0.079)	1.59 ± 0.20	1.62 ± 0.19	0.028 (−0.041; 0.097)
($N_{xtc-naives}$ = 54)		Globus pallidus	1.03 ± 0.22	1.03 ± 0.18	0.009 (−0.068; 0.086)	1.07 ± 0.20	0.99 ± 0.21	−0.083 (−0.154; −0.012)*
($N_{(future)xtc}$ = 54)		Putamen	1.30 ± 0.15	1.34 ± 0.15	0.036 (−0.021; 0.094)	1.32 ± 0.12	1.28 ± 0.18	−0.058 (−0.110; −0.006)*
		Caudate nucleus	1.25 ± 0.16	1.28 ± 0.14	0.029 (−0.027; 0.085)	1.23 ± 0.17	1.28 ± 0.18	0.028 (−0.029; 0.085)
		Dorsolateral frontal grey matter	1.67 ± 0.14	1.65 ± 0.18	−0.033 (−0.095; 0.029)	1.70 ± 0.13	1.65 ± 0.17	−0.040 (−0.091; 0.011)
		Mid-frontal grey matter	1.73 ± 0.17	1.69 ± 0.19	−0.041 (−0.111; 0.029)	1.72 ± 0.21	1.69 ± 0.19	−0.007 (−0.068; 0.055)
		Occipital grey matter	2.13 ± 0.21	2.08 ± 0.31	−0.045 (−0.145; 0.055)	2.11 ± 0.23	2.08 ± 0.29	−0.009 (−0.097; 0.078)
		Superior parietal grey matter	1.97 ± 0.18	1.93 ± 0.23	−0.032 (−0.112; 0.048)	1.99 ± 0.19	1.94 ± 0.24	−0.033 (−0.105; 0.039)
		Temporal grey matter	2.03 ± 0.20	2.00 ± 0.21	−0.026 (−0.105; 0.053)	2.03 ± 0.18	2.00 ± 0.26	−0.010 (−0.087; 0.068)

[a]Expressed as mean ± SD. Scores are uncorrected for covariates.
[b]Future ecstasy users versus persistent ecstasy-naives at baseline (*t*-test).
[c]Incident ecstasy users versus persistent ecstasy-naives at follow-up (linear regression adjusted for baseline scores).
*Significant difference, $P < 0.05$.

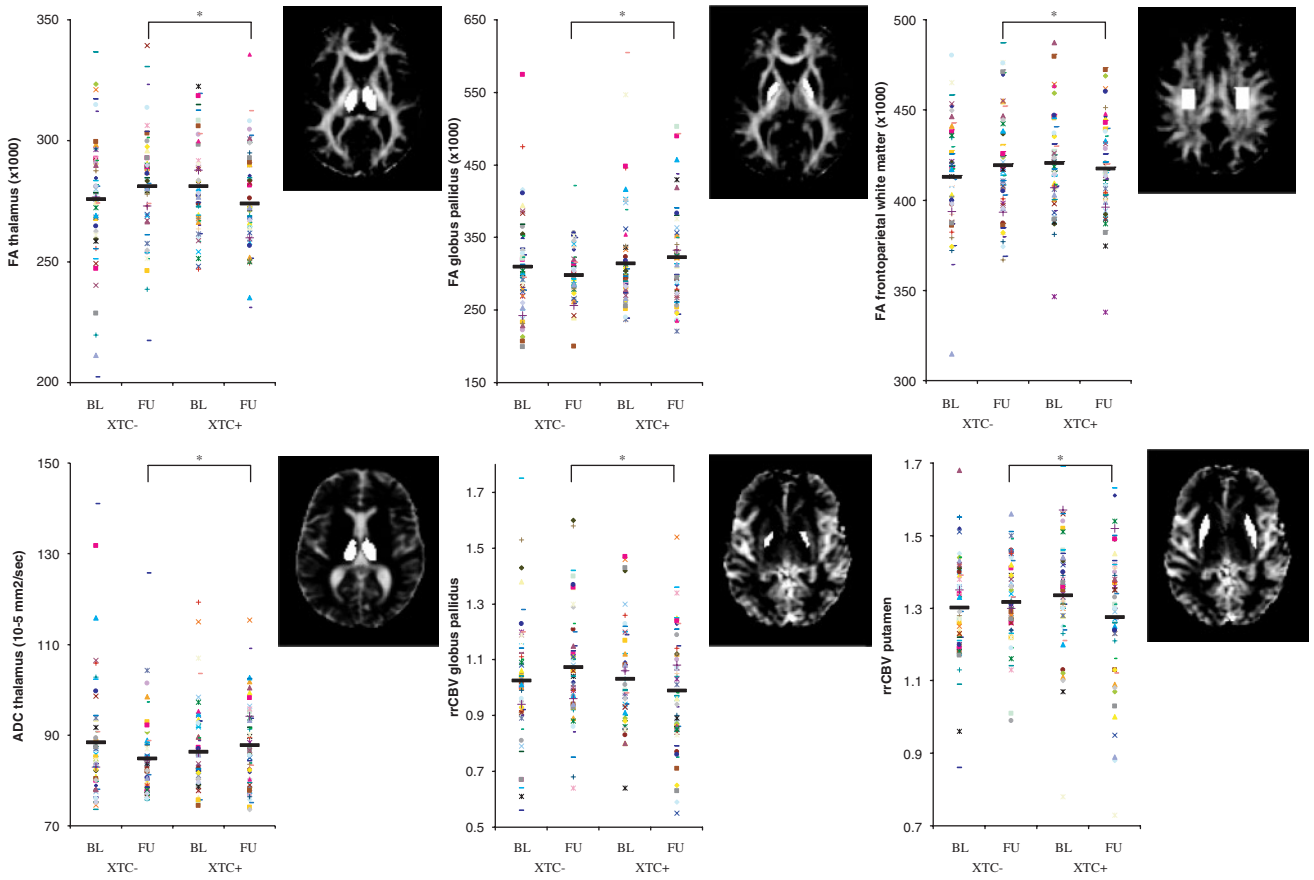

Fig. 2 Areas with significantly different findings between novel ecstasy users and persistent ecstasy-naives at follow-up ($^{*}P < 0.05$), corrected for baseline measurements. Points represent individual and mean FA values in the thalamus, globus pallidus and frontoparietal white matter, ADC values in the thalamus and rrCBV values in the globus pallidus and the putamen in ecstasy-naives (XTC−) and novel ecstasy users (XTC+) at baseline (BL) and at follow-up (FU). In the right corner of each graph, the corresponding brain regions (marked in white) drawn on the FA, ADC and rCBV maps are given. Baseline scoreswere not significantly different between both groups ($P > 0.05$). Only statistical significant results are shown, for complete results of all analyses see Table 2. Note that the vertical axis does not start at 0.

use and serotonergic-mediated brain perfusion, probably related to time since last tablet, cumulative dose, and adaptation of serotonergic transporters and receptors to the ecstasy-induced increase of serotonin in the (sub)acute stage and serotonergic depletion on the long-term.

We did not observe changes in SERTs and brain metabolites after first ecstasy use. Various studies showed that SERT reductions are dose dependent and observed no differences between moderate ecstasy users and controls (Reneman *et al.*, 2006). The same holds true for previous ^{1}H-MRS studies that showed decreased NAA and increased mI metabolite ratios in heavy users (Chang *et al.*, 1999; Reneman *et al.*, 2002*b*); whereas, others found no change in metabolite ratios in subjects with more moderate lifetime doses (Daumann *et al.*, 2004). Also, in a variety of animal species the effects of MDMA on serotonergic axons have been demonstrated to be dependent upon the dose given (Steele *et al.*, 1994). The current observation that low-dose ecstasy use has no sustained effect on central SERTs and brain metabolites is thus in line with previous studies in moderate ecstasy users. It is also possible that [^{123}I]β-CIT SPECT and ^{1}H-MRS are not sensitive enough to detect the small changes expected after low-dose ecstasy use. Previous animal studies showed for example that *in vivo* [^{123}I]β-CIT SPECT underestimated decreases in SERT densities after MDMA administration, when compared to *ex vivo* measurements of SERTs (de Win *et al.*, 2004).

No significant differences in substance use, SERT polymorphisms and imaging parameters were found between future ecstasy users and persistent ecstasy-naives at baseline. This is important, because it has been repeatedly suggested that observed differences between ecstasy users and non-users in previous studies may have been a pre-existing risk factor for ecstasy use rather than a consequence of this drug.

Taken together, most of the neuroimaging parameters of neurotoxicity showed no evidence of adverse effects of relatively low dosages of ecstasy on the brain. On the other hand, we did find some significant effects of low-dose ecstasy use on PWI and DTI, even after a mean abstinence period of 18.7 weeks. As expected with such low dosages, the effects were rather small with differences between the groups being <5% and with outcomes that are within the

normal range of the age group. The changes we found were for example much smaller than changes found in FA in diseases like Alzheimer's (−30% lower FA in compared to controls; Parente *et al*., 2008), amyotrophic lateral sclerosis (Iwata *et al*., 2008) and in leukemia survivors (17% decrease in the FA value in temporal white matter; Dellani *et al*., 2008). An important question is, therefore, whether the changes in imaging parameters are related to clinical impairments. Although we did not correlate our imaging findings with clinical measurements, a recently published article in almost the same prospective study group even after lower cumulative dose (mean 3.2; median 1.5 tablets) showed a small but significant decrease in performance of verbal memory tests in the new ecstasy users compared to the persistent ecstasy-naive controls (Schilt *et al*., 2007). Although the effects are small and within the normal range, it is worrisome that even small amounts of ecstasy seem to have measurable adverse effects. These effects may become more clinically significant with increasing age when the serotonergic function decreases physiologically (van Dyck *et al*., 2000). Moreover, our recent study with exactly the same imaging parameters showed greater effects in heavy ecstasy users, and thus the effects seem to be a dose dependent as previously also suggested by others (Reneman *et al*., 2006; McCann *et al*., 1998).

We are aware of some methodological limitations of our study, some of which have been discussed previously (de Win *et al*., 2005b; de Win *et al*., 2007). This study provides information about the relationship between ecstasy use and imaging parameters, but offers no undisputable evidence of causality. First, although prospective, the study design was not experimental, and no dose-response relationship was found. Therefore, it is possible that the observed changes are not related to ecstasy use, but to other time- or ecstasy-related variables. Confounding by other substances cannot be totally excluded, although the findings remained significant after adjusting for differences in use of these substances. Second, there was no control on purity and the amount of MDMA in the ecstasy tablets, although >95% of the tablets sold as ecstasy in the Netherlands contain MDMA (Drugs Informatie en Monitoring Systeem, 2004). Third, the study mainly comprised low-dose ecstasy users. Because expected neuronal damage after a low dose of ecstasy is small, the statistical power of this study may have been insufficient for [^{123}I]β-CIT SPECT and ^{1}H-MRS to detect changes. It may also be that these techniques are not sensitive enough to detect mild changes after low-ecstasy use. It is likely that PET (and also SPECT with a more selective radiotracer) has a higher sensitivity to detect smaller changes in SERT densities (Reneman *et al*., 2006), although no such studies have been performed yet. Related to this point, the variation in the number of pills taken within the group of incident users was quite small, most of the users (54.3%) took only one ecstasy pill or less and only 10 users (17.0%) took a cumulative dose of 10 pills or more. This could be the reason that no dose-response relationship was observed in the current study, while previous studies did report dose-response effects. Fourth, since few studies used ^{1}H-MRS, DTI and PWI to study neuronal damage in ecstasy users, little is known about sensitivity and specificity of these techniques to detect ecstasy-induced neuronal damage, although ^{1}H-MRS, DTI and PWI have been shown to be sensitive tools in various neuropsychiatric disorders (Levin *et al*., 1996; Ernst *et al*., 2000; Stanley, 2002; Lim and Helpern, 2002; Reneman *et al*., 2006). Because these techniques do not specifically measure the serotonin system, it is also possible that the changes observed with PWI and DTI are not (only) related to serotonin toxicity, but also to the changes in the dopaminergic or the noradrenergic system. On the other hand, ecstasy-induced dopaminergic neurotoxicity has only been observed in mice, and has not been demonstrated in any other species, including humans (Reneman *et al*., 2002a). Fifth, because we studied early indicators of potential brain damage and had clear *a priori* hypotheses on the effects of ecstasy based on previous studies, we did not correct for multiple comparisons to minimize the risk of false negative results (type II errors; Rothman, 1990) although this may have introduced false positive findings (type I errors). Therefore, additional research is needed to establish whether the current significant findings can be confirmed. Finally, although we found some sustained effects of ecstasy on the brain in subjects 19 weeks after last ecstasy intake, we did not measure long-term effects, and therefore it is possible that the observed effects represent reversible adaptations of the brain and not permanent neurotoxicity.

In conclusion, this study showed some small sustained effects of ecstasy on brain vasculature, white matter maturation and possibly axonal damage after low-dose ecstasy use. Because we do not know yet whether these effects are reversible or not, we cannot exclude that ecstasy even in low doses may be neurotoxic.

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