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A Prospective Cohort Study on Sustained Effects of Low-Dose Ecstasy Use on the Brain in New Ecstasy Users

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It is debated whether ecstasy use has neurotoxic effects on the human brain and what the effects are of a low dose of ecstasy use. We prospectively studied sustained effects (>2 weeks abstinence) of a low dose of ecstasy on the brain in ecstasy-naive volunteers using a combination of advanced MR techniques and self-report questionnaires on psychopathology as part of the NeXT (Netherlands XTC Toxicity) study. Outcomes of proton magnetic resonance spectroscopy (^{1}H-MRS), diffusion tensor imaging (DTI), perfusion-weighted imaging (PWI), and questionnaires on depression, impulsivity, and sensation seeking were compared in 30 subjects (12M, 21.8±3.1 years) in two sessions before and after first ecstasy use (1.8±1.3 tablets). Interval between baseline and follow-up was on average 8.1±6.5 months and time between last ecstasy use and follow-up was 7.7±4.4 weeks. Using ^{1}H-MRS, no significant changes were observed in metabolite concentrations of *N*-acetylaspartate (NAA), choline (Cho), myo-inositol (mI), and creatine (Cr), nor in ratios of NAA, Cho, and mI relative to Cr. However, ecstasy use was followed by a sustained 0.9% increase in fractional anisotropy (FA) in frontoparietal white matter, a 3.4% decrease in apparent diffusion (ADC) in the thalamus and a sustained decrease in relative regional cerebral blood volume (rrCBV) in the thalamus (−6.2%), dorsolateral frontal cortex (−4.0%), and superior parietal cortex (−3.0%) (all significant at $p<0.05$, paired *t*-tests). After correction for multiple comparisons, only the rrCBV decrease in the dorsolateral frontal cortex remained significant. We also observed increased impulsivity (+3.7% on the Barratt Impulsiveness Scale) and decreased depression (−28.0% on the Beck Depression Inventory) in novel ecstasy users, although effect sizes were limited and clinical relevance questionable. As no indications were found for structural neuronal damage with the currently used techniques, our data do not support the concern that incidental ecstasy use leads to extensive axonal damage. However, sustained decreases in rrCBV and ADC values may indicate that even low ecstasy doses can induce prolonged vasoconstriction in some brain areas, although it is not known whether this effect is permanent. Additional studies are needed to replicate these findings.
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INTRODUCTION

There is increasing evidence that ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is toxic to the human brain, especially to the serotonergic system (eg, McCann *et al*, 2000; Reneman *et al*, 2006), although the validity of these findings is still highly debated (Turner and Parrott, 2000; Grob, 2002; Kish, 2002). Many human studies are littered with methodological problems, including inadequate sampling of subjects and controls, lack of drug use analysis, and lack of baseline data (eg, Morgan, 2000; De Win *et al*, 2005). The latter argument leads to interpretative difficulties concerning causality between ecstasy use and potential toxicity, because it leaves open the possibility that differences between ecstasy users and controls were pre-existent, as discussed previously by others (Jansen and Forrest, 1999; Morgan, 1999; Dughiero *et al*, 2001). It may be possible that personality traits like impulsivity and sensation seeking, associated with substance misuse, are

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related to lower serotonergic function (Khantzian, 1997; Zuckerman and Kuhlman, 2000) or that results are biased by confounding variables such as polydrug use, gender, and lifestyle.

Few prospective studies were performed in which MDMA was administered to volunteers either with (Downing, 1986; Grob *et al*, 1996; Camí *et al*, 2000; Chang *et al*, 2000) or without prior experience with ecstasy use (Greer and Tolbert, 1986; Vollenweider *et al*, 1998; Gamma *et al*, 2000; Liechti *et al*, 2000; Liechti and Vollenweider, 2000a, b). Most of these studies focused on acute and not on sustained or permanent effects of ecstasy. These studies have led to ongoing discussion on safety and ethics of administration of potentially neurotoxic drugs to healthy humans. Several authors objected to administering a potential neurotoxic drug to humans for the purpose of science (Gijsman *et al*, 1999; McCann and Ricaurte, 2001), while others supported these experiments (Lieberman and Aghajanian, 1999; Vollenweider *et al*, 1999, 2001).

The discussion mainly persists, because it is assumed that heavy ecstasy use most probably causes adverse long-term effects (eg, McCann *et al*, 1998; Reneman *et al*, 2001a), while it is not known whether a low dose of ecstasy can cause lasting brain damage. Effects of a single dose of ecstasy in ecstasy-naive humans were only described up to 24 h after intake (Vollenweider *et al*, 1998), while persisting psychopathology after a single dose was only described in case reports (McGuire *et al*, 1994; Vaiva *et al*, 2001). In rats, neuronal damage was demonstrated in various brain areas following a single dose of MDMA (Colado *et al*, 1995; Schmued, 2003), including persistent effects on behavior (Ho *et al*, 2004). In addition, studies in primates showed serotonin depletion 2 weeks after administering a single (5 mg/kg) (Ricaurte *et al*, 1988) or two oral doses (4.3 mg/kg) (Mechan *et al*, 2006). The validity of animal data for the human situation has been questioned, however, because MDMA is usually administered to animals in higher dosages than generally used by humans. Some authors do not support the suggestion that a single oral dose at 1.7 mg/kg is likely to produce neurotoxic effects in humans (Lieberman and Aghajanian, 1999; Vollenweider *et al*, 1999, 2001). On the other hand, it has been advocated that these dosages in animals are equivalent to typical recreational dosages in humans according to the principles of interspecies scaling (Ricaurte *et al*, 2000; McCann and Ricaurte, 2001).

It is important to know whether a low dose of ecstasy is neurotoxic for at least two reasons. First, recreational use of ecstasy is common among adolescents and young adults and many of them are 'experimenters' who take ecstasy incidentally and will not become heavy or regular users (The Netherlands National Drug Monitor, 2004). Determining that the incidental use of ecstasy could cause persisting neuronal damage would have major clinical and social implications. Second, it is debated whether MDMA should become available for medical use, because MDMA may be useful as an adjunct in psychotherapy, or whether this would lead to neuronal damage (Check, 2004). This discussion is of interest, since pilots have been approved that will study therapeutic effects of MDMA on anxiety in patients with post-traumatic stress disorder (Check, 2004) and in terminally ill cancer patients (Bender, 2005). When considering ecstasy as adjunct in psychotherapy, it is important that estimations of risk are available to decide whether potential risks outweigh potential benefits.

With advanced magnetic resonance (MR) techniques, such as proton magnetic resonance spectroscopy (^{1}H-MRS), diffusion tensor imaging (DTI), and perfusion weighted imaging (PWI) it is possible to study various aspects of neuronal damage in the brain.

^{1}H-MRS allows to study certain metabolites in the brain, such as *N*-acetylaspartate (NAA), choline-containing compounds (Cho), myo-inositol (mI), and (phospho)creatine (Cr). NAA is decreased in neuronal damage and impaired cognition (Ross *et al*, 1997). Cho is increased in brain diseases that involve increased membrane breakdown, myelination, or inflammation, and it is thought to reflect cellular density (Miller *et al*, 1996). MI is a putative glial cell marker increased in diseases that involve gliosis (Ross *et al*, 1997). Cr is often used as an internal reference (Pouwels and Frahm, 1998). Previous studies in heavy ecstasy users showed decreased NAA/Cr in the prefrontal cortex (Reneman *et al*, 2002), correlated to decreased memory function (Reneman *et al*, 2001c) and increased mI/Cr in the parietal white matter (Chang *et al*, 1999). However, another study could not confirm lower NAA/Cr ratios in cortical brain regions, and observed only a tendency towards lower NAA/Cr ratios in the left hippocampus of ecstasy users (Daumann *et al*, 2004a).

With DTI it is possible to measure diffusional motion of water molecules. In the brain, the motion is restricted in amplitude and direction by cellular structures such as axons. (Sub)acute processes that involve axonal injury and ischemia can lead to a decreased apparent diffusion coefficient (ADC) of water due to cytotoxic edema (Haykin *et al*, 2005). However, in chronic stage of axonal damage, ADC can be increased and fractional anisotropy (FA) of water can be decreased due to increased extracellular water content. It is difficult to determine the time course of change in ADC. For stroke, transition from decreasing to increasing ADC values seems to occur between 18 h and 7 days after stroke onset (Copen *et al*, 2001), while delayed cytotoxic edema with restricted ADCs was described up to 6 months, after carbon monoxide poisoning (Murata *et al*, 2001; Chu *et al*, 2004), and heroin abuse (Chen *et al*, 2000). Only one article was published with ADC measurements in heavy ecstasy users, and showed increased ADC values in the globus pallidus (Reneman *et al*, 2001b).

Finally, PWI can map regional relative cerebral blood volume (rrCBV) using the dynamic susceptibility contrast (DSC) technique (Belliveau *et al*, 1990; Levin *et al*, 1996). This is of interest because serotonin is involved in regulation of brain microcirculation (Cohen *et al*, 1996). Previous publications described cerebrovascular accidents, such as cerebral infarction and hemorrhage, (Hanyu *et al*, 1995; Lee *et al*, 2003) and cerebrovascular changes (Chang *et al*, 2000; Reneman *et al*, 2000, 2001b) in ecstasy users. It is expected that ecstasy use induces a (sub)acute increase of extracellular serotonin leading to vasoconstriction, whereas the long-term effect of ecstasy use may be a decrease in extracellular serotonin and thus vasodilatation.

If a low dose of ecstasy is neurotoxic, it is important to know whether this has clinical consequences in terms of psychopathological parameters such as mood, impulsivity, and sensation seeking. Many previous studies reported

increased levels of depression (Sumnall and Cole, 2005), impulsivity (Morgan, 1998; Parrott *et al*, 2000; Tuchtenhagen *et al*, 2000; Daumann *et al*, 2001, 2004b; Bond *et al*, 2004; Butler and Montgomery, 2004), and sensation/novelty seeking (Gerra *et al*, 1998; Tuchtenhagen *et al*, 2000; Schifano, 2000; Dughiero *et al*, 2001) in ecstasy users, although it is unclear whether these associations reflect a causal relationship, that is whether ecstasy use causes changes in mood, impulsivity, and sensation seeking or whether depression, impulsivity, and sensation seeking increase the probability of (heavy) ecstasy use (see also De Win *et al*, 2006).

The aim of the present study was to assess whether a low dose of ecstasy would be neurotoxic. Using a prospective naturalistic study design, parameters of neurotoxicity measured with ^{1}H-MRS, DTI, and PWI and outcomes of psychopathological self-report inventories on depression, impulsivity, and sensation seeking were compared between a baseline session before first ecstasy use and a follow-up session after ecstasy use. Based on previous findings, we hypothesized that if a low dose of ecstasy has sustained effects on the brain MR-derived parameters and psychopathology would show relatively small changes between both sessions, that is a decrease (in the subacute stage shortly after ecstasy use) or increase (after a longer period of abstinence) in rrCBV and ADC, depending on the time since last ecstasy use; an increase in Cho (or Cho/Cr), mI (or mI/Cr), depression, impulsivity, and sensation seeking; and a decrease in FA and NAA (or NAA/Cr).

MATERIALS AND METHODS

Participants

The current study is part of the NeXT (Netherlands XTC Toxicity) study, which investigates causality, course, and clinical relevance of ecstasy neurotoxicity. A detailed description of the NeXT study and recruitment strategies can be found in a special design paper (De Win *et al*, 2005). Between April 2002 and April 2004, 188 young adults (77 M, 111 F, age 21.7 ± 3.0 years) were included in the study. They had never used ecstasy, but were selected on a relatively high probability to start using ecstasy in the near future. Subjects were recruited using a combination of targeted site sampling, advertisement through a website on the project, and snowball sampling referrals. Main inclusion criteria were intention to probably or certainly use ecstasy for the first time in near future and/or having friends who already used ecstasy. Exclusion criteria were: ecstasy use in the past (at baseline), age below 18 or above 35 years, severe physical or mental illness, use of psychotropic medications such as serotonin reuptake inhibitors, pregnancy, use of intravenous drugs, and contraindications for MRI (eg, claustrophobia, pacemaker). 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 by urine drug screening (enzyme-multiplied immunoassay for amphetamines, ecstasy, opiates, cocaine, benzodiazepine, cannabis, and alcohol).

The study was approved by the local medical ethics committee. Subjects were informed about potential negative consequences of ecstasy use and all subjects signed informed consent. Subjects received an allowance for their participation (between €100 and €150 per session).

Study Procedure and Measurements of Confounders

At baseline all 188 subjects underwent MR imaging, including ^{1}H-MRS, DTI, and PWI, and completed self-report questionnaires on depression, impulsivity, and sensation seeking. After baseline examination, subjects had to complete questionnaires (four in total) about their drug use at regular intervals over a period of approximately 18 months. For the present study, the first 31 incident ecstasy users were included in a first follow-up session, relatively soon after their first ecstasy use (after we received their first drug use questionnaire indicating use of ecstasy) and with a maximum cumulative ecstasy dose of 10 tablets. During the follow-up session, ^{1}H-MRS, DTI, PWI, and self-report questionnaires on depression, impulsivity, and sensation seeking were repeated.

At both sessions, subjects had to complete questionnaires about potential confounders, such as demographic variables and education. Various aspects of lifetime ecstasy use (frequency of use, cumulative dose, and duration of use), and last year use of alcohol (units per week), tobacco (cigarettes per week), cannabis (number of joints last year), amphetamines (number of times used last year), and cocaine (number of times used last year) were assessed using substance-use questionnaires (Van de Wijngaart *et al*, 1997). Verbal intelligence was estimated using The Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test (Nelson, 1991).

Image Acquisition

MR imaging was performed on a 1.5 T scanner (Signa Horizon, LX 9.0, General Electric Medical Systems, Milwaukee, WI, USA) using the standard head coil. The protocol included (1) an axial PD- and T2-weigthed sequence (echo time $(TE)_1/TE_2$/relaxation time $(TR) = 10/98/4000$ ms, 12 slices of 5 mm, 1.5 mm slice distance, 23 cm field of view (FOV)); (2) three ^{1}H-MRS scans with the single voxel point-resolved spectroscopy (PRESS) sequence ($TE/TR = 35/1500$ ms); (3) DTI: diffusion-weighted spin echo Echo Planar Imaging (EPI) ($TE/TR = 90/8000$ ms, 12 slices of 5 mm, 1.5 mm slice distance, 23 cm FOV; $b = 0$ and 1000 s/mm^2, 128×128 matrix); (4) PWI: gradient echo EPI first-pass dynamic T_2^*-weighted contrast-enhanced imaging ($TE/TR = 55/2000$ ms, 12 slices of 5, 1.5 mm slice distance, 23 cm FOV); and (5) a high resolution T1-weigthed 3D scan using a Fast Spoiled GRadient Echo (FSPGR) sequence ($TE/TR = 6/30$ ms, voxel size $1.0 \times 1.0 \times 1.4$ mm^3). Throughout the study positioning of subjects in the scanner and positioning of the slices and voxels were performed by the same examiner and according to a protocol to keep positioning as reproducible as possible.

The voxel size for ^{1}H-MRS was 6.5 ml ($18 \times 18 \times 20$ mm^3) and voxels were placed in the left centrum semiovale (frontoparietal white matter) and in mid-frontal and mid-occipital gray matter as in previous publications (Chang *et al*, 2000; Reneman *et al*, 2002). Shimming and water suppression were automatically performed by the scanner. Diffusion was measured in six non-collinear directions

and in the six opposite directions. For each of these 12 directions ($b = 1000\,s/mm^2$) and for a baseline measurement without diffusion weighting ($b = 0\,s/mm^2$), two acquisitions were averaged. Perfusion images were obtained at 2-s intervals for 80 s. At 6 s after the start of image acquisition, a bolus (0.12 ml/kg) with gadobutrol 1.0 mol/l (Gadovist 1.0; Schering, Berlin, Germany) was injected, using a power-injector (Spectris MR injector; Medrad, Indianpolis, PA, USA) at a rate of 5 ml/s through a cannula inserted in the antecubital vein. The gadobutrol injection was followed by a 15-ml saline flush (0.9% NaCl).

Image Analysis

Spectra derived from ^{1}H-MRS were analyzed using LCModel (Linear Combination of Model spectra) (Provencher, 1993). This is a user-independent analysis method that estimates absolute metabolite concentrations by fitting the *in vivo* spectra to a set of previously acquired *in vitro* spectra (the basis set). This procedure allows the absolute quantification of metabolite concentrations. Both absolute concentrations of NAA, Cho, mI, and Cr as well as the ratios of NAA to Cr, Cho to Cr, and mI to Cr were calculated with LCModel. Quality control of ^{1}H-MRS included checking of line-width and the percent SD of the estimated concentrations after analyses by LCModel. Unsuppressed spectra with a waterpeak line-width of more than 6 Hz were excluded. Also %SD > 20% for NAA and %SD > 50% for Cho, mI, and Cr were considered unreliable and were excluded (Srinivasan *et al*, 2004).

The DTI scans were corrected for the effects of residual eddy currents by matching the images acquired with opposite diffusion sensitizing gradients to each other with an affine transformation, and then correcting both images with the 'half' of that transformation (Bodammer *et al*, 2004). From the resulting diffusion weighted images, ADC, and FA maps were calculated as described elsewhere (Hunsche *et al*, 2001). The AMC Postprocessing Package (APP, http://amcpostpack.sourceforge.net) was used to calculate CBV maps from the PWI scans.

All image-derived parameters (FA, ADC, and CBV) were spatially normalized by registration to the Montreal Neurological Institute brain template (MNI152) in three steps. First, the scans corresponding to baseline measurements in the DTI and PWI sequences were individually matched to the T2-weighted images by 2D-non-rigid registration (program align_warp, Automated Image Registration Library, AIR, Woods *et al*, 1998). Second, the T2 scans were rigidly registered to the T1-3D scans (program flirt, fMRIB Software Library, FSL, Smith *et al*, 2004). Finally, the T1-3D scans were registered to the MNI152 brain template by a non-rigid transformation (align_warp). The transformations calculated to align the baseline measurements into T2, T2 into T1-3D, and T1-3D into MNI152 were applied to align the FA, ADC, and CBV maps to the MNI152 brain (see Figure 1 for representative images of individual FA, ADC, and CBV images after transformation to the MNI152 brain template). All images were skull-stripped (program bet, FSL, Smith, 2002). Segmentation of white and gray matter was performed using T1-3D and PD scans (program fast, FSL, Gudbjartsson and Patz, 1995). The scans were segmented into three classes of tissue (CSF, white, and gray matter), and the tissues of interest were isolated into separated binary maps (only white matter, only gray matter, and combined white and gray matter). 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.

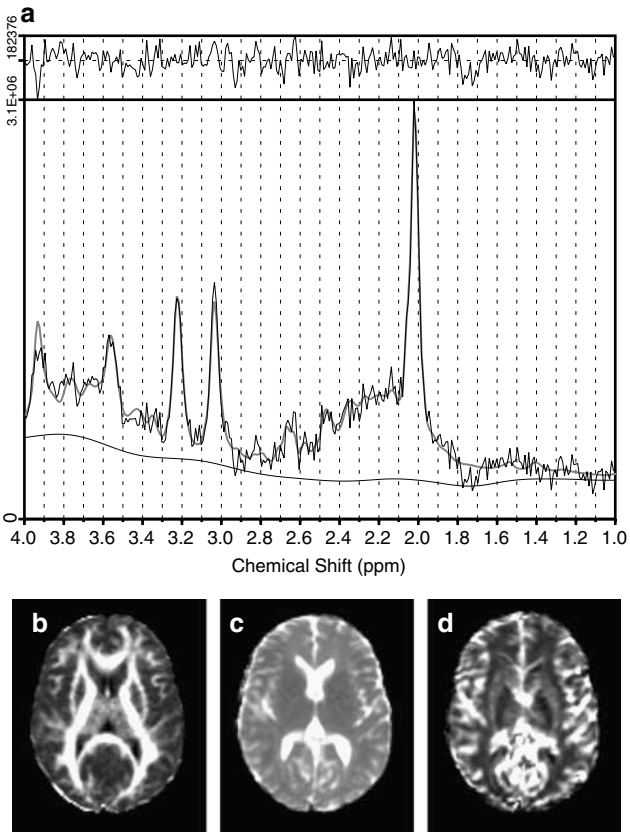

Figure 1 Representative images of an individual (a) ^{1}H-MR spectrum after analysis by LCModel and representative (b) FA, (c) ADC, and (d) CBV images after transformation to the spatially normalized MNI brain template.

Regions of interest (ROIs) were drawn on the MNI152 brain template in the thalamus, putamen, globus pallidus, head of the caudate nucleus, centrum semiovale (fronto-parietal white matter), and dorsolateral frontal, mid-frontal, occipital, superior parietal, and temporal cortex (see Figure 2). For the cortical ROIs, only voxels within the gray matter mask were included and for the ROIs of the basal ganglia only voxels within the combined white and gray matter mask were included (CSF voxels were excluded). Selection of ROIs was based on findings of previous studies, which indicated that ecstasy-induced abnormalities are most prominent in basal ganglia and cortical areas; ecstasy-induced abnormalities in white matter were rarely reported and thus not expected. As cortical gray matter has very low anisotropy, it is very difficult to get reliable FA and ADC measurements in cortical areas. For this reason only white matter and basal ganglia ROIs were taken into account in the measurements of FA and ADC. Within the ROIs, individual mean values of FA, ADC, and regional relative CBV (rrCBV) were calculated. Values of FA, ADC, and rrCBV from ROIs in left and right hemispheres were averaged.

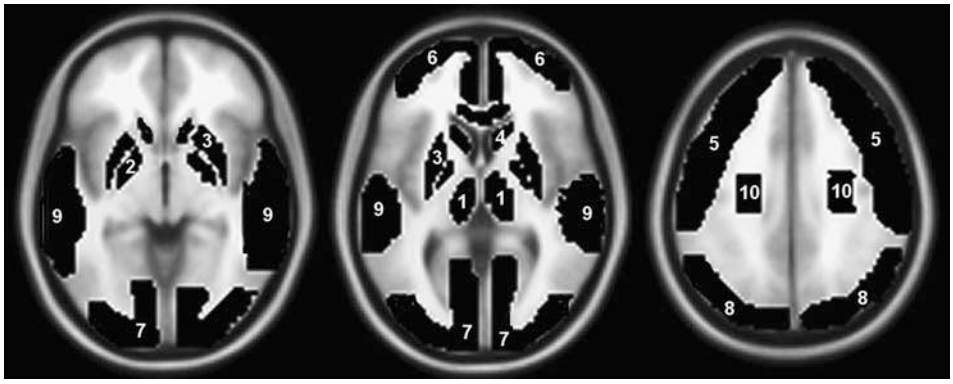

Figure 2 Region of interests used for analyses of DTI (measuring FA and ADC) and PWI (measuring rrCBV) drawn on the MRI brain template at three levels: (1) thalamus, (2) globus pallidus, (3) putamen, (4) caudate nucleus, (5) dorsolateral frontal cortex, (6) mid-frontal cortex, (7) occipital cortex, (8) superior parietal cortex, (9) temporal cortex, and (10) white matter of the centrum semiovale. Note that rrCBV was measured in all these ROIs and FA and ADC only in the white matter and basal ganglia ROIs.

For each individual scan, all steps in the post-processing and analysis were visually inspected to check the quality of image registration and segmentation.

Psychopathological Assessments

Current depressive symptoms were assessed using the Beck Depression Inventory (BDI) (Beck *et al*, 1961), a 21-item self-report inventory that measures characteristic attitudes and symptoms of depression in the week before assessment. Impulsivity was assessed using the Dutch version of the Barratt Impulsiveness Scale (BIS-11), a reliable measure of impulsiveness (Patton *et al*, 1995). The Dutch BIS-11 contains 31 self-reported items. The Spannings Behoefte Lijst (SBL), a Dutch adaptation of the Sensation Seeking Scale (Zuckerman and Link, 1968) with 51 items, was used to measure sensation seeking as it has proven to be a reliable measure for research populations (Feij *et al*, 1982; Feij and van Zuilen, 1984).

Statistical Analyses

All substance-use variables were log-transformed because they were not normally distributed. First, paired *t*-tests, uncorrected for multiple comparisons, were used to assess whether parameters of substance use, imaging, and self-report questionnaires had changed between baseline session before ecstasy use and follow-up session after ecstasy use.

Second, paired *t*-tests were repeated for imaging and psychopathology parameters excluding volunteers with increased cocaine use between both sessions ($N=4$, leaving $N=26$ for the second analysis), because paired analysis of substance use showed an increased frequency of cocaine use between baseline and follow-up sessions.

Third, previous studies showed that effects of ecstasy might be dose-dependent (McCann *et al*, 1998; Reneman *et al*, 2001a) and that females are more vulnerable for the effects of ecstasy than males (Reneman *et al*, 2001a; Buchert *et al*, 2004). Other studies suggested a relationship between brain perfusion and time since last ecstasy use and between ADC and time since onset of neuronal damage (Chang *et al*, 2000; Reneman *et al*, 2000). Therefore, we performed separate multiple linear regression analyses with follow-up measures of imaging and self-reported psychopathology as dependent variable and gender, cumulative dose of ecstasy, period of abstinence (weeks since last ecstasy tablet) and change in cocaine use (because this was the only significantly increased drug-use parameter) as independent variable and baseline measures of imaging and self-reported psychopathology as covariates.

Finally, Pearson correlations were calculated between statistically significant changes in MR outcomes and significant changes in outcomes of the psychopathology questionnaires.

The chance of a type I error (α) was set at 0.05 for all analyses. In addition, Bonferroni *post hoc* corrections were performed for the analyses of the imaging parameters, adjusting the α-level for multiple comparisons. The adjusted α-level was set at 0.006 for ^{1}H-MRS outcomes (nine comparisons), at 0.010 for FA and ADC (five comparisons each), and at 0.005 for rrCBV (10 comparisons).

All statistical analyses were performed using SPSS version 11.5; SPSS Inc., Chicago, IL, USA). Mean values reported in the result section are followed by their standard deviations (mean $\pm$ SD). In the tables mean differences between the paired measurements are reported with their 95% confidence intervals (95% CI) and in the text the percentage difference and the two-tailed significance level (*p*-values) are reported.

RESULTS

Characteristics of the Sample and Substance Use

Of the 188 ecstasy-naive subjects at baseline, 31 subjects were included in the first follow-up session relatively soon after their first ecstasy use (12 M, 19 F, age 21. 7 $\pm$ 3.1 years). One female was excluded because of a positive urine test on cocaine, leaving 30 volunteers for analysis with a mean age of 21.8 years. Characteristics of the sample and their substance use are described in Table 1. The interval between the baseline and follow-up sessions was on average 8.1 $\pm$ 6.5 months (range: 0.9–29.5 months). At this first follow-up session incident ecstasy users had used a mean of 1.8 $\pm$ 1.3 ecstasy tablets (range: 0.5–6; median 1.4 tablets). The majority had used ecstasy only once ($N=18$; 60%). Six

Table 1 Characteristics of Demographics, Use of Ecstasy and Other Substances, and Psychopathological Assessments ($N = 30$)

	Baseline before ecstasy use	Follow-up after ecstasy use	Mean of paired differences (95% CI)[a]
Gender	12 M, 18 F		NA
Age	21.8 ± 3.1	22.5 ± 3.2	0.67 (0.47; 0.87)*
Years of education	14.2 ± 2.8	14.8 ± 2.9	0.57 (0.26; 0.87)*
DART-IQ	104.6 ± 8.5		NA
Ecstasy			
Cumulative dose (tablets)	NA	1.8 ± 1.3	NA
Time since first tablet (weeks)	NA	9.8 ± 4.5	NA
Time since last tablet (weeks)	NA	7.7 ± 4.4	NA
Other substances			
Alcohol (units/week)	9.3 ± 7.3	8.7 ± 7.5	−0.10 (−0.27; 0.07)
Tobacco (cig/week)	23.4 ± 39.0	17.4 ± 30.2	−0.07 (−0.51; 0.36)
Cannabis (joints in last year)	36.2 ± 52.0	38.4 ± 52.6	−0.03 (−0.34; 0.28)
Amphetamine (number of times used last year)	0.2 ± 1.1	0.2 ± 1.1	NA
Cocaine (number of times used last year)	0.6 ± 1.8	1.4 ± 2.6	0.34 (0.01; 0.67)*
Psychopathological assessments			
Beck Depression Inventory	4.8 ± 4.0	3.4 ± 3.4	−1.37 (−2.63; −0.10)*
Barratt Impulsiveness Scale	67.5 ± 6.8	70.0 ± 7.8	2.50 (0.79; 4.21)*
SBL—Sensation Seeking Scale	13.7 ± 1.3	13.9 ± 1.2	0.23 (−0.09; 0.55)

[a]Paired *t*-test baseline *vs* follow-up, substance use log-transformed.
*Statistical significant difference between base line and follow-up.
Values expressed as mean ± SD's. Results expressed in mean of paired differences (95% CI).

subjects (20%) had used more than one ecstasy tablet at the same occasion with a maximum of two tablets per occasion. The interval between the last ecstasy use and follow-up measurements was 7.7 ± 4.4 weeks.

Table 1 shows that besides the use of ecstasy, there was a significant increase in cocaine use between sessions ($p = 0.043$), while there was no change in use of other substances.

^{1}H-MRS, DTI, and PWI

The maximum line-width of the unsuppressed spectra was 6 Hz and maximum %SD of the estimated metabolite concentrations was 20% for NAA and Cho and 25% for mI, and therefore, all spectra could be included. However, due to technical problems with the scanner it was not possible to perform ^{1}H-MRS in two subjects at the follow-up session.

Anatomical images (T1 3D scans and T2-weighted scans) were read by a neuroradiologist for atrophy or white matter lesions, and no significant abnormalities were detected. However, one subject had enlarged lateral ventricles and visual inspection showed this hampered matching to the standard brain, and therefore the measurements of FA, ADC, and rrCBV of this subject were not included. Therefore, we report comparisons between baseline and follow-up measurements of FA, ADC, and rrCBV in 29 subjects and of ^{1}H-MRS in 28 subjects.

Table 2 shows results of all measurements and Figure 3 illustrates the statistically significant findings. There were no significant changes in absolute concentrations of NAA, Cho, mI, and Cr and in ratios of NAA, Cho, or mI relative to Cr in any of the three voxels after ecstasy use. With DTI, we observed a small but significant increase of 0.9% in FA of the white matter of the centrum semiovale ($p = 0.027$) and a significant decrease of 3.4% in ADC of the thalamus ($p = 0.015$) after ecstasy use. With PWI we found significant decreases in rrCBV in the thalamus (−6.2%, $p = 0.010$), dorsolateral frontal gray matter (−4.0%, $p = 0.001$), and superior parietal gray matter (−3.0%, $p = 0.029$) (Figure 3). When adjusted for multiple comparisons using the Bonferroni correction, only the decreased rrCBV value in the dorsolateral frontal gray matter remained statistically significant.

Similar to the first set of analyses, the second set of analyses, excluding subjects with increased cocaine use between both sessions, showed no significant changes in metabolite concentrations and ratios. Similar to the first analysis, it showed a significant decrease of ADC in the thalamus (−3.9%, $p = 0.011$) and of rrCBV in the thalamus (−6.7%, $p = 0.010$), dorsolateral frontal cortex (−4.2%, $p = 0.002$), and superior parietal gray matter (−3.4%, $p = 0.026$). However, the increase in FA in the white matter of the centrum semiovale was not significant anymore ($p = 0.085$) and the second analysis showed an additional significant decrease of rrCBV in the putamen

Table 2 MRI Parameters before and after First Ecstasy Use

MR technique	Parameter	Region of interest	Baseline before ecstasy use	Follow-up after ecstasy use	Mean of paired differences (95% CI)
^{1}H-MRS ($N=28$) absolute	NAA	Mid-frontal gray matter	16.21±2.33	15.8±1.83	−0.41 (−1.55; 0.72)
		Mid-occipital gray matter	17.13±1.81	17.44±1.88	0.32 (−0.56; 1.20)
		Left centrum semiovale	13.65±1.41	13.41±1.56	−0.24 (−1.02; 0.55)
	Cho	Mid-frontal gray matter	3.10±0.55	3.23±0.57	0.13 (−0.14; 0.40)
		Mid-occipital gray matter	1.72±0.25	1.75±0.22	0.03 (−0.06; 0.12)
		Left centrum semiovale	2.98±0.40	3.03±0.52	0.05 (−0.08; 0.18)
	mI	Mid-frontal gray matter	11.93±1.93	11.41±1.79	−0.52 (−1.38; 0.34)
		Mid-occipital gray matter	9.26±1.06	9.43±0.95	0.18 (−0.41; 0.76)
		Left centrum semiovale	7.37±0.98	7.80±0.99	0.44 (−0.05; 0.92)
	Cr	Mid-frontal gray matter	13.42±1.93	12.84±1.78	−0.58 (−1.51; 0.34)
		Mid-occipital gray matter	11.59±0.77	11.49±0.72	−0.10 (−0.51; 0.31)
		Left centrum semiovale	9.02±1.05	9.13±1.09	0.11 (−0.36; 0.58)
^{1}H-MRS ($N=28$) ratio's	NAA/Cr	Mid-frontal gray matter	1.23±0.26	1.25±0.20	0.02 (−0.11; 0.15)
		Mid-occipital gray matter	1.49±0.18	1.53±0.17	0.04 (−0.06; 0.13)
		Left centrum semiovale	1.52±0.20	1.48±0.29	−0.03 (−0.17; 0.10)
	Cho/Cr	Mid-frontal gray matter	0.23±0.04	0.25±0.05	0.02 (−0.00; 0.04)
		Mid-occipital gray matter	0.15±0.02	0.15±0.02	0.00 (−0.00; 0.01)
		Left centrum semiovale	0.33±0.04	0.33±0.05	−0.00 (−0.02; 0.02)
	mI/Cr	Mid-frontal gray matter	0.90±0.14	0.90±0.15	0.00 (−0.07; 0.08)
		Mid-occipital gray matter	0.80±0.10	0.83±0.10	0.02 (−0.03; 0.07)
		Left centrum semiovale	0.83±0.13	0.86±0.16	0.04 (−0.02; 0.10)
DTI ($N=29$)	FA (×1000)	Thalamus	277±18	278±17	1.9 (−4.0; 7.7)
		Globus pallidus	303±59	295±55	−8.0 (−28.6; 12.6)
		Putamen	212±30	212±30	−0.2 (−10.4; 10.1)
		Caudate nucleus	176±39	176±29	−0.3 (−11.2; 10.7)
		Centrum semiovale	419±20	422±19	3.6 (0.4; 6.8)*
	ADC 10^{-5} mm^2/s	Thalamus	84.4±6.6	81.6±5.3	−2.8 (−5.1; −0.6)*
		Globus pallidus	72.4±4.2	72.3±2.9	−0.1 (−1.4; 1.3)
		Putamen	70.8±1.9	70.8±1.5	0.1 (−0.7; 0.8)
		Caudate nucleus	88.4±11.8	86.0±8.5	−2.4 (−5.3; 0.5)
		Centrum semiovale	70.0±1.8	70.1±2.0	0.1 (−0.4; 0.5)
PWI ($N=29$)	rrCBV	Thalamus	1.64±0.18	1.53±0.16	−0.10 (−0.18; −0.03)*
		Globus pallidus	1.00±0.14	1.06±0.16	0.07 (−0.01; 0.14)
		Putamen	1.36±0.13	1.32±0.12	−0.04 (−0.09; 0.01)
		Caudate nucleus	1.28±0.12	1.25±0.12	−0.04 (−0.08; 0.01)
		Dorsolateral frontal gray matter	1.69±0.14	1.62±0.12	−0.07 (−0.11; −0.03)*,†
		Mid-frontal gray matter	1.68±0.17	1.65±0.14	−0.02 (−0.07; 0.02)
		Occipital gray matter	2.16±0.22	2.11±0.20	−0.04 (−0.11; 0.03)
		Superior parietal gray matter	1.98±0.17	1.92±0.16	−0.06 (−0.11; −0.01)*
		Temporal gray matter	2.02±0.21	1.98±0.22	−0.04 (−0.11; 0.03)
		Centrum semiovale	0.76±0.05	0.78±0.07	0.01 (−0.01; 0.03)

*Statistical significant difference between baseline and follow-up (paired *t*-test, uncorrected for multipele comparisons).
†Statistical significant difference between baseline and follow-up (paired *t*-test, corrected for multipele comparisons).
Values expressed as mean±SD's. Results expressed in mean of paired differences (95% CI).

(−3.8%, $p=0.047$). After correction for multiple comparisons, only the rrCBV value in the dorsolateral frontal gray matter remained statistically significant.

The linear regression analyses showed a significant effect of gender on Cho/Cr in the occipital gray matter ($B=-0.02$, $p=0.038$), on Cho/Cr ($B=-0.04$, $p=0.032$), and on rrCBV

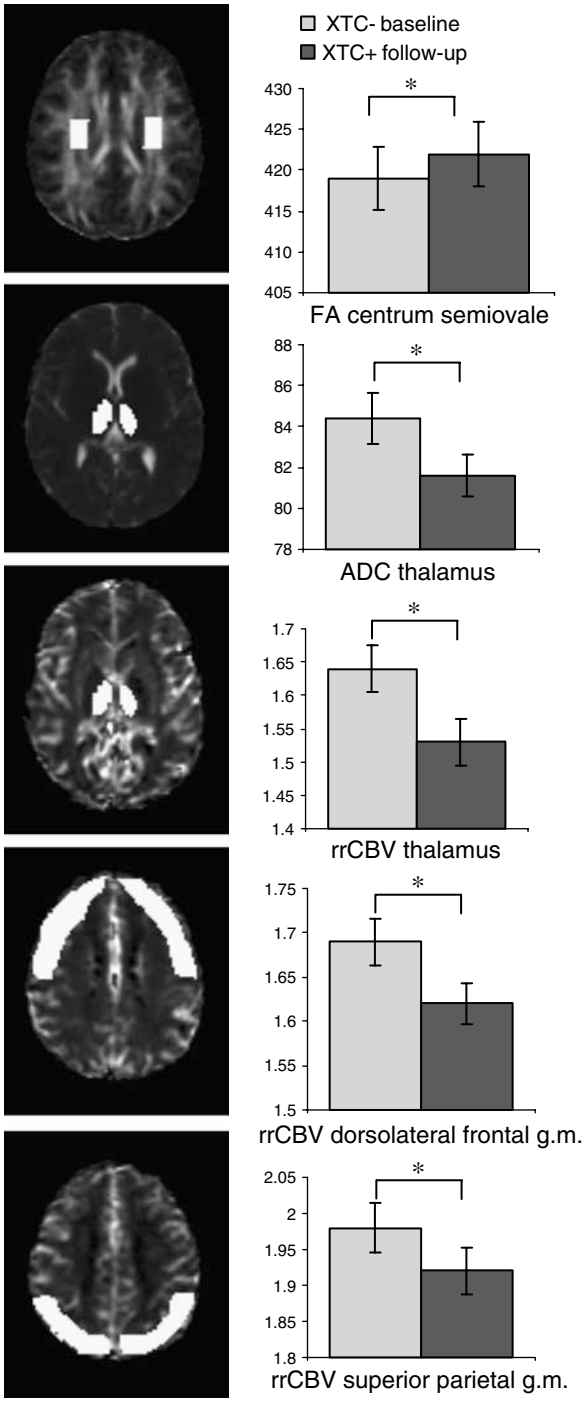

Figure 3 On the left, FA, ADC, and rCBV maps with brain regions, that significantly differed between baseline and follow-up (uncorrected for multiple comparisons), marked in white. On the right, columns reflect corresponding FA values in the centrum semiovale, ADC values in the thalamus, and rrCBV values in thalamus, dorsolateral frontal gray matter. and superior parietal gray matter at baseline before (XTC−) and at follow-up after ecstasy use (XTC +). Results represent mean ± SEM; * = $p < 0.05$. Only significant results are shown, for complete results of all analyses see Table 2. Note that the vertical axis does not start at zero.

in the temporal gray matter ($B = -0.17$, $p = 0.021$). This means that females (assigned '2' in the analysis) showed a significant larger decrease in Cho/Cr and rrCBV than males (assigned '1' in the analysis). The total amount of ecstasy tablets had only a significant positive effect on FA in white matter ($B = 3.35$, $p = 0.009$) and the time since last ecstasy use had no significant effect on any of the outcome measures at the follow-up session. Increase in cocaine use was significantly related to an increase in mI and mI/Cr in the occipital gray matter ($B = 0.23$, $p = 0.033$ and $B = 0.02$, $p = 0.019$, respectively). Of these regression analyses only the positive effect of the total amount of ecstasy tablets on FA in white matter remained significant after correction for multiple comparisons.

Psychopathological Assessments

Results of the self-report questionnaires on depression, impulsivity, and sensation seeking at baseline and follow-up sessions are shown in Table 1. After ecstasy use (at the follow-up session), subjects scored significantly lower on symptoms of depression (−28.0%, $p = 0.035$) and significantly higher on signs of impulsivity (3.7%, $p = 0.006$). No changes were observed in sensation seeking. Similar to the first analyses, the second analyses, excluding subjects with increased cocaine use between both sessions, showed no significant changes in sensation seeking, significant lower symptoms of depression (−29.8%, $p = 0.045$), and significantly higher signs of impulsivity (3.4%, $p = 0.013$).

The linear regression analyses showed a significant positive effect of increased cocaine use on sensation seeking ($B = 0.15$, $p = 0.026$). There were no significant correlations between increased depression and impulsivity scores and significant changes in MR outcomes.

DISCUSSION

To our knowledge, this is the first imaging study that prospectively examined sustained effects of a low dose of ecstasy on the human brain. Given the existing data on potential neurotoxicity, it is highly controversial to give ecstasy to ecstasy-naive individuals in a controlled experiment (Gijsman *et al*, 1999; Lieberman and Aghajanian, 1999; Vollenweider *et al*, 1999; McCann and Ricaurte, 2001). Therefore, we used a naturalistic design in which young adults with a relatively high probability for first time ecstasy use were included in a follow-up study. Only a few subjects incidentally used amphetamines and cocaine, and the use of alcohol, tobacco, and cannabis before the two sessions was very similar.

^{1}H-MRS and FA, parameters of structural elements of the brain, did not show indications of neuronal damage (ie, no decrease in NAA, NAA/Cr, FA, and no increase in Cho, Cho/Cr, mI, mI/Cr) after the first use of a low dose of ecstasy. This is not unexpected, because previous observations showed that neurotoxic effects of ecstasy are probably dose-related (McCann *et al*, 1998; Reneman *et al*, 2001a; Buchert *et al*, 2004). Previous ^{1}H-MRS studies showed decreased NAA/Cr ratios in ecstasy users with an average cumulated dose of more than 700 tablets (Reneman *et al*, 2001c, 2002), while others found no decreased NAA/Cr ratios in subjects with more moderate lifetime doses (Chang *et al*, 1999; Daumann *et al*, 2004a). Therefore, these effects probably only appear after cumulative heavy use. On the other hand,

we observed a small but significant decrease of 3.5% (Cohen's $d=-0.47$) in ADC in the thalamus after first ecstasy use (Cohen, 1988). We can speculate that this might be related to ecstasy-induced cytotoxic edema, as observed in other neurotoxic substances (Heaney *et al*, 2003; Haykin *et al*, 2005), although it also could be related to protracted vasoconstriction, since we also observed a decreased rrCBV in the thalamus (Pearson correlation between these findings is 0.65, $p<0.001$). With DTI we also encountered the unexpected finding of increased FA in the centrum semiovale related to the total amount of ecstasy tablets, although this 0.9% increase was very small (Cohen's $d=0.15$).

Functional parameters were measured with PWI and self-report questionnaires. As previously observed (Reneman *et al*, 2001b) we found an increase in rrCBV in the globus pallidus, although this effect was not significant ($p=0.09$). In addition, we found significant small to moderate decreases in rrCBV in dorsolateral frontal cortex, superior parietal cortex, and thalamus (Cohen's $d=0.36$–0.65). Decreases in cerebral blood flow (CBF), mainly in the caudate nucleus, and superior parietal and right dorsolateral frontal cortices, were previously observed after only two doses of MDMA (Chang *et al*, 2000). As this happened within 3 weeks after MDMA administering and because microcirculation in these areas has a strong relationship with serotonergic terminals (Cohen *et al*, 1996), the authors hypothesized that the decreased CBF was caused by subacute vasoconstriction due to MDMA-mediated serotonergic effects. The same study reported an increased CBF 2–3 months after MDMA intake (although only studied in two subjects) and no differences were found in CBF between controls and abstinent ecstasy users with a mean abstinence period of 6.6 months. They speculated that these findings might reflect depletion of serotonin after a longer period of abstinence and normalization of brain perfusion, respectively. Based on these results, they suggested a relationship between brain perfusion and abstinence period. Previous findings of higher rrCBVs in ecstasy users with an average period of abstinence of 14.6 weeks (globus pallidus) (Reneman *et al*, 2001b) and higher rrCBVs in former ecstasy users (globus pallidus and thalamus) than in recent ecstasy users and controls (Reneman *et al*, 2000) could be in line with this hypothesis, although study populations were small. The latter study also showed low rrCBV values in combination with lower cortical 5-HT_2 receptor densities, suggesting downregulation of 5-HT_2 receptors, in ecstasy users with a mean abstinence period of 7 weeks, and they hypothesized this was caused by excessive ecstasy-induced serotonin release. The currently observed decreased rrCBV values in subjects with a mean period of abstinence of 7.7 weeks might therefore also be related to a, probably transient, ecstasy-induced downregulation of 5-HT_2 receptors, which play an important role in the regulation of brain microcirculation (Parsons, 1991; Cohen *et al*, 1996). On the other hand, we did not find significant correlations between rrCBV and the time since last ecstasy tablet (abstinence interval). Another speculation is that decreased rrCBV values might reflect decreased brain function, because a single ecstasy dose was shown to cause degenerating neurons in parietal cortex and thalamus of rats (Schmued, 2003). In line with this, deficits in brain perfusion were reported in polydrug (Levin *et al*, 1996) and methamphetamine abusers (Iyo *et al*, 1997; Chang *et al*, 2002).

Outcomes of the self-report questionnaires after first ecstasy use showed increased impulsivity, as previously observed in other studies (Morgan, 1998). However, the magnitude of the effect is limited (3.7% increase; Cohen's $d=0.34$) and the clinical relevance is therefore questionable. Subjects also reported lower levels of depression after ecstasy use than before, an unexpected finding that might be related to a euphoric feeling about the first ecstasy experience (Korf *et al*, 1991). Also here, the clinical relevance of the reduction from 4.8 to 3.4 is questionable because the effect size is rather limited (Cohen's $d=-0.38$) and because BDI scores between 0 and 9 are considered to be within the normal range. Moreover, it should be noted that the findings were not reproduced after a longer follow-up period in a larger sample of the same baseline population (De Win *et al*, 2006).

Although this study and some other studies showed that adverse effects of a low ecstasy dose are limited (Downing, 1986; Vollenweider *et al*, 1998), there are various factors (eg, poor metabolism, hypertension, young age, simultaneous use of other substances, environmental conditions) that might contribute to individual or situational vulnerability for acute adverse effects and long-term neurotoxicity of ecstasy (Buchert *et al*, 2001; Obrocki *et al*, 2002; Green *et al*, 2004; Segura *et al*, 2005). Therefore, it is not possible to state that incidental use of ecstasy is completely safe. For example, neurocognitive data from a larger sample of the current study population suggest that even low-dose ecstasy use is associated with small but significant decreases in verbal memory relative to non-users (Schilt *et al*, 2006).

As we used multiple techniques as indicators for ecstasy-induced brain damage and multiple regions of interests, there is an increased probability of type I errors (false positive results). Therefore, additional *post hoc* Bonferroni corrections on all imaging analyses were performed. The results showed that most of the significant findings did not remain significant after Bonferroni correction. On the other hand, the Bonferroni correction may be too conservative especially because *a priori* we expected small effects as we studied early indicators of potential brain damage in subjects with only *low* cumulative doses of ecstasy use. Moreover, all imaging techniques and ROIs were chosen based on *a priori* hypothesis. Therefore, it is likely that the Bonferroni-correction induces type II errors (false negative findings). The risk of such corrections was previously discussed by Rothman (1990) who showed that they can obscure possibly important findings (Rothman, 1990). As a result of its social impact, additional research is needed to establish whether the current uncorrected significant findings can be replicated.

A limitation of the present study is the uncertainty about variances in dosage and purity of ecstasy tablets, although pill-testing confirms that in the Netherlands more than 95% of the tablets sold as ecstasy contain MDMA as the only (91.2%) or major (4.2%) component (Drugs Informatie en Monitoring Systeem, 2003; The Netherlands National Drug Monitor, 2004). The MDMA-related psychoactive substances 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA) or 3,4-methylenedioxyamphetamine (MDA) are major components in 1.5% of the ecstasy tablets and only 1% of

the ecstasy tablets contain amphetamine. The mean concentration of MDMA in an ecstasy tablet was 78 mg in 2003 in the Netherlands, but there is an increase in tablets with a dose of more than 140 mg MDMA (The Netherlands National Drug Monitor, 2004). Also the environmental circumstances under which ecstasy was taken and the simultaneous use of other substances was heterogeneous. As a result of these changing circumstances, it is possible that the observed changes in FA, ADC, rrCBV, depression, and impulsivity are not related to ecstasy use, but to other time- or ecstasy-related variables. Confounding by the use of other substances, such as alcohol, nicotine, cannabis, amphetamines, and cocaine, cannot be totally excluded, although alcohol, nicotine, cannabis, and amphetamine use did not change between sessions and most effects remained significant after exclusion of subjects with increased use of cocaine between sessions.

Another limitation is that we did not include a control group. Therefore, we cannot be completely sure about the reproducibility of our measurements. Other studies suggest that reproducibility of ^{1}H-MRS (Schirmer and Auer, 2000), DTI (Brockstedt *et al*, 1999; Cassol *et al*, 2004) and PWI (Henry *et al*, 2001) is good, although this might depend on the scanner, the scan protocol, and post-processing procedures. ROIs were drawn in the spatially normalized MNI brain template, which may have introduced additional variance due to inherent variations in mapping of individual brains to the MNI brain. On the other hand, compared to drawing ROIs for each individual subject, the current procedure is user-independent and reproducible, because the same procedure is performed for all subjects exactly in the same way. As few studies used ^{1}H-MRS, DTI, and PWI to study neuronal damage in ecstasy users, little is known about the sensitivity and specificity of these techniques to detect ecstasy-induced neuronal damage. Therefore, additional studies are needed, both in animals and in heavy human ecstasy users. As expected neuronal damage after a low dose of ecstasy is relatively small, the statistical power of this study could have been insufficient for ^{1}H-MRS and DTI to detect changes. DTI is particularly suitable for detection of white matter lesions, while ecstasy-related neuronal damage is especially expected in basal ganglia and cerebral cortex. As these areas have low FA, the sensitivity of this parameter to detect axonal dysfunction in basal ganglia might be limited. On the other hand, ^{1}H-MRS, DTI, and PWI have been shown to be sensitive tools in various neuropsychiatric disorders. For example, ^{1}H-MRS showed to be sensitive to detect changes in patients with schizophrenia, affective disorders, autism, and depression (Stanley, 2002; Kumar *et al*, 2002) and substance users (Ernst *et al*, 2000; Nordahl *et al*, 2002; Reneman *et al*, 2006). DTI showed to be sensitive in detection of early diffuse axonal injury after traumatic brain injury (Arfanakis *et al*, 2002) and various neuropsychiatric disorders (Lim and Helpern, 2002). PWI showed to be sensitive in detection of rrCBV deficits in early Alzheimer's disease (Harris *et al*, 1998; Bozzao *et al*, 2001) and in other neuropsychiatric diseases (Renshaw *et al*, 1997).

In conclusion, with the currently used techniques we found no indications for structural neuronal damage after a low dose of ecstasy use in first time ecstasy users. Therefore, these data do not support the concern that incidental ecstasy use leads to serious axonal loss, although more studies are needed to assess the sensitivity of the currently used MR techniques to detect small ecstasy-induced neuronal changes. However, our findings of decreased rrCBV and ADC may indicate that even a low dose of ecstasy can induce sustained vasoconstriction in some brain areas, although we do not know whether these findings are permanent. Therefore, and because there may be various personal and environmental factors that play a role in the occurrence of acute and long-term effects of ecstasy, it is impossible to state, based on this study, that incidental use of ecstasy is totally safe for the brain.

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