EFFECTS OF ACUTE AND CHRONIC TREATMENT WITH 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) AND THE CANNABINOID RECEPTOR AGONIST WIN55,212-2 ON BEHAVIOUR AND COGNITION IN RATS

Aus dem Institut für Hirnforschung, Abteilung Neuropharmakologie,
Zentrum für Kognitionswissenschaften

DISSERTATION

Zum Erlangen des akademischen Grades eines Doktors der Naturwissenschaften
(Dr. rer. nat.)

Vorgelegt dem Fachbereich 2 (Biologie/Chemie) der Universität Bremen

von

SYBILLE SCHULZ

Im März 2012

Universität Bremen

1. **Gutachter:** Prof. Dr. Michael Koch
2. **Gutachter:** Prof. Dr. Ursula Dicke

Kolloquium: 15.5.2012

Declaration of original authorship

I hereby declare that the thesis submitted only contains my original work except where indicated and properly acknowledged. Some parts of the practical work have been carried out by students under my supervision. No other sources or materials where used.

Sybille Schulz

Bremen, März 2012

List of publications

(*) indicates publications or manuscripts included in this thesis. Articles have been published or submitted to international scientific journals.

Articles

* **Schulz, S** (2011). MDMA & Cannabis: A Mini-Review of Cognitive, Behavioural and Neurobiological Effects of Co-consumption. *Current Drug Abuse Reviews*, 4(2):81-6.

* **Schulz, S**; Gundelach, J; Svärd, HK; Hayn, L; Koch, M (2012). Acute co-administration of the Cannabinoid receptor agonist WIN 55-212,2 does not influence 3,4-methylenedioxymetamphetamine (MDMA)-induced effects on effort-based decision making, locomotion, food intake and body temperature. *Substance Abuse* (under review).

* **Schulz, S**; Becker, T; von Ameln-Mayerhofer, A; Nagel, U; Koch, M (2012). Chronic co-administration of the cannabinoid receptor agonist WIN55,212-2 during puberty or adulthood reverses 3,4-methylenedioxymetamphetamine (MDMA)-induced deficits in recognition memory but not in effort-based decision making. *Behavioural Pharmacology* (under review).

Crunelle, CL; **Schulz, S**; de Bruin, K; Miller, ML; van den Brink, W; Booij, J (2010). Dose-dependent and sustained effects of varenicline on dopamine D2/3 receptor availability in rats. *European Neuropsychopharmacology*, 21(2):205-10.

Crunelle, CL; van de Giessen, E; **Schulz, S**; Vanderschuren, LJ; de Bruin, K; van den Brink, W; Booij, J (2011). Cannabinoid-1 receptor antagonist rimonabant (SR171416) increases striatal dopamine D2 receptor availability. *Addiction Biology*, doi: 10.1111/j.1369-1600.2011.00369.

Poster presentations

International Symposium on Drugs of Abuse, 28-29 July 2008, Sao Miguel, Azores, Portugal.
Schulz, S; Nagel, U; von Ameln-Mayerhofer, A. Repeated administration of MDMA and MDMA derivatives in rats: behavioural sensitization and neurotoxic effects.

European Graduate School in Addiction Research (ESADD), 2nd seminar week, October 2009.
Schulz, S. Psychotropic Pharmaceuticals in Germany. The „silent“ addiction.

Index

General Introduction

MDMA (3,4-methylenedioxymetamphethamine, “ecstasy”) is one of the most popular illicit recreational drugs among young adults. However, the underlying neurobiological mechanisms responsible for the physiological, behavioural and psychological effects, as well as the influence on cognitive functions such as memory and decision making are still not fully examined. Furthermore, if and to what extend this drug has neurotoxic properties is debated. Most ecstasy users are polydrug users, and the majority concomitantly consumes cannabis.

Cannabis is the most frequently consumed illegal psychoactive drug world wide. While cannabis products are generally perceived as “soft drugs” and their potential medical usefulness is progressing, cognitive impairments and long-term alterations in the brain, especially following prolonged and heavy use in adolescence, are observed.

This thesis investigates the influence of MDMA and the synthetic cannabinoid receptor agonist, WIN55,212-2 (WIN), on different forms of decision making, memory function, locomotor activity and physiological parameters such as body temperature and food intake. Within both an acute as well as chronic systemic administration schedule, effects of each substance alone as well as their combination is tested in order to mimic co-consumption of these drugs. Within chronic treatment, adult as well as pubertal rats and their respective brain myelination levels are examined to determine vulnerable periods of drug consumption or age-related differences.

MDMA

History and Use

MDMA (3,4-methylenedioxymetamphetamine) is a synthetic ring-substituted amphetamine derivative (Fig.1). Originally, it was a by-product of the synthesis of the haemostatic drug hydrastinine in 1912 by the German chemist Anton Köllsch (Benzenhofer and Passie, 2006). Mentioned as one of many chemical intermediate products, it was patented in 1912 to the pharmaceutical company MerckKGaA (Freudenmann et al., 2006) The first systematic pharmacological tests with the substance now called MDMA, transformed to a hydrochlorid salt, are documented in 1927. During the 1950s, the US military clandestinely tested MDMA, presumably for its toxic effects or as novel interrogation method (Shulgin, 1990). Official studies in animals and humans emerged another 30 years later, and only in the 1960s, its psychoactive properties were scientifically studied and reported (Benzenhofer and Passie, 2006). MDMAs' potential for use in psychotherapeutic settings, enhancing interpersonal relationships due to its empathogenic and entactogenic properties, was suggested by the American chemist Alexander Shulgin in the late 1970s (Benzenhofer and Passie, 2010). Therapists applied MDMA during their sessions until it was restricted by the drug enforcement administration (DEA) in 1985. Today its application in treatment of post-traumatic stress disorder (PTSD) is still debated (Doblin, 2002).

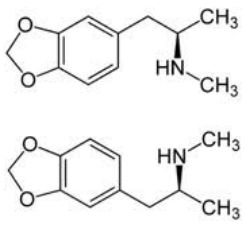

Fig.1: Chemical structure of 3,4-methylenedioxymetamphetamine (MDMA). MDMA is a racemate, consisting of a 1:1 mixture of the two enantiomeres R(-) (above) and S(-) (below). S(-) MDMA has a half-life of about 4.8 hours, and is associated with subjective and psychomotor effects. R(-) MDMAs half-life is longer (14.8 hours), thus thought to be involved in the consequences regarding mood and cognitive performance which last longer than the acute effects (Pizarro et al., 2004).

MDMA is classified as a schedule I drug in Germany and most other countries, meaning it is illegal to buy, sell, or possess, except for strictly limited research or medical purposes (UNODC, 2010). Since it was not regulated under law until 1985, the substance started to become widely distributed and was used as recreational drug mainly at clubs and dance parties among all social classes during the 1980s (Benzenhofer and Passie, 2006). Paralleling the emergence of the rave culture, popularity of MDMA increased rapidly during the 1990s and today it is the second most commonly

used illegal psychoactive drug world wide (after cannabis). The WHO estimates the global illegal production of MDMA between 55 and 133 tons annually, with most manufacture taking place in North America and Europe, followed by Brazil and Argentina, Oceania and Russia (UNODC, 2010).

Fig.2: Picture of ecstasy pills (retrieved from www.erowid.com)

MDMA is most commonly sold in the form of "ecstasy" tablets and taken orally, rarely the powder form is snorted. In Europe, most ecstasy tablets contain MDMA as their main psychoactive ingredient (EMCDDA, 2010), however other substances (e.g., other amphetamines (including MDE or MDA), ephedrine, paracetamol, caffeine) have also been found in tablets sold as “ecstasy” (Sherlock et al., 1999). In recent years, purity in terms of MDMA content has been reported to lie between 90-100% (Parrott, 2004) (Fig.2). In the following, the term “ecstasy” will be used when referring to the pills taken by human drug users, whereas “MDMA” refers to the pure chemical compound found in ecstasy tablets.

Prevalence

The prevalence of use has stayed relatively constant within the last two decades. Recent representative surveys in western countries found that 3.5% of 15-34 year olds consumed ecstasy during the last year, and 8.4% report life time prevalence (Degenhardt et al., 2010) (1.7 and 5.8% in European countries, respectively (EMCDDA, 2010)). Prevalence is higher in younger adults and in males compared to females. Most past-year users took ecstasy tablets intermittently (once or twice a year) and only a minority (5-10%) consumed ecstasy on a monthly or weekly basis. The United Nations estimate around 9 million current users world wide.

Acute effects

Psychological effects described by users include increased euphoria, energy, empathy and mood, and awareness of sensations, feelings of comfort and belonging to others, hallucinations, as well

as decreased anxiety upon consumption. Acute adverse physiological effects include decreased appetite, increased heart rate and blood pressure, sweating, bruxism, dry mouth, hyperthermia and insomnia. Difficulties concentrating, anxiety, as well as psychological addiction have been reported. Most users also report negative after-effects 24 hours to one week following ecstasy consumption (Green et al., 2003). In laboratory animals, dose-dependent alteration of body temperature, leading to hyperthermia (or hypothermia, dependent on the ambient temperature), increased heart rate, vasoconstriction and anxiogenesis (at low doses), hyperactivity upon acute administration of MDMA, and increasing locomotor activity in response to repeated administration (termed behavioural sensitization), are well known effects (Green et al., 2003). MDMA does have reinforcing properties, as rats voluntarily and repeatedly self-administer doses up to 1mg/kg intravenously (i.v.) (Schenk, 2009). These effects can be ascribed to the MDMA-induced stimulation of neurotransmitter systems, especially serotonin (5-HT) and dopamine (DA), in the brain.

Risks, long-term effects & impairments

Acute adverse side effects range from minor disturbances of the central and autonomous nervous system such as confusion, irritability, hypertension or bruxism to major negative and potentially life-threatening effects like severe hyperthermia (up to 42°C), cardiovascular collapse, arrythmias and rhabdomyolysis (breakdown of skeletal muscle) (Green et al., 2003). Statistics from the USA and EU drug monitoring centres show an increase in ecstasy-related emergency room visits and deaths during the last 20 years, paralleling increased use of the drug. Deaths involving only MDMA are rare and mostly occur in first time users (UNODC, 2010; EMCDDA, 2009).

Furthermore, delayed and possibly long-lasting neuropsychiatric effects have been found in heavy and/or chronic ecstasy-users. These include panic disorder, aggressive behaviour, major depressive disorder, and deficits in memory and cognitive functioning. While some traits, like impulsivity, sensation seeking or vulnerability to depression may be precursors, rather than consequences of ecstasy use (Butler and Montgomery, 2004), most long-term or retrospective studies so far have supported the idea that ecstasy has deleterious effects on certain brain functions. In particular, deficits in various memory domains (working, delayed, associative and verbal memory tasks), learning, executive control and attention have been shown (Gouzoulis-Mayfrank et al., 2000; McCann et al., 1999; Morgan et al., 2006; Rodgers, 2000; Morgan et al., 2002; Jager et al., 2008). Some studies found only small effects of ecstasy use on neurocognitive performance, or attributed deficits to concomitant use of other drugs, especially cannabis (Halpern et al., 2011; Medina and Shear, 2007; Daumann et al., 2004). Furthermore, altered activity in frontal and hippocampal brain areas was found in ecstasy users (Daumann et al., 2005; Jacobsen et al., 2004). However, since MDMAs' popularity only emerged 20 years ago, there are only few

well-controlled longitudinal studies looking at long-term impairments in mood and cognitive functions.

In rats, MDMA-administration is associated with long-term consequences including impaired thermoregulation at high ambient temperatures (Mechan et al*.,* 2001), increased anxiety, and deficits in social interaction. Increased anxiety, measured in emergence, elevated plus maze and social interaction tests, was evident up to three months after moderate doses of MDMA (5mg/kg) (Gurtman et al*.,* 2002; McGregor et al*.,* 2003). Lasting impairments in cognitive functioning were evident, too. For example, rats treated with an intermittent schedule during puberty later displayed deficits in short-term or working memory, measured with an object recognition task, and spatial learning and memory, measured in a Morris water maze task (Capela, 2009).

These long-term effects may correlate to MDMA-induced reduction in serotonergic functioning (see **Excursion 2: Neurotoxicity**).

Excursion 1: The Serotonergic System

Serotonin (5-hydroxytryptamine, 5-HT) is one of the main monoamine neurotransmitters in the brain. It modulates numerous processes in the central nervous system (CNS), including mood, anxiety, sleep, appetite, cognition and reward systems. In the human brain stem, cells from the rostral raphe nuclei, including dorsal and medial raphe nuclei, give rise to ascending serotonergic fibres, projecting to forebrain regions including cortex, thalamus and limbic structures, whereas caudal raphe nuclei are the origin of descending and cerebellar projections (Fig.3). The first and rate limiting step of 5-HT synthesis is hydroxylation of tryptophan to 5-hydroxytrypthophan (5-HTP) by the enzyme tryptophan hydroxylase. 5-HTP is further decarboxylated to 5-HT by L-amino-acid decarboxylase. Subsequently, 5-HT is transported into intracellular storage vesicles by a vesicular monoamine transporter (VMAT). Upon arrival of an action potential, a Ca^{2+}-mediated process leads to release of 5-HT from the terminals of the presynapse. 5-HT binds to seven main classes of 5-HT receptors (5-HT_1- 5-HT_7), which are further divided into 14 distinct 5-HT receptor subtypes based on their structural, pharmacological and chemical properties (Barnes and Sharp, 1999). All 5-HT receptors consist of seven transmembrane domains. Except for 5-HT_3, which is a ligand-gated ion channel, all receptors are members of the G-protein coupled (metabotropic) receptor family. Binding of 5-HT to the extracellular site activates a G-protein, which initiates intracellular second messenger signalling. 5-HT_1 receptors are linked to inhibitory G proteins, whereas activation of $5\text{-HT}_{2,}$ $5\text{-HT}_{4,}$ presumably 5-HT_5 and 5-HT_7 initiates stimulatory signalling. Action of 5-HT is terminated by serotonin plasma membrane transporter (SERT)-mediated reuptake into the presynapse or metabolization to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase. Considering the differential characteristics of the 5-HT receptor classes (including inhibitory autoreceptors (e.g., 5-HT_{1A}) as well as excitatory post-synaptic receptors (e.g., $5\text{-HT2}_{A/C}$)), and the abundant distribution of serotonergic neurons across the CNS, the complexity of serotonergic involvement in physiological as well as behavioural, emotional, and cognitive processes becomes obvious.

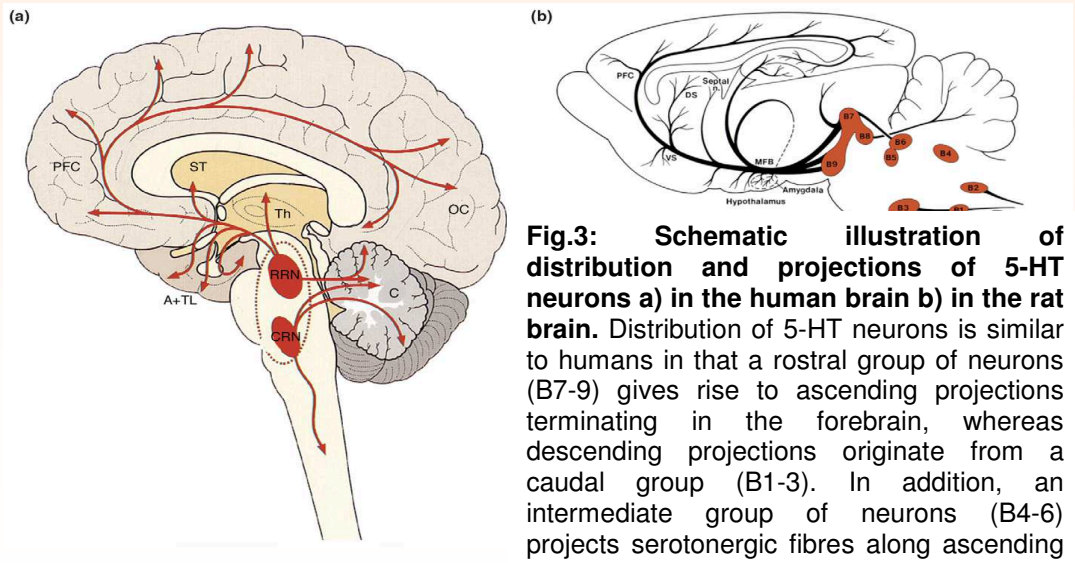

Fig.3: Schematic illustration of distribution and projections of 5-HT neurons a) in the human brain b) in the rat brain. Distribution of 5-HT neurons is similar to humans in that a rostral group of neurons (B7-9) gives rise to ascending projections terminating in the forebrain, whereas descending projections originate from a caudal group (B1-3). In addition, an intermediate group of neurons (B4-6) projects serotonergic fibres along ascending and descending pathways (adapted from Cools, 2007).

Pharmacology

Pharmacodynamics

MDMA affects the central nervous system by influencing neurotransmitter systems. More specifically, it acts as an indirect monoamine agonist. Early studies in laboratory animals demonstrate increased levels of serotonin (5-HT), to a lesser extent dopamine (DA), and noradrenaline (NA) following administration of MDMA (Gudelsky and Nash, 1996; Yamamoto and Spanos, 1988; Fitzgerald and Reid, 1990). More recently, inhibition of the monoamine transporters (SERT, DAT, NAT) was found responsible for increases in extracellular monoamines (Capela et al*.,* 2009). In comparison to other amphetamines, MDMA has increased potency to inhibit SERT, while its potencies to inhibit DAT and NAT are reduced. Fig.5 details mechanisms by which MDMA increases extra- and intracellular 5-HT levels. Some of its behavioural effects have also been ascribed to indirect activation of the DA system (Bankson and Cunningham, 2001). In addition to binding to monoamine receptors (subtypes 5-HT_2, 5-HT_1, D_1 and D_2), MDMA exerts its effects by interaction with various other neurotransmitter systems. For example, MDMA displays affinity to adrenergic, histaminergic, nicotinic and muscarinic acetylcholine receptors, leading to increased signal transduction, thereby contributing to the cardiovascular and autonomic side effects as well as abuse-potential (Capela et al*.,* 2009).

Pharmacokinetics

Due to easy diffusion across cell membranes, the lipophilic molecule MDMA is rapidly absorbed in several tissues. Controlled studies in humans demonstrated that the maximal plasma concentration of MDMA is reached between 1.5-3 hours after oral ingestion. The increase of concentration is not proportional to the dose ingested, indicating non-linear pharmacokinetics (Yang et al*.,* 2006). This is due to saturated hepatic metabolism of MDMA, as well as interaction of its metabolites with enzymes involved in the catabolism. MDMA interferes with its own metabolic pathway by inhibiting the hepatic CYP2D isoenzyme, which regulates demethylenation of MDMA during the metabolic process in humans (CYP2D6) as well as in rats (CYP2D1) (de la Torre et al*.,* 2000; Delaforge et al*.,* 1999; Heydari et al*.,* 2004). This autoinhibition lasts at least 24 hours, and is accompanied by increased pharmacological, physiological and subjective effects after repeated dosing (Farre et al*.,* 2004). In humans, about 80% of MDMA is transformed metabolically through the liver, 20% is excreted unaltered in urine. In rats, the rate of metabolism is almost linear up to a dose of 10mg/kg. Higher doses produce non-linear increases in the concentration in the brain and plasma levels. Notably, brain concentrations are generally much higher for doses of 20 or 40mg/kg compared to plasma concentrations, because MDMA accumulates in 5-HT cells (see Fig.5).

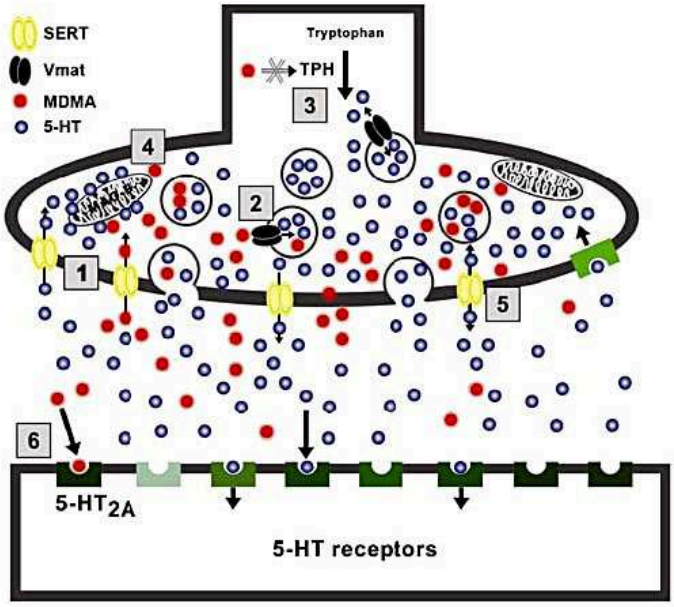

Fig.5: Pharmacological action of MDMA on a serotonergic synapse. MDMA induces increases in 5-HT concentrations via distinct mechanisms: [1] entering the cell by binding to the SERT, [2] disruption of vesicular storage, thereby increasing cytoplasmic concentrations of 5-HT, [3] inhibition of tryptophan hydroxylase (TPH), [4] inhibition of monoamine oxidase, the enzyme responsible for 5-HT degradation, [5] reversing the direction of SERT, thus more 5-HT molecules exit the cell. The first two and the fifth mechanisms involve substrate binding of MDMA to the monoamine transporters and reversing their actions, namely the SERT and the vesicular monoamine transporter (VMAT). Furthermore, MDMA directly binds to the postsynaptically located 5-HT_2 receptor subtype 5-HT_{2A} [6], which accounts for its hallucinogenic property and hyperthermic effect (adapted from Capela, 2009).

Excursion 2: Neurotoxicity of MDMA

Neurotoxicity is defined as any adverse effect which disrupts, impairs, or impedes activity of neuronal cells. This can take place by affecting the functionality (e.g., loss of signalling ability) or damaging physical structures (e.g., deterioration of cells), and effects may be permanent or reversible (Erinoff, 1995). Despite two decades of studies in humans and laboratory animals, the degree of MDMA-induced neurotoxicity and the underlying mechanisms remain inconclusive (Capela et al., 2009).

Some studies suggest that MDMA leads to general neuronal cell death (apoptosis) across brain regions, for example, in the cortex, thalamus, and hippocampus. Acutely, MDMA leads to an increased efflux of both 5-HT and DA, however, most experimental data points to or is restricted to effects on the serotonergic system. High doses of MDMA given repeatedly (20mg/kg/day, for 10 days) selectively reduced 5-HT levels in the forebrain of laboratory rats, while DA levels remained the same or increased 4 weeks after treatment (Mayerhofer et al., 2001). In neuroimaging studies with recreational MDMA users, a reduction of SERT, but not DAT, density was evident (Schouw et al., 2011; McCann et al., 2008). In the cerebrospinal fluid of MDMA users, reduced levels of 5-HIAA, the main metabolite of 5-HT, was found, and this reduction was more severe the higher the average dose(s) of MDMA (McCann et al., 1994; McCann et al., 2008).

Doses and route of administration differs between human and animal studies, and most administration schedules in rats possibly relate to rather heavy MDMA-consumption in humans. Furthermore, differences in metabolism and polydrug use in humans make a direct comparison of neurotoxic properties of MDMA difficult. However, animal experiments allow for more detailed studies regarding the underlying mechanisms of pure MDMA administration, which may also relate to humans. In rats and non-human primates, neurochemical markers for 5-HT, 5-HIAA, and SERT have shown decreased levels of the neurotransmitter and reduced availability of its transporter in various brain regions, specifically in the neocortex, striatum, and hippocampus, as well as in cerebrospinal fluid. Degeneration of serotonergic axon terminals, mainly fine diameter fibres arising from the dorsal raphe nuclei, has been postulated (Capela et al., 2009).

Mechanisms

There are many theories and experimental indications about the underlying mechanisms of MDMA-induced neurotoxicity. In the following, three major hypotheses are described in detail.

Hyperthermia

There is a positive correlation between serotonergic neurotoxicity of MDMA and hyperthermia. In a hot environment (30°C) MDMA induced greater increase in efflux of DA as well as 5-HT, and depletion of 5-HT and 5-HIAA levels was more pronounced in temperatures above 26°C (Malberg and Seiden, 1998; O'Shea et al., 2005). Neuronal cortical cells incubated with added MDMA at 40°C showed significantly higher rates of cell death compared to those at 36.5°C (Capela et al., 2006). However, rats in cold environments (10°C) still show reduced 5-HT levels. In humans, MDMA promotes increase of body temperature regardless of the ambient temperature (Freedman et al., 2005). Furthermore, drugs with a hypothermic effect, for example 5-HT_{2A} and glutamate receptor antagonists, attenuate, whereas drugs inducing hyperthermia enhance, MDMA-induced neurotoxicity. Some protective mechanisms (e.g., inhibition of nitric oxide synthase) have been shown to be effective by inducing hypothermia or counteracting hyperthermia. Therefore, the importance of

hyperthermia in the toxic process is firmly supported. Hyperthermia enhances, but is not necessary for, or the only cause of, neurotoxicity. At the same time, a hyperthermic environment might increase the metabolizing activity of enzymes, thus increasing the rate of production of possibly toxic metabolites of MDMA (Capela et al., 2006).

Metabolites of MDMA

Direct injection of MDMA into the brain of rats does not cause serotonergic neurotoxicity seen after peripheral administration (Monks et al., 2004). MDMA metabolism leads to the formation of nine potentially highly reactive main metabolites, thus MDMA metabolites are prominent candidates for neurotoxic potential. Generally, *in vivo* as well as *in vitro* studies show that the further advanced the metabolite, the more direct and faster are its toxic effects. In this line, alpha-MeDA (the major metabolite of MDMA in rats), produced long-term depletion 5-HT in the cortex, hippocampus, and striatum of rats when administered subcutaneously (s.c.), and in neuronal cell cultures when incubated for 48 hours. On the other hand, further metabolites 5-(GSH)-alpha-MeDA and 5-(NAC)-alpha-MeDA had no effect when administered intracerebroventricularly (i.c.v.), but caused long-term 5-HT depletion when directly administered intrastriatal or intracortical, as well as substantial neuronal cell death already during short incubation time (6 hours) (Capela et al., 2009). Further oxidization of these metabolites leads to the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which promote oxidative stress that can ultimately lead to neuronal cell death. Antioxidants have been shown to reduce MDMA-induced neurotoxicity, thus confirming the notion of oxidative stress being a major contributor to neurotoxicity. This hypothesis is further supported by decreased 5-HT neurotoxicity following inhibition of SERT by flouxetine, which prevents entry of MDMA or its reactive metabolites into the cell.

Monoamine oxydase (MAO)-mediated metabolism

MAO deaminates monoamine neurotransmitters inside the cells, a process during which ROS are formed. The excessive extravesicular release of 5-HT, and increase in extracellular concentrations of DA and NE by MDMA leads to increased ROS formation, presumably damaging mitochondria, eventually leading to degradation of the nerve terminal. Furthermore, MAO-mediated metabolism of 5-HT and DA results in reactive aldehyde intermediates before conversion to more stable DOPAC and 5-HIAA. Pharmacological inhibition of MAO-B, which deaminates 5-HT, has been proven to be protective against MDMA-induced neurotoxicity due to oxidative damage (Capela et al., 2009).

Current controversy

Early studies using silver staining as a marker for neuronal cell death found increased staining of axons, dendrites and few cell bodies in frontal brain areas following MDMA-administration (Jensen et al., 1993). However, doses used in these experiments were extremely high (25-150mg/kg), and nowhere near behaviourally relevant or commonly ingested doses by humans. Studies investigating the long-term effect of a neurotoxic MDMA treatment regime demonstrated region dependent recovery of serotonergic markers in most brain areas after one year, however some brain areas remained denervated or abnormally innervated with nerve fibre fractions (Scanzello et al., 1993; Sabol et al., 1996). These observations have led to the

assumption of permanent loss of serotonergic cells, specifically axon terminals due to MDMA administration. However, the problem with neurochemical markers of the serotonergic system is that they provide an indirect measure of functioning, and do not evidence structural loss of neurons. The most parsimonious conclusion from these studies is that at very high doses, MDMA leads to general neuronal degradation, not restricted to 5-HT neurons. Some recent studies tried to disentangle structural from functional loss. For example, depletion of 5-HT, but not SERT or changes in glial fibrillary acidic protein (GFAP), a marker for neuronal degeneration, were found after MDMA administration (single or repeated administration of 7.5-20mg/kg) (Wang et al., 2004; Baumann et al., 2007; Pubill et al., 2003). In contrast, the specific serotonergic neurotoxin 5,7DHT decreased 5-HT and SERT expression and increased GFAP (Wang et al., 2005). 5-HT levels could be restored two weeks later in 5,7DHT treated animals. Neuronal axotomy, either by the known 5-HT neurotoxin or presumably by MDMA, does not seem to prevent serotonergic recovery. Recent experimental data using Western blot technique demonstrates that SERT protein content in the striatum and nucleus accumbens is not changed, but SERT was relocated into cellular structures after MDMA treatment (Kivell et al., 2010). Thus, neurochemical markers attaching to extracellular SERT would reveal that its binding availability is reduced, while in fact, temporary or permanent relocation took place. Long-lasting behavioural effects in animals, for example, increased anxiety, and cognitive impairments in humans, such as memory function, have been shown in the absence of 5-HT or SERT depletion (McGregor et al., 2003; Thomasius et al., 2006), suggesting that functional changes at the receptor or transporter level may be sufficient to elicit long-term consequences. These results add to the current debate about the neurotoxicity of MDMA in that changes on the 5-HT system might be restricted to depletion of 5-HT and decreased availability of SERT in brain tissue, or lead to permanent alterations of serotonergic neuron function, rather than promoting complete cell death.

Cannabis

History and Use

The term cannabis describes the natural hemp plant (cannabis sativa/indica) and its products and derivatives, which have a long history of ritual, medicinal, industrial and recreational use. Native to central Asia, earliest narratives of use have been dated back to 3000 B.C. In the 18th century, cannabis plants and first descriptions of its medical and psychoactive potential appeared in Europe. In 1839, a comprehensive description of the medical uses of Indian hemp (cannabis indica) by an Irish doctor stationed in Calcutta sparked its use for medical purposes around Europe (see **Excursion 3**: Medical use of cannabis) (Murray et al., 2007). By the mid-20th century, chemical substances (for example, aspirin) replaced cannabis as promising pharmaceutical agent. Furthermore, its instable effects, and increasing legal restrictions lead to a decline of medical use of cannabis products (Aldrich, 1997). Today, it is almost exclusively used for recreational purposes. In fact, cannabis is the most consumed illegal psychoactive substance world wide. It is third in line of the most commonly (ab)used drugs, following the legal substances alcohol and nicotine (EMCDDA, 2008). Most common cannabis products for recreational use are marihuana (from the dried leaves and buds) and hashish (from the concentrated resin) (Fig.6).

Fig.6: Cannabis leaves of cannabis sativa (left) and hashish blocks (right). Of the three different varieties of the plant, today cannabis sativa is the most frequently grown. Marihuana is produced from the dried leaves and flowering tops, whereas hashish is made of the resin of the plant. (retrieved from www.wissen.dradio.de and www.freedrugzone.com)

The cannabis plant contains at least 60 active cannabinoids, of which delta9-Tetrahydrocannabinol (Δ9-THC, THC) is the primary psychoactive constituent and cannabidiol the major sedative compound. The major cannabinoids found in the female plant are inactive carboxyl acids, which are transformed into active phenols by heating (smoking, baking). THC is readily absorbed by inhalation and evident in the plasma within seconds. It is estimated that maximal concentration of THC (approx. 1 %) is reached in the brain 15 minutes after inhalation (Grotenhermen, 1999). Subsequently, THC exerts its actions by binding to endogenous cannabinoid receptors, while mechanisms underlying the effects of cannabidiol are not clearly established (although its binding

to a G-protein-coupled receptor, GPR555, is confirmed) (Howlett et al., 2002). The concentrations and ratios of THC, cannabidiol and other cannabinoids, and thus the effects of the drug, vary greatly according to plant breeding, cultivation, post-harvesting techniques and countries. The potency of cannabinoid drugs is usually expressed in terms of THC content. Seizured cannabis products in the EU contained between 1 and 15% of THC. Generally, the THC content is higher in hashish than in marihuana (EMCDDA, 2010).
Since cannabinoids (including THC) are lipophilic, they accumulate in fatty tissue of the body and are metabolized and secreted slowly. Therefore, THC metabolites (some of which retain pharmacological activity) can be detected up to seven days after consumption in blood samples, and up to two weeks in urine. Interestingly, some of the hepatic enzymes responsible for metabolism of THC are inhibited by canabidiol (Ashton, 2001).

Cannabis is able to grow in many environments, thus trafficking routes for marihuana are generally regional and cultivation mainly occurs indoors (e.g., within Europe). On the other hand, reports from the United Nations (UN) suggest Afghanistan and Morocco to be the biggest suppliers of hashish, grown on outdoor cultivation plants. The UN world drug report estimates the global production of cannabis products for the year 2008 between 13 000 and 66 000 tons (UNODC, 2010).

In most countries cultivation, possession, and distribution of cannabis is illegal. However, some states in the USA and also Europe (including Germany, Italy, Portugal) decriminalize possession of small amounts, such that punishment involves a fine rather than more severe penalties.

Special case- The Netherlands: In the Netherlands, the decriminalization of cannabis in 1976, and subsequent legalization of cannabis sales in “coffee shops” meeting certain set criteria (but not the possession, distribution or use elsewhere) has been ground for debate. However, when comparing cannabis use data from the Netherlands to other European countries, similar trends are observed regardless of the legal status of the drug. In the mid-90s, local communities received the opportunity to decide whether they want coffee shops or not. Since then, many coffee shops have been closed and today are mainly restricted to the large communities (EMCDDA, 2008). Recently, new legal arrangements have been put forward, including restricting cannabis use to registered members, and allowing a maximal amount of 15% THC in cannabis products sold in coffee shops.

Prevalence

Most people have tried some form of cannabis at some point in their life. First experimental contact usually occurs during adolescence or young adulthood. Some people become regular users, and

of these, a minority (10%) becomes heavy (i.e., daily) consumers. The majority of consumers stops around age 30 or older. The world drug report of the UN states the 12 months prevalence among adults (15-64 year olds) between 2.8 and 4.5%, depending on the country (Fig.7). This estimate is lower than figures from the European and German drug monitoring centres, indicating that 6.7% of European and 9.7% of German adults have used cannabis during the last year. Numbers are higher for young adults (European 15-24 year olds: 15.2%) as well as adolescents, and for males compared to females (EMCDDA, 2010).

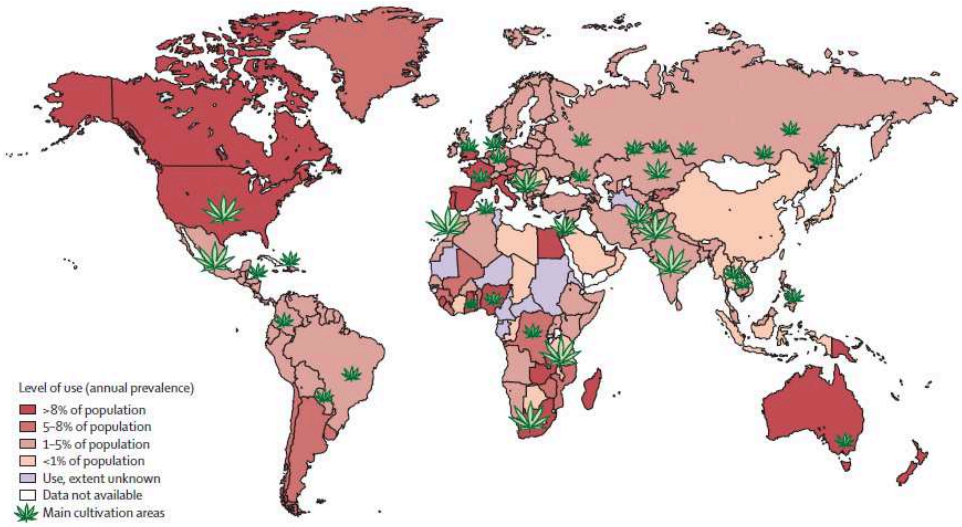

Fig.7: Cannabis consumption and main cultivation areas world wide. 12-months prevalence rates from 2009 or latest data available (UNODC, 2010). Consumption over the last 20 years has risen but recent data suggest it is currently stable in most European countries.

Effects

Acute (and desired) psychological effects upon cannabis consumption include mild euphoria and relaxation, heightened sensory perception, and increased sociability (Hall and Degenhardt, 2009). Physiologically, cannabinoids stimulate appetite and have antinociceptive as well as sedative properties. Additionally, impairments in short-term and working memory, attention, decision making, and distortions in time and space perception appear (Iversen, 2003). In terms of motor behaviour, increased motor activity may be followed by incoordination, weakness, and ataxia (Kumar et al., 2001). Adverse effects like panic attacks and anxiety (mainly in novel users) as well as increased heart rate, lowered blood pressure and dry mouth have been reported. Furthermore, high doses can lead to psychotic symptoms (Chopra and Smith, 1974; D'Souza et al., 2004).
Results from experimental studies in animals show similar effects of THC. For example, dose-dependent modulation of locomotor activity, food intake, pain, body temperature and memory was demonstrated (Ameri, 1999). More specifically, experiments in rats indicate a cannabinoid-

mediated dysfunction of the hippocampus, the brain area closely involved in learning and memory. In hippocampal cell cultures, cannabinoid agonists decreased long-term potentiation (LTP), a molecular mechanism crucial for short-term memory and storage of information (Schlicker and Kathmann, 2001; Sullivan, 2000).

Risks, long-term effects & impairments

There are no reports of deaths caused directly by cannabis consumption; the risk of an overdose is close to zero. However, traffic accidents involving cannabis have been reported: acute cannabis consumption increases the risk of motor vehicle crashes, probably due to impaired time perception, attention and slowed reaction times (Asbridge et al., 2012; Ameri, 1999).

Cannabis can lead to psychological habituation and dependence as defined by the international classification for mental disorders (DSM IV) in about 10% of regular users. Development of tolerance, i.e. the need for an increased dose to produce the same effect, is common in cannabis users, and rewarding properties of THC have been confirmed in self-administration experiments in animals (Iversen, 2003; Tanda and Goldberg, 2003). Withdrawal symptoms, including negative mood (anxiety, depression, irritability), sleep disturbance and decreased appetite have been reported by regular users who stop consumption, and replicated in animal models (Ameri, 1999).

Long-time cognitive deficits are debated. Several studies found no correlation between heavy cannabis use and IQ or cognitive deficits after 28 days of abstinence (Pope et al., 2002), while others found persistent dose- or onset-related impairments of memory, learning, attention and decision making in a gambling task (Solowij and Battisti, 2008; Bolla et al., 2005). Neuroimaging studies demonstrate changes in brain structures related to these cognitive functions. For example, diminished activity in prefrontal cortex, hippocampus and cerebellum have been found in heavy cannabis consumers (Block et al., 2002; Nestor et al., 2008). Alterations of cannbinoid binding to its receptor in the brain have also been found (Howlett et al., 2004), suggesting changes on a cellular level in response to chronic administration of cannabinoid agents.

Consumption of cannabis has been associated with an increased risk for psychiatric conditions, namely schizophrenia (Arseneault et al., 2004) and depression (Degenhardt et al., 2003). This is especially true for adolescent individuals and those with a preexisting vulnerability to psychiatric conditions. Although many retrospective studies suffer from the drawback of incomplete control groups and inadequate possibilities to control for confounding factors such as personality, well-designed longitudinal studies indicate that cannabis use is a risk factor for incident psychotic symptoms, and its continued use can contribute to the persistence of the symptoms or development of a psychotic disorder (Kuepper et al., 2011). In schizophrenic patients, cannabis use can exacerbate symptoms and increase the frequency of relapses (Kumar et al., 2001). Furthermore, an association between cannabis use and polymorphism in the gene encoding catechol-O-methyltransferase (COMT) was found in current patients (Costas et al., 2011) and

cannabis using adolescents who later developed psychosis (Caspi et al., 2005). In line with this, COMT variation was found to modulate THC-induced psychotic experiences as well as impairments in cognition (attention and memory) (Henquet et al., 2006).

In animal models, similar effects were found after administration of synthetic cannabinoid drugs. Rats treated chronically during puberty with a cannabinoid receptor agonist showed more severe and persistent deficits in short-term memory information processing and in social tasks compared to those animals treated during adulthood (Schneider et al., 2008). Furthermore, a deficit in sensorimotor gating mechanisms, a phenomenon which has been observed in neuropsychiatric disorders like schizophrenia, together with alterations in gene expression in certain parts of the brain was evident after chronic pubertal treatment (Wegener and Koch, 2009b).

The probability for persistence of the above mentioned impairments rises with increased frequency and dose of use, as well as with initiation of cannabis consumption during puberty. Occasional use, especially during adulthood, is associated with relatively low risks (Iversen, 2005).

The most robust associations were found between cannabis use and reduced educational achievement as well as increased used of other drugs, and health problems involving the respiratory system, for example, chronic bronchitis (Macleod et al., 2004). Risk of developing pharyngeal or lung cancer is increased in cannabis smokers. However, many studies caution that most of their subjects also smoke tobacco (Hall, 1998).

Excursion 3: Medical use of cannabis

For millennia, cannabis has been known for its medicinal properties. In 1839, a comprehensive description of the medical uses of Indian hemp (cannabis indica) by the Irish doctor O'Shaughnessy stationed in Calcutta sparked its use for medical purposes around Europe. Shortly afterwards, French psychiatrists discovered the potential of hashish to treat psychiatric conditions, and describe their physical and psychological effects. Between 1880 and 1900, the medical use of hashish had become widespread in Europe and the USA, and the main pharmaceutical companies manufactured cannabis products. Most important applications were against pain, migraine, asthma and insomnia, followed by symptoms of cholera. Rarely it was used to treat depression and diminished appetite (Aldrich, 1997).

The discovery of endogenous cannabinoid receptors and their ligands as well as the development of synthetic ligands in the late 20th century has further specified the medically relevant properties of cannabinoids. These include anti-emetic (preventing nausea and vomiting), analgesic (reducing acute and inflammatory pain), and orexigenic (increasing appetite and food intake) effects. Anecdotal reports from patients suffering from multiple sclerosis suggested reductions in pain and spasticity following cannabis consumption (Consroe et al., 1997). Recent experimental studies employing animal models of multiple sclerosis and pain confirmed these reports, as mice showed less tremors and spasms following administration of cannabinoids (Baker et al., 2003), and rats had diminished pain reaction in response to neuropathic and chronic pain after treatment with a cannabinoid receptor agonist (Kumar et al., 2001). On a cellular level, THC and cannabidiol have anti-inflammatory and anti-oxidant properties (Hampson et al., 1998).

Examples of implementation

Although cannabis is a scheduled drug according to USA federal government laws, 15 states have passed a law in 1996 which allows possession of a small amount of cannabis for medical purposes. Patients, mostly suffering from diseases involving chronic pain, extreme loss of body weight or chemotherapy, need written referral of a medical practitioner and obtain their share from so called “caregivers”, who legally cultivate cannabis plants for these restricted purposes. Local authorities are officially asked to de-prioritize persecution of consumers and suppliers of medicinal cannabis. In the Netherlands, cannabis can be bought in pharmacies with a prescription since 2003. Many other European countries consider altering their laws to permit cannabis use for medical purposes, depending on outstanding results of large scale clinical trials and scientifically sound research.

Neuroprotective properties

Substantial experimental evidence indicates neuroprotective properties of cannabinoids. *In vivo* administration of synthetic cannabinoid agonists (e.g., WIN55,212-2), THC, cannbidiol or endocannabinoids such as anandamide protected against global and local ischemic damage and poison-induced excitotoxicity. Studies in cultured cells have supported these findings, as the cannabinoid agonist CP55,940 protected cortical neurons from glutamatergic excitotoxicity (Sarne and Keren, 2004; Sarne and Mechoulam, 2005). These neuroprotective effects of cannabinoids have been attributed to the modulation of calcium-dependent mechanisms which play a role in neuronal damage and cell death. Thereby, cannabinoids directly (reducing free radical production) and indirectly (reducing glutamate release and subsequent excitotoxicity as well as nitric oxide synthesis) decrease cell death by attenuating intracellular calcium (Grundy et al., 2001). Nevertheless, low doses or concentrations of cannabinoids and THC given over a long period of time do have neurotoxic properties. This is of special importance when considering medical use of cannabinoid agents.

Current developments

Extensive research into the beneficial properties of cannabinoid ligands without psychoactive side-effects has been undertaken by pharmaceutical industries and independent sources. Apart from the above mentioned effects, research examines the therapeutic potential against cancer and auto-immune diseases and addiction. For example, two synthetic cannabinoid receptor agonists, dronabinol and nabilone, have already been approved for medical use since the 1980s (Manzo, 1988). Recently, sativex, a cannabis plant extract which is delivered as an oral spray, has been developed and approved for treatment of pain in patients with multiple sclerosis in Canada (Novotna et al., 2011). A second new drug is the cannabinoid receptor antagonist rimonabant, which has been developed to treat obesity and received recommendations for approval in the EU. However, high drop-out rates in the clinical trials and many side effects, for example depression, lead the USA to reject the drug (Christensen et al., 2007). Further cannabis-related drugs for various medical conditions are currently under research. Substances inhibiting endocannabinoid degradation, or new synthetic analogues which lack psychoactive side effects or exert their effects on the allosteric site of the cannbinoid receptor in the brain could prove valuable as adjunct therapeutics.

Pharmacology

Endocannabinoid system

Considering the long history of cannabis use, chemical isolation and identification of the psychoactive ingredients of the plant (Mechoulam and Gaoni, 1967), followed by the discovery of endogenous cannabinoid receptors and identification of endogenous substances acting on these receptors occurred fairly recently. The term “endocannabinoid system” includes at least two specific cannabinoid receptor types, the cannabinoid receptor1 (CB1, discovered 1988 (Devane et al., 1988)) and cannabinoid receptor2 (CB2, discovered 1992 (Munro et al., 1993)), as well as their endogenous ligands. CB1 is primarily found in the central and peripheral nervous system (CNS and PNS), whereas CB2 is mainly located in the periphery, specifically in tissues of the immune system. The most extensively researched endocannabinoids are anandaminde and 2-arachidonylglycerol (2-AG) (Piomelli, 2003). Since the discovery of the endocannabinoid system, research has determined its involvement in the modulation of analgesia, cognition, memory, locomotor activity, appetite stimuli, food intake and reward properties of drugs and immune control. The endocannabinoid system regulates synaptic neurotransmission by influencing the release of neurotransmitters. Postsynaptically synthesized and released endocannabinoids act as retrograde messangers to presynaptic CB receptors, which they activate tonically (Howlett et al., 2004). In response to a depolarization of the postsynapse, endocannabinoids increasingly stimulate CB1 receptors, which leads to inhibition of neurotransmitter release (see Fig.8B). Action of endocannabinoids at the CB1 receptor is terminated by a carrier-mediated mechanism and subsequent enzymatic action of fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (in case of 2-AG) (Wegener and Koch, 2009a).

Synthetic cannabinoid receptor ligands, for example CB1 agonists WIN55,212-2 (WIN), CP55,940, and CB1 antagonists AM251 and SR141716A (Rimonabant), have been developed to increase the understanding of the endocannabinoid system as well as to investigate the mechanisms by which exogenous cannbinoids (e.g., THC) exert their effects on a variety of physiological and psychological processes.

CB1 receptor

The CB1 is a presynaptically located G_i-protein coupled receptor which is widely distributed throughout the brain. It has also been discovered in peripheral sympathetic neurons as well as in cells of the immune system, the spleen, and testes (Pertwee et al., 2010). In the CNS, both endo- and exogenous cannabinoids act as retrograde messengers on CB1, thereby inhibiting the release of neurotransmitters (Wilson and Nicoll, 2002) (see Fig.8B). CB1 is predominantly found on

terminals of inhibitory gamma-aminobytric acid (GABA)-ergic interneurons and glutamatergic neurons, especially in the cortex, cerebellum and hippocampus. Other neurotransmitters affected are noradrenaline, DA, 5-HT and acetylcholine (Kano et al., 2009; Kawamura et al., 2006; Tsou et al., 1998). On DA and 5-HT neurons, CB1 is additionally expressed on extrasynaptic axonal and somatodendritic sites (Lau and Schloss, 2008). The CB1 forms functional heteromers with other receptors, including DA D_2 receptors in the hippocampus and cortex and opiod μ receptors in the spinal cord (Pertwee et al., 2010). Endocannabinoids are involved in depolarization-induced suppression of inhibition (in the hippocampus) and excitation (in the cerebellum) in response to local signal transmission on particular neurons. On the other hand, exogenous cannbinoids reduce neurotransmission on a larger scale due to activation of CB1 all over the brain (Wilson and Nicoll, 2002).

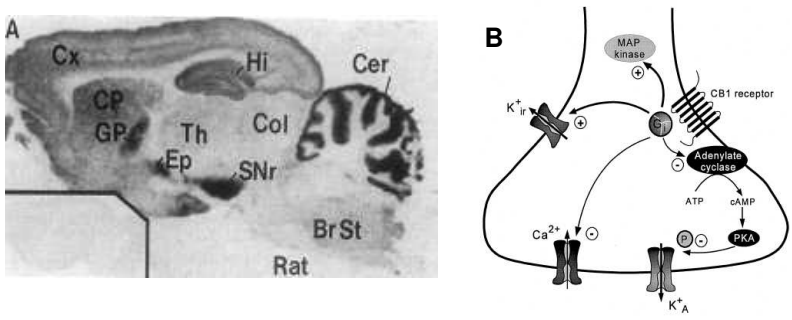

Fig.8: A) Distribution of the CB1 throughout the rat brain B) simplified schematic diagram of the presynaptic mechanisms influencing signal transduction A) Photograph of a sagittal brain slice from an early study using the radioligand [H^3]CP55,940, which binds with high affinity to the CB1 (adapted from Herkenham, 1990). Highest densities were found in the frontal cerebral cortex, anterior cingulated cortex (Cx), thalamus (Th), basal ganglia (CP, caudate putamen, GP, globus pallidus, SNr, substantia nigra), hippocampus (Hi), cerebellum (Cer) and olfactory bulb as well as spinal cord (Herkenham et al., 1990). This heterogenous pattern of distribution accounts for the effects of cannabinoid substances on cognition, memory and learning, movement control and coordination, as well as pain perception. Low density in the brainstem (BrSt), which controls many autonomic functions, probably accounts for the lack of cannabis-induced fatalities. More recent neuroimaging studies in humans and rats have supported this distribution, as the pattern of functional activation matched the behavioural profile (Howlett et al., 2004). B) Two main mechanisms by which endocannabinoids and CB1 agonists inhibit neurotransmitter release: 1) activation of K+ (A-type and inwardly rectifying) channels leads to an increased potassium efflux, reducing the presynapitc action potential and thus neurotransmitter release. This occurs by a) direct G-protein-mediated activation, and b) inhibition of adenylate cyclase activity, leading to a decrease of cAMP in the synapse and subsequent reduction of protein kinase-mediated phosphorylation of K+ channels. 2) G-protein-mediated inhibition of voltage-dependent N and P/Q-type Ca^{2+} channels, thereby reducing intracellular Ca^{2+} concentration which is necessary for neurotransmitter release. Furthermore, activation of the CB1 stimulates mitogen-activated protein (MAP) kinase pathways (Schlicker and Kathmann, 2001; Ameri, 1999).

Decision making

Individuals are faced with an ever-changing environment in which making choices is a crucial process for adaptation and associative learning in everyday life. Decision making can be defined as the cognitive process during which two or more alternatives are compared, resulting in a choice for a (behavioural) action. Decision making is a complex cognitive but mostly automatic process which involves various steps. When faced with a choice of different behavioural options, characteristics of the benefit or reward of each action are weighed against the (expected or known) costs to reach the optimal solution. Subsequently, the choice is transformed into a response and a reaction is executed. Decisions, conscious or unconscious, are cost-benefit evaluations.

Deficits in decision making have been shown in patients with focal lesions of the involved brain regions, and in individuals with psychiatric disorders, for example, schizophrenia, depression and drug addiction, who tend to choose a cost-aversive alternative, or repeat maladaptive choices despite knowing the adverse consequences (Bechara et al., 2000; Grant et al., 2000).

Two different, yet interrelated forms of cost-benefit decision making are delay-based and effort-based choice. Delay-based tasks test the ability to tolerate delays before a reward (i.e., how long an individual is willing to wait before receiving a large reward), and have been implemented as animal models of impulsive choice. Effort-based tasks measure how much physical work is invested for a large reward. In animal models differentiating between these forms of decision making, the different costs are manipulated. Delay-based paradigms usually employ 10-15 seconds of waiting time for the higher reward. Effort-based tasks increase the amount of physical work, either by increasing the number of lever presses or by inserting a barrier which the animal has to climb in order to obtain the large reward (Fig.9).

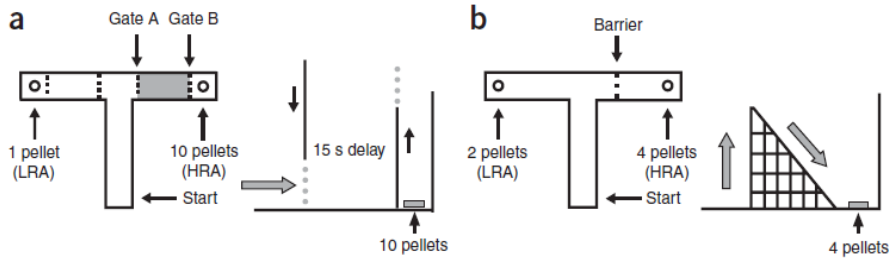

Fig.9: Schematic diagram of a) delay- and b) effort-based experimental tasks in a T-maze. Rats are placed in the start arm and choose between a high cost option (waiting time between gate A and gate B or effortful climbing of a barrier) to obtain a large reward, and a low-cost option (no delay, no barrier) to obtain a small reward. Under normal circumstances, rats trained on these tasks decide in favor of the large reward over the small reward even if its attainment is associated with a delay or effort. LRA=low reward arm, HRA=high reward arm (adapted from Rudebeck, 2006).

In line with human studies, (pre)frontal cortex regions including the anterior cingulated cortex (ACC) and the orbitofrontal cortex (OFC), but also the basolateral amygdala (BLA) as well as the nucleus accumbens (NAC) have been associated with decision making in animal models. In addition, memory-based, and motor controlled aspects of choice behaviour require recruitment of the hippocampus and cerebellum (Ernst et al., 2002). Furthermore, distinct forms of cost-benefit choices seem to depend on different frontal regions. In a human study, options involving increasing effort- or delay-based costs were discounted in the same behavioural manner, however processing in the brain could be dissociated for effort (ACC) and delay (medial PFC) devaluation (Prevost et al., 2010). Lesions of the OFC of rats led to a decrease in choices for a large reward if the cost was a delay. On the other hand, lesions of the ACC led to decreased choices for the large reward if the cost was effort (Walton et al., 2003; Floresco and Ghods-Sharifi, 2007). However, either brain region is solely involved in decision processes. Rather, they are part of a neural network, as "both ACC and OFC send afferent projections to the nucleus accumbens, albeit to distinct subregions, [and to the BLA] suggesting there may be distinct fronto-striatal loops which process effort and delay costs" (Rudebeck et al., 2006)(p.1166). In fact, disruption of the connection between ACC and the BLA decreased HRA choices in an effort-based T-maze task (Floresco and Ghods-Sharifi, 2007), and inactivation of the NAC core, but not shell subregion, reduced the choices for the high reward arm in effort- as well as delay-based tasks (Ghods-Sharifi and Floresco, 2010; Cardinal et al., 2001). Thus, these interconnected subcortical regions (representing a "limbic-motor interface" and the emotional/motivational value (Mogenson et al., 1980; Homberg, 2012)) play a crucial role in cost-benefit decision making, whereas frontal cortical brain regions differentiate between the different forms of costs.

Furthermore, dissociable neurochemical mechanisms involving monoamine transmitters mediate effort- and delay-based decisions. 5-HT modulates delay-based choices, as reduction of 5-HT

functioning leads to more impulsive behaviour in humans and animals (Cardinal, 2006). Moreover, 5-HT synthesis blocker increase the choices for the LRA. This effect may be reversed after chronic treatment with serotonergic drugs (Liu et al., 2004). On the other hand, the DA system is involved in delay- as well as effort-based decisions (Denk et al., 2005). Specifically, D_1 and D_2 receptor antagonists shift rats choices towards the small reward option (Bardgett et al., 2009).

Influence of MDMA

Retrospective studies in human MDMA users point to deficits in cognitive functions including memory, attention, and decision making.

MDMA is perceived as a "serotonergic drug", as its major behavioural and physiological effects are mediated by the influence on 5-HT activity, followed by DA and NA. On cortico-limbic structures, 5-HT neurons and receptors are present abundantly, thus the 5-HT system seems closely involved in cognitive processes (Homberg, 2012).

As mentioned above, delay-based decisions have been shown to be under control of 5-HT transmission (Denk et al., 2005). Specifically, increases in 5-HT activation (for example, by selective serotonin reuptake inhibitors, SSRIs) enables longer waiting, whereas reduced 5-HT levels lead to impulsive choices (Homberg, 2012). While MDMA causes an acute increase of extracellular 5-HT (and DA and NA) levels, chronic stimulation of the 5-HT system leads to a reduction of 5-HT synthesis via inhibition of tryptophan hydroxylase (Green et al., 2003). In line with this, SERT-deficient rats showed improved inhibitory control (Homberg et al., 2007), and studies investigating decision making and impulsive behaviour in drug users demonstrate increased impulsive choice as well as deficits in decision making in heavy, long-term MDMA users (Quednow et al., 2007; Morgan et al., 2006). A replicated finding from neuroimaging studies are reduced SERT levels in abstinent MDMA users (Cowan, 2007). However, the degree and time span of MDMA-induced serotonergic dysfunction in prefrontal or OFC regions remains inconclusive. On the other hand, studies investigating drug users reporting weak or moderate MDMA consumption found that drug use *per se*, rather than MDMA exclusively, contributes to impairments in decision making (Hanson et al., 2008), or did not find fundamental differences compared to control groups (Halpern et al., 2011). In line with this, binge administration of MDMA to rats acutely decreased inhibitory control, however the behavioural effect was not evident seven days later despite decreases in frontal cortex 5-HT levels (Saadat et al., 2006). Acute and chronic effects of MDMA on effort-based decision making remain to be investigated.
The long-term effects of MDMA on decision making seem to depend on (life time) dosage, as heavier and chronic use is associated with more severe deficits, probably attributable to functional alterations in the brain.

Influence of Cannabis

Most research examining the influence of cannabis on cognitive functions focuses, and agrees on, (short term) memory impairments following acute consumption (Morgan et al., 2010), whereas persistent memory impairment due to chronic consumption is less established (Solowij and Battisti, 2008).

In contrast to memory, cognitive processes such as decision making have only been examined scarcely. In one study employing a gambling task, the authors conclude that "[cannabis] users made more decisions that led to larger immediate gains despite more costly losses than controls" (Whitlow et al., 2004)(p.107). A recent study combining a gambling task with imaging technique found that heavy cannabis users (who were abstinent for 25 days prior to testing in order to exclude confounders such as remaining drug derivatives and withdrawal symptoms) showed maladaptive decision making coupled with decreased brain activity in the OFC (Bolla et al., 2005). However, one study found no effects of acute consumption of THC (McDonald et al., 2003), and other studies found no difference in decision making between current cannabis users and controls tasks (Quednow et al., 2007) or following 28 days of controlled abstinence (Pope et al., 2002).

In animal experiments, a complex role of the cannabinoid system in decision making was shown. In a delay-based paradigm, CB1 receptor antagonists improved inhibitory control or blocked the amphetamine-induced increase of impulsive choice, but administration of CB1 agonists also decreased impulsive choice (THC) or had no effect on delay-based choices (WIN55,212-2) (Wiskerke et al., 2011; Pattij et al., 2007). Since activation of the CB1 receptor exerts inhibitory control over the DA as well as 5-HT system, both implicated in decision making, modulation of the endocannabinoid tone or selective CB1 activation in OFC or ACC should improve knowledge about the influence of the cannabinoid system on decision making.

These results point to an association between MDMA and possibly also cannabis consumption and deficits in decision making; however, as immanent in retrospective human studies predisposition to maladaptive choices or impulsivity (two risk factors for drug abuse), as well as pre-existing brain dysfunction cannot be ruled out.

MDMA & Cannabis

Any study involving human ecstasy users inevitably faces difficulties in interpreting the results due to (the possibility of) polydrug use. This may be caused by impurity of ecstasy tablets, containing a variety of other psychoactive substances and/or stimulants, making attribution of effects to MDMA use difficult. Furthermore, polydrug use, especially among young adults, has become normality rather than rarity (EMCDDA, 2009). Specifically, MDMA use has been associated with elevated consumption of legal drugs including alcohol, cigarettes, and illegal drugs, for example cannabis as well as other psychostimulants such as amphetamine, meta-amphetamine, and cocaine (Grov et al., 2009). Cannabis is by far the most common concomitantly taken illegal substance to MDMA (Gouzoulis-Mayfrank and Daumann, 2006b). These two drugs differ markedly in their biological effects. However, research examining neurobiological, behavioural and cognitive effects of co-consumption in humans or animal models is scarce (see Review).

	MDMA	**Cannabis**
Facts		
Origin	Synthetic amphetamine derivative	Natural hemp plant: cannabis indica/sativa
First appearance	1912	3000 b.c.
Major form of intake	Oral (pills)	Inhaled (smoked)
Duration of effect	3-5 hours	1-3 hours
Half life (human)	Approx. 8-11 hours	Up to 55 hours
Metabolism	Hepatic, CYP2D enzyme	Hepatic, CYP enzymes
Neurochemical mechanism	Reuptake inhibitor/releaser of 5-HT, DA, and NE	Agonist on CB1 and CB2 receptors
LD_{50}	97mg/kg i.p. (mice), 49mg/kg i.p. (rats)	660mg/kg oral (rats)
Effects		
temperature	hyperthermic	hypothermic
oxidative stress	increasing	decreasing
activity	increasing	decreasing
food intake/appetite	hypophagia	hyperphagia
subjective effect	stimulant	relaxant , stimulant

Table 1: Comparison of facts and effects of MDMA and cannabis.

Aim of the thesis and summaries

The thesis aims to increase the understanding of changes in cognitive and behavioural functioning following acute or chronic administration of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Specifically, animal models of decision making and memory are implemented. The influence of the cannabinoid receptor agonist WIN55,212-2 as well as interactive effects between MDMA and CB1 receptor stimulation on these measures are studied in adult and pubertal rats. Following chronic treatment, histological examination of the myelination levels in various brain areas is carried out.

Review

In "MDMA & Cannabis: A Mini-Review of Cognitive, Behavioral, and Neurobiological Effects of Co-consumption" I reviewed recent studies investigating the combination of MDMA and cannabis or cannabinoid agents on cognitive functions, behavior, and neurobiological interactions. Human studies examining the effect of concomitant use on cognitive functions reveal inconsistent results. Some demonstrate cannabis-induced impairments of memory, and learning and decision making deficits attributable to MDMA use only. Others show that polydrug use leads to additive negative effects. However, different inclusion criteria (heaviness of life time MDMA use, time of abstinence, co-consumption of other drugs) and control groups (including or excluding cannabis use, abstinent or naïve MDMA user) make conclusive comparisons difficult. Furthermore, although most studies investigating polydrug users are retrospective, there is a shortage of animal studies modeling chronic co-use and its consequences. In acute animal models, the cannbinoid system has been shown to regulate the reinforcing effects of MDMA. Furthermore, disruption of working memory as well as abolishment of deficits in an object recognition task was evident with co-administration. These results point to an interaction of MDMA-induced alterations of neurotransmission, specifically the 5-HT system, and cannabinoid receptor activation in the hippocampus. Furthermore, cannabinoid agents were shown to have a modulating effect on MDMA-induced changes in body temperature and locomotion in animal models, and presumed neurotoxicity in cell cultures.

Study 1

"Acute Co-administration of the Cannabinoid Receptor Agonist WIN55-212,2 does not influence 3,4-methylenedioxymetamphetamine (MDMA)-induced Effects on Effort-based Decision making, Locomotion, Food intake and Body temperature" investigates possible interactions of MDMA (7.5mg/kg, s.c.) and WIN (1.2mg/kg, i.p.) on various behavioural tasks when administered alone

and in combination. As a measure for cognitive ability, effort-based decision making was tested in a T-maze paradigm. MDMA as well as MDMA-WIN administration induced effort-aversive choice, whereas WIN alone had no effect. Furthermore, MDMA as well as MDMA+WIN decreased food intake and increased locomotor and exploratory behaviour, whereas effects of WIN did not differ from control. The only (tentative) interaction was seen within the open filed: MDMA induced anxiety-like behaviour, which was diminished by co-administration of WIN. The underlying neurobiological alterations remain speculative. However, our data indicate that effects of acute MDMA not only include the established physiological responses like hyperthermia and increased activity, but may also immediately impair cognitive functions like decision making. Generally, co-administration of WIN did not have modulatory effects on behavioural changes induced by acute MDMA administration.

Study 2

“Chronic co-administration of the cannabinoid receptor agonist WIN55,212-2 during puberty or adulthood reverses 3,4-methylenedioxymetamphetamine (MDMA)-induced deficits in recognition memory but not in effort-based decision making“ deals with the effects of chronic administration of either MDMA (7.5mg/kg, s.c.) or WIN (1.2mg/kg, i.p.) alone or in combination, to either adult (postnatal day (PD) >80) or pubertal (PD 40-65) animals. After 25 days of irregular treatment, all MDMA-treated animals showed a significant deficit in short-term memory (tested with the object recognition task), which was not evident when WIN was co-administered. This deficit persisted in pubertally treated animals when re-tested four weeks later. MDMA and MDMA+WIN-treated adult animals showed problems in effort-based decision making, as they took longer to re-learn the task in comparison to vehicle or WIN-treated animals, whereas there were no apparent differences between the pubertally treated groups. No effects of chronic treatment were apparent for the delay-based task. Furthermore, there were no differences between the four treatment groups of both ages in terms of horizontal locomotor activity or exploratory behaviour shortly after cessation of treatment or four weeks later. WIN had no modulator effect on MDMA-induced decrease in weight change of the pubertal rats during treatment. In conclusion, regardless of treatment age, chronic CB1 receptor stimulation is capable of modulating long-term MDMA-induced deficits in basic memory performance, presumably due to altered (serotonergic) neurotransmission. This interaction is most likely localized to the hippocampus, whereas no amelioration of MDMA-induced disruption seems to take place in prefrontal areas responsible for more complex tasks like effort-based decision in rats treated during adulthood.

MDMA & Cannabis: A Mini-Review of Cognitive, Behavioral, and Neurobiological Effects of Co-consumption

Sybille Schulz

Abstract

Although the prevalence of co-use of cannabis and 3,4-methylenedioxymethamphetamine (MDMA) is very common among polydrug users in western societies, few studies have tested the consequences on behavior, cognition or neurobiology. This review examines 23 articles published between 2002 and 2010 with an explicit focus on the combination, or administration, of MDMA and cannabis or cannabinoid agents. The aim was to provide a short overview on the latest human research concerning cognitive effects of co-consumption of MDMA and cannabis, and a more elaborate picture of the state of knowledge about the interaction of cannabinoid agents and MDMA from animal studies. It was found that recent retrospective studies on cognitive functions in long-term drug abusers point to an additive negative effect on different types of memory, as well as a cannabis-independent decrease in learning and decision making in MDMA users. Behavioral experiments in rodents and *in vitro* studies investigating the combined effect of MDMA and cannabinoid agents demonstrate modulator effects of acute co-administration on measures like body temperature, conditioned reinforcement, and presumed neurotoxicity. As neural mechanism underlying these changes, an interaction between the cannabinoid system, especially cannabinoid receptor 1, and the serotonergic and dopaminergic system in the prefrontal cortex, nucleus accumbens, and hippocampus is suggested. In conclusion, there are few and somewhat contradictory studies examining the effects of co-use of these drugs on cognitive measures like impulsivity, memory and executive functions or underlying neurobiological alterations, and a shortage of animal studies examining long-term effects of chronic co-administration.

Introduction

Not only did the consumption of illicit drugs like cannabis, MDMA (3,4-methylenedioxymethamphetamine, “ecstasy”) or other psychotropic substances increase during the last decade, but particularly polydrug use among young people has augmented concerns about aversive effects and increased challenges in drug abuse research investigating the effects of any specific drug (Scholey et al., 2004; EMCDDA, 2010). For instance, reports of recreational MDMA users disclose a strong abuse of a range of other psychoactive compounds and drugs (Braida et al., 2005). In fact, 98% of MDMA users have used cannabis at some point in their life (although not all cannabis consumers become regular MDMA users) (Fox et al., 2001; Gouzoulis-Mayfrank and Daumann, 2006b). More specifically, cannabis is revealed to be the most widely taken illegal co-drug in MDMA users, especially among younger adults. Ninety to ninety-eight per cent report to concomitantly have smoked cannabis (e.g., USA: 98%, lifetime prevalence (Keyes et al., 2008; Wu et al., 2009; Grov et al., 2009); UK: 89%, 12-month-prevalence (Smith et al., 2011)), and many of them consume both drugs at the same time (Parrott et al., 2004). The decision for polydrug use might be influenced by psychological and functional aspects (Parrott et al., 2004; Degenhardt et al., 2001). In contrast to the initial entactogenic and euphoric effects upon acute administration, decreasing concentrations of MDMA in the brain can lead to anxiety, agitation, insomnia and depressive feelings. Due to its sedative properties, cannabis might be taken as remedy to relieve these negative physiological and emotional states associated with the “come-down” from ecstasy (Winstock et al., 2001) (or with serotonin (5-HT) depletion due to chronic use (Green et al., 2003; Morton, 2005). In addition, many users also report taking cannabis during the initial acute stimulatory phase in order to “improve its effects“ and obtain a more “mellow” experience on Ecstasy (Boys et al., 2001).

The behavioral and neurobiological effects of MDMA (e.g., (Green et al., 2003; Cole and Sumnall, 2003), or cannabis (e.g., (Hall et al., 2001; Howlett et al., 2004)) have been researched extensively and are well documented. However, there is inconsistency in the literature about the effects of a combined administration, both in the few human studies investigating cognitive impairments as well as in animal studies focusing on behavioral models or the interaction on neurocellular levels. Taken acutely, at different incidences, the two substances may have opposing effects (see above), whereas when taken chronically their negative effects might be additive (Parrott et al., 2007). In both cases, *interaction* of their effects might take place both on a psychological as well as neurobiological level.

The aim of this review is, firstly, to provide a short overview on the latest human research concerning cognitive effects of co-consumption of MDMA and cannabis. Although Ecstasy users ingest pills with an unknown amount of the main psychoactive compound MDMA, the amount of MDMA in Ecstasy pills has increased within the last decade (Parrott, 2004; 2010). For the sake of clarity, the term MDMA users will be used for human ecstasy users throughout this review.

Research with human polydrug users typically tests for cognitive abilities after prolonged and past drug use. There are only very few studies examining acute effects of the co-consumption of MDMA and cannabis. Secondly, a more elaborate picture of the state of knowledge about the interaction of cannabinoid agents and MDMA in animal studies will be given. Here, research predominantly focuses on the acute effects of co-administration, or on long-term consequences on a cellular level, and less studies are known which examine chronic effects on behavior or cognition.

Key learning objectives

1 Ninety to ninety eight percent of MDMA users concomitantly smoke cannabis.

2 In chronic MDMA+cannabis users, impairments in various memory functions, impulsivity and decision making have been found. Whether these drugs can be allocated to specific deficits remains unclear. Frequency, duration and pattern of co-consumption may influence the impact of either drug.

3 Experiments in rodents demonstrate various behavioural effects, presumably due to interaction of the endocannabinoid system and 5-HT/DA in various brain regions:

a) Acute rewarding effects of MDMA (number of lever presses, CPP) are most likely regulated by interaction with CB1 receptors and DA in the nucleus accumbens.

b) Disruption of memory function by co-administration of MDMA and THC, and the reversal of MDMA-induced working memory deficit by a CB1 antagonist points to an interaction of MDMA-induced neurotransmission and CB1 receptor activation in the hippocampus.

c) Hyperthermia, anxiety and depletion of 5-HT in prefrontal cortex, hippocampus, and amygdala due to MDMA administration was prevented by THC and a CB1 agonist.

This review was compiled by classical systematic literature research using the scientific search engine PubMed (search terms included “MDMA” OR “ecstasy” + “cannab*” OR “cannabis” + “cognition” OR “behavior”). Studies focusing on use or administration of only one of the substances were excluded. The following overview includes 23 studies published between 2002 and 2010 with an explicit focus on MDMA plus cannabis use or cannabinoid agent administration. Conclusions and implications for further research will be discussed.

MDMA+Cannabis: effects on higher cognitive functions

This section describes human studies focusing on cognitive functions or the impairments thereof after MDMA and cannabis co-consumption. The vast majority of studies examines long-term,

chronic (poly-) drug users in a retrospective manner, thus those will receive most attention. Furthermore, one study examining the acute effects of co-consumption is described.

In a study examining executive functions of abstinent polydrug users, multiple regression analysis yielded that severity of MDMA use was inversely correlated with performance on a working memory and analogical reasoning task, whereas severity of cannabis use predicted cognitive flexibility (Verdejo-Garcia et al., 2005). However, Morgan et al. (2006) showed that former MDMA users also perform worse on measures of impulsivity and decision making than polydrug users (any illicit drug other than MDMA) or drug-naïve controls. Furthermore, MDMA users seem to have more problems in complex learning tasks (Brown et al., 2010), processing speed (de Sola et al., 2008) and implementation of control strategies (Roberts and Garavan, 2010) than cannabis users. In a recent review examining whether certain cognitive impairments can be attributed to specific drugs, it was noted that MDMA and cannabis use has a robust effect on spatial processing and complex planning (Fernandez-Serrano et al., 2011). On the other hand, Fisk et al. (2006) found that MDMA+cannabis users performed equally to MDMA users, and both groups were worse than controls, on measures of associative learning, verbal and visual-spatial working memory and reasoning. A study with MDMA+cannabis and only cannabis users by Croft et al. (2001) showed that neuropsychological deficits in learning, memory, and verbal word fluency were related to cannabis consumption, rather than MDMA consumption, as there was no difference in performance between the two groups. Only in the Stroop test (a measure for impulsivity/speed of processing) did the MDMA+cannabis group perform worse than the cannabis only consumers. Notably, both groups performed significantly worse than the control (drug naïve) group in the different cognitive functions tested (Croft et al., 2001). Furthermore, cannabis users showed significant deficits in memory function as well as word free-call regardless of concomitant MDMA use (Dafters et al., 2004; Clark et al., 2009), and impairment on a measurement for impulsivity compared to MDMA users and controls (Clark et al., 2009). Thus, some cognitive impairments previously attributed to MDMA use in fact could be caused by (unreported) cannabis abuse (Lamers et al., 2006). In line with this, self-reported psychological impairments of MDMA users were found to be mainly associated with regular concomitant cannabis use (Daumann et al., 2004). However, MDMA use was shown to have significant dose-related negative effect on verbal delayed recall after adjusting for the use of other drugs (Schilt et al., 2008), and in a meta-analysis including 45 recent studies, it was found that MDMA users performed significantly worse in verbal short-term and working memory than polydrug control groups (Nulsen et al., 2010). In line with this, poorer performance of heavy MDMA polydrug-users on a memory test was attributed to the extent of Ecstasy use only (Gouzoulis-Mayfrank et al., 2003). One explanation of these distinct findings could be that cannabis consumers self-report problems with short-term and prospective memory (Solowij and Battisti, 2008), whereas MDMA users complain about long-term memory deficits (Murphy et al., 2009). Another explanation of these effects is drug-induced neurobiological change in distinct areas of the brain responsible for different mental capacities. Imaging studies with

humans (for review, see (Howlett et al., 2004)) and animal studies (Scallet, 1991) show structural and/or functional impairment in the hippocampus (a cannabinoid receptor type 1 (CB1) rich area implicated in memory) after chronic cannabis consumption, and serotonin (5-HT) and serotonin transporter (SERT) deterioration in the (pre)frontal cortex (implicated in planning and executive functions) of chronic MDMA users (e.g., (Reneman et al., 2001; Lundqvist, 2010)). Depending on amount and frequency of co-consumption, long-term polydrug use therefore may lead to additive neuropsychological impairment. Only one study examined the acute effects of co-administration: Dumont et al. (2010) conclude from a variety of psychomotor, memory and subjective effects tests, that overall "cognitive impairments induced by acute administration of THC was more robust compared with MDMA, and co-administration did not exacerbate single drug effects on cognitive function" (Dumont et al., 2010), yet the participants were experienced users of both drugs.

Summing up, the literature on the contribution of cannabis on long-term cognitive effects of MDMA abuse is somewhat inconsistent. Whereas some studies report memory deficits to be mostly attributable to cannabis consumption, MDMA abuse is generally associated with control-related functions (but see (Nulsen et al., 2010). It might be suggested that the pattern of cannabis consumption (light or heavy use) influences the (lifetime-consumption-dependent) MDMA-induced cognitive problems. However, most human studies cannot rule out additive effects, and different control groups between studies (i.e., drug-naïve vs. MDMA-naïve) complicate direct comparisons (but see (Nulsen et al., 2010)). In addition, amount and frequency of consumption differ between the two drugs and between drug users (Gouzoulis-Mayfrank and Daumann, 2006b) (for example, purity of Ecstasy tablets taken on any previous occasion is unverifiable). Furthermore, caution needs to be exerted when interpreting self-reported symptoms of (former) long-term drug users, as these can be related to any of the substances used, and distorted by e.g., memory problems or resulting or pre-existing psychiatric impairments. However, it is still worthwhile to consider studies in which subjects have previously used both drugs, in order to find out about the impact of the frequent combination of these drugs on complex executive functions and combine this information with animal research investigating the underlying neurological effects.

MDMA+Cannabinoid agents: behavioral effects & neurobiological interactions

In contrast to human studies, animal research allows for exact monitoring of dose and combination of unadulterated substances as well as a clearer establishment of cause-and effect. The following section describes studies examining behavioral and neurobiological effects of co-administration of MDMA and cannabis or cannabinoid agents. Most animal studies focus on acute effects of these substances, therefore those make up the majority of works depicted.

In the same way that different cannabinoid ligands are a great advantage to study detailed effects of cannabis consumption, the use of various cannabinoid agents can also be a confounding issue.

Depending on the design and aim of the study, these could be delta9-tetrahydrocannabinol (Δ9-THC, the main psychoactive ingredient derived from the cannabis plant), or synthetic cannabinoids with distinct affinities to the CB receptors, like WIN55,212-2 (a CB1 receptor agonist), CP55,940 (a CB1 and CB2 receptor agonist) or SR141716A and AM251 (CB1 receptor antagonists). Each agent only mimics part of the effects evoked by the mixture usually inhaled by human cannabis consumers, and provides specific and therefore limited information about neurobiological interaction with MDMA. Furthermore, route of administration and therefore pharmacokinetics, differ between animal and human studies (e.g.,(Green et al*.,* 2009)), and systemic administration of MDMA and any cannabinoid agent to animals only allows for speculation regarding the neurocellular mechanisms. However, *in vitro* studies have shown that MDMA and cannabis administration leads to metabolic and neurotransmission alterations in prefrontal and subcortical brain areas responsible for distinct components of executive functioning, (Nagayasu et al*.,* 2010; Fiaschi and Cerretani, 2010; Sarne and Mechoulam, 2005; De Petrocellis and DiMarzo, 2010) presumably by interaction of CB1 receptor activity and serotonin (5-HT) or dopamine (DA). For example, in the prefrontal cortex, 1/3 of axon terminals co-express CB1 receptors. Acute administration of SR141716A increases extracellular release of 5-HT and DA, whereas subchronic or chronic treatment with WIN55,212-2 causes a persistent reduction of DA in the rat prefrontal cortex (Lopez-Moreno et al*.,* 2008). In the nucleus accumbens, Δ9-THC significantly decreased 5-HT and glutamate release, and this effect was reversed by the CB1 antagonist SR141716A (Sano et al*.,* 2008). In contrast, DA release was increased in this brain structure upon intravenous administration of WIN55,212-2 (Lopez-Moreno et al*.,* 2008).

In rodents, some evidence shows regulative effects of separate or co-administration on various behavioral measures. In CB1 knock-out mice, pre-treatment with Δ9-THC prevented MDMA-induced hyperthermia and decreased acute responses to MDMA (increased locomotor activity, body temperature, and anxiousness). CB1 knock-out mice still showed a place preference for MDMA, however they failed to self-administer any dose up to 0.25 mg/kg (Tourino et al*.,* 2010). Another study demonstrated that a low dose (0.1mg/kg) of WIN55,212-2 increased rewarding effects of MDMA, whereas a higher dose (0.5mg/kg) decreased MDMA-induced conditioned place preference in mice (Manzanedo et al*.,* 2010).

One review describes some of the interactive effects of MDMA with cannabinoid agents in rats. In intracerebroventricular (i.c.v.) self-administration studies, CP55,940 decreased the number of lever pressings to obtain MDMA (Sala and Braida, 2005). In contrast, pre-treatment with SR141716A increased MDMA-associated lever pressings and also MDMA-induced conditioned place preference (Braida et al*.,* 2005; Braida and Sala, 2002). It seems likely that the endocannabinoid system influences the mechanisms regulating the reinforcing effects of MDMA, possibly via modulation of DA transmission by presynaptically located inhibitory CB1 receptors (Braida and Sala, 2002). Overlapping of CB receptors and DA receptors has been shown, for example in the nucleus accumbens, and stimulation of CB1 receptors might produce additional reinforcing effects.

However, acute attenuation of short-term memory via stimulation of CB1 receptors in the hippocampus could also result in a reduction of time spent in the compartment previously associated with MDMA. Taken together, these studies point to an endocannabinoid control of the acute rewarding effects of MDMA (Braida and Sala, 2002). Interestingly, MDMA alone affects rodent species in distinct ways. In mice, acute administration leads to a strong release of DA and to a less pronounced release of 5-HT in various brain regions, whereas the opposite is true for rats (Green et al*.,* 2003). Neurobiological markers of presumed neurotoxicity, for example for 5-HT neurons (Stone et al*.,* 1987), further support this distinction. For example, Kindlundh-Högberg et al., 2007, found reduced binding of dopamine transporter (DAT) of mice, and decreased SERT density in rats in the nucleus accumbens shell after repeated intermitted MDMA administration (3x5mg/kg). In terms of behaviour rats, but not mice, showed reduced anxiety in the open field test (Kindlundh-Högberg et al*.,* 2007). However, there is no difference in measures like body temperature and activity (Green et al*.,* 2003). These differential effects of MDMA on brain neurotransmission could also influence the outcome of co-administration of cannabinoid agents in these species.

Although memory performance is widely investigated as a primary measure of cognitive capacity in human polydrug users, there is only one animal study systematically examining the effect of acute co-administration of MDMA and Δ9-THC on mnemonic function (Young et al*.,* 2005). Low and moderate dosages (0.25mg/kg Δ9-THC plus 1.25mg/kg MDMA, 0.5mg/kg Δ9-THC plus 2.5mg/kg MDMA, respectively) tested in rats disrupted working memory in a double Y-maze task, even though the low doses had no effect when administered alone. Another study investigated cannabinoid influence on object recognition task performance during withdrawal after repeated treatment with MDMA (10mg/kg; daily for seven days) (Nawata et al*.,* 2010). It was found that MDMA-induced memory impairment was prevented by co-administration of AM251, as well as by single pre-treatment with the CB1 receptor antagonist on the 7th day of withdrawal. Furthermore, MDMA- treated CB1 knock-out mice did not show impairments in the object recognition task. These two studies point to an interaction of MDMA- induced neurotransmission and CB1 receptor changes in the hippocampus. However, they contradict the above mentioned human study (Dumont et al*.,* 2010) finding more pronounced acute impairments of memory due to Δ9-THC, regardless of co-consumption of MDMA.

An interaction between the presumably neurotoxic effects of MDMA and neuroprotective potential of cannabinoid agents in rats has been demonstrated by Morley et al., 2004, who showed acute reversion of MDMA-induced hyperthermia to hypothermia and a potential decrease of MDMA-induced hyperactivity by co-administration of Δ9-THC or the synthetic cannabinoid receptor agonist CP55,940. Weeks after the treatment, a high-performance liquid chromatography (HPLC) analysis showed that both Δ9-THC as well as CP55,940 partially prevented the MDMA-induced depletion of 5-HT and its metabolite 5-HIAA in some brain regions, speculatively by counteracting oxidative

stress reactions. Co-administration of the CB1 antagonist SR 141716A, did not have a modulator effect (Morley et al., 2004).

The majority of studies described so far employ a study design adequate to investigate acute effects, either of the combination administered simultaneously via injection or infusion, or effects of MDMA after pre-treatment with a cannabinoid agent. However, since human studies are usually based on retrospective accounts of long-term, chronic co-users, it is worthwhile to take a look at animal models employing chronic treatment. There are some studies reporting the effects, both behavioral as well as neurological, of prolonged administration of either substance alone and with varying treatment regimes (e.g., (Rubino and Parolaro, 2008; Meyer et al., 2008; Wegener and Koch, 2009b; Piper et al., 2010)). However, as to current knowledge, there is no realistic chronic treatment model co-administering MDMA+cannabis or cannabinoid agents to investigate cognitive deficits and underlying causes.

Future research questions

Future research could address the following points:

1. In human studies, special care should be taken to rule out confounding drug-use other than MDMA and/or cannabis. Research focusing on the acute effects of co-consumption would yield insight into immediate changes and possibly endangering impairments. Furthermore, more research examining neurological effects of chronic co-consumption on prefrontal and hippocampal brain areas via in vivo imaging techniques would be desirable.

2. Behavioural animal studies should focus on long-term and moderate to heavy co-administration, and examine a) cognitive deficits via paradigms testing memory, impulsivity and decision making, in order to clarify contradictory results from retrospective human studies and b) neurocellular changes, especially in regions implicated in the respective cognitive functions, focusing on the interaction of serotonin, dopamine and the endocannabinoid system.

Summing up, the endocannabinoid system, and especially the cannabinoid receptor type 1, has been shown to mediate several effects of psychostimulant drugs of abuse. Animal experiments provide substantial evidence for modulator or interactive effects of acute co-administration of MDMA and cannabinoid agents both in behavioral paradigms like conditioned place preference and body temperature as well as in studies examining neurobiological/-transmitter changes. Chronic models of co-administration are sparse.

Conclusion

There is some research on the physiological processes involved in simultaneous use of MDMA and cannabis regarding acute interaction, but little consensus exists about impairments of higher cognitive functions. The contribution of each substance, and their combination, to deficits in memory, mood, impulsivity and psychopathologies in human polydrug users is unclear. Studies reporting on only MDMA or only cannabis use might focus on drug-specific neuropsychological tests and therefore disregard the potential effect of the combination of these drugs. Furthermore, especially when it comes to measuring the long-term effects of any drug, it is nearly impossible to rule out confounding variables in humans, e.g., purity of ingested substances, or previously existing cognitive impairments. Animal studies, on the other hand, not only allow for exact monitoring of administration (including modeling polydrug use), but also for equalizing external influences. Current literature does express concerns about the differences in frequency of dosing, route of administration and dosage exposure between rodent models and human patterns of use (Green et al., 2009). Naturally, animal models of consumption and behavioral as well as neurobiological changes can only provide indications which have to be confirmed on retrospective human studies. But retrospective user reports and cognitive test results are usually based on long-term co-consumption patterns. However, as of current knowledge, no chronic intermittent animal models of the combination MDMA+cannabis in animals exist. Especially changes in higher cognitive functions as a consequence of dual intake of these substances, and the possible underlying cellular mechanisms have not been investigated in suitable long-term animal studies.

Acute Co-administration of the Cannabinoid Receptor Agonist WIN55-212,2 does not influence 3,4-methylenedioxymetamphetamine (MDMA)-induced Effects on Effort-based Decision making, Locomotion, Food intake and Body temperature

Sybille Schulz, Jannis Gundelach, Heta K. Svärd, Linda Hayn, Michael Koch

Abstract

3,4-methylenedioxymethamphetamine (MDMA) and cannabis are illegal drugs that are frequently co-consumed in western societies. This study investigated the acute behavioural effects of co-administration of MDMA and the synthetic cannabinoid receptor agonist WIN55,212-2 (WIN) in rats. Four treatment groups were tested: MDMA (7.5.mg/kg, s.c.), WIN (1.2mg/kg, i.p.), combined administration (MDMA+WIN), and vehicle control. Following pre-training, animals were tested in a T-maze effort-based decision making task. Body temperature was measured at three time points. Separated by one week wash-out periods, rats were tested for locomotor and exploratory activity in an open field, as well as food intake under the same between subjects treatment schedule. Acute MDMA administration impaired effort-based choice behaviour, indicating immediate effects of consumption on decision making ability. MDMA and MDMA+WIN treatment increased body temperature to a similar extent, whereas WIN alone increased temperature at an earlier time point. Locomotor activity and exploratory behaviour, as well as food intake was similar (and significantly different from vehicle controls) in MDMA and MDMA+WIN-treated animals. MDMA-induced decreased exploration anxiety behaviour in the open field was diminished by WIN, whereas WIN alone had no effect. In summary, acute co-administration of a cannabinoid receptor agonist did not substantially modulate MDMA-induced behavioural effects in rats.

Introduction

Polydrug use has become a challenging issue in research involving human drug users (EMCDDA, 2010). For example, most 3,4-methylenedioxymetamphetamine (MDMA) users are polydrug users (Danielsson et al., 2011). Cannabis is the most frequently taken illegal co-drug: 98% of a representative sample of MDMA users concomitantly use cannabis (Wu et al., 2009; Parrott et al., 2007). Combined effects on physiological, behavioural and cognitive measures are difficult to disentangle and may depend on frequency, duration, and amount of co-consumption. In addition, many human studies involve chronic and/or heavy polydrug users, thus causal relationships between single drugs and their effects are hard to discern. Many MDMA users report smoking cannabis concomitantly to enhance positive sensations (for example, euphoria, empathy, prosocial behaviour, energy) or some time after MDMA consumption to alleviate adverse effects (anxiety, depression, anhedonia and agitation) of the "come-down" (Parrott et al., 2004; Boys et al., 2001).

Acute MDMA administration leads to a transient, dose-dependent release and reuptake inhibition of the neurotransmitters serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) and to a lesser extent noradrenaline (NA) and acetylcholine (ACh) (Cole and Sumnall, 2003), particularly in the medial prefrontal cortex (mPFC), striatum and hippocampus (Green et al., 2003; Mechan et al., 2002). Increased 5-HT transmission contributes to the behavioural effects of MDMA in a complex way which additionally seems to depend on the interaction with DA transmission (Bankson and Cunningham, 2001). In accordance with reported effects in human users, acute effects of MDMA-administration in animals include hyperthermia (Docherty and Green, 2010), hyperlocomotion (Morley et al., 2004), anxiety (Cole and Sumnall, 2003), and hypophagia (Frith et al., 1987; DeSouza et al., 1997). Few studies have tested the acute effects on more complex behaviour. A decrease in impulsivity in humans (Vollenweider et al., 1998) as well as a dose-dependent increase of lever pressings for reinforcement in animals (Byrne et al., 2000) was shown after MDMA administration.

In contrast, the main psychoactive compound of cannabis, delta9-tetrahydrocannabinol (delta9-THC, THC) acts as an inhibitory transmitter on presynaptic cannabinoid type 1 (CB1) and type 2 (CB2) receptors within the endocannabinoid system. Activation of CB1 receptors seems to modulate neurotransmission (Wilson and Nicoll, 2002). In the CNS, a high abundance of CB1 receptors has been found in basal ganglia, cerebellum, olfactory bulb and hippocampus, moderate density appears in cortical areas (Herkenham et al., 1991). Results from acute studies in animals employing THC, or one of the more potent synthetic CB1 receptor agonists (e.g., WIN55-212,2, CP55,940) or antagonists (e.g., SR171416) support reports obtained from human studies in that cannabinoids are implicated in the acute regulation of several physiological processes. For example, acute consumption of THC increases heart rate and, impulsive responding on certain tasks, decreases postural stability and alertness and influences time-estimations in humans

(McDonald et al., 2003; Zuurman et al., 2009). In animals, activation of CB receptors increases food intake and heart rate, dose-dependently affects locomotor behaviour and decreases body temperature (Elphick and Egertova, 2001; Ameri, 1999; Iversen, 2003).

Behavioural and neurobiological effects of sole administration of MDMA or cannabis are well documented (for review see (Green et al., 2003; Morton, 2005; Howlett et al., 2004)) and some studies examine the long-term cognitive consequences of co-consumption of these drugs (Croft et al., 2001; Rodgers et al., 2001; Daumann et al., 2004). Literature on the consequences of acute co-consumption is less thorough. One human study demonstrated mixed effects: co-administration of cannabis prolonged the onset and duration of MDMA-induced increases in temperature, but had no additive influence on deficits in memory. THC induced psychomotor impairments independently of MDMA (Dumont et al., 2009). Positive subjective ratings were increased for the combination of the drugs compared to each drug effect alone (Dumont et al., 2010). Some evidence from rodent studies shows regulatory effects of co-administration on locomotion, body temperature, and reinforcing effects. For example, prevention of MDMA-induced hyperthermia, anxiety, and a decrease of MDMA-induced hyperactivity due to of THC was observed in rats (Morley et al., 2004). Impairment of working memory upon co-administration of low and medium doses of MDMA plus THC was shown (Young et al., 2005). In terms of reinforcing properties, intracerebroventricular (i.c.v.) self-administration studies demonstrate a modulation of MDMA-induced reinforcing effects (self-administration or conditioned place preference (CPP)) by cannabinoid agents (Sala and Braida, 2005; Braida and Sala, 2002). In mice, exposure to WIN in adolescence later facilitated MDMA-induced CPP (Rodriguez-Arias et al., 2010) or potentiated the rewarding effects of MDMA (Manzanedo et al., 2010). In the latter study, the CB1 antagonist SR171416 did not block the effects of WIN. Furthermore, in CB1-knock-out mice, pre-treatment with THC still prevented MDMA-induced acute responses (Tourino et al., 2008), pointing towards a CB1-independent mechanism of interaction. The effects of acute co-administration on cognitive tasks, for example choice behaviour, have not been investigated.

This study aimed to further elucidate the interactive effects seen after acute co-administration of MDMA and Cannabis. The synthetic specific CB receptor agonist WIN55,212-2 (WIN) was used to investigate interactions of MDMA and CB receptor stimulation, aiming to narrow the range of effects of THC in the central nervous system. For the first time, the effect of acute MDMA administration as well as the combined administration of MDMA and a cannabinoid agonist on effort-based choice behaviour is investigated, in addition to locomotion, food intake and body temperature measures.

Materials & Methods

Animals

In total, 39 adult naive male Wistar rats (Harlan, Borchen, Germany) weighing 230-300 grams were used in these experiments. Upon arrival, animals were allowed to habituate for 4-5 days in a vivarium under standardized conditions (4-6 animals per Makrolon type IV cage; tap water *ad libitum*; 12 hour light/dark circle, lights on at 7am; temperature 22 +/-2°C) and were handled regularly. During the habituation and handling period, standard lab chow was available *ad libitum*. Controlled feeding (12g/animal/day) started two days before the first training session. All animal experiments were conducted in accordance with the principles of animal care and the international laws on animal experiments (Directive 2010/63/EU) and were approved by the local authorities.

Drugs

All drugs were prepared freshly before administration and were injected in a volume of 1ml/kg. WIN55,212-2 (SIGMA-Aldrich, Steinheim, Germany) was dissolved in 2% Tween®80 (Serva, Heidelberg, Germany) and 98% NaCl solution (0.9% NaCl, Fresenius Kabi GmbH, Bad Homburg, Germany) and injected at a dose of 1.2mg/kg, intraperitoneally (i.p.). MDMA hydrochloride (synthesized in the Institute of Inorganic Chemistry, Prof. Nagel, University of Tübingen. Identity and chemical purity was verified) was dissolved in phosphate buffered saline (PBS), stabilizing a well-tolerated pH-value of the solution, and injected at a dose of 7.5mg/kg, subcutaneously (s.c.). PBS injection served as vehicle control. Single doses which have been shown to be behaviourally relevant (Young et al., 2005; Drews et al., 2005) were chosen in order to minimize the number of animals used in this study.

Behavioural tests

Behavioural testing was conducted in a between subjects design. Each treatment group consisted of 10 rats, except the combination (MDMA+WIN) group (n=9). Each animal underwent the same training and testing procedures. However, the sequence of tests was altered in order to minimize the influence of repeated substance administration and previous behavioural tasks. All animals started with training and subsequent testing in the effort-based decision making task. Half of each treatment group was tested in an open field one week later, followed by a food preference test another week later. The second half of the group underwent the food preference task first, followed by the open field tests. After each test, a wash-out period of seven days was allowed for all animals. Contrary to other reports (Chaperon and Thiebot, 1999), there were no observable

adverse effects upon first, or subsequent, exposure to the cannabinoid agonist. MDMA was administered 30 minutes and WIN 10 minutes prior to testing, thereby allowing examination of behaviour during the peak time of effect. The combined treatment was timed in such a way that the peak time of the effects accumulated at the time of testing. Half of the control (vehicle) group (n=5) was injected 10 minutes, the other half (n=5) 30 minutes prior to testing.

Effort-based decision making

The effects of MDMA, WIN, and the combination of both drugs on effort-based decision making were tested in a T-maze paradigm. This task allows monitoring of cost-benefit choice behaviour, i.e., how much effort the animal is willing to exert to obtain a (larger) reward. At the end of the reward arms of the T-maze (measurements of each arm: 60 cm x 15 cm x 30 cm (L x W x H)) either two or four pellets (Bio-Serv©, UK Dustless Precision Pellets©, 45mg) were placed in a metal food well. While the arm containing two pellets (low reward arm, LR) was freely accessible for the rats, a 30 cm barrier made of wire mesh was placed in the arm containing four pellets (high reward arm, HR). The HR was the right side T-maze arm for half of the animals, and left side for the other half. All animals were habituated to the apparatus as well as to increasing heights of the barrier and pre-trained until as a group they reached baseline level of ≥ 80% choice of HR for three consecutive days. Intertrial interval (ITI) was 1 min. Habituation and pre-training sessions took place once a day for an average of 16 days (for further detail on apparatus and training method see (Walton et al., 2002), adapted from the original study by Salamone (1994)). For the test the animals received drugs or vehicle as described above and performed two forced choice runs (one to each arm, in pseudo-randomized order), prior to ten free choice runs. The percentage of choices for the HR arm was calculated for each treatment group.

Body temperature

Temperature was measured with an in-ear thermometer (Thermoscan, IRT3020 CO, Braun, Switzerland) at three different time points: A baseline measurement one day before the first testing in the effort-based task was done in order to rule out effects of the injection procedure. A second measurement was done before the animals were tested (i.e., at the respective peak times of effects of the substances, T1), and a third time 1 hour after the test (T2). At each time point, temperature was measured three consecutive times, and the mean of these three measures was considered the temperature value for that time point.

Locomotor activity

Animals were placed in infrared beam-controlled acrylic glass chambers (ActiMot-system; TSE, Bad Homburg, size: 44.7 cm x 44.7 cm x 44 cm) measuring horizontal and vertical locomotion. Locomotion and exploratory behaviour was automatically recorded by a PC (ActiMot Software;

TSE, Bad Homburg) for 35 minutes and stored as aggregated data in seven intervals. Parameters analysed were number of rearings, total activity (%), and time spent in centre (%) per 5-minute interval.

Food preference test

Animals were placed in a standard Makrolon type II cage with two glass food wells each containing pellets (Bio-Serv©, UK Dustless Precision Pellets©, 45mg) or breeding chow (Altromin, Lage, Germany). Breeding chow and pellets only differed in palatability, not in protein (22.5%, 18.7%, respectively) or fat (5%, 5.6%, respectively) content. Animals were allowed to free-feed for 10 minutes. Animals had not eaten for 20 (+/-2) hours when testing was conducted. Mass of eaten food was weighed for each animal.

Data analysis

For statistical analysis, analyses of variance (ANOVAs) were conducted with SigmaStat2.03 for Windows (SPSS Inc., Chicago, IL, USA), followed by post hoc Tukey tests for pairwise multiple comparisons. For all measurements, $p<0.05$ was considered a significant difference.

Results

Effort-based decision making

One animal from the MDMA+WIN group was unable to complete the task and therefore was excluded, thus yielding n=10 for MDMA, WIN and vehicle groups, and n=8 for the MDMA+WIN group for the statistical analysis. MDMA- and MDMA+WIN-treated animals chose the high reward arm (HR) less often compared to the WIN and vehicle groups (51% and 57% vs. 82% and 95%, respectively, Fig.1). A one-way ANOVA yielded a significant difference of HR choices between treatment groups [$F(3,34)= 3.50$, $p=0.026$]. The post-hoc test revealed a significant difference between the vehicle and MDMA group (mean HR choice= 95%, 51%, respectively, $p=0.034$). While the combined treatment (MDMA+WIN) reduced choices for the HR, this effect failed to reach significance ($p=0.117$).

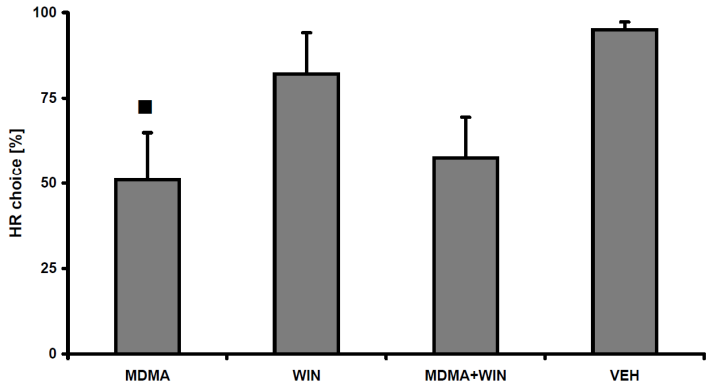

Fig.1: Effects of acute MDMA (n=10), WIN (n=10), MDMA+WIN (n=8) and vehicle (n=10) administration on effort-based decision making. Bars depict the percentage means (+SEM) of HR choices (large reward obtained by climbing the 30cm barrier) during the behavioral test sessions. Closed square denotes $p<0.05$ compared to vehicle.

Body temperature

One hour after behavioural testing (T2), body temperature was increased in rats treated with MDMA (mean: 37.4°C) and MDMA+WIN (mean: 37.5°C) compared to baseline (BL) and to measurements 30 minutes after administration (T1). WIN-treated animals showed an increase in temperature 10 minutes after administration (37.3°C, BL: 36.6.°C; Fig.2). A two-way ANOVA yielded a significant effect of time point of measurement [$F(2,44)=15.41$, $p<0.001$] as well as a significant interaction of time point x substance ($F(6,44)= 3.40$, $p=0.008$]. Post-hoc test revealed significant differences comparing treatment groups MDMA+WIN vs. WIN measured before the first run (T1) ($p=0.007$). Furthermore, significant differences were found within MDMA and MDMA+WIN groups between measurements taken one hour after behavioural testing (T2) and BL ($p=0.001$ and $p<0.001$) as well as between T1 and T2 ($p=0.017$ and $p<0.001$). Within the WIN group, the difference between T1 and BL was significant ($p<0.001$).

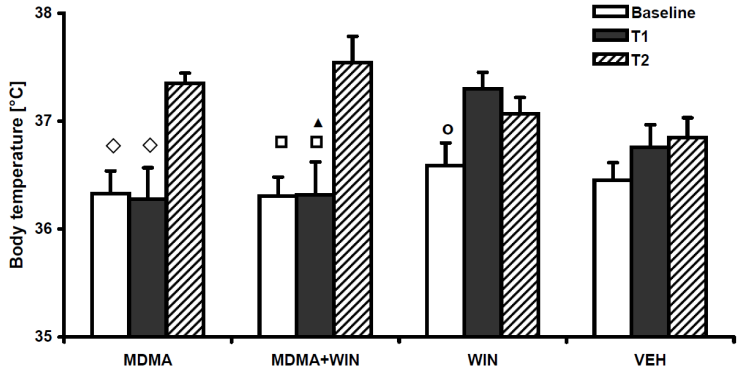

Fig.2: Effect of administration of MDMA (n=10), WIN (n=10), MDMA+WIN (n=9) and vehicle (n=10) on body temperature (°C). Data are means (+SEM) of three measurements for each animal at each time point. Closed triangle denotes significant difference ($p<0.05$) compared to WIN at T1; open symbols represent differences within the same treatment group compared to T1 (circle) or T2 (rhombus and square) ($p<0.001$).

Locomotor activity and exploratory behaviour

MDMA and MDMA+WIN-treated animals showed an increase in activity (mean: 63% and 61%, respectively) as well as in number of rearings (mean: 53 and 43, respectively), compared to the WIN (20% and 12) or vehicle (20% and 18) group (Fig.3). In addition, MDMA-treated rats spent more time in the centre of the open field than the remaining three treatment groups (mean: 15% vs. 8% (MDMA+WIN), 4% (WIN) and 6% (vehicle)). For locomotor activity, a two-way ANOVA yielded significant effects of treatment group [$F(3,244)= 310.74$, $p<0.001$], time interval [$F(6,244)= 27.46$, $p<0.001$], and interaction of substance x time interval [$F(18,244)= 2.18$, $p=0.004$]. Post-hoc test revealed significant differences between MDMA and MDMA+WIN groups compared to WIN- and vehicle-treated groups ($p<0.001$) over all 35 minutes, as well as within each 5-minute time interval ($p<0.001$). WIN- and vehicle-treated animals significantly reduced their activity between the first and all subsequent intervals ($p<0.001$), as well as between the second and the second last (WIN, $p=0.05$) or the third last (vehicle, $p=0.011$) intervals.

A two-way ANOVA comparing the number of rearings yielded significant effects for substance [$F(3,244)= 73.56$, $p<0.001$] and time interval [$F(6,244)= 3.24$, $p=0.004$]. Post-hoc test revealed significantly increased number of rearings for the MDMA group compared to the other three treatment groups ($p<0.001$ (WIN and vehicle), $p=0.021$ (MDMA+WIN)), as well as for the MDMA+WIN group compared to the WIN and vehicle groups ($p<0.001$).

In terms of differences within time intervals, MDMA-treated animals reared significantly more often than WIN- and vehicle-treated groups in all but the very first time interval ($p<0.001$ to $p=0.006$). MDMA+WIN-treated animals reared significantly more often than both WIN- and vehicle-treated animals in the last three intervals ($p<0.001$) and the third interval ($p=0.029$; $p=0.044$, respectively),

as well as compared to WIN during the second and fourth interval ($p=0.003$ and $p=0.042$, respectively). Vehicle-treated animals showed a reduction in the number of measured rearings between the first and third to seventh time interval ($p<0.001$ to $p=0.025$) (For significant differences ($p<0.001$) within the time intervals, see Fig.3). A two-way ANOVA analysing the time spent in the centre of the open field yielded a significant effect for substance [$F(3,244)=17.88$, $p<0.001$]. Post-hoc test confirmed that MDMA-treated animals spent significantly more time in the centre compared to all three remaining treatment groups ($p<0.001$).

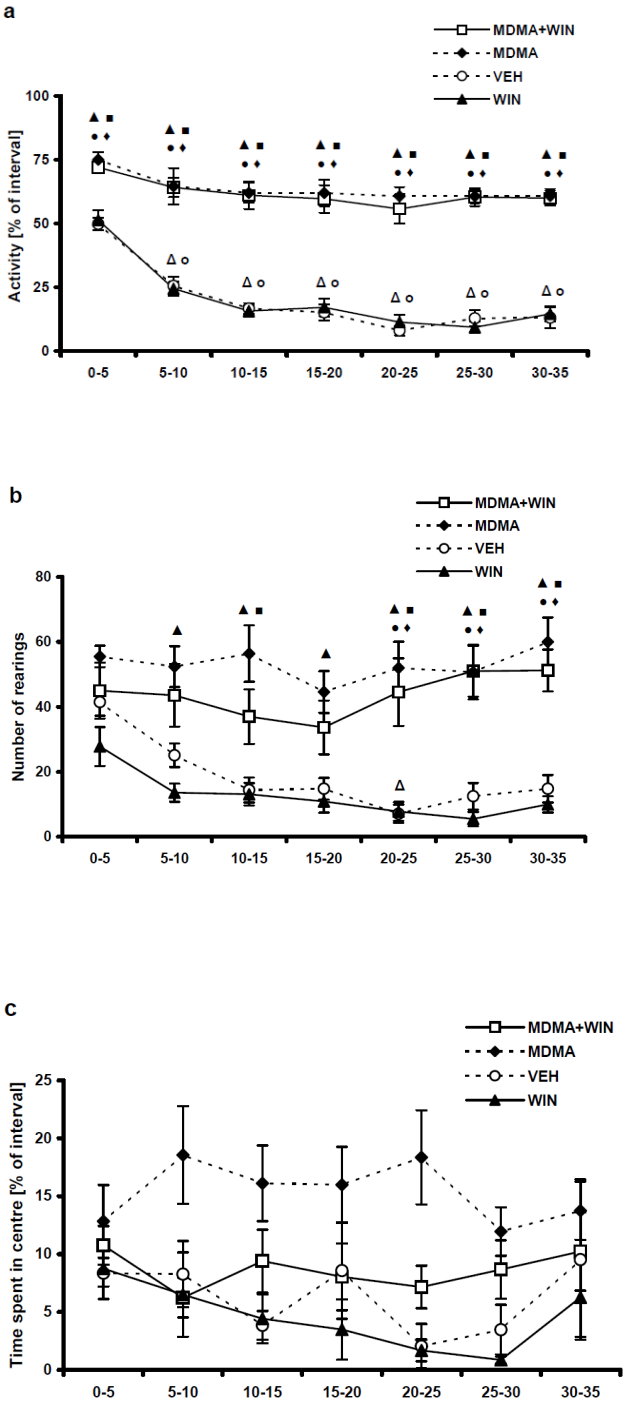

Fig.3: Effects of MDMA, WIN, MDMA+WIN and vehicle (n=10;10;9;10, respectively) on locomotion (a), exploratory (b) and anxiety-like (c) behaviour. (a) and (c) are depicted as % of time of 5-minute intervals for a total of 35 minutes, (b) as absolute number of rearings per interval. Significant differences of $p<0.001$ are depicted only. Open triangles and open circles indicate differences between the first and the denoted time interval within vehicle and WIN groups, respectively. Closed triangles and squares denote differences between MDMA vs. WIN and vehicle groups, respectively. Closed circles and rhombuses indicate differences between MDMA+WIN and WIN and vehicle groups, respectively.

Food preference test

All animals consumed more pellets than breeding chow. When comparing the amount of pellets consumed, MDMA- and MDMA+WIN-treated animals ate less than the vehicle and WIN-treated groups (mean: 4.5 and 2.8 grams vs. 6.0 and 6.8 grams, respectively) (Fig.4). A two-way ANOVA yielded significant effects for type of food [$F(1,70)=167.66$, $p<0.001$], substance [$F(3,70)=6.14$), $p<0.001$], and the interaction substance x type of food [$F(3,70)= 4.88$, $p=0,004$]. Post-hoc tests revealed that all animals preferred pellets over breeding chow ($p<0.001$). Rats from the vehicle group consumed significantly more pellets than the MDMA ($p=0.017$) and MDMA+WIN group ($p<0.001$). WIN-treated animals consumed significantly more pellets than the MDMA+WIN group ($p<0.001$). When comparing total amount of food intake, vehicle and WIN-treated groups ate significantly more than MDMA+WIN-treated animals ($p=0.004$ and $p=0.002$, respectively). MDMA+WIN-treated animals consumed less food than MDMA-treated animals (2.8grams vs. 4.7 grams), however this difference was not statistically significant.

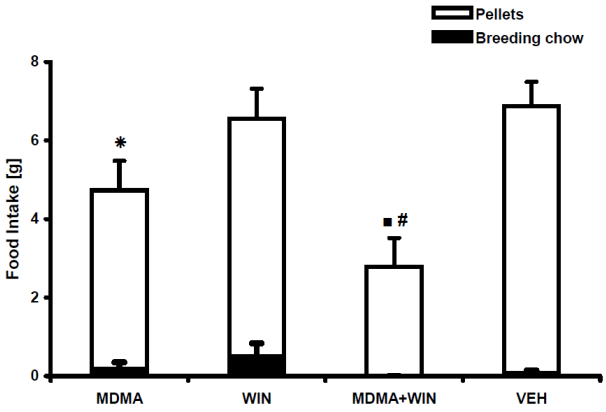

Fig.4: Effects of acute administration of MDMA, WIN, MDMA+WIN and vehicle (n=10;10;9;10, respectively) on food preference and intake. Closed square shows significant difference ($p<0.005$) in total food intake compared to vehicle and WIN groups; hash symbol denotes difference ($p<0.001$) in amount of pellets consumed compared to vehicle and WIN groups; asterisk shows difference ($p<0.05$) in amount of pellets consumed compared to vehicle group.

Discussion

Various studies suggest an interaction of MDMA influence on different neurotransmitters and the inhibitory effects of CB receptors on transmitter release in a range of brain regions. For example, the synthetic CB1 receptor antagonist SR141617A increased serotonergic and dopaminergic neurotransmission, especially in the mPFC (Darmani et al., 2003), whereas THC decreases 5-HT neurotransmission in the nucleus accumbens (Sano et al., 2008; Lopez-Moreno et al., 2008).

Furthermore, CB1 receptors are expressed on 5-HT and DA neurons not only presynaptically, but also on dendrites, and may interfere with the serotonin transporter (SERT) and dopamine transporter (DAT) (Lau and Schloss, 2008). The abundant and overlapping distribution of CB1 receptors may indirectly alter activity of dopaminergic and serotonergic neurons by influencing GABAergic inhibition of DA neurons (Pistis et al., 2001). An effect of WIN on glutamatergic (Shen et al., 1996) and cholinergic (Gessa et al., 1998) synapses has been shown as well, offering further sites for interaction. Therefore, the DA and 5-HT release and reuptake inhibition properties of MDMA would hereby interact with the inhibitory effects of WIN via the CB1 receptor and result in opposing, additive or regulative effects in motivation and effort, as well as temperature regulation and locomotor behaviour.

Effort based decision making

MDMA impaired choice behaviour based on effort irrespective of co-administration of WIN. As far as to current knowledge, no previous study has investigated the effect of acute MDMA administration on effort-based choice behaviour. Exact mechanisms on how MDMA-induced alterations in 5-HT and DA release could influence the fronto-striatal circuitry (Floresco et al., 2008) or DA release in the nucleus accumbens (Assadi et al., 2009) responsible for regulating effort-based choice remain speculative. The impact of central 5-HT release on general aspects of behaviour, e.g. motivation, may be important here. Furthermore, acute MDMA administration did not impair locomotion, but decreased food intake (see below). Therefore, increased 5-HT release might have decreased appetite and thus, motivation for climbing the barrier to obtain the high reward. Whatever the underlying causes, our data indicates that acute MDMA effects do not only include physiological responses like hyperthermia or increased activity, but may also immediately impair cognitive functions like decision making.

Although only MDMA-alone significantly differed from the control group, responses of the combined treatment group closely paralleled those of MDMA-treated animals. The lack of a significant effect is probably due to side effects observed in the MDMA+WIN-treated animals such as increased head waving, defecation, salivation and overall slow responses and movements. One MDMA+WIN-treated animal was excluded from the analysis due to inability to move further than the decision point of the T-maze. These impairments may have been adverse effects upon the first simultaneous administration of MDMA and WIN, as this behavioural pattern was not, or to a lesser extent, observed in subsequent experiments. Young et al. (2005) reported similar behavioural impairments upon combined administration of THC (1mg/kg) and MDMA (5mg/kg) in a within subjects design. As in our study, no major adverse effects of administration of either MDMA or the cannabinoid agonist alone were evident, thus observed impairments seem to be due to the combination, not the single doses, of the substances. Overall, and especially taking into account the observed side effects of co-administration, it is not possible to draw firm conclusions about the

influence of the combined consumption on effort-based decision making based on these results. However, MDMA-administration seems to have an effect on effort-based choice behaviour with or without WIN co-administration.

Our results show no effect of WIN (1.2mg/kg) on effort-based decision making in rats. This contrasts with an earlier study employing operant chambers showing that acute treatment with 1.2mg/kg or 1.8mg/kg WIN significantly reduced the number of lever presses for pellets ("break point") in a progressive-ratio task compared to a lower dose of WIN (0.6mg/kg) or vehicle treatment. Moreover, a significant decrease in the total number of lever presses was detected, indicating a complex role of cannabinoids in reward-related behaviour (Drews et al*.,* 2005). However, although both paradigms aim at investigating the influence of CB1 activation on effort-based reward obtainment, there are of course differences between T-maze- and instrumental tasks, which preclude a direct comparison. However, our data indicate no influence of CB1 receptor activation on this behavioural paradigm.

Body temperature

The present results support previous studies demonstrating an increase in body temperature upon consumption of MDMA in humans (Freedman et al*.,* 2005; Mohamed et al*.,* 2011) and rats (Green et al*.,* 2003). The peak temperature measured 1 hour after behavioural testing contrasts with previous findings showing significant increases compared to baseline levels at 20-30 minutes after administration of MDMA (12.5mg/kg, i.p.) (Mechan et al*.,* 2002). However, depending on the route of administration (s.c. or i.p. injection) hyperthermic effects can vary in time due to different absorption and metabolizing rates. Furthermore, according to Green at al. (2003) peak temperatures are also observed 40-60 minutes after i.p. administration.

Co-administration of WIN did not influence MDMA-induced hyperthermia. Significantly higher temperature compared to both baseline and pre-test was measured 1 hour after behavioural testing for the MDMA+WIN group. In fact, temperature changes were akin to those seen in the MDMA group. These results are in contrast with a finding by Morley (2004), showing that THC and CP55,940 prevent MDMA-induced hyperthermia in rats. However, these cannabinoid agents were administered according to a different injection scheme (4 x 2.5mg/kg). A study in humans demonstrated that THC co-administration does not prevent MDMA-induced temperature increase (Dumont et al*.,* 2009). Our results do not support the modulatory role of acutely administered CB1/2 receptor ligands on MDMA-induced hyperthermia. Rather, a modulation of the WIN-induced rise in temperature by MDMA appears.

WIN (1.2mg/kg) led to a significant increase in body temperature 10 minutes after administration compared to baseline. Earlier studies on cannabinoid effects on body temperature found that low doses of THC (0.05 and 0.1 mg/kg) caused hyperthermia, while doses of 1.0, 2.0 and 5.0mg/kg induced hypothermia (Taylor and Fennessy, 1977). In contrast to our study, cannabinoid receptor

agonists WIN55,212-2 and CP55,940 led to hypothermia, which was reversed by the selective CB1 receptor antagonist SR141716 (Chaperon and Thiebot, 1999). On the other hand, it was recently shown that the endogenous cannabinoid anandamide increases temperature when administered intracerebroventricularly (i.c.v.), an effect which is reduced by co-administration of a CB1 receptor antagonist (Fraga et al., 2009). Furthermore, low doses of THC significantly reduce, but doses of 1.0, 2.0 and 5.0mg/kg increase the levels of 5-HT metabolites in the whole brain (Taylor and Fennessy, 1977). The dose used in the current study may have had an indirect increasing effect on body temperature by elevating 5-HT levels.

Locomotor activity and exploratory behaviour

MDMA as well as MDMA+WIN treatment significantly increased locomotor activity compared to the WIN- as well as vehicle- group (Fig.3a). These effects were stable over the 35 minutes test duration and support other studies demonstrating hyperactivity upon acute MDMA-administration (Spanos and Yamamoto, 1989; Green et al., 2003). Rats treated with 1.2 mg/kg WIN showed activity levels akin to the vehicle group. Co-administration of WIN therefore does not have an attenuating effect on MDMA-induced increases in locomotor activity.

Compared to MDMA-treated animals, MDMA+WIN administration led to a significant overall reduction in exploratory behaviour (number of rearings) over the 35 minute measurement (Fig.3b). No habituation was observed over time. However, there was no difference when comparing any of the individual time intervals. Therefore, co-administration of WIN seems to have a small, if any, modulating effect on MDMA-induced exploratory behaviour. In contrast to these results, rodent studies administering THC and MDMA found that the cannabinoid had an attenuating effect on MDMA-induced hyperactivity in rats (Morley et al., 2004) and mice (Tourino et al., 2008). The discrepancy between the previously described and our results may be due to the different test paradigms, dose-dependent biphasic effects of cannabinoids (Chaperon and Thiebot, 1999), and a more unspecific effect of THC compared to WIN.

In terms of the time spent in the centre of the open field, a measure for exploration anxiety, MDMA+WIN-, WIN- and vehicle-treated animals spent significantly less time in the centre than MDMA-treated animals (see Fig.3c). MDMA seems to have an anxiolytic effect. In contrast, increased anxiety levels were found in MDMA-only treated rats (Morley and McGregor, 2000) and mice (Ferraz-de-Paula et al., 2011) on various anxiety-related measures. However, this result is congruent with Morley et al. (2005) demonstrating decreases of MDMA-induced anxiety by THC, measured in an emergence test. The attenuating effect of WIN on MDMA-induced decreased exploration anxiety is not due to differences in locomotion as the MDMA+WIN group displayed equal levels of hyperactivity to the MDMA group whilst not differing from vehicle group in the anxiety measure. Future studies seeking to elucidate the influence of CB1 agonism on (MDMA-induced) anxiety should employ a more direct measure as well as various CB agents.

Rats treated with 1.2mg/kg WIN showed activity, vertical exploratory behaviour as well as exploration anxiety levels akin to the vehicle group. Furthermore, there were no differences in habituation, i.e. a reduction of activity over the measured intervals within the 35 minutes occurred in WIN- and vehicle-treated animals equally. As noted previously, CB1 agonists may have biphasic effects according to dose, inducing hyperactivity at low doses and severe motor deficits at larger doses (Chaperon and Thiebot, 1999). In line with this, locomotor activity in the open field has been reported to be increased by 0.6mg/kg, but not by higher doses of WIN (Drews et al*.,* 2005). A dose of 1mg/kg does not affect either ambulation or the frequency of rearings, while higher doses (3 or 5.6mg/kg) reduce both measures (Jarbe et al*.,* 2006). Various reports point to involvement of CB1 antagonism (for example, by SR141716), but not agonism, in anxiogenic effects (Moreira et al*.,* 2009; Rodgers et al*.,* 2005; Patel et al*.,* 2005; Haller et al*.,* 2004). In the current experiment, WIN-only treatment did not affect any of the measures.

Food preference

MDMA reduced intake of pellets, which is congruent with previous studies demonstrating that MDMA consumption reduces food intake and appetite in humans (Vollenweider et al*.,* 1998; Kirkpatrick et al*.,* 2011) and animals (Frith et al*.,* 1987; Jean et al*.,* 2007). Co-administration of WIN does not seem to have an effect on MDMA-induced hypophagia, as MDMA+WIN treated animals consumed even less than the MDMA group (means of total food intake: 2.7 vs. 4.5 grams), but this difference was not statistically significant. Food intake is a process mediated by stimulation of 5-HT receptors (Lam et al*.,* 2010; Merroun et al*.,* 2009). Although i.c.v. administration of WIN has been shown to decrease extracellular 5-HT and 5-HIAA in hypothalamic brain areas (Merroun et al*.,* 2009), this effect seems to be overruled by the strong MDMA-induced increase of 5-HT release and the associated reduction in food intake (Lam et al*.,* 2010).

WIN-treated animals did not differ from the vehicle-treated group in terms of food consumption. This result was somewhat unexpected since previous studies demonstrated an increase in food intake following THC or WIN administration compared to vehicle groups (Kirkham, 2005). For example, i.p. administration of WIN at doses of 0.5, 1 and 2mg/kg caused a significant increase in food intake from 1 hour - 6 hours after injection (Merroun et al*.,* 2009). However, Merroun et al. (2009) did not find significant differences when comparing the non-cumulative amounts of food intake between vehicle- and WIN- (1 or 2mg/kg) treated animals. As with activity levels and body temperature, activation of CB1/2 receptors tends to evoke dose-dependent biphasic responses. WIN at doses of 1 and 2mg/kg promotes hyperphagia, whereas administration of a higher dose (5mg/kg) significantly inhibited food intake in partially satiated rats (Merroun et al*.,* 2009). Drews et al. (2005) even found a significant reduction in the amount of pellets consumed by animals treated with 1.8mg/kg WIN. If orexigenic effects of the CB receptor agonist appear subsequent to maximal

blood concentration levels, hyperphagia may have been evoked only partially in the current study as testing took place 10 minutes after administration.

Conclusion

MDMA led to decreased choices of the HR choice in an effort-based decision making task. Furthermore, previously well-documented increases in activity and body temperature as well as decreased food intake were replicated. Overall, our behavioural tests do not support a modulatory role of WIN regarding MDMA-induced acute effects. Apart from an augmenting effect on body temperature, WIN administration alone did not yield effects distinct from vehicle treatment. Although there was a wash-out period of seven days between each test, additive or habituation effects cannot be completely ruled out. Future studies could vary the administration schedule and doses. In the current study, we used doses which in other animal studies have been shown to be behaviourally relevant (Drews et al., 2005; Young et al., 2005). However, since the dosage used in these experiments was relatively high, a lower dose of MDMA could reveal a putative potentiating effect of WIN. Many MDMA users consume cannabis concomitantly to enhance positive sensations or some time after MDMA consumption to alleviate adverse effects of the “come-down” (Winstock et al., 2001; Schulz, 2011). The neurobiological mechanisms underlying behavioural effects of MDMA, as well as co-consumption of cannabis, remain somewhat unclear as a complex interplay between 5-HT and DA release as well as activation of different 5-HT subtypes must be considered. Administration of specific CB1 agonists and manipulation of certain 5-HT receptors, and/or verification of DA and 5-HT-transmitter levels in brain areas known to be involved in behavioural responses, could further elucidate underlying pharmacological mechanisms. From these experimental tests in rats, we conclude that acute co-administration of a CB agonist does not substantially attenuate the MDMA-induced behavioural effects.

Chronic co-administration of the cannabinoid receptor agonist WIN55,212-2 during puberty or adulthood reverses 3,4-methylenedioxymetamphetamine (MDMA)-induced deficits in recognition memory but not in effort-based decision making

Sybille Schulz, Thorsten Becker, Ulrich Nagel, Andreas von Ameln-Mayerhofer , Michael Koch

Abstract

Cannabis and 3,4-methylenedioxymetamphetamine (MDMA, “ecstasy”) are the most frequently combined illegal drugs among young adults in western societies. This study examined the effects of chronic co-administration of the cannabinoid receptor agonist WIN55,212-2 (WIN) and MDMA on working memory and effort-based decision making in rats. Treatment consisted of MDMA (7.5mg/kg), WIN (1.2mg/kg), a combination of these substances (MDMA+WIN) or vehicle over a period of 25 days during puberty (PD40-65) or adulthood (PD80-105). Ten days after the last treatment, WIN reversed MDMA-induced working memory deficits in the object recognition test in animals treated during adulthood or puberty, but had no influence on impairment of adult rats in the effort-based T-maze task. No differences were observed between groups of pubertally treated rats in the decision making tasks. During a subsequent acute drug challenge MDMA and MDMA+WIN decreased high reward choices in both age groups, indicating MDMA-induced cost-aversive choice. Differential long-term interactions on the neuronal level in the hippocampus and MDMA-induced disturbances in cortico-limbic connections are suggested.

Introduction

The illicit drug 3,4-methylenedioxymetamphetamine (MDMA, “ecstasy”) is consumed for its stimulant effects including euphoria, increased energy and sensory awareness. MDMA is a potent monoamine releaser which dose-dependently influences the neurotransmitters serotonin (5-HT), dopamine (DA), and noradrenaline (NA) and binds to and reverses the direction of their respective reuptake transporters. Chronic and/or heavy administration has been associated with dysfunction and presumable selective neurotoxicity of serotonergic neurons and depletion of 5-HT levels in a variety of brain regions in animals (Green et al*.,* 2003; Hatzidimitriou et al*.,* 1999; Baumann et al*.,* 2007; Mayerhofer et al*.,* 2001). Decreased densities of cortical serotonin transporter (SERT) were found in current MDMA users, however 5-HT neurotoxicity is debated in humans (Reneman et al*.,* 2001; Thomasius et al*.,* 2006; Cowan, 2007). Recreational use of MDMA has been shown to impair learning and memory, for example, reduced immediate and delayed word recall (Dumont et al., 2010), disturbed verbal and visuo-spatial memory retrieval (Kuypers and Ramaekers, 2007; Murphy et al., 2009) and working memory (Nulsen et al., 2010). In addition, deficits in decision making processes, for example, elevated impulsivity (Morgan et al., 2006) and impaired decision making cognition in a gambling task (Quednow et al., 2007) were found. Especially after chronic and heavy consumption, cognitive impairments are exacerbated (Croft et al., 2001; Gouzoulis-Mayfrank et al., 2003), and still persist even after one year of abstinence (Reneman et al*.,* 2001; Zakzanis and Young, 2001; Gouzoulis-Mayfrank and Daumann, 2006a).

Although studies in laboratory animals confirm acute effects of MDMA administration such as increased locomotor activity (Green et al*.,* 2003; Cole and Sumnall, 2003), body temperature (DeSouza et al., 1997; Docherty and Green, 2010), social interaction (Morley and McGregor, 2000) and appetite suppression (O'Shea et al., 1998), studies of long-term effects after chronic administration in models of cognitive ability, such as memory tasks or more complex decision making are scarce. For example, Meyer et al. (2008) found deficits in an object recognition memory test in adult rats after intermittent exposure during adolescence. Three times 3 or 6mg/kg still impaired object recognition two weeks later (Rodsiri et al., 2011), and one week after a period of self-administration, object recognition memory was impaired, but recovered after 70 drug-free days (Schenk et al., 2011). A common form of decision making are cost-benefit choices, in which an individual needs to evaluate different options (involving costs) in terms of their outcome value (benefits) and weigh them against each other. The medial frontal cortex, specifically the anterior cingulate cortex (ACC), and interactions between prefrontal and sub-cortical brain structures are crucially involved in this form of decision making (Walton et al*.,* 2002; Schweimer and Hauber, 2006; Floresco and Ghods-Sharifi, 2007). However, effects of MDMA on effort-based decision making have not been examined.

The majority of ecstasy users also consume other drugs, and cannabis is the most frequently consumed and combined illegal drug. Approximately 95% of MDMA users report taking cannabis concomitantly (Gouzoulis-Mayfrank and Daumann, 2006b; Wu et al., 2009; Grov et al., 2009), mostly for its relaxant properties, thereby counteracting dysphoric symptoms of the "come down" from MDMA (Boys et al., 2001; Winstock et al., 2001). Delta9-tetrahydrocannabinol (THC), the main psychoactive compound of cannabis, acts on endogenous cannabinoid type1 (CB1) and cannabinoid type2 (CB2) receptors. CB receptors play a role in the regulation of a variety of physiological processes such as body temperature, food intake, heart rate and locomotion (Elphick and Egertova, 2001; Ameri, 1999; Iversen, 2003). Stimulation of CB1 receptors generally inhibits neurotransmitter release. Human studies controlling for polydrug use, either via statistical means or arbitrary cut off criteria for cannabis use, reveal contradictory results. Some conclude that most cognitive and neurological effects cannot be allocated to long-term ecstasy use only, and may be in part due to concomitant consumption of cannabis (Lamers et al., 2006; Croft et al., 2001), or other drugs (Hanson et al., 2008). On the other hand, some studies comparing ecstasy and ecstasy+cannabis users with non-users suggest cognitive deficits to be attributable to ecstasy (Schilt et al., 2008), or found that participants using both drugs perform worse than ecstasy-only users on a range of learning and memory tasks (Fisk et al., 2006). In polydrug users, ecstasy use has been dose-dependently associated with impairments in executive control and decision making functions (Morgan et al., 2006), which is in line with predominantly cortically altered 5-HT transmission, and reported problems with long-term memory (Murphy et al., 2009). Deficits in short-term and prospective memory tasks are more robustly attributed to cannabis consumption (Solowij and Battisti, 2008), which supports the notion of long-lasting effects of stimulation of inhibitory CB1 receptors abundant in the hippocampus (Kano et al., 2009). Research in animals examining co-administration of MDMA and cannabinoid agents debates whether there are additive, interactive or regulatory effects on physiological, behavioural, and neurochemical measures. In rats, acute low and moderate doses of THC and MDMA disrupted performance in a working memory task (Young et al., 2005), whereas the CB1 antagonist AM251 ameliorated deficits in object recognition during withdrawal from MDMA (Nawata et al., 2010). Co-administration of either THC or the CB1 agonist CP55,940 reversed MDMA-induced hyperthermia and hyperactivity and prevented decreases in 5-HT concentration in hippocampus, amygdala, and prefrontal cortex (Morley et al., 2004). In CB1 knock-out mice, acute effects of MDMA (hyperthermia, increased locomotion, anxiety) were lower or absent compared to wild-type animals, indicating CB1 receptor involvement in these basic physiological processes (Tourino et al., 2008). Research examining long-lasting effects on behaviour after chronic co-consumption is scarce (Schulz, 2011). One recent study demonstrated that chronic co-administration of THC (5mg/kg, once a day) during adolescence counteracted MDMA (2 x 10mg/kg, every 5th day)-induced decreases in exploratory behaviour as well as reductions of 5-HT and serotonin transporter (SERT) levels in frontal, parietal

cortex and striatum, but not in the hippocampus. THC did not affect the loss of body weight (Shen et al., 2011).

Apart from co-consumption of other drugs, age is another aspect of vulnerability to long-term effects of substance abuse. Most MDMA and cannabis users are young adults or adolescents (EMCDDA, 2009; Wu et al., 2009), and drugs may have differential effects on adolescent brain function (Spear, 2000). Specifically, impairments in left hippocampus functioning were associated with memory deficits in adolescent MDMA users, suggesting an increased vulnerability to cognitive and neuronal impairments during this age period (Jacobsen et al., 2004). Dauman et al. (2005) found similar diminished hippocampus activity in adult MDMA polydrug users, albeit without decreased memory performance. Whether this discrepancy is due to age-related changes or possibly compensating effects of other drugs remains speculative. Rodent experiments have shown an influence of early exposure to MDMA on behaviour and serotonergic functioning. In pubertal female rats, repeated exposure to 3 and 10mg/kg MDMA lead to a transient increase in locomotor activity, whereas stimulatory effects in adult animals were continuous (Wiley et al., 2011). Juvenile rats (postnatal day (PD) 25-30) showed a moderate vulnerability to MDMA-induced decreases in 5-HT binding, whereas adult rats (PD90), where maximally susceptible (Kelly et al., 2002). Furthermore, exposure to cannabinoid agents during adolescence can lead to persisting changes in brain functioning as well as behavioural and cognitive performance (Rubino and Parolaro, 2008; Wegener and Koch, 2009b). For example, chronic treatment with the CB1 agonist WIN55,212-2 during adolescence, but not adulthood, lead to deficits in recognition memory and impairments in a progressive ratio task (Schneider and Koch, 2003). Long-term effects of chronic MDMA-administration, as well as of the combination of MDMA and a CB1 receptor agonist, during adolescence and adulthood on memory performance and complex decision making have not been investigated.

This study addresses two questions: First, how does chronic MDMA administration affect working memory and effort-based decision making, and does co-administration of WIN55,212-2 alter these effects? Second, are these effects age-dependent, i.e. different when administered during puberty or adulthood?

Materials & Methods

Animals

Forty-eight adult naive male Wistar rats (Harlan, Borchen, Germany) weighing 230-300 grams upon start of the experiment were used. Upon arrival, animals were allowed to habituate for 4-5 days in a vivarium under standardized conditions (4-6 animals per Makrolon type IV cage; tap water *ad libitum*; 12:12 hour light/dark circle, lights on at 7am; temperature 22+/- 2°C) and were handled regularly. In addition, 48 pubertal rats were taken from the in-house breeding facility. On postnatal day (PD) 21 they were weaned and afterwards housed under the same conditions as the adult rats. During the habituation and handling period, standard lab chow was available *ad lib* for all animals. For adult rats, controlled feeding (12g/animal/day) started two days before the first training session and for pubertal rats ten days after the last drug treatment. All animal experiments were conducted in accordance with the principles of animal care and the international laws on animal experiments (Directive 2010/63/EU) and were approved by the local authorities.

Drugs

All drugs were prepared freshly every third injection day before administration and injected in a volume of 1ml/kg. WIN55,212-2 (SIGMA-Aldrich, Steinheim, Germany) was dissolved in 2% Tween®80 (Serva, Heidelberg, Germany) and 98% NaCl solution (0.9% NaCl, Fresenius Kabi GmbH, Bad Homburg, Germany) and injected at a dose of 1.2mg/kg, intraperitoneally (i.p.). (±)3,4-methylenedioxymetamphetamine hydrochloride (synthesized in the Institute of Inorganic Chemistry, Prof. Nagel, University of Tübingen, Germany. Identity and chemical purity was verified) was dissolved in phosphate buffered saline (PBS), and injected at a dose of 7.5mg/kg, subcutaneously (s.c.). PBS injection (1ml/kg) served as vehicle control.

Treatment

Adult as well as pubertal rats were divided into four groups, respectively (n=12): MDMA group (receiving only MDMA injections), WIN group (only WIN injections), MDMA+WIN group (receiving both MDMA and WIN injections) and the control group (receiving PBS injections). Half of the control group animals received two vehicle injections to control for any effect two injections might have. There was no apparent or statistical effect in any of the measures, therefore, all 12 animals receiving PBS injections constituted the control group. The injection schedule was pseudo randomized over a period of 25 days, with adult rats receiving treatment from approximately PD80-105 (three weeks after arrival), and pubertal rats from PD40-65. Animals received no treatment on 10 days, one treatment per day on 10 days, and two treatments per day on 5 days, summing up to

a total of 20 injections. On days with two treatments, these were done at least 4 hours apart from each other.

Behavioural tests

Behavioural testing was conducted in a between subjects design. Adult as well as pubertal rats underwent the same training and testing procedure. Six animals per treatment group were pre-trained in the effort-based or delay-based decision making task for approximately 14 days prior to drug treatment and tested two weeks after the last injection. All remaining behavioural tests were conducted after long-term treatment with n=12.

Object recognition test

Based on recognition of a previously explored object, the object recognition task allows measurement of working memory without training procedures (Ennaceur and Delacour, 1988). Ten days after the last treatment, animals (n=12) were singly placed into a Makrolon type II cage and allowed to habituate to the environment for 30 minutes. On the following day, they explored the first object (object1) for 5 minutes. The time during which the animal was actively exploring the object (sniffing, licking, gnawing, pushing/pulling, handling) was recorded. After 25 minutes, a novel object (object2) was placed into the Makrolon type II cage together with object1 (now object 1B) in order to directly compare exploration times. For half of the animals, the first object was a small beaker and the second object a closing tap of a water dispenser, for the other half the order of objects was reversed. For the pubertally treated rats, the same testing procedure was repeated four weeks after the last treatment in order to monitor long-term effects as well as to compare their performance at the same age as adult rats were tested at.

Effort-based decision making

This task allows monitoring of effort-related decision making choice behaviour, i.e., how much effort the animal is willing to exert to obtain a larger reward. At the end of the arms of the T-maze (measurements of each arm: 60 cm x 15 cm x 30 cm (L x W x H)) either two or four pellets (Bio-Serv©, UK Dustless Precision Pellets©, 45mg) were placed in a metal food well. While the arm containing two pellets (low reward arm, LR) was freely accessible for the rats, a 30 cm barrier made of wire mesh was placed in the arm containing four pellets (high reward arm, HR). The HR was the right side T-maze arm for half of the animals, and left side for the other half. Pre-training sessions took place once a day for an average of 14 days. All animals (n=6) were habituated to the apparatus as well as to increasing heights of the barrier, and were trained until all animals reached a mean baseline level of ≥ 80% choice of HR for three consecutive days. Some animals reached baseline level prior to 14 days; they remained included in the group training. Inter-trial interval (ITI)

was one minute (for further detail on apparatus and training method see (Walton et al., 2002), adapted from the original study by Salamone (1994)). Post-treatment sessions started 12 days after the last injection day. The first session consisted of 10 forced trials only, i.e. animals were hindered entering the HR or LR arm in a pseudo randomized order by one grey sliding door in the choice area. During 10 following training sessions, animals could freely choose which reward arm they entered. On post-training day 11, animals absolved the testing under acute substance influence as a drug challenge was conducted, during which rats received the same substances they had been treated with chronically.

Delay-based decision making

This task allows examining one aspect of impulsivity, namely the ability to tolerate delays of reward. It was conducted in the same T-maze apparatus as the effort-based decision task. Removable, non-transparent guillotine doors were placed at the entry of the starting way, at the choice area, and at the entry of each goal area. The food wells in the goal areas contained either 2 pellets (low reward arm, LR, no delay), or 10 pellets (high reward arm, HR, 10 seconds delay). Pre-training consisted of habituation of the animals (n=6) to the apparatus, sliding doors, as well as to increasing waiting times for the HR and training until as a group they reached baseline level of ≥70% choice of HR for three consecutive days (for further details on training and testing procedure see for example, (Hadamitzky et al., 2009; Bizot et al., 1999)). Post-treatment training and challenge followed the same procedure as in the effort-based task.

Locomotion

Animals (n=12) were gently placed into infrared beam-controlled acrylic glass chambers (ActiMot-system; TSE, Bad Homburg, size: 44.7 cm x 44.7 cm x 44 cm) measuring horizontal (locomotion) and vertical (rearings) activity. This test was performed on day 11 days after cessation of the drug treatment. Locomotion and exploratory behaviour was automatically recorded by a PC (ActiMot Software; TSE, Bad Homburg) for 35 minutes and stored as aggregated data in seven 5-minute intervals. Parameters analysed were number of rearings, total activity (%), and time spent in centre (%). This test was repeated four weeks later.

Body weight

The body weight of the animals was measured using a laboratory scale on each of the 15 treatment days before injection as well as on the substance challenge day before the T-maze tests.

Statistical analysis

For data analysis, analyses of variance (ANOVAs) were conducted with SigmaStat2.03 for Windows (SPSS Inc., Chicago, IL, USA), followed by post hoc Tukey tests for pairwise multiple comparisons. For all measurements, $p<0.05$ was considered significant.

Results

Object recognition test

Rats treated with MDMA during adulthood explored object1, object1B and object2 for a similar amount of time, an effect which was not apparent for MDMA+WIN, WIN, or vehicle-treated animals. ANOVA confirmed that there was a significant difference in the time spent exploring the objects for the control group [$F(2,22)=10.30$, $p<0.001$]), the WIN group [$F(2,22)=8.61$, $p=0.002$], and the MDMA+WIN group [$F(2,22)=10.17$,$p=0.006$]. Post-hoc test revealed a decrease in the time spent exploring the first object (object1) when it was presented for the second time (object1B), compared to the first presentation of the same object ($p=0.002$ to $p=0.003$) as well as to the presentation of the second object (object2, $p=0.002$ to $p=0.009$). There was no statistical difference in exploration times of the objects within the MDMA group ($p=0.398$, Fig.1a). Ten days after the last treatment, exploration times of object1, object1B and object2 did not differ for rats treated with MDMA during puberty. This effect was not apparent for MDMA+WIN, WIN, or vehicle-treated animals. ANOVA revealed that there was a trend towards a significant difference between the exploration times of the objects for the control group [$F(2,22)=2.11$, $p=0.14$], as well as significant differences in exploration times for the WIN [$F(2,22)=20.17$, $p<0.001$] as well as MDMA+WIN-treated groups [$F(2,22)=8.07$, $p=0.002$]. Post-hoc Tukey test showed that animals spent significantly more time exploring object1 compared to object1B (WIN: $p<0.001$, MDMA+WIN: $p=0.002$). Animals treated with MDMA showed no difference in exploring the objects [$F(2,20)=0.71$, $p=0.50$] (Fig.1b). Four weeks later, the between group differences persisted, in that all but the MDMA-treated group showed significant differences between the objects (control: [$F(2,22)=3.87$, $p=0.036$], WIN: [$F(2,22)=7.93$, $p=0.003$], MDMA+WIN: [$F(2,22)=5.60$, $p=0.011$]) In particular, animals explored object1 longer than object1B (control: $p=0.031$, WIN: $p=0.002$, MDMA+WIN: $p=0.010$) (Fig.1c).

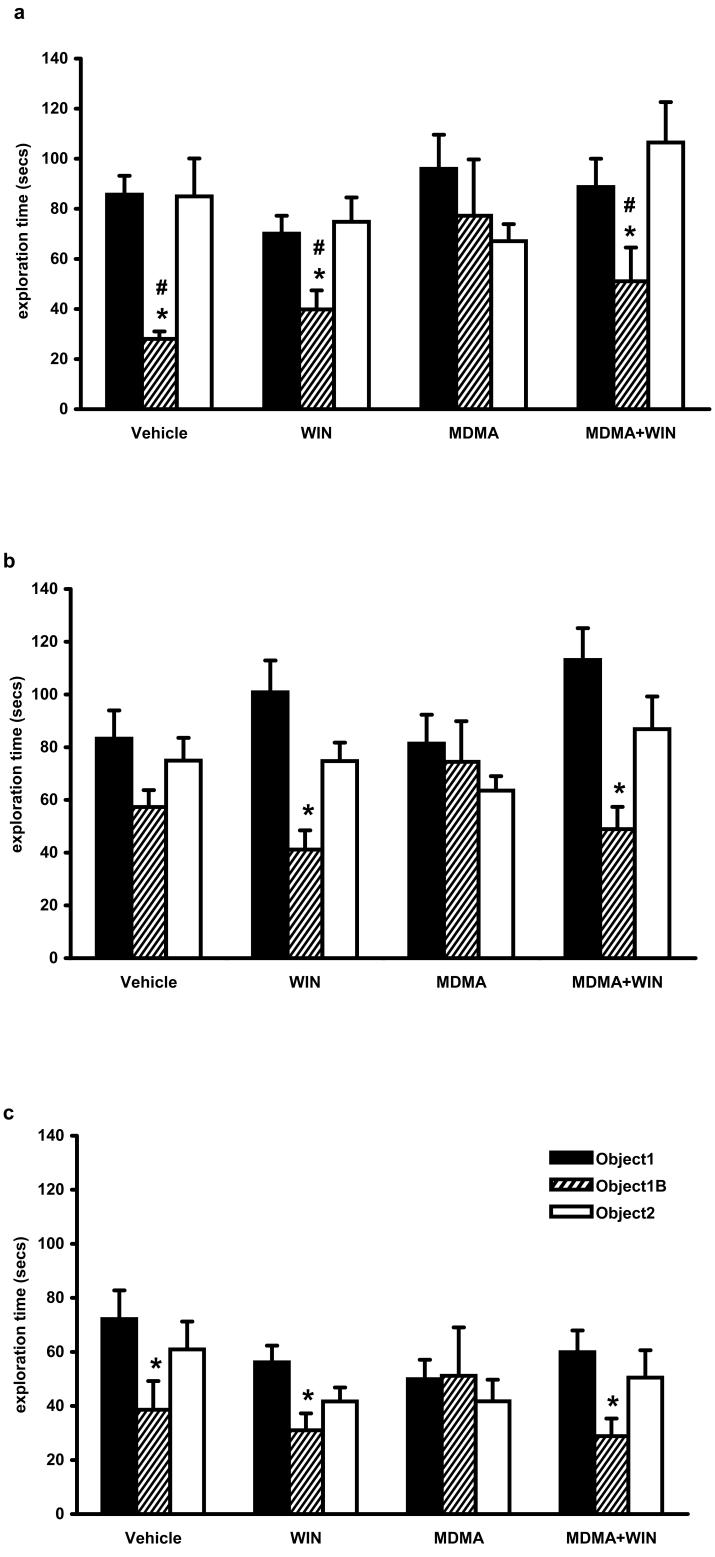

Fig.1: Object recognition test. Mean time (+SEM) exploring object1, object1B, and object2 during the object recognition test. Rats treated during adulthood and puberty were tested 10 days after the last treatment (**a**- upper panel and **b**- lower panel, respectively), pubertally treated rats were re-tested again 4 weeks later (**c**). All symbols $p<0.05$. * denotes significant differences in exploration times between object1 and object1B, # denotes significant differences between object1B and object2.

Effort-based decision making

MDMA+WIN as well as MDMA-treated adult rats took longer to resume baseline level (>80% HR arm choices) than WIN and vehicle-treated rats, and chose the HR arm less often during the acute challenge. ANOVA confirmed significant effects for the factors treatment [$F(3,217)=12.94$, $p<0.001$] and post-training day [$F(10,217)$, $p<0.001$]. Post-hoc test revealed that control- and WIN-treated animals chose the HR significantly more often than both MDMA- and MDMA+WIN-treated rats ($p<0.001$). Overall, animals chose the HR significantly less often on post-training day 11 (challenge day) compared to days 6-8 ($p=0.011$, $p=0.029$, $p=0.031$, respectively). MDMA-treated animals chose the HR significantly more often on post training days 5 and 6 compared to day 1 ($p=0.038$, $p=0.011$, respectively). MDMA+WIN-treated animals chose the HR significantly less often on day 11 compared to days 8-10 ($p=0.026$ and $p=0.037$). No treatment x day interaction was apparent for control and WIN-treated animals. On the first post-training day, both control and WIN-treated animals chose the HR significantly more often than MDMA-treated animals ($p=0.033$, $p=0.011$, respectively), whereas on the third day, control and WIN-treated animals chose the HR more often than MDMA+WIN-treated animals ($p=0.024$, $p=0.017$, respectively). On the second post-training day, WIN animals chose the HR more often compared to the MDMA+WIN group ($p=0.033$). On post-training day 11 (challenge day) control group animals preferred the HR arm compared to the MDMA+WIN group ($p=0.022$), and there was a trend toward significance for the difference of HR choice between the WIN and the MDMA+WIN group ($p=0.075$) (Fig.2a).

Drug treatment during puberty did not influence resumption of baseline level for any group, however during the acute challenge, MDMA administration led to less HR arm choices. ANOVA showed a significant effect for the factor post-training day [$F(10,209)=2.66$, $p=0.005$] and a strong trend toward significance for treatment [$F(3,209)=2.49$, $p=0.061$]. Post-hoc Tukey test revealed a higher percentage of overall HR choices on day 10 compared to day 2 ($p=0.033$). Within the MDMA-treated group a trend towards a significant difference ($p=0.091$) was revealed when comparing day 10 to day 11 (challenge day). Control animals chose the HR arm significantly more often than MDMA+WIN-treated animals on post-training day 1 and more often than MDMA-treated animals on day 11 ($p=0.030$, $p=0.013$, respectively) (Fig.2b).

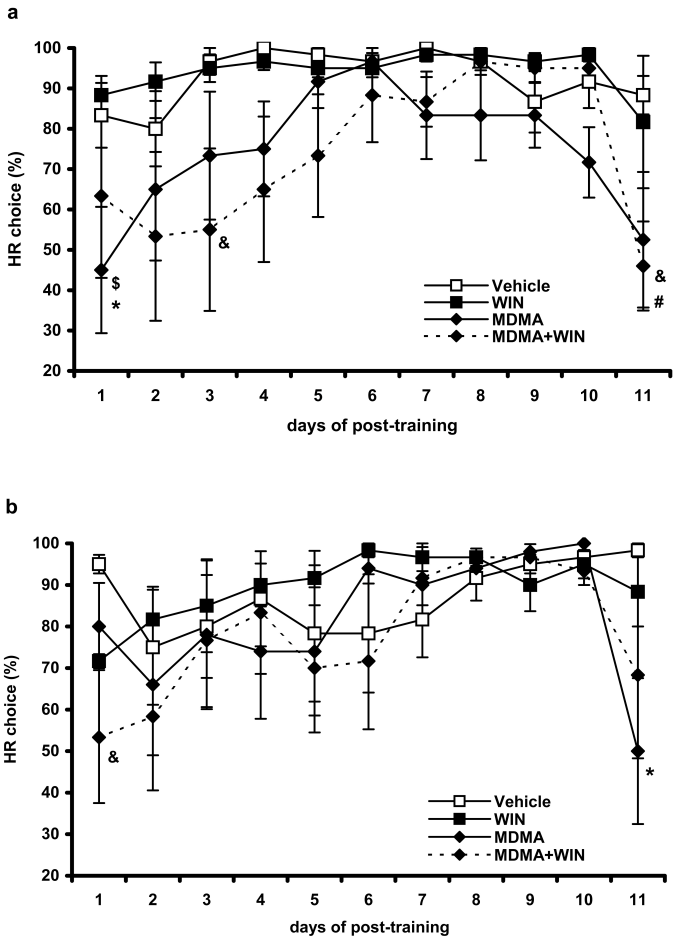

Fig.2: Post-training phase of the effort-based decision making task and drug challenge. Percent choice of the high reward (HR) arm (%)(+/-SEM) during ten days of post-training (day 1=13th day after last treatment) and challenge day (day 11) after chronic treatment (n=6) for **a** rats treated during adulthood. $^{\#}$ denotes significant differences within the MDMA+WIN group compared to training days 8-10, $^{\&}$ denotes significant differences between MDMA+WIN group and control group, $^{\$}$ denotes differences within the MDMA-group compared to training days 5 and 6, * denotes significant difference between MDMA and control animals. **b** pubertally treated rats, where * denotes significant difference between MDMA and control groups, $^{\&}$ denotes significant differences between MDMA+WIN group and control groups. All symbols $p<0.05$.

Delay-based decision making

No effects for substance or post-training day were found for rats treated during adulthood.

Data from rats treated during puberty showed significant effects for the factors substance [$F(3,220)=4.04$, $p=0.008$] as well as post-training day [$F(10,220)=2.23$,$p=0.017$]. Post-hoc test revealed overall less HR choices for animals treated with WIN compared to MDMA+WIN ($p=0.027$) and MDMA ($p=0.035$) treatment. Furthermore, animals chose the HR arm significantly more often on days 9 and 10 compared to day 1 ($p=0.022$, $p=0.036$, respectively). No further interaction was found (data not shown).

Locomotion

Chronic drug treatment during adulthood did not influence horizontal or vertical locomotor activity 11 or 39 days after the last administration. A two way repeated measures ANOVA confirmed no effect for treatment or time point of measurement for the parameters activity (seconds), distance (meters), or number of rearings (all $p>0.78$). A significant treatment effect was found when comparing the time spent in the centre of the open field [$F(3,88)$,$p=0.036$], and post-hoc test revealed that WIN treated rats spent significantly less time in the centre compared to MDMA+WIN-treated rats ($p=0.038$), as well as a trend towards a significant difference between WIN- and MDMA-treated rats ($p=0.081$) (Table 1).

Chronic drug treatment during puberty did not influence horizontal activity or anxiety-like exploration 11 or 39 days after the last administration, however, the number of rearings increased with increasing age. ANOVA confirmed no significant effect for the factor treatment or time point of measurement for the parameters activity (secs) and time spent in the centre (%) (all $p>0.05$). There was a significant effect of time point of measurement for the parameter rearings [$F(1,43)=19.63$, $p<0.001$), and post-hoc tests revealed all treatment groups reared more often during the second measurement (four weeks following the first). This difference was significant for MDMA-, MDMA+WIN-treated as well as control animals ($p=0.025$, $p=0.022$, $p=0.034$, respectively; WIN: $p=0.055$). In terms of distance (meters), the time point of measurement showed a significant effect [$F(1,43)=8.53$,$p=0.006$], with post-hoc test revealing control animals running more meters during the second measurement ($p=0.032$), and WIN treated animals showing a strong trend towards significance ($p=0.070$) (Table 1).

Treatment during adulthood	Activity (secs)		Number of rearings		Distance (meter)		Time in centre (%)	
Vehicle	63,70 (5,85)	64,42 (6,08)	17,54 (2,48)	19,42 (2,33)	22,05 (2,43)	23,00 (2,68)	6,27 (1,47)	3,38 (0,74)
MDMA	66,20 (5,95)	62,09 (7,33)	18,01 (3,04)	15,98 (2,82)	23,37 (2,54)	22,86 (2,59)	4,84 (1,64)	2,94 (0,81)
WIN	61,79 (9,20)	61,58 (8,40)	16,27 (3,36)	17,73 (3,69)	22,73 (3,96)	22,09 (3,63)	7,10 (2,97)	8,40* (3,02)
MDMA+WIN	58,29 (5,59)	59,17 (6,17)	16,35 (3,07)	17,71 (3,64)	21,18 (2,83)	20,71 (2,94)	3,58 (1,45)	2,80 (0,55)
Treatment during puberty								
Vehicle	60,58 (4,32)	66,50 (4,53)	13,78 (1,80)	18,30# (1,77)	20,97# (2,02)	25,18 (2,23)	3,20 (1,58)	6,64 (2,23)
MDMA	50,73 (3,74)	56,91 (2,35)	10,33 (1,50)	15,33# (1,20)	17,86 (2,17)	18,30 (0,31)	1,95 (0,85)	4,07 (1,95)
WIN	62,33 (3,70)	62,83 (4,31)	15,32 (1,05)	19,39 (2,00)	20,05 (1,58)	23,58 (2,36)	5,12 (1,98)	4,10 (0,97)
MDMA+WIN	61,58 (4,54)	65,83 (3,4-6)	13,25 (1,85)	18,14# (1,45)	20,52 (1,92)	23,57 (1,35)	2,25 (0,58)	4,05 (0,72)
Time point of measurement	11 days	39 days	11 days	39 days	11 days	39 days	11 days	39 days

Table1: Locomotor activity and exploratory behaviour. Mean activity, number of rearings, distance travelled and time spent in the centre (SEM) measured on two time points (11 and 39 days after the last treatment) is shown for animals treated during adulthood or puberty. #denotes a significant difference within treatment groups between time points of measurement, *denotes difference compared to MDMA+WIN-treated animals. All symbols indicate $p<0.05$.

Body weight

For rats treated during adulthood, significant effects were found for injection day [$F(15,45)=26.55$, $p<0.001$] as well as for treatment [$F(3,45)=8.37$, $p<0.001$]. Post-hoc tests revealed a significant decrease in weight between day 2 and day 14 ($p=0.032$ to $p<0.001$) as well as increased weight on day 16 (challenge day) compared to days 3-15 ($p=0.014$ to $p<0.001$). Furthermore, MDMA-, WIN-treated and control animals weighed significantly more than MDMA+WIN-treated rats (all $p<0.001$) (data not shown).

All pubertal animals gained weight during the course of treatment; however MDMA as well as MDMA+WIN treated rats gained less than the WIN and vehicle groups. ANOVA revealed significant effects for treatment [$F(3,655)=37.13$, $p<0.001$] as well as for injection day [$F(15,655)=294.58$, $p<0.001$]. Post-hoc test showed control and WIN-treated animals weighing significantly more than MDMA+WIN- and MDMA-treated rats (all $p<0.001$). Specifically, WIN-treated animals weighted significantly more than MDMA and MDMA+WIN-treated animals on injection days 8 to 15 ($p=0.015$ to $p<0.001$), and control animals weighted significantly more than MDMA+WIN animals on day 7, 9-11, 14 and 15, and more than MDMA-treated animals on days 9 and 13-15 ($p=0.049$ to $p=0.005$) (Fig.3). No significant weight difference was observed on the challenge day.

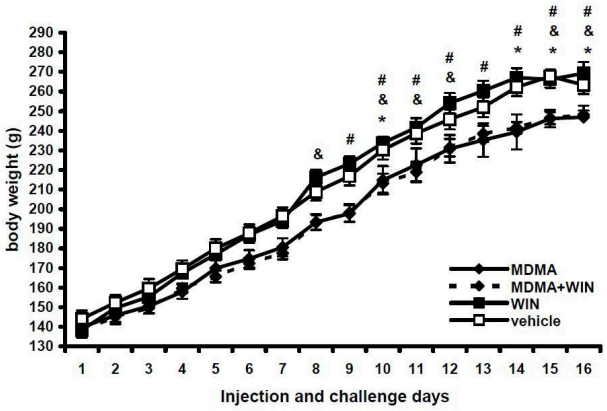

Fig.3: Weight change of pubertal animals on injection and challenge days. Effect of chronic treatment with WIN, MDMA, MDMA+WIN, or vehicle (n=12) on weight gain of pubertal rats fed *ad libitum*. Mean body weight (grams) (+/-SEM) are depicted for fifteen injection days and the challenge day (16). # denotes significant difference between WIN- versus MDMA- and MDMA+WIN-treated animals, * and & denote significant difference of control versus MDMA and MDMA+WIN groups, respectively. All symbols indicate $p<0.05$.

Discussion

In rats chronically treated with MDMA, memory deficits in working memory (object recognition test) as well as in long-term memory (re-learning of the effort-based decision making task) were apparent. This is in line with human and animal studies observing deficits in memory after long-term administration. Abnormal functioning of the left hippocampus during a working memory task was found in adolescent MDMA users (Jacobsen et al., 2004), and impairments in working memory in adult rats intermittently treated with MDMA during puberty (Meyer et al., 2008). In contrast to studies proposing increased vulnerability of adolescents to the effects of cannabinoids (Schneider and Koch, 2003; Rubino and Parolaro, 2008), long-term CB1 receptor stimulation did not influence performance in the object recognition test or the more complex effort-related decision making, as WIN-treated animals behaved similar to control rats. The most interesting result here is the reversal of the MDMA-induced deficit in object recognition for adult as well as pubertal treated rats by co-administration of WIN. The fact that WIN attenuated MDMA-induced deficits in the memory task but not in the effort-based decision making task points to a long-term neurobiological interaction of these substances within brain regions involved in short-term memory, rather than in the execution of the more complex decision making tasks. A likely candidate for the location of interactive effects on working memory is the hippocampus, which has a high vulnerability to toxic events, including presumable MDMA-induced deterioration of 5-HT nerve fibres (Hatzidimitriou et al., 1999; Green et al., 2003). However, Rodsiri (2011) found impairments of object recognition

memory without reduction of 5-HT levels in frontal cortical or hippocampus areas two weeks after 3 or 6mg/kg MDMA binges, indicating neurotoxicity-independent mechanisms for long-lasting memory deficits. This is in line with studies indicating that MDMA-induced deficits in recognition memory are not due to serotonergic alterations (Nawata et al., 2010; Piper and Meyer, 2004). Nevertheless, 3, 6, and 15mg/kg MDMA increased extracellular concentrations of 5-HT in the hippocampus one hour after acute administration (Rodsiri et al., 2011; Mechan et al., 2002). Repeated MDMA administration enhanced long-term potentiation (LTP) (Morini et al., 2011), probably due to postsynaptic DA- as well as presynaptic 5-HT_2 receptors (Rozas et al., 2011). Importantly, the hippocampus is an area expressing CB1 receptors in high levels (Pertwee et al., 2010; Herkenham et al., 1991), and might therefore be particularly sensitive to cellular changes due to chronic MDMA administration and CB1 agonist treatment. Specifically, in contrast to MDMA, CB1 receptor agents impair LTP (Schlicker and Kathmann, 2001; Collins et al., 1995), thus possibly preventing MDMA-induced long-term alterations on a cellular level. Another mechanism could be neuroprotection by cannabinoids. Although chronic cannabinoid administration has previously been shown to induce deficits in learning and memory tasks as well as morphological changes in the brain, *in vitro* studies demonstrate the protective effects of cannabinoid agents, specifically WIN, against excitotoxicity via a decrease of glutamate release (Shen and Thayer, 1998). In line with our results, Shen (2011) found a reduction of MDMA-related behavioural changes and attenuation of reduced 5-HT levels and SERT binding after chronic co-administration of THC. Other studies found a deficit in working memory when MDMA and THC were co-administered (Young et al., 2005), or a similar ameliorating effect on recognition memory during MDMA-withdrawal when a CB1 receptor antagonist was co-administered (Nawata et al., 2010). However, these studies investigated memory impairment after acute, rather than chronic administration, or used mice in which MDMA predominantly affects the DA system, rather than rats (O'Shea et al., 2001; O'Shea et al., 1998). In MDMA-treated mice, THC has been shown to reduce DA terminal loss, presumably due to CB1 receptor activation (Tourino et al., 2010), pointing to a neuroprotective mechanism of CB1 agonists. Since the current study did not assess neurotransmitter levels, this interpretation is based on behavioural interactions only. Our results nevertheless indicate strong regulatory effects of chronic co-treatment with a cannabinoid agonist during puberty as well as adulthood on long-lasting MDMA-induced working memory deficits, supporting the notion of WIN having long-term protective effects on neurobiological changes in the hippocampus evoked by MDMA administration.

Deficits in effort-based decision making were observed for both MDMA- and MDMA+WIN-treated adult rats during the first five days of post-training. Chronic MDMA-treatment seems to have an effect on memory retrieval, rather than inability to complete the task, and this effect is more pronounced if treated during adult age. Pubertally treated animals needed similar training time to resume to near-baseline levels irrespective of treatment, whereas within the adult group, MDMA as

well as MDMA+WIN treated animals took longer to re-learn compared to control and WIN-treated animals. Repeatedly administered MDMA has been shown to enhance LTP, a cellular mechanism crucial to the formation of long-term memory (Peng et al., 2011). Brain areas implicated in this process, as well as in retrieval of previously learned information are the hippocampus and its connections to cortical areas, specifically frontal, anterior cingulate and temporal cortex (Bontempi et al., 1999), which exhibit only moderate density of CB1 receptors (Pertwee, 1997). Therefore, CB1 receptor activation might be insufficient to attenuate damage to prefrontal areas due to chronic MDMA-administration during adulthood. In contrast, frontal areas responsible for storage and retrieval of long-term memory and effort-based decision making are among the last to mature (Sowell et al., 1999). Therefore, if neuronal transmission is disturbed by MDMA-administration during puberty, compensative mechanisms might take effect, whereas damage to adult rats may be irreversible. In line with this, adult rats generally are even more vulnerable to MDMA-induced long-term depletion of 5-HT (Piper, 2007). Acute effects of MDMA, WIN and their combination on decision making are discussed elsewhere (Schulz et al., submitted). However, when challenged with the respective substances, MDMA or MDMA+WIN-treated animals in both age groups chose the HR arm less often than WIN and control animals, indicating effort-aversive decision making upon acute MDMA administration irrespective of WIN co-administration and previously learned strategies. In another study, acute MDMA-administration in adult rats induced increases in reference memory errors, i.e., impairments in re-using the strategy from a previous trial to solve a task (Kay et al., 2010). Furthermore, the well described MDMA-induced 5-HT and DA release might influence fronto-striatal circuitry (Floresco et al., 2008) or nucleus accumbens function (Kurniawan et al., 2011). However, the specific role of these neurochemical effects in regulating effort-based choice remains elusive.

No effects of chronic treatment with either substance could be observed for the delay- based task, an animal model of impulse control action. Rats pubertally treated with WIN showed an overall decrease of HR choices compared to MDMA as well as MDMA+WIN-treated animals, however no differences to the control group were found. This task is generally more difficult to acquire than the effort-based task, as observed during pre-training. All animals, including controls, took longer or failed to recover baseline levels after treatment, although the number of overall HR choices increased toward the last two post-training days. Furthermore, no differences were found during an acute challenge for any age or treatment group. In line with this, Saadat (2006) found no long-term changes in behavioural inhibition after a high dose administration of MDMA, despite significant depletion of cortical 5-HT. The absence of an effect of chronic treatment on delay-based choice points to more pronounced MDMA-induced alterations in the medial prefrontal cortex, specifically, the ACC, involved in effort-based choices, compared to the orbitofrontal cortex, which has been shown to regulate delay-based decisions.

The drug effects on learning and memory are not due to impairments in locomotion, as there where no differences in terms of activity, rearings or distance travelled between the treatment groups. The increased time adult rats previously treated with MDMA or MDMA+WIN spent in the centre compared to WIN-treated rats probably reflects a slight decrease in exploration anxiety rather than an increase of anxiety in WIN-treated rats, as the latter did not differ from controls. The significantly increased number of rearings of all pubertally treated rats most likely reflects age-dependent, rather than treatment-related, differences (cf. animals treated during adulthood). Adding to the well-established effect of hyperactivity after acute MDMA treatment (Green et al., 2003), and amelioration thereof by cannabinoid agents (Morley et al., 2004), our results indicate that chronic administration of MDMA does not result in long-term hyperactivity.

With respect to body weight, adult MDMA animals kept on a restricted feeding regime weighed slightly more than controls and WIN-treated animals, probably because they were kept under restricted feeding conditions in one cage with MDMA+WIN animals, which had a reduced food intake, so that MDMA-treated rats were left with more available food. Results are clearer for pubertal animals which were fed *ad libitum*, where an anorexic effect of MDMA is apparent. Reduced food intake can influence food reward-based tasks such as delay- or effort-based choices; however, animals treated during puberty with MDMA or MDMA+WIN did not differ from their controls in these tasks. WIN-treatment did not ameliorate MDMA-induced decrease in weight change, indicating no additive or interactive effect of the substances on this physiological measure (see Shen at al, 2011).

Conclusion

Chronic WIN co-treatment during puberty or adulthood did not have additive or regulatory effects on MDMA-induced behavioural changes in locomotion or body weight. However, it resulted in long-lasting recovery of MDMA-induced deficits in recognition memory, presumably by interaction of altered 5-HT neurotransmission and CB1 receptors in the hippocampus. MDMA-induced disturbances of decision making after chronic administration are not modulated by CB1 receptor agonism in adult rats.

Histological verification of the density of myelinated axons following chronic treatment with MDMA, WIN55,212-2, or their combination

MDMA has been shown to be neurotoxic, and behavioural observations after chronic treatment in pubertal as well as adult animals point to neuronal changes at least in the hippocampus (see Study 2). On the other hand, cannabinoid agents are anti-oxidant and anti-inflammtory, and thus might be able to block or prevent cellular changes induced by MDMA-administration. Therefore, histological examination of the brains from the animals used in Study 2 was the logical subsequent step. As an indication of structural changes underlying treatment-induced behavioural changes, myelination density was examined in various brain areas. Myelination is an insulation sheath (formed by oligodendrocytes in the CNS) around axons which serves as an accelerator for signal transmission in the nervous system (Hartline and Colman, 2007). Many studies have demonstrated disturbed or degenerated myelination in patients suffering from psychiatric or inflammatory disorders, such as schizophrenia or multiple sclerosis (MS) (Emery, 2010; Bartzokis, 2012).

Method: Gold-chloride staining

From each treatment group (MDMA, WIN, MDMA+WIN and Vehicle), six rats were randomly chosen. Following transcardial perfusion (250 ml phosphate buffered saline followed by 500ml 4% paraformaldehyde in 0.1 PB), brains of the animals were removed and frozen at -17°C until they were cut on a cryostat (Jung CM 3000, Leica Instrument GmbH, Nussloch, Germany). Every sixth slice (40µm) was mounted onto a gelatinised glass slide and stained with 0,2% gold-chloride solution (AuCl4, Roth, Karlsruhe, Deutschland) for 1-3 hours. Staining was terminated upon articulated visibility of the hippocampus as red-brown structure. Slices were rinsed in aqua dest. and fixated in freshly prepared 2,5% Na-Thiosulfate solution for 5 minutes. After rinsing for 30 minutes under running tap water and aqua dest., slices were dehydrated in an ascending ethanol series (50, 70, 80, 96, 100%) and cover-slipped in Terpineol and Rotihistol. Myelination levels in different brain areas were microscopically analysed by an experimenter blind to the treatment conditions using image analysing software MetaMorph (Version 4.6, Universal Imaging Corp., Downington PA 19335, USA). In the digital image taken at hundredfold magnification, contrast was enhanced in order to enable the software to detect and label stained fibres. Within the area of interest (according to the rat brain atlas of Paxinos and Watson, 1998), myelination levels were calculated as % stained.

Results

Overall, MDMA+WIN treatment resulted in the lowest levels of myelination for both age groups in most analyzed areas. Brains of animals treated during adulthood showed decreased myelination after WIN treatment compared to vehicle and MDMA-treatment, whereas for the pubertally treated animals myelination levels after WIN treatment did not differ from vehicle or MDMA-treatment. Out of the 21 brain areas chosen to compare the density of myelination, one-way ANOVA revealed significant differences between treatment groups within seven regions. For rats treated during adulthood these include: Nucleus accumbens core [$F_{(3,19)}$=4.56; p=0.014], nucleus accumbens shell [$F_{(3,19)}$=6.16; p=0.004)], hippocampal areas CA3 [$F_{(3,19)}$=8.16; p=0.001] and CA1+2 [$F_{(3,18)}$=3.45; p=0.039], as well as the dorsal [$F_{(3,15)}$=3.39; p=0.046], medial [$F_{(3,15)}$=6.87; p=0.004] and caudal [H=13.57; p=0.004] raphe nuclei. Pubertally treated rats showed differences within the basolateral amygdala [$F_{(3,20)}$=11.23; p< 0.001], central amygdala [$F_{(3,20)}$=6.48; p=0.003], hippocampal areas CA3 [$F_{(3,18)}$ =19.26; p<0.001] and CA1+2 [$F_{(3,16)}$ =5.10; p=0.012], as well as

dorsal [$F_{(3,16)}$=3.73; p=0.033], medial [$F_{(3,15)}$=4.57; p=0.018] and caudal [$F_{(3,14)}$=11.10; p<0.001] raphe nuclei. Results of the post-hoc (Tukey) tests according to age group are shown in Fig.10.

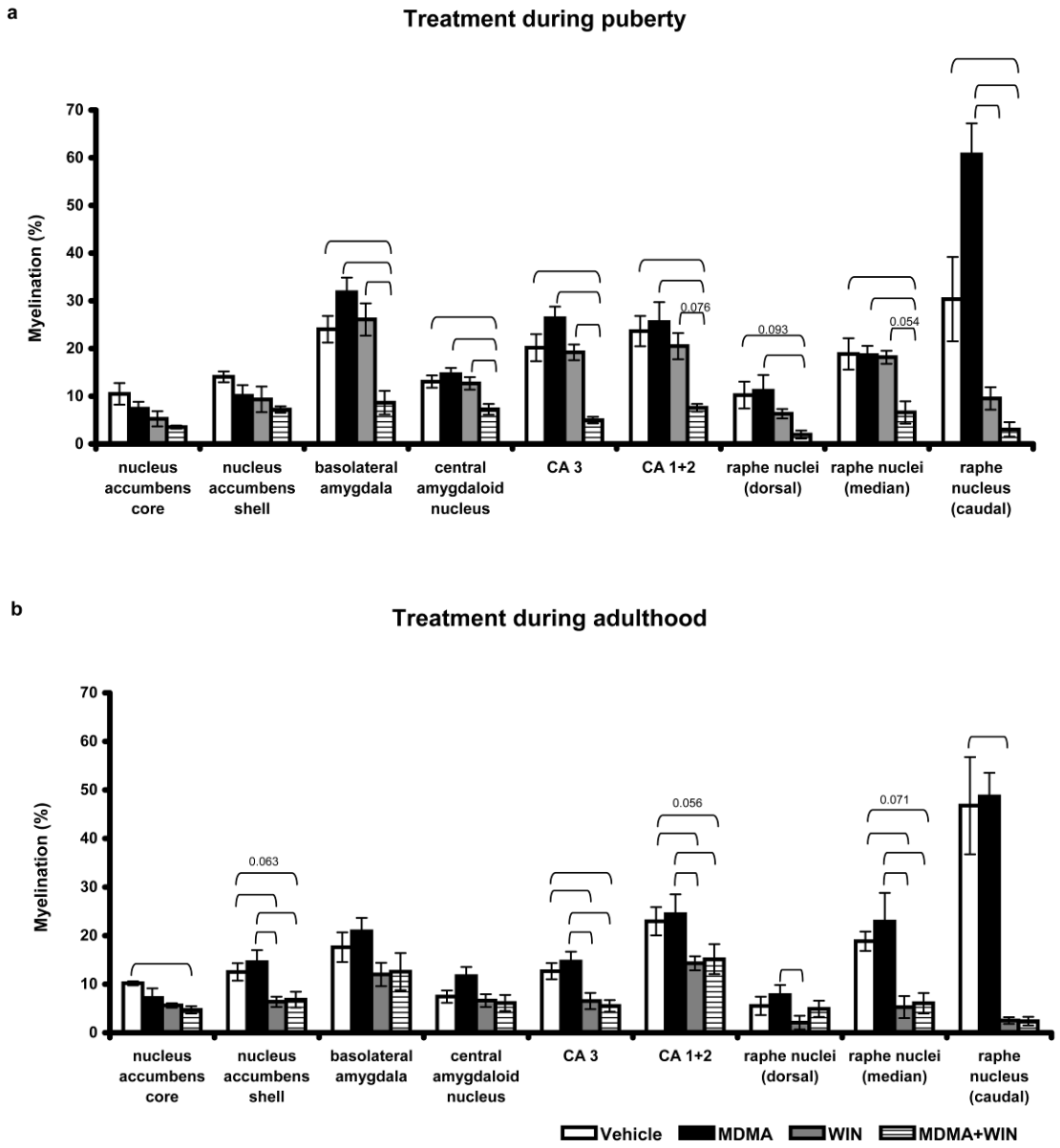

Fig.10: Mean density of myelination (%) according to treatment and brain region for rats treated during a) puberty or b) adulthood. Depicted are data for nine brain areas within which significant differences were found for either age group. Square brackets denote p<0.05 unless otherwise indicated. [Frontal brain areas (including the orbitofrontal and limbic cortex) were also examined, however data are omitted here because of very low amount of white matter and very similar (high signal-to-noise ratio) myelination levels irrespective of treatment group.]

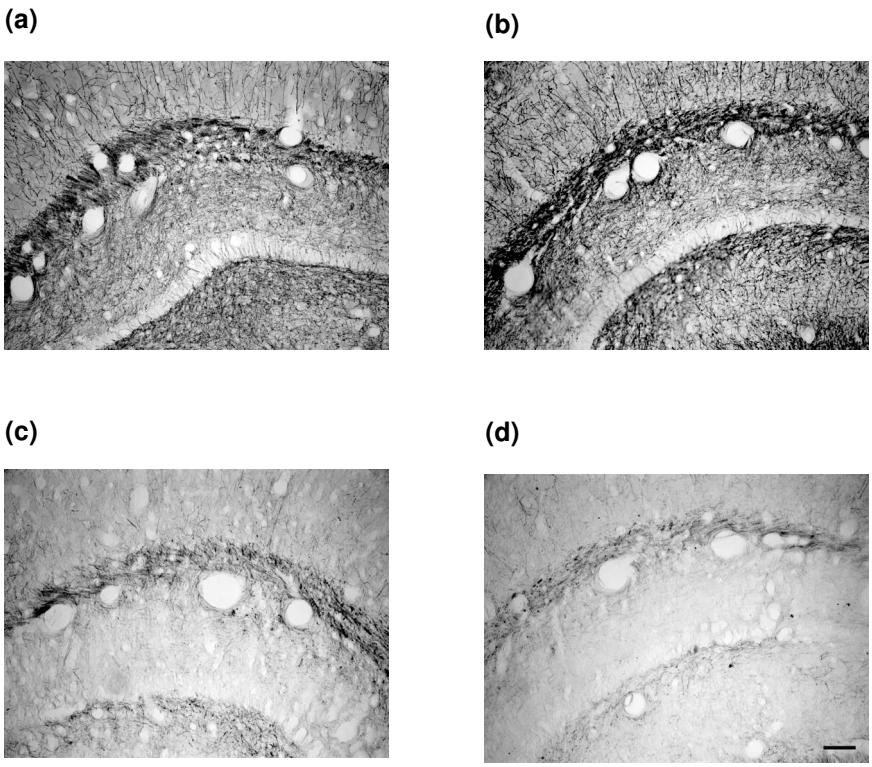

Fig.11: Hippocampus CA 1+2 area. Representative photographs of adult brain slices (-2.56 mm relative to Bregma) after chronic Vehicle (a), MDMA (b), MDMA+WIN (c) or WIN (d) treatment. Scale bar = 100µm.

Discussion

Chronic MDMA+WIN treatment reduced myelination in all brain regions examined. This effect seems to be independent from age during treatment. WIN-treatment during adulthood decreased myelination levels to equal amounts than the combination MDMA+WIN. Thus, the cannabinoid receptor agonist seems to have a degenerating effect on white matter, at least in hippocampal areas and raphe nuclei of adult rat brains. This is in line with studies indicating that chronic, low doses of cannabinoids might be neurotoxic, whereas acute doses have neuroprotective effects (Sarne and Keren, 2004), and long-term alterations on the cellular level after chronic administration of cannabinoid receptor agonists (Wegener and Koch, 2009b). On the other hand, symptoms and disease progression of MS in animal models, and reduction of spasticity in human patients can be attenuated by cannabinoids (THC, canabidiol, WIN), presumably due to anti-inflammatory effects (de Lago et al., 2012; Sanchez and Garcia-Merino, 2012). However, whereas cannabis-based medicines only produce few side effects (Rog et al., 2005; Aragona et al., 2009), self-medication may lead to dose-dependent decrease in cognitive functioning in MS patients (Honarmand et al., 2011). Myelination levels of rats treated with WIN during puberty are akin to those after treatment with vehicle or MDMA. Therefore, although puberty is a developmental period highly vulnerable to drug-induced neuronal, behavioural and cognitive alterations (Schneider, 2008), chronic treatment with the cannabinoid agonist WIN55,212-2 during puberty did not reduce myelination levels. Since the endocannbinoid system is most

active during puberty and plays a major role in the maturation process of neurons, presumable neurotoxic effects could have been overlaid, or recuperation after treatment occurred. This distinct pattern can be observed best when comparing myelination levels in the hippocampal areas (CA 3 and CA 1+2) and the median raphe nuclei (Fig.10). Furthermore, rats treated during puberty show a comparable pattern in the amygdala; adult-treated rats in the nucleus accumbens shell. Interestingly, brain myelination of MDMA- and vehicle-treated animals did not differ in any of the examined regions. Contrary to many human and animal studies indicating neurodegenerative properties of MDMA, there was no indication of decreases in myelination levels due to chronic MDMA-treatment during either adulthood or puberty. Although gold-chloride staining is a fast and effective method to investigate myelination levels (Wahlsten et al., 2003), it is not without limitations. Staining intensity can vary between the samples, and arbitrary definition of the threshold reduces the reliability of this method. Furthermore, staining of white matter is universal, therefore no conclusions about damage to unmyelinated fibers, or functional alterations can be drawn. However, the present results point to three indications which deserve further investigation: 1. MDMA+WIN treatment robustly reduces myelination levels. 2. MDMA administration alone does not change myelination levels. Both 1. and 2. are age- independent effects. 3. WIN reduced myelination levels in adult-, but not pubertally treated rats.

General discussion

Drug abuse and its consequences contribute to enormous costs in terms of health care and social welfare. However, underlying mechanisms and effects of two of the most commonly consumed illicit recreational drugs world wide, namely ecstasy (MDMA) and cannabis, are still not fully understood. Most ecstasy users are young adults as well as polydrug users, and the majority concomitantly consumes cannabis. Consumption of cannabis, especially during adolescence, has been associated with subsequent consumption of "harder" drugs and addiction and the cannabinoid system has been implicated in the reinforcing properties of psychostimulant drugs. Activation of cannabinoid receptors in the brain influences various neurotransmitter systems by inhibiting neurotransmitter release, thus potentially modulating the effect MDMA has on 5-HT transmission and resulting behaviour.

This thesis set out to investigate effects of these drugs on cognition and behaviour in rats. The Review examined the state of knowledge regarding polydrug use (MDMA+cannabis or cannabinoid agents). Acute (Study 1) and chronic (Study 2) effects of MDMA, the cannabinoid receptor agonist WIN and the combined administration in pubertal and adult rats were investigated. In the following, the main findings are summarized and discussed.

MDMA

As expected, acute MDMA administration increased the body temperature and motor activity, and decreased food intake. In addition, Study 1 as well as the acute challenge following chronic treatment in Study 2 demonstrated an acute influence on decision making: **Acutely, MDMA induced cost-aversive choice in the effort-based task, indicating immediate alterations on decision making cognition.** The most likely neurobiological mechanism is the alteration of 5-HT and/or DA transmission. MDMA increases extracellular 5-HT levels, particularly in the medial prefrontal cortex, the striatum and the hippocampus (Gudelsky and Nash, 1996; Mechan et al., 2002). 5-HT increase has been shown to reduce choices for high reward-high cost options (Homberg, 2012).

In Study 2, cognitive deficits were evident after long-term administration of MDMA: **Chronic administration of MDMA induced persistent deficits in recognition memory as well as in effort-based decision making.** Alterations within brain areas involved in memory and decision making processes, such as the hippocampus and ACC are the likely causes of these effects. Unexpectedly, locomotor and exploratory behaviours in drug-treated rats were not different from controls, indicating MDMA-induced alterations of activity to be acute only. In the additional

histological examination, no difference in terms of myelination levels was found between MDMA-treated rats and controls. Considering the bulk of evidence pointing towards structural alteration following chronic MDMA consumption, this result was unexpected. However, recent evidence proposes serotonergic dysfunction, rather than structural loss of neurons (Baumann et al., 2007; Kivell et al., 2010; Wang et al., 2004). The technique of gold-chloride staining only assesses white matter, thus structural changes in grey matter or functional alterations of neurotransmission are not asserted. Both acute and chronic administrations of MDMA lead to behavioural as well as cognitive changes. Therefore, lack of alterations of involved brain areas is quite unlikely and should be assessed using other methods.

WIN55,212-2

In earlier studies, systemic and intracerebral administration of the cannabinoid receptor agonist WIN55,212-2 induced dose-dependent alterations in motor activity, prepulse inhibition, operant behaviour and social interaction (Drews et al*.,* 2005; Schneider et al*.,* 2008; Wegener et al*.,* 2008). In the present studies, systemically administered WIN did not alter decision making or the majority of the behavioural patterns examined. **Acutely, WIN administration lead to an unexpected increase of body temperature. Furthermore, it had no effect on food intake or motor activity.** This contrasts with studies finding dose-dependent alterations of locomotion, hypothermia and hyperphagia in response to CB1 receptor agonists (Kirkham, 2005; Taffe, 2012; Razdan, 1986; Little et al*.,* 1988). This discrepancy can be explained by differences in dosage, route of administration, species, rat strains and substances used (see below).

Interestingly, various studies suggest lasting impairments in learning and memory in response to chronic treatment with WIN (Abush and Akirav, 2012) or THC (Steel et al., 2011). However, in the present study **administered chronically, WIN did not induce memory deficits or impairments of decision making**. Considering the frequency of administration and the long half-life of cannabinoids, the dose of WIN was realistically high.

Although all chronically treated animals showed a behavioural profile indistinguishable from controls, age-related differences in myelination levels were observed, indicating reduced myelination in adult, but not pubertal rats. Whether this is due to neurotoxic properties of long-term administration of WIN (Sarne and Mechoulam, 2005), or increased white matter due to CB1 activation during puberty, remains elusive.

WIN55,212-2 is one of the most widely used and well established synthetic cannbinoid agonist. Its systemic administration to animals mimics exogenous cannabinoid actions more closely than

restricted local application. WINs pharmacological, cognitive and physiological effects resemble, though are not identical to, those of THC. However, some disadvantages of experimental application appear. First, it binds to both CB1 and CB2, and to CB2 with a slightly higher affinity (Pertwee, 2008). By comparison, THC binds equally to CB1 and CB2 receptors, and has less efficacy at the latter. WIN elicits cannbinoid-like effects (antinociception, hypoactivity) independently of CB1 receptors, making differentiation of its effects on the brain and the periphery difficult. Second, exogenous cannabinoids have a distinct selective profile of pharmacological action within the brain and body compared to endogenous cannbinoids, which have been shown to activate vanilloid transient receptor potential (TRPV1) receptors and GPR55 in addition to CB1 and CB2 (Pertwee, 2008; Pertwee, 2010). Therefore, WIN has to be considered as a somewhat limited pharmacological agent.

MDMA & WIN

The results of Study 1 demonstrate that the cannabinoid receptor agonist WIN has little influence on MDMA-induced impairments in decision making, increased body temperature and locomotion when administered acutely. The only exception, attenuation of MDMA-produced anxiolytic behaviour, was neither statistically clear or obtained from an explicit anxiety test. In other words, **acute co-consumption of a cannabiniod receptor agonist does not seem to counteract MDMA-produced effects**. Rather, behavioural and cognitive effects seen following co-administration were similar or more pronounced, indicating additive negative effects.

Retrospective studies involving human long-term polydrug users reveal inconsistent results. Some attribute impairments in decision making and memory to MDMA use, others hold cannabis responsible. General agreement is reached regarding additive negative consequences of chronic MDMA+cannabis consumption on decision making and memory (Schulz, 2011). In contrast, Study 2 demonstrates a task-dependent influence on cognition: **whereas chronic co-administration of WIN reversed MDMA-produced deficits in working memory, there was no interaction in terms of effort-based choice**, body weight, or locomotion. Cannabis-independent deficits in executive functions such as decision making in long-term MDMA users has been demonstrated by several human studies (Morgan et al., 2006; Quednow et al., 2007), and further supports the notion of a limited interaction of these substances to certain brain regions.

Additional histological examination of myelination suggests that there is no attenuation of presumed MDMA-induced neurotoxicity by WIN. On the contrary, chronic MDMA+WIN administration robustly resulted in low myelination levels in all brain regions examined compared to the control group, and this result was evident for both adult as well as pubertal rats.

Future studies could narrow down the mechanism and site of interaction by 1) co-administering specific CB1 agonists or cannabinoid transporter inhibitors, 2) microinject WIN or a cannabionoid receptor agonist into the hippocampus while administering MDMA systemically and 3) examine 5-HT content or more conclusive markers for neurotoxicity after chronic co-administration.

Conclusion

This thesis provides the first account of effects of MDMA in an animal model of decision making. Both acute and chronic administrations lead to impairments in effort-based choice. No apparent structural alterations in the brain in terms of myelination were found, however the method is limited to white matter and thus functional changes, especially within the 5-HT system, cannot be excluded. Furthermore, reports of memory deficits in long-term MDMA users could be supported. Overall, detrimental consequences of acute and chronic MDMA administration were observed, which support findings of reduced cognitive capacity in long-term human MDMA users and indicate immediate reductions in decision making ability.

The minimal effects of WIN on cognition or behaviour, despite possible alterations on a cellular level as indicated by diminished myelination for chronically treated adult rats, was somewhat unexpected. However, differences between the synthetic CB receptor agonist and exogenous cannabinoids found in cannabis exist, and make direct comparisons difficult. At the same time, having equal (null) effects as the vehicle group on decision making tasks demonstrate WIN-only administration to be an appropriate control and adds value to the restricted interaction with MDMA on memory.

Study 2 implements an animal model of chronic (co-)administration of MDMA and a cannabinoid agonist, thereby adding to the knowledge about long-term effects of these drugs on cognition and behaviour in adult and pubertal rats. The only interaction of WIN with MDMA was observed after chronic treatment in the object recognition memory task. Otherwise, no modulatory action of CB1 stimulation on MDMA- induced impairments was evident.

With exception of the impairments in recognition memory, adult rats seemed to be more sensitive to the impairments induced by MDMA and MDMA+WIN administration. However, pubertally treated rats were not unaffected, and functional brain changes cannot be ruled out.
Generalization of these results to human drug users is limited due to differences in doses ingested, route of administration and metabolism. However, if any conclusion can be derived for human

MDMA users, it should be noted that co-consumption of cannabinoid agents does not seem to reduce, but probably exacerbates, MDMA-induced impairments.

Deutsche Zusammenfassung

Einleitung

MDMA (3,4-methylenedioxymetamphethamin, “Ecstasy”) ist eine der meist verbreiteten illegalen Drogen. Allerdings ist der Einfluss von sowohl akutem wie auch chronischem Konsum auf kognitive Funktionen wie Gedächtnis und Entscheidungsverhalten, sowie die den physiologischen und psychologischen Effekten zugrunde liegenden neurobiologischen Mechanismen noch nicht vollständig geklärt. Des Weiteren wird unter Forschern debattiert, ob und in welchem Ausmaß MDMA neurotoxische Wirkung besitzt.
MDMA ist ein synthetisches Amphetaminderivat, welches 1912 als Nebenprodukt der Herstellung eines blutstillenden Medikamentes des pharmazeutischen Konzerns MerckKGaA patentiert wurde und heutzutage den Hauptbestandteil von „Ecstasy“ Tabletten ausmacht. Die überwiegend innerhalb der „Rave“Kultur eingenommenen Pillen führen zu Euphorie, Empathie, Energie sowie pro-sozialen Empfindungen. Negative Wirkungen sind unter anderem Hyperthermie, stark erhöhte Herzschlagrate und Blutdruck, Schwitzen, Schlaflosigkeit und depressive Verstimmung sowie Angst und Konzentrationsschwierigkeiten während des „come downs“. Auch Langzeitbeeinträchtigungen von z. B. Gedächtnisleistung und Entscheidungsverhalten wurden bereits in Studien mit MDMA Konsumenten gezeigt. Eine große Rolle spielt hierbei die durch MDMA hervorgerufene erhöhte Ausschüttung und Wiederaufnahmehemmung der monoaminen Neurotransmitter Serotonin (5-HT), Dopamine (DA) und Noradrenaline (NA). Desweiteren könnte häufige und/oder andauernde MDMA Gabe zu spezifischer Neurotoxizität (bzw. strukturellen/funktionalen Veränderungen) auf serotonerge Axonterminale in frontalen Gehirnarealen führen.
Die Mehrheit der Ecstasykonsumenten sind junge Erwachsene, und fast alle (90-98%) nehmen begleitend andere Drogen, hauptsächlich Cannabis.

Cannabis ist die am häufigsten konsumierte illegale psychoaktive Droge weltweit. Produkte der Hanfpflanze (Cannabis sativa/indica) werden schon seit Jahrtausenden für industrielle und medizinische Zwecke verwendet. Aufgrund neuroprotektiver sowie entzündungshemmender Eigenschaften wird derzeit die medizinische Anwendung von (synthetischen) Cannabisprodukten erforscht; hauptsächlich wird Cannabis aber als Droge konsumiert. Die Herauslösung des psychoaktiven Hauptbestandteils von Cannabis, delta9-Tetrahydrocannabinol (Δ9-THC, THC) im Jahr 196vier führte zur Entdeckung des endocannabinoiden Systems, welches endogene Cannabinoide und deren Rezeptoren einschließt. Aktivierung von cannbinoiden Rezeptoren, speziell Typ1 (CB1) im zentralen Nervensystem trägt maßgeblich zur Steuerung von Reizweiterleitung bei; größtenteils findet eine Hemmung der Ausschüttung von GABA, Glutamat, 5-HT, DA und NA statt. Die Wirkung von Cannabis umfasst, abhängig vom THC Gehalt,

Stimulation, milde Euphorie, Entspannung, erhöhte sensorische Wahrnehmung, reduziertes Schmerzempfinden und Appetitsteigerung. Sowohl akut als auch nach mehrfachem Konsum können negative Effekte wie Verschlechterung der Kurzzeit- und Arbeitsgedächtnisleistung, Konzentration, Aufmerksamkeit, motorische Beeinträchtigung, und psychotische Symptome entstehen. Studien mit Cannabis Konsumenten sowie Verabreichung von cannabinoiden Substanzen an Labortiere zeigten strukturelle und funktionale Veränderungen in bestimmten Gehirnarealen, z. B. im Hippocampus, welcher mit Lernen und Gedächtnis assoziiert ist. Obwohl Cannabisprodukte generell als „weiche Drogen“ wahrgenommen werden, und ihre medizinische Nutzung voranschreitet, wurden Beeinträchtigungen kognitiver Leistung sowie langzeitliche Veränderungen im Gehirn, vor allem nach längerem und schwerem Konsum, nachgewiesen.
Die psychopharmakologische Forschung hat sich sowohl mit MDMA als auch Cannabis beschäftigt - dagegen sind Studien zu Co-Konsum spärlich. Bisherige Ergebnisse deuten auf einen modulierenden Einfluss akuter Verabreichung von cannabinoiden Agonisten auf MDMA-induzierte Verhaltensweisen hin, während chronischer Co-Konsum womöglich zu zusätzlichen Beeinträchtigungen führt. Allerdings sind (Langzeit)Studien in Tiermodellen, welche den Einfluß der jeweiligen Substanz alleine und/oder in Kombination auf Verhaltens- und Gedächtnisleistung sowie kognitive Funktionen testen, rar.

Die vorliegende Arbeit untersucht den Einfluß von MDMA und dem synthetischen cannabinoiden Rezeptor Agonist WIN55,212-2 (WIN), auf verschiedene Formen des Entscheidungsverhaltens, Gedächtnisleistung, physische Aktivität und physiologische Parameter wie Körpertemperatur und Fressverhalten. Innerhalb eines sowohl akuten als auch chronischen systemischen Verabreichungsmodell werden die Effekte beider Substanzen einzeln und in Kombination getestet, um Co-Konsum zu simulieren und interaktive Reaktionen festzustellen. Innerhalb der chronischen Behandlung werden adulte sowie pubertäre Tiere und der Myelinisierungsgrad der zugehörigen Gehirne untersucht, um etwaige vulnerable Perioden für Drogenkonsum oder altersabhängige Unterschiede festzustellen.

Review

In “MDMA & Cannabis: A Mini-Review of Cognitive, Behavioral, and Neurobiological Effects of Co-consumption” untersuchte ich 23 kürzlich veröffentlichte Studien (zwischen 2002 und 2010), welche sich mit der Auswirkung einer Kombination von MDMA und Cannabis oder cannabinoiden Substanzen auf kognitive Funktionen, Verhalten, und neurobiologische Interaktionen befassen. Humanstudien zu kognitiven Funktionen ergeben ein uneinheitliches Bild. Einige zeigen eine Cannabis-abhängige Verringerung der Gedächtnisleistung, und schreiben MDMA Konsum Defizite in Lern- und Entscheidungsverhalten, sowie kognitiver Kontrolle zu. Andere bestätigen einen additiven negativen Effekt gleichzeitiger Einnahme. Eine Möglichkeit, die diskrepanten Ergebnisse

zu erklären ist, dass die Menge und Häufigkeit von begleitendem Cannabiskonsum für die Ausprägung der durch MDMA hervorgerufenen Schwierigkeiten entscheidend sein kann. Allerdings erschweren unterschiedliche Einschlusskriterien (Menge und Häufigkeit des MDMA Missbrauchs, Zeitraum der Abstinenz, Konsum weiterer Drogen) und Kontrollgruppen (Cannabis Konsum einschließend oder ausschließend, abstinente oder naive MDMA User) eindeutige Vergleiche oder Resultate. Die meisten Forschungsarbeiten involvieren Langzeit- „Polydrug"-Konsumenten und sind retrospektiver Natur, während Tierstudien, welche chronischen Co-Konsum modellieren, spärlich sind. In akuten Tiermodellen hingegen wurde z. B. ein regulativer Effekt des cannabinoiden Systems auf die verstärkende/belohnende Wirkung von MDMA, sowie eine Störung des Arbeitsgedächtnisses bzw. Aufhebung der durch MDMA verursachten Defizite im Wiedererkennungstest, bei gleichzeitiger Gabe gezeigt. Diese Studien deuten auf eine Interaktion zwischen veränderter Neurotransmission durch MDMA, speziell der Wirkung auf das serotonerge System, und Aktivierung cannabinoider Rezeptoren im Hippocampus hin. Des weiteren wurden in Tierstudien und Zellkulturen modulierende Effekte von akuter Co-Gabe cannabinoider Liganden auf MDMA-induzierte Veränderungen der Körpertemperatur, Aktivität, und Neurotoxizität gezeigt.

Studie 1

"Acute Co-administration of the Cannabinoid Receptor Agonist WIN55-212,2 does not influence 3,4-methylenedioxymetamphetamine (MDMA)-induced Effects on Effort-based Decision making, Locomotion, Food intake and Body temperature" untersucht Existenz und Ausmaß der akuten Wechselwirkung von MDMA (7.5mg/kg, s.c.) und WIN (1.2mg/kg, i.p.) bei verschiedenen Verhaltensweisen. Die Wirkung dieser Substanzen auf die kognitive Fähigkeit des Entscheidungsverhaltens wurde mittels dem Aufwands-basiertem („effort-based") Paradigma in einem T-Labyrinth geprüft. Sowohl MDMA als auch MDMA+WIN Verabreichung führte zu einer Verringerung der Entscheidungen für den hohen Aufwand-hohe Belohnung-Arm und der Nahrungsaufnahme und zu einer Steigerung der motorischen Aktivität sowie der Exploration (Anzahl der Aufrichtungen), während WIN hier keinen Effekt hervorrief. WIN induzierte eine unerwartete kurzfristige Erhöhung der Körpertemperatur, während sowohl MDMA als auch MDMA+WIN erst eine Stunde nach den Verhaltenstests einen signifikanten Anstieg produzierten. Die einzige (instabile) Interaktion fand sich im Open Field: MDMA führte zu angstähnlichen Verhalten, welches durch Co-Administration von WIN verringert wurde. Die zugrunde liegenden neurobiologischen Mechanismen dieser Effekte bleiben spekulativ. Allerdings zeigen die Ergebnisse, dass akute MDMA Gabe über die bekannten physiologischen Veränderungen (Hyperthermie, erhöhte Aktivität, verringerter Appetit) hinaus auch sofortige Beeinträchtigungen bestimmter kognitiver Funktionen, nämlich Entscheidungsverhalten, hervorruft. Ingesamt hatte akute Co-Administration von WIN kaum modulierende Wirkung auf MDMA-induzierte Verhaltensweisen.

Studie 2

In “Chronic co-administration of the cannabinoid receptor agonist WIN55,212-2 during puberty or adulthood reverses 3,4-methylenedioxymetamphetamine (MDMA)-induced deficits in recognition memory but not in effort-based decision making“ werden die Effekte chronischer Verabreichung von MDMA (7.5mg/kg, s.c.) und WIN (1.2mg/kg, i.p.), alleine oder in Kombination, an adulten (postnataler Tag (PT) >80) oder pubertären (PT 40-65) Ratten untersucht. Nach 25 Tagen irregulärer Behandlung zeigten alle MDMA-behandelten Tiere deutliche Defizite im Objektwiedererkennungstest, einer Aufgabe mittels derer das Kurzzeitgedächtnis getestet wird. Diese Beeinträchtigung war bei pubertären Tieren vier Wochen später noch vorhanden. In der Gruppe, welche MDMA+WIN erhielt, wurde keine solche Verringerung der Gedächtnisleistung gemessen. Des weiteren benötigten sowohl MDMA als auch MDMA+WIN, aber nicht WIN-behandelte adulte Tiere mehr Zeit um optimales aufwands-basiertes („effort-based“) Entscheidungsverhalten wiederzuerlernen. Innerhalb der Tiergruppe, welche während der Pubertät behandelt wurde, zeigten sich dagegen keine Unterschiede. In einer weiteren Form des Entscheidungsverhaltens, dem verzögerungsbasiertem (“delay-based“) Paradigma, waren nach chronischer Verabreichung keinerlei Differenzen erkennbar. Darüber hinaus zeigten alle vier Behandlungsgruppen unabhängig vom Alter ähnliche motorische Aktivität und Explorationsverhalten sowohl kurz nach Beendigung der chronischen Gabe als auch vier Wochen später. Während der Behandlung mit MDMA und MDMA+WIN zeigten pubertäre Ratten verringerte Gewichtszunahme; WIN hatte hier keinen modulierenden Effekt. Zusammenfassend läßt sich sagen, dass chronische MDMA Gabe robuste Langzeitdefizite in Gedächtnisleistung produziert. Dieser Effekt gründet sich mutmaßlich auf veränderte (serotonerge) Neurotransmission und kann durch chronische Stimulation des CB1 Rezeptors aufgehoben werden. Die Interaktion auf neuronaler Ebene ist höchstwahrscheinlich im Hippocampus lokalisiert, wohingegen scheinbar keine Verbesserung von MDMA-induzierten Störungen in präfrontalen Hirngebieten, welche komplexere Aufgaben wie Entscheidungsverhalten steuern, stattfindet.

Fazit

MDMA

Diese Arbeit beschreibt das erste Rattenmodell zu Entscheidungsverhalten unter dem Einfluß von MDMA. Sowohl akute als auch chronische Verabreichung führte zu einer Verschlechterung der aufwandsbasierten Wahl. Zwar wurden keine strukturellen Veränderungen im Gehirn (Myelinisierungsgrad) gemessen, allerdings ist diese Färbemethode auf das Erkennen von weißer Substanz (Axone) begrenzt. So können funktionale Abweichungen, vor allem innerhalb des 5-HT Systems, nicht ausgeschlossen werden. Desweiteren wurde nach Langzeitkonsum von MDMA ein robustes Defizit in Gedächtnisleistung gezeigt. Insgesamt wurden nachteilige und nachhaltige

Konsequenzen von chronischer MDMA Gabe gezeigt, welche somit Ergebnisse von Humanstudien validieren, welche reduzierte kognitive Fähigkeiten bei (langzeit-) MDMA Konsumenten fanden. Zusätzlich weist Studie 1 auf eine sofortige Reduzierung der Fähigkeit, optimale Entscheidungen zu treffen, nach akutem MDMA Konsum hin.

WIN

Die Verabreichung von WIN führte zu unerwartet wenigen Verhaltensänderungen, trotz verringerter Myelinisierung in verschiedenen Gehirnbereichen bei chronisch behandelten adulten (jedoch nicht pubertären) Ratten. Allerdings existieren Differenzen zwischen diesem synthetischen cannabinoid Rezeptor Agonisten und exogenen Cannabinoiden, wie sie in Cannabis vorkommen, daher ist eine Generalisierung der Ergebnisse schwierig. Gleichzeitig unterstützt das Ausbleiben eines WIN-induzierten Effektes auf Entscheidungsverhalten die Rolle des CB1 Rezeptor Agonisten als Kontrolle bzw. modulierende Substanz ohne eigene Wirkung auf diese kognitiven Funktionen. Daher wird die eingeschränkte Interaktion mit MDMA auf Gedächtnisleistung um so bedeutsamer.

MDMA+WIN

In Studie 2 wird ein Tiermodell zur chronischen (Co)-Gabe von MDMA und einem cannabinoiden Agonisten eingeführt. Hiermit wurde das Wissen um die Langzeiteffekte dieser Substanzen auf Verhalten und Kognition in adulten und pubertären Ratten erweitert. Da die einzige Interaktion von WIN mit MDMA innerhalb des Gedächtnistests deutlich wurde, konnte der Hippocampus als eine vulnerable Gehirnregion bestätigt werden. Anderweitig wurden keine modulierenden Effekte der MDMA-induzierten Beeinträchtigungen durch CB1 Stimulation gemessen. Mit Ausnahme der Verschlechterung im Wiedererkennungstest scheinen adulte Ratten sensibler auf durch MDMA und MDMA+WIN Verabreichung hervorgerufene Defizite zu reagieren. Andererseits waren Ratten, die während der Pubertät behandelt wurden, ebenfalls betroffen, und funktionale Schäden im Gehirn zusätzlich zu der Verringerung des Myelinisierungsgrades können nicht ausgeschlossen werden.

Eine Generalisierung dieser Ergebnisse auf menschliche Drogenkonsumenten scheint nicht angemessen, da die Unterschiede, z. B. in Dosierung, Art der Verabreichung, und Metabolismus zu groß sind. Falls ein Rückschluß gezogen werden sollte, läßt sich höchstens feststellen, daß Co-Konsum eines cannabinioden Stoffes MDMA-induzierte Beeinträchtigungen nicht reduziert, sondern wahrscheinlicher, verschlimmert.

References

World Drug Report 2010 of the United Nations Office on Drugs and Crime. 2010. United Nations Publication. Ref Type: Report

Abush H, Akirav I (2012). Short- and long-term cognitive effects of chronic cannabinoids administration in late-adolescence rats. *PLoS One* 7:e31731.

Aldrich M (1997). History of therapeutic cannabis. In: *Cannabis in medical practise: A legal, historical and pharmacological overview of the therapeutic use of marijuana.* Mathre ML (editor). Jefferson, USA: McFarland & Company, Inc. pp. 35-55.

Ameri A (1999). The effects of cannabinoids on the brain. *Prog Neurobiol* 58:315-348.

Aragona M, Onesti E, Tomassini V, Conte A, Gupta S, Gilio F, Pantano P, Pozzilli C, Inghilleri M (2009). Psychopathological and cognitive effects of therapeutic cannabinoids in multiple sclerosis: a double-blind, placebo controlled, crossover study. *Clin Neuropharmacol* 32:41-47.

Arseneault L, Cannon M, Witton J, Murray RM (2004). Causal association between cannabis and psychosis: examination of the evidence. *Br J Psychiatry* 184:110-117.

Asbridge M, Hayden JA, Cartwright JL (2012). Acute cannabis consumption and motor vehicle collision risk: systematic review of observational studies and meta-analysis. *BMJ* 344:e536.

Ashton CH (2001). Pharmacology and effects of cannabis: a brief review. *Br J Psychiatry* 178:101-106.

Assadi SM, Yucel M, Pantelis C (2009). Dopamine modulates neural networks involved in effort-based decision-making. *Neurosci Biobehav Rev* 33:383-393.

Baker D, Pryce G, Giovannoni G, Thompson AJ (2003). The therapeutic potential of cannabis. *Lancet Neurol* 2:291-298.

Bankson MG, Cunningham KA (2001). 3,4-Methylenedioxymethamphetamine (MDMA) as a unique model of serotonin receptor function and serotonin-dopamine interactions. *J Pharmacol Exp Ther* 297:846-852.

Bardgett ME, Depenbrock M, Downs N, Points M, Green L (2009). Dopamine modulates effort-based decision making in rats. *Behav Neurosci* 123:242-251.

Bartzokis G (2012). Neuroglialpharmacology: Myelination as a shared mechanism of action of psychotropic treatments. *Neuropharmacology*.

Baumann MH, Wang X, Rothman RB (2007). 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. *Psychopharmacology (Berl)* 189:407-424.

Bechara A, Tranel D, Damasio H (2000). Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. *Brain* 123 (Pt 11):2189-2202.

Benzenhofer U, Passie T (2006). [The early history of "Ecstasy"]. *Nervenarzt* 77:95-99.

Benzenhofer U, Passie T (2010). Rediscovering MDMA (ecstasy): the role of the American chemist Alexander T. Shulgin. *Addiction* 105:1355-1361.

Bizot J, Le Bihan C, Puech AJ, Hamon M, Thiebot M (1999). Serotonin and tolerance to delay of reward in rats. *Psychopharmacology (Berl)* 146:400-412.

Block RI, O'Leary DS, Hichwa RD, Augustinack JC, Boles Ponto LL, Ghoneim MM, Arndt S, Hurtig RR, Watkins GL, Hall JA, Nathan PE, Andreasen NC (2002). Effects of frequent marijuana use on memory-related regional cerebral blood flow. *Pharmacol Biochem Behav* 72:237-250.

Bolla KI, Eldreth DA, Matochik JA, Cadet JL (2005). Neural substrates of faulty decision-making in abstinent marijuana users. *Neuroimage* 26:480-492.

Bontempi B, Laurent-Demir C, Destrade C, Jaffard R (1999). Time-dependent reorganization of brain circuitry underlying long-term memory storage. *Nature* 400:671-675.

Boys A, Marsden J, Strang J (2001). Understanding reasons for drug use amongst young people: a functional perspective. *Health Educ Res* 16:457-469.

Braida D, Iosue S, Pegorini S, Sala M (2005). 3,4 Methylenedioxymethamphetamine-induced conditioned place preference (CPP) is mediated by endocannabinoid system. *Pharmacol Res* 51:177-182.

Braida D, Sala M (2002). Role of the endocannabinoid system in MDMA intracerebral self-administration in rats. *Br J Pharmacol* 136:1089-1092.

Brown J, McKone E, Ward J (2010). Deficits of long-term memory in ecstasy users are related to cognitive complexity of the task. *Psychopharmacology (Berl)* 209:51-67.

Butler GK, Montgomery AM (2004). Impulsivity, risk taking and recreational 'ecstasy' (MDMA) use. *Drug Alcohol Depend* 76:55-62.

Byrne T, Baker LE, Poling A (2000). MDMA and learning: effects of acute and neurotoxic exposure in the rat. *Pharmacol Biochem Behav* 66:501-508.

Capela JP, Carmo H, Remiao F, Bastos ML, Meisel A, Carvalho F (2009). Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: an overview. *Mol Neurobiol* 39:210-271.

Capela JP, Meisel A, Abreu AR, Branco PS, Ferreira LM, Lobo AM, Remiao F, Bastos ML, Carvalho F (2006). Neurotoxicity of Ecstasy metabolites in rat cortical neurons, and influence of hyperthermia. *J Pharmacol Exp Ther* 316:53-61.

Cardinal RN, Pennicott DR, Sugathapala CL, Robbins TW, Everitt BJ (2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science* 292:2499-2501.

Cardinal RN (2006). Neural systems implicated in delayed and probabilistic reinforcement. *Neural Networks* 19:1277-1301.

Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A, Arseneault L, Williams B, Braithwaite A, Poulton R, Craig IW (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry* 57:1117-1127.

Chaperon F, Thiebot MH (1999). Behavioral effects of cannabinoid agents in animals. *Crit Rev Neurobiol* 13:243-281.

Chopra GS, Smith JW (1974). Psychotic reactions following cannabis use in East Indians. *Arch Gen Psychiatry* 30:24-27.

Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A (2007). Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *The Lancet* 370:1706-1713.

Clark L, Roiser JP, Robbins TW, Sahakian BJ (2009). Disrupted 'reflection' impulsivity in cannabis users but not current or former ecstasy users. *J Psychopharmacol* 23:14-22.

Cole JC, Sumnall HR (2003). The pre-clinical behavioural pharmacology of 3,4-methylenedioxymethamphetamine (MDMA). *Neurosci Biobehav Rev* 27:199-217.

Collins DR, Pertwee RG, Davies SN (1995). Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. *Br J Pharmacol* 115:869-870.

Consroe P, Musty R, Rein J, Tillery W, Pertwee R (1997). The perceived effects of smoked cannabis on patients with multiple sclerosis. *Eur Neurol* 38:44-48.

Costas J, Sanjuan J, Ramos-Rios R, Paz E, Agra S, Tolosa A, Paramo M, Brenlla J, Arrojo M (2011). Interaction between COMT haplotypes and cannabis in schizophrenia: a case-only study in two samples from Spain. *Schizophr Res* 127:22-27.

Cowan RL (2007). Neuroimaging research in human MDMA users: a review. *Psychopharmacology (Berl)* 189:539-556.

Croft RJ, Mackay AJ, Mills AT, Gruzelier JG (2001). The relative contributions of ecstasy and cannabis to cognitive impairment. *Psychopharmacology (Berl)* 153:373-379.

D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT, Braley G, Gueorguieva R, Krystal JH (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* 29:1558-1572.

Dafters RI, Hoshi R, Talbot AC (2004). Contribution of cannabis and MDMA ("ecstasy") to cognitive changes in long-term polydrug users. *Psychopharmacology (Berl)* 173:405-410.

Danielsson AK, Wennberg P, Hibell B, Romelsjo A (2011). Alcohol use, heavy episodic drinking, and subsequent problems among adolescents in 23 European countries: does the prevention paradox apply? *Addiction*.

Darmani NA, Janoyan JJ, Kumar N, Crim JL (2003). Behaviorally active doses of the CB1 receptor antagonist SR 141716A increase brain serotonin and dopamine levels and turnover. *Pharmacol Biochem Behav* 75:777-787.

Daumann J, Fischermann T, Heekeren K, Henke K, Thron A, Gouzoulis-Mayfrank E (2005). Memory-related hippocampal dysfunction in poly-drug ecstasy (3,4-methylenedioxymethamphetamine) users. *Psychopharmacology (Berl)* 180:607-611.

Daumann J, Hensen G, Thimm B, Rezk M, Till B, Gouzoulis-Mayfrank E (2004). Self-reported psychopathological symptoms in recreational ecstasy (MDMA) users are mainly associated with regular cannabis use: further evidence from a combined cross-sectional/longitudinal investigation. *Psychopharmacology (Berl)* 173:398-404.

de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R, Roset PN, Segura J, Cami J (2000). Non-linear pharmacokinetics of MDMA ('ecstasy') in humans. *Br J Clin Pharmacol* 49:104-109.

de Lago E, Moreno-Martet M, Cabranes A, Ramos JA, Fernandez-Ruiz J (2012). Cannabinoids ameliorate disease progression in a model of multiple sclerosis in mice, acting preferentially through CB(1) receptor-mediated anti-inflammatory effects. *Neuropharmacology*.

De Petrocellis L, DiMarzo V (2010). Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. *J Neuroimmune Pharmacol* 5:103-121.

de Sola LS, Miguelez-Pan M, Pena-Casanova J, Poudevida S, Farre M, Pacifici R, Bohm P, Abanades S, Verdejo GA, Langohr K, Zuccaro P, de la TR (2008). Cognitive performance in recreational ecstasy polydrug users: a two-year follow-up study. *J Psychopharmacol* 22:498-510.

Degenhardt L, Hall W, (editors) (2010). The health and psychological effects of "ecstasy" (MDMA) use. *National drug and alcohol research centre, NDARC Monograph No 62*.

Degenhardt L, Hall W, Lynskey M (2001). The relationship between cannabis use and other substance use in the general population. *Drug Alcohol Depend* 64:319-327.

Degenhardt L, Hall W, Lynskey M (2003). Exploring the association between cannabis use and depression. *Addiction* 98:1493-1504.

Delaforge M, Jaouen M, Bouille G (1999). Inhibitory metabolite complex formation of methylenedioxymethamphetamine with rat and human cytochrome P450. Particular involvement of CYP 2D. *Environ Toxicol Pharmacol* 7:153-158.

Denk F, Walton ME, Jennings KA, Sharp T, Rushworth MF, Bannerman DM (2005). Differential involvement of serotonin and dopamine systems in cost-benefit decisions about delay or effort. *Psychopharmacology (Berl)* 179:587-596.

DeSouza I, Kelly JP, Harkin AJ, Leonard BE (1997). An appraisal of the pharmacological and toxicological effects of a single oral administration of 3,4-methylenedioxymethamphetamine (MDMA) in the rat. *Pharmacol Toxicol* 80:207-210.

Devane WA, Dysarz FA, III, Johnson MR, Melvin LS, Howlett AC (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 34:605-613.

Doblin R (2002). A clinical plan for MDMA (Ecstasy) in the treatment of posttraumatic stress disorder (PTSD): partnering with the FDA. *J Psychoactive Drugs* 34:185-194.

Docherty JR, Green AR (2010). The role of monoamines in the changes in body temperature induced by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and its derivatives. *Br J Pharmacol* 160:1029-1044.

Drews E, Schneider M, Koch M (2005). Effects of the cannabinoid receptor agonist win 55,212-2 on operant behavior and locomotor activity in rats. *Pharmacology Biochemistry and Behavior* 80:145-150.

Dumont G, Van Hasselt J, de Kam M, van Gerven J, Touw D, Buitelaar J, Verkes R (2010). Acute psychomotor, memory and subjective effects of MDMA and THC co-administration over time in healthy volunteers. *J Psychopharmacol*.

Dumont GJ, Kramers C, Sweep FC, Touw DJ, van Hasselt JG, de Kam M, van Gerven JM, Buitelaar JK, Verkes RJ (2009). Cannabis coadministration potentiates the effects of "ecstasy" on heart rate and temperature in humans. *Clin Pharmacol Ther* 86:160-166.

Elphick MR, Egertova M (2001). The neurobiology and evolution of cannabinoid signalling. *Philos Trans R Soc Lond B Biol Sci* 356:381-408.

EMCDDA (2008). A cannabis reader: global issues and local experiences. Perspectives on cannabis controversies, treatment and regulation in Europe. *European Monitoring Centre for Drugs and Drug Abuse*.

EMCDDA (2009). Selected issue. Polydrug use:patterns and responses. *European Monitoring Center for Drugs and Drug Abuse*.

EMCDDA (2010). The EMCDDA annual report 2010: the state of the drugs problem in Europe. *Euro Surveill* 15.

Emery B (2010). Regulation of oligodendrocyte differentiation and myelination. *Science* 330:779-782.

Ennaceur A, Delacour J (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* 31:47-59.

Erinoff L (1995). General considerations in assessing neurotoxicity using neuroanatomical methods. *Neurochemistry International* 26:111-114.

Ernst M, Bolla K, Mouratidis M, Contoreggi C, Matochik JA, Kurian V, Cadet JL, Kimes AS, London ED (2002). Decision-making in a risk-taking task: a PET study. *Neuropsychopharmacology* 26:682-691.

Farre M, de la TR, Mathuna BO, Roset PN, Peiro AM, Torrens M, Ortuno J, Pujadas M, Cami J (2004). Repeated doses administration of MDMA in humans: pharmacological effects and pharmacokinetics. *Psychopharmacology (Berl)* 173:364-375.

Fernandez-Serrano MJ, Perez-Garcia M, Verdejo-Garcia A (2011). What are the specific vs. generalized effects of drugs of abuse on neuropsychological performance? *Neurosci Biobehav Rev* 35:377-406.

Ferraz-de-Paula V, Stankevicius D, Ribeiro A, Pinheiro ML, Rodrigues-Costa EC, Florio JC, Lapachinske SF, Moreau RL, Palermo-Neto J (2011). Differential behavioral outcomes of 3,4-methylenedioxymethamphetamine (MDMA-ecstasy) in anxiety-like responses in mice. *Braz J Med Biol Res* 44:428-437.

Fiaschi AI, Cerretani D (2010). Causes and effects of cellular oxidative stress as a result of MDMA abuse. *Curr Pharm Biotechnol* 11:444-452.

Fisk JE, Montgomery C, Wareing M, Murphy PN (2006). The effects of concurrent cannabis use among ecstasy users: neuroprotective or neurotoxic? *Hum Psychopharmacol* 21:355-366.

Fitzgerald JL, Reid JJ (1990). Effects of methylenedioxymethamphetamine on the release of monoamines from rat brain slices. *Eur J Pharmacol* 191:217-220.

Floresco SB, Ghods-Sharifi S (2007). Amygdala-prefrontal cortical circuitry regulates effort-based decision making. *Cereb Cortex* 17:251-260.

Floresco SB, St Onge JR, Ghods-Sharifi S, Winstanley CA (2008). Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. *Cogn Affect Behav Neurosci* 8:375-389.

Fox HC, Parrott AC, Turner JJ (2001). Ecstasy use: cognitive deficits related to dosage rather than self-reported problematic use of the drug. *J Psychopharmacol* 15:273-281.

Fraga D, Zanoni CI, Rae GA, Parada CA, Souza GE (2009). Endogenous cannabinoids induce fever through the activation of CB1 receptors. *Br J Pharmacol* 157:1494-1501.

Freedman RR, Johanson CE, Tancer ME (2005). Thermoregulatory effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology (Berl)* 183:248-256.

Freudenmann RW, Oxler F, Bernschneider-Reif S (2006). The origin of MDMA (ecstasy) revisited: the true story reconstructed from the original documents. *Addiction* 101:1241-1245.

Frith CH, Chang LW, Lattin DL, Walls RC, Hamm J, Doblin R (1987). Toxicity of methylenedioxymethamphetamine (MDMA) in the dog and the rat. *Fundam Appl Toxicol* 9:110-119.

Gessa GL, Casu MA, Carta G, Mascia MS (1998). Cannabinoids decrease acetylcholine release in the medial-prefrontal cortex and hippocampus, reversal by SR 141716A. *Eur J Pharmacol* 355:119-124.

Ghods-Sharifi S, Floresco SB (2010). Differential effects on effort discounting induced by inactivations of the nucleus accumbens core or shell. *Behav Neurosci* 124:179-191.

Gouzoulis-Mayfrank E, Daumann J (2006a). Neurotoxicity of methylenedioxyamphetamines (MDMA; ecstasy) in humans: how strong is the evidence for persistent brain damage? *Addiction* 101:348-361.

Gouzoulis-Mayfrank E, Daumann J, Tuchtenhagen F, Pelz S, Becker S, Kunert HJ, Fimm B, Sass H (2000). Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). *J Neurol Neurosurg Psychiatry* 68:719-725.

Gouzoulis-Mayfrank E, Thimm B, Rezk M, Hensen G, Daumann J (2003). Memory impairment suggests hippocampal dysfunction in abstinent ecstasy users. *Prog Neuropsychopharmacol Biol Psychiatry* 27:819-827.

Gouzoulis-Mayfrank E, Daumann J (2006b). The confounding problem of polydrug use in recreational ecstasy/MDMA users: a brief overview. *J Psychopharmacol* 20:188-193.

Grant S, Contoreggi C, London ED (2000). Drug abusers show impaired performance in a laboratory test of decision making. *Neuropsychologia* 38:1180-1187.

Green AR, Gabrielsson J, Marsden CA, Fone KC (2009). MDMA: On the translation from rodent to human dosing. *Psychopharmacology (Berl)*.

Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol Rev* 55:463-508.

Grotenhermen F (1999). [Some practice-relevant aspects of the pharmacokinetics of THC]. *Forsch Komplementarmed* 6 Suppl 3:37-39.

Grov C, Kelly BC, Parsons JT (2009). Polydrug use among club-going young adults recruited through time-space sampling. *Subst Use Misuse* 44:848-864.

Grundy RI, Rabuffetti M, Beltramo M (2001). Cannabinoids and neuroprotection. *Mol Neurobiol* 24:29-51.

Gudelsky GA, Nash JF (1996). Carrier-mediated release of serotonin by 3,4-methylenedioxymethamphetamine: implications for serotonin-dopamine interactions. *J Neurochem* 66:243-249.

Gurtman CG, Morley KC, Li KM, Hunt GE, McGregor IS (2002). Increased anxiety in rats after 3,4-methylenedioxymethamphetamine: association with serotonin depletion. *Eur J Pharmacol* 446:89-96.

Hadamitzky M, Feja M, Becker T, Koch M (2009). Effects of acute systemic administration of serotonin2A/C receptor ligands in a delay-based decision-making task in rats. *Behav Pharmacol* 20:415-423.

Hall W (1998). The respiratory risks of cannabis smoking. *Addiction* 93:1461-1463.

Hall W, Degenhardt L (2009). Adverse health effects of non-medical cannabis use. *Lancet* 374:1383-1391.

Hall WD, Degenhardt LJ, Currow D (2001). Allowing the medical use of cannabis. *Med J Aust* 175:39-40.

Haller J, Varga B, Ledent C, Freund TF (2004). CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. *Behav Pharmacol* 15:299-304.

Halpern JH, Sherwood AR, Hudson JI, Gruber S, Kozin D, Pope HG, Jr. (2011). Residual neurocognitive features of long-term ecstasy users with minimal exposure to other drugs. *Addiction* 106:777-786.

Hampson AJ, Grimaldi M, Axelrod J, Wink D (1998). Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci U S A* 95:8268-8273.

Hanson KL, Luciana M, Sullwold K (2008). Reward-related decision-making deficits and elevated impulsivity among MDMA and other drug users. *Drug and Alcohol Dependence* 96:99-110.

Hartline DK, Colman DR (2007). Rapid conduction and the evolution of giant axons and myelinated fibers. *Curr Biol* 17:R29-R35.

Hatzidimitriou G, McCann UD, Ricaurte GA (1999). Altered serotonin innervation patterns in the forebrain of monkeys treated with (+/-)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. *J Neurosci* 19:5096-5107.

Henquet C, Rosa A, Krabbendam L, Papiol S, Fananas L, Drukker M, Ramaekers JG, van Os J (2006). An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology* 31:2748-2757.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, De Costa BR, Rice KC (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 11:563-583.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, De Costa BR, Rice KC (1990). Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A* 87:1932-1936.

Heydari A, Yeo KR, Lennard MS, Ellis SW, Tucker GT, Rostami-Hodjegan A (2004). Mechanism-based inactivation of CYP2D6 by methylenedioxymethamphetamine. *Drug Metab Dispos* 32:1213-1217.

Homberg JR (2012). Serotonin and decision making processes. *Neurosci Biobehav Rev* 36:218-236.

Homberg JR, Pattij T, Janssen MC, Ronken E, De Boer SF, Schoffelmeer AN, Cuppen E (2007). Serotonin transporter deficiency in rats improves inhibitory control but not behavioural flexibility. *Eur J Neurosci* 26:2066-2073.

Honarmand K, Tierney MC, O'Connor P, Feinstein A (2011). Effects of cannabis on cognitive function in patients with multiple sclerosis. *Neurology* 76:1153-1160.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 54:161-202.

Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ (2004). Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* 47 Suppl 1:345-358.

Iversen L (2003). Cannabis and the brain. *Brain* 126:1252-1270.

Iversen L (2005). Long-term effects of exposure to cannabis. *Curr Opin Pharmacol* 5:69-72.

Jacobsen LK, Mencl WE, Pugh KR, Skudlarski P, Krystal JH (2004). Preliminary evidence of hippocampal dysfunction in adolescent MDMA ("ecstasy") users: possible relationship to neurotoxic effects. *Psychopharmacology (Berl)* 173:383-390.

Jager G, de Win MM, van dT, I, Schilt T, Kahn RS, van den BW, van Ree JM, Ramsey NF (2008). Assessment of cognitive brain function in ecstasy users and contributions of other drugs of abuse: results from an FMRI study. *Neuropsychopharmacology* 33:247-258.

Jarbe TU, Ross T, DiPatrizio NV, Pandarinathan L, Makriyannis A (2006). Effects of the CB1R agonist WIN-55,212-2 and the CB1R antagonists SR-141716 and AM-1387: open-field examination in rats. *Pharmacol Biochem Behav* 85:243-252.

Jean A, Conductier G, Manrique C, Bouras C, Berta P, Hen R, Charnay Y, Bockaert J, Compan V (2007). Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens. *Proc Natl Acad Sci U S A* 104:16335-16340.

Jensen KF, Olin J, Haykal-Coates N, O'Callaghan J, Miller DB, de Olmos JS (1993). Mapping toxicant-induced nervous system damage with a cupric silver stain: a quantitative analysis of neural degeneration induced by 3,4-methylenedioxymethamphetamine. *NIDA Res Monogr* 136:133-149.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M (2009). Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* 89:309-380.

Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, Ohno-Shosaku T, Kano M (2006). The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J Neurosci* 26:2991-3001.

Kay C, Harper DN, Hunt M (2010). Differential effects of MDMA and scopolamine on working versus reference memory in the radial arm maze task. *Neurobiol Learn Mem* 93:151-156.

Kelly PA, Ritchie IM, Quate L, McBean DE, Olverman HJ (2002). Functional consequences of perinatal exposure to 3,4-methylenedioxymethamphetamine in rat brain. *Br J Pharmacol* 137:963-970.

Keyes KM, Martins SS, Hasin DS (2008). Past 12-month and lifetime comorbidity and poly-drug use of ecstasy users among young adults in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Drug Alcohol Depend* 97:139-149.

Kindlundh-Högberg AMS, Schiöth HB, Svenningsson P (2007). Repeated intermittent MDMA binges reduce DAT density in mice and SERT density in rats in reward regions of the adolescent brain. *NeuroToxicology* 28:1158-1169.

Kirkham TC (2005). Endocannabinoids in the regulation of appetite and body weight. *Behav Pharmacol* 16:297-313.

Kirkpatrick MG, Gunderson EW, Perez AY, Haney M, Foltin RW, Hart CL (2011). A direct comparison of the behavioral and physiological effects of methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology (Berl)*.

Kivell B, Day D, Bosch P, Schenk S, Miller J (2010). MDMA causes a redistribution of serotonin transporter from the cell surface to the intracellular compartment by a mechanism independent of phospho-p38-mitogen activated protein kinase activation. *Neuroscience* 168:82-95.

Kuepper R, van Os J, Lieb R, Wittchen HU, Hofler M, Henquet C (2011). Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. *BMJ* 342:d738.

Kumar RN, Chambers WA, Pertwee RG (2001). Pharmacological actions and therapeutic uses of cannabis and cannabinoids. *Anaesthesia* 56:1059-1068.

Kurniawan IT, Guitart-Masip M, Dolan RJ (2011). Dopamine and effort-based decision making. *Front Neurosci* 5:81.

Kuypers KP, Ramaekers JG (2007). Acute dose of MDMA (75 mg) impairs spatial memory for location but leaves contextual processing of visuospatial information unaffected. *Psychopharmacology (Berl)* 189:557-563.

Lam DD, Garfield AS, Marston OJ, Shaw J, Heisler LK (2010). Brain serotonin system in the coordination of food intake and body weight. *Pharmacol Biochem Behav* 97:84-91.

Lamers CT, Bechara A, Rizzo M, Ramaekers JG (2006). Cognitive function and mood in MDMA/THC users, THC users and non-drug using controls. *J Psychopharmacol* 20:302-311.

Lau T, Schloss P (2008). The cannabinoid CB1 receptor is expressed on serotonergic and dopaminergic neurons. *Eur J Pharmacol* 578:137-141.

Little PJ, Compton DR, Johnson MR, Melvin LS, Martin BR (1988). Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J Pharmacol Exp Ther* 247:1046-1051.

Liu YP, Wilkinson LS, Robbins TW (2004). Effects of acute and chronic buspirone on impulsive choice and efflux of 5-HT and dopamine in hippocampus, nucleus accumbens and prefrontal cortex. *Psychopharmacology (Berl)* 173:175-185.

Lopez-Moreno JA, Gonzalez-Cuevas G, Moreno G, Navarro M (2008). The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. *Addict Biol* 13:160-187.

Lundqvist T (2010). Imaging cognitive deficits in drug abuse. *Curr Top Behav Neurosci* 3:247-275.

Macleod J, Oakes R, Copello A, Crome I, Egger M, Hickman M, Oppenkowski T, Stokes-Lampard H, Davey SG (2004). Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. *Lancet* 363:1579-1588.

Malberg JE, Seiden LS (1998). Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. *J Neurosci* 18:5086-5094.

Manzanedo C, Rodriguez-Arias M, Daza-Losada M, Maldonado C, Aguilar MA, Minarro J (2010). Effect of the CB1 cannabinoid agonist WIN 55212-2 on the acquisition and reinstatement of MDMA-induced conditioned place preference in mice. *Behav Brain Funct* 6:19.

Manzo M (1988). Dronabinol and nabilone ease cancer chemotherapy. *Nursing* 18:81.

Mayerhofer A, Kovar KA, Schmidt WJ (2001). Changes in serotonin, dopamine and noradrenaline levels in striatum and nucleus accumbens after repeated administration of the abused drug MDMA in rats. *Neurosci Lett* 308:99-102.

McCann UD, Mertl M, Eligulashvili V, Ricaurte GA (1999). Cognitive performance in (+/-) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") users: a controlled study. *Psychopharmacology (Berl)* 143:417-425.

McCann UD, Ridenour A, Shaham Y, Ricaurte GA (1994). Serotonin neurotoxicity after (+/-)3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy"): a controlled study in humans. *Neuropsychopharmacology* 10:129-138.

McCann UD, Szabo Z, Vranesic M, Palermo M, Mathews WB, Ravert HT, Dannals RF, Ricaurte GA (2008). Positron emission tomographic studies of brain dopamine and serotonin transporters in abstinent (+/-)3,4-methylenedioxymethamphetamine ("ecstasy") users: relationship to cognitive performance. *Psychopharmacology (Berl)* 200:439-450.

McDonald J, Schleifer L, Richards JB, de Wit H (2003). Effects of THC on behavioral measures of impulsivity in humans. *Neuropsychopharmacology* 28:1356-1365.

McGregor IS, Clemens KJ, Van der PG, Li KM, Hunt GE, Chen F, Lawrence AJ (2003). Increased anxiety 3 months after brief exposure to MDMA ("Ecstasy") in rats: association with altered 5-HT transporter and receptor density. *Neuropsychopharmacology* 28:1472-1484.

Mechan AO, Esteban B, O'Shea E, Elliott JM, Colado MI, Green AR (2002). The pharmacology of the acute hyperthermic response that follows administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to rats. *Br J Pharmacol* 135:170-180.

Mechan AO, O'Shea E, Elliott JM, Colado MI, Green AR (2001). A neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) to rats results in a long-term defect in thermoregulation. *Psychopharmacology (Berl)* 155:413-418.

Mechoulam R, Gaoni Y (1967). The absolute configuration of delta-1-tetrahydrocannabinol, the major active constituent of hashish. *Tetrahedron Lett* 12:1109-1111.

Medina KL, Shear PK (2007). Anxiety, depression, and behavioral symptoms of executive dysfunction in ecstasy users: contributions of polydrug use. *Drug Alcohol Depend* 87:303-311.

Merroun I, Errami M, Hoddah H, Urbano G, Porres JM, Aranda P, Llopis J, Lopez-Jurado M (2009). Influence of intracerebroventricular or intraperitoneal administration of cannabinoid receptor agonist (WIN 55,212-2) and inverse agonist (AM 251) on the regulation of food intake and hypothalamic serotonin levels. *Br J Nutr* 101:1569-1578.

Meyer JS, Piper BJ, Vancollie VE (2008). Development and characterization of a novel animal model of intermittent MDMA ("Ecstasy") exposure during adolescence. *Ann N Y Acad Sci* 1139:151-163.

Mogenson GJ, Jones DL, Yim CY (1980). From motivation to action: functional interface between the limbic system and the motor system. *Prog Neurobiol* 14:69-97.

Mohamed WM, Hamida SB, Cassel JC, de Vasconcelos AP, Jones BC (2011). MDMA: Interactions with other psychoactive drugs. *Pharmacol Biochem Behav*.

Monks TJ, Jones DC, Bai F, Lau SS (2004). The role of metabolism in 3,4-(+)-methylenedioxyamphetamine and 3,4-(+)-methylenedioxymethamphetamine (ecstasy) toxicity. *Ther Drug Monit* 26:132-136.

Moreira FA, Grieb M, Lutz B (2009). Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Pract Res Clin Endocrinol Metab* 23:133-144.

Morgan CJ, Schafer G, Freeman TP, Curran HV (2010). Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study: naturalistic study [corrected]. *Br J Psychiatry* 197:285-290.

Morgan MJ, Impallomeni LC, Pirona A, Rogers RD (2006). Elevated impulsivity and impaired decision-making in abstinent Ecstasy (MDMA) users compared to polydrug and drug-naive controls. *Neuropsychopharmacology* 31:1562-1573.

Morgan MJ, McFie L, Fleetwood H, Robinson JA (2002). Ecstasy (MDMA): are the psychological problems associated with its use reversed by prolonged abstinence? *Psychopharmacology (Berl)* 159:294-303.

Morini R, Mlinar B, Baccini G, Corradetti R (2011). Enhanced hippocampal long-term potentiation following repeated MDMA treatment in Dark-Agouti rats. *Eur Neuropsychopharmacol* 21:80-91.

Morley KC, Li KM, Hunt GE, Mallet PE, McGregor IS (2004). Cannabinoids prevent the acute hyperthermia and partially protect against the 5-HT depleting effects of MDMA ("Ecstasy") in rats. *Neuropharmacology* 46:954-965.

Morley KC, McGregor IS (2000). (+/-)-3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') increases social interaction in rats. *Eur J Pharmacol* 408:41-49.

Morton J (2005). Ecstasy: pharmacology and neurotoxicity. *Curr Opin Pharmacol* 5:79-86.

Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365:61-65.

Murphy PN, Wareing M, Fisk JE, Montgomery C (2009). Executive working memory deficits in abstinent ecstasy/MDMA users: a critical review. *Neuropsychobiology* 60:159-175.

Murray RM, Morrison PD, Henquet C, Di Forti M (2007). Cannabis, the mind and society: the hash realities. *Nat Rev Neurosci* 8:885-895.

Nagayasu K, Kitaichi M, Shirakawa H, Nakagawa T, Kaneko S (2010). Sustained exposure to 3,4-methylenedioxymethamphetamine induces the augmentation of exocytotic serotonin release in rat organotypic raphe slice cultures. *J Pharmacol Sci* 113:197-201.

Nawata Y, Hiranita T, Yamamoto T (2010). A cannabinoid CB(1) receptor antagonist ameliorates impairment of recognition memory on withdrawal from MDMA (Ecstasy). *Neuropsychopharmacology* 35:515-520.

Nestor L, Roberts G, Garavan H, Hester R (2008). Deficits in learning and memory: parahippocampal hyperactivity and frontocortical hypoactivity in cannabis users. *Neuroimage* 40:1328-1339.

Novotna A, Mares J, Ratcliffe S, Novakova I, Vachova M, Zapletalova O, Gasperini C, Pozzilli C, Cefaro L, Comi G, Rossi P, Ambler Z, Stelmasiak Z, Erdmann A, Montalban X, Klimek A, Davies P (2011). A randomized, double-blind, placebo-controlled, parallel-group, enriched-design study of nabiximols* (Sativex((R))), as add-on therapy, in subjects with refractory spasticity caused by multiple sclerosis. *Eur J Neurol* 18:1122-1131.

Nulsen CE, Fox AM, Hammond GR (2010). Differential effects of ecstasy on short-term and working memory: a meta-analysis. *Neuropsychol Rev* 20:21-32.

O'Shea E, Escobedo I, Orio L, Sanchez V, Navarro M, Green AR, Colado MI (2005). Elevation of ambient room temperature has differential effects on MDMA-induced 5-HT and dopamine release in striatum and nucleus accumbens of rats. *Neuropsychopharmacology* 30:1312-1323.

O'Shea E, Esteban B, Camarero J, Green AR, Colado MI (2001). Effect of GBR 12909 and fluoxetine on the acute and long term changes induced by MDMA ('ecstasy') on the 5-HT and dopamine concentrations in mouse brain. *Neuropharmacology* 40:65-74.

O'Shea E, Granados R, Esteban B, Colado MI, Green AR (1998). The relationship between the degree of neurodegeneration of rat brain 5-HT nerve terminals and the dose and frequency of administration of MDMA ('ecstasy'). *Neuropharmacology* 37:919-926.

Parrott AC (2004). Is ecstasy MDMA? A review of the proportion of ecstasy tablets containing MDMA, their dosage levels, and the changing perceptions of purity. *Psychopharmacology (Berl)* 173:234-241.

Parrott AC, Gouzoulis-Meyfrank E, Rodgers J, Solowij N (2004). Ecstasy/MDMA and cannabis: the complexities of their interactive neuropsychobiological effects. *J Psychopharmacol* 18:572-575.

Parrott AC, Milani RM, Gouzoulis-Mayfrank E, Daumann J (2007). Cannabis and Ecstasy/MDMA (3,4-methylenedioxymethamphetamine): an analysis of their neuropsychobiological interactions in recreational users. *J Neural Transm* 114:959-968.

Patel S, Cravatt BF, Hillard CJ (2005). Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala. *Neuropsychopharmacology* 30:497-507.

Pattij T, Janssen MC, Schepers I, Gonzalez-Cuevas G, de Vries TJ, Schoffelmeer AN (2007). Effects of the cannabinoid CB1 receptor antagonist rimonabant on distinct measures of impulsive behavior in rats. *Psychopharmacology (Berl)* 193:85-96.

Peng S, Zhang Y, Zhang J, Wang H, Ren B (2011). Glutamate receptors and signal transduction in learning and memory. *Mol Biol Rep* 38:453-460.

Pertwee RG (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther* 74:129-180.

Pertwee RG (2008). Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addict Biol* 13:147-159.

Pertwee RG (2010). Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. *Curr Med Chem* 17:1360-1381.

Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di M, V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB and CB. *Pharmacol Rev* 62:588-631.

Piomelli D (2003). The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 4:873-884.

Piper BJ (2007). A developmental comparison of the neurobehavioral effects of ecstasy (MDMA). *Neurotoxicol Teratol* 29:288-300.

Piper BJ, Ali SF, Daniels LG, Meyer JS (2010). Repeated intermittent methylenedioxymethamphetamine exposure protects against the behavioral and neurotoxic, but not hyperthermic, effects of an MDMA binge in adult rats. *Synapse* 64:421-431.

Piper BJ, Meyer JS (2004). Memory deficit and reduced anxiety in young adult rats given repeated intermittent MDMA treatment during the periadolescent period. *Pharmacol Biochem Behav* 79:723-731.

Pistis M, Porcu G, Melis M, Diana M, Gessa GL (2001). Effects of cannabinoids on prefrontal neuronal responses to ventral tegmental area stimulation. *Eur J Neurosci* 14:96-102.

Pizarro N, Farre M, Pujadas M, Peiro AM, Roset PN, Joglar J, de la TR (2004). Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites in human samples including the catechol-type metabolite (3,4-dihydroxymethamphetamine). *Drug Metab Dispos* 32:1001-1007.

Pope HG, Gruber AJ, Hudson JI, Huestis MA, Yurgelun-Todd D (2002). Cognitive measures in long-term cannabis users. *J Clin Pharmacol* 42:41S-47S.

Prevost C, Pessiglione M, Metereau E, Clery-Melin ML, Dreher JC (2010). Separate valuation subsystems for delay and effort decision costs. *J Neurosci* 30:14080-14090.

Pubill D, Canudas AM, Pallas M, Camins A, Camarasa J, Escubedo E (2003). Different glial response to methamphetamine- and methylenedioxymethamphetamine-induced neurotoxicity. *Naunyn Schmiedebergs Arch Pharmacol* 367:490-499.

Quednow BB, Kuhn KU, Hoppe C, Westheide J, Maier W, Daum I, Wagner M (2007). Elevated impulsivity and impaired decision-making cognition in heavy users of MDMA ("Ecstasy"). *Psychopharmacology (Berl)* 189:517-530.

Razdan RK (1986). Structure-activity relationships in cannabinoids. *Pharmacol Rev* 38:75-149.

Reneman L, Lavalaye J, Schmand B, de Wolff FA, van den BW, den Heeten GJ, Booij J (2001). Cortical serotonin transporter density and verbal memory in individuals who stopped using 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy"): preliminary findings. *Arch Gen Psychiatry* 58:901-906.

Roberts GM, Garavan H (2010). Evidence of increased activation underlying cognitive control in ecstasy and cannabis users. *Neuroimage* 52:429-435.

Rodgers J (2000). Cognitive performance amongst recreational users of "ecstasy". *Psychopharmacology (Berl)* 151:19-24.

Rodgers J, Buchanan T, Scholey AB, Heffernan TM, Ling J, Parrott A (2001). Differential effects of Ecstasy and cannabis on self-reports of memory ability: a web-based study. *Hum Psychopharmacol* 16:619-625.

Rodgers RJ, Evans PM, Murphy A (2005). Anxiogenic profile of AM-251, a selective cannabinoid CB1 receptor antagonist, in plus-maze-naive and plus-maze-experienced mice. *Behav Pharmacol* 16:405-413.

Rodriguez-Arias M, Manzanedo C, Roger-Sanchez C, Do Couto BR, Aguilar MA, Minarro J (2010). Effect of adolescent exposure to WIN 55212-2 on the acquisition and reinstatement of MDMA-induced conditioned place preference. *Prog Neuropsychopharmacol Biol Psychiatry* 34:166-171.

Rodsiri R, Spicer C, Green AR, Marsden CA, Fone KC (2011). Acute concomitant effects of MDMA binge dosing on extracellular 5-HT, locomotion and body temperature and the long-term effect on novel object discrimination in rats. *Psychopharmacology (Berl)* 213:365-376.

Rog DJ, Nurmikko TJ, Friede T, Young CA (2005). Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. *Neurology* 65:812-819.

Rozas C, Loyola S, Ugarte G, Zeise ML, Reyes-Parada M, Pancetti F, Rojas P, Morales B (2011). Acutely applied MDMA enhances long-term potentiation in rat hippocampus involving D1/D5 and 5-HT2 receptors through a polysynaptic mechanism. *Eur Neuropsychopharmacol*.

Rubino T, Parolaro D (2008). Long lasting consequences of cannabis exposure in adolescence. *Mol Cell Endocrinol* 286:S108-S113.

Rudebeck PH, Walton ME, Smyth AN, Bannerman DM, Rushworth MF (2006). Separate neural pathways process different decision costs. *Nat Neurosci* 9:1161-1168.

Saadat KS, Elliott JM, Green AR, Moran PM (2006). High-dose MDMA does not result in long-term changes in impulsivity in the rat. *Psychopharmacology (Berl)* 188:75-83.

Sabol KE, Lew R, Richards JB, Vosmer GL, Seiden LS (1996). Methylenedioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. Part I: Synaptosomal uptake and tissue concentrations. *J Pharmacol Exp Ther* 276:846-854.

Sala M, Braida D (2005). Endocannabinoids and 3,4-methylenedioxymethamphetamine (MDMA) interaction. *Pharmacol Biochem Behav* 81:407-416.

Sanchez AJ, Garcia-Merino A (2012). Neuroprotective agents: cannabinoids. *Clin Immunol* 142:57-67.

Sano K, Mishima K, Koushi E, Orito K, Egashira N, Irie K, Takasaki K, Katsurabayashi S, Iwasaki K, Uchida N, Egawa T, Kitamura Y, Nishimura R, Fujiwara M (2008). Delta 9-tetrahydrocannabinol-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens due to the action of glutamate-containing neurons. *Neuroscience* 151:320-328.

Sarne Y, Keren O (2004). Are cannabinoid drugs neurotoxic or neuroprotective? *Med Hypotheses* 63:187-192.

Sarne Y, Mechoulam R (2005). Cannabinoids: between neuroprotection and neurotoxicity. *Curr Drug Targets CNS Neurol Disord* 4:677-684.

Scallet AC (1991). Neurotoxicology of cannabis and THC: a review of chronic exposure studies in animals. *Pharmacol Biochem Behav* 40:671-676.

Scanzello CR, Hatzidimitriou G, Martello AL, Katz JL, Ricaurte GA (1993). Serotonergic recovery after (+/-)3,4-(methylenedioxy) methamphetamine injury: observations in rats. *J Pharmacol Exp Ther* 264:1484-1491.

Schenk S (2009). MDMA self-administration in laboratory animals: a summary of the literature and proposal for future research. *Neuropsychobiology* 60:130-136.

Schenk S, Harper DN, Do J (2011). Novel object recognition memory: measurement issues and effects of MDMA self-administration following short inter-trial intervals. *J Psychopharmacol* 25:1043-1052.

Schilt T, de Win MM, Jager G, Koeter MW, Ramsey NF, Schmand B, van den BW (2008). Specific effects of ecstasy and other illicit drugs on cognition in poly-substance users. *Psychol Med* 38:1309-1317.

Schlicker E, Kathmann M (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci* 22:565-572.

Schneider M (2008). Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addict Biol* 13:253-263.

Schneider M, Koch M (2003). Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology* 28:1760-1769.

Schneider M, Schomig E, Leweke FM (2008). Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addict Biol* 13:345-357.

Scholey AB, Parrott AC, Buchanan T, Heffernan TM, Ling J, Rodgers J (2004). Increased intensity of Ecstasy and polydrug usage in the more experienced recreational Ecstasy/MDMA users: a WWW study. *Addict Behav* 29:743-752.

Schouw ML, Gevers S, Caan MW, Majoie CB, Booij J, Nederveen AJ, Reneman L (2011). Mapping serotonergic dysfunction in MDMA (ecstasy) users using pharmacological MRI. *Eur Neuropsychopharmacol*.

Schulz S (2011). MDMA & Cannabis: A Mini-Review of Cognitive, Behavioral, and Neurobiological Effects of Co-Consumption. *Curr Drug Abuse Rev* 4:81-86.

Schweimer J, Hauber W (2006). Dopamine D1 receptors in the anterior cingulate cortex regulate effort-based decision making. *Learn Mem* 13:777-782.

Shen EY, Ali SF, Meyer JS (2011). Chronic administration of THC prevents the behavioral effects of intermittent adolescent MDMA administration and attenuates MDMA-induced hyperthermia and neurotoxicity in rats. *Neuropharmacology*.

Shen M, Piser TM, Seybold VS, Thayer SA (1996). Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J Neurosci* 16:4322-4334.

Shen M, Thayer SA (1998). Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. *Mol Pharmacol* 54:459-462.

Sherlock K, Wolff K, Hay AW, Conner M (1999). Analysis of illicit ecstasy tablets: implications for clinical management in the accident and emergency department. *J Accid Emerg Med* 16:194-197.

Shulgin AF (1990). History of MDMA. In: *Ecstasy: The Clinical, Pharmacological and Toxicological Effects of the Drug MDMA*. Boston: Kluwer Academic Publishing. pp. 1-20.

Smith GW, Farrell M, Bunting BP, Houston JE, Shevlin M (2011). Patterns of polydrug use in Great Britain: findings from a national household population survey. *Drug Alcohol Depend* 113:222-228.

Solowij N, Battisti R (2008). The chronic effects of cannabis on memory in humans: a review. *Curr Drug Abuse Rev* 1:81-98.

Sowell ER, Thompson PM, Holmes CJ, Jernigan TL, Toga AW (1999). In vivo evidence for post-adolescent brain maturation in frontal and striatal regions. *Nat Neurosci* 2:859-861.

Spanos LJ, Yamamoto BK (1989). Acute and subchronic effects of methylenedioxymethamphetamine [(+/-)MDMA] on locomotion and serotonin syndrome behavior in the rat. *Pharmacol Biochem Behav* 32:835-840.

Spear LP (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* 24:417-463.

Steel RW, Miller JH, Sim DA, Day DJ (2011). Learning impairment by Delta(9)-tetrahydrocannabinol in adolescence is attributable to deficits in chunking. *Behav Pharmacol* 22:837-846.

Stone DM, Hanson GR, Gibb JW (1987). Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats. *Neuropharmacology* 26:1657-1661.

Sullivan JM (2000). Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. *Learn Mem* 7:132-139.

Taffe MA (2012). Delta9-Tetrahydrocannabinol attenuates MDMA-induced hyperthermia in rhesus monkeys. *Neuroscience* 201:125-133.

Tanda G, Goldberg SR (2003). Cannabinoids: reward, dependence, and underlying neurochemical mechanisms--a review of recent preclinical data. *Psychopharmacology (Berl)* 169:115-134.

Taylor DA, Fennessy MR (1977). Biphasic nature of the effects of delta9-tetrahydrocannabinol on body temperature and brain amines of the rat. *Eur J Pharmacol* 46:93-99.

Thomasius R, Zapletalova P, Petersen K, Buchert R, Andresen B, Wartberg L, Nebeling B, Schmoldt A (2006). Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users: the longitudinal perspective. *J Psychopharmacol* 20:211-225.

Tourino C, Ledent C, Maldonado R, Valverde O (2008). CB1 cannabinoid receptor modulates 3,4-methylenedioxymethamphetamine acute responses and reinforcement. *Biol Psychiatry* 63:1030-1038.

Tourino C, Zimmer A, Valverde O (2010). THC Prevents MDMA Neurotoxicity in Mice. *PLoS One* 5:e9143.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83:393-411.

UNODC (2010). World Drug Report of the United Nations office on drugs and crime. *United Nations Publication*.

Verdejo-Garcia AJ, Lopez-Torrecillas F, Aguilar dA, Perez-Garcia M (2005). Differential effects of MDMA, cocaine, and cannabis use severity on distinctive components of the executive functions in polysubstance users: a multiple regression analysis. *Addict Behav* 30:89-101.

Vollenweider FX, Gamma A, Liechti M, Huber T (1998). Psychological and cardiovascular effects and short-term sequelae of MDMA ("ecstasy") in MDMA-naive healthy volunteers. *Neuropsychopharmacology* 19:241-251.

Wahlsten D, Colbourne F, Pleus R (2003). A robust, efficient and flexible method for staining myelinated axons in blocks of brain tissue. *J Neurosci Methods* 123:207-214.

Walton ME, Bannerman DM, Alterescu K, Rushworth MF (2003). Functional specialization within medial frontal cortex of the anterior cingulate for evaluating effort-related decisions. *J Neurosci* 23:6475-6479.

Walton ME, Bannerman DM, Rushworth MF (2002). The role of rat medial frontal cortex in effort-based decision making. *J Neurosci* 22:10996-11003.

Wang X, Baumann MH, Xu H, Morales M, Rothman RB (2005). (+/-)-3,4-Methylenedioxymethamphetamine administration to rats does not decrease levels of the serotonin transporter protein or alter its distribution between endosomes and the plasma membrane. *J Pharmacol Exp Ther* 314:1002-1012.

Wang X, Baumann MH, Xu H, Rothman RB (2004). 3,4-methylenedioxymethamphetamine (MDMA) administration to rats decreases brain tissue serotonin but not serotonin transporter protein and glial fibrillary acidic protein. *Synapse* 53:240-248.

Wegener N, Koch M (2009a). Neurobiology and systems physiology of the endocannabinoid system. *Pharmacopsychiatry* 42 Suppl 1:S79-S86.

Wegener N, Kuhnert S, Thuns A, Roese R, Koch M (2008). Effects of acute systemic and intra-cerebral stimulation of cannabinoid receptors on sensorimotor gating, locomotion and spatial memory in rats. *Psychopharmacology (Berl)* 198:375-385.

Wegener N, Koch M (2009b). Behavioural disturbances and altered Fos protein expression in adult rats after chronic pubertal cannabinoid treatment. *Brain Research* 1253:81-91.

Whitlow CT, Liguori A, Livengood LB, Hart SL, Mussat-Whitlow BJ, Lamborn CM, Laurienti PJ, Porrino LJ (2004). Long-term heavy marijuana users make costly decisions on a gambling task. *Drug Alcohol Depend* 76:107-111.

Wiley JL, Evans RL, Grainger DB, Nicholson KL (2011). Locomotor activity changes in female adolescent and adult rats during repeated treatment with a cannabinoid or club drug. *Pharmacol Rep* 63:1085-1092.

Wilson RI, Nicoll RA (2002). Endocannabinoid signaling in the brain. *Science* 296:678-682.

Winstock AR, Griffiths P, Stewart D (2001). Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. *Drug Alcohol Depend* 64:9-17.

Wiskerke J, Stoop N, Schetters D, Schoffelmeer AN, Pattij T (2011). Cannabinoid CB1 receptor activation mediates the opposing effects of amphetamine on impulsive action and impulsive choice. *PLoS One* 6:e25856.

Wu LT, Parrott AC, Ringwalt CL, Yang C, Blazer DG (2009). The variety of ecstasy/MDMA users: results from the National Epidemiologic Survey on alcohol and related conditions. *Am J Addict* 18:452-461.

Yamamoto BK, Spanos LJ (1988). The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. *Eur J Pharmacol* 148:195-203.

Yang J, Jamei M, Heydari A, Yeo KR, de la TR, Farre M, Tucker GT, Rostami-Hodjegan A (2006). Implications of mechanism-based inhibition of CYP2D6 for the pharmacokinetics and toxicity of MDMA. *J Psychopharmacol* 20:842-849.

Young JM, McGregor IS, Mallet PE (2005). Co-administration of THC and MDMA ('ecstasy') synergistically disrupts memory in rats. *Neuropsychopharmacology* 30:1475-1482.

Zakzanis KK, Young DA (2001). Memory impairment in abstinent MDMA ("Ecstasy") users: a longitudinal investigation. *Neurology* 56:966-969.

Zuurman L, Ippel AE, Moin E, van Gerven JM (2009). Biomarkers for the effects of cannabis and THC in healthy volunteers. *Br J Clin Pharmacol* 67:5-21.

Acknowledgements

Many thanks to …

… Prof. Michael Koch, for giving me the opportunity to spend the last three years on this fascinating topic, and for very helpful comments and improvements. Furthermore, for allowing me to pursue extracurricular activities (addiction research and psychotherapy) for which I am very grateful.

… Prof. Ursula Dicke for being the second referee of my thesis.

…the working group Neuropharmacology. Lunch hours were always great! Especially worth mentioning are Malte and Sybille K., for general, professional and personal talks and comments, as well as Maja Brand and Simone Sikora, for their help with histology and animal testing.

…my fabulous Master students Thorsten and Jannis for their hard and independent work. I am grateful to all other students helping me with my experiments, including Heta Svärd, Linda Hayn, Sergej Penner and Sven Büttner.

… my former supervisor, Dr. Andreas von Ameln-Mayerhofer, for sparking my interest in and providing MDMA.

… my parents, whose emotional and financial support brought me all the way.

… my brother, whose positive attitude towards life made me take some things less seriously (though he still thinks I am leading a “students´” life).

… my very best friend Stef, who never doubted me or any of my decisions during the last 12 years.

… my scraggy, wonderful boyfriend Jannis, who probably kept me sane by driving me insane, and relaxed by making me laugh all those times.