Functional MRI studies in human Ecstasy and cannabis users

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Functionele MRI studies in ecstasy- en cannabis gebruikers
(met een samenvatting in het nederlands)

Proefschrift

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"Words are, of course, the most powerful drug used by mankind"

Rudyard Kipling (1865-1936)

Voor Frank

Table of contents

Chapter 1
Introduction

1.1 General introduction

The aim of the research presented in this thesis is to increase the understanding of the long-term effects of two popular recreational drugs, ecstasy and cannabis on human cognitive brain function.

There is increasing evidence from animal studies[1, 2] and human studies[3-5] that ecstasy might be neurotoxic to serotonin axons in the brain. Serotonin is important for many physiological, psychological and cognitive processes, such as vasoconstriction, thermoregulation, regulation of sleep and mood, and memory and learning[6-8], so serotonergic dysfunction could potentially lead to serious functional sequelae. However, 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 yet been answered, mainly because of methodological limitations in 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[9-12]. The use of other substances, such as cannabis, amphetamines, cocaine, tobacco and alcohol, could especially be major confounders 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 dysfunction. In order to overcome the limitations in current ecstasy research and to provide answers to the remaining questions concerning causality, course and clinical relevance of the potential neurotoxicity of ecstasy, the Netherlands Research and Development Program on Substance Use and Addiction in 2001 supplied a grant for the Netherlands XTC Toxicity (NeXT) study. 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 provide better scientific knowledge regarding the neurotoxicity of ecstasy that can be used in prevention messages, clinical decision making, and the development of an (inter)-national evidence based ecstasy policy.

The studies presented in this thesis are part of the NeXT study and will focus entirely on the application of functional magnetic resonance imaging (fMRI) in studying the sustained effects of the use of ecstasy and its most commonly used co-drug cannabis on cognitive brain function.

1.2 Cannabis and Ecstasy

Background

Cannabis is a natural drug that has been used for over thousands of years across many cultures. It has been a drug employed in religious rites and as a medicine in the ancient Middle East. Much later, in the nineteenth century it reached a position of prominence within Western medicine, but then fell in disgrace in the twentieth century. Despite worldwide suppression, recreational cannabis use has gained enormous popularity since the 1960s and the drug has remained easily obtainable in most countries ever since[13]. In recent years, canna-

bis has been rediscovered as a drug with therapeutic potential in the treatment of a series of neurological and other diseases[14-16].

3,4-Methylenedioxymethamphetamine(MDMA), popularly known as "ecstasy" is a synthetic drug that produces hallucinogenic and stimulant effects. MDMA was patented by Merck in 1914 in Germany. It has been suggested that it was developed as an appetite suppressant, but other sources report it was tested by the US military as a drug for interrogation purposes. However, ecstasy was never marketed and although it was used as a therapeutic adjunct in psychotherapy in the US and in Switzerland, for the general public the drug went relatively unnoticed for many years until it became popular as a recreational drug at all-night dance parties (raves) in the mid 1980s. In recent years, although the popularity of the raves has declined, the use of ecstasy has shifted to nightclubs and discos, and also to private home gatherings with friends. Despite its illegality it remains the second most commonly used illicit drug, after cannabis, in the Western world[13]. Similar to cannabis, there is a current revival of interest in the possible medical applications of MDMA, for example as a therapeutic adjunct in patients with post-traumatic stress disorder or in the symptomatic treatment of last stage cancer patients to reduce anxiety and agitation[17, 18].

Cannabis and cognitive brain function

The main feature of recreational use of cannabis is that it produces a euphoric effect or 'high'. Accompanying the 'high', cannabis produces perceptual changes, cognitive impairment (mainly in learning and memory), motor disturbances, and anxiolytic, sedative, analgesic and psychedelic effects. However, cannabis can also produce dysphoric reactions, including severe anxiety and panic, and sometimes paranoia and psychosis.

In the 1960s the major active constituent was identified, delta-9-tetrahydrocannabinol or delta-9-THC, which lead to the discovery of specific endogenous CB-1 and CB-2 cannabinoid receptors on which cannabis exerts its effect. In turn, the presence of specific cannabinoid receptors led to the discovery of endogenous ligands (anandamide, 2-arachidonoyl glycerol and 2-arachidonyl glyceryl ether), indicating a whole central nervous signaling system of cannabinoid receptors, endogenous ligands and interactions with exogenous ligands such as cannabis[19, 20].

Despite the impressive number of studies on a broad variety of issues concerning cannabis use and the human brain, to date little is known about the long-term effects of cannabis use on cognitive brain function. Cognitive impairments of various types are readily demonstrable during acute cannabis intoxication, and there is a small but growing body of evidence indicating residual effects on memory, information processing, and executive functions. However, the lack of consistency in the results of different studies do not permit a decision yet as to whether long-lasting or permanent functional losses can result from chronic, heavy cannabis use[21]. In part, this is due to lack of control over a number of methodological variables that may affect the reliability, validity and clinical relevance of the results obtained. The most obvious

methodological challenges are small sample sizes, imperfectly controlled for the use of other substances than cannabis (e.g. alcohol or other stimulant or sedative drugs) and elevated rates of neuropsychiatric disorders in cannabis users. Other main methodological challenges to be faced are sufficient duration and time between last use of cannabis and assessment of cognitive brain function and questionable reliability of self-reported drug use histories and / or abstinence without confirmation by urine drug screening[22-24].

Ecstasy and cognitive brain function

The acute psychological effects of ecstasy include an increased emotional sensitivity and empathy, elevated self-confidence, reduced anxiety and heightened sensory awareness. Ecstasy users also consume the drug for its stimulant properties, which enables them to dance for hours at parties and nightclubs. Ecstasy is also known for its acute adverse effects, including depersonalization, cognitive disturbances, elevated anxiety, trismus and other acute and sometimes severe physiological problems such as elevated blood pressure and heart rate, hyperthermia, renal failure, intracranial haemorrhage or cerebral infarction. MDMA exerts its effect by raising synaptic levels of monoamines: it has major effects on serotonin (5-HT) pathways, but also affects the dopamine (DA) and norepinephrine (NE) neurotransmitter systems in the brain. MDMA causes an acute and rapid increase in extracellular 5-HT, but this results in a marked depletion of 5-HT in the hours following drug administration. Although the 5-HT levels recover within 24 hours after a single dose of MDMA, higher or repeated doses of MDMA

can cause sustained depletion of 5-HT, a decrease in the number of 5-HT transporter sites and receptors, as well as a loss of fine axons projecting from the dorsal raphe nucleus in the brain of several animal species, including non-human primates[19, 25]. Based on the latter findings, there has been concern about the neurotoxic potential of ecstasy almost from the start of its popularity as a recreational drug in the 1980s, because on the basis of these animal data it is likely that ecstasy might damage the axons of serotonin neurons in the human brain too. However, in humans, evidence for neurotoxicity is less convincing. Neuroimaging studies (PET, SPECT) using radiotracers that bind to serotonin transporters (SERT) at the axon terminals have reported reduced SERT densities in the brains of ecstasy users 3. However, these effects may be transient in most brain regions, as reversibility was shown in former ecstasy users[26].

Cognitive consequences of ecstasy use have been examined more extensively. Numerous cross-sectional studies reported impairments of learning and memory in moderate to heavy recreational users[8]. Little is known about the effects of ecstasy on the neural systems involved in cognition. Although there was a steep increase in the number of publications on the effects of ecstasy on the brain since the 1990s when modern non-invasive neuroimaging techniques became available, it was not until 2003 that the first fMRI study in ecstasy users appeared[27]. Since then, some work has been done on the sustained effects of heavy ecstasy use on working memory and episodic memory[28-32], but there is no suitable evidence yet available to permit a conclusive message concerning the effects of ecstasy on

brain activity patterns and specifically affected brain areas.

1.3 Functional Magnetic Resonance Imaging

Background

In the early nineties, the first studies were published reporting that activation of the brain could be visualized with an MRI scanner. It was demonstrated that the MR signal in the vicinity of blood vessels and in perfused brain tissue decreased with a decrease in blood oxygenation. Therefore, this technique was called 'Blood-Oxygen-Level Dependent' functional MRI (BOLD-fMRI), as it used the oxygenation level of blood as a natural agent for detecting brain activation[33, 34]. FMRI allows researchers in cognitive neuroscience to visualize brain activation in awake subjects with a moderate time resolution (i.e. seconds) and a high spatial resolution (i.e. millimeters) resulting in a precise mapping of brain activity. A big advantage of fMRI is that it is non-invasive (no need to inject radioactive ligands (PET, SPECT), allowing for repeated measures (i.e. more scans within one session, and multiple scan sessions). Another important advantage of fMRI in examining cognitive brain function is the powerful combination of behavioral and brain activation measures. More specifically, fMRI can reveal abnormalities in the organization of brain networks involved in cognitive processing, which may occur as an adaptive (compensatory) response to brain damage and which may be difficult to detect in behavior. However, there are some drawbacks as well. First, subjects are not allowed to move their body and especially their head during the scan session. Movement, although partially corrected for through re-alignment of the functional time-series, can dramatically affect the outcome of the statistical analysis and can result in activation artifacts. The restriction of movement is not only unpleasant for the subject, it also limits the type of paradigms suitable for fMRI, as subjects are not free to act out activities often involved in cognitive testing, notably giving a verbal or a manual response.

How does BOLD-fMRI work?

Functional MRI images are obtained using an MRI scanner, which is basically a large magnet. The strength of the magnet generally varies from 1.5 Tesla (all scans for the research presented in this thesis were made on a 1.5 Tesla scanner) to 3.0 Tesla (rapidly becoming the standard in neuroscience) or even 7.0 Tesla (the next upgrade aimed at). The acquisition of scans typically involves continuous series of scans, each lasting between one and a few seconds and covering much or all of the brain. A scan consists of several thousand data points, each of which is derived from a cube of brain tissue (also called a voxel). The series of scans is stored as a time-series of 3D volumes, where each voxel is associated with a series of intensity values (the fMRI-signal). The basis of the signal originates in protons. When a subject is placed inside the scanner (i.e. inside the magnetic field), a slight minority of all the protons will align with the field (B_0). The signal measured by an MRI scanner is based on the emission of electromagnetic radiation from the nuclei of these protons (hydrogen atoms), which are excited by a radio frequency (RF) pulse. After excitation, the protons will fall back into their normal state.

The scan type, i.e. the type of pulse sequence, determines the particular set of factors that affect the basis of the signal. Some sequences are differentially sensitive to the type of tissue the protons are in, for instance gray matter, white matter, cerebrospinal fluid or blood. In fMRI, the pulse sequence is sensitive to blood dynamics, i.e. blood flow, blood volume and oxygenation state. Transient changes in blood dynamics affect the fMRI-signal, which can be detected by a receiver coil placed as close as possible to the head of the subject. Thus, the essence of fMRI is that it enables visualization and measurement of transient changes in blood dynamics in the brain[35].

Neuronal activity and BOLD-fMRI

In general, fMRI images are referred to as images representing brain activation. Many studies have shown that fMRI yields activation patterns that agree with what is known from human and animal studies about functional topography. Therefore, fMRI is now the main tool to visualize 'the brain in action', i.e. to localize and assess cerebral cognitive functions such as memory and attention[35, 36]. However, in interpreting fMRI results it is important to realize that BOLD-fMRI does not allow for absolute measurement of neuronal activity but actually measures relative changes in the distribution of (de)oxygenated blood. Basically, the mechanism can be described as follows: changes in brain activation are accompanied by changes in blood flow, causing the oxygen level in the blood to rise in brain region concerned. As oxygenated hemoglobin is diamagnetic, i.e. it exerts little effect on the regional magnetic field, and deoxygenated blood is paramagnetic, i.e. it disturbs the regional magnetic field, the changes in relative levels of oxygen in the blood can be effectively measured with fMRI.[37]. However, the exact relationship between neuronal activity and the fMRI signal, also known as neurovascular coupling, is quite complicated, involving multiple vascular, metabolic and neuronal processes, some of which are even now not well understood[38]. Still, our knowledge of the relationship between the fMRI signal and the underlying neural activity has substantially increased in recent years, thanks to the work of researchers like Logothetis et al.[39-40]. Logothetis experiments involved a new method of simultaneous recordings of the fMRI signal and various measures of the electrical activity of neurons in monkeys during visual stimulation (viewing checkerboard patterns). With their electrical recording techniques the researchers were able to distinguish between several aspects of neuronal activity, i.e. action potentials and local field potentials (LFP). Action potentials are the all-or-nothing firing rates of neurons which occur immediately after stimulus presentation and reflect neuronal output, whereas LFP are the more slowly varying gradient potentials that arise from the input to the dendrites of neurons and are associated with local information processing. Logothetis results showed that the fMRI signal was mainly determined by the local field potentials, meaning that brain activation as measured by fMRI, predominantly reflects the input to a brain area rather than the output from the area. This pioneering work has had major implications for the interpretation of fMRI. The good news was that one can with greater confidence ascribe the BOLD signal to a change in local field potentials in post-

synaptic neurons. Thus, the fMRI signal represents definable alterations in neuronal activity. However, Logothetis results also held a warning for cognitive neuroscientists: the signal-to-noise ratios of neural recordings directly from the brain were much greater than the accompanying BOLD signal. This implicates that the absence of an fMRI signal does not necessarily mean that no neural activity is going on in a particular brain area[36, 39-40].

1.4 Research questions and hypotheses

In line with the larger NeXT project, the main objectives of the fMRI studies presented in this thesis were to study 1) the causal role of ecstasy in observed brain pathology in humans; 2) the long-term course of such brain pathology; and 3) the clinical relevance of reported brain pathology in ecstasy users. An important fourth objective was to study the neurotoxic consequences of ecstasy use in relation to the use of other drugs such as cannabis, amphetamine, cocaine, alcohol and tobacco. Cannabis was of primary interest, because cannabis is the most commonly used co-drug in ecstasy users, and thus an important confounder in ecstasy research.

The first question on the causality of ecstasy use and observed brain pathology is addressed in the prospective cohort study in novice ecstasy users and persistent ecstasy-naive controls. Novice users were examined before and after first ecstasy use on cognitive brain function in three cognitive domains, i.e. working memory, selective attention and long-term memory, with fMRI, and were compared with

controls, also measured twice and matched for gender, age, verbal IQ and cannabis use with the novice users. Assuming that the reported changes in cognitive function in ecstasy users are a consequence of, instead of predating, the use of ecstasy, we hypothesized an interaction between test session (baseline, follow-up) and group (novice users, persistent ecstasy-naive controls). It was expected that novice users would show alterations in cognitive brain function after a first episode of ecstasy use, compared to the controls, whereas both groups would not differ during baseline measurements. Because the cumulative dose of ecstasy in the novice users would be relatively low and because the cognitive deficits reported in heavy ecstasy users are relatively mild, although significant, it seemed unlikely that task performance would be reduced in incident users. However, effects of low dose ecstasy could be reflected by adaptive or compensatory changes in brain activity patterns.

The second question on the long-term course of observed brain pathology in ecstasy users is addressed in the retrospective cohort sub-study in a representative sample of lifetime users and matched controls. We expected that lifetime users would vary on cumulative dose, duration and frequency of ecstasy use and time since last ecstasy use. Control subjects would be matched as good as possible with the lifetime users on possible confounding factors such as gender, age, educational level, use of other substances and psychopathology. After matching, we hypothesized that potential abnormalities in cognitive brain function in lifetime users compared to controls would be in part explained by the variation on ecstasy

use parameters. For example, if negative effects of ecstasy use on cognitive brain function (performance and/or brain activity patterns) are (in part) reversible after cessation of the drug, one can expect an inverse relationship between the magnitude of the effects of ecstasy and time since last use.

In order to estimate the clinical relevance of reported brain pathology in ecstasy users we studied functional consequences for cognitive brain function of ecstasy-induced damage to the serotonergic system in the human brain. To clarify, some reported findings in ecstasy users, such as changes in serotonin transporter densities or changes in cerebral perfusion, may not reflect long-term damage but only transient effects of the use of the drug without functional sequelae. Although the term clinical relevance is somewhat ambiguous, we expected clinically relevant effects on cognitive brain function to be expressed in significant effects of ecstasy use both at the level of behavior (poorer task performance out of the normal range) and at the neurophysiological level (abnormal patterns of brain activity).

Concerning our fourth objective, to study the specific effects of ecstasy on cognitive brain function in the context of poly-substance use, we hypothesized that after adjustment for the confounding use of other drugs, mainly cannabis, amphetamine, cocaine, alcohol and tobacco, there would still be statistically significant effects of ecstasy use on cognitive brain function, especially in heavy users.

With regard to the selective effects of cannabis use on cognitive brain function, our hypotheses were less specific. The previously reported effects on cognition and cognitive brain function are less consistent than with ecstasy. The most consistent sustained effect of frequent cannabis use on cognitive function is mild impairment of memory and learning. Based on this we hypothesized that if cannabis use would affect cognitive brain function measured with fMRI, it would most likely surface in the associative memory task. As the reported cognitive deficits are subtle, even in very heavy cannabis users, we did not expect task performance to be reduced, but we hypothesized alterations in brain activity patterns in the associative memory system, especially in the (para)hippocampal areas, which appear to be a likely target due to its high density of CB-1 receptors.

1.5 Outline of the thesis.

Chapter 2 describes the Netherlands XTC Toxicity (NeXT) study, adapted from a special design paper[41]. In Chapter 2.1 and 2.2 the background, objectives and general design of the NeXT study are explained. Chapter 2.3 gives an introduction to the fMRI studies that were part of the NeXT study: 1) a cross-sectional sub-study on the sustained effects of cannabis (the most commonly used co-drug in recreational ecstasy users and therefore an important confounder in ecstasy research) on cognitive brain function; 2) a cross-sectional sub-study among heavy poly-substance ecstasy users to specify the specific effects of ecstasy on cognitive brain function in the context of poly-drug use; 3) a prospective cohort study in novice ecstasy users to

study the causality question and to determine the effects of relatively low cumulative dosages of ecstasy on cognitive brain function; and 4) a retrospective cohort study in a representative sample of lifetime ecstasy users and a matched control group (selected from the longitudinal 'Zuid-Holland study') to study the course and the clinical relevance of the potential neurotoxicity of ecstasy. Unfortunately, there was some delay in recruitment and data-acquisition with regard to the retrospective cohort study. Therefore, no chapter in the current thesis is dedicated to the results of the retrospective cohort study, but findings will be published later.

Chapter 2.4 gives an overview of the fMRI task paradigms that were used in the different sub-studies.

The cross-sectional study on the sustained effects of cannabis on cognitive brain function resulted in two papers on the effects of frequent cannabis use on working memory and attention and the specific effects of frequent cannabis use on associative memory respectively. These studies are reported in Chapters 3 and 4. Then, switching the focus to ecstasy, Chapter 5 describes the cross-sectional fMRI study in heavy poly-substance ecstasy users regarding the questions on course and clinical relevance. Chapter 6 presents the results of the prospective fMRI study on the effects of low dose ecstasy use on cognitive brain function in novice ecstasy users regarding the issue of causality. Finally, chapter 7 provides with a summary and general discussion.

Chapter 2
The Netherlands XTC
Toxicity (NeXT) study

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2.1 Background

Despite the vastly growing number of studies 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 yet been answered. Research in this area suffers from methodological problems for which there are no easy solutions. Most human studies contend with interference of many potential confounders, such as poly-substance use, inadequate sampling of subjects and controls, small samples, lack of drug-use analysis, short follow up periods, and the lack of baseline data[10,12,42].

For one, recreational ecstasy users are characterized by a large heterogeneity. Experienced ecstasy users not only consume more ecstasy than novice users but they are also more likely to consume other drugs such as cannabis, amphetamine, cocaine, LSD and psilocybin (mushrooms)[43]. Consequently, cumulative ecstasy use almost invariably correlates highly with use of other drugs, making it almost impossible to differentiate between effects of ecstasy and of the other drugs. Indeed, some studies have shown that indicators of neurotoxicity in ecstasy users may well be associated with poly-drug use in general or with the use of drugs other than ecstasy such as cannabis and amphetamine[44].

A second important issue is the lack of baseline data, which 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 using cross-sectional studies with retrospective assessment of ecstasy use and potential confounders. However, this leaves the possibility that observed differences between ecstasy users and controls might actually predate and place individuals at risk for drug abuse rather than being the result of abuse.

Third, little is known about the functional consequences and the clinical relevance of observed serotonergic changes in the human brain. Functional abnormalities seen in ecstasy users include cognitive impairment, mainly memory disturbance, depression, impulsivity, and other neuropsychiatric disorders in which brain serotonin has been implicated[11,45-48].

Therefore, it is not only important to study the effects of ecstasy on serotonergic neurons and their axons, but also to study the potential functional consequences related to damage of these axons.

Finally, our understanding of dose-response characteristics and vulnerability factors that may predispose some individuals to experience more negative effects following ecstasy use is limited. For example, it is important to know 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[49, 50], while at the same time others advocate the controlled use of MDMA as a therapeutic adjuvant for psychotherapy[17]. Furthermore, it has been suggested that time intervals between subsequent ecstasy exposures, circumstances during ecstasy use (e.g. heat, noise, dehydration, exhaustion, stress)[51] and the combination with other substances (e.g. alcohol, cannabis,

amphetamines)[29, 44, 52-54] might be modifiers of ecstasy-induced brain damage. Moreover, there are presumably important biological and psychobiological risk factors such as age, gender, neurotransmitter polymorphisms, 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[55].

In 2001 the Netherlands Research and Development Program on Substance Use and Addiction supplied a grant for The Netherlands XTC Toxicity (NeXT) study. The purpose of this research project was to extend our knowledge on the issues described in the previous paragraphs, and to find answers to (at least some) questions concerning the effects of recreational ecstasy use on brain and brain function.

2.2 General design of the NeXT study

Only a longitudinal prospective study of brain 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 questionable. Therefore, the current project studied causality, course, and outcome of various indicators of brain pathology (e.g. neuroimaging), and possibly related clinically relevant symptoms (e.g. neurocognitive and psychiatric symptoms and disorders) of ecstasy neurotoxicity in a combination of three sub-studies. The study includes

(1) a cross-sectional sub-study in heavy ecstasy users and controls with variation in amount and type of drug use that provides information on potential neurotoxic consequences of ecstasy use in the context of poly-substance use, (2) a prospective cohort sub-study in ecstasy-naive volunteers with a high risk for future first ecstasy use that gains new knowledge about the causality and short-term course of ecstasy use and potential neurotoxicity, especially for low exposure levels, and (3) a retrospective (historical) cohort sub-study 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 sub-studies will provide us with the highest possible level of certainty regarding the neurotoxicity of ecstasy in humans. Because the most common other psychoactive drug consumed by recreational ecstasy users is cannabis, we extended the NeXT study with an fMRI sub-study on the specific effects of cannabis on cognitive brain function.

The NeXT research program is a joint venture of the Academic Medical Center of the University of Amsterdam, the Bonger Institute of Criminology of the University of Amsterdam and the University Medical Center of the University of Utrecht (The Netherlands), and is expected to result in more than twenty scientific papers: part of them already published, the others submitted or in preparation. Together, these studies will yield four dissertations between 2006 and 2008, of which the present thesis on functional MRI studies is the first.

2.3 Functional MRI studies

Cross-sectional sub-study among moderate to frequent cannabis users

The main objective of the cross-sectional fMRI study among cannabis users is to provide information on the specific sustained effects of cannabis use on cognitive brain function. This is of interest because cannabis is the most commonly used co-drug in recreational ecstasy users and therefore is a potential confounder in ecstasy research. The non-acute effects of cannabis on cognitive brain function were retrospectively investigated with fMRI. We compared moderate to frequent cannabis users who were abstinent for at least one week with drug-naive control subjects on working memory and attention (10 users, 10 controls), and associative memory (20 users, 20 controls; including the users and controls from the study on working memory and attention).

Cross-sectional sub-study among heavy ecstasy users

The two main objectives of the cross-sectional fMRI study among heavy ecstasy users were (a) to specify potential neurotoxic consequences of ecstasy use in the context of poly-substance use and (b) to validate the fMRI technique for ecstasy research, which to date has been used in only very few studies. The specific effects of heavy ecstasy use on cognitive brain function were investigated with a retrospective assessment of drug use history and by comparing behavioral and brain activity outcomes in a carefully composed stratified sample of 71 subjects with substantial variation in the amount and type of drugs that were used. Multiple regression analysis with ecstasy and other drugs as separate regressors was applied to investigate the specific effects of ecstasy on three domains of cognitive functioning: working memory, selective attention and associative memory using fMRI. Overall, subjects could be classified according to four different profiles: (1) heavy ecstasy users (both poly-drug users and selective ecstasy users); (2) poly-drug users with a history of heavy amphetamine, cocaine and/or cannabis use but very limited use of ecstasy; (3) ecstasy-naive cannabis users; and (4) drug-naive controls. Some of the cannabis and drug-naive controls were age-matched subjects taken from the baseline population of the prospective cohort sub-study (see below).

Prospective cohort sub-study

To study the causal nature of ecstasy use on neuroimaging, cognitive, 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 (18-35 years of age) with a relatively high probability to start using ecstasy in the near future was followed during a period of 12 - 24 months. Of these 188 volunteers, 96 participated in the fMRI part of the research program. 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 website on the project and an internet campaign; and word-of-mouth recruiting. Main criteria for inclusion were intention to probably or certainly use ecstasy for the first time in the near future and/or having one or more friends who already used ecstasy.

After baseline examination subjects had to complete questionnaires sent to them by mail about their drug use every three months during a follow-up period of one year. Besides assessing drug use through these questionnaires, fMRI outcome parameters (performance and brain activity data) were assessed up to three times: (1) directly following recruitment, i.e. before first ecstasy use (N=96); (2) as soon as possible following first ecstasy use in the incident ecstasy users (N=27); (3) between 12 and 24 months after baseline assessment in incident ecstasy users (N=18), and in a control group (N=24) of persistent ecstasy-naive subjects (matched on gender, age, IQ and cannabis use with the novice ecstasy users) from the initial cohort of N=96. To study whether a low dose of ecstasy affects cognitive brain function, changes in fMRI outcome parameters were compared between the incident ecstasy users soon after their first ecstasy use and a matched group of persistent ecstasy-naive subjects.

Retrospective cohort sub-study
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 sub-study. The participants of this sub-study are selected from the longitudinal 'Zuid-Holland study'. This study started in 1983 with 2600 subjects of Dutch nationality, aged 4 - 16 years (birth cohorts 1967 - 1979), randomly selected from the municipal registers from the Dutch province of Zuid-Holland, with both urbanized and rural areas. Of these, 2076

(84%) participated in the first measurement in 1983[56]. Since then the sample was reassessed five times, most recently in 1997[57] when 1578 subjects still participated (76.0% of the original sample of 2076). Of these 1578 subjects 98 indicated in 1997 during psychiatric assessment with the Composite International Diagnostic Interview[58] 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. Matching variables included age, gender, use of cannabis, and internalizing (e.g. anxiety, depression) or externalizing (e.g. conduct disorder, ADHD) problems.

FMRI scanning and cognitive testing in these groups started in May 2003 and was finished by the end of 2005. To assess whether lifetime ecstasy users of a representative sample differ on indicators of neurotoxicity 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 analyzed to study the course and dose-response relationship of potential ecstasy-induced detrimental effects on cognitive brain function.

The retrospective cohort study is currently in the data-analysis stage. Results will therefore not be presented in the current thesis but will be published later.

2.4 Assessment of working memory, attention and associative memory with fMRI

Changes in cognitive performance and/or brain activation patterns measured with fMRI in human ecstasy and/or cannabis users can be interpreted as reflecting functional consequences of the neurotoxic effects of these drugs. The cognitive domains of interest, i.e. working memory, attention and long-term episodic memory, were selected based on findings reported in neuropsychological literature[8, 46, 59, 60]. FMRI paradigms were designed to assess behavioral performance and brain activity in these domains, i.e. working memory, selective attention and episodic associative memory. All three tasks are described in detail and illustrated in chapters 3 – 6. A concise description is given below:

1. Working memory was assessed using a modified Sternberg item recognition task (denoted STERN): This task assesses capacity and flexibility of the working memory system and has two parts, i.e. a training session to accomplish automatization of cognitive processing involving working memory, and an fMRI scanning session to acquire neurophysiological measures of working memory. The task involves memorizing sets of consonants, and deciding whether subsequently presented letters belong to the set or not. The task has two versions, i.e. a practiced version (trained prior to scanning) with a fixed set and a novel version with a variable set. In healthy volunteers cognitive processing during this task consistently activates a well-defined fronto-parietal network of brain regions[61, 62].

2. Selective attention was measured using a selective attention task (denoted SAT). The SAT is a visuo-auditory oddball detection task. It involves detection of tones with a higher or lower pitch than a baseline tone, and similarly, detection of dots with a larger or smaller size than a baseline dot. A threshold for detecting differences in pitch and dot-size was determined individually before the scan session, by adjusting it until the subject detected 80% of the deviant stimuli. Stimulus-invoked changes in brain activity during tone or dot detection reflects a robust measure for selective attention. In addition, the parametric characteristics of the task allow for examination of the dynamics within the attention system, as the difference between tone - and dot detection reflects the ability to switch attention from the auditory to the visual modality and vice versa.

3. Associative memory was assessed using a pictorial memory fMRI-paradigm (denoted PMT) that involves three tasks. First, an associative learning phase is conducted which requires subjects to remember a specific combination of pictures and to establish a meaningful connection between the two pictures. In the next phase simple pictures have to be judged, which serves as a control task. Finally, in a retrieval phase, subjects have to retrieve specific combinations previously presented during associative learning. In healthy volunteers this task reliably reveals brain activity in the hippocampus and the (para)-hippocampal gyrus bilaterally, especially during the associative learning condition[63].

Chapter 3
Long-term effects of frequent cannabis use on working memory and attention: an fMRI study

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Abstract

Rationale

Excessive use of cannabis may have long-term effects on cognitive abilities. Mild impairments have been found in several cognitive domains, in particular in memory and attention. It is not clear, however, whether these effects also occur with moderate, recreational use of cannabis. Furthermore, little is known about underlying brain correlates.

Objectives

To assess brain function in frequent but relatively moderate cannabis users in the domains of working memory and selective attention.

Methods

Functional magnetic resonance imaging (fMRI) was used to examine verbal working memory and visuo-auditory selective attention in 10 frequent cannabis users (after one week of abstinence) and 10 non-using healthy controls. Groups were similar in age, gender and estimated IQ.

Results

Cannabis users and controls performed equally well during the working memory task and the selective attention task. Furthermore, cannabis users did not differ from controls in terms of overall patterns of brain activity in the regions involved in these cognitive functions. However, for working memory, a more specific region-of-interest analysis showed that, in comparison to the controls, cannabis users displayed a significant alteration in brain activity in the left superior parietal cortex.

Conclusion

No evidence was found for long-term deficits in working memory and selective attention in frequent cannabis users after one week of abstinence. Nonetheless, frequent cannabis use may affect brain function, as indicated by altered neurophysiological dynamics in the left superior parietal cortex during working memory processing.

Introduction

Except for alcohol and tobacco, cannabis is the most widely used psychoactive substance in the western world[64]. Acute effects of cannabis include disruption of episodic memory, learning, working memory and attention[65, 66]. In addition, cannabis consumption induces acute deficits in motor skills, verbal expression, mathematical abilities and alterations in time perception[67, 68]. Whether cannabis causes consistent long-lasting changes in cognitive functions, is less clear. Studies on long-term effects of cannabis on cognition have failed to find proof of gross abnormalities, but there is some evidence for mild cognitive impairments, in particular in the domain of attention, memory and executive functions[66, 67, 69-74]. In a recent meta-analysis effect sizes were estimated for the potential long-term effects of cannabis use on eight cognitive domains (simple reaction time, attention, verbal/language, executive functioning, perceptual motor, simple motor, learning and forgetting/retrieval). With the exception of learning and forgetting, no statistically reliable deficits were observed, but even in the statistically significant domain the average effect sizes were small (-.21, -.27)[59].

More recently, functional imaging techniques such as PET and fMRI have also been employed to study the effects of cannabis on cognition and brain function. Two PET-studies on executive functioning in abstinent heavy cannabis users reported persistent alterations in brain activity. On a modified Stroop task, 25-days abstinent cannabis users showed hypoactivity in the left perigenual anterior cingulate cortex (ACC) and the left lateral prefrontal cortex (l-LPFC) and hyperactivity in the hippocampus bilaterally[75]. Bolla et al.[76] found hy-peractivity in the left cerebellum and hypoactivity in the right lateral orbitofrontal cortex (r-OFC) and the right dorsolateral prefrontal cortex (r-DLPFC) in a group of 28-days abstinent heavy cannabis users, while performing a decision-making task (Iowa Gambling Task). An fMRI study on spatial working memory indicated an association between heavy cannabis use (lifetime use: mean 19,200 joints; range 5,100 – 54,000 joints) and increased activation in the prefrontal and anterior cingulate cortex. Furthermore, the heavy cannabis users showed more widespread brain activity, as they recruited additional regions in the basal ganglia. Increased brain activity may reflect an increase in required neurophysiological effort, i.e. having to "work harder" to overcome subtle neurophysiological deficits[77]. A second fMRI study[78] compared adolescent cannabis users (number of days of cannabis use: mean 282 days; range 24 – 1460 days) with two groups of controls (tobacco smokers and non smokers) on a sustained attention task and a working memory task. Adolescent cannabis users performed less accurately than controls during both tasks. Across working memory task conditions (two levels task difficulty) adolescent cannabis users failed to deactivate the right hippocampus, as opposed to both control groups.

While these studies are indicative of persistent, residual effects of excessive cannabis use on neurocognitive functioning, there are some limitations and unanswered questions. First, few studies take acute pharmacological effects and withdrawal into account. Secondly, there is a tendency in cannabis research to focus on extremely heavy users (smoking several joints per day, over periods of many years).

Impairments found in this group may not be fully representative for the majority of recreational cannabis users, who are characterized by less frequent and shorter-duration use (i.e., once per week or even less, for a period of months to a few years only)[64, 79]. Also, there is currently a growing interest in the possible medical applications of cannabis. For example as a treatment for multiple sclerosis-related symptoms such as muscle spasm and tremor, as an appetite stimulant or anti-emetic agent in cancer patients, or as an analgesic in patients with neuropathic pain disorders[14-16, 80]. Therefore, a better understanding of the long-term behavioral and neurophysiological effects of moderate and controlled use of cannabis is of great importance to make informed decisions about its use.

In this paper we investigated whether frequent but relatively moderate cannabis users displayed impairments in selective attention and/or working memory after one week of abstinence, both in terms of performance and brain function. Two fMRI paradigm were used guided by their ability to invoke reliable activity in networks of regions involved in working memory and attention (specifically the dorsolateral prefrontal and parietal regions, and the anterior cingulate cortex) and by proven efficacy in yielding reliable maps of brain activity in individual subjects[61, 62]. Based on previous observations we anticipated several possible outcomes. The first possibility is that both task performance and brain activity are impaired in chronic cannabis users. This could then be explained by mild damage to (part of) the working memory and/or attention system, though serious enough to lead to reduced functioning. A second possibility is that performance in cannabis users is within normal range, but the brain activity data are different from the non-using controls. Differences in brain activity may take two forms. Cannabis users may activate the involved brain regions in a similar way as the controls, but normal performance levels are obtained at 'a higher cost'. In this case, we expect an increase in activity in the regions involved in working memory and attention.

Alternatively, differences in brain activity between cannabis users and controls may pertain to changes in relative levels of activity in the networks, where intact brain regions 'compensate' for compromised information processing in other regions.

Methods

Subjects

Ten subjects who frequently used cannabis (lifetime use: median 1,300 joints; range 675 – 5,400 joints, last year use: median 350 joints; range 75 – 900 joints) were compared to ten (almost) non-using healthy control subjects (lifetime use: median 2 joints; range 0 – 15 joints, last year use: median 0; range 0 - 4). To minimize differences in lifestyle, control subjects were recruited at the same locations as the cannabis users. Moreover, six out of ten control subjects had incidentally experimented with cannabis use, but their lifetime exposure to cannabis was negligible in comparison with the cannabis users. All Subjects participated in a larger research project "NeXT" (The Netherlands XTC Toxicity study)[41]. Participants were recruited through advertisements on the

internet, at locations such as 'coffee shops' where people can buy cannabis products, universities and colleges, and through word of mouth. The groups did not differ in age, gender or estimated IQ (see Table 1). Exclusion criteria were: (1) major medical, neurological, and neuropsychiatric disorders; (2) current use of psychotropic medications; (3) pregnancy; (4) the use of intravenous drugs; (5) left-handedness and (6) contraindications for MRI. Written informed consent was obtained from all participants. All procedures were performed with approval of the ethical committee from the University Medical Center Utrecht. Drug - and alcohol use were assessed by self-report questionnaires, and the Substance Abuse Scales from the M.I.N.I. Plus, Mini International

statistically non-significant) alcohol consumption than the control subjects. Lifetime exposure to substances other than cannabis, alcohol or tobacco, was negligible in both groups (Table 1). Cannabis using subjects were currently smoking 2 – 17 (median 7) joints per week at the time of entry into the study. To exclude acute pharmacological effects of cannabis use on the main outcome parameters, subjects had to abstain from drug use, including alcohol, for at least one week prior to testing. This was checked by urine drug screening (enzyme-multiplied immunoassay for amphetamine, ecstasy, opiates, cocaine, benzodiazepine, cannabis and alcohol (Jellinek Laboratory, Amsterdam, The Netherlands). Negative urine drug screening served as objective support for self-reported abstinence.

Table 1 Demographic features and patterns of use of psychoactive substances in cannabis users and controls

	Cannabis users N=10	Control subjects N=10	p-value[a]
Male/female , N	7/3	7/3	
Age (years), mean (SD)	22.7 (4.2)	22.8 (2.9)	ns
IQ (DART-score), mean (SD)	104.9 (8.3)	106.1 (6.1)	ns
Cannabis use lifetime (nr of joints), median (range)	1300 (675 – 5400)	2 (0 – 15)	0.000
Cannabis use last year (nr of joints), median (range)	350 (75 – 900)	0 (0 – 4)	0.000
Duration of cannabis use (years), mean (SD)	7.1 (3.9)	0 (0)	
Tobacco smoking (nr of cigarettes/week), median (range)	8 (0 - 100)	0 (0 - 1)	0.055
Alcohol consumption (nr of units/week), median (range)	17 (7 - 40)	7 (0 -25)	ns
MDMA use lifetime (nr of pills), median (range)	0 (0)	0 (0)	-
Amphetamine use lifetime (nr of occasions), median (range)	0 (0 – 2)	0 (0)	ns
Cocaine use lifetime (nr of occasions), median (range	0 (0 – 5)	0 (0)	ns

[a]Significance of differences calculated using t-tests and nonparametric Kolmogorov-Smirnov Z test, two-tailed.
ns = non-significant

Neuropsychiatric Interview for DSM IV clinical disorders (Translated Dutch version 5.0.0[81]). On average, consumption of tobacco was significantly higher in cannabis users than in controls. Cannabis users also reported greater (but

Assessment of Working Memory and Selective Attention
Two fMRI tasks were administered: a modified Sternberg item recognition task (denoted STERN) and a visuo-auditory selective attention

task (denoted SAT). The STERN task has the following basic format (see also[61, 62]): a set of five consonants is shown for 5340 ms (the target-set). After this a series of ten consonants is displayed in sequence (Figure 1). A new set of five consonants is then shown, followed by ten new trials presented with an interval of 2670 ms. Subjects are instructed to memorize the target-set, and subsequently press a button as quickly as possible when a consonant belongs to the target-set (50% were targets). Two experimental tasks were administered, which differed only with regard to the target-set(s): a novel set and a practiced set. In the practiced set task (PT) the same set was used repeatedly. In the novel set task (NT), the composition of the target-set was changed after every run of 10 trials. The target-set and set of non-targets for the NT were chosen from the ten remaining consonants that were not used for the PT. During the training-session, which lasted 21 minutes, only the PT was presented in 5 series of 100 stimuli. In the scanner, both tasks were presented in 8 epochs of 10 stimuli each. Each epoch lasted approximately 30 sec. Also, an additional reaction time control task (CT, same duration and number of epochs and stimuli) was included where subjects had to press the button as fast as possible when the symbol '< >' appeared, as well as 8 rest periods of equal epoch duration. The sequence of the three tasks and rest periods was randomized. Reaction time for all correctly identified targets and accuracy for all stimuli were recorded. The tasks contained equal numbers of targets.

The second task SAT is a visuo-auditory odd-ball detection task. It involves detection of tones with a higher or lower pitch than a base-line tone, and similarly, detection of dots with a larger or smaller size than a baseline dot. A threshold for detecting differences in pitch and dot-size was determined individually before the scan session, by adjusting it until the subject detected 80% of the deviant stimuli. The 200 ms tones and dots (both series of stimuli contained 20% deviants randomly distributed) were presented simultaneously with a variable inter-stimulus interval (mean 1.0 s). Tones and dots were presented in 16 epochs of 29 s each. Epochs differed only with regard to the task instruction. At the start of each epoch, subjects were instructed to attend either to the tones while ignoring the dots (TO), or to the dots and to ignore the tones (DO). Also, 8 rest periods (RS) of equal epoch duration were included. The sequence of the two tasks and rest periods was randomized. Accuracy for all correctly identified deviants (hits) and incorrect responses for all other stimuli (intrusions) was recorded.
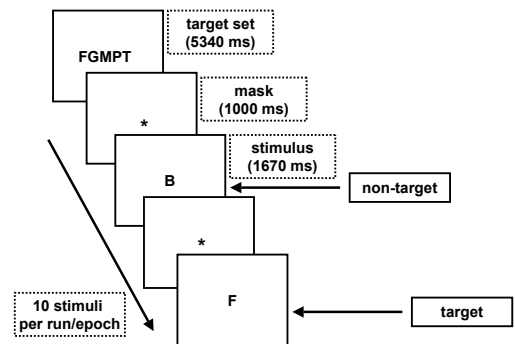

Figure 1 The temporal sequence of events is shown for the STERN task. Each epoch starts with presentation of the target-set, and is followed by ten trials. Subjects have to press a button as fast as possible, if the letter belongs to the target-set. Details are described in the Methods section.

Procedure

Practice session

During a practice session before scanning, subjects were given instructions for the tasks, were trained on the PT from the STERN for 21 min[61, 62, 103] and detection thresholds for deviants were established for SAT. In addition, alcohol and drug use questionnaires and the M.I.N.I Plus Interview were administered, and urine samples were collected.

FMRI data acquisition

To minimize head movement during the scan session, subjects were fixated by a strap around their forehead and by the use of foam padding. The computerized tasks were projected onto a through-projection screen positioned near the feet and visible to the subjects via a mirror attached to the head coil. Subjects wore a set of headphones, connected to an audio set outside the scanner room. Subjects responded by pressing button(s) with their right thumb on an air-pressure button box device. For fMRI a standard scan protocol was used (navigated 3D PRESTO[82] on a clinical 1.5 Tesla scanner. For the STERN task a single run of 384 scans was acquired over a period of 18 minutes. Functional images were obtained with the following parameter settings: FOV 208 x 256 x 120, TR 24.5 ms, TE 35.5 ms, flip angle 10.5°, voxelsize 4 mm isotropic, matrix 52 x 64 x 30, scantime 2800 ms (transaxial orientation). Each epoch spanned 12 scans and lasted 33.6 s. This was followed by one volume with a flip angle of 30° for registration purposes, and a 3D anatomical scan for spatial localization (FOV 256 x 256, TR 30 ms, TE 4.6 ms, matrix 128 x 128 x 130, flip angle 30°, slice thickness 1.2 mm, duration 7 minutes). For the SAT task a single run of 360 scans was acquired over a period of 12 min-utes. Functional images were obtained with the following parameter settings: FOV 208 x 256 x 88, TR 23.7 ms, TE 35.7 ms, flip angle 10.5°, voxelsize 4 mm isotropic, matrix 52 x 64 x 22, scantime 2000 ms (transaxial orientation. Each epoch spanned 15 scans and lasted 30 s. One volume was acquired with a flip angle of 30° for registration purposes.

Data analysis

Performance data

Outcome measures included performance accuracy (for STERN and SAT) and reaction times (STERN only). Accuracy of STERN performance was assessed by computing percent of targets correctly identified (percent hits) and percent of non-targets incorrectly identified as targets (percent false alarms). Accuracy of SAT was assessed by dividing the number of correctly identified deviants by the total number of trials (percent correct). To test for effects of group (being a cannabis user or nonuser), repeated measures analysis was applied with task condition, accuracy and reaction times as within-subject factors. T-tests were used to test for differences in threshold values (detecting 80% of the deviant stimuli during TO and DO respectively) between users and non-users.

FMRI data

After reconstruction, functional and anatomical data were processed off-line using PV-wave® and Matlab® processing software.

For STERN data analysis consisted of three stages, following the same strategy as in two previous studies on this task[61, 62]. First, after motion

correction, statistical activity maps were generated for each subject, for each of the three tasks (NT, PT and CT each compared to the rest condition by means of multiple regression[83]). Next, these maps were smoothed (FWHM 8 mm) and normalized into standard MNI space[84]

ues across all contained voxels for each subject. These final variables were entered into an analysis of variance with task load (CT versus NT, and NT versus PT) and region (listed in Table 2) as within-subject factors and group as between-subject factor.

Table 2 Regions of working memory-related brain activity in the STERN task

Region	Brodmann area	Number of voxels	X	Y	Z	Maximum z-value
Left dorsolateral prefrontal cortex l-DLPFC	9/46	164	42	5	28	9.64
Left superior parietal cortex l-SPC	7	164	34	-59	44	10.05
Anterior cingulate cortex ACC	6/24	49	6	5	56	7.48
Right superior parietal cortex r-SPC	7	15	-34	-63	44	5.65
Left fusiform gyrus l-FuG	37	16	42	-67	-12	5.25

MNI coordinates are shown for the five regions where activity during NT is significantly increased compared to CT, i.e. the working memory-specific regions. The coordinates X, Y and Z represent location of the voxels with the highest z-value in the group map. Corresponding names and Brodmann areas are obtained from the location of voxels with the highest z-value.

and were analyzed for the whole group contrasting NT to CT and using z-statistics (for details see[62]). When compared to NT, CT requires the same amount of perceptual processing and the same motor response, but it lacks the working memory component. Therefore, the contrast NT-CT eliminated activity that was not directly involved in working memory, and yielded several working memory-specific regions at a threshold of z=4.5 (p<0.05, corrected) with a cluster size of at least 10 voxels. These regions of interest (ROIs) were marked and were used for the third stage of analysis where we focused on a) the magnitude of working memory activity (high load NT versus no load CT) and b) the dynamic aspects of working memory, i.e. how brain activity within the ROIs differs between NT and PT. For this purpose, for each of the ROIs, mean activity values were obtained for CT, NT and PT, by averaging z-val-

For SAT regions of interest (ROIs) were defined by contrasting scans acquired during tone detection to scans acquired during rest periods (TO-RS), and similarly by contrasting scans acquired during dot detection to scans during rest (DO-RS). Both contrasts yielded a similar network of regions of activity at a threshold of z=6.0 (p<0.05 corrected) with a cluster size of at least 10 voxels. Therefore, only ROIs from the TO-RS contrast were marked and used for further analysis. For each of the ROIs, two mean activity values were obtained for TO and DO, that were entered into the analysis of variance with task (TO, DO) and region (listed in Table 4) as within-subject factors and group as between-subject factor. A second analysis focused on regions where brain activity was modulated by attention (i.e. attending to either tone – or dot detection), contrasting TO with DO at a threshold of z=4.5, (p<0.05 cor-

rected) This contrast yielded regions in the auditory and visual cortex involved in the dynamics of selective attention, that is, the magnitude of brain activity in these regions depends on whether attention is directed to tone - or dot detection respectively. Again, for each of these ROIs, mean activity values were obtained for TO and DO, both entered into the analysis of variance with task and region (listed in Table 6) as within-subject factors and group as between-subject factor.

caine (nr of occasions) and alcohol and tobacco consumption (units/week, cigarettes/week) (see Table 1). T-tests and nonparametric Kolmogorov-Smirnov Z-tests were used to calculate differences between cannabis users and non-users. To detect possible confounding effects of differences in tobacco and alcohol consumption between cannabis users and controls, bivariate correlations were used to explore associations between tobacco – or alcohol consumption and any of the task

Table 4 Regions of selective attention-related brain activity in the SAT task

Region	Brodmann area	Number of voxels	X	Y	Z	Maximum z-value
Right dorsolateral prefrontal cortex r-DLPFC	9/46	78	-46	13	36	8.77
Anterior cingulate cortex ACC	6/24	90	-2	13	48	11.34
Right primary – & secondary auditory cortex r-AUD	22/42	137	-58	-31	4	11.93
Left primary – & secondary auditory cortex l-AUD	22/42	122	58	-23	8	13.17
Right inferior frontal gyrus r-IFG	47	78	-46	21	-4	9.63

MNI coordinates are shown for the five regions where activity during TO is significantly increased compared to RS, i.e. the regions involved in visuo-auditory selective attention. The coordinates X, Y and Z represent location of the voxels with the highest z-value in the group map. Corresponding names and Brodmann areas are obtained from the location of voxels with the highest z-value.

Table 6 Regions where brain activity is modulated by attention during tone detection in the SAT task

Region	Brodmann area	Number of voxels	X	Y	Z	Maximum z-value	Δt - TOminusDO	
							Drug Naive	Cannabis
Right middle temporal gyrus r-MTG	22	32	-58	-27	0	6.30	0.82 (0.28)	0.35 (0.34)
Left superior temporal gyrus l-STG	42	5	58	-31	8	4.75	0.42 (0.30)	0.74 (0.32)
Right occipital lobe, cuneus-I r-CUN-I	19/30	44	-6	-67	8	5.41	0.82 (0.32)	0.47 (0.21)
Right occipital lobe, cuneus-II r-CUN-II	19	7	-2	-83	32	4.82	0.53 (0.25)	0.55 (0.30)

MNI coordinates are shown for the four regions where activity during TO is significantly increased compared to DO, i.e. the regions where brain activity is modulated by attention, depending on stimulus-modality. The coordinates X, Y and Z represent location of the voxels with the highest z-value in the group map. Corresponding names and Brodmann areas are obtained from the location of voxels with the highest z-value. The magnitude of the modulatory effect on activity is shown by Δt – TO minus DO (± SEM) for both groups.

Drug use data

Drug use data included lifetime and last year use of cannabis (nr of joints), lifetime use of MDMA (nr of tablets), amphetamines and co-

performance - or brain activity outcome parameters. Because no significant correlations were found, we did not adjust for tobacco- or alcohol consumption into further analyses.

Results

None of the participants had a positive urine drug screening for cannabis, other drugs or alcohol on the day of testing.

Performance Data

STERN Across task versions (CT, PT and NT), cannabis users performed equally fast and accurate as non-using controls ($F(4,14) = 1.51$, ns). Figure 2 shows mean reaction times and error scores for both groups.
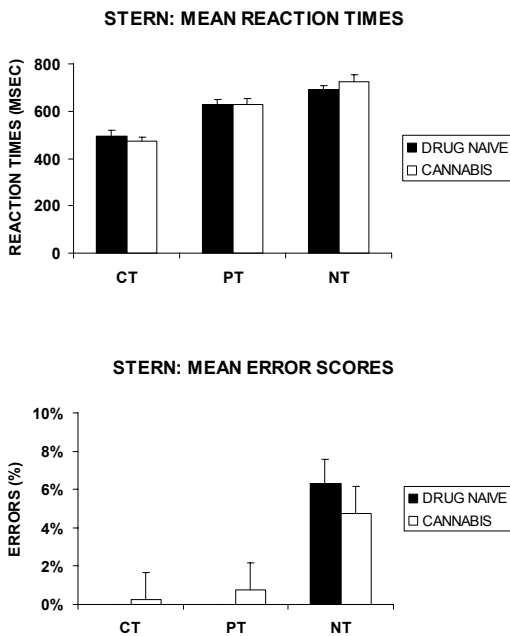
STERN: MEAN REACTION TIMES

STERN: MEAN ERROR SCORES

Figure 2 Graphs show behavioral performance data during STERN. Mean reaction times (± SEM) of correct responses on targets for both groups, and mean percentage of errors (misses and false alarms) as percent of all trials (± SEM).

SAT Groups did not differ in thresholds for detecting 80% of the deviant tones ($F(1,18)$ 2.88, ns) or the deviant dots ($F91,18) = .03$, ns). On average, the cannabis users needed a 9 Hz (SD 2 Hz) pitch difference to detect a deviant tone, compared to an 11 Hz (SD 5 Hz) difference for the non-using controls. To detect deviant dots, both groups needed on average a difference of 3 pixels (SD 1 pixel). Accuracy (percentage correctly identified deviant stimuli) during SAT did not differ between groups ($F(1,18) = .32$, ns) (Figure 3). However, a positive correlation was found between accuracy and the number of intrusions/false alarms. This could be related to a certain cognitive bias or decision criterion where relatively high accuracy is reached at the cost of more false alarms and vice versa. Therefore, we also tested for differences in accuracy between cannabis users and controls, using the number of intrusions as a covariate. This did not change the finding of equal performance during SAT for both groups.

Taken together, behavioral performance data indicate normal capacity and efficiency of the working memory and attention system in the cannabis users.
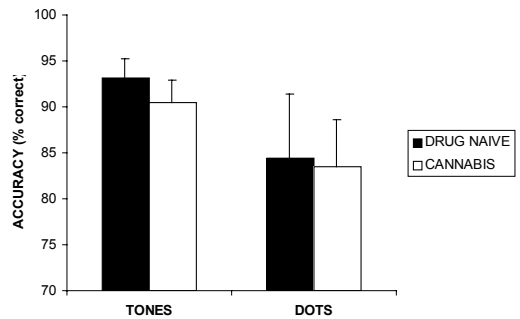

Figure 3 Graph shows behavioral performance data during SAT: The percentage correctly identified targets during tone or dot detection (± SEM) for both groups.

Magnitude and patterns of brain activity

To identify the regions of interest (ROIs) for both task paradigms, individual activity maps

(NT-CT contrast for STERN, and TO-RS contrast for SAT) of all subjects were combined into one group map for each task. For STERN five regions reached significance in the left fusiform gyrus (l-FuG), the left dorsolateral prefrontal cortex (l-DLPFC), the left – and right superior parietal cortex (l-SPC; r-SPC) and the anterior cingulate cortex (ACC) (Table 2). For SAT five areas in the left – and right auditory cortex (l-AUD; r-AUD), the right inferior frontal gyrus (r-IFG), the right dorsolateral prefrontal cortex (r-DLPFC) and the anterior cingulate cortex (ACC) reached significance (Table 4). Two regions were active in both tasks, i.e. the dorsolateral prefrontal cortex (BA 9/46) and the anterior cingulate cortex (BA 6/24). Task-specific regions included the superior parietal cortex (BA 7) bilaterally and the left fusiform gyrus during STERN and areas in the right inferior frontal gyrus (BA 47) and auditory cortex bilaterally (BA 22/42) during SAT. Repeated measures analyses of the fMRI signal in the ROIs were conducted for both tasks separately.

STERN Magnitude of working memory activity: Repeated measures analysis (five ROIs and task load (NT versus CT) as within-subjects factors, and group as between-subjects factor) revealed no significant differences between groups (see Table 3 for brain activity levels in both groups). This indicated comparable levels and patterns of overall brain activity in the whole brain network of regions involved in working memory, in both groups. Even when we compared the groups on the difference between high load (NT) and control (CT) levels for each ROI separately, we found no significant effects of cannabis use.

Dynamics of working memory: Repeated measures analysis (five ROIs and task load (NT versus PT) as within-subjects factors, and group as between-subjects factor) also revealed no significant differences between groups (Table 3). Thus, we found no evidence for more pronounced activity or compensatory shifts of activity across the five ROIs in the cannabis users. In a subsequent region-of-interest analysis we focused on those brain areas most consistently activated in verbal working memory studies[61, 62, 85, 86]. These areas involved the left dorsolateral prefrontal cortex (l-DLPFC), regions encompassing the anterior cingulate cortex (ACC) and the left superior parietal cortex (l-SPC). Restricted to these three areas, repeated measures analysis yielded a significant Task x Region x Group interaction ($F(2,17) = 4.49$, $p < 0.05$). Post-hoc analysis of variance (ANOVA) indicated that, whereas the controls showed a consistent decrease in activity in the l-SPC in response to a decrease of working memory load following practice, cannabis users failed to do so ($F(1,18) = 5.67$, $p < 0.05$) (Figure 4). Importantly, in cannabis users the difference in activity between NT and PT varied between a decrease, on average no decrease or even an increase. The difference in activity between NT and PT correlated negatively with last year exposure to cannabis ($r = -0.47$, $p < 0.05$) (Figure 5). Although the association between the difference in activity and cannabis use lifetime failed to reach significance ($p = 0.20$, 2-tailed) the correlation was also negative ($r = -0.30$). Thus, recent cannabis use correlated with a smaller decrease or no decrease at all in activity in the left superior parietal cortex after practice, suggesting abnormal dynamics in (part of) the working memory system.

Table 3 Brain activity in working memory-related regions in the STERN task for cannabis users and non-using controls.

Region	DRUG NAIVE Average t Value			CANNABIS Average t Value		
	CT	PT	NT	CT	PT	NT
l-DLPFC	-0.11 (0.11)	0.12 (0.07)	0.25 (0.08)	-0.03 (0.15)	0.04 (0.14)	0.28 (0.19)
l-SPC	-0.25 (0.13)	0.01 (0.10)	0.43 (0.14)	-0.07 (0.09)	0.36 (0.10)	0.30 (0.21)
ACC	0.45 (0.24)	0.64 (0.15)	0.85 (0.15)	0.40 (0.17)	0.61 (0.21)	0.76 (0.28)
r-SPC	-0.12 (0.23)	-0.20 (0.23)	0.30 (0.25)	0.09 (0.29)	0.12 (0.17)	0.39 (0.28)
l-FuG	0.40 (0.18)	0.23 (0.24)	0.22 (0.28)	0.44 (0.22)	0.84 (0.25)	0.96 (0.31)

This shows the average t value (± SEM) for both groups within each region of interest. CT = control task; PT = practiced task; NT = novel task; see Table 2 for abbreviations of regions of interest
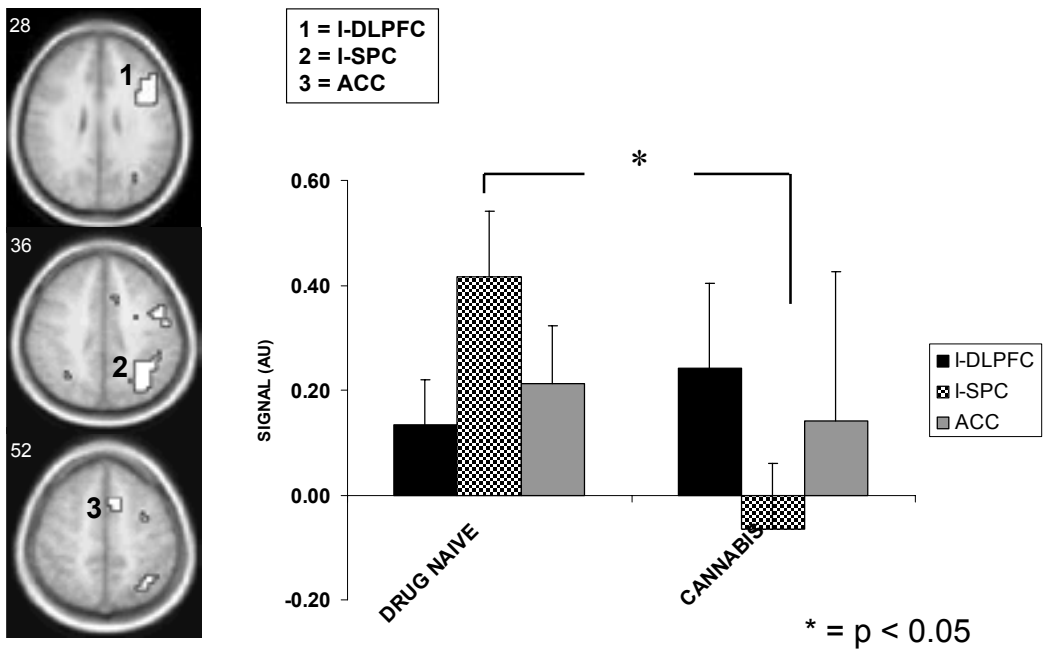
Figure 4 Effect of practice in the subset of STERN working memory regions corresponding to areas commonly reported in literature: left dorsolateral prefrontal cortex (l-DLPFC), left superior parietal cortex (l-SPC) and anterior cingulate cortex (ACC). The numbers above the slices indicate the MNI z-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa). The graph shows the decrease in activity in the l-DLPFC, the ACC, and the l-SPC, in response to a diminished working memory load after practice. The vertical axis is scaled in arbitrary units (AU) and represents the reduction of brain activity (novel task minus practiced task). A larger value reflects a larger reduction of brain activity.
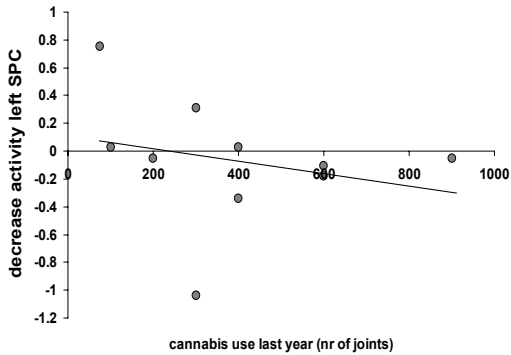
Figure 5 The correlation between last year cannabis use (nr of joints) and the magnitude of the decrease in activity in the left superior parietal cortex (l-SPC, r = -.47), following task load reduction.

SAT   Repeated measures analysis (with ROIs and task as within-subjects factors and group as between-subjects factor) revealed a significant Task x Region interaction ($F_{(4,15)}$ = 3.21, $p < 0.05$), indicating that brain activity in frontal - , auditory - and visual cortices was differentially affected during selective tone detection in comparison to selective dot detection. This reflects the expected modulation of brain activity through attention, i.e. the difference between stimulus processing facilitated by overt attention versus passive stimulus processing. More interestingly, no significant

Table 5 Brain activity in selective attention-related regions in the SAT task for cannabis users and non-using controls.

Region	DRUG NAIVE Average t Value		CANNABIS Average t Value	
	TO	DO	TO	DO
r-DLPFC	1.22 (0.28)	1.11 (0.31)	1.21 (0.20)	1.27 (0.24)
ACC	1.68 (0.16)	1.32 (0.19)	1.06 (0.23)	0.91 (0.27)
r-AUD	1.29 (0.24)	0.64 (0.25)	1.06 (0.21)	0.87 (0.13)
l-AUD	1.20 (0.21)	0.80 (0.23)	0.88 (0.26)	0.67 (0.22)
r-IFG	1.05 (0.26)	0.90 (0.22)	1.08 (0.19)	0.85 (0.21)

This shows the average t value (± SEM) for both groups within each region of interest. TO = tone detection task; DO = dot detection task; see Table 4 for abbreviations of regions of interest

Table 6 Regions where brain activity is modulated by attention during tone detection in the SAT task

Region	Brodmann area	Number of voxels	X	Y	Z	Maximum z-value	Δt - TOminusDO Drug Naive	Cannabis
Right middle temporal gyrus r-MTG	22	32	-58	-27	0	6.30	0.82 (0.28)	0.35 (0.34)
Left superior temporal gyrus l-STG	42	5	58	-31	8	4.75	0.42 (0.30)	0.74 (0.32)
Right occipital lobe, cuneus-I r-CUN-I	19/30	44	-6	-67	8	5.41	0.82 (0.32)	0.47 (0.21)
Right occipital lobe, cuneus-II r-CUN-II	19	7	-2	-83	32	4.82	0.53 (0.25)	0.55 (0.30)

MNI coordinates are shown for the four regions where activity during TO is significantly increased compared to DO, i.e. the regions where brain activity is modulated by attention, depending on stimulus-modality. The coordinates X, Y and Z represent location of the voxels with the highest z-value in the group map. Corresponding names and Brodmann areas are obtained from the location of voxels with the highest z-value. The magnitude of the modulatory effect on activity is shown by Δt – TO minus DO (± SEM) for both groups.

effects of group were found. Thus, frequent cannabis users and controls displayed comparable levels and patterns of overall brain activity in the regions involved in selective attention (see Table 5 for brain activity levels in both groups). To test for abnormalities in the dynamics of selective attention, individual activity contrast maps (TO-DO contrast) of all subjects were combined into a group map to identify the regions where brain activity, related to modality-specific stimulus processing, was modulated through attention. This yielded four regions in the primary and secondary auditory and visual cortices bilaterally (Table 6). Repeated measures analysis did not reveal any differences between groups (F(3,16) = .98, ns). In conclusion, no evidence was found for alterations in the attention system due to cannabis use, neither at the behavioral, nor at the neurophysiological level.

Discussion

The present fMRI study did not find evidence for robust long-term deficits in working memory and selective attention in frequent but relatively moderate cannabis users after one week of abstinence. Both users and nonusers performed equally well on a verbal working memory task and a visuo- auditory selective attention task. Furthermore, cannabis users did not differ from non-using controls in terms of overall patterns of activity in the brain regions involved in these cognitive functions. Both tasks activated the anterior cingulate - and the dorsolateral prefrontal cortex, areas that have been postulated to operate as an attention ex-

ecutive system[87]. This system is engaged when information processing requires attention and has a modulating role in activating or deactivating other regions involved in task-specific cognitive processing[88, 89]. Based on the current findings, it seems unlikely that this attention executive system is compromised by cannabis use. Nonetheless, we cannot completely rule out effects of cannabis on brain function, because the cannabis users displayed an abnormality in the left superior parietal cortex, a region well known to be involved in working memory. This area plays a role in short-term storage and retrieval of verbally coded material[62, 90, 91], a notion supported by evidence from human lesion studies showing that left posterior parietal lesions can impair verbal short-term memory[92, 93]. Therefore, higher levels of activity during the practiced task in the superior parietal region in frequent cannabis users might reflect a stronger need for reactivating the stored set of consonants. Regarding our hypotheses, this finding tentatively supports the "hyperactivity" notion, i.e. cannabis users perform equally well and appear to activate the working memory system in a similar way as controls, but normal performance levels are obtained at 'higher neurophysiological cost' to meet the demands of the task[94, 95]. When more challenging tasks are used, such compensatory mechanisms may no longer be sufficient and as a consequence task performance could deteriorate. Indeed, deficits in memory of word lists and other verbal memory tasks have been reported in cannabis users by several neuropsychological studies[69-72]. Both Bolla et al.[76] and Eldreth et al.[75] reported persistent dose-related alterations in brain activity (measured with PET)

in abstinent heavy cannabis users, in frontal areas, the hippocampus and the cerebellum. In addition, Kanayama et al.[77] and Jacobsen et al.[78] reported disruptive effects of cannabis on brain function (measured with fMRI), in frontal areas and the right hippocampus. With regard to brain activity, the present study did not replicate any of the above-described findings. This may be explained by differences in subject samples and methods used. For one, the cannabis users (N=11) participating in the studies of Bolla et al.[76] and Eldreth et al.[75] had smoked a mean of 41 joints per week (range 8 – 84) for a mean duration of 7.9 years (range 4 – 22). In the study of Kanayama et al.[77], cannabis users (N=12) had smoked a mean of 19,200 joints and were smoking at least seven times per week at the time of entry in the study. Compared to this, lifetime exposure in our group of users was moderate (median 1,300 joints). Deficits in working memory and attention may only surface in excessive users. In addition, use of other drugs of abuse may have contributed to the results of Kanayama et al.[77], as they included heavy cannabis users who also reported use of ecstasy, hallucinogens and cocaine. Thirdly, Jacobsen et al.,[78] studied a small group of cannabis users (N=7) with a different age range in comparison to the current sample, namely recently abstinent teenagers with a lifetime history of substantial cannabis use. However, both human and animal data suggest that early onset of cannabis use might result in more severe and/or more persistent deficits[96, 97], implicating that negative consequences on brain function from early onset cannabis use in adolescents cannot be extended to adult users with later onset.

Fourth, in the present study cannabis users were abstinent for at least one week before testing, whereas in Kanayama's study, subjects were scanned between 6 – 36 hours after last use. Their urine samples, collected at the time of imaging, showed positive levels of the cannabinoid metabolite 11-nor-9-carboxy-delta 9-tetrahydrocannabinol (THCCOOH). Curran et al.[65] investigated the acute and residual cognitive effects of delta(9)-tetrahydrocannabinol (THC) in infrequent cannabis users. Participants were assessed before and 1, 2, 4, 6, 8, 24 and 48 hours after drug administration. The results showed that only after 24 hours or more following ingestion of oral delta(9)-THC, effects of cannabis were undetectable. Therefore, the 6 – 36 hours of abstinence in Kanayama's study were too short to rule out the influence of acute pharmacological – and withdrawal effects. In the present study, confounding acute pharmacological effects are unlikely as the urine toxicology tests were negative for all users. Finally, inconsistencies in findings between the current study and Jacobsen et al.[78] can be attributed to the use of different methods for fMRI data analyses. Whereas Jacobsen et al.[78] focused their region-of-interest analysis on the hippocampus, the present study did a whole brain analysis. The clear advantage of the latter approach is that complex cognitive functions such as attention and working memory rely on a network of functionally interconnected brain regions. As a consequence, the integrity of such brain systems should be studied as a whole, whereas focusing on a particular region of interest can serve as an additional strategy. For example, compensatory shifts or reorganizations may occur within the network of

regions involved, as a consequence of an adaptive response to neuronal dysfunction in one region.

The results of this study should be interpreted with several limitations in mind. For one, sample size was relatively small. Furthermore, we used a retrospective design and therefore cannot rule out the possibility of pre-existing neurophysiological differences between the cannabis users and the non-using healthy controls. Nevertheless, although this finding needs replication, the dose-effect relationship that was found between cannabis consumption during the last year and the abnormality in the left parietal cortex, is difficult to reconcile with the notion of pre-existing neurophysiological differences. Another possible limitation of the current study is the difference in tobacco and alcohol consumption between the cannabis users and the non-using controls, with the cannabis users on average drinking more and smoking more tobacco cigarettes. However, we found no relation between alcohol or tobacco consumption and any of the neurocognitive outcome parameters. Finally, although the groups were carefully screened to exclude subjects with substantial use of other drugs or alcohol, current psychiatric or medical disorders, use of psychoactive medications, and were recruited at the same locations, the cannabis users may represent a specific subculture among the population of young adults. Although we minimized this potential bias by recruiting controls from the same pop-

ulation, we cannot exclude the possibility that the groups differed on some other factor than cannabis use itself.

In contrast to most studies on the long-term effects of cannabis use on cognition and brain function, we focused in the present study on frequent but relatively moderate users. This may account for the absence of behavioral impairments in working memory and attention, which may only surface in excessive users when tested with more challenging tasks. Nevertheless, we do not think that the study design and the used task paradigms lack sensitivity to detect deficits in cognitive brain function in cannabis users. Both STERN and SAT are highly suitable to assess multiple aspects of working memory and selective attention[61, 62]. The difference between the novel and the control task during STERN is a robust measure of working memory capacity and efficiency, whereas the parametric characteristics of the task (i.e., the difference between novel and practiced) enable us to study working memory from a dynamical perspective. Comparably, in SAT, stimulus-invoked changes in brain activity during tone or dot detection reflects a robust measure for selective attention. Again, the parametric characteristics of the task allow for examination of the dynamics within the attention system, as the difference between tone - and dot detection reflects the ability to switch attention from the auditory to the visual modality and vice versa.

Finally, the growing interest in the possible medical benefits of cannabis in certain patient groups such as neurological disorders, pain, musculoskeletal disorders, cancer and anorexia/cachexia[14-16, 80], stresses the necessity and relevance of cannabis research including populations with moderate and controlled patterns of use.

This study has focused on working memory and attention. Therefore, it cannot be taken as evidence for the absence of any long-term effects of frequent cannabis use on cognition. Also, this paper did not address questions with regard to causality and course of possible long-term effects of cannabis on brain function. These topics clearly need more study, which emphasizes the need for future longitudinal prospective and – retrospective studies with repeated observations on the same subjects at sufficiently long intervals, investigating a broad range of cognitive abilities.

Chapter 4
Effects of frequent cannabis use on hippocampal activity during an associative memory task

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Abstract

Interest is growing in the neurotoxic potential of cannabis on human brain function. We studied non-acute effects of frequent cannabis use on hippocampus-dependent associative memory, investigated with fMRI in 20 frequent cannabis users and 20 non-users matched for age, gender and IQ. Structural changes in the (para)hippocampal region were measured using voxel-based morphometry (VBM). Cannabis users displayed lower activation than non-users in brain regions involved in associative learning, particularly in the (para)hippocampal regions and the right dorsolateral prefrontal cortex, despite normal performance. VBM-analysis of the (para)hippocampal regions revealed no differences in brain tissue composition between cannabis users and non-users. No relation was found between (para)hippocampal tissue composition and the magnitude of brain activity in the (para)hippocampal area. Therefore, lower brain activation may not signify neurocognitive impairment, but could be the expression of a non-cognitive variable related to frequent cannabis use, for example changes in cerebral perfusion or differences in vigilance.

Introduction

Cannabis[1] is the most popular of all illicit drugs[64, 98]. Although cannabis has long been considered to be more benign than other drugs, interest in the neurotoxic potential of cannabis on the brain and brain function is growing. Until now, there is very limited proof for structural brain abnormalities in frequent cannabis users. Although ex vivo studies in rat hippocampal neurons in culture have revealed delta9-THC-induced cell death with shrinkage of neurons and DNA fragmentation[99, 100], in humans the picture is less clear. Several studies failed to demonstrate morphometric changes of the brain as a whole and the hippocampus in particular, in long-term cannabis users[101, 102]. On the other hand, recent findings from a voxel-based morphometry (VBM) study suggest that cannabis is neurotoxic to human hippocampal neurons, with lower gray matter tissue densities bilaterally in the hippocampal regions in frequent cannabis users compared to non-users, and indications for subtle structural changes in other brain regions as well[103]. Evidence for functional, i.e. cognitive abnormalities after extended and heavy use of cannabis is somewhat stronger. Acute effects include impairments of perceptual-motor and cognitive tasks, especially memory and learning[65-67, 104]. Effects of long-term use of cannabis are less consistent: some studies did not find proof for persistent effects of cannabis use[21, 105], while others reported subtle impairments of memory and learning, executive functions and attention[59, 69, 70, 75, 106-108]. It is important to note that only few studies investigated persistent, long-term effects of cannabis use on cognition with adequately long monitored abstinence periods (approximately one month)[70, 75, 107, 108]. In other studies abstinence periods were much shorter (17-28 hours), in which case reported effects were likely to reflect subacute, possibly transitory effects of cannabis use on cognition[69, 106].

It has been suggested that cannabis affects certain aspects of memory more profoundly than others. Evidence for this hypothesis comes from a study where infrequent cannabis users showed impaired episodic memory and learning in a dose-dependent manner, whereas implicit memory and working memory were unaffected[65]. The question arises whether impaired episodic memory is accompanied by altered brain function, and if so, whether specific brain regions are involved. Differential involvement of brain regions in different types of memory is well documented based on functional neuroimaging studies (for a review see[85, 109]). For example, prefrontal areas play an important role in working memory[86], whereas formation and retrieval of episodic memory, specifically associative memory, rely more heavily on temporal brain regions such as the hippocampus and the parahippocampal gyrus[63, 110, 111]. Interestingly, the highest densities of cannabinoid receptors (CB-1) are found in the hippocampus, cerebellum and striatum[112, 113]. Therefore, the hippocampal region can be regarded as the most likely candidate region for the observed impairments in memory and learning in heavy cannabis users. Results from imaging studies using a cognitive task challenge indeed indicate sustained changes in regional cerebral blood flow (rCBF) and neurophys-

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[1] With the term 'cannabis' we refer to substances with as major psychoactive compound delta9-tetrahydrocannabinol (THC), i.e. marijuana, weed etcetera.

iological abnormalities in (para)hippocampal regions (among other brain regions) in frequent cannabis users[75, 78, 106]. However, these previous studies have some methodological limitations. First, findings may reflect sub-acute effects from lingering cannabis intoxication[106]. Second, the choice for a particular cognitive paradigm was motivated by the cognitive function under investigation, i.e. working memory[78] and executive functioning[75]; functions in which the hippocampus is not necessarily involved. To illustrate, in a recent study from our own laboratory on the long-term effects of cannabis use on working memory and attention, we found evidence for involvement of the dorsolateral prefrontal and the anterior cingulate cortex (consistent with many other fMRI studies on working memory and attention), but no evidence for hippocampal involvement[114]. It is clear that examination of sustained effects of cannabis on (para)hippocampal function requires a paradigm that activates this region. An appropriate paradigm for this is one that addresses associative memory, which has been shown previously to activate the hippocampus[63, 111].

The purpose of the current study is to provide a comprehensive assessment of potential sustained effects of cannabis on hippocampus-dependent associative memory function. Behavioral, functional and structural measures of the (para)hippocampal region are acquired from frequent, but abstinent cannabis users and matched controls, using fMRI. The primary objective is to answer the following research questions. First, is performance on a specific, hippocampal-dependent associative memory task affected in frequent, but abstinent cannabis users compared to non-using controls? Second, are there differences in brain activity in the network of regions involved in associative memory between these users and the controls. If this is the case, then which area(s) exhibit abnormal function, and is the hippocampus involved? Third, is there evidence for structural abnormalities in the (para)hippocampal brain region in frequent cannabis users.

Experimental procedures

Subjects

Twenty frequent cannabis users (lifetime use: median 1,900 joints; range 675 – 10,150 joints) were compared to twenty (almost) drug naive healthy control subjects (lifetime use: median 0 joints; range 0 – 30 joints). The groups were matched for age, gender and verbal IQ. Half of the subjects (10 users, 10 controls) were selected from a prospective study on ecstasy neurotoxicity[42] and a study on the long-term effects of cannabis use on working memory and attention[114]. The remaining subjects were recruited through advertisements in newspapers or Internet, at locations where cannabis is sold, at university colleges and through word of mouth. Substance - and alcohol use was assessed by self-report questionnaires and the Substance Abuse Scales of the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders (M.I.N.I: Translated Dutch Version 5.0.0[81]). Verbal intelligence was estimated using the Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test[115]. Inclusion criteria were (1) right-handedness, (2) age between 18 and 35 years, (3) estimated lifetime use of 500 joints or more

for the cannabis users, and (4) willingness to abstain from cannabis and alcohol (all subjects) for at least 7 days prior to testing. Compliance was checked by urine drug screening (enzyme-multiplied immunoassay for amphetamine, ecstasy, opiates, cocaine, benzodiazepine, cannabis and alcohol (Jellinek Laboratory, The Netherlands). Participants were excluded if they reported (1) major medical, neurological or neuropsychiatric illnesses that might affect cognitive function, (2) current use of psychotropic medication, (3) pregnancy, (4) use of other substances than cannabis, alcohol and tobacco on more than five occasions lifetime, or (5) contraindications for MRI.

the combination. In the next task single pictures have to be classified (SC), which serves as a control task. Finally, in the retrieval task (RE) subjects have to recognize specific combinations previously presented during associative learning. Half of the stimuli were new combinations and half were combinations presented during the AL task. The RE-task provides a performance measure. In healthy volunteers this task reliably reveals brain activity in the hippocampus and parahippocampal gyrus bilaterally, especially during associative learning[63]. When compared to AL, SC requires the same amount of perceptual processing and the same motor response, but it lacks the associative memory

Table 1 Demographic features and drug use of cannabis users and control subjects

	Cannabis users N=20	Control subjects N=20	P[a]
Male/Female, N	13 / 7	13 / 7	
Age (years), mean (SD)	24.5 (5.2)	23.6 (3.9)	0.82
IQ (DART-score), mean (SD)	107 (8.0)	103 (8.4)	0.58
Cannabis use lifetime (nr of joints), median (range)	1900 (675 – 10,150)	0 (0 – 30)	0.000
Cannabis use last year (nr of joints), median (range)	332.5 (10 – 1,450)	0 (0 – 8)	0.000
Ecstasy use lifetime (nr of tablets), median (range)	0 (0 – 4)	0 (0)	0.978
Amphetamines (nr of occasions), median (range)	0 (0 – 2)	0 (0)	1.000
Cocaine (nr of occasions), median (range)	0 (0 – 5)	0 (0 – 1)	0.819
Tobacco smoking (nr of cigarettes/week), median (range)	10.0 (0 – 100)	0 (0 – 100)	0.003
Alcohol consumption(nr of units/week), median (range)	10.0 (0 – 40)	5.5 (0 – 25)	0.17

[a]Significance of differences calculated using nonparametric Kolmogorov-Smirnov Z test, two-tailed

Assessment of associative memory

Associative memory was assessed using a pictorial memory fMRI-paradigm (denoted PMT), based on a task paradigm from Henke et al.[63] that involves three tasks. First, an associative learning task (AL) is conducted which requires subjects to establish a meaningful connection between two pictures and to memorize

component. Therefore, the contrast AL-SC eliminates activity that is not directly involved in associative memory. Figure A (page 100) depicts a schematic example of the PMT task. Each picture contains two photographs on a white background, and is presented for 5000 ms, followed by a 2340 ms fixation cross. Each task is presented in 4

epochs (duration 65 sec) of 8 stimuli (picture + fixation cross). Also, four rest periods (RS) are included of equal epoch duration. A fixed order sequence of a rest period and the three tasks, i.e. RS, AL, SC and RE, is repeated four times, resulting in a total task duration of 18 min. Subjects respond by using a button box device and are instructed to respond as accurately as possible without stressing speed of response.

Voxel-based morphometry

To assess structural abnormalities in (para)hippocampal regions, voxel-based morphometry (VBM) was applied. VBM is an automated and non-biased procedure that identifies regional differences in tissue composition (gray matter or white matter) on a voxel-by-voxel basis using a measure of tissue density[116]. Changes in brain tissue composition, i.e. relative changes in white matter and/or gray matter densities could have implications for interpreting functional findings from fMRI studies. As the BOLD-signal is a heamodynamic measure of changes in neuronal activity, mechanisms behind changes in white – or gray matter densities, such as apoptosis, neuronal shrinkage, changes in ratio neurons/glial cells or reduced synaptic density, could affect the BOLD-signal.

Procedure

Image acquisition

Image acquisition was performed on a clinical Philips ACS-NT 1.5 Tesla MR-scanner with fast (PT6000) gradients. To minimize head movement during the scan session, subjects were fixated by a strap around their forehead and by the use of foam padding. Subjects wore earplugs to reduce scanner noise. For fMRI, the computerized task was projected to a through-projection screen positioned near the feet and was visible to the subjects via a mirror attached to the head coil. Subjects responded by pressing buttons with their right thumb on an air-pressure button box device. A standard scan protocol was used (navigated 3D PRESTO[82]). A single run of 432 scans was acquired over a period of 18 minutes (scan parameters: TE 37 ms TR 24.4 ms, flip angle 9°, FOV 208 x 256 x 104, matrix 52 x 64 x 26, voxelsize 4.0 mm isotropic, time per scan 2340 ms, 26 slices, scan orientation parallel to the long axis of the hippocampus). Each epoch spanned 27 scans and lasted 65 s. This was followed by one volume that was acquired with a flip angle of 30° for registration purposes. In addition, a volumetric T1-weighted MR anatomical scan was acquired for spatial localization and for VBM analysis. Acquisition parameters were FOV 256 x 256, TR 30 ms, TE 4.6 ms, matrix 128 x 128 x 130, flip angle 30°, with 150 slices of 1.2 mm thickness.

Data analysis

Performance

Performance accuracy during PMT was computed for SC (percentage correctly identified stimuli) and recall accuracy for RE by averaging the mean percentage 'hits' (percentage of picture pairs correctly identified as seen previously) and 'correct negatives' (percentage of picture pairs correctly identified as not seen before). To test for differences between cannabis users and non-users, ANOVA was applied, with group as between-subject factor.

fMRI

After reconstruction, functional and anatomical data were processed off-line using PV-wave® and Matlab® processing software. First, after motion correction, statistical activity maps were generated for each subject, for each of the three tasks (AL, SC and RE, each compared to the rest condition) by means of multiple regression analysis[83]. Next, these maps were smoothed (FWHM 8 mm) and normalized into standard stereotaxic MNI space[84]. The normalized activity maps were analyzed for the whole group, contrasting AL to SC and using z-statistics. To check whether the cannabis users activated additional regions in comparison to the controls, we defined regions of interest (ROIs) for both groups separately (AL-SC contrast, z=5.0 (p<0.05 corrected). This revealed highly similar patterns of activity for both groups. Therefore, the analyses were continued on the basis of the brain activity maps of all subjects (N=40) combined. The AL-SC contrast eliminated activity that was not directly involved in associative learning, and yielded several regions of activity at a threshold of z=5.0 (p<0.05, corrected) with a cluster size of at least 10 connected voxels. These ROIs were marked and were used for the next stage of analysis. For each of the ROIs, mean activity values were obtained for AL, SC and RE, by averaging z-values across all contained voxels for each subject. These final variables were entered into the analysis of variance with task (AL, SC, RE) and region (listed in Table 2) as within-subject factors, and group as between-subject factor. ROIs included, among other regions, clusters of voxels located in the hippocampus and parahippocampal gyrus bilaterally.

The size of the (para)hippocampus ROIs was large, with activity extending into the fusiform gyrus. As the analysis of primary interest concerned the (para)hippocampal regions sec, we added a second analysis to improve specificity by limiting the analysis to anatomically defined regions (right and left hippocampus, right and left parahippocampal gyrus). For this purpose, a segmentation procedure was applied based on individual anatomical scans, yielding four separate clusters (right and left hippocampus, right and left parahippocampal gyrus) marked for each subject separately. Then, per individual, two statistical activity contrast-maps based on an AL-SC and RE-SC contrast respectively, z=3.0 (p<0.05 corrected), were generated. After this, mean activity values within the four clusters for both contrast-maps were obtained for each subject. Finally, these variables were entered in GLM repeated measures analyses with cluster as a within subject factor and group as a between subject factor.

Table 2 Regions of associative learning-related brain activity in the PMT task

Region	Brodmann area	Number of voxels[a]	X	Y	Z	Maximum z-value
Right parahippocampal gyrus r-PHG	37/36	163	-34	-51	-20	12.49
Right middle occipital gyrus r-MOG	18/19	125	-34	-91	12	9.67
Left dorsolateral prefrontal cortex l-DLPFC	9/46	95	42	9	32	8.95
Left parahippocampal gyrus l-PHG	37/36	84	38	-51	-20	11.14
Anterior cingulate cortex ACC	6	26	2	29	40	7.00
Right dorsolateral prefrontal cortex r-DLPFC	9/46	17	-46	29	24	6.75

MNI coordinates are shown for the six regions where activity during AL is significantly increased compared to SC, i.e. the associative learning-specific regions. The coordinates X, Y and Z represent location of the voxels[a] with the highest z-value in the group map. Corresponding names and Brodmann areas are obtained from the location of voxels[a] with the highest z-value. [a] Voxelsize is 4 mm isotropic

VBM

Structural scans were analyzed using whole brain VBM implemented in Statistical Parametric Mapping (SPM2, Wellcome Department of Imaging Neuroscience, London, UK)[117, 118]. The structural MR images were spatially normalized to standard stereotaxic MNI space and were re-sampled to an isotropic voxel size of 1 mm. After this, images were segmented using a modified mixture cluster analysis technique to produce separate images of gray and white matter for each subject. The gray and white matter images were then spatially normalized to the MNI gray matter or white matter template. Normalized gray and white matter images were smoothed with a 12 mm³ isotropic Gaussian kernel. As sample size was relatively small (20 users, 20 controls), we restricted the VBM-analysis to the (para)hippocampal regions, using a small volume correction for improved statistical power (for details see[119]). Then, relative differences in voxel density values between cannabis users and controls were determined within (para)hippocampal regions (threshold value z=3.76 (p<0.05, corrected) with GLM in SPM2. Posthoc ANOVAs were used to evaluate increased or decreased tissue density values between groups.

Potential confounders

Because of apparent differences between the cannabis and the control group in the number of alcoholic drinks and the number of cigarettes smoked per week (see Table 1), we tested for correlations between these variables and the dependent variables (i.e. performance, fMRI parameters and VBM parameters). In case of significant correlations (p<0.20)[120], further analyses were corrected for the potential confounding effects of these variables.

Results

Sample characteristics and drug use

Demographics and drug-use history of users and controls are presented in Table 1. Cannabis users and controls did not differ in age, gender distribution and DART- IQ. On average, cannabis users had a marginally higher consumption of alcohol (median alcoholic drinks per week for cannabis users was 10, versus 5.5 for the controls, p=0.17). However, none of the subjects reached clinical criteria for alcohol abuse or dependence. Cannabis users also smoked more cigarettes than controls (median 10 per week versus 0 per week, p<0.01). Alcohol

consumption (number of drinks per week) was significantly correlated with recall accuracy (r=0.33, p<0.05, two-tailed) and tobacco smoking (number of cigarettes per week) correlated significantly with brain activity during AL (r= -0.31, p<0.10). Therefore, we included alcohol and tobacco consumption as covariates in all further analyses. Lifetime exposure to substances other than cannabis, alcohol or tobacco, such as ecstasy, amphetamines or cocaine was negligible in both groups (Table 1). Compliance to abstinence was supported by a negative urine toxicology test on all substances, including cannabis, for all participants.

Performance

Cannabis users did not differ from non-users on accuracy during the simple classification (SC) and the recall (RE) task, indicating normal simple classification processing and normal associative memory. Figure 1 shows mean accuracy for both groups. Adding alcohol or tobacco as a covariate did not change this finding. However, although as a group cannabis users performed within the normal range, recall accuracy within the group of cannabis users (N=20) was negatively correlated with the extent of last year cannabis use (r= -0.44, p=0.05, two-tailed) and also with the extent of lifetime cannabis use (r= -0.77, p<0.001, two-tailed) (Figure 2).
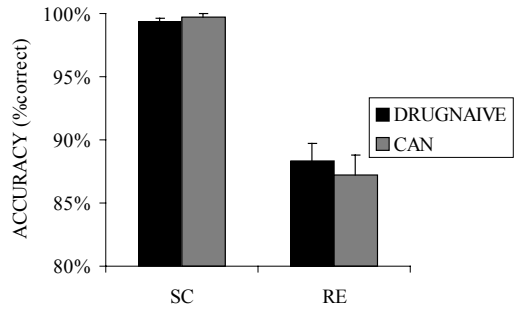

Figure 1 Graph shows behavioral performance data during PMT: Recall accuracy during simple classification (SC) and retrieval (RE) (± SEM) for both groups.
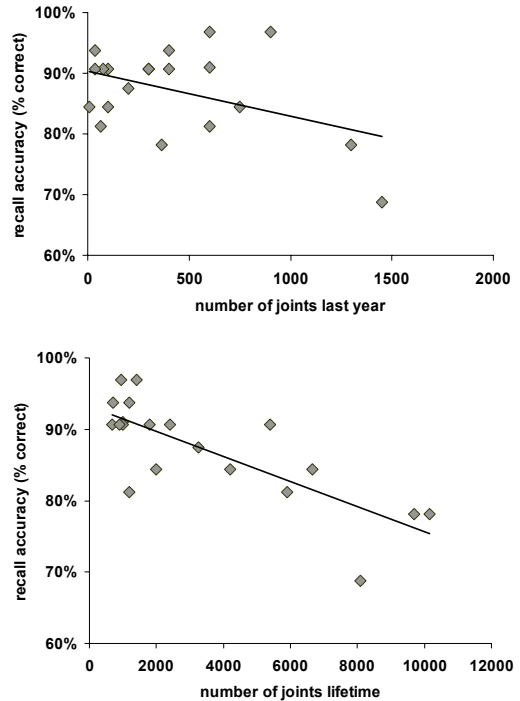

Figure 2 The correlation between last year cannabis use (number of joints) and task performance, and between lifetime cannabis use (number of joints) and task performance: r= -0.44, p<0.05 and r= -0.77, p<0.001, twotailed.

Brain activity

Whole brain analysis

To identify the regions specifically involved in associative learning, a group map (N=40) was generated for the associative learning minus simple classification contrast (AL-SC) (z=5.0, p<0.05 corrected). Six areas were activated, namely the dorsolateral prefrontal cortex bilaterally, the anterior cingulate cortex, the right medial occipital gyrus, and the parahippocampal/fusiform gyrus bilaterally (Table 2; Figure B, page 101). Analysis of these 6 ROIs revealed a significant 2-way interaction between task (AL, SC, RE) and group (F(2,37)=4.93, p<0.05); cannabis users exhibited an overall lower magnitude of activity in the ROIs than controls. Including alcohol or tobacco as a covariate did not alter the main finding (F(2,34)=6.14, p<0.05). Post-hoc ANOVA revealed that during AL, hypo-activity was most pronounced in the left and right parahippocampal area (F(1,38)=12.14, p<0.01, and F(1,38)=6.23, p<0.05 respectively) and in the right dorsolateral prefrontal cortex (F(1,38)=6.02, p<0.05). During RE, hypo-activity was most pronounced in the anterior cingulate cortex (F(1,38)=7.31, p<0.05). During SC no significant differences in magnitude of activity were observed between groups. Figure 3 shows levels of activity in the ROIs during AL and RE for cannabis users and controls.
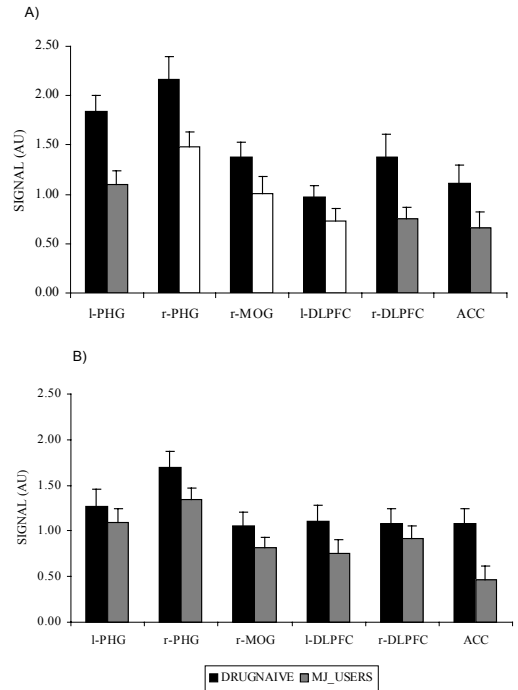
Figure 3 Abbreviations: l-PHG = left parahippocampal gyrus; r-PHG = right parahippocampal gyrus; r-MOG = right middle occipital gyrus; l-DLPFC = left dorsolateral prefrontal cortex, r-DLPFC = right dorsolateral prefrontal cortex; ACC = anterior cingulate cortex. A) Graph shows mean brain activity in the regions of interest during associative learning for both groups. B) Graph shows mean brain activity in the regions of interest during retrieval for both groups. The vertical axis is scaled in arbitrary units (AU). *; significance of differences calculated using ANOVA, p<0.05.

Region of interest analysis

Repeated measures analysis of brain activity in four anatomically defined (para)hippocampal clusters (right and left hippocampus, right and left parahippocampal gyrus) confirmed that during AL cannabis users displayed an overall lower magnitude of activity in the parahippocampal regions (F(1,37)=4.51, p<0.05).

Post-hoc ANOVA indicated that hypo-activity was more distinct in the left hippocampus and parahippocampal gyrus than in the right side ($F(1,37)=4.54$, $p<0.05$, and $F(1,37)=3.47$, $p=0.07$ respectively). During RE, activity in the parahippocampal regions did not differ between groups. In contrast to task performance, we did not find a relationship between the extent of lifetime or recent cannabis use and (para)hippocampal activity.

Voxel Based Morphometry

(Para)hippocampal gray and white matter density

The results of the VBM analysis revealed no statistical significant differences in (para)-hippocampal gray or white matter density values between cannabis users and controls. (Para)hippocampal VBM parameters were not significantly associated with the extent of lifetime or current cannabis use.

Correlations between (para)hippocampal VBM-outcome and fMRI parameters

There were no significant correlations (positive or negative) between gray matter density values in the anatomically defined (para)-hippocampal clusters and task performance or (para)hippocampal brain activity.

Discussion

In the present fMRI study we investigated the effects of frequent cannabis use on brain activity during hippocampus-dependent associative memory. Evidence was found for lower brain activity in abstinent cannabis users in the (para)hippocampal region bilaterally and the right dorsolateral prefrontal cortex (most pronounced during associative learning), in spite of normal memory performance. VBM-analysis yielded no evidence for structural changes in the (para)hippocampal region in cannabis users. Furthermore, gray and white matter density values in the cannabis users were unrelated to the extent of lifetime or recent cannabis use. Also, we found no relation between lifetime exposure to cannabis and brain activity measured with fMRI. Thus, lower activation during associative learning is associated with frequent cannabis use, but not necessarily with the cumulative extent of cannabis use. On average, task performance was within normal range and was unrelated to the level of brain activity. Therefore, lower activation may be the neurophysiological expression of a non-cognitive behavioral or physiological variable related to frequent cannabis use, for instance vigilance or mental attitude during a cognitive challenge. To illustrate, there is some evidence that individuals who differ in the magnitude of their blood pressure response to a cognitive stressor (Stroop interference task) also differ in their stressor-induced functional brain activity. High responders (larger magnitude increase in systolic blood pressure to the Stroop task) showed a larger increase in activity in the posterior cingulate cortex (a brain region implicated in vigilance) than low responders[121]. In addition, another study reported correlations between fMRI time series and changes in skin conductance response over time, in a network of brain regions that is activated independent of cognitive state, i.e. also during resting state[122]. It is therefore conceivable that lower activation in

cannabis users reflects similar effects of behavioral or physiological variables, either pre-existent or as a consequence of frequent cannabis use, but independent from the cognitive process being studied.

Several considerations are important in understanding the current results. First, the finding of overall lower activation in the network of brain regions involved in associative memory in cannabis users is at odds with previous findings. Abnormalities in brain activity in memory-related regions in cannabis users have been reported, but the most frequent finding is higher activation, or a combination of lower activity in some regions with higher activity in others. For example, Kanayama et al.[77] reported overall increased cortical activation in long-term heavy cannabis users and even recruitment of ancillary brain regions during spatial working memory, measured with fMRI. Using PET and a verbal memory task, Block et al.[106] found decreased memory-related blood flow in the prefrontal cortex, but increased blood flow in the cerebellum and altered lateralization in hippocampus. The discrepancy between the current findings and those of Kanayama et al.[77] and Block et al.[106] could be related to differences in abstinence periods and lifetime exposure to cannabis. Whereas cannabis users in the current study were abstinent for at least 7 days, the users in the other studies were abstinent for shorter periods (6-36 hours in Kanayama et al[77], and an average of 28 hours in Block et al.[106]). These studies probably measured more acute effects whereas the current findings could be classified as sub-acute or sustained effects. It is possible that after a limited abstinence period of one week, fre-

quent users still are in a state of withdrawal or sub-clinical intoxication that affects brain activity levels, without compromising task performance. Nonetheless, although we cannot exclude the possibility of withdrawal effects, sub-clinical intoxication seems unlikely, because all cannabis users had a negative urine toxicology test.

With regard to the extent of use, the cannabis users in the present study were classified as 'frequent' users, i.e., regular, almost daily users, who had been using for at least 3 years or more (mean 8.4 ± 4.8 years, median 1,900 joints; range 675 – 10,150). Duration of use is comparable to the cannabis users in the study from Block et al.[106] (daily users for on average 3.9 ± 0.4 years), but the extent of cannabis use is very moderate compared to users participating in the study of Kanayama et al.[77], with a mean of 19,200 occasions of cannabis use (range 5,100 – 54,000). Combined effects (lower and higher activity) of cannabis use on cognitive brain function are also reported in two papers reporting on the same subjects in a PET-study, where heavy cannabis users (mean 34.7 joints per week for on average 7.5 years) were abstinent for 25 days. These users displayed hypo-activity in the anterior cingulate cortex and the lateral prefrontal cortex during a Stroop task, but hyperactivity in the hippocampus bilaterally despite normal task performance in one study[75]. In the other paper on the same subjects, Bolla et al.[76] demonstrated additional differences between the most heavy and more moderate users during a decision-making task. Relative to moderate users, heavy users showed hypo-activity in the medial orbitofrontal cortex and hyperactivity in the cer-

ebellum, parahippocampal gyrus and the posterior cingulate. Finally, a recent study from our own laboratory, including some of the subjects participating in the current study, indicated that compared to controls, frequent cannabis users failed to show a decrease in activation in the superior parietal cortex after practice during a working memory task. However, brain activity in the prefrontal and the anterior cingulate cortex did not differ between groups[114]. Thus, the dorsolateral prefrontal cortex was activated both during working memory and (in the current study) during associative memory. Interestingly, cannabis users displayed normal levels of activity in this region during working memory, whereas they showed lower activation during associative learning.

In general, hyperactivity can be interpreted as stronger 'neurophysiological' effort to maintain normal behavioral performance (e.g.[94]). Relative hypo-activity in some regions contrasted to relative hyperactivity in other areas could be accounted for in terms of a compensatory mechanism to overcome focal, cannabis-induced brain dysfunction. Following this line of reasoning, overall lower activation might suggest a 'beneficial effect' of cannabis on neurophysiological efficiency, as users appear to perform as well as non-users with less neurophysiological effort, and without indications for compensatory mechanisms. However, we consider such an explanation unlikely. Although there is no conclusive evidence yet available to permit a conclusion that chronic heavy use of cannabis results in long-lasting or permanent functional losses[21], there is also no evidence for the opposite notion, i.e. that cannabis use fa-

cilitates cognitive brain function. The latter is not plausible, because we observed an inverse dose-response relationship between cannabis use lifetime and recall accuracy within the group of cannabis users. Thus, more cannabis consumption was accompanied by poorer memory performance (although still within the normal range), which is hard to reconcile with the notion of enhanced neuronal efficiency.

An alternative explanation for overall lower activation in the associative memory network is that reduced activation is secondary to other global or focal effects of cannabis on the brain, such as subtle cerebrovascular changes or subtle structural changes in neural tissue. There is supportive evidence for effects of prolonged cannabis use on cerebral perfusion. A recent study from Herning et al.[123] indicated that reduced cerebral blood flow in heavy users persisted over an extended period of monitored abstinence (28-30 days). Because the cannabis users in the present study were abstinent for at least one week, but probably not much longer, we cannot exclude the possibility that reduced perfusion related to withdrawal has affected the BOLD-signal. Regarding structural changes, a study from Matochik et al.[103] suggested that longer duration of cannabis use (in years) is related to abnormalities in gray matter tissue composition in the parahippocampal gyrus, the precentral gyrus, the right thalamus and the left parietal lobe in heavy cannabis users. The current results do not replicate the finding of (para)hippocampal changes in gray matter density values in frequent cannabis users. At present, the possibility of atrophy seems unlikely, since there is hardly any evidence for volumetric changes (which is not equivalent

to VBM, since a change in tissue composition can occur without a corresponding volumetric change) in the brain of cannabis users[101,102,124].

There are several limitations to this study that have to be taken into account when interpreting its results. First, due to the cross-sectional design it cannot be determined whether the differences between groups in brain activity may have preceded the initiation of cannabis use. Despite a careful screening of the groups to exclude subjects with substantial use of other drugs or alcohol, current psychiatric or medical disorders, use of psychoactive medications, we cannot exclude the possibility that the groups differed on some other factor than cannabis use itself. A second issue is sample size. Although sample size is quite sufficient for fMRI, VBM generally requires larger groups to meet the stringent statistical assumptions and facilitate noise reduction. Therefore, the VBM results should be considered with some caution.

In conclusion, the present results provide evidence for non-acute or sustained differences between frequent cannabis users and cannabis naive controls in brain activity dur-ing an associative learning task, especially in the (para)hippocampal region and the right dorsolateral prefrontal cortex. Lower (para)hippocampal activation was not accompanied by alterations in brain tissue composition in this region, and was unrelated to memory performance. Therefore, we think that lower brain activation in frequent cannabis users compared to cannabis naive controls may not signify neurocognitive impairment, but could be related to a behavioral or physiological variable independent from associative memory, for instance differences in vigilance. Whether such differences precede

or are caused by frequent use of cannabis is unclear. From this point of view, the present findings offer a new avenue in cannabis research. Future studies should focus on the relation between heterogeneous functional imaging findings and yet undefined non-cognitive variables on which frequent cannabis users may differ from non-users, for instance vigilance, eagerness to perform well, impulsivity or self-monitoring. The use of convergent methods and larger samples will facilitate interpretation of the different outcomes.

Chapter 5
Sustained effects of Ecstasy on cognitive brain function in the context of poly-substance use in humans

Gerry Jager, Maartje M.L. de Win, Ingeborg van der Tweel, Thelma Schilt, René S. Kahn, Wim van den Brink, Jan M. van Ree, Nick F. Ramsey

Abstract

Background

Heavy ecstasy use has been associated with neurocognitive deficits in various behavioral and brain imaging studies. However, this association is not conclusive due to the unavoidable confounding factor of poly-substance use.

Methods

The present study, as part of the Netherlands XTC Toxicity (NeXT) study, investigated specific effects of ecstasy on working memory, attention and associative memory, in a stratified sample with variation in amount and type of drug used. Effects of the use of ecstasy, amphetamine, cocaine and cannabis were assessed and separated with multiple regression analyses, in a sample of 33 heavy ecstasy users (mean 322 pills lifetime) and 38 controls.

Results

Use of drugs was associated with reduced performance and altered brain activity for associative memory, but had little effect on working memory and attention. Memory performance was affected by amphetamine much more than by ecstasy. Both drugs affected brain activity, but the effects were consistently in opposite directions.

Conclusions

These findings suggest that reported sustained effects of ecstasy on memory may rather be due to concomitant use of amphetamine. Both drugs affect brain activity, but in opposite directions, suggesting that different mechanisms are at play, possibly associated with serotonin versus dopamine systems.

Ecstasy (3,4-methylenedioxymethamphet-amine, MDMA) is a popular recreational drug, despite the fact that there is considerable concern about its neurotoxic potential. Studies in animals have shown deleterious effects of MDMA on the serotonin system, indicated by decreases in the number of central serotonin transporters (SERT) and a significant depletion of serotonin in various cortical and subcortical regions[2]. On the basis of these animal data it is likely that ecstasy might damage serotonin neurons in the human brain too. However, in humans, evidence for neurotoxicity is less convincing. Neuroimaging studies (PET, SPECT) using radiotracers that bind to SERT at the axon terminals have reported reduced SERT densities in the brain of ecstasy users[3]. However, these effects may be transient in most brain regions, as reversibility was shown in former ecstasy users[26]. Cognitive consequences of ecstasy use have been examined more extensively. Numerous cross-sectional studies reported impairments of learning and memory in moderate to heavy recreational users[8]. However, little is known about the effects of ecstasy on the neural systems involved in cognition. A few studies have examined working memory but generated inconclusive results concerning the effects of ecstasy on brain activity patterns and which specific brain areas are affected[27, 29, 31, 32]. One study investigated episodic memory function and suggested that memory deficits in ecstasy users arise from a hippocampal dysfunction[30]. Summarizing, there is some evidence of effects of ecstasy use on neurocognitive brain function, but this issue is clearly in need of further investigation.

Research in this area suffers from methodological problems for which there are no easy solutions[12]. Most human studies contend with interference of many potential confounders, such as poly-substance use and large heterogeneity in recreational ecstasy users. Experienced ecstasy users not only consume more ecstasy than novice users but they are also more likely to consume other drugs such as cannabis, amphetamine, cocaine, LSD and psilocybin (mushrooms)[43]. Consequently, cumulative ecstasy use almost invariably correlates highly with use of other drugs, making it almost impossible to differentiate between effects of ecstasy and of the other drugs. Indeed, several studies have indicated that signs of neurotoxicity in ecstasy users may well be associated with poly-drug use in general or with the use of other drugs such as cannabis and amphetamine[44]. In some studies an effort was made to control for the use of other drugs, either by including a group of 'pure' ecstasy users[125], a group of ecstasy-naive poly-substance users[126], or by statistically adjusting for poly-drug use[127]. Although statistical techniques help to tease apart the effects of ecstasy and of other drugs to some extent, they generally do not suffice if recruitment of the subjects is performed randomly, given the fact that ecstasy use almost invariably correlates with poly-drug use.

The present study aims to clarify the specific effects of ecstasy on neurocognitive brain function. For this purpose a large, stratified sample (n=71) with substantial variation in the amount and type of drug used was carefully composed. Multiple regression analysis with ecstasy and other drugs as separate regressors was applied to investigate the specific effects of ecstasy on

working memory, selective attention and associative memory, measured with functional magnetic resonance imaging (fMRI). We hypothesized that if deficits in memory or attention and/or related brain function would surface, ecstasy use would be the primary but not the only factor accounting for these abnormalities. We expected that amphetamines, cannabis and/or cocaine would contribute to possible neurocognitive deficits.

Methods and Materials
This study is part of the Netherlands XTC Toxicity (NeXT) study, design and objectives of which is provided in another paper[41]. Besides fMRI subjects underwent SPECT and MR imaging and cognitive testing; results of these measurements will be reported in separate publications.

Subjects
In total 71 subjects were included based on variations in the amount and type of drug used in order to keep correlations between drugs as limited as possible. The sample consisted of (1) heavy ecstasy poly-substance users; (2) selective ecstasy and cannabis users; (3) poly-substance users with a history of frequent amphetamine and/or cocaine and/or cannabis but very limited ecstasy use (< 5 tablets lifetime); (4) ecstasy-naive cannabis users, and (5) drug-naive (except for alcohol and tobacco) controls. For exclusion criteria see Table 1. Participants agreed to abstain from use of all psychoactive drugs for at least two weeks and from alcohol for at least one week before examinations. Compliance to abstinence was checked with urine drug screening. Participants underwent a semi-structured diag-

nostic psychiatric interview (Mini International Neuropsychiatric Interview for DSM-IV clinical disorders) to screen for current axis I psychiatric disorders. Drug use histories were collected using self-report questionnaires. Verbal intelligence was estimated using the Dutch version of the national adult reading test[115].

The study was approved by the local medical ethics committee and conformed to the Helsinki Declaration.

Table 1 Exclusion criteria subjects

Left-handedness
Severe physical or psychiatric illness
Current use of psychotropic medication
Use of intravenous drugs
Pregnancy
Contra-indications for MRI

FMRI
Three fMRI tasks were administered: a working memory task based on Sternberg's item-recognition paradigm (denoted STERN) (for details see Jansma et al.[62]) (see fig. 1), a visuo-auditory selective attention task (SAT) (for details see Ramsey et al.[61]) (see fig. 2), and a pictorial associative memory task (PMT) that depends on (para)hippocampal brain function (for details see Jager et al.[128]) (see fig. A, page 100). Each of these tasks has been presented in previous studies and yield clear maps of brain activity. The tasks consisted of several conditions to control for non-specific activity: The STERN consisted of a control condition CT (pressing a left or right button corresponding to a visual cue), a low WM load condition (practiced task, PT) and a high WM condition (novel task, NT). The SAT consisted of an 'attend to tones' (TO)

and an 'attend to dots' (DO) condition. The PMT consisted of an associative learning condition (AL), a simple classification (SC) and a recognition (RE) condition.

Scans were made on a clinical Philips ACS-NT 1.5 Tesla MR-scanner with PT 6000 gradients, using a standard scan protocol (navigated 3D PRESTO[82]). For further details on the scan procedure and scan parameters we refer to Jansma et al.[62], Ramsey et al.[61] and Jager et al.[128]
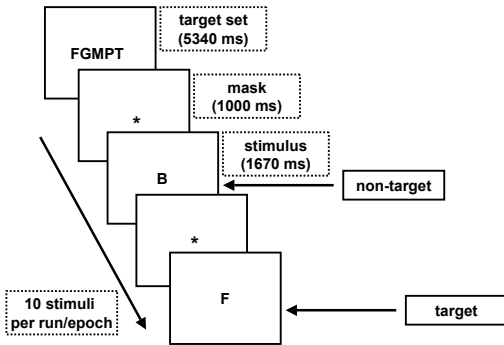

Figure 1 The temporal sequence of events is shown for the STERN task. Each epoch starts with presentation of the target-set, and is followed by ten trials. Subjects have to press a button as fast as possible, if the letter belongs to the target-set.
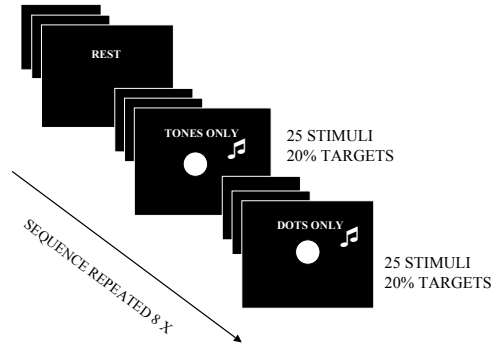
Figure 2 The temporal sequence of events is shown for the SAT task. Each epoch (duration 29 s) starts with an instruction slide (5 sec), indicating 'rest', 'attend to the tones only' or 'attend to the dots only'. Both during 'tones only' and 'dots only' the instruction slide is followed by a series of 25 stimuli (simultaneous asynchronous presentation of tones and dots at a variable inter-stimulus interval rate) of which on average 20% deviant (targets). In case of a target, subjects have press a button as fast as possible. Prior to fMRI scanning the difference between standard and deviant tones and dots is determined for each individual by changing the contrast until a performance of 80 % correct is obtained.

Dependent and independent variables

Drug use

Drug use data included self-reported lifetime use of ecstasy (number of tablets), cannabis (number of joints), amphetamine and cocaine (number of occasions), and alcohol and tobacco consumption (drinks/week versus cigarettes/week). However, self-report histories may be inaccurate and in addition there is imprecision arising from variation in drug content in ecstasy tablets. As a result, inaccuracies in dosage calculations are likely to undermine the validity of dose-response measures[129]. Therefore, drug use variables were dichotomized using a cut-off score to maximize the contrast between users and non-users (see Table 2).

fMRI

Analysis of fMRI data involved generating statistical activity maps for each individual (details are described in the given references for each task). We then identified regions of activity in the group-maps for each task, thresholded at $p<0.05$ corrected for total volume. These group-maps were based on the following contrasts. For STERN the Regions of Interest (ROI's) were derived from the difference between NT and CT. For SAT the ROI's were obtained from the contrast between TO and rest. For PMT the ROI's were based on the contrast between AL and SC.

For regression analyses (based on drug use) we used the mean levels of activity in each of these ROI's for each subject, for each task and for each condition within tasks, relative to resting state.

Statistics

Multiple Regression Analyses

Specific effects of ecstasy use and the relative contributory effects of other substances (amphetamines, cocaine, cannabis, tobacco and alcohol) on task performance and brain activity was assessed using multiple regression analyses with the different drugs as separate regressors. As non-drug predictors verbal IQ and gender were added to the model.

The strength of the effect of ecstasy use was estimated using two stepwise regression models. Model 1 estimated the upper bound effect of ecstasy on neurocognitive function, i.e., after adjustment for the effects of gender and IQ but without correction for the effects of other drugs. In the first step gender and IQ were entered, then in a second step, ecstasy. The independent effect of ecstasy is quantified as the R-square change between the first and the second step of the model. This model resembles the approach in previous studies where ecstasy-users were compared with non-users without accounting for polydrug use. Model 2 estimated the lower-bound effects of ecstasy, where all other substance-use (except for ecstasy), gender and IQ were entered into the model first. Ecstasy use was entered as the second step, and its effect was expressed as the R-square change between the first and the second step.

Table 2 Demographic features and drug usage patterns

	N = 71	Mean (SD)	median	range
Male/Female	44 / 27			
Age (years)		23 (3.8)	22	18 - 37
IQ (DART-score)		101 (7.7)	100	83 - 122
Ecstasy users (> 5 tablets lifetime)	33	322 (354)	250	15 - 2000
Amphetamine users (> 10 occasions lifetime)	18	151 (154)	120	15 - 600
Cocaine users (> 10 occasions lifetime)	22	72 (70)	43	12 - 300
Cannabis users (> 50 joints lifetime)	41	1260 (1633)	700	56 - 6650
Alcohol users (> 10 drinks per week)	36	22 (12)	18	12 - 60
Tobacco users (> 10 cigarettes per week)	32	83 (46)	80	17 - 200

Mean (SD), median and range for the different drugs show scores from the users only

Table 3 Regions of interest

Task	Region	Brodmann area	Number of voxels[a]	X	Y	Z	Maximum z-value
STERN	l-SPC	7	190	34	-63	48	12.65
	l-DLPFC	9/46	168	46	5	32	12.50
	ACC	6/24	69	6	6	56	12.17
	l-FuG	37	60	46	-39	-12	9.06
	r-SPC	7	55	-30	-67	44	9.19
SAT	r-IFG	47	138	-46	21	-4	16.99
	r-AUD	41/42/22	121	-58	-31	4	16.58
	l-AUD	41/42/22	86	60	-23	8	17.45
	ACC	6/24	58	-2	21	44	17.77
PMT	r-PHG	37/36	220	-26	-47	-16	16.33
	l-PHG	37/36	136	34	-47	-20	14.42
	r-MOG	19	103	-34	-87	8	11.52
	l-DLPFC	9	82	50	17	28	10.39
	r-DLPFC	9/46	65	-46	29	24	8.26
	r-IFG	47	38	-42	21	-4	8.39
	ACC	6/24	34	2	29	40	9.22
	l-MOG	18	33	30	-91	8	9.21
	l-IFG	24/6	33	50	21	-4	7.53

MNI coordinates are shown for the regions of interest where for working memory (STERN), selective attention (SAT) and associative memory (PMT). The coordinates X, Y and Z represent location of the voxels with the highest z-value in the group map. Corresponding names and Brodmann areas are obtained from the location of voxels with the highest z-value. Abbreviations: 'l-' and 'r-' stand for left and right respectively, SPC = superior parietal cortex, DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, FuG = fusiform gyrus, IFG = inferior frontal gyrus, AUD = auditory cortex, PHG = parahippocampal gyrus, MOG = middle occipital gyrus. [a]Voxel size = 4mm isotropic.

Table 4 Phi correlations between dichotomised substance use variables, gender and verbal IQ in the whole sample (N = 71)

	Gender	IQ	Alcohol	Tobacco	Ecstasy	Amph	Cocaine	Cannabis
Gender		ns	ns	ns	ns	ns	ns	-0.23
DART-IQ			ns	ns	ns	ns	ns	ns
Alcohol				ns	ns	ns	ns	0.38
Tobacco					0.40	ns	0.31	0.41
Ecstasy						0.43	0.54	ns
Amphetamine							0.45	ns
Cocaine								ns
Cannabis								

Phi correlations (p<0.05, two-tailed) between gender (0 = male, 1 = female) and the dichotomised drug use variables (0 = below cut-off value, 1 = above cut-off value). See Table 2 for classification criteria used. ns = not significant

Table 5 Effects of drug use on performance for STERN, SAT and PMT

	R^2 change of Ecstasy		Model 2: standardized β coefficients							
	Model 1	Model 2	Ecstasy	Amphetamine	Cocaine	Cannabis	Alcohol	Tobacco	Gender	IQ
STERN	4%	4.7%	.29	-.41 ** ‡	.04	.10	-.13	.04	.13	.11
SAT	1.3%	3.9%	.25	.19	-.32 ** ‡	-.16	.11	-.20	.07	.00
PMT	5.0%	1.0%	-.13	**-.39 **	.16	.18	.03	-.12	.19	.05

'*' 0.10 < P <0.05; * P < 0.05; ** P < 0.01; *** P < 0.001

Model 1 (upper bound): R^2 change with XTC as predictor, corrected for gender and DART-IQ; Model 2 (lower bound): R^2 change with XTC as predictor, corrected for other substances, gender and DART-IQ

‡ significant β-coefficient, whereas the regression model (model2) did not reach significance

Goodness-of-fit statistics were used to quantify the fit of the model and standardized regression coefficients β were used to indicate the predictive power of the different regressors. Phi correlations were used to explore associations between dichotomized substance variables and demographic variables (Table 4). The Variance Inflation Factor (VIF), a measure for multicollinearity, for the complete sample was 1.1 (as a rule of thumb any VIF that exceeds 10 is a reason for concern[130]), indicating that correlations between variables in the model did not cause over-specification of the regression model, allowing for reliable estimation of the effects of the various drugs.

Results

As data from a few tasks were lost for some subjects due to technical malfunction, results are reported for each task separately with the number of subjects included between brackets. Sample characteristics are shown in Table 2, and regression variables are shown in Table 4.

The effects of drug use on performance are shown in Table 5. There was one significant effect: amphetamine use predicted a reduction in RE performance in the PMT task.

Table 6 Effects of drug use on brain activity during STERN and PMT

		R^2 change of Ecstasy		Model 2: standardized β coefficients							
		Model 1	Model 2	Ecstasy	Amphetamine	Cocaine	Cannabis	Alcohol	Tobacco	Gender	IQ
STERN	l-DLPFC	12.8%*	1.4%	-.16	-.18	-.01	.13	-.07	.27 *	.25 *	-.22 '*'
PMT	r-MOG	11.0%**	11.0% **	.43 **	-.34 *	.24 '*'	.10	-.14	-.23 '*'	.09	-.10
(AL-related	l-DLPFC	1.0%	6.0% *	-.32 *	.21	.37 *	-.10	-.07	-.17	-.02	.03
activity)	r-DLPFC	0.2%	0.2%	-.06	.48 ***	-.18	-.12	-.19	-.05	.08	-.20
	l-PHR	5.0% '*'	0.3%	.23	-.28 '*'	.17	.12	-.19	.05	.09	-.11

'*' 0.10 < P <0.05; * P < 0.05; ** P < 0.01; *** P < 0.001

Model 1 (upper bound): R^2 change (%) with Ecstasy as predictor, corrected for gender and DART-IQ; Model 2 (lower bound): R^2 change (%) with Ecstasy as predictor, corrected for other substances, gender and DART-IQ. For abbreviations of regions see Table 3

fMRI

The regions of interest obtained with the contrast described in the methods are shown in figure C (color illustrations, page 102), and details are given in Table 3. The results of regression analyses are summarized in Table 6. The key results are as follows: There were no significant effects of drug use on brain activity in the STERN (n=70) or the SAT (n=69) tasks. However, there was a clear effect of tobacco use and gender on DLPFC in the STERN task: being a smoker was associated with higher levels of brain activity in this brain area during working memory processing, as was being female. Use of drugs did affect AL-related activity during the PMT (n=69) task. Ecstasy use predicted lower magnitude of activity in the left DLPFC, whereas in the right MOG it was the opposite, i.e. stronger magnitude of brain activity. Amphetamine use predicted lower magnitude of activity in the right MOG, higher activity in the right DLPFC and marginally lower activity in the l-PHG. Cocaine use predicted higher brain activity in the left DLPFC and marginally higher activity in the right MOG. Finally, use of tobacco predicted marginally lower activity in the right MOG. Strength and direction of the effects of drug use on AL-related brain activity are depicted in figure D (color illustrations, page 103).

Discussion

The present study found working memory and attention to be intact in poly-substance ecstasy users, but they performed less than ecstasy-naive controls on an associative memory task. Interestingly, when effects of drugs were teased apart, it was the use of amphetamine, and not the use of ecstasy, that largely accounted for reduced memory performance. Ecstasy use was related to altered brain activity patterns during associative learning in the left dorsolateral prefrontal cortex and the right middle occipital gyrus. These effects were independent from those of cannabis and alcohol use, and appeared to be independent from those of amphetamine, cocaine or tobacco use.

The specific effects of ecstasy on brain activity during associative learning may reflect sustained, possibly long-term adaptation or compensatory reorganization in a fronto-visual network. Whether or not this signifies serotonin neurotoxicity in terms of neuronal damage cannot be concluded from the present findings. However, our results do not support the notion of widespread loss of serotonin neurons, as the effects of ecstasy use were moderate, and selective for associative memory. It is therefore more likely that the network involved in associative memory is more sensitive to the effects of ecstasy on cognitive activation than other networks. Several underlying mechanisms may be involved. For one, ecstasy use could compromise serotonin function. There is evidence of serotonergic modulation of functional brain activity in the prefrontal cortex and limbic structures during a cognitive challenge[131, 132]. In one study, acute tryptophan depletion significantly reduced brain activation in the right orbito-inferior prefrontal cortex, whereas it increased activation in the superior and medial temporal cortex. It was suggested that reduced prefrontal activation reflects low serotonin turnover, whereas the increased engagement of temporal brain regions could

reflect compensatory mechanisms[132]. These findings bear a resemblance to the current observations of reduced activation of the dorsolateral prefrontal cortex, increased activation of the middle occipital gyrus during associative learning. Thus, it is conceivable that heavy ecstasy use induces sustained reduced serotonin turnover in the prefrontal cortex. However, this does not explain why lower prefrontal activation in ecstasy users was selective to one cognitive domain, i.e. associative memory; the left dorsolateral prefrontal cortex, activated in all tasks (see fig. C, page 102, ROI 'B' for STERN and PMT, data not shown for SAT), was only affected during associative memory.

Another mechanism could be mild damage to (part of) the associative memory system due to ecstasy use, though serious enough to lead to reduced functioning. In this case, the (para)hippocampal region is the most plausible candidate as this region was only activated during associative memory, and not during working memory or attention. However, our results suggest that the (para)hippocampal region may not be a prime target for ecstasy-related neurotoxicity[30], as we found only tentative evidence for enhanced brain activity in the left parahippocampal area.

In addition to the specific effects of ecstasy, the effects of amphetamine on activity in the network engaged during associative memory are of special interest as most of the heavy ecstasy users also take amphetamine. Amphetamine use was related to altered brain activity in the left parahippocampal region, the right middle occipital cortex and the right dorsolateral prefrontal cortex. The effects of ecstasy and amphetamine were different and in opposite directions in the prefrontal and posterior regions, suggesting that different mechanisms are at play, possibly associated with serotonin and dopamine systems. At present, there is no compelling evidence to suggest that ecstasy use damages dopamine neurons in the human brain[133], but there is evidence from studies in detoxified methamphetamine abusers indicating changes in dopaminergic brain circuits[134].

Our findings of impaired memory performance in (poly-substance) ecstasy users are consistent with many neuropsychological studies reporting memory deficits. It is important to note, however, that our results showed that lower associative memory performance in ecstasy users was largely due to amphetamine, and not ecstasy use. As previous studies in heavy ecstasy users often contend with confounding effects of poly-substance use (including amphetamine), at least part of the previously observed impairments in memory could be attributed to concomitant use of amphetamine. Furthermore, our brain activity data challenge the notion that memory impairments in poly-substance ecstasy users reflect hippocampal dysfunction due to a specific vulnerability of this brain region to the neurotoxic effects of ecstasy[30, 31]. The present findings merely indicate a tentative effect of ecstasy on associative learning-related brain activity in the left (para)hippocampal region, whereas amphetamine use more convincingly affected (para)hippocampal activity.

With regard to working memory and attention our results are less straightforward. We

found no evidence for robust effects of ecstasy or other drugs on performance or brain activity in the network engaged during working memory or selective attention. This seems at odds with findings from several previous fMRI studies on working memory in ecstasy users in which both decreased and enhanced brain activity has been reported in ecstasy users in a variety of brain regions, including the frontal and temporal cortex[27-29, 31, 32]. There are several possible explanations for the discrepancy between the current and previous findings: First, activity in brain areas other than those consistently reported as being activated in working memory studies in healthy controls, i.e. parietal and frontal areas[135], may result from specific characteristics of the fMRI task paradigm used. Consequently, observed differences between ecstasy users and non-users in, for example, hippocampal activity, may not be specifically related to working memory, but may surface because the task paradigm used involves other (episodic) memory processes as well. Second, differences in statistical analyses impede comparisons between the studies. In the present study we used a whole brain analysis correcting for multiple comparisons, whereas other studies used more liberal statistical thresholds[27] or performed a region-of-interest analysis restricted to the (para)hippocampal region[31]. This may have biased results to certain brain areas that are not involved in working memory per se.

Several limitations of the current study should be noted. For one, a consistent critique of a cross-sectional design is that neurocognitive abnormalities might actually predate and place individuals at risk for drug abuse rather than being the result of abuse. In this regard, it is important to note that animal research has reported MDMA-induced neurotoxicity in several species, including primates[2]. Furthermore, several human studies have demonstrated dose-effect relationships between cumulative lifetime ecstasy use and memory deficits, which support the idea of ecstasy use causing neurocognitive impairments[136]. A second limitation is we had to rely on statements by the subjects themselves on their current and earlier consumption habits, with questionable reliability. Unfortunately, in a naturalistic design there is no obvious solution to this problem. A third potential weakness is that sample stratification was not completely satisfactory. The resulting correlations between use of ecstasy and other illicit drugs such as amphetamine and cocaine were reduced but still substantial and statistically significant. Thus, we cannot claim perfect orthogonality between predictors and this may have weakened the validity of the regression model. However, by using a conservative model, i.e. entering ecstasy use into the model after controlling for the effects of all other predictors, we probably underestimated the actual strength of the effect of ecstasy use. Importantly, the association between ecstasy use and its most commonly used co-drug cannabis was successfully removed as a result of sample stratification, thereby controlling for an important confounder.

In conclusion, this study shows no strong effects of use of ecstasy or other drugs in the domains of working memory and attention. However, heavy ecstasy users exhibit reduced associative

memory performance, but this impairment is largely due to concomitant amphetamine use, and not ecstasy. Ecstasy and amphetamine have differential effects on brain activity in the network engaged during associative memory. These effects are in opposite directions, suggesting that different mechanisms are at play, possibly associated with differential effects on serotonin and dopamine systems. The effects of ecstasy and amphetamine are not specific for brain structures; the dorsolateral prefrontal cortex, activated in all tasks, is only affected during associative memory. It seems more likely that that the network as a whole is affected, and that the dorsolateral prefrontal cortex responds differently to alterations in serotonergic versus dopaminergic neurotransmission within certain brain systems.

Chapter 6
Incidental use of Ecstasy: no evidence for harmful effects on cognitive brain function in a prospective fMRI study

Gerry Jager, Maartje M.L. de Win, Hylke K.E. Vervaeke, Thelma Schilt, René S. Kahn, Wim van den Brink, Jan M. van Ree, Nick F. Ramsey

Abstract

Heavy ecstasy [3,4-methylenedioxymeth-amphetamine (MDMA)] use in humans has been found to be associated with cognitive impairments and with changes in cognitive brain function, supposedly due to damage to the central serotonergic system. There is concern that even a single dose of MDMA may be neurotoxic, but very little is known about the consequences of a low dose of ecstasy for cognitive brain function. We therefore prospectively studied, as part of the NeXT (Netherlands XTC Toxicity) study, sustained effects of a low dose of ecstasy on brain function in 25 subjects before and after their first episode of ecstasy use (mean 2.0 ± 1.4 ecstasy pills, on average 11.1 ± 12.9 weeks since last ecstasy use), compared to 24 persistent ecstasy-naive controls, also measured twice and matched with the novice users on age, gender, IQ, and cannabis use. Cognitive brain function was measured in the domains of working memory, selective attention and associative memory, using functional magnetic resonance imaging (fMRI). The study yielded no firm evidence for sustained effects of a low dose of ecstasy on working memory, selective attention or associative memory, neither at the behavioral level nor at the neurophysiological level.

Ecstasy (3,4-methylenedioxymethamphet-amine or MDMA) is a popular recreational drug. A large body of evidence indicates that MDMA has the potential to damage brain serotonin neurons in various animal species[1, 137]. There is still controversy, however, whether similar serotonergic damage does occur in human ecstasy users[42, 138-140]. Nonetheless, ecstasy use has been frequently associated with a variety of functional sequelae, including psychological problems and cognitive impairments[141, 142]. Functional neuroimaging studies in ecstasy users have reported changes in cognitive brain function as well. Both decreased and enhanced brain activity related to memory function has been observed in various brain regions, including frontal, temporal, visual, and limbic areas[27, 29, 30, 32]. However, some important questions remain unanswered. For one, most studies concern heavy ecstasy users, but there is concern that even a single dose of MDMA might be neurotoxic[49, 50]. For example, animal studies indicate that even after a single dose of MDMA, damage can occur in the serotonin system[143-145]. However, whether these animal findings can be extrapolated to humans is still debated[137]. Second, interpretation of human data is hampered by the lack of baseline data[42, 140]. Only a few prospective studies have been performed in ecstasy-naive volunteers, but these studies invariably focused on acute instead of sustained or long-term effects of ecstasy[9]. There are compelling reasons why more research is needed on the sustained effects of low dose ecstasy use in humans. First, the majority of recreational ecstasy users are incidental or moderate users[43, 146]. Second, interest

is growing in the possible beneficial actions of MDMA in posttraumatic stress disorder and in late stage cancer to reduce anxiety and agitation (MAPS Research Information[2]). Ideally, only a longitudinal prospective study in ecstasy-naive subjects randomly assigned to MDMA or placebo and conducted in a laboratory setting, could answer the question whether ecstasy is neurotoxic in humans. However, given the potential neurotoxicity of MDMA, such a study is ethically not acceptable. Recently, it has been advocated to start longitudinal prospective studies in specific groups of young people who are at an increased risk for use of ecstasy[10]. The current study is the first that succeeded in this approach. We investigated the effects of initial use of ecstasy on working memory, selective attention and associative memory, measured with functional magnetic resonance imaging (fMRI), using a prospective naturalistic design. Based on previous results with the same fMRI task paradigms in heavy poly-substance ecstasy users[147] we hypothesized that first use of ecstasy would affect associative memory in terms of performance and of brain function, but not working memory and selective attention.

Materials and Methods

This study is part of the Netherlands XTC Toxicity (NeXT) study. A detailed description of the design and objectives of the NeXT study is provided in a paper on the methods[41]. Besides fMRI, subjects underwent SPECT, MR imaging and cognitive testing; results of these measurements will be reported in separate publications.

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[2] MAPS Research Information at http://www.maps.org/mdma/ for information on approved phase I and II studies.

Subjects

Between April 2002 and April 2004, a total of 188 young adults (18-35 y) were included in the prospective cohort study of the NeXT project. Of this cohort, 96 participated in the present fMRI study. At the time of inclusion, none of the subjects had ever used ecstasy, but they were selected for being at high risk of initiating the use of ecstasy in the near future (see below). Subject recruitment consisted of a combination of targeted site sampling, advertisement[3] and snowball sampling referrals. For details on recruitment, inclusion and exclusion criteria we refer to De Win et al.[41]. Main inclusion criterion was a high probability to start using ecstasy based on the intention to probably or certainly use ecstasy for the first time in the near future and/or ecstasy use by peers. All subjects were right-handed and were excluded if they reported: major medical or psychiatric disorders; current use of psychotropic medications; use of intravenous drugs; pregnancy; and contra-indications for MRI. Except for smoking which was allowed until 2 hours before scanning, subjects had to abstain from psychoactive substances for at least two weeks and from alcohol for at least one week prior to testing. Compliance to abstinence was checked by urine drug screening (enzyme-multiplied immunoassay for amphetamines, ecstasy, opiates, cocaine, benzodiazepines, cannabis and alcohol).

Subjects were fully informed about the potential risk of ecstasy use in the study information letter, and gave their written consent according to the Helsinki Declaration. The local ethics committee approved the study. Subjects were paid for their participation (€150 per session of 2 days).

Procedure

At baseline all 96 subjects underwent fMRI scanning and completed validated self-report questionnaires about their drug use[148]. They were screened for axis I psychiatric disorders using the Dutch version of the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders[149]. Urine samples were collected and pre-morbid verbal intelligence was estimated using the Dutch version of the National Adult Reading Test[150]. After baseline examination, subjects were approached at regular intervals to fill out a questionnaire concerning drug use (four in total during a follow-up period of approximately 18 months). Within this follow-up period, 27 subjects started to use ecstasy. These subjects were invited for a second fMRI scan relatively soon after their first ecstasy use and with a maximum cumulative ecstasy dose of 10 tablets. As a consequence of the latter criterion, one subject who has used 20 tablets had to be excluded from the current study, leaving 26 novice ecstasy users for the analysis. The control group, also scanned a second time, consisted of 24 subjects selected from the initial baseline sample who did not use ecstasy within the follow-up period, based on age, gender, IQ, and history of cannabis use (for individual matching with the users). Urine drug screening and drug use questionnaires were repeated.

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[3] Advertisements were placed on the internet, on a special website for this study from the Academic Medical Center Amsterdam and by a pop-up advertising campaign on the Microsoft MSN Network.

Assessment of working memory, selective attention and associative memory

Three fMRI tasks were administered: a working memory task based on Sternberg's item-recognition paradigm (denoted STERN), a visuo-auditory selective attention task (SAT), and a pictorial associative memory task (PMT) that depends on (para)hippocampal brain function.

The STERN task involves memorizing sets of 5 letters (Fig. 1). Subjects have to decide whether subsequently presented letters belong to the set or not. Prior to scanning, subjects practiced on a specific set of letters for 21 minutes. During scanning, two experimental tasks were administered, which differed only with regard to the target-set(s): a novel set and a practiced set. In the practiced set task (PT) the specific set was used repeatedly. In the novel set task (NT), the composition of the target-set was changed after every run of 10 trials. An additional reaction time control task (CT) was included, as well as rest periods of equal epoch duration (for further details on the STERN we refer to Jansma et al.[62, 94] and Ramsey et al.[61]).

The SAT is an oddball detection task (Fig. 2) and involves detection of deviant stimuli (either tones deviant in pitch from a baseline tone, or dots deviant in size from a baseline dot). A threshold for detecting differences in pitch and dot-size was determined individually before the scan session, by adjusting it until the subject detected at least 80% of the deviant stimuli. Tones and dots were presented simultaneously during two experimental tasks in which subjects were instructed to attend either

to the tones while ignoring the dots (TO), or vice versa (DO) (for details we refer to Jager et al.[114]). Rest periods (RS) were intermixed.

The PMT is a pictorial memory task, modified from a paradigm from Henke et al.[63]. It involves three experimental tasks (Fig. A, page 100): (1) an associative learning task (AL), where subjects have to encode an association between two pictures; (2) a classification task, in which single item pictures have to be classified (SC); and (3) a retrieval task (RE), where subjects have to recall specific picture pairs previously presented during the associative learning task (for details we refer to Jager et al.[128]).
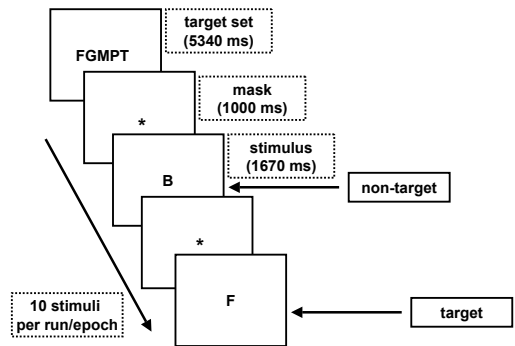

Figure 1 The temporal sequence of events is shown for the STERN task. Each epoch starts with presentation of the target-set, and is followed by ten trials. Subjects have to press a button as fast as possible, if the letter belongs to the target-set.
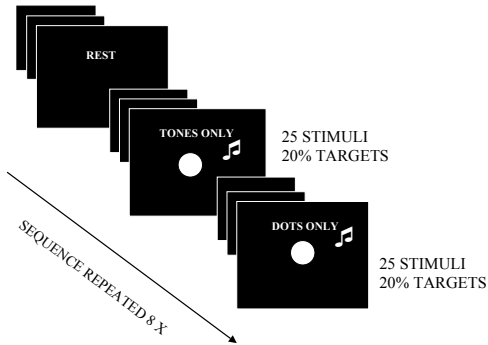
Figure 2 The temporal sequence of events for the SAT task. Each epoch (duration 29 s) starts with an instruction slide (5 sec), indicating 'rest', 'attend to tones only' or 'attend to dots only'. Both during 'tones only' and 'dots only' the instruction slide is followed by a series of 25 stimuli (simultaneous asynchronous presentation of tones and dots at a variable inter-stimulus interval rate) of which on average 20% deviant (targets). In case of a target, subjects have to press a button as fast as possible. Prior to fMRI scanning the difference between standard and deviant tones and dots is determined for each individual by changing the contrast until a performance of 80 % correct is obtained.

FMRI data acquisition

Scans were made on a Philips ACS-NT 1.5 Tesla MR-scanner with PT 6000 gradients, using a standard scan protocol (navigated 3D PRESTO[82]). Further details on the scan procedure and scan parameters for STERN, SAT and PMT have been previously described in Jansma et al.[62], Ramsey et al.[61] and Jager et al.[114, 128].

Data and data analysis

Demographic and drug use data

Various aspects of ecstasy use were assessed (frequency of use, cumulative number of tablets, duration of use (months between first and last ecstasy use) and abstinence (number of weeks since last ecstasy use). Additional drug use data included lifetime and last year use of cannabis (number of joints), amphetamine and cocaine (number of occasions), and last year alcohol and tobacco consumption (drinks/week versus cigarettes/week). Demographic variables included age, gender, and verbal IQ (see Table 1).

Table 1 Demographics and characteristics of ecstasy use and use of other drugs (mean ± SD)

	Ecstasy users (N = 25)		Controls (N = 24)	
	Baseline	Follow-up	Baseline	Follow-up
Gender	9M, 16F		8M, 16F	
Age[a+b]	21.8 ± 2.5	22.8 ± 2.7	21.2 ± 1.8	23.0 ± 1.9
DART-IQ[a+b]	103.4 ± 7.4		103.6 ± 10.0	
Ecstasy				
Cumulative dose (tablets)		2.0 ± 1.4		
Time since last use (weeks)		11.1 ± 12.9		
Duration of use (months)		1.2 ± 2.4		
Other substances (last year)[c+d]				
Alcohol (drinks/week)	8.9 ± 7.2	9.1 ± 7.6	8.9 ± 7.0	7.2 ± 5.5
Tobacco (cigarettes/week)	37.6 ± 50.8	23.8 ± 37.6	22.9 ± 42.1	22.5 ± 35.4
Cannabis (number of joints)	25.1 ± 38.5	31.6 ± 53.6	19.8 ± 31.9	22.4 ± 64.3
Amphetamine (number of occasions)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cocaine (number of occasions)	1.0 ± 2.2	1.4 ± 2.6	0.75 ± 2.0	0.25 ± 1.2

[a] baseline novice users vs controls p < 0.05 (t-test); [b] follow-up novice users vs controls p < 0.05 (t-test); [c] baseline and follow-up, novice users vs controls p < 0.05 (Kolmogorov-Smirnov Z); [d] novice users and controls, baseline vs follow-up p < 0.05 (Kolmogorov-Smirnov Z). Except for ecstasy use, no significant differences were found between groups or between sessions

Performance data

Outcome measures included performance accuracy (error rate for STERN, number correctly identified deviant stimuli for SAT, and percentage correct responses during RE for PMT) and reaction time (STERN only; measurements of mean reaction time during CT, PT and NT).

fMRI data

For all three tasks (STERN, SAT and PMT) data analysis included several steps. First, individual activity maps were generated for each of the task conditions compared to the rest condition by means of multiple regression analysis[83]. After registration to the standard Montreal Neurological Institute (MNI) brain[84], these individual maps were combined into a group map, creating a contrast of interest for each task and using z-statistics (for details see Jansma et al.[62]). For all contrasts, the statistical threshold for significant signal change was adjusted to yield separate regions of interest (ROIs) but always met the p < 0.05 level, corrected for the total

number of voxels in the brain. Using higher thresholds was necessary because with the large sample size (n = 49), ROIs merge when using the p < 0.05 threshold. For STERN, the contrast NT-CT eliminated activity not directly involved in working memory, and yielded several working memory-specific regions at a threshold of z = 6.0 (p < 0.01, corrected) with a cluster size of at least 10 voxels. For each of the ROIs, mean activity values were obtained for CT, PT and NT.

For SAT, ROIs were defined based on the TO-RS contrast z = 6.0 (p < 0.01, corrected, clusters > 10 voxels), and mean activity values were obtained for TO and DO.

For PMT, the contrast AL-SC eliminated activity not directly involved in associative learning, and yielded several ROIs at a threshold of z = 5.0 (p < 0.01, corrected, clusters > 10 voxels). For each of the ROIs, Δt-variables were computed, reflecting a measure for associative learning (contrasting AL with SC) and one for retriev-

al (contrasting RE with SC). Δt-variables were entered into statistical analyses.

The size of the (para)hippocampal ROIs was large, with activity extending into the fusiform gyrus. Because the (para)hippocampal regions are critically involved in associative memory processing, we added a second analysis to improve specificity by limiting the analysis to anatomically defined regions (right and left hippocampus, right and left parahippocampal gyrus). For this purpose, a segmentation procedure[151] was applied based on individual anatomical scans, yielding four separate clusters (right and left

hippocampus, right and left parahippocampal gyrus) marked for each subject separately (for details we refer to Jager et al.[128]). Then, per individual, two statistical activity contrast-maps based on an AL-SC and RE-SC contrast respectively, z = 3.0 (p < 0.05 corrected), were generated. After this, mean activity values within the four clusters for both contrast-maps were obtained for each subject.

ROIs for STERN, SAT and PMT are listed in Table 2. For each task separately, mean activity values and Δt-variables were entered into GLM repeated measures analyses.

Table 2 Brain regions of interest engaged in STERN, SAT and PMT respectively

Task	Region	Brodmann area	Number of voxels	X	Y	Z	Maximum z-value
STERN	l-SPC	7	213	30	-63	44	14.88
	l-DLPFC	9/46	192	42	5	28	13.03
	ACC	6/24	73	6	9	52	13.82
	l-FuG	37	64	46	-63	-12	10.03
SAT	r-IFG	47	224	-42	21	0	12.95
	l-AUD	41/42/22	157	58	-23	8	13.97
	ACC	8/24	130	-2	21	44	15.41
	r-AUD	41/42/22	98	-58	-31	8	13.66
	l-PcG	6	36	50	-3	44	9.94
	l-INS	13	12	10	34	21	9.55
SAT-attention [a]	VIS	23	252	-10	-71	8	8.01
	l-IFG	9/47	43	50	17	20	6.27
	l-AUD	42	33	60	-31	8	5.51
	r-AUD	42	23	-58	-27	0	6.13
PMT	r-PHG	37/36	153	-26	-47	-12	13.05
	l-PHG	37/36	134	26	-47	-12	12.29
	l-DLPFC	9	116	46	13	28	11.89
	l-MOG	19	98	34	-87	16	10.01
	r-MOG	18	95	-34	-87	12	10.76
	r-DLPFC	46	41	-50	29	20	7.37
	ACC	8/24	34	2	25	44	6.91
	r-IFG	47	27	-42	26	-8	6.62
	l-IFG	47	22	50	21	-8	6.54

MNI coordinates[304] are shown for the regions of interest involved in working memory (STERN), selective attention (SAT) and associative memory (PMT).
[a] shows the MNI coordinates for the regions where activity is modulated through attention during SAT.
The coordinates X, Y and Z represent location of the voxels with the highest z-value in the group map. Corresponding names and Brodmann areas are obtained from the location of voxels with the highest z-value. Abbreviations: 'l-' and 'r-' stands for left and right, SPC = superior parietal cortex, DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, FuG = fusiform gyrus, IFG = inferior frontal gyrus, AUD = auditory cortex, PcG = Precentral gyrus, INS = insula, PHG = parahippocampal gyrus, MOG = middle occipital gyrus, VIS = visual cortex.

Statistical analyses

One incident ecstasy user was excluded based on a positive urine test for cocaine, leaving 25 novice users for the analysis. Incidental technical malfunction of the MR-scanner or the computer used for task presentation, resulted in some missing or incomplete data. As a consequence datasets were not complete for each of the included 49 subjects on each task paradigm, and results are reported for each task separately with the number of subjects that were included within brackets.

To test whether ecstasy users differed from ecstasy-naive controls at baseline and at follow-up in terms of age, verbal IQ and use of other substances than ecstasy (cannabis, amphetamine, cocaine, tobacco and alcohol), t-tests and non-parametric Kolmogorov-Smirnov tests were applied.

To assess effects of ecstasy on task performance (accuracy, reaction times), GLM repeated measures analysis was applied for each task separately, with group as fixed factor, and with session (baseline, follow-up) and task condition as within-subject factors.

Effects of ecstasy on brain activity were also tested using GLM repeated measures analysis, with brain activity as dependent variable and session, task condition and ROIs (listed for all three tasks STERN, SAT and PMT in Table 2) as within-subject factors.

Dose-response measures were examined using correlation analyses (Pearson's r) in the group of novice ecstasy users with cumulative number of ecstasy tablets, duration of use, and duration of abstinence as predictor variables, and performance and brain activity as outcome variables. All analyses were performed using SPSS version 12.0.1 (SPSS Inc., Chicago, IL, USA).

Results

Sample characteristics and drug use data

Table 1 shows baseline and follow-up characteristics on demographics and substance use of subjects that participated in the follow-up session. Incident ecstasy users used 2.0 tablets on average (range: 0.5 – 6, median 1.5 tablets) in a mean period of 6.7 (SD 9.4) weeks during an average follow-up period of 15.9 (SD 4.6) months. The majority of novice ecstasy users had used ecstasy only once (N = 15/25; 60%). At baseline, the two groups of future ecstasy users and persistent ecstasy naive controls were similar in terms of gender distribution, age (p = 0.34) and verbal IQ (p = 0.93). Also, the two groups did not differ in terms of smoking, use of alcohol, cannabis, amphetamines, and cocaine (all p-values > 0.50). With the exception of ecstasy use, at follow-up, incident ecstasy users and controls were still very similar in terms of age and use of other drugs (all p-values > 0.40).

Performance Data

Performance data are summarized in figure 3. Working memory (N = 44; 20 incident ecstasy users, 24 persistent ecstasy-naive controls) Repeated measures analyses were performed on speed and accuracy of task performance

as dependent variables, group (novice users, controls) as fixed factor and session (baseline, follow-up) and task (CT, PT and NT) as within-subject factors. These analyses yielded no significant group differences. Apart from a marginally significant 3-way interaction for reaction time, between session, task and group $(F(1,41) = 2.65, p < 0.10)$, all other main or interaction effects of initial ecstasy use showed p-values > 0.30.

Selective attention (N = 43: 24 incident ecstasy users, 19 persistent ecstasy-naive controls)
Repeated measures analysis revealed no significant effects of incident ecstasy use on thresh-

old-values for detecting 80% of the targets. Performance accuracy was similar for both groups (all p-values > 0.20).

Associative memory (N = 45; 24 incident ecstasy users, 21 persistent ecstasy-naive controls)
Repeated measures analysis with performance accuracy as dependent variables, group as fixed factor, and session and task (SC, RE) as within-subject factor revealed no significant effects of group (p > 0.25), indicating that simple classification and recall accuracy were not affected by incident ecstasy use.
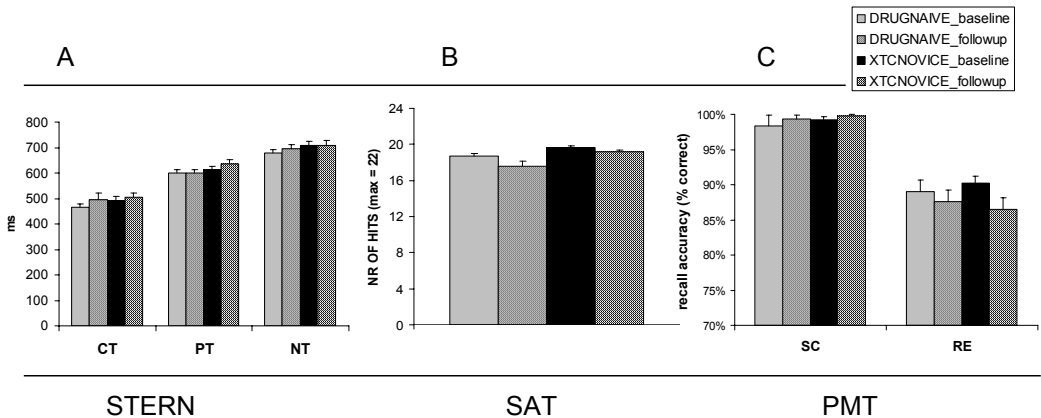

Figure 2 Task performance on the STERN, SAT and PMT during baseline and follow-up for initial ecstasy users and persistent ecstasy-naive controls. A = reaction time (milliseconds) for reaction time control task (CT), the practiced task (PT) and the novel task (NT). B = accuracy during SAT (number of correctly identified targets during tone and dot detection). C = accuracy during PMT (percentage correct answers) for the simple classification control task (SC) and the recognition task (RE).

Brain Activity

For STERN (N = 49), based on the NT-CT contrast, four ROIs reached significance in the frontal, parietal, and the fusiform gyrus. For SAT (N = 47), based on the TO-RS contrast, six areas in the frontal, auditory and visual cortex were activated above threshold. In addition, the TO-DO contrast yielded four areas in the inferior frontal, visual and auditory cortices where brain activity was modulated by attention. Finally, for PMT (N = 49) the group contrast map (AL-SC) yielded nine ROIs in the (para)hippocampal regions bilaterally, the frontal and the occipital cortex. There was some overlap in ROIs between tasks. The anterior cingulate cortex was activated during all three tasks, whereas the right inferior frontal gyrus and the left dorsolateral prefrontal cortex was activated both during STERN and PMT. Table 2 and figure E (color illustrations, page 104) shows ROIs, their MNI coordinates (X, Y, Z) and peak activity values for each task. GLM repeated measures analyses of the fMRI signal in the ROIs were conducted for each task separately.

Working memory-related brain
activity (STERN)
Whole brain analysis
Two contrasts were examined. First, working memory activity (NT versus CT) was compared between groups, with session and ROIs (Table 2) as within-subject factors. There were no significant differences between groups (all p-values > 0.20), indicating that incident ecstasy use did not affect patterns of brain activity in the engaged network. Next, the effect of practice (NT versus PT, reflecting flexibility

of the WM system[62] was compared. This also revealed no significant differences between groups, indicating that ecstasy did not affect the way practice reduces activity in the WM network.

Selective attention-related brain
activity (SAT)
Whole brain analysis
Selective attention activity (based on the contrast TO versus RS) was compared between groups, with session and ROIs (Table 2) as within-subject factors. We found no significant effects of group (all P-values > 0.50) indicating that initial ecstasy use did not affect patterns of brain activity in the involved network. Then, the modulating effect of attention on brain activity was compared between groups, with session and SAT-attention ROIs (Table 2) as within-subject factors. Again, no significant differences between groups were found (all P-values > 0.50).

Associative memory-related brain
activity (PMT)
Whole brain analysis
Similar to STERN, for PMT also two contrasts were examined. First, associative learning-related activity (AL versus SC) was compared between groups, with session and ROIs (Table 2) as within-subject factors. No significant differences between groups were found (all p-values > 0.50). Second, brain activity related to recall (RE versus SC) was compared, also yielding no significant differences between groups (all p-values > 0.20). This indicates that novice ecstasy use did not affect associative memory activity in the engaged network.

Region of interest analysis

Next, analysis was restricted to four anatomically defined regions, i.e. right and left hippocampus, and right and left parahippocampal gyrus. Again, two contrasts were examined: AL versus SC and RE versus SC, also not yielding significant differences between initial ecstasy users and persistent ecstasy-naive controls (p-values > 0.20).

Correlations between ecstasy use and brain activity

Within the group of novice ecstasy users (N = 25) we examined possible associations between two indices of ecstasy use, cumulative dose (total number of ecstasy tablets at follow-up) and weeks since last ecstasy use, and outcome variables (performance and brain activity). To avoid type I errors the level of significance was Bonferroni corrected, i.e. using a more stringent p-value adjusted for the number of ROIs. Duration of use was not included as an ecstasy use variable, because the majority of subjects had used ecstasy only once (N=15, 60%) and thus scored zero on this variable.

Task performance

No significant correlations were found between performance and cumulative dose or weeks since last use for all three tasks.

Brain activity

In line with the results on performance, we found no significant correlations between cumulative dose and weeks since last ecstasy use, and brain activity in any of the regions of inter-est for STERN, SAT and PMT.

To examine whether the exclusion of one novice ecstasy user with a cumulative dose of 20 ecstasy tablets at follow-up did affect the results, all above described statistical analyses were re-run, this time including this particular subject. The main findings remained unchanged, as no significant effects of initial ecstasy use on performance and brain activity for all three tasks were found. There was only one tentative effect observed regarding the region of interest analysis for PMT. Including this subject with a cumulative dose of 20 tablets yielded a marginally significant effect ($F(1,48)=2.80$, p= 0.10) of initial ecstasy use on overall brain activity in the four anatomically defined hippocampal and parahippocampal regions. Ecstasy users tended to reveal slightly lower levels of brain activity in these regions than controls.

Discussion

The present fMRI study prospectively examined the non-acute effects of a single or low dose of ecstasy on cognitive brain function. We assessed task performance and brain activity patterns during working memory, selective attention, and associative memory in novice (but at least two weeks abstinent) ecstasy users before and after a period of first ecstasy use and compared these data with the same baseline and follow-up measurements of matched controls who did not use ecstasy during the follow-up period. We did not find firm evidence for sustained effects of initial ecstasy use

on task performance in the domains of memory and attention. Also, no effect of incident ecstasy use was found on brain activity in the brain systems engaged in working memory, attention, or associative memory.

To date, this is the first prospective fMRI study on sustained effects of low dose ecstasy use on cognitive brain function. Therefore, we can only compare our results with findings from studies investigating the (mainly acute) effects of MDMA on human neuropsychological function. In a recent review, Dumont and Verkes[9] reported on all placebo-controlled studies that administered MDMA to healthy humans. Eleven tests in the attention domain were evaluated but none of them had generated any significant effect. In the executive function domain the literature search yielded only one study by Lamers et al.[152], which reported no effects of MDMA on visual search, planning or retrieval from semantic memory. Surprisingly, there are no studies about the acute effects of MDMA on memory, even though cross-sectional studies into the long-term effects of ecstasy on human cognition, most consistently report this domain to be impaired[8].

We are aware of several limitations of the current study. For one, although prospective, the study design was naturalistic and not experimental. Therefore, there is uncertainty about dosage and purity of ecstasy tablets and we had to rely on statements of the subjects themselves. However, because both novice users and persistent ecstasy-naive controls were included for follow-up testing from the initial baseline cohort, we have no reason to question the truthfulness of their statements. Furthermore, pill-testing confirms that in The Netherlands more than 95% of the tablets sold as ecstasy contain MDMA as the sole (91.2%) or main (4.2%) psychoactive component[153]. Another limitation is that the sample was not randomly selected. Therefore, we cannot claim it to be statistically representative of the population of young people on the brink of experimenting with ecstasy. Yet we do believe it is sufficiently varied, since we contacted candidates in many different places and ways. Moreover, in view of the specific demands of the study (a fairly demanding research project, including extensive brain imaging and blood sampling), even an initially random sample would have almost certainly reached a selective group in the end. Thirdly, the pattern of use, the environmental circumstances during ecstasy consumption (ambient heat, dehydration) and the possible interaction with other substances, for instance alcohol, nicotine, cannabis, cocaine or amphetamine (although use of the latter two was minimal), were not investigated. Therefore, we cannot exclude the possibility of confounding interactions between ecstasy and other drugs. More specifically, it has been suggested that cannabis attenuates the neurotoxic effects of ecstasy, as cannabis and ecstasy may have opposite effects on oxidative stress[155]. Ecstasy is thought to cause increased oxidative stress, thereby enhancing the risk for serotonergic neurotoxicity[1], whereas cannabis may act as an antioxidant[154] and therefore possibly has neuroprotective effects[155].

Finally, our failure to detect effects of a low dose of ecstasy on memory and attention and

related brain function may be due to insufficient sensitivity and specificity of the task paradigms and the fMRI technique that was used. In this regard, it is important to note that in a previous study from our own laboratory, we did observe decreased memory performance and altered patterns of brain activity in various brain regions involved in associative memory in (poly-substance) frequent ecstasy users, applying exactly the same fMRI task paradigms and scanning procedures[147]. FMRI has been shown to be a sensitive tool in detecting neurocognitive impairments at the very early stage of multiple sclerosis[156], in patients with various neuropsychiatric disorders, i.e. schizophrenia and obsessive compulsive disorder[94, 157, 158], and in substance users, i.e. cannabis users, tobacco smokers and alcohol users[76, 159, 160].

In conclusion, the present results provide no firm evidence for sustained effects of a low dose of ecstasy use on working memory, selective attention, and associative memory, neither at the behavioral level nor at the neurophysiological level. The present findings are relevant for the development of prevention and harm reduction strategies, i.e. prevention of any drug use might be the preferred objective, but in many cases harm reduction might be the only realistic option. It should be noted, however, that the result of a single prospective study using just one of the many available neurotoxicity detection techniques in a population with a rather narrow dose range is not sufficient for an evidence based harm reduction strategy. Therefore, monitoring of the current sample is worthwhile, because the expected increase in variation in dosage, frequency and duration of ecstasy use within this group of novice users presents us with a unique opportunity for further research. In addition, more research with fMRI and other neuroimaging techniques into the acute and sustained effects of MDMA on cognitive brain function is needed.

Chapter 7
Summary and
discussion

In this final chapter, a summary and general discussion is provided of the findings of the current fMRI study, followed by some methodological considerations. The chapter continues with implications and directions for future research and ends by placing the fMRI studies in a broader context, by giving a short overview of the preliminary findings of the NeXT study at large.

Research questions

Cannabis and ecstasy (MDMA) are among the most widely used recreational illicit drugs in the world, especially by young people[13]. Both substances have in common that they have generated substantial concern about their neurotoxic potential for brain and brain function. On the other hand, in recent years we have witnessed a revival of interest in the possible beneficial actions of both drugs in several serious medical conditions, i.e. as an analgesic or antiemetic drug (cannabis)[14, 15] or to reduce anxiety, tension or agitation (ecstasy) in patients with post-traumatic stress disorder or in last stage cancer patients[17, 161].

In the past twenty years, numerous (animal and human) studies have reported on the neurotoxicity of cannabis and ecstasy on the brain (for review see[3,9, 21, 42, 162]).

However, some crucial questions regarding the causality, course and clinical relevance remain unanswered and few fMRI studies have been done on the effects of ecstasy and/or cannabis on human cognitive brain function. The aim of this thesis was to investigate whether ecsta-sy and its most commonly used illicit co-drug cannabis causes sustained effect on cognitive brain function in humans, and to this purpose we employed functional magnetic resonance imaging (fMRI). In line with the larger NeXT research project (Netherlands XTC Toxicity study) the main objectives were to study 1) the causal role of ecstasy in reported brain pathology in humans; 2) the long-term course of such brain pathology and related clinical characteristics in ecstasy users; and 3) the clinical relevance of reported brain pathology in ecstasy users. An important fourth objective of this thesis was to study the potential neurotoxic consequences of ecstasy use in relation to the use of other drugs.

Summary

Cannabis
We studied the sustained effects of moderate to frequent cannabis use in at least one-week abstinent cannabis users (median 1,300 joints; range 675 – 5,400 joints lifetime) on working memory and attention, both in terms of performance and related brain activity in the network of brain regions involved in these cognitive processes. No firm evidence was found for long-term deficits in working memory and attention. However, for working memory a subtle abnormality was found in the dynamics of brain activation in response to working memory load in the left superior parietal cortex, in that cannabis users, compared to controls, failed to reduce activity in this region in response to practice, suggesting that they tend to over-activate this region. Given that the effect was subtle, it requires replication before

we can interpret this finding. In addition, we found sustained effects of frequent cannabis use (median 1,900 joints; range 675 – 10,150 joints lifetime) on hippocampus-dependent associative memory function, and there was some indication of structural changes in the (para)hippocampal brain region in cannabis users. The (para)hippocampal region is critically involved in this type of memory, and due to its high density of cannabinoid receptors is thought to be specifically vulnerable to potential neurotoxic effects of cannabis. We found that although the cannabis users performed equally well as non-using controls during the associative memory task, they showed significantly lower levels of brain activity in the regions involved in associative memory, most explicitly in the (para)hippocampal regions and the right dorsolateral prefrontal cortex. However, lower brain activation was not correlated with changes in (para)hippocampal tissue composition and was unrelated to task performance. We therefore argue that lower brain activation may not signify neurocognitive impairment, but could be the expression of a non-cognitive variable related to frequent cannabis use, for example sustained changes in cerebral perfusion or differences in vigilance.

Ecstasy
Heavy ecstasy users invariably use other drugs as well, which makes it difficult to disentangle the effects of ecstasy from the effects of other drugs. We investigated the sustained effects of ecstasy on human cognitive brain function in the context of poly-substance use, i.e. in subjects using cannabis as well as amphetamine, cocaine, alcohol and tobacco, in addition to ecstasy (median 250 tablets; range 15 – 2000

tablets lifetime). A large stratified sample with considerable variation in type and amount of drugs that were used, was examined on working memory, attention and associative memory brain function after at least two weeks of abstinence. In the statistical analyses we included all drugs mentioned, together with non-drug variables such as gender and IQ as separate predictors in a regression model to predict variation in task performance and brain activity. The findings indicated that drug use was associated with reduced performance and altered brain activity for associative memory, but had little effect on working memory and attention. Importantly, associative memory performance was affected by amphetamine more than by ecstasy. Both drugs affected brain activity, but the effects were consistently in opposite directions. These findings suggest that previously reported sustained effects of ecstasy on memory might be due to concomitant use of amphetamine rather than to ecstasy. The finding that ecstasy and amphetamine use affects brain activity differently indicates that separate mechanisms are at play, possibly associated with their differential effects on serotonin (ecstasy) versus dopamine (amphetamine) systems.

Apart from negative sequelae of frequent ecstasy use reported in literature there is concern that even a single or a low dose of ecstasy may be neurotoxic. We designed a prospective fMRI study where we examined sustained effects of a low dose of ecstasy on cognitive brain function in 25 subjects before and after their first episode of ecstasy use. During follow-up they had consumed an average of 2.0 ecstasy pills (range 1 – 6) and last use was on average 11 weeks before follow-up testing. They were

compared with 24 persistent ecstasy-naive controls, also measured twice, and matched with the novice users on age, gender, IQ, cannabis use and use of other substances (cocaine, amphetamine, alcohol, tobacco). With this particular prospective design we solved an important methodological problem that affects many previous studies on ecstasy, namely the lack of ecstasy-naive baseline data. Our results showed no firm evidence for sustained effects of a low dose of ecstasy on working memory, selective attention or associative memory, neither at the behavioral nor at the neurophysiological level.

General discussion

Despite previous reports on ecstasy-induced serotonergic neurotoxicity in animals and humans, the results of the studies presented in this thesis showed surprisingly little evidence for functional consequences in terms of abnormal cognitive brain function, even after consumption of considerable amounts of ecstasy (mean 250 tablets in the study among heavy users). Regarding memory performance, we found that even in heavy ecstasy users, the effects of ecstasy were quite moderate in comparison with those of amphetamine, which significantly reduced associative memory performance and in addition altered brain activity patterns. These findings suggest that animal studies may not reflect circumstances of human ecstasy use, and that human reports on memory deficits in heavy ecstasy users may have been confounded with the effects of amphetamine use. Another explanation might be that fMRI is not the most sensitive technique

to detect ecstasy-related damage to the brain. Taken together, we have no grounds to conclude that ecstasy is entirely free of harmful effects since it did affect memory-related brain activity in heavy users (which may be an early marker of cognitive deficits later in life). The fact that these effects are distinctly different from those of amphetamine most likely reflects the different neurotransmitter systems these drugs act upon. With regard to concomitant cannabis use, the studies presented in this thesis yielded no support for the notion that cannabis may be (in part) responsible for the observed cognitive deficits or changes in brain activity patterns in recreational ecstasy users. Thus, our studies indicate that the cognitive decline reported in ecstasy users is more likely to be caused by concomitant amphetamine use, as opposed to ecstasy or cannabis.

The primary aim of the studies described in this thesis was to expand our knowledge concerning yet unanswered questions about the causality, course and clinical relevance of the potential neurotoxicity of ecstasy.

The question of causality is one of the most difficult ones in this type of research. The typically employed cross-sectional and retrospective designs cannot contribute much to this question because they lack baseline data acquisition (required to assess drug-induced changes) as well as means to control potential confounders adequately. The present prospective fMRI study in incident ecstasy users and persistent ecstasy-naive controls yielded no clear effects of a low dose of ecstasy on cognitive brain function. Then how does this study contribute to the debate of ecstasy neurotoxicity? Firstly, the design excludes

several confounds that could cause effects by themselves, resulting in apparent effects of ecstasy: subjects were matched for age, gender, verbal IQ, use of cannabis and other drugs. Second, the study did not lack power, because subjects were their own controls (repeated fMRI scans), and groups were of considerable size (25 novice ecstasy users, 24 persistent ecstasy-naive controls). Nevertheless, neurotoxic effects may be below detection threshold, and may emerge only after an accumulation of doses. We intend to follow up this prospective cohort sample to address this question, since it is expected that the variation in dosage, frequency and duration of ecstasy use will increase in time in this group of novice users.

Concerning the research question on the course of the potential neurotoxicity of ecstasy for brain function, abstinence periods of approximately two weeks in the study on heavy ecstasy use were too short to allow for assessment of the permanence of changes in brain function. In the prospective cohort study time since last ecstasy use was somewhat longer (11 weeks on average with a minimum of 2 weeks), resulting in a somewhat larger timeframe. However, a firmer conclusion on the course of ecstasy-induced effects on cognitive brain function must await future work on the data from the retrospective fMRI study and is therefore beyond the scope of this thesis.

With regard to clinical relevance it can be concluded that the observed effects of ecstasy on cognitive brain function reported in this thesis are quite moderate, even after consumption of on average 250 tablets. In two out of three cognitive domains, i.e. working memory and attention, we could not demonstrate any functional effects of ecstasy on task performance or related brain activity patterns, whereas the observed reduction in performance during the associative memory task was largely due to amphetamine rather than to ecstasy use. The effects of ecstasy on memory-related brain activity in a fronto-visual network were statistically significant and may reflect long-term adaptation or compensatory reorganization. However, as the alterations in brain activity did not result in a reduced performance, the clinical relevance of this finding seems limited. It is worth mentioning, however, that we do not know whether these alterations in brain activity are temporary or may persist, even after long-term cessation of the drug. In the latter case, these alterations in brain activation may in time gain clinical relevance by adding to the decline in cognitive brain function due to normal aging. This combination might result in premature cognitive aging in (former) frequent ecstasy users.

Methodological considerations

The NeXT study is driven by the determination to design the experiments in such a way that the experiments produce a significant contribution to the questions that are posed. We went to great length to select subjects on stringent criteria, and to obtain adequate numbers of participants for detection of subtle effects. Given the significant emphasis we put on design of the studies, we will discuss methodological strengths and weaknesses in greater detail. After all, methodology determines to a great extent the validity of the findings.

The most prevalent methodological problems in naturalistic human studies on potentially harmful effects of drugs of abuse pertain to subject selection (homogeneity of drugs used, quantities, abstinence periods, matched controls), study design, choice of dependent variables, sample size, and reliability of self-reports on use. The fact that only cross-sectional and retrospective designs are used which lack baseline data complicates interpretation in that many confounding factors cannot be accounted for[9]. This list of methodological problems can serve as a framework to summarize the strengths and limitations of the present study.

Subject Selection

All groups of subjects were carefully screened to exclude subjects with substantial use of alcohol or other drugs than those under investigation in a particular study, current psychiatric or medical disorders, use of psychoactive medications such as serotonin reuptake inhibitors (SSRI). Furthermore, all subjects were recruited from the same social settings and networks to facilitate matching of the control subjects to the drug users on factors such as lifestyle, age and gender distribution, and educational level. Nonetheless, we cannot exclude the possibility that users and non-users differed on some other factors than the substance use itself. Also, samples were not randomly selected. For the sub-study on effects of heavy ecstasy use in the context of poly-substance use, we intentionally searched for the proverbial 'needle in a haystack', i.e. heavy ecstasy users without the typical poly-drug use pattern or poly-drug using controls with (almost) no previous ecstasy use, to meet the requirements of an orthogonal regression model. For the prospec-

tive cohort sub-study, recruitment strategies included a combination of targeted site sampling at locations such as universities, colleges, dance events, discotheques, youth fairs and parks; advertisement through a website on the project and an Internet campaign; and word-of-mouth recruiting[41]. Therefore, we cannot claim samples to be statistically representative of the population of young people on the brink of experimenting with ecstasy. Yet we do believe it is sufficiently varied, since we contacted candidates in many different places and ways. Moreover, in view of the specific demands of the NeXT study (a fairly demanding research project, including extensive brain imaging and blood sampling), even a completely random sampling would most likely yield a selective group in the end.

Abstinence periods previous to testing varied between studies from at least one week for cannabis and alcohol in both studies in selective cannabis users (chapters 3 and 4) to at least two weeks for ecstasy, cannabis and other psychoactive drugs, and one week for alcohol, in the ecstasy studies (chapters 5 and 6). Compliance to abstinence was checked by urine drug screening on the day of testing. Although longer abstinence periods might have been preferable in examining the long-term effects of cannabis and ecstasy on cognitive brain function, the choice for abstinence periods of at least one or two weeks was also motivated by practicality and feasibility, in view of the limited willingness among frequent users of a particular substance to abstain from drug use in order to participate in the study. Nevertheless, based on evidence from previous studies we believe that the abstinence

periods were sufficiently long to rule out acute pharmacological effects of ecstasy, cannabis or other psychoactive drugs taken by the subjects. This was supported by negative urine tests for all subjects included in data-analysis. Nonetheless, in terms of investigating long-term effects on cognitive brain function, an abstinence period of at least one or two weeks has its restrictions, hence our use of the terms 'sustained' or 'sub-clinical' effects.

Study design

Clearly, one of the strengths is the study design. To illustrate, in the cross-sectional study in heavy ecstasy users, we applied an unusual approach to deal with the confounding problem of poly-drug use. Innovative was the careful composition of a large stratified sample of subjects with substantial variations in the amount and type of drugs used instead of a random recruitment among heavy ecstasy users. The subsequent multiple regression model with ecstasy and other drugs as separate predictors of outcome variables, enables one to disentangle the effects of ecstasy and the effects of other drugs, and solves the problem of finding relatively large numbers of proper controls for drugs used other than ecstasy. Also, the prospective study in novice ecstasy users is characterized by a unique design. The idea of acquiring baseline data in a sample of subjects with a relatively high risk for first ecstasy use in the near future, in combination with a longitudinal follow-up may not be new, but as far as we know, the NeXT project is the first to succeed in this approach. Our approach in identifying a cohort of young people who were not yet ecstasy users but belonged to a high-risk group for future ecstasy use, paid off.

A high risk for future ecstasy use was defined as a 'positive' intention to use ecstasy for the first time in the near future if an opportunity did occur, and/or having friends who had previous experience with ecstasy use. Moreover, the NeXT project, in being a joint venture of three institutions, anticipated on and provided for the necessary testing capacity to carry out such an arduous study.

Dependent variables

A further strength of the studies presented in this thesis is the use of fMRI to assess the effects of ecstasy and cannabis use on cognitive brain function. One of the important advantages of fMRI over behavioral measures of cognitive brain processes is that fMRI can reveal abnormalities in the organization of brain networks, that may occur as an adaptive response to brain abnormalities and may be difficult to detect in behavior. That fMRI fulfilled its promise in the present studies is illustrated by the findings of changes in brain activity in frequent cannabis and ecstasy poly-drug users who were able to maintain normal task performance. However, a drawback of the use of fMRI is that to date, it has been sparsely applied in the field of ecstasy research. Therefore, our failure to detect effects of a low dose of ecstasy on cognitive brain function may be due to insufficient sensitivity and specificity of the technique and/or the fMRI-task paradigms used. In this regard however, it is worth mentioning that fMRI has been shown to be a sensitive tool in detecting neurocognitive impairments at the very early stage of multiple sclerosis[156], in patients with various neuropsychiatric disorders, i.e. schizophrenia and obsessive compulsive disorder, e.g.[94, 157, 158], and also

in substance users, i.e. cannabis users, tobacco smokers and alcohol users[75, 76, 159, 160].

Sample size

In at least three out of four studies presented in this thesis sample size is adequate regarding the issue of proper statistical power. Twenty subjects or more per group is quite sufficient for fMRI[167]. However, we could have done better in the study on the effects of cannabis on working memory and attention where we compared relatively small samples of 10 users with 10 controls. Furthermore, for other analyses techniques incidentally used, for instance voxel-based morphometry (VBM) in general larger samples are required. As a result, the VBM findings have to be considered with caution, as has already been discussed in chapter 4.

Reliability of self-reports

Verification of drug usage - In the studies presented in this thesis, with a naturalistic as opposed to an experimental design, it was impossible to determine exactly which drug had been used and in which dose. Drug urine screening supported compliance to the required abstinence from psychoactive substances in the one or two weeks immediately prior to testing. But for drug use beyond those one or two weeks before testing we had to rely on self-reported data from both frequent cannabis users and heavy ecstasy (poly-substance) users. Unfortunately, in a naturalistic design there is no obvious solution to this problem. Also in the prospective sub-study we had to rely on self-report and there was no external validation of dosage and purity of ecstasy tablets. However, because both novice users and persistent ecstasy-naive controls

were included for follow-up testing from the initial baseline cohort, we have no reason to question the truthfulness of their statements (i.e. subjects were told that ecstasy use did not affect the chance to be called in for re-testing). Furthermore, in The Netherlands the chemical composition of an ecstasy tablet is fairly well known at the time of the study, because of the Drugs Information and Monitoring System (DIMS), a project dedicated to pill testing and monitoring the ecstasy market. Over the period 2001-2004 more than 95% of the tablets sold as ecstasy contained MDMA as the sole (91.2%) or main (4.2%) psychoactive component, whereas the proportion of pills containing (also) another psychoactive substance such as amphetamine, decreased[153].

Restricted dose ranges – When recruitment started in 2001 no fMRI studies were yet published on the effects of ecstasy on cognitive brain function. Therefore, to maximize our chances to detect clear interpretable effects of ecstasy with fMRI, we decided on an inclusion criterion of a cumulative dose of at least 100 tablets lifetime for heavy users. In contrast, the amount of ecstasy consumed by the incident users in the prospective fMRI sub-study was very low (range 1-6 tablets). As a consequence, this thesis lacks a study of the potential effects of intermediate dosages of ecstasy on cognitive brain function, i.e. a cumulative dose between 10-100 pills. Whether this would have changed our main conclusions is unclear. Considering prevention messages, clinical decision making, and the development of an (inter)national ecstasy policy, this intermediate dose range is of interest because many of the recreational ecstasy users nowadays,

i.e. those with a more moderate and regulated pattern of use but who continue use for some time, will probably reach a lifetime cumulative dose within this range of 10-100 pills.

Implications and future directions based on the fMRI findings

This thesis started with the assumption that ecstasy and cannabis have a neurotoxic potential. Consequently, functional sequelae including cognitive disorders ought to be expected in humans following ecstasy or cannabis-induced neurotoxic brain damage. If the results from the present study had confirmed the presence of functional consequences for cognitive brain function, then the implications would have been clear. In that case, prevention messages and clinical decision-making should all be directed at warning young people to stay away from ecstasy and cannabis, and the clinical trials with MDMA and cannabis in certain patient groups should be reconsidered. However, the results of the studies presented in this thesis were to some extent surprising and contrary to our expectations. In the following section the focus will be on ecstasy, but many of the implications and directions of future research also apply to cannabis.

Despite claims of MDMA-induced neurotoxicity and associated cognitive deficits in ecstasy users, the results of the present fMRI studies showed less evidence for functional consequences in terms of abnormal cognitive brain function than we hypothesized, even in subjects who consumed considerable amounts of ecstasy. Therefore, the studies presented in this thesis cannot bring the existing controversy concerning MDMA neurotoxicity or MDMA-induced cognitive deficits in humans[42, 140] to an end.

Nevertheless, some implications can be formulated. For one, research on MDMA-induced neurotoxicity and associated cognitive deficits is not exempt from the rules of scientific scrutiny, particularly with regard to confounding effects of other drugs of abuse, and to the clinical significance of findings. Reports of abnormalities in ecstasy users are often used in public media without due regard for these issues, fuelled by emotional or political judgment in which some messages are more welcome than others. As Lyvers[140] put it, " - uncritically reported claims of ecstasy-induced brain damage - as an approach to preventing illicit drug use serves only to undermine the credibility of the relevant authorities -."

Second, our results are based on samples of young, healthy, generally well-educated volunteers without any serious psychological or medical problems. It may be harder to detect subtle abnormalities in cognitive brain function after MDMA exposure in this specific population of youngsters, as they may have more cognitive reserve and show resistance to damage brought about by ecstasy use, or at least to the functional consequences associated with serotonin neurotoxicity[163-165]. More research is needed to gain further insight in the so-called protective and risk factors for potential neurotoxicity of ecstasy, and future studies could be directed in a number of different directions. For example, an aspect that has already

received some attention in the literature is functional polymorphisms of genes involved in coding for serotonin transporters in the brain[55, 166]. Some alleles may increase the particular risk for ecstasy-induced neurotoxicity whereas others could have some protective properties. Other risk factors include the setting in which ecstasy consumption takes place (ambient temperature, physical exertion, dehydration), different dosage schemes, in particular 'binge' schemes, or the interaction between ecstasy and other illicit or prescribed drugs.

A third implication is for future efforts and funding in ecstasy research to focus on longitudinal and prospective studies instead of a continuation of studies in samples that retrospectively investigate the possible long-term effects of ecstasy in humans. Also, more human studies are needed on the acute and long-term effects of incidental or low to medium dose ecstasy use with different neuroimaging techniques and neuropsychological and psychiatric assessment to facilitate convergence of evidence.

Studies in humans can and sometimes must be strengthened by studies in animals due to legal and ethical restrictions in human research on ecstasy, but we should keep in mind the complexities of interspecies scaling and differences in interspecies pharmacokinetics.

Finally, clinical trials with MDMA in patients (despite the ongoing and fierce debate on whether it is ethically justified and/or safe to do so) can also be of value in extending our knowledge on the effects of regulated and low dose ecstasy use on the human brain and brain function.

Findings from the NeXT study at large

The fMRI studies presented in this thesis are part of the NeXT study and besides fMRI, the same subjects underwent extensive neuroimaging using other techniques, i.e. structural MRI, [123]β-CIT SPECT (measuring serotonin transporters); ^{1}H-MR spectroscopy; diffusion tensor imaging (DTI); perfusion weighted imaging (PWI), and neuropsychological and psychiatric assessment of memory, depression, and personality. Results based on these techniques are or will be published in separate papers. To place the fMRI findings in the broader context, the main preliminary findings from these other neuroimaging and neuropsychological studies are summarized below.

In the study with heavy ecstasy users, neuropsychological testing indicated that verbal memory was affected by ecstasy. A reduction of performance was observed that was statistically significant, and correlated with estimated amount of ecstasy used. The clinical relevance is not clear because performance of heavy ecstasy users remained within the normal range, but the reduction in performance might be an indication of a more severe deficit later in life (e.g. an accelerated ageing effect). Brain imaging indicated an effect of heavy ecstasy use on the thalamus: a reduction of serotonin transporters, an increase in blood volume and a reduction of fractional anisotropy. These converging multimodal findings could indicate a change in the neurochemical make-up of the thalamus, with structural and functional sequelae after extensive use of ecstasy. In the prospective study, several differences were observed between novice users and controls. Again, only verbal memory was affected, albeit in an indirect way: inci-

dental ecstasy use affected the way performance changes as a consequence of the test-retest learning effect (i.e., statistical interactions between ecstasy use and test-retesting). Brain imaging results indicated a reduction of fractional anisotropy in the thalamus and various other effects in other regions. The relevance of the latter findings is not clear yet, because they were not found in the study involving heavy ecstasy users. Thus, it appears that ecstasy affects verbal memory performance, and induces changes in the thalamus.

If these findings are to be taken at face value, one would have to conclude that recreational ecstasy use (even incidental) should be strongly discouraged until future studies prove otherwise, and clinical trials with MDMA in patients ought to be reconsidered in light of this new evidence. However, given the fact that the NeXT study entails an interconnected set of studies with a heavy emphasis on methodology, a more sophisticated discussion is warranted to fully exploit the body of results.

It is clear that the findings (and consequently the main conclusions based on these findings) of the fMRI studies and the other neuroimaging and neuropsychological findings do not converge, causing a dilemma with regard to answering the primary question of the NeXT study: is ecstasy neurotoxic in recreational use? The fMRI prospective study reports no robust effects of low dose ecstasy use on cognitive brain function, neither at the behavioral nor at the neurophysiological level, and can be taken to endorse prevention messages that aim at harm reduction by preventing excessive ecstasy use or use with a risky pattern ('binge-use')

instead of prevention of any recreational ecstasy use. In addition, the absence of clear negative effects on cognitive brain function is interpreted as encouraging news considering the clinical trials with MDMA in patients with serious psychiatric or medical diseases, to investigate its possible beneficial properties in psychotherapy or symptom alleviation. This seems to be at odds with the observations of sustained changes in the thalamus and a decline in verbal memory function, even after a single or low dose of ecstasy. The different perspectives touch upon the key issues that dominate the discussion in literature. Although the NeXT study is still in progress, and a comprehensive final conclusion has not been yet drawn, we can comment on the points of debate.

For one, there is effect size. What does the size of effects mean for the concept of neurotoxicity? The effects of ecstasy on the thalamus were subtle, with the exception of the reduction of serotonin transporter density in heavy users. Moreover, there were no clear behavioral effects of ecstasy use, considering that heavy users still performed within what is regarded as the normal range on the verbal memory test (the only test that showed a significant effect). Without robust functional consequences, even after extensive use of ecstasy, in terms of clinically relevant impaired cognition, mood disorders or any other brain function in which the serotonin system is involved, the question remains whether this signifies irreversible damage to the brain. If one does regard this as evidence for neurotoxicity, then how does one express the severity of that effect? One way of doing so is to draw a comparison with another popular, but socially accepted, psychoactive

substance like alcohol. Alcohol, even in moderate social drinkers, is known to be toxic to neurons in the brain, but obviously that does not stop people from using it. It is clear that the only way to quantify neurotoxicity in the context of consequences for human mental health is to assess behavioral and psychopathological effects in elderly (former) users, to make up the balance. This is not to say we should wait and see what becomes of the current young recreational ecstasy users. Ecstasy became popular as a recreational drug in the 1980s, meaning that the first generation of elderly (former) users now enters the stage. This population may serve as a window into the future of the current users regarding the severity and clinical relevance of the observed neurotoxic effects of ecstasy on the serotonin system.

Second, there is the issue of statistics. One of the strengths of the NeXT study is the combination of many different tests and techniques in order to investigate the same questions. However, the drawback of this multitude of methods and tests is the problem of how to correct for multiple comparisons or choosing a statistical threshold of significance. A conservative correction for multiple comparisons or tests increases the risk for false negative results, something one wants to avoid when expected effects are small and the issue under investigation is of high social and clinical relevance. On the other hand one can argue that because this is a socially and clinically important field of research, it should be subjected to an even more rigorous standard to avoid erroneous conclusions. So, what to do? In the fMRI studies we chose to apply multivariate repeated measures analysis (recognizing the interde-

pendency between different task conditions and multiple measurements) and corrected for multiple comparisons, for instance the number of regions of interest in the brain. Had we applied a less conservative statistical threshold, we might have found more. For the other neuroimaging techniques and neuropsychological data no corrections for multiple comparisons were performed, because the expected effects were small. More stringent corrections for multiple comparisons might have resulted in fewer significant effects, which would have lessened the differences between the fMRI findings and the other neuroimaging or neuropsychological findings.

Finally, with regard to the neuroimaging techniques applied there is the question about sensitivity and specificity. For fMRI, this has already been recognized and discussed in this chapter. It is evident that replications in other fMRI studies would strengthen our findings and would enhance confidence in our conclusions.

To conclude, the rapid development and increasing availability of modern imaging techniques such as fMRI or the other imaging techniques applied in the NeXT study, has generated a wealth of findings with regard to the effects of ecstasy on brain structure, physiology and neurophysiology. Without a clear functional correlate, however, the term 'serotonergic neurotoxicity' is hard to relate to human mental health issues. Hopefully, this relationship will become one of the key issues in future ecstasy research, because it is of great importance to the scientists working in this field, to the practitioners in the field of prevention and treatment, and to the people who use this drug.
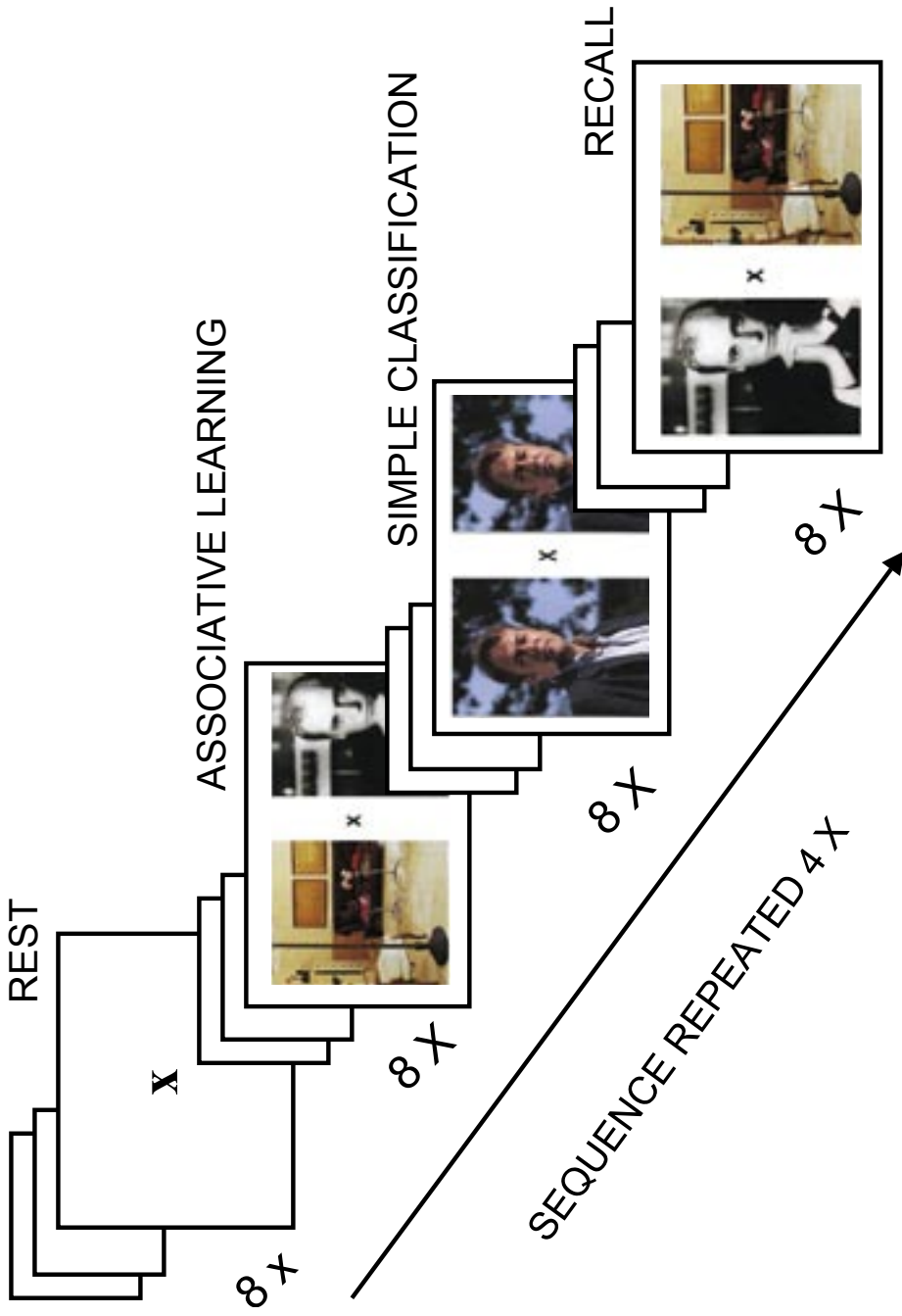
Chapter 8
Color Figures A-E

Figure A The temporal sequence of events is shown for the PMT task. Each epoch starts with an instruction slide (5 sec) followed by a fixation cross (2.25 sec). This is followed by 8 trials of 7.25 sec each (picture pair 5 sec, fixation cross 2.25 sec). Subjects have to respond to the task by pressing one out of two buttons, according to the instruction in each task condition.
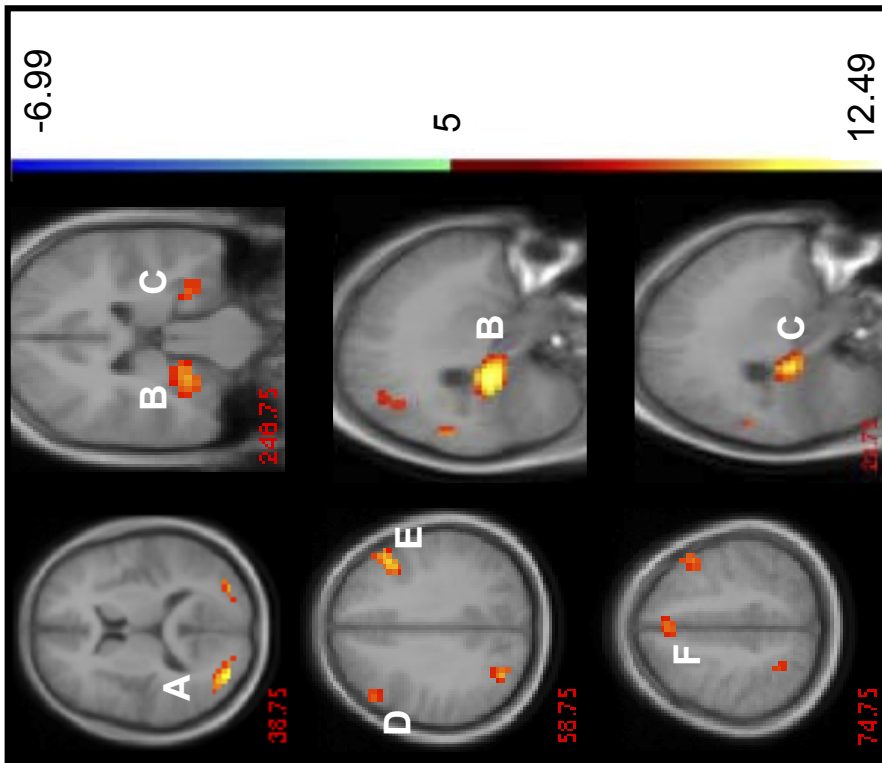
Figure B Display in transversal, coronal and saggital orientation of the six regions that emerged from the group-contrast between AL and SC, thresholded at p < 0.05, corrected for multiple tests (i.e., z = 5.0): A) the right middle occipital gyrus (r-MOG), B and C) the right and left parahippocampal gyrus (r-PHG and l-PHG), D and E) the right and left dorsolateral prefrontal cortex (r-DLPFC and l-DLPFC) and F) the anterior cingulate cortex (ACC). The numbers above the slices indicate the MNI z,x,y-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa). The color bar on the right represents z values.
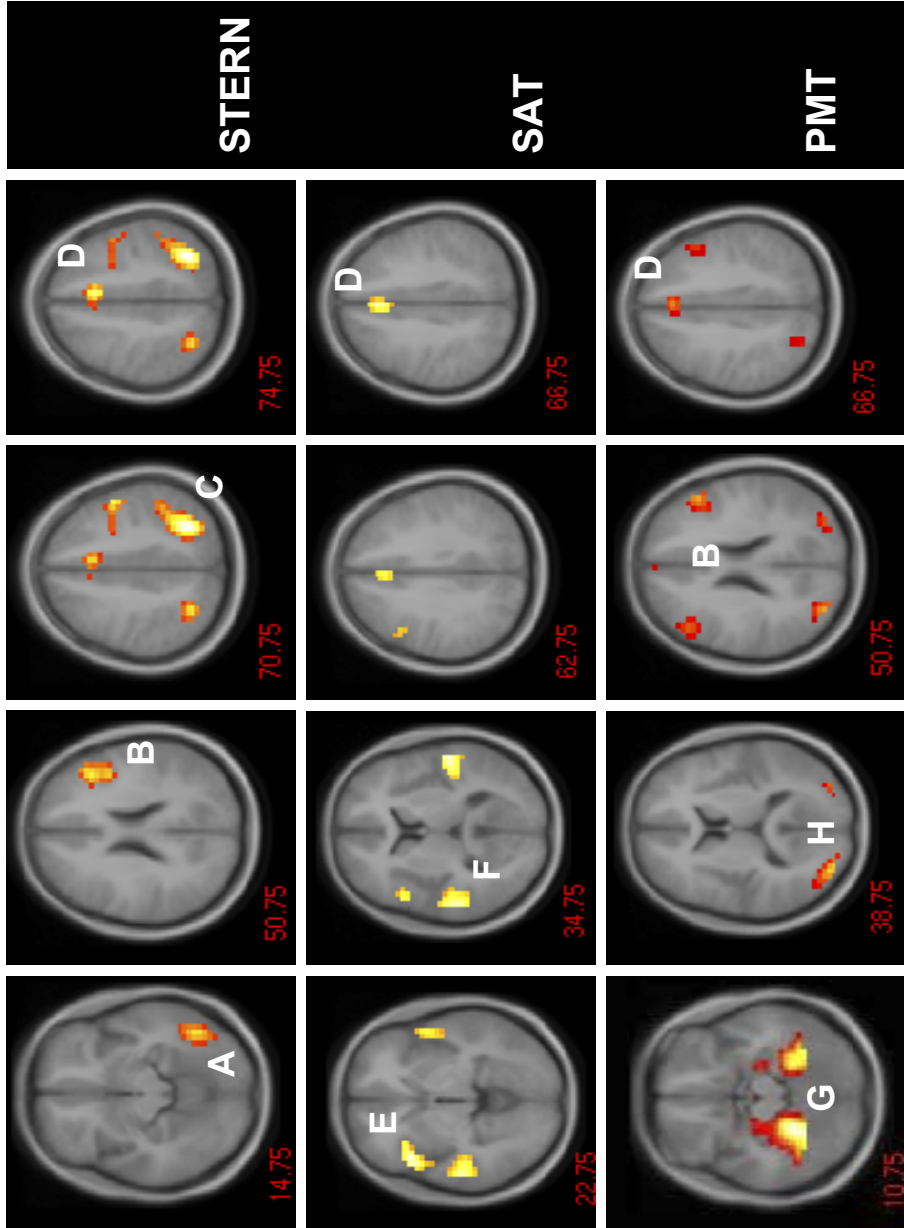
Figure C Regions of Interest for STERN, SAT and PMT: A = fusiform gyrus, B = dorsolateral prefrontal cortex, C = superior parietal cortex, D = anterior cingulate cortex, E = inferior frontal cortex, F = auditory cortex, G = (para)hippocampal region, H = middle occipital gyrus. The numbers beneath the slices indicate the MNI z-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa).
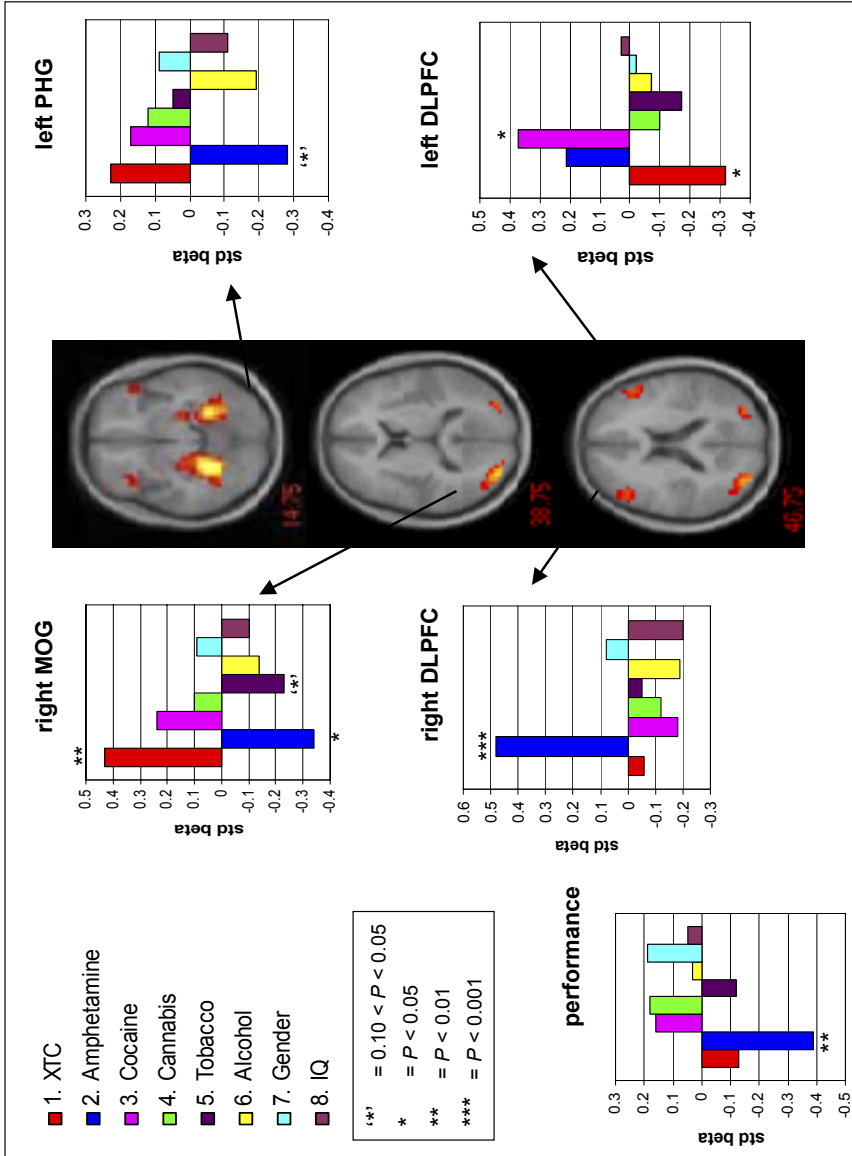
Figure D Overview of the results from the multiple regression analyses for PMT. The vertical axis of the graphs represents standardized β-coefficients.Values reflect strength and direction of the association between use of the drug and magnitude of brain activity in a brain region. On the horizontal axis coloured bars represent the different drugs (see legend: number 1 – 8 displayed from left to right in the graphs). Abbreviations: L-PHR = left parahippocampal region, L-DLPFC = left dorsolateral prefrontal cortex, R-DLPFC = right dorsolateral prefrontal cortex, R-MOG = right middle occipital gyrus. '*' = P-values between 0.05 – 0.20, * = P < 0.05. The numbers beneath the slices indicate the MNI z-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa).
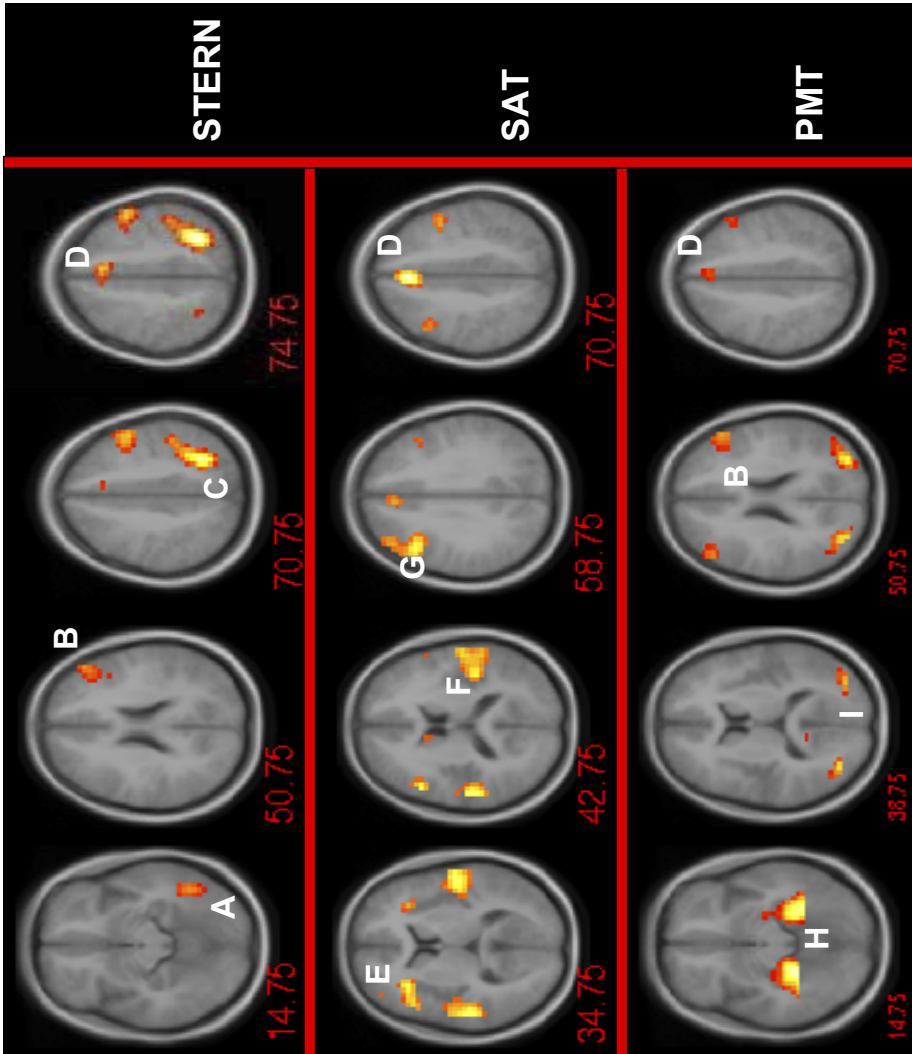
Figure E Regions of Interest for STERN, SAT and PMT: A = fusiform gyrus, B = dorsolateral prefrontal cortex, C = superior parietal cortex, D = anterior cingulate cortex, E = inferior frontal cortex, F = auditory cortex, G = pre-central gyrus, H = (para)hippocampal region, I = middle occipital gyrus. The numbers beneath the slices indicate the MNI z-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa).

List of abbreviations

Δ9/delta-9-THC	delta-9-tetrahydrocannabinol		MTG	middle temporal gyrus
5-HT	serotonin		NART	National Adult Reading Test
ACC	anterior cingulate cortex		NE	norepinephrine
ADHD	attention deficit hyperactivity disorder		NeXT	Netherlands XTC Toxicity study
			ns	non-significant
AL	associative learning		NT	novel task
ANOVA	analysis of variance		PcG	Precentral gyrus
AU	arbitrary units		PET	Positron Emission Tomography
AUD	auditory cortex		PHG	parahippocampal gyrus
BA	Brodmann area		PMT	pictorial memory task
BOLD	blood-oxygen-level-dependent		PT	practiced task
CB1 / 2	cannabinoid receptor type 1 versus type 2		PWI	perfusion weighted imaging
			rCBF	regional cerebral blood flow
CT	control task		rCBV	regional cerebral blood volume
CUN	cuneus		RE	retrieval
DA	dopamine		ROI	Region of Interest
DART	Dutch Adult Reading Test		RS	rest
DLPFC	dorsolateral prefrontal cortex		SAT	selective attention task
DO	dots		SC	simple classification
DSM-IV	Diagnostic Statistic Manual-IV		SD	standard deviation
DTI	diffusion tensor imaging		sec / s	seconds
fMRI	functional Magnetic Resonance Imaging		SEM	standard error of means
			SERT	serotonin transporter
FOV	field of view		SPC	superior parietal cortex
FuG	fusiform gyrus		SPECT	Single Photon Emission Computed Tomography
FWHM	full-width-half-maximum			
GLM	general linear model		SSRI	selective serotonin reuptake inhibitor
Hz	Hertz			
IFG	inferior frontal cortex		STERN	Sternberg item-recognition task
INS	insula		STG	superior temporal gyrus
IQ	intelligence quotient		TE	echo time
l- / r-	left / right		THCCOOH	carboxy-tetrahydrocannabinol
LFP	local field potentials		TO	tones
MDMA	3,4-Methylenedioxymethamphetamine		TR	repetition time
min	minutes		VBM	voxel-based-morphometry
MNI	Montreal Neurological Institute		VIF	variance inflation factor
MOG	middle occipital cortex		VIS	visual cortex
MRI	Magnetic Resonance Imaging		WM	working memory
ms	milliseconds		XTC	ecstasy

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Nederlandse samenvatting

In dit proefschrift worden vier studies beschreven naar de lange termijn effecten van de psychoactieve drugs ecstasy en cannabis op cognitieve hersenfuncties (geheugen, concentratievermogen). De studies zijn uitgevoerd met behulp van functionele magnetische resonantie imaging (fMRI), een moderne beeldvormingtechniek waarmee het mogelijk is door middel van hersenscans de 'hersenen in actie' te bestuderen. Op basis van de resultaten van de studies in dit proefschrift lijkt het frequente en langdurige gebruik van de partydrug ecstasy niet zonder risico voor het lange termijn geheugen en dus af te raden. Daarentegen leverde de studie naar lange termijn effecten van een eenmalige of incidentele lage dosis ecstasy geen solide aanwijzingen op voor schadelijke gevolgen voor het geheugen en het concentratievermogen. In de twee studies naar de lange termijn effecten van cannabis gebruik werden geen sterke aanwijzingen gevonden voor schadelijke gevolgen van cannabis voor geheugen en concentratievermogen en de bijbehorende hersenfuncties.

Achtergrond

Cannabis (hennep, wiet, hasjiesj) en ecstasy (XTC) behoren wereldwijd tot de meest gebruikte illegale drugs. Uit de Nationale Drugs Monitor 2005 van het Trimbos Instituut blijkt dat 17% van de Nederlanders ooit wel eens cannabis heeft gebruikt, terwijl 3% van de bevolking (ongeveer 500.000 mensen) aangaf in de afgelopen drie maanden nog cannabis te hebben gebruikt. Voor ecstasy geldt dat 3% van de Nederlanders deze drug ooit wel eens heeft gebruikt, terwijl 0,5 % (ongeveer

80.000 mensen) in de afgelopen drie maanden nog ecstasy had gebruikt. Cannabis en ecstasy zijn psychoactieve drugs, middelen die het bewustzijn veranderen doordat ze bepaalde biochemische processen in de hersenen beïnvloeden. Naast recreatief gebruik, is er de afgelopen jaren een toenemende interesse in de mogelijke medicinale toepassingen van zowel cannabis als ecstasy. Cannabis (medicinale wiet) heeft onder andere pijnstillende en eetlustbevorderende effecten en wordt soms voorgeschreven aan patiënten met ernstige neurologische aandoeningen, zoals multiple sclerose. Ecstasy heeft mogelijk een gunstig effect op het verminderen van angstgevoelens, spanning of onrust, bijvoorbeeld bij patiënten met een posttraumatische stress stoornis, of bij patiënten in een vergevorderd stadium van kanker.

Er zijn aanwijzingen uit wetenschappelijk onderzoek dat ecstasy en cannabis 'neurotoxische' eigenschappen hebben, dat wil zeggen, ecstasy en cannabis veroorzaken mogelijk schade aan de neuronen (zenuwcellen) in de hersenen, wat functionele stoornissen tot gevolg zou kunnen hebben, zoals psychiatrische symptomen (depressie, psychose), maar ook cognitieve stoornissen (stoornissen in mentale processen zoals het geheugen of het concentratievermogen).

In de afgelopen twintig jaar is er veel onderzoek gedaan naar de effecten van cannabis en ecstasy op de hersenen en hersenfuncties, waardoor de kennis op dit gebied enorm is toegenomen. Het voert te ver om alle bevindingen van eerdere studies uitgebreid te beschrijven, maar de belangrijkste bevindingen

op het gebied van cognitieve hersenfuncties (waar dit proefschrift over gaat) zijn als volgt:

In de acute fase, dat wil zeggen wanneer iemand onder invloed is van cannabis, veroorzaakt cannabis verstoringen van het leervermogen en het geheugen. Echter, de lange termijn effecten van langdurig en frequent cannabis gebruik zijn minder duidelijk; sommige studies laten zien dat forse cannabis gebruikers, ook langere tijd nadat ze gestopt zijn met deze drug, subtiele verstoringen van het geheugen en de hogere uitvoerende mentale functies (plannen, redeneren, probleem oplossend vermogen, organiseren van gedrag) hebben. Echter, er zijn ook studies waarin geen lange termijn effecten van cannabis gebruik op cognitieve hersenfuncties werden gevonden. De bevindingen zijn dus tegenstrijdig. Bovendien is er nog maar weinig bekend van de effecten van cannabis op de patronen van hersenactiviteit in de delen van het brein die bij specifieke cognitieve hersenfuncties, zoals het geheugen, betrokken zijn.

Het gebruik van ecstasy heeft in de acute fase invloed op cognitieve hersenfuncties, in het bijzonder op leren, geheugen en aandachtsfuncties. Daarnaast zijn er aanwijzingen voor lange termijn effecten (enkele dagen tot enkele maanden na het laatste ecstasy gebruik) op de hersenfysiologie. Onderzoeken waarbij gebruik wordt gemaakt van moderne beeldvormingtechnieken hebben aangetoond dat frequent ecstasy gebruik tot langdurige veranderingen kan leiden in het zogenaamde 'serotonine systeem' in de hersenen. Serotonine is een neurotransmitter, een chemische stof die signalen tussen de hersencellen doorgeeft.

Serotonine is betrokken bij de regulatie van tal van belangrijke functies zoals stemming, slaap en waakritme, en belangrijke cognitieve functies zoals leren en geheugen. Het is onduidelijk of deze veranderingen in het serotonine systeem permanent zijn of dat ze na verloop van tijd weer verdwijnen.

De gevolgen van ecstasy gebruik voor cognitie zijn uitgebreid onderzocht. Uit een vergelijking en weging van de bevindingen van een groot aantal studies (een zogenaamde meta-analyse) komt als meest consistente bevinding naar voren dat frequent ecstasy gebruik kan leiden tot lichte verstoringen van het verbale (talige) geheugen en het verbale leervermogen. Het is echter nog onduidelijk welke gebieden in de hersenen betrokken zijn bij deze verstoringen in het verbale geheugen en wat er precies verandert of misgaat in het functioneren van deze gebieden onder invloed van ecstasy.

Ondanks de toenemende kennis is een aantal belangrijke vragen tot nu toe onbeantwoord gebleven. Deze vragen hebben betrekking op een viertal onderwerpen:

> 1 Causaliteit, ofwel oorzaak en gevolg; wanneer er een samenhang wordt gevonden tussen ecstasy gebruik en bijvoorbeeld depressieve symptomen dan zijn er in principe twee mogelijkheden: de depressieve symptomen zijn het gevolg van ecstasy gebruik, of ecstasy gebruik is een gevolg van de depressieve symptomen. In dit laatste geval is het denkbaar dat mensen die zich somber voelen misschien sneller geneigd zijn ecstasy te gaan gebruiken om zich prettiger te voelen.

2 Lange termijn verloop van de neuro-toxische effecten: Wat is de aard en het verloop van bijvoorbeeld de lichte geheugenstoornissen die zijn aangetoond bij zware ecstasy gebruikers? Zijn de stoornissen op den duur omkeerbaar, wanneer mensen stoppen met het gebruik van de drug, en zo ja, hoe lang duurt dat dan, of zijn de effecten blijvend?

3 Klinische relevantie: Een vraag die betrekking heeft op de ernst en de betekenis van de effecten. Een statistisch significant verschil is pas betekenisvol indien het verschil voor de klinische praktijk van wezenlijk belang is ofwel klinische relevantie heeft. Een voorbeeld: onderzoek heeft aangetoond dat frequent ecstasy gebruik tot langdurige veranderingen leidt in het serotonine systeem in de hersenen. Serotonine is een neurotransmitter, een chemische stof die signalen tussen de hersencellen doorgeeft, en is betrokken bij de regulatie van tal van belangrijke functies zoals stemming, maar ook het geheugen. Langdurige veranderingen in het serotonine systeem zouden zich kunnen uiten in bijvoorbeeld stemmingsstoornissen of geheugenstoornissen, maar dit hoeft niet noodzakelijkerwijs zo te zijn, bijvoorbeeld als er sprake is van voldoende reservecapaciteit in het serotonine systeem of wanneer andere systemen worden ingeschakeld ter compensatie. In dit geval is er sprake van een duidelijk effect van ecstasy (verminderde serotonine functie), maar omdat dit niet gepaard gaat met functionele stoornissen (geen

stemmingsstoornissen of een slechter geheugen) is de klinische relevantie van dit effect onduidelijk.

4 Gecombineerd drugsgebruik: ervaren ecstasy gebruikers gebruiken naast ecstasy vrijwel altijd ook andere psychoactieve drugs, waarvan cannabis de meest genoemde is. Maar ook gebruik van amfetamine (speed), cocaïne, alcohol, tabak en andere middelen naast ecstasy komt veel voor. Er is nog weinig bekend over de specifieke effecten van ecstasy in de context van het gebruik van andere middelen.

Het gegeven dat juist deze onderzoeksvragen niet of slechts gedeeltelijk beantwoord zijn komt voort uit het feit dat het stuk voor stuk vragen zijn die lastig te onderzoeken zijn. Neem bijvoorbeeld de causaliteitsvraag. De enige manier om onomstreden vast te stellen of ecstasy gebruik de oorzaak is van een verminderde geheugenfunctie in plaats van andersom, is een studie waarbij onder gecontroleerde omstandigheden ecstasy of een placebo (niet-werkzame stof) wordt toegediend aan een groep vrijwilligers die nog nooit eerder ecstasy hebben gebruikt (waarbij het lot bepaalt wie wat krijgt), en die in alle andere opzichten (leeftijd, geslacht, opleidingsniveau, enzovoort) zoveel mogelijk op elkaar lijken. Na afloop van het experiment wordt dan de geheugenfunctie van de groep mensen die ecstasy heeft gekregen vergeleken met die van de placebo groep. Echter, een dergelijke studie in mensen is in ethisch opzicht discutabel omdat er aanwijzingen zijn dat ecstasy een neurotoxische stof is. Het alternatief is een zogenaamde 'natural-

istische' studie, waarbij een grote groep vrijwilligers met een relatief hoog risico voor eerste ecstasy gebruik in de nabije toekomst (bijvoorbeeld omdat ze vrienden hebben die al ecstasy gebruikt hebben) getest wordt vóór hun eerste ecstasy gebruik. Vervolgens wordt deze groep enkele jaren gevolgd, in de verwachting dat een deel van hen inderdaad vrijwillig zal gaan experimenteren met ecstasy. Dit zijn omvangrijke (lees kostbare) en tijdrovende studies om uit te voeren. Hetzelfde geldt voor het bestuderen van het verloop van effecten van ecstasy gebruik. Ook hier betreft het dure, tijdrovende, logistiek uitdagende studies waartoe niet altijd de middelen en de mogelijkheden beschikbaar zijn.

Het doel van de vier functionele MRI studies beschreven in dit proefschrift was een bijdrage te leveren aan het beantwoorden van de nog openstaande onderzoeksvragen zoals boven beschreven, en het bestuderen van de lange termijn effecten van ecstasy en cannabis op cognitieve hersenfuncties, met behulp van functionele magnetische resonantie imaging (fMRI). Er worden MRI scans van de hersenen gemaakt terwijl de proefpersoon een mentale taak uitvoert (bijvoorbeeld een geheugentaak). Op deze manier kan men twee maten van cognitieve hersenfuncties met elkaar in verband brengen. Enerzijds zijn er de gedragsdata; hoe goed voert de proefpersoon de taak uit (percentage fouten, reactietijden), anderzijds la-ten de fMRI scans zien welke delen van de hersenen actief worden bij het uitvoeren van (delen van) de taak en hoe het patroon van hersenactiviteit er uitziet.

De fMRI studies beschreven in dit proefschrift maken deel uit van een groter onderzoek-sproject, de NeXT studie (Nederlandse XTC Toxiciteit studie), dat in samenwerking met het Academisch Medisch Centrum Amsterdam en het Bonger Instituut voor Criminologie van de Universiteit van Amsterdam werd uitgevoerd tussen 2001 en 2006.

Resultaten fMRI studies

De eerste twee studies beschrijven de lange termijn effecten van matig tot frequent cannabis gebruik op het korte termijn geheugen en aandacht, en op het lange termijn geheugen. In de eerste studie naar het korte termijn geheugen en aandacht werden 10 matige tot frequente cannabis gebruikers (gemiddeld totaal gebruik 1300 joints, wat vergelijkbaar is met 5 - 7 joints per week voor een periode van ongeveer 4 jaar) vergeleken met 10 controle deelnemers die nooit of hooguit enkele keren cannabis hadden gebruikt. Alle deelnemers mochten vanaf 1 week voor de testdatum geen alcohol gebruiken, en de cannabis gebruikers ook geen cannabis of andere drugs. Alle deelnemers voerden twee taken uit; een korte termijn geheugentaak en een aandachtstaak. Taakuitvoering en bijbehorende patronen van hersenactiviteit werden gemeten. De resultaten lieten geen duidelijke verschillen zien in korte termijn geheugen en concentratievermogen tussen de cannabis gebruikers en de controles.

In de tweede studie werden de lange termijn effecten van frequent cannabis gebruik (gemiddeld totaal gebruik 1900 joints, vergelijkbaar met 5 – 7 joints per week voor een periode van 6 jaar, of 10 – 14 joints per week voor een

periode van 3 jaar) op het lange termijn geheugen onderzocht. De resultaten lieten zien dat de cannabis gebruikers als groep minder sterke activiteit vertoonden in de hersengebieden die betrokken waren bij de uitvoering van de lange termijn geheugentaak. Ook waren er subtiele aanwijzingen voor veranderingen in de structuur van het hersenweefsel in de hippocampale gebieden. De hippocampus is een gebied in de hersenen dat van groot belang is voor bepaalde geheugenprocessen, en de hippocampale zenuwcellen hebben een zeer hoge dichtheid aan cannabinoïde receptoren (dat deel van de cel waar de stof cannabis op aangrijpt). Er wordt gedacht dat de hippocampus hierdoor extra gevoelig zou kunnen zijn voor de mogelijk schadelijke gevolgen van frequent cannabis gebruik. Desondanks de lagere activiteit in het netwerk van hersengebieden betrokken bij de geheugentaak, werd er geen enkele relatie gevonden tussen de lagere activiteit en bijvoorbeeld de mate van cannabisgebruik, het succes waarmee de taak werd uitgevoerd of de subtiele veranderingen in de structuur van hersenweefsel bij cannabis gebruikers. Het ontbreken van dergelijke relaties ontkracht het idee dat de verlaagde hersenactiviteit in cannabis gebruikers gerelateerd is aan cognitieve processen in het brein. Het is waarschijnlijker dat de verlaagde hersenactiviteit een uiting is van een andere niet-cognitieve variabele die samenhangt met frequent cannabis gebruik, bijvoorbeeld langdurige of blijvende veranderingen in de doorbloeding van het brein tengevolge van cannabis, of systematische verschillen in gedrevenheid of alertheid tijdens het uitvoeren van de taak tussen cannabis gebruikers en niet-gebruikers.

Wanneer de bevindingen uit de eerste en tweede studie worden samengenomen dan kan geconcludeerd worden dat er geen sterke aanwijzingen gevonden zijn voor lange termijn effecten (1 week of langer na het laatste gebruik) van cannabis op cognitieve hersenfuncties.

De derde en de vierde studie in dit proefschrift beschrijven de langere termijn effecten van ecstasy gebruik op het korte termijn geheugen, aandacht en het lange termijn geheugen, gemeten met fMRI.

In de derde studie worden de lange termijn effecten (minimaal twee weken na het laatste ecstasy gebruik) van frequent en langdurig ecstasy gebruik op de bovengenoemde cognitieve functies onderzocht in de context van polidrug gebruik, dat wil zeggen, naast ecstasy (gemiddeld 250 pillen totaal, range tussen 15 en 2000 pillen) gebruikten de deelnemers ook één of meerder andere drugs zoals cannabis, amfetamines, cocaïne, alcohol en/of tabak. De 71 proefpersonen die deelnamen aan deze studie varieerden aanzienlijk in de soort en hoeveelheid drugs die gebruikt werden. In de statistische analyses werden ecstasy, de diverse andere drugs, en een aantal non-drugs gegevens zoals geslacht en IQ, in een regressiemodel gebruikt als aparte voorspellers voor taakuitvoering en hersenactiviteit. De resultaten lieten zien dat het gebruik van drugs gepaard ging met een slechtere prestatie en veranderingen in hersenactiviteit tijdens de lange termijn geheugentaak, terwijl het korte termijn geheugen en het concentratievermogen niet of nauwelijks werden beïnvloed. Opvallend was echter, dat de taakuitvoering op de lange termijn

geheugentaak sterker werd beïnvloed door het gebruik van amfetamines dan door het gebruik van ecstasy. Dit suggereert dat conclusies uit eerdere studies ten aanzien van effecten van ecstasy op geheugenfuncties, waarbij niet gecorrigeerd is voor het gebruik van amfetamine naast ecstasy, mogelijk vertekend zijn door het gebruik van amfetamine of andere drugs naast ecstasy. Het patroon van hersenactiviteit in de huidige studie werd zowel door ecstasy als door amfetamine gebruik beïnvloed, maar deze effecten waren steeds in tegenovergestelde richting. Ter illustratie: ecstasy gebruik hing samen met een relatief lagere hersenactiviteit in de linker frontale hersenschors, terwijl amfetamine gebruik juist gepaard ging met een relatief verhoogde activiteit in dit gebied. De bevinding dat ecstasy en amfetamine hersenactiviteit op een verschillende manier beïnvloeden wijst erop dat verschillende mechanismen een rol spelen, mogelijk gerelateerd aan de neurotransmittersystemen waarop deze drugs inwerken; het serotonine systeem (ecstasy) en het dopamine systeem (amfetamine).

Afgezien van de negatieve effecten van frequent ecstasy gebruik die in de wetenschappelijke literatuur worden gerapporteerd, bestaat het vermoeden dat zelfs een eenmalige dosis of een enkele malen herhaalde lage dosis ecstasy neurotoxisch kan zijn. In de vierde studie werd prospectief onderzocht of een lage dosis ecstasy effect had op cognitieve hersenfuncties, gemeten met fMRI, in 25 vrijwilligers vóór en ná een eerste episode van ecstasy gebruik. Tijdens de nameting hadden de vrijwilligers gemiddeld 2 ecstasy pillen gebruikt (range tussen de 1 en 6 pillen), en het laatste ecstasy gebruik was gemiddeld 11 wek-

en geleden. De nieuwe gebruikers werden vergeleken met 24 controles die nooit ecstasy hadden gebruikt. De controles werden ook tweemaal getest, en kwamen overeen met de nieuwe gebruikers in leeftijd, geslacht, IQ, het gebruik van cannabis en andere drugs. Dit zogenaamde 'prospectieve' design is een oplossing voor een belangrijk methodologisch probleem dat veel eerdere ecstasy studies parten speelt, namelijk het ontbreken van basisgegevens van vóór het eerste ecstasy gebruik. De resultaten van deze studie leverden geen solide aanwijzingen op voor langere termijn effecten van een lage dosis ecstasy op de taakuitvoering of de hersenactiviteit gerelateerd aan het korte termijn geheugen, het concentratievermogen of het lange termijn geheugen.

Discussie

Ondanks eerdere observaties in zowel dieronderzoek als humane studies van neurotoxische effecten op het serotonine systeem van ecstasy, laten de resultaten van de studies beschreven in dit proefschrift verbazingwekkend weinig bewijs zien voor functionele gevolgen van een potentieel beschadigd serotonine systeem in de zin van stoornissen in cognitieve hersenfuncties. Met betrekking tot de taakuitvoering op de lange termijn geheugentaak, vonden we dat zelfs na aanzienlijk ecstasy gebruik (gemiddeld 250 pillen totaal) de effecten van ecstasy relatief mild waren in vergelijking met de effecten van amfetamine. De discrepantie tussen onze bevindingen en die uit eerder gepubliceerd onderzoek kan meerdere verklaringen hebben. Mogelijk zijn dierstudies naar de neurotoxische effecten van ecstasy niet

rechtstreeks te vertalen naar de menselijke situatie, en verder is het mogelijk dat eerder gerapporteerde geheugenstoornissen in zware ecstasy gebruikers (deels) vertekend zijn door de effecten van andere middelen, in het bijzonder amfetamines. En andere verklaring zou kunnen zijn dat de techniek van functionele MRI niet gevoelig genoeg is om ecstasy gerelateerde schade aan de hersenen te detecteren. Alles bij elkaar genomen, kunnen we niet concluderen dat ecstasy volledig veilig en vrij van schadelijke bijwerkingen is, omdat het wel een effect had in zware gebruikers op de hersenactiviteit tijdens lange termijn geheugenprocessen en dit zou een eerste signaal kunnen zijn van cognitieve stoornissen later, als de effecten van ecstasy gebruik als het ware gaan optellen bij de effecten van normale veroudering op cognitieve functies.

Met betrekking tot het gebruik van cannabis, naast ecstasy, leveren de studies in dit proefschrift geen bewijs op voor het idee dat een deel van de geobserveerde cognitieve verstoringen of veranderingen in hersenactiviteit in recreatieve ecstasy gebruikers, toegeschreven zouden moeten worden aan het gebruik van cannabis in plaats van ecstasy.

Het doel van de studies beschreven in dit proefschrift was het uitbreiden van onze kennis met betrekking tot de nog openstaande vragen van causaliteit (oorzaak/gevolg), het verloop en de klinische relevantie van de potentiële neurotoxiciteit van ecstasy. In hoeverre is dit gelukt?

De studie in nieuwe ecstasy gebruikers, gemeten voor en na eerste ecstasy gebruik, liet geen duidelijke effecten zien van een lage dosis ec-stasy op cognitieve hersenfuncties. Echter, hierdoor is de causaliteitsvraag irrelevant geworden; waar geen effect is, kan ook geen oorzaak/gevolg relatie vastgesteld worden. Wel zijn er een aantal factoren 'uitgeschakeld' die mogelijk een rol kunnen spelen bij de verklaring van eerder gevonden verschillen in cognitieve functies tussen ecstasy gebruikers en niet-gebruikers, zoals leeftijd, geslacht, IQ en het gebruik van cannabis.

Echter, het is mogelijk dat neurotoxische effecten van ecstasy op cognitieve hersenfuncties pas naar voren komen na langer gebruik en/of hogere doses. Daarom zal de huidige groep beginnende ecstasy gebruikers verder gevolgd worden. Naar verwachting zal na verloop van tijd de variatie in doses, frequentie en de duur van het ecstasy gebruik toenemen in deze groep.

Wat betreft het lange termijn beloop van de neurotoxische effecten van ecstasy hebben de studies in dit proefschrift maar beperkt bijgedragen aan een antwoord op deze vraag omdat de tijd tussen het testen en het laatste ecstasy gebruik varieerde van ongeveer twee weken (zware gebruikers) en gemiddeld 11 weken met een minimum van 2 weken (beginnende gebruikers). Dergelijke abstinentie periodes zijn te kort om een betrouwbare uitspraak te kunnen doen over het lange termijn beloop.

Met betrekking tot de vraag over de klinische relevantie kan geconcludeerd worden dat de effecten van ecstasy op cognitieve hersenfuncties beschreven in dit proefschrift tamelijk mild zijn, zelfs na consumptie van gemiddeld 250

pillen. In twee van de drie onderzochte cognitieve functies, namelijk korte termijn geheugen en aandacht, werden helemaal geen functionele effecten van ecstasy gebruik op de taakuitvoering of hersenactiviteit aangetoond, terwijl de gevonden slechtere prestatie op de lange termijn geheugentaak grotendeels toegeschreven moest worden aan amfetamine gebruik in plaats van ecstasy. De gevonden effecten van ecstasy op de hersenactiviteit tijdens lange termijn geheugenprocessen was echter wel significant en kan wijzen op langdurige aanpassing of compensatoire mechanismen. Maar omdat de veranderingen in hersenactiviteit niet gepaard gingen met een slechtere taakuitvoering, lijkt de klinische relevantie van deze bevinding gering. Desondanks is het belangrijk te vermelden dat we niet weten of de veranderingen in hersenactiviteit tijdelijk of blijvend zijn, zelfs na langdurig afzien van het gebruik van ecstasy. In het laatste geval kan de klinische relevantie van deze veranderingen in hersenactiviteit toenemen, bijvoorbeeld onder invloed van de achteruitgang in cognitieve hersenfuncties tengevolge van normale veroudering. Deze combinatie zou kunnen resulteren in een vroegtijdige cognitieve veroudering bij frequente (ex) ecstasy gebruikers.

Conclusie

Op basis van de bevindingen van de studies in dit proefschrift lijkt het frequente en langdurig recreatief gebruik van ecstasy niet zonder risico en dus af te raden. Ten aanzien van de medische toepassingen van cannabis en ecstasy, waarbij de afweging van de baten en de risico's een andere is dan bij jonge gezonde mensen, lijken de bevindingen met betrekking tot de lange termijn effecten van cannabis en ecstasy op cognitieve hersenfuncties vooralsnog geen aanleiding te geven om het onderzoek naar de mogelijke medicinale eigenschappen van cannabis en ecstasy te ontmoedigen.

List of Publications

Journal articles

For this thesis
Jager G, de Win MML, Vervaeke HKE, Schilt T, Kahn RS, van den Brink W, van Ree JM, Ramsey NF. Incidental use of Ecstasy: no evidence for harmful effects on cognitive brain function in a prospective fMRI study. Submitted

Jager G, de Win MML, van der Tweel I, Schilt T, Kahn RS, van den Brink W, van Ree JM, Ramsey NF. Sustained effects of Ecstasy on cognitive brain function in the context of poly-substance use in humans. Submitted

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Related topics
Schilt T, de Win MML, Jager G, Koeter M, Ramsey NF, Schmand B, van den Brink W. Specific effects of ecstasy and other illicit drugs on cognition in poly-substance ecstasy users. Submitted

De Win MML, Jager G, Booij J, Reneman L, Schilt T, Lavini C, Olabarriaga SD, den Heeten GJ, van den Brink W. Sustained effects of ecstasy on the human brain: a prospective neuroimaging study in novel users. Submitted

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"The meeting of two personalities is like the contact of two chemical substances:
if there is any reaction, both are transformed... "

Carl Jung (1875-1961)

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Curriculum Vitae

Gerry Jager was born on May 9, 1966 in Rotterdam. She graduated from the Christelijke Scholengemeenschap Comenius Capelle aan den IJssel in 1984. From 1984 - 2001, she worked as an operation theater nurse in several hospitals. In 2002, she completed cum laude a degree in psychology at Utrecht University, specializing in neuropsychology. After graduating, she started a PhD project at the Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, University Medical Center Utrecht, where as an undergraduate student she had worked with the Functional Neuroimaging group of Dr. Nick F. Ramsey. After defending her thesis on October 31, 2006, she will remain at the University Medical Center in Utrecht as a postdoctoral fellow in neuroscience, working on a Dutch-US collaboration project on the effects of cannabis use on the development of human cognition and brain function. In the course of 2006, she has spent several weeks at the Department of Health of the University of Iowa (Iowa City, USA), working with Prof. Robert I. Block and his colleagues. She will continue working on this project during 2007.

Gerry Jager werd op 9 mei 1966 geboren te Rotterdam. Ze behaalde in 1984 het eindexamen aan de Christelijke Scholengemeenschap Comenius te Capelle aan den IJssel. Van 1984 tot 2001 was ze werkzaam als operatieassistente chirurgie in diverse ziekenhuizen. In 2002 studeerde ze cum laude af in de Neuropsychologie aan de Universiteit Utrecht. Ze begon een promotie onderzoek bij het Rudolf Magnus Instituut voor Neurowetenschappen, afdeling Psychiatrie, Universitair Medisch Centrum Utrecht, waar ze als student stage had gelopen bij de Functionele Neuroimaging groep van Dr. Nick F. Ramsey. Na de verdediging van dit proefschrift op 31 oktober 2006 blijft ze werkzaam in het Universitair Medisch Centrum Utrecht als postdoc onderzoeker bij een samenwerkingsproject tussen Nederland en de Verenigde Staten, met als onderwerp cannabis en de ontwikkeling van de menselijke cognitie en hersenfunctie. In de loop van 2006 heeft voor dit project enkele weken doorgebracht aan de Universiteit van Iowa (Iowa City, USA) om samen te werken met Prof Robert I. Block en zijn medewerkers. Haar werkzaamheden als postdoc onderzoeker voor dit project zullen doorlopen tot eind 2007.