Aus dem Zentrum für Neurologie und Psychiatrie der Universität zu Köln
Klinik und Poliklinik für Psychiatrie und Psychotherapie
Direktor: Universitätsprofessor Dr. med. F. Jessen

Hat der Konsum von Ecstasy und Amphetaminen eine Verminderung der grauen Hirnsubstanz zur Folge? Eine kombinierte tract-based spatial statistics und voxel-based morphometry Analyse

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Katrin Schöllhorn
aus Wangen im Allgäu

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Dekan: Universitätsprofessor Dr. med. Dr. h.c. Th. Krieg

1. Berichterstatterin: Frau Professor Dr. med. E. Gouzoulis-Mayfrank
2. Berichterstatter: Professor Dr. rer. medic. M. G. A. Hellmich

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Contents

Glossary

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
5-HTT	5-HT transporter, SERT
ACh	Acetylcholine
ADHD	Attention deficit hyperactivity disorders
BA	Brodman area
BDNF	Brain derived nerve growth factor
BET	Brain Extraction Tool
CNS	Central nervous system
CSF	Cerebrospinal fluid
DA	Dopamine
DAT	Dopamine transporter
df	Degrees of freedom
DSM-IV	Diagnostic and Statistical Manual of the American Psychiatric Association, 1994
DTI	Diffusion tensor imaging
ESPAD	European School Survey Project on Addiction and other Drugs
FA	Fractional anisotropy
FDT	FMRIB's Diffusion Toolbox
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional Magnetic Resonance Imaging
FMRIB	Functional Magnetic Resonance Imaging of the Brain

FNIRT	FMRIB's Nonlinear Image Registration Tool
FSL	FMRIB Software Library
FWHM	Full width at half maximum
GLM	General linear model
GLU	Glutamate
GM	Gray matter
MDMA	3,4-methylenedioxymethamphetamine
METH	Methamphetamine
MRI	Magnetic resonance imaging
NE	Norepinephrine
PE	Phenylethylamine
TBSS	Tract-based spatial statistics
TE	Echo time
TFCE	Thresholdfree cluster enhancement
THC	Δ9-tetrahydrocannabinol
TPH	Tryptophane hydroxylase
TR	Repetition time
SERT	Serotonin transporter
SNR	Signal-to-noise ratio
SPECT	Single photon emission computed tomography
VBM	Voxel-based morphometry
WM	White matter

A. Introduction

1 General information and epidemiology

3,4-methylenedioxymethamphetamine (MDMA), commonly known as "Ecstasy", "XTC", "Adam" or simply "E", is a synthetic amphetamine analogue that has gained significant popularity as a recreational drug. Particularly young people (aged 18 to 30) reported to have tried this illicit substance, often being taken at "rave" or "techno" parties (Green et al., 2003). Common recreational doses are 30 – 150 mg/pill, although the purity of the street drug is notoriously poor (Hall and Henry, 2006). While most ecstasy tablets sold in Europe and the United States were found to contain MDMA, the content of MDMA and other additives in these pills may vary (Cole et al., 2002; Parrott, 2004). Most commonly, ecstasy is taken orally as a capsule or tablet, but it can also be injected, smoked or absorbed as a suppository (Hurley et al., 2002).

The other substance of interest is amphetamine. Associated slang names of amphetamine are: Amp, Bennies, Black beauties, Browns, Crank, Fives, Goey, Hearts, Louee, Speed, Uppers, Whiz or Pep (Greene et al., 2008). Amphetamine tends to be more common than methamphetamine (METH) in Germany (Pfeiffer-Gerschel et al., 2009), the latter being widely-used in Asia, Oceania and North America (United Nations Office on Drugs and Crime, 2007). Amphetamine and methamphemtamine are available in different forms, most of which consist of powders. They may be ingested, snorted, inhaled and, less commonly, injected (European Monitoring Centre for Drugs and Drug Addiction, 2008).

As is the case with all illicit drugs, ecstasy and amphetamine use is often combined with that of other agents, principally alcohol, cannabis and other psychostimulants (Fox et al., 2001; Winstock et al., 2001; Scholey et al., 2004).

In the early nineties, studies suggest a substantial increase in the use of both ecstasy and related substances as well as hallucinogens particularly in younger age groups and women (Schuster et al., 1998). Since 1997 though, the lifetime prevalence of the consumption of ecstasy has remained almost unchanged and ecstasy is now second in popularity to cannabis as an illicit drug (Pope et al., 2001). According to the na-

tional report 2009 of the European Monitoring Centre for Drugs and Drug Addiction (Pfeiffer-Gerschel et al., 2009), 1.1 percent of the 12 to 17 year olds and 2.0 percent of the 18 to 64 year olds have experience with ecstasy. However, the prevalence strongly depends on the analyzed population: Tossmann et al. (2001) showed a significantly greater prevalence of ecstasy use among German techno party attendees. 44% of the 12 to 25 year olds have gained experience with ecstasy compared to 5% in a representative sample of this study. In addition, the use of other illegal substances like cannabis, amphetamine, hallucinogens and cocaine is significantly more widespread in the techno party scene (Tossmann et al., 2001).

The lifetime prevalence of amphetamine is 0.7 percent of the 12 to 17 year olds and 2.5 percent of the 18 to 64 year olds (Pfeiffer-Gerschel et al., 2009). According to the results of the European School Survey Project on Addiction and other Drugs (ESPAD), amphetamines were the most commonly tried illicit drugs except for cannabis among pupils. Six percent of the teenagers had taken amphetamine at least once in a lifetime (Pfeiffer-Gerschel et al., 2009). Nowadays, amphetamine gains a more and more positive image as it corresponds to the "Zeitgeist". Thus, for example, in Frankfurt's party scene speed has taken on the predominant role among hard drugs (Pfeiffer-Gerschel et al., 2009).

2 MDMA (3,4-methylenedioxymethamphetamine)

2.1 History of MDMA

First patented by the German pharmaceutical company Merck in 1914 as precursor agent for therapeutically active compounds, MDMA was used as an appetite suppressant for German soldiers in World War I (Hurley et al., 2002). In 1970s, it became an adjunct for psychotherapy in the United States (Cohen, 1998). MDMA was ultimately placed on Schedule I of controlled substances in 1985 by the Drug Enforcement Agency (Klein and Kramer, 2004) and found its way into dance clubs or ''raves''

in the mid 1990s.

2.2 Chemical structure of MDMA

3,4-methylenedioxymethamphetamine is a ring-substituted amphetamine derivative that is also structurally related to methamphetamine and its hallucinegenic compound mescaline with which it shares the capacity to alter perception (cf. Figure A.2) . The chiral nature of the MDMA molecule gives rise to a pair of enantiomers, each of which is biologically active (cf. Figure A.1). The racemic mixture is the one consumed; the S-MDMA enantiomer is primarily responsible for empathic effects and stimulation and the R-MDMA enantiomer for hallucinogenic effects (de la Torre et al., 2004).

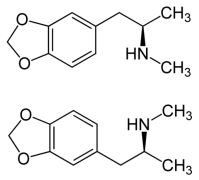


Figure A.1: *The (R)-enantiomer (top) and the (S)-enantiomer of MDMA.*

It is interesting to know that specifically adult females are more sensitive to the acute and subacute physical and psychological effects of MDMA and long-term alterations in aspect of psychological functioning than males. Conversely, males are more sensitive to the acute physiological effects of MDMA than women (Allott, 2007).

With respect to the acute consequences of MDMA used for recreational purposes, there are two major consequences which follow its ingestion that will be discussed in more detail below: psychological and somatic effects, the former are the main reason for taking the drug (Green et al., 2003).

2.3 Psychological effects of MDMA

Psychological effects occur after 20 to 60 minutes after ingestion, reach their peak after 60 to 90 minutes and last for three to five hours (Green et al., 2003). Most MDMA

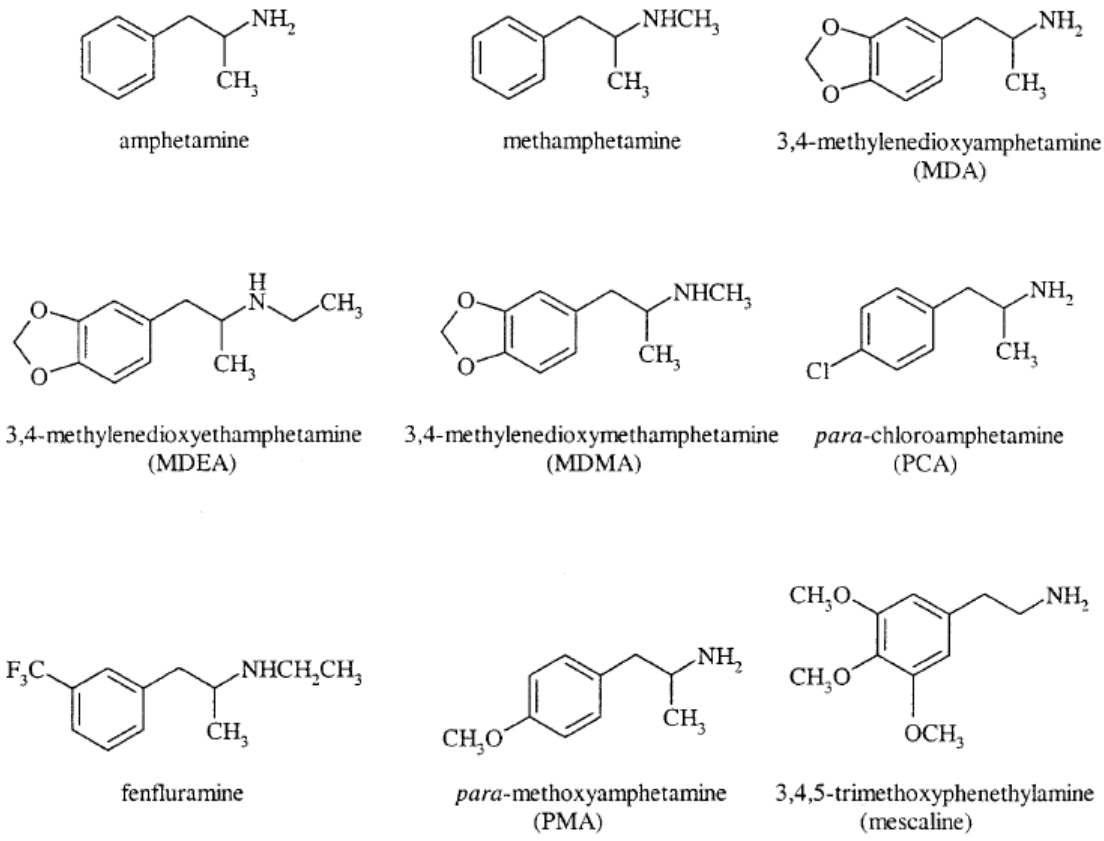


Figure A.2: *Chemical structures of amphetamine and some of its derivatives, including MDMA and mescaline (Green et al., 2003).*

users report several positive mood and emotional effects, particularly in their relation to others such as greater capacity for empathy and communication, increased sociability, extroversion, reduction of negative thoughts and a decrease in inhibitions (Peroutka et al., 1988; Hegadoren et al., 1999; Liechti and Vollenweider, 2000; de la Torre et al., 2004). Euphoria, increased self-esteem, high physical and emotional energy, mild perceptual disturbances, changed perception of colors and sounds, relaxation, dissociation (Semple et al., 1999) and an increase in sexual desire and satisfaction (Tancer and Johanson, 2001) were also reported .

However, MDMA can produce acute adverse psychological effects like anxiety, moderate thought disorder, poor coordination, impaired decision making ability and impulsive or aggressive behavior (El-Mallakh and Abraham, 2007; de la Torre et al.,

2004). Furthermore, the use of MDMA is associated with memory and cognitive impairment as well as the development of dependence on the drug in some consumers (Legendre et al., 2010).

2.4 Somatic effects of MDMA

The somatic effects of Ecstasy vary from almost universal minor symptoms to those that are rare but potentially life-threatening. Within four to six hours, MDMA taken orally produces short-term effects on cardiovascular function resulting from sympathomimetic stimulation like dose-dependent increases in heart rate and systolic and diastolic blood pressure (Kolbrich et al., 2008). Other minor clinical symptoms are jaw clenching (trismus) and tooth grinding (bruxism), dry mouth, mydriasis, nystagmus and sweating (Hall and Henry, 2006; Peroutka et al., 1988). Delayed effects that may last up to seven days include midweek depression and a prolonged "hangover" with muscle aches, fatiguability, lack of energy, depressed mood, insomnia, drowsiness, irritability, anxiety and difficulty concentrating (Curran and Travill, 1997; Peroutka et al., 1988; de la Torre et al., 2004).

Severe effects are relatively rare and include the serotonin syndrome (increased muscle rigidity, hyperreflexia and hyperthermia) (de la Torre, 2004 et al.), as well as sudden death, rhabdomyolysis and multi-organ failure, hyperpyrexia, isolated liver failure and hyponatraemia with cerebral oedema (Hall and Henry, 2006).

2.5 Neurochemical and neurotoxic effects of MDMA

Short after administration, MDMA causes the release of serotonin (5-hydroxytryptamine; 5-HT) from pre-synaptic 5-HT neurons in the central nervous system (CNS) with concomitant inhibition of reuptake (Johnson et al., 1986; McKenna and Peroutka, 1990; Nishisawa et al., 1999; Schmidt et al., 1986). MDMA also rapidly increases dopamine (DA) and norepinephrine (NE) release from cerebral tissue (Gough et al., 1991; Gudelsky et al., 1994; Nixdorf et al., 2001; Sabol and Seiden, 1998; Hall

and Henry, 2006), but the main focus of attention still seems to fall on 5-HT as the primary transmitter involved in both the psychological and physical effects of MDMA. For these reactions, MDMA binds to membrane transporters dealing with neurotransmitter reuptake and vesicular storage systems (de la Torre et al., 2004). Johnson et al. (1986) were the first who demonstrated an acute release of 5-HT from rat hippocampal slices by MDMA and reported that there had been no significant difference in the releasing effects of the two MDMA enantiomers (Johnson et al., 1986). There is also a dose-related increase in cortical acetylcholine (ACh) release, but the primary transmitters associated with acute psychological effects of MDMA are still 5-HT and dopamine (Colado et al., 2004; Liechti and Vollenweider, 2000). In contrast, dopamine does not seem to contribute to the physiological effects of MDMA, indicating a contribution of serotonin and norepinephrine (Liechti and Vollenweider, 2000). It is known that MDMA administration inhibits the activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme required for 5-HT synthesis (Stone et al., 1987a; b; 1988; Schmidt and Taylor, 1988). These effects can be very long-lasting or even permanent in animal studies (Hatzidimitriou et al., 1999; Fischer et al., 1995).

The neurochemical mechanism of action of MDMA is well studied in animals and has been shown to be neurotoxic upon central serotonergic systems when administered at high or repeated doses (Green et al., 2003). Neurotoxicity derives from damaging fine-diameter unmyelated serotonergic axons, presumably araising from the dorsal raphe nucleus, with sparing of cell bodies (Green et al., 2003; Lyles and Cadet, 2003). This leads to widespread reductions of serotonin, its metabolite 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT transporter (5-HTT, SERT) binding in brain tissue (Ricaurte et al., 1988; 1992; Stone et al., 1987b). Yet, there is some evidence for normalization of 5-HTT levels with prolonged abstinence of MDMA (Green et al., 2003).

However, given the fact, that most ecstasy users are polydrug users and most human studies are littered with methodological problems, including inadequate subject sampling, retrospective design, lack of baseline data before first ecstasy use and inclusion

of only moderate and heavy ecstasy users (Lyvers, 2006), the neurotoxicity of MDMA is still debated, although most researchers agree that MDMA is to some extent toxic to human serotonergic (and perhaps dopaminergic) neurons. They also agree that some of the toxicity may be long-lasting in humans as well (McCann et al., 1998; 2005; Reneman et al., 2001b). Concerning this matter, neuropsychological, neuroendocrine, and neuroimaging studies reported subtle abnormalities in MDMA exposed humans that may reflect funcional sequelae of long-term, neurotoxic modifications in serotonergic pathways (Green et al., 2003). Semple et al. (1999), using single photon emission computed tomography (SPECT) detected a reduction in 5-HT transporter density, but found no evidence of a reduction in dopamine transporter densitiy associated with the use of ecstasy at the same time. In contrast to that, a similar study only displayed a reduction in 5-HT transporter densitiy in female "heavy" users that perhaps indicates a higher susceptibility of women (Reneman et al., 2001a). The same authors suggest that exclusive use of ecstasy does not appear to be neurotoxic to dopaminergic neurones in humans (Reneman et al., 2002a). This observation is in accordance with previous animal studies which failed to find any damage to DA neurones following MDMA treatment (Steele et al., 1994; Green et al.; 1995). However, the combined use of amphetamine and MDMA seems be associated with a reduction in the density of nigrostriatal DA transporters (DAT) (Reneman et al., 2002a).

3 Amphetamine

3.1 History of amphetamine

For thousands of years amphetamines have been administered as plant products, more precisely ephedrine, the active constituent of *Ephedra* which is known as *Ma huang ("looking for trouble")* in traditional Chinese medicine. It has been used as an herbal remedy for asthma, hay fever and the common cold (Abourashed et al., 2003). Amphetamine was first synthesized in 1887 by the Romanian chemist Lazăr Edeleanu

(1862-1941, a.k.a. Edeleano) at the University of Berlin. He named the compound phenylisopropylamine. Chemists have since developed a wide range of derivatives, among them the psychopharmacologist Gordon Alles who resynthesized amphetamine in 1927 and first described its stimulant effects (Alles, 1933). In 1932, the pharmaceutical firm Smith, Kline and French introduced the volatile base form of the drug as a decongestant inhaler and four years later as tablet under the trade name Benzedrine. In the following years amphetamine was widely used as a specific therapy for neurotic depression and promoted as treatment of schizophrenia, opiate addiction, infantile cerebral palsy, seasickness, radiation sickness, and persistent hiccups (Bett, 1946). At the same time, it became more and more popular among students, artists, truck drivers and others due to word-of-mouth reports about its stimulant properties (Sulzer et al., 2005). During World War II amphetamine was extensively used by the military to promote alertness in soldiers (Sulzer et al., 2005). Finally, amphetamine became a Schedule II drug under the Controlled Substances Act in 1971 (Green et al., 2003). Nowadays, it is mainly used in the treatment of narcolepsy and attention deficit hyperactivity disorders (ADHD) (Seiden et al., 1993; Seeman and Madras, 1998).

3.2 Chemical structure of amphetamine

The chemical structure of amphetamine consists of an unsubstituted phenyl ring, a two-carbon side chain between the phenyl ring and nitrogen, an alpha-methyl group, and a primary amino group (Biel and Bopp, 1978). Amphetamines are derived from phenylethylamine (PE) and feature structural similarities to adrenaline (cf. Figure A.3).

Phenylethylamines are trace amines such as tyramine and tryptamine. These are endogenous amine compounds that are related to the classic neurotransmitters dopamine, serotonin, and noradrenaline by structure, metabolism and tissue distribution (Philips, 19984). Phenylethylamines have been postulated to function primarily as central ner-

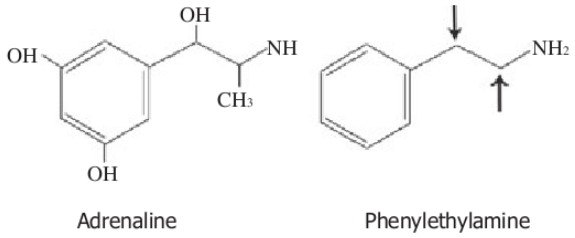


Figure A.3: *Chemical structure of adrenaline and phenylethylamine (Greene et al., 2008).*

vous system stimulants (Greene et al., 2008). In electrophysiological studies, PE has two effects on neuronal activity: an indirect sympathomimetic effect and a modulatory effect on neuronal responses to catecholamines (Lundberg et al., 1985).

3.3 Psychological and somatic effects of amphetamine

Like MDMA, amphetamine and its more potent analogue methamphetamine, are effective psychostimulants causing euphoria, alertness, increased libido and self-confidence that can lead to abuse as well as psychological and physical addiction (Anglin et al., 2000). Further psychological effects can include mood and anxiety disorders, irritability, aggressiveness, cognitive deficits and with chronic and/or high doses, amphetamine psychosis can occur (Darke et al., 2008).

The somatic effects of this recreational drug, including reduced appetite, increased or distorted sensations, hyperactivity, dry mouth, erectile dysfunction, sweating, impaired speech, uncontrollable movements or shaking, vasoconstriction, palpitations and arrhythmia are nearly similar to the clinical picture associated with MDMA. The administration of amphetamine can lead to an overdrive of the adrenergic system that ends up in a sympathomimetic toxidrome of tachycardia, hyperthermia, tachypnoea, hypertension, mydriasis and a wide range of CNS states (hyperarousal, agitation, paranoia, hallucinations, disinhibition, seizures and coma) (Henry et al., 1992; Derlet et al., 1989).

3.4 Neurochemical and neurotoxic effects of amphetamine

Similar to MDMA, stimulant amphetamines act as substrates for transporters associated with the uptake of the biogenic amines serotonin, dopamine and norepinephrine. Amphetamines also induce acute release from storage vesicles and reuptake inhibition into vesicles of 5-HT and DA, thus increasing the cytoplasmatic concentrations of the neurotransmitters and making them more readily available for reverse transport. In addition, amphetamines increase the release of glutamate (GLU), which supposably contributes to the neurodegenerative properties of these substances (Nash and Yamamoto, 1992; Rocher and Gardier; 2001).

MDMA and amphetamine/METH vary in their affinities for monoamine transporters: MDMA has a greater affinity for 5-HT transporters compared to DA transporters than amphetamine or METH (Rothman and Baumann, 2003).

As well as MDMA, amphetamines were shown to be neurotoxic in a number of animal studies (Seiden and Sabol, 1996). High and/or repeated doses of amphetamine or methamphetamine cause long-term destruction of presynaptic serotonergic and dopaminergic axon terminals, which subsequently leads to 5-HT and DA depletion and to lower 5-HT and DA transporter densities in the CNS (Seiden and Sabol, 1996; Hanson et al., 2004; McCann and Ricaurte, 2004). Another aspect related to amphetamine induced neurotoxicity involves excitotoxic damage following glutamate release and activation of glutamate receptors (Yamamoto and Bankson, 2005; Nash and Yamamoto, 1992).

4 The main neurotransmitters: serotonin and dopamine

4.1 Serotonin

Serotonin, a biogenic amine, is present in significant amounts in numerous structures of the CNS. It is involved in the control and regulation of a wide variety of physiological functions, such as sensory (e.g. visual processing and orientation) and motor functions, memory, mood, cognition, vegetative functions and neuroendocrine secretion of hormones. It has also been implicated in the etiology of a range of psychiatric disorders including anxiety, depression, and eating disorders, along with other conditions such as obesity and migraine (Ayala, 2009). Several reports suggest that some recreational MDMA users display selective abnormalities in most of these functional domains (Green et al., 2003). The most consistent findings have been on impaired cognitive performances in learning und memory tasks (Bolla et al., 1998; Parrott and Lasky, 1998; Gouzoulis-Mayfrank et al., 2000; 2003).

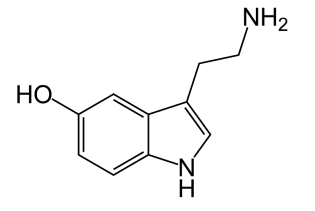


Figure A.4: *Structure of serotonin.*

Serotonin has important effects on the cytoskeleton, including coupling to brain neurotrophic factors (S-100 beta and BDNF/brain derived nerve growth factor) implicated in the regulation of neuronal morphology and apoptosis (Duman et al., 2001; Azmitia, 2002). Furthermore, it affects vasoconstriction via innervation of intracranial blood vessels (Marco et al., 1999) which can lead to vascular ischemia or hemorrhage. These effects in addition to transmitter-mediated toxicity can potentially result in absolute or relative reductions in gray matter (Cowan et al., 2003).

4.2 Dopamine

Dopamine is a key regulator in the coordination and integration of the different aspects of behaviour and cognition (Nieoullon, 2002). This member of the catecholamine family is involved in the modulation of voluntary movement, hormonal secretion, mood, working memory, learning and the adaption of emotion, motivation, and attention. Associated with the pleasure system of the bain, dopamine provides feelings of enjoyment, reinforcement and reward (Keltikangas-Jaervinen and Salo, 2009).

These effects are mediated via three main dopaminergic pathways in the brain: the mesocorticolimbic DA system which is involved in emotion-related behavior, the tuberoinfundibular system, and the mesodiencephalic DA system including the negrostriatal system which plays an essential role in the control of voluntary movement (Arias-Carrion and Poeppel, 2007).

Even though, neurotoxic dopaminergic lesions can cause dysfunction in motion, cognition and psychopathological sequelae in theory (Gouzoulis-Mayfrank and Daumann, 2006), studies on long-term psychopathological failures associated with stimulant drug use are inconclusive (Riehman et al., 2002). However, deficits in short-term memory, frontal executive control and planning abilities have been demonstrated at the same time (Ornstein et al., 2000; Salo et al., 2002). This is where the chicken and the egg issue arises, as it is unclear whether these shortcomings are an implication of the use of stimulants or whether they reflect pre-existing low cognitive means (Gouzoulis-Mayfrank and Daumann, 2006).

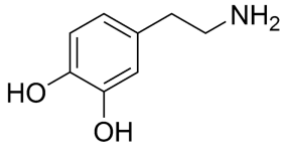


Figure A.5: *Chemical structure of dopamine.*

5 Structural alterations

5.1 MDMA-related structural alterations

MDMA-induced changes in brain structure are based upon several parameters including the 5-HT reuptake transporter, the 5-HT2A receptor and regional brain volume (Cowan et al., 2008). Given the fact that MDMA produces 5-HT axon damage, drug associated alterations are expected to take place in brain regions with a high density of serotonergic innervations. In animal studies, neurotoxic effects are mainly observed in the frontal cortex and hippocampus (Reneman et al., 2001b). These regions are known to play a pivotal role in cognitive function and memory (Reneman et al., 2001b).

Miscellaneous studies made use of 5-HTT radionuclide ligand binding to detect serotonergic transporter loss across multiple brain regions. MDMA exposed humans consistently showed lower 5-HTT levels (Semple et al., 1999; Reneman et al., 2001c; McCann et al., 1998; 2005). However, it is unclear whether reduced ligand binding actually depends on actual 5-HT axon loss or on other effects, such as long-standing decrease in expression of the 5-HTT (Cowan et al., 2008).

5-HT2A receptors that are post-synaptic to 5-HT axons (Cowan et al., 2008), are enriched in many areas of the cortex (Pazos et al., 1987; Hoyer et al., 1986). To date, one study observed reduced 5-HT2A receptor levels in multiple cortical regions in the group of ongoing/acute MDMA users compared to controls by using possibly single-photon emission computed tomography (SPECT) (Reneman et al., 2002b). These effects tend to be based on ongoing MDMA-mediated increases in 5-HT release. However, ex-MDMA users showed an upregulation of 5-HT2A receptors in the occipital cortex, which is possibly a result of MDMA-induced 5-HT depletion (Reneman et al., 2002b).

As mentioned above, the serotonin transmitter system and psychotropic drugs that act on serotonin, affect certain growth factors, such as S-100 beta and BDNF, via 5-HT

receptor-binding (Duman et al., 2001; Azmitia, 2002). Loss of serotonergic coupling can lead to changes in neuronal plasticity as well as loss and damage of neurons, which can be detectable as reduced regional brain volume (Cowan et al., 2003).

De Win et al. (2008) suggest that even low dosages of MDMA lead to sustained effects on brain microvasculature, white matter maturation and possibly axonal damage. In this regard, de Win et al. (2008) showed decreased regional relative cerebral blood volume in the globus pallidus and putamen, decreased fractional anisotropy (FA) in thalamus and frontoparietal white matter, increased FA in globus pallidus and increased apparent diffusion coefficient in the thalamus in novel ecstasy users. There was no evidence for changes in serotonin transporter densities (de Win et al., 2008).

One co-registered SPECT and magnetic resonance imaging (MRI) study has reported a negative correlation between duration of MDMA use and individual global brain volume, although there was no difference in mean global brain volumes between the ecstasy and control groups (Chang et al., 2000). Further abnormal imaging findings in globus pallidus and subcortical white matter have been reported in ecstasy users who have survived initial toxic reactions (Bitsch et al., 1996; Bertram et al., 1999; Spatt et al., 1997).

Finally, MDMA users may have differences in brain structure (e.g. cortical gray matter volume reductions) or function that may be a biological marker for the risk of addictive behavior and accordingly MDMA use (Cowan et al., 2008; Bartzokis et al., 2000).

5.2 Amphetamine-related structural alterations

In case of amphetamines, structural abnormalities could be observed in young recreational amphetamine users compared to drug-naïve controls in a MRI study (Bartzokis et al., 2000). Beside age-related gray matter volume reductions of the frontal lobe, which occured in all examined groups, the amphetamine group had significantly smaller temporal lobe gray matter volumes compared to the normal control

group. Other regions of interest than the frontal and temporal lobe were not quantified in this study (Bartzokis et al., 2000).

Yet another MRI study investigating structural deficits in the human brain detected less gray matter in chronic methamphetamine abusers in comparison with age-matched, healthy controls (Thompson et al., 2004). These deficits were located in the cingulate, limbic and paralimbic cortices. MRI-based maps were consistent with previous suggestions that chronic methamphetamine use causes brain damages that may lead to impaired memory performances (Berman et al., 2008).

Using voxel-based morphometry (VBM), Kim et al. (2006) compared differences in gray and white matter density between 29 methamphetamine users and 20 control subjects on the one hand and former methamphetamine users who were abstinent for either less than six months (n=11) or more than six months (n=18) on the other hand. The authors reported lower gray matter density in the right middle frontal gyrus (Brodman area 10) of methamphetamine abusers, as compared to control subjects. Abstinent methamphetamine users had prefrontal gray matter density decrease, whereas short-term abstinent abusers showed higher deficits, that is why the authors suggest at least a partial recovery with long-term abstinence. There were no significant abnormalities in white matter detectable (Kim et al., 2006).

6 Current state of research

To date, there are only two voxel-based morphometry (VBM) studies investigating neuronal integrity in MDMA users. Cowan et al. (2003) compared regional brain gray and white matter concentration of 31 MDMA polydrug users (participants had abused MDMA at least five times) and 29 non-MDMA users. The authors reported significantly smaller gray matter densities in multiple brain regions of MDMA polydrug users compared to controls. These reductions were localized to neocortex in bilateral Brodman area (BA) 18 in the occipital lobe, left BA 45 in the frontal lobe, and left BA 21 in the temporal lobe (cf. Figure A.6). In addition, the bilateral cerebellum

and the midline brainstem also revealed decreased gray matter concentration. However, no detectable differences in white matter integrity could be found (Cowan et al., 2003).

The study findings are consistent with the previously existing evidence that MDMA may be neurotoxic upon serotonin fibers, since MDMA is known to only damage the fine-diameter serotonergic axons araising from the dorsal raphe nucleus (Ricaurte et al., 1988). Furthermore, these outcomes are in agreement with clinical findings, which demonstrated several cognitive impairments in verbal and auditory memory (Bolla et al., 1998; Verkes et al., 2001; Reneman et al., 2000), as well as in learning (Herholz et al., 2001). These kinds of tasks demand activations in aforementioned brain regions (Lee et al., 2002; Booth et al., 2002; Friederici et al., 2000).

The other study was just like the current study part of a more comprehensive investigation of the department of psychiatry and psychotherapy at the University of

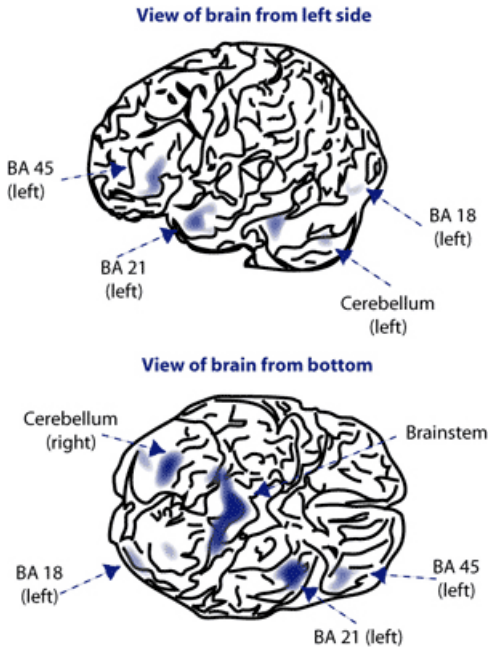


Figure A.6: *Preliminary results from voxel-based morphometry suggest reduced gray matter densities in some brain areas of MDMA polydrug users. Affected areas include the bilateral Brodman area (BA) 18, the left BA 45 and BA 21, as well as the bilateral cerebellum and midline brainstem (Cowan et al., 2003).*

Cologne. By applying tract-based spatial statistics (TBSS) and voxel-based morphometry, 42 polydrug users with very limited experience concerning the consumption of MDMA and amphetamine, 20 heavy users and 16 drug-naïve controls underwent high resolution magnetic resonance imaging (Daumann et al., 2011). In comparison to the group of beginning users, heavy users exhibited multiple regions of statistically significant reductions of gray matter bilaterally in the gyrus frontalis medialis and the left frontal pole, as well as in the left gyrus rectus and the right gyrus paracingularis. However, low exposure users with a lifetime dose less than five units did not show alterations in gray matter integrity compared to drug-naïve controls. Just like Cowan and colleagues (Cowan et al., 2003), they could not detect any differences in white matter integrity between the two groups in this investigation (Daumann et al., 2011).

7 The current study

The primary aim of the current study was to investigate neuronal integrity in nineteen low exposure users of the illicit drugs MDMA and amphetamine in comparison with a matched group of sixteen drug-naïve volunteers. Due to preexisting findings (Daumann et al., 2011; Ricaurte et al., 1988), we assumed that there would be no discrepancy in white matter integrity between drug users and controls (Cowan et al., 2003). Data analysis was performed similarly as in the study of Daumann et al., 2011. In difference to Daumann et al., 2011, the current study used a different sample of users, thereby validating previous findings with different consumption parameters. Nevertheless, we used tract-based spatial statistics (TBSS) to detect possible alterations in white matter integrity in a first step. In a second step, we applied voxel-based morphometry in order to detect drug-related reductions in gray matter on a voxel-by-voxel basis. Because of the different severities of ecstasy and speed use of the nineteen participants of the user group, we additionally searched for correlations between gray matter density and drug use characteristics among this group.

As shown above, MDMA can affect the brain in a variety of ways. Cowan et al. (2003)

created the thesis that decreased gray matter concentration is a result of serotonin-mediated trophic effects. Previous findings hypothesized that the initial use of either MDMA or amphetamine does not lead to lasting altered neurophysiological processes. However, chronic and heavy consumption impairs brain areas with a high vulnerability to the effects of either of these substances (Cowan et al., 2003). Only de Win et al. (2008) indicated some small lasting effects of ecstasy on brain microvasculature, white matter maturation and possibly axonal damage after low-dose ecstasy use. As long as it is unclear whether these effects are reversible or not, ecstasy may be neurotoxic even in low doses (de Win et al., 2008). Some authors suggest that perhaps even a single dose of MDMA may be neurotoxic (Gijsman et al., 1999; McCann and Ricaurte, 2001).

B. Materials & Methods

1 Participants

The present study was part of a more comprehensive investigation by the department of psychiatry and psychotherapy at the University of Cologne which also assessed the effects of the synthetic drugs ecstasy and amphetamine on cognitive performance, psychological wellbeing and neuroanatomic changes. Data were collected between 2007 and 2009.

Nineteen low exposure users (15 men, 4 women; mean age: 24.6 years ± 5.1) were recruited directly in the dance scene by students who were involved in the study, via word-of-mouth and via the internet. Low exposure users were included to the study if they were at least 18 years old and had taken one of the investigated drugs at least once. The use of methamphetamine is not common in West Germany and was absent in the study sample, leaving MDMA, amphetamines and cannabis as the primarily investigated substances.

This group of low exposure users was compared to sixteen drug-naïve controls (9 men, 7 women; mean age: 26.3 years ± 4.1). Controls had no previous or current history of alcohol or drug use. This sample of drug-naïve controls was also used in the study of Daumann et al., 2011.

Exclusion criterion for the low exposure users was the use of ecstasy or speed of more than 35 units. Given the fact that daily moderate cannabis use was part of the life style of many subjects (Gouzoulis-Mayfrank und Daumann, 2006) and because cannabis screens may remain positive for several weeks after the last use, we accepted that subjects would only abstain from cannabis on the study day. Further exclusion criteria for both examinations were a history of current or previous severe alcohol consumption (according to DSM-IV criteria, APA 1994), metallic objects in the body, pregnancy, claustrophobia, psychological or neurological disorder or any other general medical

condition requiring pharmacological treatment.

2 Procedure

All users agreed to abstain from drug use for at least seven days prior to the study, with the exception of cannabis. Drug screens for stimulant amphetamines, MDMA, cocaine and its metabolites, marijuana, benzodiazepines, barbiturates and opiates (enzyme-multiplied immunoassay, von Minden GmbH) were performed on the study day. In addition, all participants underwent a drug history interview assessing time since last use, mean and maximum frequency of use, mean and maximum quantity intake per occasion and lifetime use of amphetamines, MDMA, cannabis and other substances on the study day. Results of hair analyses from randomly chosen subjects were collected by the Institute of Legal Medicine of the University of Cologne and showed no evidence for mismatch between self reported drug questionnaires and actual drug consumption. Potential confounders such as demographic variables and education were measured.

The study was carried out in accordance with the Helsinki Declaration of 1975 and was approved by the local ethics committee at the Medical Faculty of the University of Technology Aachen and the Federal Health Administration (Bundesinstitut für Arzneimittel und Medizinprodukte, Bundesopiumstelle, Berlin). Written informed consent was obtained from all subjects after we described the experimental procedures in detail and explained that they may withdraw from the study at any time without having to explain the reasons.

3 Image acquisition

Magnetic resonance imaging (MRI) was performed on a clinical Siemens Magnetom Trio Tim whole body MRI system. The data were acquired using a standard quadrature head coil to obtain images with signal intensities being as homogeneous as possible. Head movements were minimized in all subjects using foam pads and Velcro

straps. Subjects with any observable structural abnormalities were excluded from the study (exclusion criterion).

For VBM, three-dimensional T1-weighted structural images were obtained on a clinical Siemens Magnetom Trio Tim whole body MRI system operating at 3.0 Tesla. The imaging parameters for T1-weighted images were: flip angle = 18°, repetition time (TR) = 1930 ms, echo time (TE) = 5.8 ms, slice thickness = 1.25 mm, voxel size = 1.00 x 1.00 x 1.25 mm, SNR = 1 and matrix size = 256 mm.

Diffusion tensor images were obtained in the same session with parameters of: TR = 9000 ms, TE = 87 ms, slice thickness = 1.7 mm, voxel size = 1.7 x 1.7 x 1.7 mm, SNR = 1 and matrix size = 220 mm in six blocks, each containing ten diffusion directions and one unweighted b0 image. Additionally, magnetic field mapping was performed with the identical slice positions and geometric parameters to account for local field inhomogeneities in DTI volumes.

4 Data analysis

This study is based on a combination of structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI). Localised statistical testing of diffusion data was carried out using tract-based spatial statistics (TBSS). The alignment-invariant white matter tract representations acquired in this analysis were also used to alleviate the characteristic distortion introduced by non-linear co-registration of subjects in the VBM analysis.

The analysis of diffusion tensor and structural brain imaging data was performed by the tools of FSL 4.1.4 (FMRIB Software Library, Oxford, UK). Statistical analysis was carried out by the software tool Randomise that is implemented to FSL as well.

The statistical analysis of the demographic data was performed by means of χ^2- Test and unpaired t-test, both part of SPSS 17 (SPSS inc., Chicago III). Differences in brain

imaging data and demographic data were considered significant when $p < 0.05$.

5 TBSS

Voxelwise statistical analysis of the FA data was carried out using TBSS (tract-based spatial statistics), part of FSL (Smith et al., 2004). The measure of the fractional anisotropy quantifies how strongly directional the local tract structure is by assessing the diffusion of water. It is highest in major white matter tracts (maximum theoretical value 1) and lower in gray matter while approaching 0 in cerebro-spinal fluid (CSF) (Smith et al., 2006) (cf. Figure B.2).

Prior to running TBSS analysis we created FA images from our diffusion study data. Therefore diffusion images with no weighting were differentiated into brain and non-brain tissue using BET (Brain Extraction Tool) (cf. FigureB.1) and corrected for the effects of eddy currents. Unweighted b0 images were extracted and used as a reference for head motion correction of the same subjects weighted images with 12 degrees of freedom. Afterwards, the corrected images were registered to standard space. Using the gradient directions and b-values, fractional anisotropy (FA) images of all participants were created by fitting a tensor model to the raw diffusion data using FDT (FMRIB's Diffusion Toolbox).

After obtaining the FA images (cf. Figure B.3), all data were brain-extracted using the FSL brain extraction tool (Smith, 2002) and then aligned into a 1x1x1 mm standard space (FMRIB58_FA) using the nonlinear registration tool FNIRT (Andersson et al., 2007a;b), which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). This standard space was made up of a high-resolution average of 58 well-aligned good quality FA images from healthy male and female subjects at the age of 20 to 50.

Finally, this was followed by a linear registration of the nonlinear transformations found in the previous stage with the same standard to bring them ideally into standard space.

Next, the mean of all aligned FA images was created and thinned to create a mean FA skeleton which represents the centres of all tracts common to the subjects involved in the study. Each subject's aligned FA data was then projected onto this skeleton by filling the skeleton with FA values from the nearest relevant tract centre and the resulting data fed into voxelwise cross-subject statistics. Smoothing was performed with full width at half maximum (FWHM) of 7.05 mm.

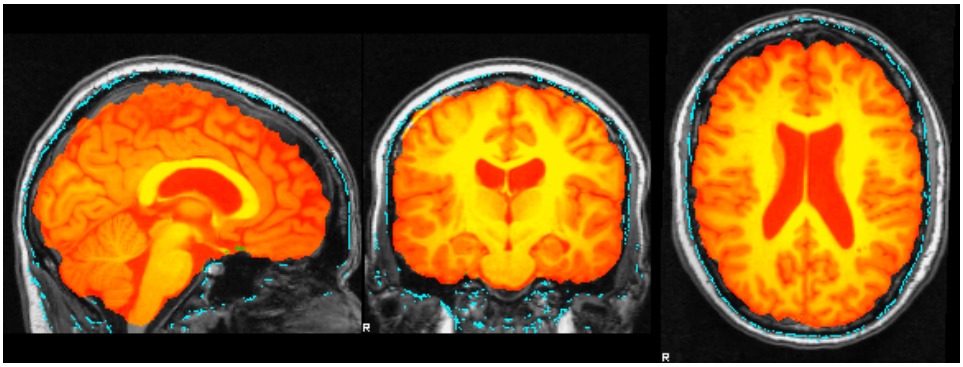


Figure B.1: *T1 image segmented into brain and non-brain tissue by BET.*

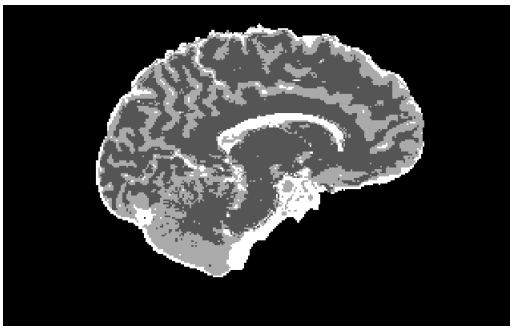


Figure B.2: *Segmented T1 image.*

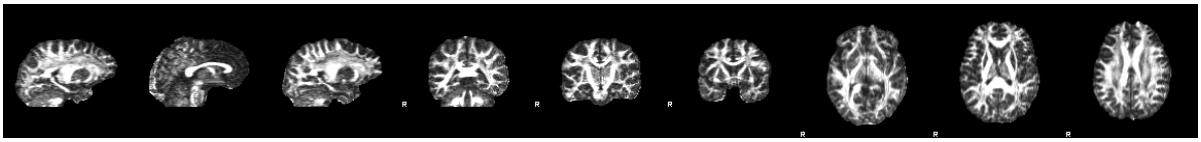


Figure B.3: *Example of FA image of one subject.*

6 Voxel-based morphometry (VBM)

6.1 Primary comparison

Structural data were analyzed with FSL-VBM, a voxel-based morphometry style analysis (Ashburner and Friston, 2000; Good et al., 2001) carried out with FSL tools (Smith et al., 2004). VBM is a tool for studying patterns of brain change in development or disease and neuroanatomical correlates of subjects' characteristics (Ridgway et al., 2008).

This essentially involves voxel-wise statistical analysis of pre-processed structural MR images. The inputs to VBM's statistical analysis are derived from structural MR images using tissue segmentation, spatial normalization and smoothing (Ridgway et al., 2008). To optimize inter-subjects brain registrations, registration matrices from the TBSS analysis were used to bring all T1 weighted images into FA space. For this purpose, all T1 images were registered to the same subjects FA image (9 df), followed by a linear (12 df) and then a non-linear registration of this image with FA standard space.

Subsequently, the VBM standard protocol was applied: The T1-weighted images in FA space were brain-extracted by means of FSL brain extraction tool (BET) (Smith, 2002). Tissue-type segmentation into gray matter (GM), white matter (WM), cerebrospinal fluid (CSF) was carried out using FAST4 (Zhang et al., 2001). The resulting gray-matter partial volume images were then aligned to MNI152 (Montreal Neurological Institute) standard space using the affine registration tool FLIRT (Jenkinson and Smith, 2001) with seven degrees of freedom, followed by nonlinear registration using FNIRT. The resulting images were averaged to create one study-specific template, using the same number of subjects in both groups to avoid any bias during the

registration step that would have consisted in favouring one of the groups. The native gray matter images were then non-linearly re-registered to this template. Afterwards, the registered partial volume images were modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field.

The next image-processing step is smoothing the segmentations through convolution with a FWHM of 7.05 mm and accordingly with a FWHM of 9.42 mm in an additional procedure. The approximation to Gaussian distribution of the data is prerequisite to the followed statistic analysis.

The final step of a VBM analysis involves a voxel-wise statistical analysis to determine voxels with statistically significant differences in gray matter composition. This employs the general linear model (GLM), a flexible framework that allows a variety of different statistical tests such as group comparisons and correlations with covariates of interest. GLM was applied to each voxel of raw statistical TBSS and VBM images using permutation-based non-parametric testing, threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009) and family wise error correction ($p<0.05$). TFCE-based thresholding takes a raw statistic image and produces an output image in which the voxel-wise values represent the amount of cluster-like local spatial support. To verify the findings, a second more liberal approach for displaying FSL-VBM results was applied, namely cluster-based thresholding.

The output of these methods is a statistical map showing regions where gray matter concentration differs significantly between the two groups (Ashburner and Friston, 2000).

6.2 Secondary comparison

Former studies of experienced users of amphetamine-type stimulants revealed differences in gray matter concentration merely in frontal brain areas (Daumann et al., 2011). Therefore, we payed particular attention on these regions by creating a frontal mask to detect significant difference between consumers and non-consumers in a

more sensitive additional VBM analysis (cf. Figure B.4).

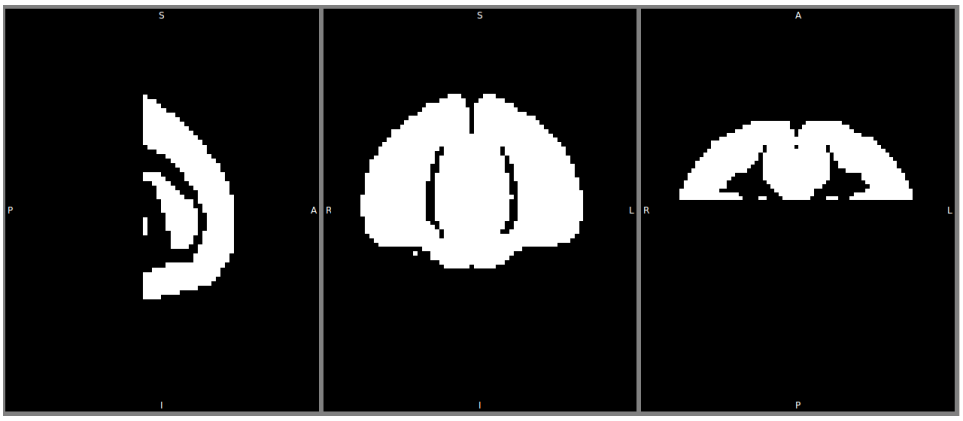


Figure B.4: *Frontal mask.*

As drug use parameters diverged highly within the group of beginning users, we performed a secondary comparison to detect possible correlations between gray matter concentration and the variables listed below.

Drug use characteristics for MDMA:

1. Lifetime dose MDMA (pills)
2. Total dose MDMA of the last year (pills)
3. Daily dose MDMA (pills per occasion)
4. Maximum dose MDMA (pills per occasion)
5. Frequency of intake of MDMA (pills per month)
6. Maximum frequency of intake of MDMA (pills per month)
7. Elapsed time since last intake of MDMA (days)

Drug use characteristics for amphetamine:

1. Lifetime dose amphetamine (grams)

2. Total dose amphetamine of the last year (grams)
3. Daily dose amphetamine (milligrams per occasion)
4. Maximum dose amphetamine (milligrams per occasion)
5. Frequency of intake of amphetamine (days per month)
6. Maximum frequency of intake of amphetamine (intakes per month)
7. Elapsed time since last intake of amphetamine (days)

Drug use characteristics for cannabis:

1. Lifetime dose cannabis (grams)
2. Total dose cannabis of the last year (grams)
3. Daily dose cannabis (joints per occasion)
4. Maximum dose cannabis (joints per occasion)
5. Frequency of intake of cannabis (days per month)
6. Maximum frequency of intake of cannabis (intakes per month)
7. Elapsed time since last intake of cannabis (days)

C. Results

1 Demographics and drug use patterns

Sociodemographic data of the 35 subjects are presented in Table C.1. The 19 low exposure users and the 16 drug-naïve controls did not differ significantly in key demographic variables of age and sex. Subjects ranged in age from 19 to 36 years with a mean age of 24.6 years old (standard deviation ± 5.1) in the user group and a mean age of 26.3 years old (standard deviation ± 4.1) in the non-consumer group. On average, the drug user group was about two years younger than the control group, but the differences were not statistically significant (t = 1.061, df = 33, p = 0.297).

The consumer group consisted of 15 males and 4 females whereas the non-consumer group consisted of 9 males and 7 females. The proportion of males and females was similar in groups (χ^2 = 0.150). The subjects of both groups were all right-handed (inclusion criterion).

The years of education showed significant differences between the two groups, although the levels of education were similar in the ecstasy/amphetamine group compared to the group of abstinent controls (t-test: p = 0.016 and Mann–Whitney U-test: p = 0.107).

The patterns of drug use of the beginning users are presented in Table C.3. Sixteen of the nineteen subjects had gained experiences with ecstasy, nineteen subjects had gained experiences with amphetamine and sixteen of them had gained experience with both substances (cf. Table C.3).

The average extent of use was about 1.14 (± 1.35) ecstasy pills per occasion, which is a typical recreational and not a very heavy use. The average estimated total dose was 7.51 (±8.58) ecstasy tablets. Most of the subjects reported a sporadic use of ecstasy, only one subject noted a regular use of five months. Amphetamine was consumed at an average of 5.06 (±5,01) grams and accordingly 422.22 (±342.66) milligrams per occasion. Four participants consumed amphetamine at regular intervals for some time, whereas the rest of the group of beginning users never had taken amphetamine continuously.

In general, a higher extent of ecstasy use was associated with a higher extent of

	Beginning consumers (N = 19)	Non-consumers (N = 16)	t/χ^2	p
Present age (years)[1]	24.6 (± 5.1)	26.3 (± 4.1)	1.061	0.297
Gender (m:f)[2]	15 : 4	9 : 7	2.076	0.150
Level of education[3]:				0.107
Certificate of general education	2	0		
Certificate of General Education ordinary level	3	1		
Certificate of General Education advanced level	13	12		
University degree	1	2		
Years of education[1]	14.9 (± 3.1)	17.5 (± 2.7)	2.545	0.016

Table C.1: *Demographic features of beginning ecstasy and amphetamine users and non-consumers: mean (± standard deviation). [1]t-values were calculated using unpaired t-test; [2]comparison tested with chi[2]; [3] comparison tested with Mann-Whitney U-test.*

Experience with...	N
Ecstasy	16
Amphetamine	19
Cannabis	19
Cocaine	5
Hallucinogenic drugs	5
Sedative and hypnotic drugs	1
Opiates	1
Solvents and sniffing agents	0
Last use of...	Days
Ecstasy	180.5 (± 286.8)
Amphetamine	40.3 (± 63.8)
Cannabis	15.1 (± 40.3)

Table C.2: *Previous experiences with substances and elapsed time since last use of the 19 beginning users.*

amphetamine and a lower extent of cannabis use, although the correlation between MDMA and cannabis use was not particularly high (Pearson's correlation coefficients: r = 0.727 for amphetamine and r = -0.009 for cannabis). Amphetamine and cannabis

use were also correlated negatively (Pearson's correlation coefficient: r = -0.123).

The group of beginning consumers of MDMA and amphetamine showed a moderate use of cannabis (lifetime dose: mean = 916.7 ± 1149.3 grams; daily dose: mean = 3.5 ± 2.68 joints; maximum dose: mean = 17.87 ± 12.85 joints; one joint = about 0.25 grams). The control group denied any use of drugs.

	Mean	Median	Variance	Range	Minimum	Maximum
Lifetime dose MDMA (pills)	7.51 (± 8.58)	5.00	73.70	35.00	0.00	35.00
Lifetime dose amph (grams)	3.0 (± 1.6)	3.0	2.5	4.8	0.2	5.0
Lifetime dose cannabis (grams)	916.7 (± 1149.3)	4.8	1320836.6	4039.5	0.5	4040.0
Daily dose MDMA (pills/occasion)	0.64 (± 0.64)	0.75	0.41	2.00	0.00	2.00
Daily dose amph (milligrams/occasion)	422.2 (± 342.6)	425.0	117418.3	1000.00	0.00	1000.00
Daily dose cannabis (joints/occasion)	1.27 (± 0.96)	1.00	0.93	4.30	2.00	4.50
Maximum dose MDMA (pills/occasion)	1.14 (± 1.35)	1.00	1.83	5.00	0.00	5.00
Maximum dose amph (milligrams/occasion)	611.7 (± 475.4)	580.0	225979.4	1500.00	0.00	1500.00
Maximum dose cannabis (joints/occasion)	3.50 (± 2.58)	3.00	7.19	9.80	0.20	10.00

Table C.3: *Drug use characteristics of the beginning ecstasy and amphetamine users: mean (± standard deviation).*

2 Brain imaging

2.1 Primary comparison

The primary aim of this study was to compare gray and white matter integrity between low exposure MDMA/amphetamine users and completely abstinent controls with a statistical threshold of $p < 0.05$ controlling for multiple comparisons.

As expected, no significant differences in white matter integrity between the two groups could be discovered in TBSS analysis.

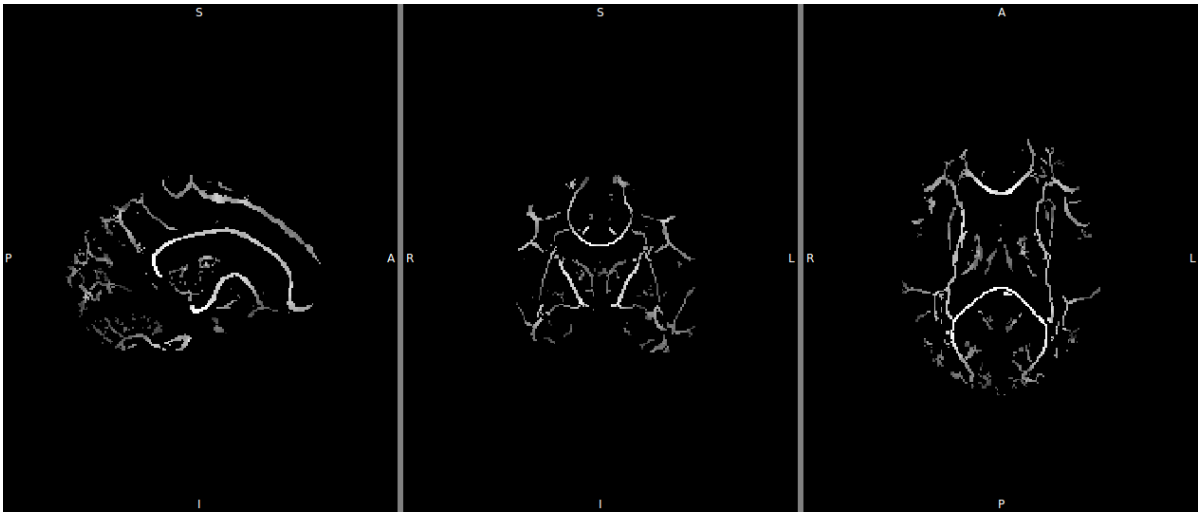


Figure C.1: *Acquired white matter skeleton on fractional anisotropy standard image used for inter-subject brain alignment.*

At this significance threshold of $p = 0.05$, VBM detected no significant reductions in gray matter concentration. Oppositely, no significant increase in white and gray matter could be located in this study.

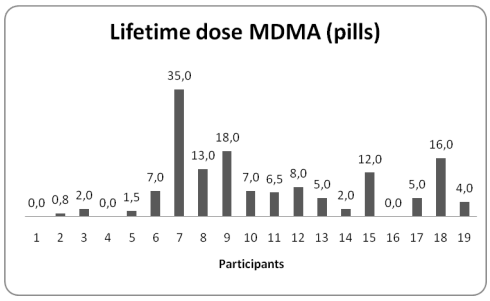
	p-value
TBSS	0.32
VBM s 3mm	0.48
VBM s 4 mm	0.20

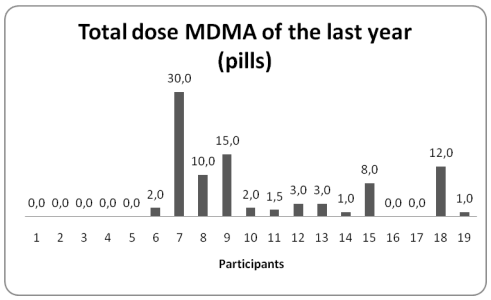
Table C.4: *Results of the TBSS analysis (corrected for multiple comparisons) to detect differences in white matter integrity and of the VBM analysis to detect differences in gray matter density smoothed with an isotropic Gaussian kernel with a sigma of 3 mm and 4 mm .*

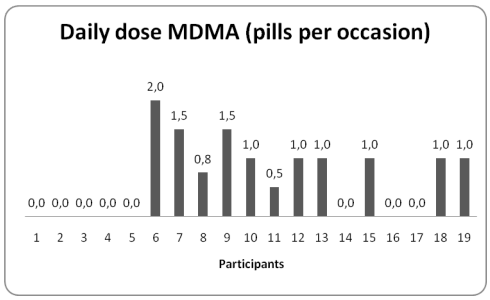
2.2 Secondary comparison

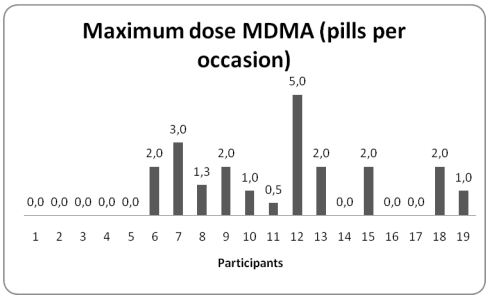
In a secondary comparison, we analyzed other variables that might lead to reduced gray matter concentrations of low exposure users. Although drug use characteristics of the nineteen MDMA and amphetamine users are heterogeneous within this group (cf. Figure C2-4), no correlations between the single drug use parameter and gray matter density could be detected.

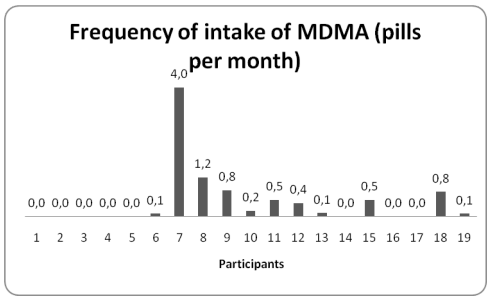
The five participants with a lifetime dose of MDMA of at least 10 pills and the ten low-level consumers with a lifetime dose of less than 5 pills showed no differences in gray matter integrity at a statistic threshold of $p < 0.05$. Sociodemographic data of the nineteen subjects are presented in Table C.5. The group of the subjects with a higher level of intake and the low-level consumers did not differ significantly in age, sex and education.






Daily dose MDMA (pills per occasion)
0,0 0,0 0,0 0,0 0,0 2,0 1,5 0,8 1,5 1,0 0,5 1,0 1,0 0,0 1,0 0,0 0,0 1,0 1,0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants


Maximum dose MDMA (pills per occasion)
0,0 0,0 0,0 0,0 0,0 2,0 3,0 1,3 2,0 1,0 0,5 5,0 2,0 0,0 2,0 0,0 0,0 2,0 1,0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants


Frequency of intake of MDMA (pills per month)
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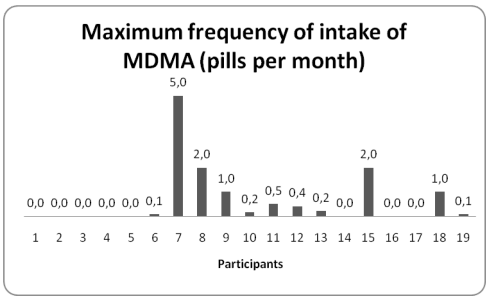
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants


Maximum frequency of intake of MDMA (pills per month)
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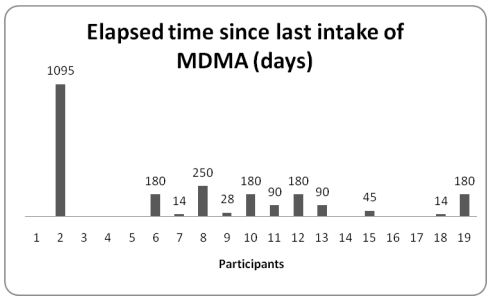
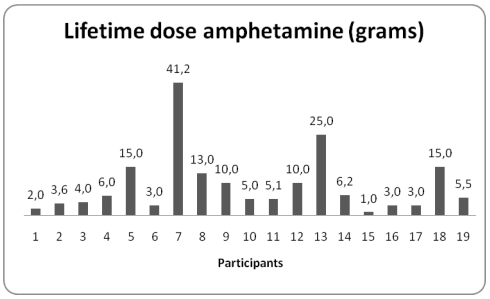
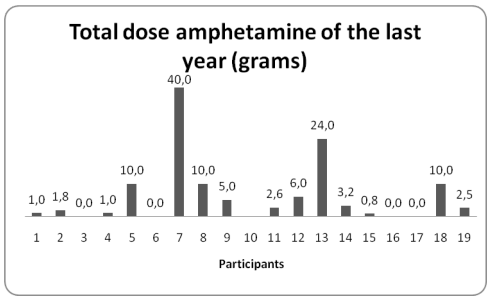
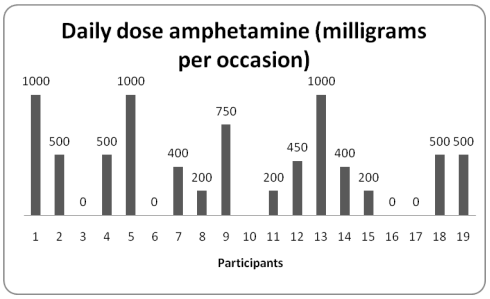
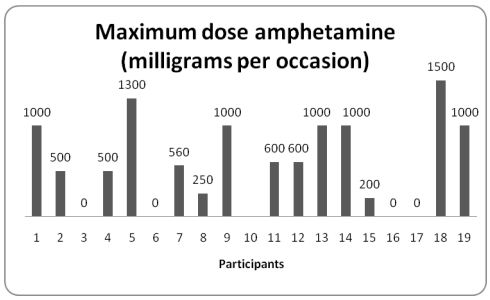
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

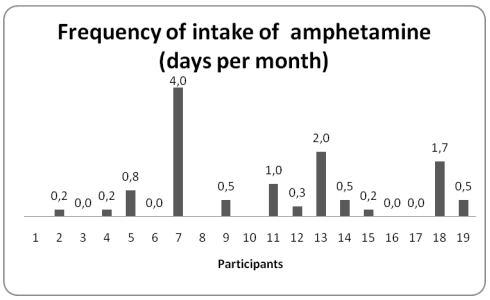

Elapsed time since last intake of MDMA (days)
1095 180 14 250 28 180 90 180 90 45 14 180
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

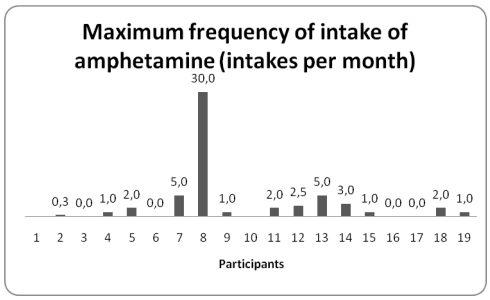

Lifetime dose amphetamine (grams)
2,0 3,6 4,0 6,0 15,0 3,0 41,2 13,0 10,0 5,0 5,1 10,0 25,0 6,2 1,0 3,0 3,0 15,0 5,5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

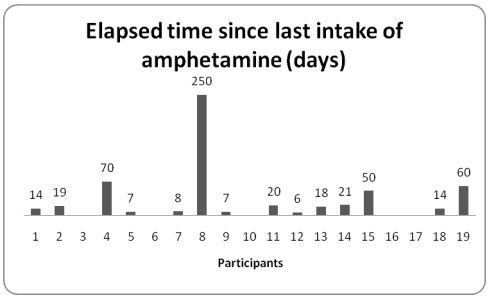

Total dose amphetamine of the last year (grams)
1,0 1,8 0,0 1,0 10,0 0,0 40,0 10,0 5,0 2,6 6,0 24,0 3,2 0,8 0,0 0,0 10,0 2,5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

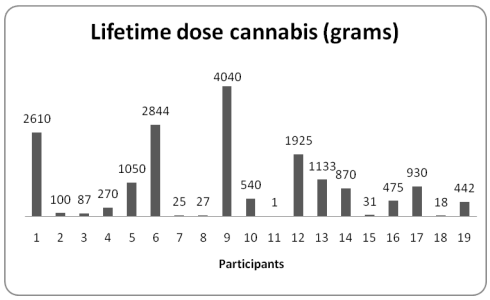

Daily dose amphetamine (milligrams per occasion)
1000 500 0 500 1000 0 400 200 750 200 450 1000 400 200 0 0 500 500
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

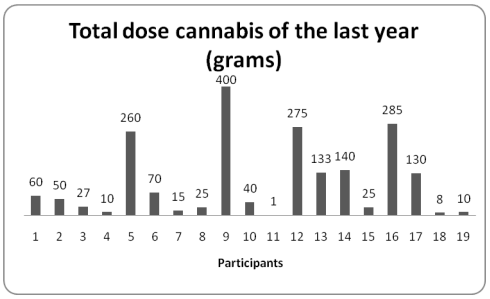

Maximum dose amphetamine (milligrams per occasion)
1000 500 0 500 1300 0 560 250 1000 600 600 1000 1000 200 0 0 1500 1000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

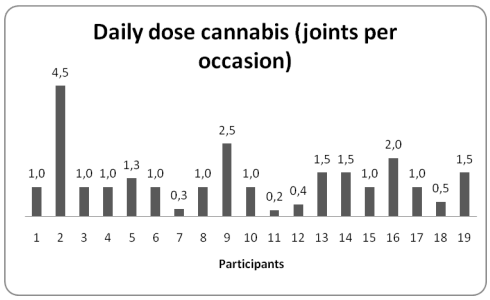

Frequency of intake of amphetamine (days per month)
0,2 0,0 0,2 0,8 0,0 4,0 0,5 1,0 0,3 2,0 0,5 0,2 0,0 0,0 1,7 0,5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

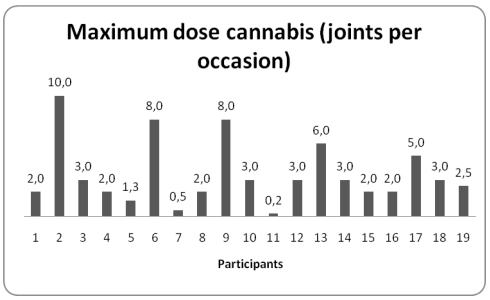

Maximum frequency of intake of amphetamine (intakes per month)
0,3 0,0 1,0 2,0 0,0 5,0 30,0 1,0 2,0 2,5 5,0 3,0 1,0 0,0 0,0 2,0 1,0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

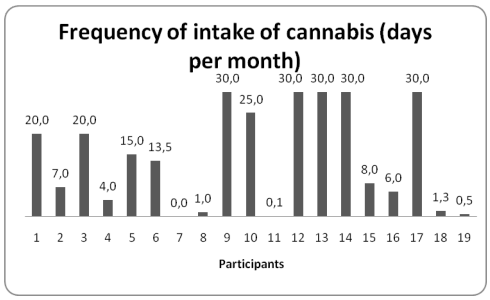

Elapsed time since last intake of amphetamine (days)
14 19 70 7 8 250 7 20 6 18 21 50 14 60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

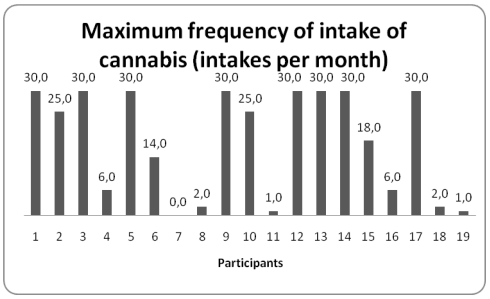

Lifetime dose cannabis (grams)
2610 100 87 270 1050 2844 25 27 4040 540 1 1925 1133 870 31 475 930 18 442
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants

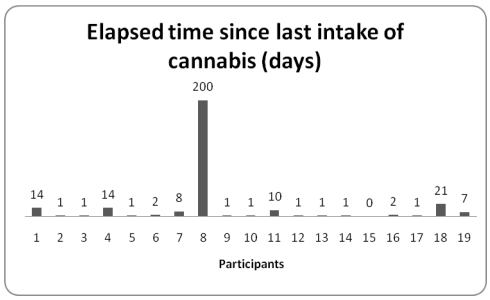

Total dose cannabis of the last year (grams)
60 50 27 10 260 70 15 25 400 40 1 275 133 140 25 285 130 8 10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants


Daily dose cannabis (joints per occasion)
1,0 4,5 1,0 1,0 1,3 1,0 0,3 1,0 2,5 1,0 0,2 0,4 1,5 1,5 1,0 2,0 1,0 0,5 1,5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants


Maximum dose cannabis (joints per occasion)
2,0 10,0 3,0 2,0 1,3 8,0 0,5 2,0 8,0 3,0 0,2 3,0 6,0 3,0 2,0 2,0 5,0 3,0 2,5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants


Frequency of intake of cannabis (days per month)
20,0 7,0 20,0 4,0 15,0 13,5 0,0 1,0 30,0 25,0 0,1 30,0 30,0 30,0 8,0 6,0 30,0 1,3 0,5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants


Maximum frequency of intake of cannabis (intakes per month)
30,0 25,0 30,0 6,0 30,0 14,0 0,0 2,0 30,0 25,0 1,0 30,0 30,0 30,0 18,0 6,0 30,0 2,0 1,0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Participants



	Lifetime dose of MDMA of at least 10 pills (N=5)	Lifetime dose of MDMA of less than 5 pills (N=10)	t/χ^2	p
Present age (years)[1]	26.6 (± 5.5)	24.1 (± 5.4)	t = - 0.838	0.417
Gender (m:f)[2]	4:1	8:2	χ^2= 0.000	1.000
Level of education[3]:				0.883
Certificate of general education	0	1		
Certificate of General Education ordinary level	2	1		
Certificate of General Education advanced level	7	3		
University degree	1	0		
Years of education[1]	16.0 (± 3.2)	14.5 (± 3.4)	t = - 0.783	0.448

Table C.5: *Demographic features of users with a higher and a lower level of consume: mean (± standard deviation). [1]t-values were calculated using unpaired t-test; [2]comparison tested with χ^2; [3] comparison tested with Mann-Whitney U-test.*

2.3 Linear relationships between gray matter volume and drug use patterns

Finally, we applied an explorative post-hoc correlation analysis for individual gray matter volume and drug use patterns of the subjects of the consumer group. This analysis has to be regarded with suspicion as it includes no correction for multiple comparisons.

Thereby, one significant correlation between gray matter volumes and the elapsed time since regular use of amphetamine could be found. All the other correlations including the elapsed time since the last use of MDMA and cannabis were not significant at a statistical threshold of $p < 0.05$ (cf. Table C.6).

Drug use parameters	Correlation	p-value
Lifetime dose MDMA (pills)	-0.050	0.838
Total dose MDMA of the last year (pills)	-0.063	0.797
Time since last use of MDMA (days)	0.082	0.791
Daily dose MDMA (pills per occasion)	0.001	0.997
Maximum dose MDMA (pills per occasion)	0.025	0.918
Frequency of intake of MDMA (pills per month)	-0.103	0.791
Maximum frequency of intake of MDMA (pills per month)	-0.186	0.445
Period of regular use of MDMA (months)	-0.155	0.612
Time since regular use of MDMA (days)	-0.629	0.576
Lifetime dose amphetamine (grams)	-0.024	0.922
Total dose amphetamine of the last year (grams)	-0.038	0.876
Time since last use of amphetamine (days)	-0.331	0.247
Daily dose amphetamine (milligrams per occasion)	0.100	0.694
Maximum dose amphetamine (milligrams per occasion)	0.329	0.182
Frequency of intake of amphetamine (days per month)	-0.024	0.928
Maximum frequency of intake of amphetamine (intakes per month)	-0.355	0.162
Period of regular use of amphetamine (months)	-0.187	0.540
Time since regular use of amphetamine (days)	-0.953	0.012
Lifetime dose cannabis (grams)	0.095	0.699
Total dose cannabis of the last year (grams)	0.072	0.770
Time since last use of cannabis (days)	-0.218	0.370
Daily dose cannabis (joints per occasion)	0.103	0.675
Maximum dose cannabis (joints per occasion)	0.183	0.453
Frequency of intake of cannabis (days per month)	-0.170	0.486
Maximum frequency of intake of cannabis (intakes per month)	-0.259	0.285
Period of regular use of cannabis (months)	-0.226	0.352
Time since regular use of cannabis (days)	-0.267	0.377

Table C.6: *Correlations of individual gray matter values and specific drug use parameters (one joint corresponds to 0.25 grams).*

D. Discussion

1 General findings and comparison to previous studies

The current study investigated potential structural abnormalities in the central nervous system as a result of neurotoxicity to serotonin and dopamine neurons after sporadic MDMA and amphetamine intake. We compared neuronal integrity of white and gray matter between nineteen low exposure users of these illicit substances and a matched group of sixteen drug-naïve controls by means of tract-based spatial statistics and voxel-based morphometry. The two examined groups were comparable in demographic characteristics like age, gender distribution and levels of education. However, in terms of the years of education the two groups were different. On average, the non-consumers spent more years in educational institutions than the consumers.

1.1 White matter integrity

The TBSS analysis of this imaging study in low exposure ecstasy and amphetamine users and drug-naïve controls suggests no significant differences in white matter integrity between the two groups. This conclusion confirms to former studies: Ricaurte et al. (1988) ascertained the fact that MDMA affects primarily fine-diameter unmyelinated axons arising from the dorsal raphe nucleus. This suggests the implication that white matter integrity does not differ between the two groups. Both Cowan et al. (2003) and Daumann and colleagues (2011) could find no detectable alterations in white matter integrity as well. However, de Win (2008) showed some effects on white matter maturity in a neuroimaging study in novel ecstasy users.

1.2 Gray matter integrity

To investigate gray matter integrity, we applied voxel-based morphometry in order to examine drug-related effects on a voxel-by-voxel basis. Generally, our data support the theory that low doses and an irregular use of these substances do not affect gray

matter integrity (Daumann et al., 2011; de Win et al., 2007). This theory is based on previous investigations which ascertained that neurotoxic effects of ecstasy are probably dose-related (McCann et al., 1998; Reneman et al., 2001a; Buchert et al., 2004). In contrast, experienced users have been found to display decreased gray matter volume in medial frontal regions (Daumann et al., 2011; Cowan et al., 2003).

1.3 Comparison to previous investigations

Only one single neuroimaging study indicated some sustained effects of MDMA on brain microvasculature, white matter maturation and possibly axonal damage after low-dose use (de Win et al., 2008). This combined functional and structural investigation revealed decreased regional relative cerebral blood volume in the globus pallidus and putamen, decreased FA in thalamus and frontoparietal white matter and increased diffusion coefficient in the thalamus of novel low-dose ecstasy users compared to ecstasy-naïves (de Win et al., 2008). The ecstasy group consisted of 59 participants that consumed six pills on average (range 0.5 to 80; median 2 pills) in a mean period of 20.4 (± 23.8) weeks (de Win et al., 2008). The 56 ecstasy-naïves had not been completely abstinent but had reported intake of cannabis, amphetamine and cocaine (de Win et al., 2008). In our study, nineteen both MDMA and amphetamine users with an average lifetime dose of 7.93 pills (range 0 to 35; median 5.75 pills) were examined. Hence, both studies are not comparable in terms of group size, mean intake, period of intake and consume parameters of the control group.

1.4 Correlation of drug use patterns and gray matter integrity

Irrespective of the different drug use characteristics, no correlation to gray matter integrity could be revealed. The small sample size of this additional analysis makes it difficult to draw definite conclusions. Anyhow, this result has to be considered as coherent with former studies: Daumann and colleagues (Daumann et al., 2011) could show lower gray matter volumes only on subjects with an average intake of 398 pills

of MDMA. Low-exposure subjects with a lifetime dose of 2.89 pills remained unremarkable (Daumann et al., 2011). However, de Win et al. (2008) detected alterations in brain vasculature, white matter maturation and possibly axonal damage even in low-dose ecstasy users with a mean intake of six pills.

1.5 Individual gray matter volumes

The post-hoc correlation analysis for individual gray matter volume and drug use patterns of the subjects of the group of MDMA and amphetamine users that we applied in a final step has to be interpreted as an additional and explorative attempt to detect any correlations. Only a correlation between gray matter volume and the elapsed time since regular use of amphetamine could be detected. Especially since the data concerning the elapsed time remained fragmentary it should be emphasized that this relationship does not imply causation and could be a mere coincidence.

1.6 The special role of cannabis

It is remarkable that all of the participants of the user group had smoked cannabis at least once in their lifetime. In the current study, no correlation between gray matter integrity and the use of cannabis regarding lifetime dose, total dose of the last year, daily and maximum dose, frequency and maximum frequency of intake as well as elapsed time since last intake could be found. The co-use of cannabis may have major confounding effects on the long-term effects of ecstasy use (Gouzoulis-Mayfrank and Daumann, 2006). Neurotoxic effects of cannabis have never been demonstrated in humans, only in animals (Chan et al., 1998). In contrast, cannabinoids may act as neuroprotective agents, since they were revealed to own anti-excitotoxic, antioxidant and anti-inflammatory properties at a cellular level (Hampson et al., 1998; 2000; Grundy et al., 2001; Mechoulam et al., 2002; van der Stelt et al., 2002; van der Stelt and Marzo, 2005). The main psychoactive ingredient of cannabis, Δ9-tetrahydrocannabinol (THC), attenuated MDMA-induced acute hyperthermia as well as long-term 5-HT depletion

and anxiety in male rats (Morley et al., 2004). Despite these properties, THC might also increase psychological problems in MDMA users with concomitant cannabis use (Daumann et al., 2001; 2004). Self-reported subclinical psychopathology such as obsessive-compulsive behavior, interpersonal sensitivity, depression and paranoid ideation is mainly attributed to the extent of cannabis rather than MDMA use (Daumann et al., 2004). However, other investigations found no association between psychopathology and the extent of cannabis use or even less psychological impairments in MDMA users with additional cannabis use (Milani et al., 2002; 2005). Our study design allows no such conclusions regarding neuroprotective properties of cannabis. Hypothetically it is possible that our neuroimaging results are caused by the neuroprotective effects of cannabis co-use. All in all, the complex interactions between cannabis and MDMA make it difficult to discern the trigger for both psychopathological and neuronal concerns.

2 Relation to functional investigations

2.1 Cognitive alterations

In a prospective fMRI study (Jager et al., 2007) human cognitive brain function remains unaffected of incidental use of ecstasy. Jager and colleagues (2007) investigated the impact of low doses of ecstasy on working memory, selective attention and associative memory. Functional neuroimaging studies came to the conclusion that heavy ecstasy use is associated with deficits in learning, memory and higher cognitive processing (Parrott, 2001; Chen and Lin, 2009). Also decision-making deficits of amphetamine (Rogers at al., 1999) and MDMA users (Quednow et al., 2007; Koechlin and Hyafil, 2007; Koenigs et al., 2007) are reported. Brain regions which are associated with decision-making match to the findings of structural analyses. These regions are located to the orbitofrontal/ventromedial and/or dorsolateral/dorsomedial prefrontal cortices (Daumann et al., 2011; Quednow et al., 2007). However, Hanson and

colleagues (2008) could not find any differences in decision-making between MDMA polydrug users and non-MDMA polydrug users. This leads to the hypothesis that not specifically MDMA use, but polydrug use may be associated with long-term impairments in cognitive behavior (Hanson et al., 2008).

2.2 Psychopathological alterations

The following issue refers to psychopathological alterations in MDMA and amphetamine users. De Win et al. (2006) found no correlation between low-dose ecstasy use and depression and impulsivity but in certain aspects of sensation seeking. Though, heavy use of MDMA goes along with elevated behavioral impulsivity (Butler and Montgomery, 2004; Quednow et al., 2007; Hanson et al., 2008; Hoshi et al., 2007). Furthermore, Butler and Montgomery (2004) found higher levels of venturesomeness and novelty seeking behavior in low- and high-dose MDMA users compared with abstinent controls. Impulsiveness involves disinhibition that is associated with 5-HT function (Puumala and Sirvioe, 1998; Evenden, 1999). Functional imaging studies localized brain regions associated with inhibition of reactions to the prefrontal, the inferior parietal, and the cingulated cortices (Liddle et al., 2001; Garavan et al., 2002; Watanabe et al., 2002).

2.3 Chicken or the egg?

It is once again unclear, whether decision-making deficits and elevated impulsivity are the cause or the effect of extensive drug use (Hanson et al., 2008). Kelly et al. (2006) suggest that these behavior patterns are predisposing factors for starting ecstasy use: Impulsive sensation seeking young adults showed greater sensitivity to the reinforcing effects of amphetamine and therefore, a greater vulnerability to the abuse potential of d-amphetamine than low impulsive sensation seekers on self-report measures (Kelly et al., 2006). However, de Win and colleagues (2006) disproved this hypothesis. 188 ecstasy-naïve volunteers with high probability for future ecstasy use underwent

self-report questionnaires concerning depression, impulsivity and sensation seeking. 59 of them started to use ecstasy and were compared to 61 matched persistent ecstasy-naïve volunteers by a follow-up measurement. All in all, the differences in the scores of the questionnaires were not significant, suggesting that depression, impulsivity and sensation seeking are no predisposing factors (de Win et al., 2006).

2.4 Sustainability

The question is, whether these effects are long-lasting or not. Whereas several observations detected potential recovery in brain serotonin neurons (Battaglia et al., 1988; Scanzello et al., 1993), psychopathological effects seem to be long-lasting (Bolla et al., 1998; Reneman et al., 2000). One recent study reported impairments in verbal memory perfomance in former ecstasy users with long-term abstinence, but recovery in reduced SERT availability after a reduction in ecstasy use (Thomasius et al., 2003). However, another longitudinal investigation reported stable memory performance of polyvalent MDMA users over the course of 18 months irrespective of continued use or abstinence (Gouzoulis-Mayfrank et al., 2005). Also, Zakzanis and Young (2001) examined possible impairment in memory due to neurotoxic potential of ecstasy use over the period of one year. They could find a decrease in the most of the aspects of memory function (Zakzanis and Young, 2001).

2.5 Addiction

Another aspect that links functional and structural alterations is addiction. The disability to guide appropriate decisions in consideration of the own expectations is typical for drug addiction (Schoenbaum et al., 2006). The orbitofrontal cortex - which revealed lower gray matter volumes in high-dose users of ecstasy and amphetamine - is a brain region that has been associated with the generation of expectations by valuing different outcomes and learning (Schoenbaum et al., 2006; Schoenbaum and Roesch, 2005). Therefore, this part of the cortex has been linked to the initiation and

adherence of addictive behavior (Schoenbaum et al., 2006; Kalivas and Volkow, 2005). A number of studies revealed prefrontal structural and functional alterations among psychostimulant users (Kim et al., 2006; Matochik et al., 2003; Tanabe et al., 2009; Dom et al., 2005) that might be associated with addictive behaviors (Schoenbaum et al., 2006). On the neurophysiological side, the dopamine system plays a crucial role in psycholstimulant addiction (Hill and Sofuoglu, 2007; Ritz and Kuhar, 1993) and rewarding effects of these substances (Mieszkiel et al., 2011). Psychostimulants like MDMA and amphetamine are proved to inhibit the dopamine transporter and/or increase the release of dopamine (Sabol and Seiden, 1998; Seiden et al., 1993; Gudelsky et al., 1994). The other neurotransmitter affected by psychostimulants is serotonin as MDMA is known to inhibit 5-HT reuptake (Mieszkiel et al., 2011). These drug-dependent neuroadaptions might enhance the risk of developing uncontrolled and high levels of intake (Schenk, 2011).

Neuronal deficits may be pre-existing to drug use initiation and might ultimately elevate vulnerability for developing and maintaining addiction. Schoenbaum and colleagues (2006) reasoned that maladaptive decision-making and accordingly addiction is in part a consequence of drug-induced orbitofrontal lesions. The results of our study could be interpreted both ways: On the one hand, our participants might have developed no sustained substance dependence with a recreational intake due to their lacking neuronal abnormalities. On the other hand, they might have shown no addictive behavior with a higher level of consumption because they did not exhibit drug-induced changes in prefrontal regions. This leads to the hypothesis that an excessive use of MDMA and amphetamine at the beginning of drug intake may elevate the risk of future substance dependence.

3 Limitations

3.1 Methodical limitations

Potential limitations of the current study relevant to our major findings have to be acknowlegded.

First of all, the retrospective design and the naturalistic uncontrolled conditions of the study alleviate the validity of the results. Participants were not situated in equal living conditions. It is unclear whether a potential causality between ecstasy and amphetamine use and the neuroimaging outcome parameters underlies pre-existing differences between drug users and controls. Another limitation arises from our recruitment strategy: Subjects were recruited directly in the dance scene and continued their usual way of life, which is why environmental circumstances could have affected the findings of the study. Although the two comparison groups did not differ significantly in terms of gender, they both consist mainly of male subjects for which reason the results cannot be generalized to women. The influence of possible known and unknown confounders, such as alcohol, cocaine and nicotine is uncertain, particularly because of the unbalanced intake parameters of these substances. And although drug use of most of the participants of the consumer group was limited to the use of MDMA and amphetamine, polydrug use is not completely screened out.

Furthermore, the reliability of the retrospective self-reported records of drug use and abstinence in the past using drug-history questionnaires is uncertain. Hence, we performed hair analyses from randomly chosen subjects by means of the Institute of Legal Medicine of the University of Cologne which supported the plausibility of the self-reported data on ecstasy and amphetamine consumption. These kinds of self-reports are known to have sufficient reliability and validity to confirm individual drug histories (Dickson et al., 2009). However, patterns of substance use, i.e. cumulative lifetime dose, maximum dose and frequency of intake, could not been verified by this. However, most ecstasy tablets sold in Europe and the United States were found to contain

MDMA as the main component, variations in dosage and purity of MDMA tablets and amphetamine have to be assumed (Cole et al., 2002; Parrott, 2004).

Another limitation of the study might be the relatively small sample size. Although 19 subjects in the experimental and 16 subjects in the control group might be viewed as a sufficient large sample in MRI-studies, effect sizes might not be strong enough to detect small differences in gray or white matter integrity. Cowan et al. (2003) compared structural MRI scans of 31 MDMA polydrug users versus 29 non-MDMA users. The other relevant investigation of Daumann et al. (2011) included 42 polydrug users, 20 heavy users and 16 drug-naïve controls. With a sample size of 20 subjects in the group of heavy users, which is very close to the sample size of the current study, reductions of gray matter could be detected (Daumann et al., 2011).

Finally, heterogeneous drug use parameters within the group of beginning ecstasy and amphetamine users make it difficult to detect significant drug-related effects in neuronal integrity. Prolonged recreational use of MDMA and amphetamine evidentially induces alterations in brain morphology (Cowan et al., 2003; Daumann et al., 2011), thus, making it hard to draw a threshold between dosages of MDMA and amphetamine that do affect white and gray matter integrity and those that do not.

With regard to possible at least partial restitution of the serotonergic lesion after long-term abstinence of ecstasy of minimum twelve months (Thomasius et al., 2003), results of this study should be interpreted with caution. Due to the elapsed time since the last use of the investigated substances, potential effects of low doses might be recovered. Reneman et al. (2002b) reported reduced cortical post-synaptic 5-HT (2A) receptor densities in recent ecstasy users and increased densities in former users with a drug-free interval of minimum two months. This corroborates studies in rats which showed temporary down-regulation of postsynaptic 5-HT2 receptors resulting from high-synaptic 5-HT concentration for up to one month after chronic treatment with MDMA (Scheffel et al., 1992). For example, Battaglia et al. (1988) demonstrated full recovery of cortical 5-HT uptake sites in rats by 12 months after MDMA administration, following an initial loss of 90% of the uptake sites. Scanzello et al. (1993) reported

nearly complete recovery of the levels of 5-HT neurons in rats 32 weeks after MDMA treatment. All in all, whether and to which point of time recovery occurs depends on dose, dosing regime, the studied brain region and the species (Hegadoren et al., 1999; Battaglia et al., 1988).

3.2 Limitations in analysis methods

Voxel-based morphometry is a standardized method for comparisons of local concentrations of gray matter (Ashburner and Friston, 2000; Good et al., 2001). Like all image analysis techniques, the method of voxel-based morphometry implies some inherent limitations (Bookstein, 2001). The first problem includes the partial volume effect, which means that a single voxel of the MR image could represent more than one kind of tissue type. This results in spatial blurring of the intensity distinction at the border of gray and white matter. The second shortcoming is the arbitrary amount of smoothing that can lead to very different results (Jones et al., 2005). In this study, we decided to use a conservative smoothing filter with a FWHM of 7.05. Jones et al. (2005) drew the conclusion that filter sizes greater than 6 mm might affect fractional anisotropy.

4 Conclusion

In conclusion, we found no indications for alterations in neuronal integrity in low exposure MDMA and amphetamine users compared to drug-naïve controls. Therefore, the present study supports the hypothesis that occasional ecstasy and amphetamine use does not lead to neurotoxic effects (Jager et al., 2007; de Win et al., 2007). Due to the study design, it remains uncertain whether the findings depend on the effects of polydrug use especially concomitant cannabis use. Therefore, and because of the miscellaneous personal and environmental factors that may affect long-term effects of MDMA and amphetamine use, even infrequent intake of these substances may dam-

age neuronal integrity. More studies are needed to confirm the absence of discrete MDMA- or amphetamine-induced structural changes and to assess an approximative threshold value to which recreational intake of these substances is definitely harmless. Besides, improved methodical conditions are needed to reduce confounding problems of polydrug use. To investigate the specific pathophysiological correlation between brain differences and the use of MDMA and amphetamine is of particular importance. The most common hypothesis comprises that reductions in gray matter integrity reflect MDMA- and amphetamine-induced neurotoxicity or altered neural plasticity but there is still some lack of clarity.

E. Summary

Amphetamine and its synthetic analogue 3,4-methylenedioxymethamphetamine (MDMA), commonly known as "Ecstasy", have gained significant popularity as recreational drugs due to their psychostimulant effects and moved more and more in the public eye. Thus, the question whether irregular consumption of low doses leads to lasting alterations of the central nervous system gained importance. Both substances are neurotoxic by inducing a release of serotonin (5-hydroxy-tryptamine; 5-HT) from pre-synaptic 5-HT neurons in the central nervous system (CNS) leading to possible reversible widespread reductions of serotonin and finally to damaging of fine-diameter unmyelated serotonergic axons. The current study investigated potential structural abnormalities in the central nervous system in connection with neurotoxicity after incidental MDMA and amphetamine intake. Therefore, we compared neuronal integrity of white and gray matter between nineteen low exposure users of these illicit substances and a matched group of sixteen drug-naïve controls by means of tract-based spatial statistics and voxel-based morphometry. In terms of white matter integrity, no significant differences between the two groups could be revealed. Besides, low exposure users showed no alterations in gray matter volumes compared to drug-naïve controls. While we are aware of the limitations of our study, our data support the theory that low doses and an unregular use of these substances does not affect gray matter integrity.

F. Zusammenfassung

Amphetamin und dessen synthetisch hergestelltes Derivat 3,4-Methylendioxymethamphetamin (MDMA), auch bekannt als “Ecstasy”, haben in den letzten Jahren durch ihre aufputschende Wirkung zunehmend an Beliebtheit als Gelegenheitsdroge gewonnen und rückten somit mehr und mehr ins Licht der Öffentlichkeit. Dadurch erlangte die Frage, ob schon geringe Einnahmedosen und -häufigkeiten anhaltende Wirkung auf das zentrale Nervensystem haben könnten, zunehmend an Wichtigkeit. Beide Substanzen besitzen neurotoxische Eigenschaften, indem sie die Ausschüttung von Serotonin (5-Hydroxy-Tryptamin; 5-HT) aus präsynaptischen 5-HT Neuronen induzieren. Dies hat eine möglicherweise reversible Verminderung von Serotonin und schließlich eine Schädigung der dünnen, unmyelinisierten, serotonergen Axone zur Folge.

Die vorliegende Studie untersuchte strukturelle Auffälligkeiten des zentralen Nervensystems im Zusammenhang mit MDMA- und Amphetamin-induzierter Neurotoxizität. Wir verglichen die neuronale Integrität der weißen und grauen Hirnsubstanz zwischen neunzehn Neukonsumenten dieser illegalen Substanzen und einer angepassten Vergleichsgruppe mit sechzehn Nichtkonsumenten mit Hilfe von Trakt-basierten Analysen (tract-based spatial statistics) und Voxel-basierter Morphometrie (voxel-based morphometry). Der Vergleich der weißen Substanz wies keine signifikanten Unterschiede zwischen beiden Gruppen auf. Zudem zeigten die Neukonsumenten keine Auffälligkeiten bezüglich der grauen Hirnsubstanz im Vergleich zur Kontrollgruppe. Somit bestätigen unsere Untersuchungen die Annahme, dass niedrige Einnahmedosen und ein unregelmäßiger Konsum dieser Drogen die Integrität der grauen Hirnsubstanz nicht beeinflussen

G. References

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