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The Netherlands XTC Toxicity (NeXT) study: objectives and methods of a study investigating causality, course, and clinical relevance

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ABSTRACT

This paper describes the objectives and methods of The Netherlands XTC Toxicity (NeXT) study focussing on the causality, course, and clinical relevance of ecstasy neurotoxicity. Previous studies suggest that ecstasy (3,4 methylenedioxymethamphetamine, MDMA, XTC) is toxic toward brain serotonin axons, but most of these studies have serious methodological limitations. The current study is a combination of different approaches with three substudies: (1) a cross-sectional substudy among heavy ecstasy users and controls with variation in drug use, which will provide information about potential neurotoxic consequences of ecstasy in relation to other drugs; (2) a prospective cohort substudy in ecstasy-naive subjects with high risk for future ecstasy use, which will provide information on the causality and short-term course of ecstasy use and potential neurotoxicity, and (3) a retrospective cohort substudy in lifetime ecstasy users and matched controls of an existing epidemiological sample that will provide information on long-term course and outcome of ecstasy use in the general population. Neurotoxicity is studied using (a) different imaging techniques (β-CIT SPECT, ^{1}H-MR spectroscopy, diffusion tensor imaging, perfusion weighted imaging and functional magnetic resonance imaging), and (b) neuropsychological and psychiatric assessments of memory, depression, and personality. The combined results will lead to conclusions that can be used in prevention messages, clinical decision making, and the development of an (inter)national ecstasy policy. Copyright © 2005 John Wiley & Sons, Ltd.

Key words: ecstasy, MDMA, serotonin, brain imaging, neurotoxicity

Introduction

Ecstasy (3,4-methelenedioxymethamphetamine, MDMA[1], XTC) was introduced as a recreational drug in the early 1980s. In the early 1990s a steep increase in ecstasy use occurred when the substance became popular as a dance and party drug in many European

[1] In this paper, the term 'MDMA' is used for ecstasy known to contain pure MDMA (laboratory conditions); the term 'ecstasy' is used for tablets/powder thought to be ecstasy although containment of MDMA only was not confirmed (general practice).

countries. In the last few years, both incidence and prevalence of ecstasy use have stabilized. According to the most recent national general population survey among Dutch residents of 12 years and older in 2001, annual incidence was 0.5%, lifetime prevalence was 2.9%, and last-month prevalence was 0.5% with higher prevalence among residents of Amsterdam (8.7% and 1.1%, respectively). Among a population of 16 million inhabitants this means that in the Netherlands approximately 70,000 people are monthly users of ecstasy. Nationally, lifetime and last-month prevalence were highest among young adults between 20 and 24 years old (13.6% and 2.5%, respectively) (Abraham et al., 2002). A national school survey among Dutch students aged 12–18 indicated a peak in lifetime prevalence of ecstasy use in the second half of the 1990s, followed by a decrease with a lifetime prevalence of 2.9% and last-month prevalence of 1.2% in 2003 (Monshouwer et al., 2004). Surveys among clubbers and ravers in Amsterdam also indicated that ecstasy use is over its peak; lifetime prevalence was 50.0% in 1995, 65.6% in 1998, and 52.7% in 2003; last-month prevalence was 32.9%, 41.3%, and 19.4% respectively (Korf et al., 2004). Prevalence rates were higher among males than females in the general population (Abraham et al., 2002), as well as among students (Monshouwer et al., 2004). Despite the widespread use of ecstasy among young adults, ecstasy use does not seem to constitute an addiction problem: on a yearly basis only 250–300 ecstasy users (0.4% of 70,000) seek advice or help for their ecstasy use at the addiction consultation and treatment centres in the Netherlands (Drugs Informatie en Monitoring Systeem, 2003).

On the other hand, there is increasing evidence from animal studies (such as Hatzidimitriou et al., 1999; Ricaurte et al., 2000) and human studies (such as McCann et al., 1998; Semple et al., 1999; Reneman et al., 2000a; Reneman et al., 2001a; Buchert et al., 2004) that the use of ecstasy might be toxic to serotonin axons in the brain. Serotonin is important for many physiological and neuropsychological processes, such as vasoconstriction, thermoregulation, memory, and learning (for example, Cohen et al., 1996; Meneses, 1999), so this could potentially lead to serious functional sequelae (see, for example, Gerra et al., 2000; Reneman et al., 2000a; Verbaten, 2003; De Win et al., 2004).

Despite the vastly growing scientific literature on the effects of ecstasy on the human brain some crucial questions regarding the causality, course, and clinical relevance of the potential neurotoxicity of ecstasy have not been answered yet, mainly because of methodological limitations of most studies. These limitations include inadequate sampling of subjects and controls, small samples, lack of drug-use analysis, restricted dose ranges, short follow up periods, and the use of cross-sectional and retrospective designs with lack of baseline data and inadequate control of potential confounders (Morgan, 2000; Turner and Parrott, 2000; Boot et al., 2000). Especially the use of other substances, such as amphetamines, cocaine, cannabis, alcohol, and tobacco, could be major confounders in almost all existing ecstasy studies, because most ecstasy users are poly-drug users. Other important potential confounders are gender, age, lifestyle, serotonin transporter genotype, pre-existing psychiatric morbidity and pre-existing cognitive dysfunctioning.

First, the lack of baseline data leads to interpretative difficulties concerning the causality between ecstasy use and potential toxicity. Because of ethical and legal issues, most research on ecstasy-induced neurotoxicity in humans has been performed with cross-sectional study designs including retrospective assessment of ecstasy use. This leaves the possibility that observed differences between ecstasy users and controls were pre-existent (Jansen and Forrest, 1999; Morgan, 1999; Dughiero et al., 2001; Lieb et al., 2002) or that results were biased by confounding variables such as poly-drug use, gender, and lifestyle. In some of the better studies, at least some measures were taken to reduce selection bias through the recruitment of control subjects from the same population as the ecstasy users (for example, Reneman et al., 2001a; Reneman et al., 2001b). However, pre-existing differences in serotonergic functioning are still relevant because some of the serotonin-related subject characteristics (e.g. sensation seeking, impulse-related disorders) could probably be considered as predisposing factors for ecstasy use.

A second important issue about the consequences of ecstasy use that has not been elucidated is the course and long-term outcome of the assumed ecstasy-induced neurotoxicity. It is important for clinicians and policymakers to know whether changes in the serotonergic system are temporary and thus reversible, or lasting and thus irreversible. In non-human primates, MDMA produces reductions in serotonergic axon terminal markers that last for months or even

years after cessation of drug exposure (Ricaurte et al., 1992; Hatzidimitriou et al., 1999). However, few human studies are available on the long-term effects of ecstasy use and the results are inconsistent and therefore inconclusive. Two studies reported normal densities of serotonin transporters (SERTs) in former ecstasy users (Reneman et al., 2001a; Buchert et al., 2003), whereas other studies (even in the same study population) reported long-lasting effects on memory function and symptoms of depression in ecstasy users who had stopped ecstasy use for at least one year (Gerra et al., 2000; Reneman et al., 2001b; De Win et al., 2004).

Third, little is known about the clinical relevance of observed serotonergic changes in humans. If ecstasy does damage serotonergic axons in humans, what functional consequences could be expected? Functional abnormalities seen in ecstasy users include memory disturbance, depression, impulsivity, and other neuropsychiatric disorders in which brain serotonin has been implicated (Morgan, 1998; Morgan, 2000; Parrott et al., 2000; Reneman et al., 2000a; Verbaten, 2003; De Win et al., 2004). Therefore, it is not only important to study the effects of ecstasy on serotonergic axons, but also to study the potential clinical consequences related to damage of these axons. Furthermore, changes in cerebral perfusion and cerebrovasculature of ecstasy users have been described (Chang et al., 2000; Reneman et al., 2000b; Reneman et al., 2001c). Moreover, besides damage to the serotonin axons, several case reports have linked ecstasy use with the onset of Parkinsonism in humans, suggesting potential damaging effects of ecstasy on the dopamine system (Mintzer et al., 1999; Kuniyoshi and Jankovic, 2003; O'Suilleabhain and Giller, 2003), although the currently available evidence for dopaminergic damage is not convincing (Kish, 2003). In addition, tablets sold as 'ecstasy' may contain substances that are toxic to neuronal systems other than the serotonin system (such as the dopamine system). Some of the observed clinical consequences of ecstasy use, however, may not reflect long-term damage but only transient effects of the use of the drug. Therefore, studies comparing (long-term abstinent) former users and ecstasy-naive controls on brain pathology, cognitive functioning, and clinical symptoms are of crucial importance to estimate its clinical relevance.

Finally, our understanding of dose-response characteristics and vulnerability factors, which may predispose some individuals to experience more negative effects following ecstasy use, is very limited. For example, it is important to find out whether brain pathology observed in heavy ecstasy users also occurs in less frequent users. Some researchers have argued that even a single moderate oral dose of MDMA might be neurotoxic in humans (Gijsman et al., 1999; McCann and Ricaurte, 2001), whereas others advocate the controlled use of MDMA as a therapeutic adjuvant for psychotherapy (for example, Doblin, 2002). Furthermore, it has been suggested that time intervals between subsequent ecstasy exposures, environmental circumstances during ecstasy use (such as temperature, noise, dehydration, exhaustion, stress) (Parrott, 2004), and the combination with other substances (such as alcohol, cannabis, amphetamines) (Butler and Montgomery, 2004; Daumann et al., 2004a; Roiser and Sahakian, 2004) could modify ecstasy-induced brain damage. Moreover, there are presumably important biological and psychobiological risk factors such as age, gender, neurotransmitter polymorphism, and pre-existing psychiatric morbidity that are related to individual differences in serotonergic functioning and to differences in vulnerability for the neurotoxic effects of ecstasy.

Because of limitations in current ecstasy research and the accompanying unanswered questions about its potential neurotoxicity, the Netherlands Research and Development Program on Substance Use and Addiction supplied a grant for the current Netherlands XTC Toxicity (NeXT) study addressing this important public health issue. The identification of specific health risks, such as cognitive impairment and brain damage, would provide a cogent argument for consumers to make informed decisions about recreational drug use. Ultimately, the NeXT study would help to predict future demands on healthcare. In the next paragraphs, the objectives and methods of this study are described and discussed.

Objectives

The overall objective of the NeXT study is to come to better informed scientific knowledge regarding the neurotoxicity of ecstasy that can be used in prevention messages, clinical decision making, and the development of an (inter)national ecstasy policy.

Primary objectives are:

1. To study the causality of ecstasy use in observed brain pathology in humans.

2. To study the long-term course of brain pathology and related clinical characteristics in ecstasy users.
3. To study the clinical relevance of observed brain pathology in ecstasy users.

Secondary objectives are:

4. To study the dose-response characteristics of ecstasy use in the causation of brain pathology.
5. To study vulnerability and protective factors in the causation of brain pathology among ecstasy users;
6. To study potential neurotoxic consequences of ecstasy use in relation to the use of other drugs.
7. To study the presence of functional or structural damage to neurotransmitter systems other than serotonin following ecstasy exposure.

Design

General design of the NeXT study

Only a long-term prospective study of serotonergic function in ecstasy-naive individuals randomly assigned to MDMA or placebo conditions could determine decisively whether recreational use is neurotoxic to human beings and whether these toxic effects are reversible or not. However, given the existing data on brain abnormalities in MDMA-treated animals and in human ecstasy users, such a study is ethically not acceptable. The NeXT study therefore studies causality, course, and outcome of various indicators of brain pathology (for example, neuroimaging) and possible related clinically relevant symptoms (such as neurocognitive and psychiatric symptoms and disorders) of ecstasy neurotoxicity in a combination of three substudies. The outlines of the three sub-studies are summarized in the Figures 1, 2, and 3. The NeXT study includes

- a cross-sectional substudy of heavy ecstasy users and controls with variation in amount and type of drug use, which will provide information on potential neurotoxic consequences of ecstasy use in relation to the use of other drugs;
- a prospective cohort substudy in ecstasy-naive subjects with a high risk for future first ecstasy use, which will provide information on the causality and short-term course of ecstasy use and potential neurotoxicity, especially for low exposure levels; and
- a retrospective (historical) cohort substudy in lifetime ecstasy users and matched controls of an existing epidemiological sample that will provide information on long-term course and outcome of ecstasy use in the general population and thus on potential public health consequences of ecstasy use in a Western society.

The combination of the three substudies with the use of similar assessment procedures in all substudies will provide important additional information regarding the neurotoxicity of ecstasy use in humans.

The total inclusion period for all three substudies was between April 2002 and June 2005 and final results are expected in the first half of 2006. All subjects had to be between 18 and 35 years of age. Exclusion criteria were: presence of a severe medical or neuropsychiatric disorder (for example, depression, psychosis, parkinsonism), use of psychotropic medications affecting the serotonin system such as selective serotonin reuptake inhibitors (SSRIs), pregnancy, intravenous drug use, and contraindications for MRI (such as claustrophobia or wearing a pacemaker). Subjects had to abstain from the use of psychoactive substances for at least 2 weeks prior to examinations and from alcohol for at least 1 week prior to examinations. Subjects were paid for their participation.

Design and study samples of the substudies

Cross-sectional substudy among heavy ecstasy users

The two main objectives of the cross-sectional substudy among heavy ecstasy users are

- to specify potential neurotoxic consequences of ecstasy use in relation to the use of other drugs; and
- to validate various imaging techniques for ecstasy research, especially ^{1}H-MR spectroscopy, diffusion tensor imaging, perfusion weighted imaging, and functional MRI (see imaging parameters), which have only been used in a very few studies.

The potential neurotoxicity of heavy ecstasy use is investigated with a retrospective assessment of drug use history and by comparing neuroimaging, neurocognitive, and psychopathological outcomes in a stratified sample of 71 subjects (Figure 1). Overall, subjects can be classified according to five different profiles or 'groups' with variations in the amount and type of drug use:

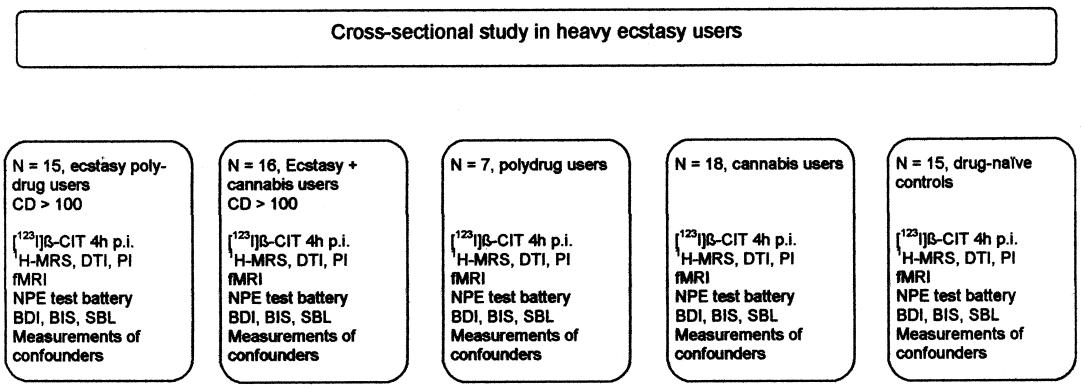

Figure 1. Outline of the cross-sectional substudy among heavy ecstasy users.

1. A group of 15 heavy ecstasy poly-drug users.
2. A group of 16 selective ecstasy and cannabis users.
3. A group of seven poly-drug controls with a history of heavy amphetamine and/or cocaine and cannabis use but very limited ecstasy use.
4. A group of 18 ecstasy-naive cannabis users.
5. A group of 15 drug-naive controls.

All subjects were included between October 2002 and January 2005. They were recruited through advertisements at dance- and drug-related sites on the Internet and in newspapers, through flyers at locations such as dance events, discotheques, youth fairs, universities, and colleges, and through word of mouth. Additional inclusion criteria for heavy ecstasy users (groups 1 and 2) were a cumulative dose (CD) of at least 100 ecstasy tablets and use of the last ecstasy tablet less than 6 months ago. The poly-drug controls (group 3) had a history of regular use of amphetamines and/or cocaine, but a very limited use of ecstasy (maximum CD of 10 tablets). The ecstasy-naive cannabis users (group 4) were matched to the heavy ecstasy users (groups 1 and 2) on gender, age, and CD of cannabis use. The drug-naive controls (group 4) had never used psychoactive drugs, although they were allowed to have experience with the use of alcohol and/or tobacco just like the other groups. Part of the cannabis and drug-naive controls (groups 4 and 5) were age-matched subjects taken from the baseline population of the prospective cohort study (see prospective cohort substudy).

In order to specify whether ecstasy users differ from non-users on indicators of neurotoxicity, including clinical characteristics, outcome parameters of neurotoxicity will be compared between ecstasy users and ecstasy-naive subjects. The comparisons will also indicate which imaging technique is most sensitive for detecting neurotoxicity in ecstasy research. In addition, separate effects of various drugs (lifetime use of ecstasy, cannabis, amphetamine, and cocaine) on the outcome parameters will be assessed to examine whether drugs other than ecstasy contribute to the potential effect of ecstasy on indicators of neurotoxicity (see statistical paragraph).

Prospective cohort substudy

To study the causal nature of ecstasy use on neuroimaging, neurocognitive, and clinical abnormalities observed in ecstasy users and to determine the effect of relatively low cumulative dosages of ecstasy, a sample of 188 ecstasy-naive young adults (aged 18 to 35 years) with a relatively high probability to start using ecstasy in near future was followed during a period of 12 to 24 months (Figure 2). They were actively recruited between March 2002 and April 2004, using a combination of targeted site sampling at locations such as dance events, discotheques, youth fairs, universities, colleges, and parks; advertisement through a Web site on the project and an Internet campaign; and snowball sampling referrals. Main criteria for inclusion were intent (probable or certain) to use ecstasy for the first time in the near future (3–5 points on a 5-points scale; 1 = certainly not; 2 = probably not; 3 = undecided; 4 = probably yes; 5 = certainly yes) and/or having one or more friends who already use ecstasy.

After baseline examination subjects had to complete questionnaires sent to them by mail about their drug use

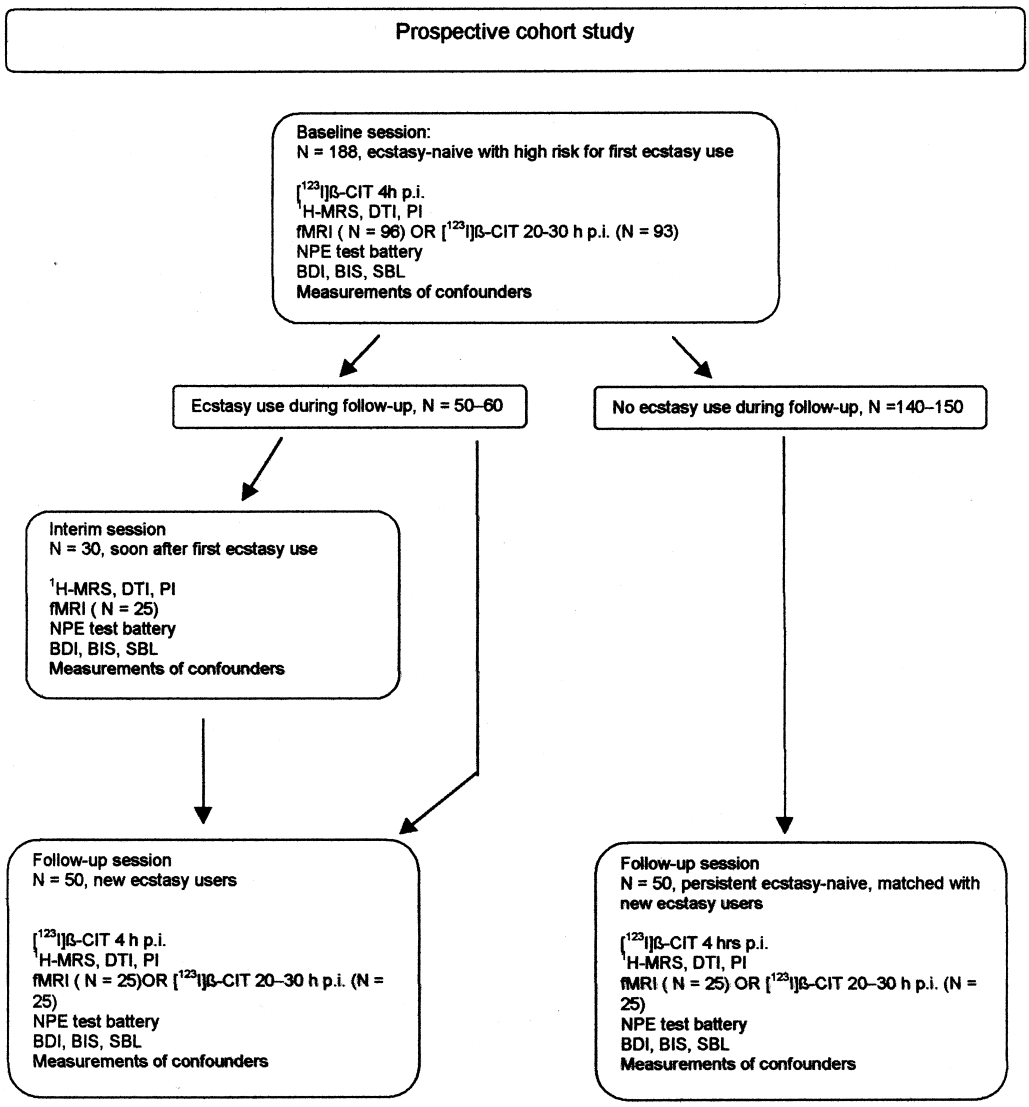

Figure 2. Outline of the prospective cohort substudy.

every 3 months during a follow-up period of 1 year. Besides assessing drug use through these questionnaires, the main outcome parameters were assessed up to three times: (T1) directly following recruitment, i.e. before first ecstasy use, in the total cohort (N = 188); (T2) soon after first ecstasy use in the first 30 incident ecstasy users; (T3) between 12 and 24 months after baseline assessment in (T3a) all incident ecstasy users (N = 50–60), and in (T3b) an individually matched (gender, age, DART-IQ, cannabis use) control group of persistent ecstasy-naive subjects (N = 50–60). Single photon emission computed tomography (SPECT) imaging was only performed twice because of radiation exposure (at the first and third session). Follow-up measurements were finished in June 2005.

To study whether a low dose of ecstasy use is neurotoxic, outcome parameters of neurotoxicity will be compared between the first follow-up session soon after first ecstasy use in 30 incident cases (T2) and their baseline sessions before first ecstasy use (T1). We

will also investigate whether ecstasy users differ from ecstasy-naive subjects on indicators of neurotoxicity and if so, whether differences were present before or developed after the first use of ecstasy. In order to examine this, indicators of neurotoxicity of incident ecstasy users (T3a) will be compared with persistent ecstasy-naive subjects (T3b) and both groups will be compared with their own baseline data (T1). Moreover, to assess whether certain variables (such as higher levels of depression, impulsivity and sensation seeking) can be considered as risk-factors for future ecstasy use in ecstasy-naive young adults, baseline data of incident ecstasy users (i.e. before first ecstasy use) will be compared with baseline data of persistent ecstasy-naive subjects. Finally, dopamine transporter (DAT) densities will be compared before and after ecstasy use in a subgroup of incident ecstasy users to specify possible effects of ecstasy use on the dopamine neurotransmitter system.

Retrospective cohort substudy

To examine the potential public health consequences of ecstasy use in a Western society, a representative sample of lifetime ecstasy users and a matched control group of ecstasy-naive individuals were included in the retrospective (historical) cohort substudy. The participants of this substudy are selected from the longitudinal 'Zuid-Holland study' (Figure 3). This study started in 1983 with 2,600 subjects of Dutch nationality, aged four to 16 years (birth cohorts 1967–79), randomly selected from the municipal registers from the Dutch province of Zuid-Holland, with both urbanized and rural areas. Of these, 2,076 (84%) participated in the first measurement in 1983 (Verhulst et al., 1985). Since then the sample was reassessed five times, most recently in 1997 (Hofstra et al., 2002) when 1,578 subjects still participated (76.0% of the original sample of 2,076). Of these 1,578 subjects 98 indicated in 1997 during a psychiatric assessment with the Composite International Diagnostic Interview (World Health Organization, 1992) that they had used ecstasy at least five times lifetime.

The group of lifetime ecstasy users and an individually matched control group of ecstasy-naive subjects were approached to participate in the current study. Outcome assessments in these groups started in May 2003 and finished in July 2005. The control group of

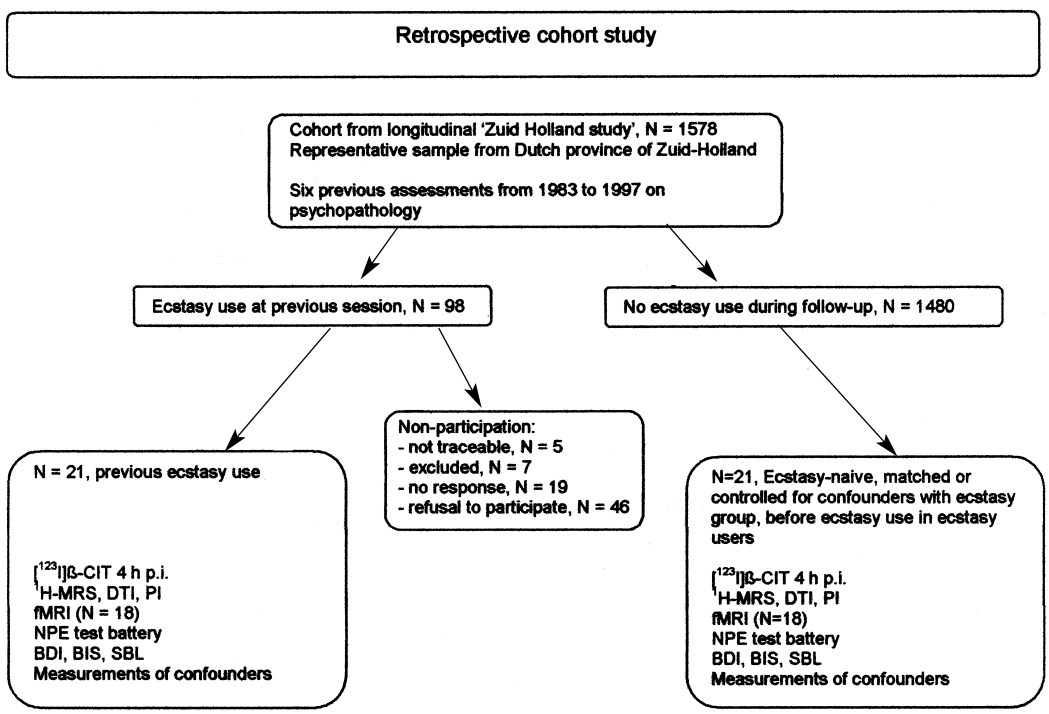

Figure 3. Outline of the retrospective cohort substudy.

ecstasy-naive subjects was matched for potential confounders that were assessed prior to the first use of ecstasy. Matching variables include age, gender, use of cannabis, and internalizing (for example, anxiety, depression) or externalizing (for example, conduct disorder, ADHD) problems at age 4 to 16 measured with the Child Behavior Checklist (CBCL) (Achenbach, 1991).

To assess whether lifetime ecstasy users of a representative sample differ on indicators of neurotoxicity, including clinical characteristics, from matched controls that never used ecstasy, outcome parameters will be compared between lifetime ecstasy users and non-users, while controlling for potential confounders. Moreover, correlations between characteristics of ecstasy use (e.g. lifetime CD, duration of abstinence) and outcome parameters will be analysed in order to study the course and dose-response relationship of potential ecstasy-induced neurotoxicity.

Assessments

Exposure to ecstasy and other substances

Variables such as dose, dosing pattern, and circumstances under which ecstasy is used (such as temperature, noise, dehydration, exhaustion, stress) can influence the severity of neurotoxicity in animals (Huether et al., 1997; Malberg and Seiden, 1998; Morton et al., 2001). Since this might also be true for humans (Parrott, 2004), we assessed these various aspects of ecstasy use with validated drug-use questionnaires (Van de Wijngaart et al., 1997). The questionnaires were also used to assess use and frequency of use of other substances such as cannabis, alcohol, tobacco, amphetamines and cocaine. To exclude acute pharmacological effects of substance use on the main outcome parameters, subjects had to abstain from drug use for at least 2 weeks and from alcohol for at least 1 week prior to testing. This was checked through urine drug screening (enzyme-multiplied immunoassay for amphetamines, ecstasy, opiates, cocaine, benzodiazepines, cannabis, and alcohol). The absence or presence of prior ecstasy use and prior use of related substances such as amphetamines, MDA and MDEA will be checked in hair of all ecstasy users and of a random sample of 25% of the ecstasy-naive controls, using gas chromatography/mass spectroscopy analysis.

Outcome parameters (indicators of neurotoxicity)

In the NeXT study indicators of neurotoxicity were assessed using a combination of neuroimaging, neurocognitive, and psychiatric assessments with techniques that already proved to be effective in detecting different aspects of serotonin-related neurotoxicity. In addition, currently known potential confounders (age, substance use, personality, depression, cognitive functioning, serotonin and dopamine transporter genotype) were assessed.

Imaging parameters

Potential ecstasy-induced neurotoxicity can be studied *in vivo* in humans using various imaging techniques that assess different aspects of the structural and functional brain and provide complementary information. Most ecstasy-related imaging studies have been performed using positron emission tomography (PET) or single photon emission computed tomography with radiotracers that bind to serotonin transporters (SERTs) at the pre-synaptic terminal of the serotonergic axon. Because of radiation exposure these techniques are not suitable for multi-session follow-up studies. Multi-session follow-up studies are possible with magnetic resonance imaging (MRI) techniques. Moreover, the use of advanced MRI techniques makes it possible to study various other aspects of neuronal damage such as neuronal density and viability using proton magnetic resonance spectroscopy (^{1}H-MRS), axonal integrity using diffusion tensor imaging (DTI), and consequences for cerebrovasculature using perfusion weighted imaging (PWI). Furthermore, the neurophysiological correlates of cognitive brain functions such as working memory and attention can be studied using blood oxygen level dependent functional MRI (BOLD fMRI). As there is limited experience in ecstasy research with the various MRI techniques it is not known yet what indicators are most sensitive to detect ecstasy-induced serotonergic damage.

Single photon emission computed tomography

Damage to the serotonergic axon can be studied in the living human brain by measuring the presynaptic SERT density. Single photon emission computed tomography is a structural element of the presynaptic membrane and has been shown to be a reliable marker of MDMA-induced serotonergic neurotoxicity (Scheffel et al., 1992). The radiotracer ^{123}iodine-2β-carbomethoxy-3β(4-iodophenyl)tropane ([^{123}I]β-CIT)

that binds with high affinity to SERTs and dopamine transporters (DATs) (Laruelle et al., 1993) can be used in combination with SPECT to assess SERT (4 hours after injection of the tracer) and DAT (20 to 30 hours after injection of the tracer) densities (Reneman et al., 2002a; De Win et al., 2005). Previous [^{123}I]β-CIT SPECT studies have shown reduced SERTs in subjects with a history of ecstasy use (Semple et al., 1999; Reneman et al., 2001a; Reneman et al., 2001b).

In the NeXT study [123I]β-CIT SPECT was performed 4 hours post injection (p.i.) to measure SERT densities in subjects of all three substudies, once in the cross-sectional heavy user study and in the retrospective cohort study and twice (at baseline and at 12 to 24 months follow-up) in the prospective cohort study. Single photon emission computed tomography scanning was also performed 20 to 30 hours p.i. to assess DAT densities in a subgroup of subjects from the prospective cohort study who were not selected for fMRI assessment. For detailed description of the SPECT procedure the reader is referred to De Win et al. (2005).

Magnetic resonance imaging: ^{1}H-MRS, DTI and PWI

^{1}H-MRS, DTI, and PWI were performed in a single scanning session on a 1.5 Tesla MRI scanner.

Single voxel ^{1}H-MRS

^{1}H-MRS allows studying of certain metabolites in the brain *in vivo,* such as N-acetylaspartate (NAA), choline-containing compounds (Cho), myo-inositol (mI) and creatine plus phosphocreatine (Cr). N-acetylaspartate exists almost exclusively within the neuronal cell bodies and axons and reductions in NAA are therefore associated with neuronal damage and impaired cognition (Ross et al., 1997). Choline is increased in brain diseases that involve increased membrane breakdown, myelination or inflammation and is thought to reflect cellular density (Miller et al., 1996). Myo-inositol is a putative glial cell marker (Ross et al., 1997). The creatine peak is thought to be relatively constant between individuals and in most brain diseases (Pouwels and Frahm, 1998) and it is therefore often used as an internal reference to calculate ratios. Previous studies in ecstasy users showed decreased NAA/Cr ratios in the frontal grey matter (Reneman et al., 2002b), correlated to impaired memory performance (Reneman et al., 2001d), and increased mI/Cr ratios in the parietal white matter (Chang et al., 1999). However, the decreased NAA/Cr ratio was not confirmed by another recent study (Daumann et al., 2004b).

In the NeXT study, single voxel ^{1}H-MRS was performed in three voxels of interest placed in left parietal white matter, in mid-frontal grey matter and in mid-occipital grey matter. Relative (using Cr as a reference) and absolute metabolite concentrations of NAA, Cho, and mI will be calculated.

Diffusion tensor imaging and Perfusion weighted imaging

With DTI it is possible to quantitatively measure diffusional motion of water molecules in the brain. In the normal situation this motion is restricted in amplitude and direction by cellular structures such as axons. Therefore the apparent diffusion coefficient (ADC) is lower and the fractional anisotropy (FA) is higher in the brain than in bulk water. Processes that disturb structural elements of the brain tissue can result in increased ADC and decreased FA. Only one previous article reported preliminary findings of ADC measurements in ecstasy users, finding significantly increased ADC values in the globus pallidus of ecstasy users (Reneman et al., 2001c).

Serotonin is involved in the regulation of brain microcirculation (Cohen et al., 1996) and cerebrovascular accidents were described in ecstasy users (Hanyu et al., 1995; Lee et al., 2003) so it is of particular interest to study the cerebral microcirculation in ecstasy users, which is possible with PWI using the dynamic susceptibility contrast (DSC) technique. Previous studies already indicated that exposure to ecstasy may lead to cerebrovascular changes (Chang et al., 2000; Reneman et al., 2000b; Reneman et al., 2001c).

Functional MRI

Functional MRI is a relatively novel imaging technique aimed at localizing and assessing cerebral functions, including memory and attention. Brain activity patterns that correspond with cognitive functions are obtained by contrasting experimental conditions with control conditions within the same session. Changes in performance and/or brain reactivity patterns on these tasks are expected to reflect the severity of ecstasy's neurotoxic effects. Cognitive domains of interest are selective/sustained attention, working memory, and long-term memory (McCann

et al., 1999; Parrott, 2000). One of the important advantages of fMRI over behavioural measures of brain functioning is that fMRI can reveal abnormalities in the organization of brain networks, which may occur as an adaptive response to brain damage and which may be difficult to detect in behaviour. This added value of fMRI has been supported by some recently published papers on the neurotoxicity of ecstasy (Daumann et al., 2004c; Daumann et al., 2005), which reported neurophysiological changes in the brains of heavy ecstasy users while task performance was normal. However, the results have been inconsistent in showing ecstasy-related long-term neuronal effects in humans, as the same research group could not demonstrate statistically significant differences between ecstasy users and controls in two previous fMRI studies (Daumann et al., 2003a; Daumann et al., 2003b).

In the NeXT study, fMRI was performed in all right-handed volunteers of the cross-sectional study among heavy ecstasy users, in all right-handed subjects of the retrospective cohort study, and in a subgroup (right-handed) of the subjects from the prospective cohort study (see Figure 2). Based on previous findings in neuropsychological literature the fMRI protocol was designed to focus on three cognitive domains: working memory, long-term memory, and selective attention. Verbal working memory was assessed using a modified Sternberg item-recognition task (see for details Ramsey et al., 2004). Long-term memory was investigated using a visual associative memory task, adapted from Henke et al. (1997). Thirdly, selective attention was measured using a visuo-auditory attention paradigm. All three tasks were presented in the scanner, and fMRI scans were acquired during performance of the tasks and during control tasks. With post-processing analysis of the fMRI scans, brain activity patterns are assessed for each subject. Use of control tasks avoids the potential confound of changes in basic brain perfusion.

Neuropsychological and psychopathological parameters

As serotonin modulates many neuropsychological processes, it can be expected that ecstasy-induced damage to serotonin axons leads to impairment of functions in which serotonin is involved, such as impulsivity, mood disorders, and memory function. Previous research on the functional consequences of serotonergic neurotoxicity induced by ecstasy showed converging evidence of impairment in memory (Gouzoulis-Mayfrank et al., 2000; Reneman et al., 2001b; Verbaten, 2003). However, studies on the effect of ecstasy use on mood, impulsivity, and sensation seeking are less conclusive because there are indications that symptoms of increased depression, impulsivity, or sensation seeking might be pre-existing or even predispose subjects to ecstasy use (Bardo et al., 1996; Lieb et al., 2002; De Win et al., 2004). They could be thought of as influencing memory deficits as well. In the NeXT study subjects were assessed on a battery of tests on various aspects of cognitive functioning and with self-report questionnaires on depression and personality traits.

Neuropsychological examination

The neuropsychological test battery used in the NeXT study includes tests that have proven to be sensitive to ecstasy-related neurotoxicity and tests related to functions or brain areas that are thought to be affected by ecstasy use (for example, prefrontal cortex, occipital cortex, hippocampus). Moreover, tests were selected by their sensitivity to detect subtle impairments in younger persons. The following cognitive domains were tested: working memory, verbal memory, visual memory, visuospatial ability, and verbal intelligence:

- *Working memory/executive functioning*. Impaired function of working memory in ecstasy users was found in several studies (see, for example, McCann et al., 1999; Wareing et al., 2000). The Paced Auditory Serial Addition Test (PASAT) (Gronwall, 1977) was administered in the current study to measure working memory and information processing accuracy. Subjects have to add numbers presented by a recorded male voice to a preceding number. A Dutch adaptation of the Digit Span (Wechsler D, subtest of Wechsler Adult Intelligence Scale- revised WAIS-R) was used to measure attention and working memory (Lindeboom and Matto, 1994). The version of Lindeboom gives a more reliable difference score between repeating digits in forward and in backward order by offering subjects one series of digits extra per length. Previous studies found decreased scores on the Digit Span in ecstasy users (Gouzoulis-Mayfrank et al., 2000; McCardle et al., 2004) while others did not (Bhattachary and Powell, 2001). Finally, we used the Iowa Gambling Task to measure decision-making and risk-taking

behaviour (Bechara et al., 1994). It provides participants with choices from four decks of cards, each associated with a specific degree of reward or punishment.

- *Verbal memory*. The most substantial evidence for cognitive deficits in ecstasy users is on impaired functioning of ecstasy users on verbal memory tasks (Bolla et al., 1998; Reneman et al., 2001b; Thomasius et al., 2003; Verbaten, 2003). Verbal memory can be measured using the Rey Auditory Verbal Learning Test (RAVLT) (Rey, 1964). In the current study a Dutch version was administered (Rey, 1964; Van den Burg et al., 1985). Subjects have to memorize a series of 15 words in five learning trials. Immediate recall is tested after each trial. Delayed recall and recognition are measured after 20 minutes.
- *Visual memory*. Previous studies on non-verbal memory reported inconclusive results (Parrott, 2001; Back-Madruga et al., 2003). We used a computerized adaptation of the Memory for Designs test (Graham and Kendall, 1960). The original test with 14 figures was split in two separate tests to obtain a parallel version. After a slide show of seven figures, 5 s each, subjects have to draw the figures from memory. The show is repeated five times. Delayed reproduction is measured after 15 minutes.
- *Visuospatial functioning*. Also studies on visuospatial functioning produced contradictive results (Parrott, 2001; Back-Madruga et al., 2003), although there are indications that brain areas such as the parieto-occipital and occipital cortex, involved in visuospatial functioning, are affected by ecstasy use (Reneman et al., 2001a). In the current study the first test to measure visuospatial functioning was the Mental Rotation Task (Shepard and Metzler, 1971). Participants were presented with 20 pairs of block designs drawn from different points of view. Within 3 minutes they had to judge whether pairs of designs are identical or different. A computerized and adapted version of the Judgement of Line Orientation (JOLO) (Benton et al., 1978) was used to test visuospatial working memory. The JOLO requires subjects to identify which two of 11 lines presented in a semicircular array have the same orientation in two-dimensional space as two target lines. The target lines in our assessments are only shown for one second, directly followed by the 11 lines.
- *Verbal intelligence*. The Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test (Nelson, 1991), was administered to estimate premorbid verbal intelligence (DART-IQ) as it is relatively insensitive to cognitive impairment caused by neurological disorders (Schmand et al., 1991).

Psychopathological parameters

Current depression was assessed using the Beck Depression Inventory (BDI) (Beck et al., 1961). The BDI is a 21-item self-report rating inventory that measures characteristic attitudes and symptoms of depression in the week prior to assessment. The BDI has proven to be a reliable and valid indicator of depression (Beck and Steer, 1984; Bouman et al., 1985). Increased BDI scores were reported in recent and former ecstasy users (Thomasius et al., 2003; De Win et al., 2004).

Also, increased impulsivity scores were reported in ecstasy users (Morgan, 1998; Bond et al., 2004). The Dutch version of the Barratt Impulsiveness Scale (BIS-11) was used in the current study to assess impulsivity (Patton et al., 1995). The Dutch BIS-11 contains 31 self-report items that have to be scored from 1 to 4. Total scores and subscale scores on attentional impulsivity ('difficulty in concentrating'), motor impulsivity ('acting without thinking'), and non-planning impulsivity ('thinking about the present rather than the future') will be calculated.

The Spannings Behoefte Lijst (SBL), a Dutch adaptation of the Sensation Seeking Scale (Zuckerman and Link, 1968), was used to measure sensation seeking (Feij et al., 1982; Feij and Van Zuilen, 1984). The SBL contains 51 sensation-seeking items, for which respondents have to indicate on a five-point scale to what extent they (dis)agree with the statements. Both total scores and scores for subscales on thrill and adventure seeking (TAS), experience seeking (ES), boredom susceptibility (BS), and disinhibition (DIS) will be calculated. Increased sensation/novelty seeking in ecstasy users was reported in various studies (Schifano, 2000; Dughiero et al., 2001; Gerra et al., 2004).

Potential confounders

Various potential confounders that have been identified in literature were assessed in all subjects included in the substudies:

- *Substance use other than ecstasy*. Almost all ecstasy users use other substances as well, so the use and frequency of use of cannabis, alcohol, tobacco, amphetamines, cocaine, LSD, mushrooms, and other substances, were assessed using questionnaires described in the 'exposure to ecstasy and other substances' paragraph.
- *Demographic variables*. Demographic variables such as age, gender, level of education of subjects and their parents, ethnicity, and lifestyle were documented.
- *Gonadal hormones*. Detailed data regarding menstrual cycle and usage of oral contraceptives were obtained because it has been suggested that female ecstasy users are more vulnerable for subjective and neurotoxic effects of ecstasy (Liechti et al., 2001; Reneman et al., 2001a; Buchert et al., 2004). Therefore, in all female subjects of the prospective and the retrospective cohort sub-studies who did not use oral contraceptives, 17-(-oestradiol and progesterone were measured because these hormones modulate some functional aspects of the serotonergic system (McQueen et al., 1997).
- *Serotonin transporter genotype*. A genetic contribution to the expression of SERTs has been described, in which the activity of the long allele of the SERT promoter region has been shown to be twice that of the short allele (Lesch et al., 1996). The serotonergic polymorphism is assessed in the participants of the prospective and the retrospective cohort substudies to investigate the effect of pre-existing (genetic) differences in serotonergic function between ecstasy users and non-users.
- *Pre-existing psychiatric morbidity and cognitive dysfunctioning*. These potential confounders were assessed by the neuropsychological test battery and the psychiatric self-report questionnaires as described above.

Statistics

Power analysis

Single photon emission computed tomography, ^{1}H-MRS, DTI, PWI, and memory performance

Previous SPECT findings (Reneman et al., 2001a) indicated that 8 females (effect size d= 0.16) and 31 males (d=0.08), thus a group of 39 subjects per group, would provide ample power to demonstrate a difference in SERT densities between pre-post assessments (prospective study) and between lifetime ecstasy users and ecstasy-naive controls (retrospective studies), if such a difference exists. This power estimate is in general agreement with two other imaging studies conducted with ecstasy users, which showed that 25 subjects were needed per group to demonstrate a difference in serotonergic transporter densities using PET (males and females together) (McCann et al., 1998) and 32 using SPECT (only males) (Semple et al., 1999). The sample sizes in all three substudies would also be big enough to detect effect sizes of 28%, 21%, and 31% on outcome parameters measured with ^{1}H-MRS, PWI, and memory performance (especially on RALVT) respectively, as indicated by previous studies (Reneman et al., 2001b; Reneman et al., 2001c; Reneman et al., 2002b).

Functional MRI

Previous studies indicated that changes in cognitive abilities are small but significant after moderate ecstasy use. For reliable measurement of cognition-related functional brain activity patterns a sample size of about 10 to 12 subjects is required. To detect differences reliably between ecstasy users and controls, 10-12 subjects would be required per group. As brain activity patterns might differ between male and female ecstasy users, 20 to 24 subjects per group would be required in order to obtain representative samples for both genders.

Statistical analyses

We hypothesized *a priori* that if ecstasy use is indeed neurotoxic, ecstasy users would differ on various imaging parameters (for example, increase of ADC, rCBV, Cho, mI and decrease of [^{123}I]β-CIT uptake ratios, FA, NAA), on BOLD fMRI parameters (increased activity or alterations in patterns of activation), as well as on parameters of neurocognitive functioning (such as decreased memory) and psychopathology (such as increased depression, impulsivity, sensation seeking) compared with non-users (cross-sectional substudy and retrospective cohort substudy) or compared with their own baseline values prior to first ecstasy use (prospective cohort substudy).

For the cross-sectional substudy among subjects with variations in amount and type of drugs used, parameters of neurotoxicity will be assessed using linear multiple regression analysis with lifetime use of ecstasy,

cannabis, amphetamine, and cocaine as separate regressors. It is expected that this will provide information about the relative contributions of the various drugs on the main outcome parameters. The regression model will also control for factors other than drug use, such as gender, age, and DART-IQ.

For the prospective cohort substudy, follow-up data will be compared between incident ecstasy users and persistent ecstasy-naive subjects using (multivariate) analysis of variance (ANOVA/MANOVA), including baseline measurements and significant confounders (such as age, gender, use of cannabis, amphetamines and cocaine) as covariates (ANCOVA, MANCOVA). In order to prevent the loss of subjects due to incomplete data, general linear mixed models could be applied in the analysis of the longitudinal data.

For the retrospective cohort study, parameters of neurotoxicity will be compared cross-sectionally between lifetime ecstasy users and matched non-users. An analysis of covariance will be used with main confounders (such as age, gender, cumulative dose of ecstasy, use of cannabis, internalizing and externalizing psychopathology at age 4–16 measured with the CBCL, prior to first ecstasy use in the group of lifetime ecstasy users) as covariates. Correlations between characteristics of ecstasy use (such as lifetime CD, duration of abstinence) and outcome parameters will be analysed using a linear regression analysis.

Ethical considerations

The NeXT study was approved by the local medical ethics committee. To rule out any suggestion that we approve of or stimulate the use of ecstasy (especially in ecstasy-naive subjects) volunteers were informed about potential negative consequences of ecstasy use. In addition, each participant had to sign a document giving informed consent, which states that participation was voluntary, that ecstasy is potential harmful and that the examiners do not have the intention to stimulate the use of ecstasy.

Discussion and conclusion

This article described the objectives, design, study populations, assessments, and statistical issues of the NeXT study with its focus on causality, course, and clinical relevance. To our knowledge this is the first large-scale ecstasy study using various imaging techniques and a combination of both cross-sectional and longitudinal (prospective and retrospective) approaches. It includes novel users with low CD as well as heavy users with high CD of ecstasy and adequate controls for confounders (partly measured prior to first ecstasy use).

The first substudy, including two groups of heavy ecstasy users (both poly-drug users and selective ecstasy users), two comparison groups (poly-drug users and cannabis users), and a drug-free control group, is especially designed to assess the potential neurotoxic consequences of heavy ecstasy use in relation to other drug use. Although some previous studies indicated that signs of neurotoxicity in ecstasy users might not be related to merely ecstasy use but rather to the use of different other psychoactive drugs (Morgan et al., 2002; Butler and Montgomery, 2004; Roiser and Sahakian, 2004; Daumann et al., 2004a), only very few studies adequately controlled for use of other substances. The advance of the current study over previous studies is that we recruited a specific sample (N =71 in total) with specific variations in amount and type of drugs used in such a way that they are virtually uncorrelated, allowing for multiple regression analysis to tease out drug-specific effects while benefiting from the statistical power of a large total sample size. The results will give insight in the relative contributions of the different drugs on the cognitive impairments and serotonin-related neurotoxicity found in heavy ecstasy users. In addition, this study will provide information about the sensitivity and suitability of different imaging techniques, especially the MRI techniques such as ^{1}H-MRS, DTI, PWI, and BOLD fMRI, in studying the potential neurotoxicity of ecstasy. Most previous imaging studies used positron emission tomography (PET) or single photon emission computed tomography (SPECT) with radiotracers that bind to SERTs at the terminal of the serotonin axon. However, the use of imaging techniques without radiation involved (for example, ^{1}H-MRS, DTI, PWI and BOLD fMRI) would make it possible to perform multisession follow-up studies in future. Moreover, these techniques enable us to study different aspects of neuronal damage, complementary to the assessment of SERT densities as measured with PET or SPECT techniques. Despite the advantages of these techniques, few studies on the neurotoxicity of ecstasy using functional MRI (Daumann et al., 2003a; Daumann et al., 2003b; Daumann et al., 2005), DTI (Reneman et al., 2001c) or PWI (Reneman et al., 2000b; Reneman et al., 2001c) have been published to date and previous studies using ^{1}H-MRS were

inconsistent (Chang et al., 1999; Reneman et al., 2001d; Reneman et al., 2002b; Daumann et al., 2004b). The current study aims to fill this gap with an exploration of the specific opportunities and limitations of these new methods for ecstasy research.

The prospective substudy, using a naturalistic approach, will enable us to test the causal role of ecstasy use in serotonergic damage, to study a possible dose-response relationship, and to establish the short-term course and outcome of (various) indicators of brain pathology and possibly related clinical relevant symptoms after ecstasy use. To our knowledge, this is the first prospective study on ecstasy neurotoxicity comparing neuroimaging and neurocognitive assessments before and after first ecstasy use. It is therefore the most innovative substudy of the three and offers major methodological advantages over most previous studies. Assessment of main outcome parameters in both incident ecstasy users and persistent ecstasy-naive subjects will enable us to control for several potential confounding effects (use of drugs other than ecstasy, personality, lifestyle, and so forth). The sampling technique of subjects with high risk for first time ecstasy use will lead to enough incident cases of first ecstasy use with generally low exposure levels and a few cases with higher levels of ecstasy exposure. The interval measurements relatively soon after the first ecstasy use in a subgroup of 30 subjects make it possible to study the effects of a single or low-dose of ecstasy on the brain. Although the issue of single or low-dose use of ecstasy and its effects on the brain has received relatively little attention in research until now, this issue is highly relevant. Only 20% to 30% of the ecstasy users use ecstasy on a regular basis (CD > 25 lifetime) (Netherlands National Drug Monitor, 2003), while most ecstasy users do so at a low continuation rate and probably quit ecstasy use after a certain period of time. Moreover, there is a growing interest in the possible medical benefits of low-dose ecstasy administration in certain groups of patients. Recently, the Food and Drug Administration (FDA) of the United States has approved two pilot studies using ecstasy as a therapeutic agent. South Carolina researchers study the effects of ecstasy in 20 patients suffering from post-traumatic stress disorder (Check, 2004). In addition, Harvard researchers will study whether ecstasy can help terminally ill cancer patients by reducing their fears, pain, and stress (Bender, 2005). In this context, the importance of our substudy on the effects of a single or low dose ecstasy use is evident. On the other hand, because of the sampling technique of subjects with high risk for first time ecstasy use, this group will not be representative for all ecstasy users. Moreover, given the relatively short follow-up period (maximum 24 months) this cohort will not provide data on long-term ecstasy abstainers and subsequently this cohort will not provide answers to the course and (long-term) outcome of neurotoxicity in ecstasy-users.

To overcome these lacunae, the retrospective cohort substudy is performed with a representative sample of lifetime ecstasy users and a matched control group of ecstasy-naive individuals. The most important advance over previous studies is that the research population is more representative of general ecstasy use in the Western society than most previous studies that mainly involved heavy ecstasy users. Therefore, this substudy will provide optimal data on the potential public health consequences of ecstasy use in a Western society. Moreover, because these subjects were involved in a longitudinal cohort study from childhood, we are able to retrieve potential confounders from available data that were acquired prior to first use of ecstasy. The group of lifetime ecstasy users in this cohort will predominantly consist of experimental and low level recreational users. Given the age range of this cohort and the low continuation rate of ecstasy use, the majority of the ecstasy users in this cohort will have stopped the use of ecstasy years before the current assessment. As a result, this cohort is very suitable to study the long-term course and outcome of the various indicators of brain pathology and possible symptoms related to ecstasy use in the general population.

The combination of the three substudies assessing different samples with the same combination of neuroimaging, neuropsychological, and psychopathological instruments to study various indicators of neurotoxicity is needed to answer the research questions and obtain a comprehensive understanding of the use of ecstasy and its potential hazards. Because the same parameters are used in all three substudies this will improve the comparability of the different results which is essential for explaining and interpreting the results from the three substudies.

However, there will be some limitations involved. First, many potential confounders are involved in the effects of ecstasy on the brain. With the combined design of the three substudies we try to assess most of the known confounders, such as use of amphetamines,

cocaine, cannabis, alcohol, and tobacco, baseline serotonergic functioning, gender, age, demographics, gonadal hormones, serotonergic transporter genotype, and pre-existing psychiatric morbidity and cognitive dysfunctioning. However, sample sizes of the three substudies are probably too small to correct adequately for all of these confounders simultaneously, especially in both retrospective substudies. Moreover, as the cumulative doses of ecstasy used in the prospective and retrospective cohort substudies will be relatively low, the potential effects on the brain are probably smaller than in heavy users. Therefore, the samples sizes of 21 per group in the retrospective study, but even the relatively big sample sizes of about 50 per group in the prospective substudy might be relatively small for the purpose of detecting potential effects.

Inherent in the non-experimental approach is uncertainty about variances in dosage and purity of the ecstasy tablets taken by the subjects, although surveys in the Netherlands confirm that in 2002 95% of the tablets sold as ecstasy mainly contained MDMA or a related compound (MDA or MDEA) (Drugs Informatie en Monitoring Systeem, 2003). These percentages were even higher in 2003 and in 2004. There will also be some confounding introduced by biased sampling or poly-drug interactions, although the designs of the substudies are aimed to control for confounders as effectively as possible. Even in the prospective substudy it is possible that the incident ecstasy users are more likely to use other substances at baseline and at follow-up than the persistent ecstasy-naïves, although both groups are from the same baseline group, recruited in the same way, and both with the intention to use ecstasy in near future. Moreover, the environmental circumstances under which ecstasy was taken and the simultaneous use of other substances will be heterogeneous. As it is not ethical to provide ecstasy tablets to humans in an experimental setting, there is still a need for separate animal studies to study some of the aspects of ecstasy neurotoxicity (such as vulnerability and protective factors, and the risk of neurotoxicity when ecstasy is used in combination with other substances) in a controlled setting.

Conclusion

The NeXT study uses a combination of cross-sectional and longitudinal (retrospective and prospective) approaches and a combination of various imaging techniques, and neuropsychological and psychopathologic examinations to study the causality, course, and clinical relevance of potential ecstasy-related neurotoxicity in humans. The combined results on course and outcome of brain pathology and related symptomatology are expected to result in scientific knowledge that can be used in prevention messages, clinical decision making, and development of (inter)national ecstasy policy.

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