# Antidepressant-like effects of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy)

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## Abstract

3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is a popular club drug that is abused worldwide. The main subjective effects of the drug include enhanced mood and self-esteem. Due to these effects, ecstasy may be used at higher rates by people with pre-existing mood disorders, or a predisposition to depression, in order to 'self-medicate' their state. This, in turn, may lead to more regular drug use, and, hence, a higher risk of side effects and negative impact on health. Moreover, some mechanisms of MDMA action in the brain are similar to those of clinically prescribed antidepressants, as the drug primarily affects the serotonin (5-HT) system. This suggests that the drug may have antidepressant-like activity.

The studies reported here, both preclinical and clinical, were designed to investigate possible antidepressant-like effects of MDMA in subjects with a predisposition to depression.

In the animal study, the effects of MDMA following single and repeated administration were compared between Sprague-Dawley and the Flinders Sensitive Line rat strains, the latter being a putative model of depression. The drug's effects on behaviour were assessed in the Forced Swimming Test, which is widely used to detect the depressive-like state in laboratory animals. Acute MDMA administration had a dose-dependent antidepressant-like effect that was more evident in the Flinders Sensitive Line animals. This effect was diminished following 3 weeks of repeated drug injection, possibly due to the development of tolerance. The chosen dosing regime didn't affect the cortical levels of 5-HT and its metabolite.

40 current ecstasy users participated in the clinical study. Predisposition to depression was assessed using a questionnaire (Brief Symptom Inventory) that determines the rates of distress in various psychological spheres. Mood scores and depressive symptoms were assessed when participants were drug-free and when they attended a social gathering. Twenty participants, with and without a predisposition to depression, who voluntarily chose to take a pill at a social gathering, were assessed 1 hour after drug consumption, and the mood disturbance and depressive symptoms were compared with participants who abstained from pill consumption. Ecstasy users with a predisposition to depression reported higher mood disturbance and more prominent depressive symptoms when they were not under the influence of the drug. At the party, mood was improved in all participants irrespective of whether they chose to consume a pill, whereas subjects predisposed to depression reported a relative decrease in depressive symptoms only after pill consumption, which may be considered as an antidepressant-like effect of the drug. Certain variants of the 5-HT transporter gene polymorphism were associated with higher depressive scores.

Analysis of the effects of different previous ecstasy exposure revealed that subjects with a greater number of pills consumed in their lifetime report more prominent positive effects following ecstasy consumption, which may explain their more frequent use.

In sum, an immediate antidepressant-like effect of MDMA was evident both in an animal model of depression and in users predisposed to depression. This may suggest the self-medicating potential of MDMA in subjects with a predisposition to depression.

## Declaration

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Irina Majumder

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# Abbreviations

$^{0}$C – degrees Celsius

$^{14}$C – radioactive carbon isotope

3α,5α-THP – 3α,5α-tetrahydroprogesterone

5-HIAA – 5-hydroxyindoleacetic acid

5-HT – serotonin (5-hydroxytryptamine)

5-HTT – serotonin transporter

5-HTTLPR – serotonin transporter linked polymorphic region

8-OH-DPAT – 8-hydroxy-2-(di-n-propylamino)tetralin

ACTH – adrenocorticotropic hormone

AMPT – alpha-methylparatyrosine

ANOVA – analysis of variance

AUC – area under the curve

BDI – Beck Depression Inventory

BDNF – brain-derived neurotrophic factor

BSI – Brief Symptom Inventory

cAMP – cyclic adenosine monophosphate

CBF – cerebral blood flow

$CH_3OH$ – methanol

$CH_3COONa$ - sodium acetate

Ci – curie

cm – centimetre

$C_{max}$ – peak concentration

CNS – central nervous system

COMT – catechol-O-methyltransferase

CREB – cAMP response element binding

CRF – corticotropin-releasing factor

CSF – cerebrospinal fluid

CYP – cytochrome P450

DA – dopamine

DAT – dopamine transporter

DFP – diisopropyl fluorophosphate

dGTP – deoxyguanosine triphosphate

DMT – dimethyltryptamine

DNA – deoxyribonucleic acid

dNTP – deoxyribonucleoside 5'-triphosphate

DOPAC – 3,4-dihydroxyphenylacetic acid

DSM-IV – Diagnostic and statistical manual of mental disorders (IV edition)

EDTA – ethylenediaminetetraacetic acid

FH – Fawn-Hooded

FRL – Flinders Resistant Line

FSL – Flinders Sensitive Line

FST – Forced Swimming Test

g – gram

GABA – gamma-aminobutyric acid

GHB – gamma-hydroxybutyric acid

h – hour

HHA – 3,4-dihydroxyamphetamine

HHMA – 3,4-dihydroxymethamphetamine

HMA – 4-hydroxy-3-methoxyamphetamine

HMMA – 4-hydroxy-3-methoxymethamphetamine

HPA – hypothalamo-pituitary axis

HPLC – high performance liquid chromatography

HPLC-ECD – high performance liquid chromatography with electrochemical detection

HVA – homovanillic acid

i.p. – intraperitoneal

IFN-α – interferon-alpha

IL – interleukin

IMVS – Institute of Medical and Veterinary Science

kg – kilogram

LC-MS – liquid chromatography mass spectrometry

LMM – Linear Mixed Model

LSD – lysergic acid diethylamide

M – mol/litre

MAO – monoamine oxidase

MAOI – monoamine oxidase inhibitor

MDA – 3,4-methylenedioxyamphetamine

MDD – major depressive disorder

MDEA – 3,4-methylenedioxyethylamphetamine

MDMA – (±)-3,4-methylenedioxymethamphetamine (ecstasy)

METH – methamphetamine

mg – milligram

MHPG – 3-methoxy-4-hydroxyphenylglycol

min – minute

ml – millilitre

mm – millimetre

mRNA – messenger ribonucleic acid

nA – nanoamper

NA – noradrenaline

NaCl – sodium chloride

$NaH_2PO_4$ – sodium dihydrogen phosphate

NAT – noradrenaline transporter

ng – nanogram

NMe5-HT – N-methyl-serotonin

NPY – neuropeptide Y

OSA – octanesulphonic acid

p.o. – per os

PBS – phosphate buffered saline

PCA – perchloric acid

PCR – polymerase chain reaction

PMA – para-methoxyamphetamine

POMS – Profile of Mood States

PPP – platelet-poor plasma

PRP – platelet-rich plasma

PTSD – posttraumatic stress disorder

REM – rapid eye movement

RM – Repeated Measures

ROS – reactive oxygen species

s – second

SCL-90 – Symptom Checklist-90

SD – Sprague-Dawley

SEM – standard error of mean

SMH – self-medication hypothesis

SNRI – serotonin-noradrenaline reuptake inhibitor

SSRI – selective serotonin reuptake inhibitor

T½ – half-life

TBE – tris/borate/EDTA

TCA – tricyclic antidepressant

TH – tyrosine hydroxylase

Tmax – time when peak concentration is reached

TMD – Total Mood Disturbance

TNFα – Tissue Necrosis Factor alpha

TPH – tryptophan hydroxylase

Trp – tryptophan

U – unit

V – volt

$VMAT_2$ – vesicular monoamine transporter type 2

VNTR – variable-number-tandem-repeat

VTA – ventral tegmental area

WKY – Wistar Kyoto

y – year

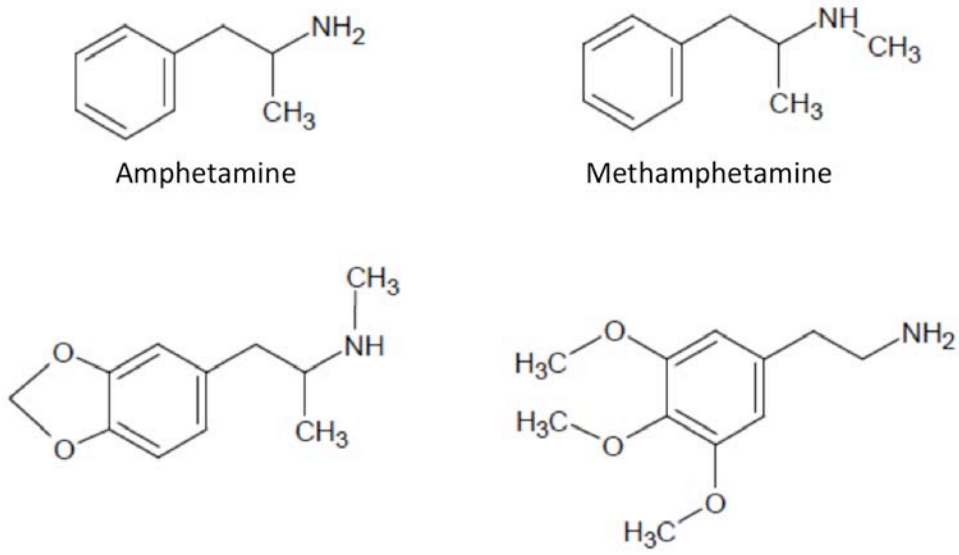
µl – microlitre

# Chapter 1. Background

## 1.1. MDMA: an overview

3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is a psychostimulant club drug that is used and abused worldwide. Its popularity has increased in the last two decades. By chemical structure it belongs to a group of ring-substituted amphetamines (Fig. 1.1). MDMA is also related to mescaline by structure and effects.

***Figure 1.1 Chemical structures of amphetamine, methamphetamine, MDMA and mescaline***




MDMA was first synthesised and patented in 1912 in Germany as a precursor of therapeutically active drugs, such as haemostatic agents, but it was not used at that time as a drug itself (Shulgin 1986; Freudenmann, Oxler et al. 2006). In the 1950s, the

toxic effects of MDMA and other mescaline analogues were investigated at the University of Michigan in a study funded by the Army Chemical Center (Hardman, Haavik et al. 1973). This study used different laboratory species, such as mice, rats, guinea pigs, dogs and monkeys, and assessed mean lethal doses of MDMA and related substances. The psychoactive properties of MDMA alone were first reported by Shulgin and Nichols almost 30 years later: the authors reported that MDMA produces an "easily controlled altered state of consciousness with emotional and sensual overtones" (Shulgin and Nichols 1978). Later on, MDMA's potential as an addition to psychotherapy was investigated in clinical settings (Shulgin 1986; Greer and Tolbert 1998).

MDMA has many street names, including ecstasy, Adam, XTC, rolls and beans. The drug is sold in tablets of various colours and labels. However, tablets sold as ecstasy rarely contain only MDMA and contents may include other amphetamine derivatives, such as methylendioxyethylamphetamine (MDEA), methylenedioxyamphetamine (MDA), paramethoxyamphetamine (PMA), methamphetamine (METH)), as well as caffeine, ketamine or ephedrine (Sondermann and Kovar 1999; Cole, Bailey et al. 2002; Camilleri and Caldicott 2005; Giraudon and Bello 2007). Some pills don't contain any amphetamine-type substances at all. There are various online resources dedicated to collecting information on the contents of the pills sold as ecstasy (http://www.ecstasydata.org ; http://www.pillreports.com).

Due to the potential for abuse and health hazards, MDMA has been declared illicit in many countries. MDMA was declared a Class A drug by the *Misuse of Drugs Act 1971* in the UK in 1977 (1971). Following several publications on neurotoxic potential of

MDMA in laboratory studies, the drug was also included in Schedule I of the *Controlled Substances Act* in the USA in 1985 (Ricaurte, Bryan et al. 1985; Schmidt, Levin et al. 1987; Peroutka, Newman et al. 1988). In 1986, ecstasy was assigned to Schedule 9 in Australia. Schedule 9 drugs comprise prohibited substances that "may be abused or misused, the manufacture, possession, sale or use of which should be prohibited by law except when required for medical or scientific research, or for analytical, teaching or training purposes with approval of Commonwealth and/or State or Territory Health Authorities" (National Drugs and Poisons Schedule Committee 2009). Despite being illegal, the popularity of ecstasy grew significantly among teenagers and young adults in mid 1980s.

Since then, MDMA use has spread worldwide. According to the United Nations World Drug Reports in recent years, the highest per capita ecstasy consumption has been consistently reported in Australia (United Nations 2006; United Nations 2008; United Nations 2009). Although data included in World Drug Reports are collected using different approaches, the difference between per capita use in Australia and other countries remains stable. Data on ecstasy consumption is usually collected via self-reported questionnaires, surveys and interviews, which may not always be objective. One objective and reproducible method of estimating drug use in different regions, which has been proposed, is measuring the levels of different drugs in wastewater (Zuccato, Chiabrando et al. 2008; Banta-Green, Field et al. 2009). The Department of Pharmacology of the University of Adelaide is currently running a project aimed to assess the levels of amphetamines and other drugs in South Australian wastewater (Irvine, Kostakis et al. 2009).

According to the recent National Health Strategy Household Survey ecstasy is the second most popular illicit drug after cannabis among Australians aged 14 years and older (Australian Institute of Health and Welfare 2008). Almost 9% of Australians aged 14 and older have tried ecstasy at least once in their life, with the highest prevalence of use among people aged 20-29 years. Lifetime, as well as recent use (in the previous 12 months), of ecstasy has been steadily increasing from 1993, when 3.1% of participants reported to have previously used ecstasy and 1.2% of respondents were recent users. In 2007, these figures were 8.9% and 3.5%, respectively. The mean age of first use was 22.6 years, and the average number of pills consumed per occasion was 1.6. 46% of respondents reported use of ecstasy once or twice a year, 8.3% were daily or weekly users. Alcohol was the most popular drug consumed with ecstasy. Ecstasy was mainly used at raves and dance parties, but a significant proportion of respondents also reported ecstasy use at private parties and at home (Australian Institute of Health and Welfare 2008). According to the same survey, ecstasy use seems to be switching from the rave scene to private social gatherings.

In New Zealand, the percentage of people who have tried ecstasy at least once in their lifetime grew from 3.1% in 1998 to 8.0% in 2007, according to a recent survey (Wilkins and Sweetsur 2008).

In the USA, MDMA use was increasing until 2001, followed by a slight decline (Martins, Mazzotti et al. 2005). The annual prevalence of ecstasy use among people aged 15-64 years in the USA was 1.1% in 2007, whereas between 2001 and 2002 the estimated prevalence of ecstasy use in the general population was 3.6%, with 7.5% of young adults 19-28 years old who reported using the drug.

In Germany, lifetime exposure to ecstasy in the general population was 1-1.2% in 2001-2002, and declined to 0.4% in 2006 (United Nations 2003; United Nations 2009).

Differences in the rates of ecstasy use in different countries may be attributable to the difference in drug availability and severity of drug possession charges. For instance:

- In South Australia, the maximum penalty for the possession or consumption of ecstasy is two years imprisonment, a $2,000 fine or both (section 33L(1), Controlled Substances Act 1984 (SA)) (1984);
- In the United Kingdom, the maximum penalty for the possession or consumption of ecstasy is seven years imprisonment, an unlimited fine or both (section 5(2)(b), schedule 4 Misuse of Drugs Act 1971 (UK)) (1971);
- In California, the maximum penalty for the possession of a "personal amount use" of ecstasy is one year imprisonment (California Penal Code sections 18, 1000) (2008).

Ecstasy is usually consumed orally in tablets. Less common ways of administration include intranasal, or “snorting the powder”, and intravenous. The latter is more common among polydrug users. One ecstasy tablet may contain, on average, 50-150 mg of MDMA (Kalant 2001). Usually, these doses are used in studies investigating different effects of ecstasy in the controlled settings (Vollenweider, Gamma et al. 1998; de la Torre, Farre et al. 2000; Liechti, Baumann et al. 2000; Liechti and Vollenweider 2000; Farre, de la Torre et al. 2004; Mueller, Kolbrich et al. 2009). The total number of ecstasy tablets consumed may vary. The most common amount taken during a party course is 1 to 2 tablets, but the number may increase up to 10 tablets

consumed by some users (Parrott 2001). A larger number of pills ingested, especially at the same time, increases the risk of severe side effects (McGuire and Fahy 1991).

The MDMA molecule has a chiral centre in its structure, and two stereoisomers. In ecstasy pills the substance most commonly comes in a racemic mixture. The S(+) enantiomer is more potent in causing euphoria and other desired effects, whereas R(-) MDMA predominantly has hallucinogenic, mescaline-like effects (Fantegrossi, Godlewski et al. 2003).

## 1.2. Pharmacology of MDMA

### 1.2.1. Pharmacokinetics and metabolism of MDMA

The metabolic pathways, pharmacokinetics and disposition of MDMA have been extensively investigated in various species, including mice, rats, monkeys and humans.

The pharmacokinetics of MDMA follow a non-linear pattern, in which the administration of MDMA produces disproportionally greater increases in plasma and brain drug concentrations than would normally be expected from the dose administered (Chu, Kumagai et al. 1996; de la Torre, Farre et al. 2000). When consumed orally, MDMA is readily absorbed from the gastrointestinal tract and the plasma concentration of the drug reaches its peak in approximately 2 hours. The half-life of MDMA is approximately 8 hours after administration of 50-125 mg of the drug (de la Torre, Farre et al. 2004).

Several studies of MDMA pharmacokinetics in humans have been done in controlled settings (Mas, Farre et al. 1999; de la Torre, Farre et al. 2000; Farre, de la Torre et al. 2004; Kolbrich, Goodwin et al. 2008; Kolbrich, Goodwin et al. 2008; Mueller, Kolbrich

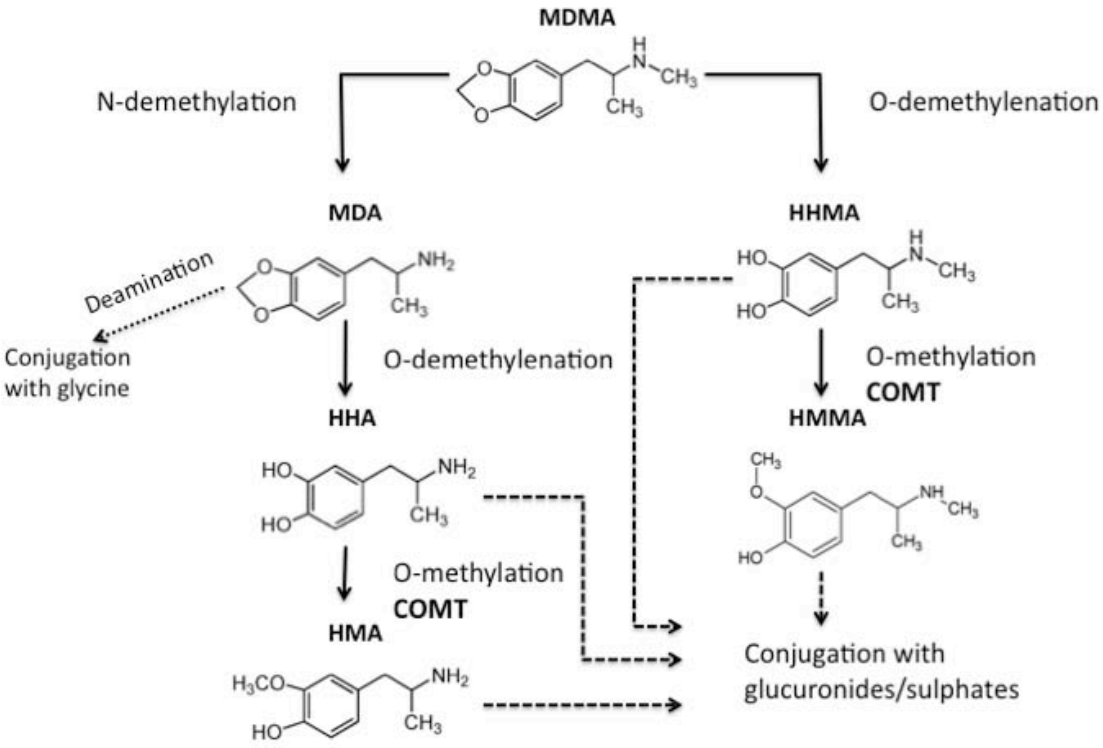
et al. 2009). After a single dose of MDMA, 95% of the drug is eliminated from the body 40 hours after administration. However, if the drug is taken repeatedly, changes in its metabolism occur due to the inhibition of the key enzyme by MDMA itself (de la Torre, Farre et al. 2004; Farre, de la Torre et al. 2004). In a double-blind, placebo-controlled study on the effects of repeated MDMA administration, where 2 doses were given 24 hours apart, an increase in plasma levels of MDMA and MDA, one of the main MDMA metabolites, was observed after the second dose. The pharmacological effects were more potent after the second dose as well (Farre, de la Torre et al. 2004).

MDMA pharmacokinetics and biotransformation were also studied in various laboratory animals, including rats, mice and primates. In rats, pharmacokinetic profiles of MDMA were done using various doses, ranging from 2 mg/kg, which is comparable to doses consumed by humans in recreational settings, up to 40 mg/kg, which is associated with marked neurotoxic effects of the drug (Chu, Kumagai et al. 1996; Baumann, Zolkowska et al. 2009). In one recent study, Baumann and colleagues explored the pharmacokinetics of (±)-MDMA after administration of either 2 mg/kg or 10 mg/kg via different routes (Baumann, Zolkowska et al. 2009). In this study, peak plasma MDMA concentrations after the lower dose were achieved 30 minutes after drug administration via per os route (p.o.) and approximately 8 minutes following intraperitoneal injection (i.p.) The half-life and Tmax of MDMA are shorter in laboratory animals than in humans, perhaps due to the differences in weight. After the administration of a 10 mg/kg dose, peak MDMA concentrations were detected more than 1 hour post-dose. A non-linear pharmacokinetic pattern was observed after administration of a higher MDMA dose: the difference between areas under the curve (AUC) between the doses was disproportionate. The maximal concentrations achieved

after the administration of a 2 mg/kg dose were comparable to those detected after administration of similar doses to humans (Mas, Farre et al. 1999; de la Torre, Farre et al. 2000).

In humans and laboratory animals, the drug is mainly metabolised in the liver (Fig. 1.2) (Adapted from (de la Torre, Farre et al. 2000; de la Torre, Farre et al. 2004; Baumann, Zolkowska et al. 2009)). There are two main pathways of MDMA breakdown in the human liver: O-demethylenation and N-demethylation. O-demethylenation leads to the generation of 3,4-dihydroxymethamphetamine (HHMA), the metabolite that has been linked with the neurotoxic potential of the drug (Segura, Ortuno et al. 2001; Jones, Duvauchelle et al. 2005). HHMA then undergoes a catechol-O-methyltransferase (COMT)-catalysed O-methylation, followed by conjugation with sulfates and glucuronides. In the second pathway N-demethylation leads to the generation of methylenedioxyamphetamine, MDA, which is an active metabolite of MDMA and contributes to the pharmacodynamics of the drug. MDMA and MDA undergo O-demethylation: MDMA is transformed into HHMA, and 3,4-dihydroxyamphetamine (HHA) is generated from MDA. COMT-catalysed O-methylation then takes place to produce 4-hydroxy-3-methoxymethamphetamine (HMMA) from HHMA and 4-hydroxy-3-methoxyamphetamine (HMA) from HHA. The resulting compounds are conjugated with sulphates or glucuronides, and are mainly found in urine and plasma in such a conjugated form (de la Torre, Farre et al. 2004). Substantially higher levels of MDA are metabolised from MDMA in rats than in humans (de la Torre, Farre et al. 2004). HHMA and HMMA are the main metabolites in urine in human subjects.

***Figure 1.2. Metabolic pathways of MDMA***




The initial stages of MDMA and MDA biotransformation are regulated by the cytochrome P450 system (CYP). In humans, the main isoform involved in the metabolism of MDMA is CYP2D6 (Tucker, Lennard et al. 1994), whereas in rats, the CYP2D1 isoenzyme has an equivalent role (Kumagai, Lin et al. 1994). The involvement of other isoenzymes has been shown for MDMA enantiomers: while CYP2D6 is the primary isoform involved in the metabolism of racemic and R-MDMA, CYP2C19 plays an important role in the metabolism of S-MDMA, an enantiomer that causes characteristic subjective effects of the drug (Meyer, Peters et al. 2008).

Inhibition of the activity of CYP2D6 may play an important role in potentiation of MDMA effects, when the drug is taken recreationally. Some users consume ecstasy

along with antidepressants, such as selective serotonin reuptake inhibitors (SSRI), in order to enhance the immediate effects of ecstasy and to decrease the negative effects of a 'come down' (Copeland, Dillon et al. 2006). Although pre-treatment with fluoxetine or paroxetine was shown to have some neuroprotective effect against potential toxic effects of MDMA in animal studies (Hekmatpanah and Peroutka 1990; Sanchez, Camarero et al. 2001; Upreti and Eddington 2008; Li, Huang et al. 2009), in humans, the concurrent use of antidepressants and ecstasy may lead to an increased risk of serious adverse effects from the latter, including the serotonin syndrome, a potentially lethal condition (Gillman 1999; Copeland, Dillon et al. 2006). Paroxetine and fluoxetine are potent inhibitors of the CYP2D6 activity, and it has been shown that pre-treatment with SSRIs leads to an increase in MDMA and MDA concentrations, but also less prominent subjective and physiological MDMA effects, thus providing a basis for more risky patterns of ecstasy use, when a higher number of pills would be consumed by users in order to achieve the desired effects (Farre, Abanades et al. 2007; Tancer and Johanson 2007; Upreti and Eddington 2008).

As noted previously, CYP2D6 and COMT are the key enzymes in MDMA metabolism in humans. Gene polymorphism, which had been described for both enzymes, can lead to decreased expression and activity of the enzyme, and thus to a slower metabolism, which, in turn, can lead to an increased risk in acute toxic effects of the drug, including the serotonin syndrome (Tucker, Lennard et al. 1994; Schilt, Koeter et al. 2009). The CYP2D6 polymorphism is heterogenous, and so-called 'poor metabolisers' who have decreased levels of the enzyme activity, may be at increased risk of acute negative and chronic neurotoxic effects of MDMA (Tucker, Lennard et al. 1994). A study with a limited number of subjects (n=10) showed that a 'poor metaboliser' genotype is

associated with increased concentrations of MDMA after a single dose in comparison with other genotypes. The HHMA concentrations were similar between the genotypes, however, a larger study is warranted to further investigate whether a 'poor metaboliser' genotype plays a role in neurotoxicity of MDMA (de la Torre, Farre et al. 2005).

Furthermore, the neurotoxicity of MDMA may be associated with the activity of the drug metabolites, as direct injection of MDMA into brain matter does not result in any long-term toxic effects (Esteban, O'Shea et al. 2001). Non-human primates, including squirrel monkeys, have been extensively used to study the neurotoxic effects of MDMA (Mechan, Yuan et al. 2006; Mueller, Kolbrich et al. 2009). In monkeys, some similarities in the metabolic pathways with humans have been observed (Mueller, Kolbrich et al. 2009). The half-life of MDMA was found to be longer in squirrel monkeys than in humans; Cmax values were similar in humans and squirrel monkeys after administration of 1.6 mg/kg and 0.8 mg/kg, respectively, whereas AUC values were comparable between species after oral administration of the doses, which were estimated to be equivalent in both species using an interspecies dose-scaling equation (1.6 mg/kg for humans and 5.7 mg/kg for squirrel monkeys). The COMT activity was likely to be greater in monkeys, as the levels of HMMA conjugates were higher than in humans, but MDA and HHMA concentrations were similar in both species.

Further sections are focussed on the mechanisms of MDMA action and the effects of the drug in humans and laboratory animals.

### 1.2.2. The pharmacological action of MDMA in the brain

The pathways of MDMA action in the central nervous system (CNS) have been well characterised. The drug affects the monoamine brain systems, primarily serotonin (5-HT) as well as dopamine (DA) and noradrenaline (NA). It interacts in various ways with normal monoamine neurotransmission functioning, resulting in a rapid and considerable increase in the neurotransmitter levels, primarily serotonin, in the synaptic cleft and consequent activation of receptors on postsynaptic membranes. This section briefly describes the main mechanisms of the neurochemical action of MDMA in the brain.

The 5-HT system has been a main area of investigation of the effects of MDMA. The drug causes changes in the serotonergic system in rats, non-human primates and humans (Ricaurte, Bryan et al. 1985; Fischer, Hatzidimitriou et al. 1995; Croft, Klugman et al. 2001; Baumann, Wang et al. 2007; McCann, Szabo et al. 2008). In mice, however, MDMA primarily affects the dopaminergic system (Logan, Laverty et al. 1988; Colado, O'Shea et al. 2004), therefore, this species may not be suitable as a model of MDMA's effects in humans.

MDMA affects the functioning of the serotonergic system in different ways. Firstly, it stimulates release of 5-HT from vesicles in various brain regions. This action has been shown in the *in vitro* studies with rat whole brain synaptosomes (Nichols, Lloyd et al. 1982; Berger, Gu et al. 1992) and *in vivo* studies, with a focus on striatal levels of the neurotransmitter (Schmidt, Levin et al. 1987). Release of 5-HT and other neurotransmitters by MDMA is mediated by the substrate-type mechanism (Rothman, Baumann et al. 2001; Rothman and Baumann 2002). Serotonin, dopamine and

noradrenaline are substrates for the vesicular monoamine transporter type 2 ($VMAT_2$), which relocates them into the vesicles within the nerve terminals (Partilla, Dempsey et al. 2006). MDMA has been shown to inhibit the activity of $VMAT_2$ *in vitro* and *ex vivo* (Bogen, Haug et al. 2003; Partilla, Dempsey et al. 2006). MDMA binds to the 5-HT, DA and NA transporters (5-HTT, DAT, NAT), thus inhibiting the reuptake of neurotransmitters from and inducing their release into the synaptic cleft (Schmidt, Levin et al. 1987; Rothman, Baumann et al. 2001; Jayanthi and Ramamoorthy 2005; Capela, Carmo et al. 2009). The potency of MDMA-induced inhibition of monoamine transporters is higher for 5-HTT and NAT than for DAT (Rothman, Baumann et al. 2001; Han and Gu 2006). In support of this action, experiments showed that the non-selective and selective 5-HT transporter inhibitors, imipramine and fluoxetine, respectively, block the 5-HT release caused by MDMA (Berger, Gu et al. 1992; Rudnick and Wall 1992). MDMA enters the nerve terminal via the reuptake transporter or, in some cases, by diffusion, and subsequently interacts with the vesicular transporter, which leads to a characteristic rapid and substantial release of 5-HT into the synaptic cleft.

Furthermore, MDMA interacts with the activity of two enzymes involved in serotonin synthesis and breakdown in the CNS. Firstly, the drug also inhibits tryptophan hydroxylase (TPH), an enzyme that catalyses conversion of tryptophan to the 5-HT precursor 5-hydroxytryptophan, thus limiting 5-HT synthesis (McKenna and Peroutka 1990). This can lead to depletion of 5-HT levels once the stores have been exhausted due to substantial release of 5-HT into the intrasynaptic space induced by MDMA. A decrease in available 5-HT stores may lead to the development of subacute effects of ecstasy (Verheyden, Hadfield et al. 2002). On the other hand, ecstasy inhibits the 5-HT

breakdown by blocking the activity of monoamineoxidase (MAO), the key enzyme for monoamine metabolism, within the nerve terminal (Leonardi and Azmitia 1994).

Moreover, MDMA's interaction with postsynaptic 5-$HT_{2A}$, 5-$HT_{2C}$ and 5-$HT_{1B}$ receptors potentially induces DA release and subsequently causes an increase in locomotor activity and other behavioural changes (Schmidt, Taylor et al. 1991; Bankson and Cunningham 2001). Thus, some effects of MDMA on the dopaminergic system are mediated by the drug's interaction with the 5-HT system. Induction of DA release from the vesicles was shown *in vitro* a few years after it has been described for the serotonergic system (Schmidt, Levin et al. 1987).

The dopaminergic mesocorticolimbic and negrostriatal neural pathways receive some innervation from 5-HT neurons (Bankson and Cunningham 2001). DA efflux in striatum and nucleus accumbens underlies several behavioural effects of psychostimulants, including induction in locomotor activity, and it also plays an important role in the reinforcing effect of cocaine and amphetamine derivatives (Melega, Williams et al. 1995; Jones, Lee et al. 1996; Jones, Joseph et al. 1999). In rats, MDMA causes release of DA from the nerve terminals in the striatum and nucleus accumbens through activation of 5-$HT_2$ receptors (Gudelsky, Yamamoto et al. 1994; Ball and Rebec 2005). This is consistent with the data that endogenous 5-HT stimulates DA release *in vivo* (Yadid, Pacak et al. 1994) and that 5-$HT_2$ receptor antagonists hinder the synthesis of DA (Schmidt, Taylor et al. 1991). Extracellular levels of DA in hippocampus are increased following MDMA administration (Shankaran and Gudelsky 1998). MDMA, although not as potent reinforcer as amphetamine or cocaine, can induce conditioned place preference behaviour in rats (Bilsky, Montegut et al. 1998). MDMA-induced

increase in 5-HT and DA levels in the nucleus accumbens underlies the euphoric effects of the drug, similarly to other stimulants, opiates and alcohol (White, Obradovic et al. 1996).

MDMA was also shown to induce the release of NA from hippocampal samples in vitro (Fitzgerald and Reid 1990). The interaction of MDMA with the NAT results in the NA efflux (Verrico, Miller et al. 2007). The drug also has a potential to activate $\alpha_1$- and $\alpha_2$-adrenergic receptors (Lavelle, Honner et al. 1999; Selken and Nichols 2007).

All in all, there are some similarities between the pathways of MDMA action with those of clinically prescribed antidepressants (See Section 1.6.3 of this Chapter). This may lead to the antidepressant-like activity of ecstasy.

The findings of numerous studies on the mechanisms of MDMA action in humans and animals provide the basis for explaining the psychological and physiological effects of ecstasy that are described in the following section.

## 1.3. Immediate effects of MDMA in humans

### 1.3.1. Psychological MDMA effects

Ecstasy is mainly used for its positive effects on mood and self-esteem. The subjective effects of MDMA include enhanced mood, elation, increased self-esteem, a feeling of closeness to other people, a more acute perception of colours and sounds, alleviation of fatigue and anxiety, feelings of depersonalisation and derealisation and sexual arousal (Peroutka, Newman et al. 1988; Liechti, Baumann et al. 2000; Baylen and Rosenberg 2006; Sumnall, Cole et al. 2006). Due to these effects, ecstasy is sometimes referred to as a ‘”love drug” or a “hug drug”. The term ‘entactogen’ was proposed for

MDMA and related amphetamine psychostimulants that improve mood and facilitate social interaction (Nichols 1986).

The immediate psychological and physiological effects of MDMA have been replicated in controlled settings with healthy volunteers (Vollenweider, Gamma et al. 1998; Liechti, Baumann et al. 2000). Doses administered usually ranged from 0.25 to 2 mg/kg (Grob, Poland et al. 1996; Vollenweider, Gamma et al. 1998; Cami, Farre et al. 2000; Liechti, Baumann et al. 2000; Tancer and Johanson 2007; Randall, Johanson et al. 2009). MDMA at a dose of 1 mg/kg is comparable to the dose that pills sold as ecstasy usually contain (Dumont and Verkes 2006). In one of the studies, a single dose of MDMA at 1.5 mg/kg was administered to healthy volunteers, who were not current ecstasy users and 2 hours following, psychometric tests were administered (Liechti, Baumann et al. 2000). Consistent with experiences reported by ecstasy users, MDMA in this dose produced mood enhancement, self-confidence, intensification of perception of sensory stimuli, slight psychomotor activation, thought disorder without anxiety, and feelings of derealisation. Pretreatment with citalopram reduced the intensity of the psychological effects of the drug, thus supporting the underlying activation of 5-HT function. Another randomised double-blind placebo-controlled study in ecstasy-naïve volunteers used a dose of 1.7 mg/kg with assessment 75-120 minutes after intake. Using visual-analogue scales (VAS), they obtained results similar to experiences of ecstasy users (Vollenweider, Gamma et al. 1998). VAS are the most frequently used psychometric instruments when assessing the immediate effects of ecstasy (Vollenweider, Gamma et al. 1998; Cami, Farre et al. 2000; Liechti and Vollenweider 2000; Tancer and Johanson 2001; Farre, de la Torre et al. 2004; Dumont and Verkes 2006; Tancer and Johanson 2007). Similar results were obtained in a study

with recreational ecstasy users: 1.5 mg/kg of MDMA induced positive effects that were attenuated by pretreatment with fluoxetine (Tancer and Johanson 2007).

Apart from being a "party drug", MDMA has been proposed as an addition to psychotherapy as it increases self-esteem (Parrott 2007). MDMA was used in psychotherapy to facilitate interpersonal communication, but also included in sessions assisting chronic cancer-related pain and post-traumatic stress disorder (PTSD) (Shulgin 1986; Greer and Tolbert 1998). Greer and Tolbert described the therapeutic sessions they were conducting. The doses of MDMA used in psychotherapeutic sessions were 75-150 mg, sometimes an additional dose of 50 mg was offered 2 hours after the initial drug administration. MDMA-assisted psychotherapy showed a potential beneficial effect, however, due to the classification of MDMA as an illicit drug, this kind of use was discontinued, until recently. At present, a few clinical trials are being carried out to investigate the positive effects of MDMA in treatment of PTSD (Doblin 2002; Bouso, Doblin et al. 2008) and anxiety in terminal stage cancer patients (Allen 2006; Johansen and Krebs 2009). PTSD and other anxiety disorders are treated with a combination of psychotherapy and pharmacotherapy. The latter includes drugs affecting the functioning of the serotonergic system. As MDMA enhances the release of 5-HT, it may be beneficial in the treatment of anxiety disorders. The initial findings of current clinical trials support MDMA's potential as an aid to psychotherapy (Bouso, Doblin et al. 2008).

Oxytocin is a hormone secreted in the hypothalamus and released into bloodstream from the pituitary gland. Apart from its endocrine function as an inductor of uterine contraction, oxytocin facilitates social interaction and increases feelings of trust

towards other people (Ross and Young 2009). MDMA was shown to induce release of oxytocin in rats by interacting with 5-$HT_{1A}$ receptors in the hypothalamus (Thompson, Callaghan et al. 2007). This mechanism may contribute to the development of feelings of closeness to others reported by users following ecstasy consumption, and assist in psychotherapy by facilitating interpersonal bonding and communication.

Another mechanism of MDMA action in brain that may support its role in psychotherapy is the induction of the release of NA and cortisol (White, Obradovic et al. 1996; Johansen and Krebs 2009). Fear elimination techniques are important in treating PTSD, and MDMA-mediated release of cortisol and NA may facilitate remembering emotions associated with previous trauma and consequently assist in coping with them (Parrott 2007).

The following sections overview the negative acute, subacute and long-term effects of ecstasy consumption.

### 1.3.2. Tolerance to the subjective effects of MDMA

Repeated consumption of ecstasy may lead to a decline in the magnitude of desired effects. In one of the earlier studies of the subjective effects of ecstasy in recreational users, 49% of subjects who reported taking MDMA on 2-5 different occasions observed a decline of positive subjective effects of the drug over time, whereas two-thirds of those who took MDMA on 6 or more occasions experienced a decrease in the desired effects and an increase in negative effects following repeated consumption (Peroutka, Newman et al. 1988). This may be consistent with the development of tolerance to the drug's effects. A decrease in desirable drug effects often leads to dose escalation (Fox, Parrott et al. 2001; Parrott 2005). As has been previously found, heavy ecstasy users

report lower levels of negative acute effects of the drug, which is consistent with their continuous pattern of drug consumption (Topp, Hando et al. 1999). Those subjects who experience a decline in the intensity of the desired effects of ecstasy tend to discontinue their use after several doses, or use the drug less frequently. Ecstasy dependence has been reported and linked with the use of escalating doses, an increase in the frequency of use, and the presence of withdrawal symptoms when not under the influence (Jansen 1999; Abdallah, Scheier et al. 2007; Bruno, Matthews et al. 2009). These features, however, are not as marked as those for drugs with higher dependence potential, such as amphetamine, cannabis and opioids (Degenhardt, Bruno et al. 2009).

The mechanisms of neuroadaptation and receptor desensitisation are associated with dysregulation in the mesolimbic dopanimergic reward pathways due to repeated exposure to drugs with addiction potential (Ochoa, Li et al. 1990; Koob 2006). Psychostimulants, including MDMA, affect the dopaminergic pathway via activation of 5-$HT_2$ receptors (Bankson and Cunningham 2001; Kuroki, Meltzer et al. 2003). MDMA's addiction potential has been studied in preclinical settings. The reinforcing effect of MDMA is supported by the findings of studies in mice, rats and primates, which are able to self-administer the drug (Beardsley, Balster et al. 1986; Cornish, Shahnawaz et al. 2003; Trigo, Renoir et al. 2007; Banks, Czoty et al. 2008; Shin, Qin et al. 2008; Reveron, Maier et al. 2009). This effect is, however, not as pronounced as that of cocaine or other psychostimulants. The difference in the mechanisms of action between MDMA and other psychostimulant drugs may explain the lower addiction potential of the former: one of the mechanisms of MDMA's action leads to attenuation of dopaminergic pathway activity, possibly due to serotonergic activation by the drug.

Moreover, attenuation of the reinforcing effects of other psychostimulants when co-administered with MDMA has been previously reported (Clemens, McGregor et al. 2007; Diller, Rocha et al. 2007).

The behavioural sensitisation and cross-sensitisation of animals to the effects of MDMA can explain its addiction potential and its potential role in subsequent use of other drugs by humans. When drug is administered to laboratory animals, the behavioural response to subsequent administrations of MDMA is more pronounced (Colussi-Mas and Schenk 2008). Similarly, previous exposure to MDMA enhances the reinforcing effects of other stimulants (Kalivas, Duffy et al. 1998). This may underlie some pathways of drug use in people: ecstasy use is associated with subsequent use of other drugs, including cocaine and heroin (Martins, Ghandour et al. 2007).

### 1.3.3. Physiological and side effects of MDMA

Although ecstasy is perceived by some recreational users as a relatively 'safe' drug, and there is even discussion on re-evaluating drug scheduling and assigning MDMA to a group of less dangerous drugs, reports of ecstasy-related deaths, although infrequent, still emerge yearly from different parts of the world (Cohen 1996; Schifano 2004; Kaye, Darke et al. 2009). MDMA is known to cause a variety of side effects, some of which under certain circumstances can lead to significant morbidity and mortality.

Perceptions of the risks associated with MDMA use have been studied previously (Gamma, Jerome et al. 2005; Murphy, Wareing et al. 2006; White, Degenhardt et al. 2006). Users commonly obtain information on the effects of ecstasy from friends or on dedicated websites, like Erowid (http://www.erowid.org), and 60-70% of respondents

associate ecstasy use with some degree of risk. The side effects of ecstasy are briefly outlined in this section.

MDMA has some sympathomimetic properties, which are associated with some immediate physiological effects of the drug, including hypertension and tachycardia, tremor, muscle tension, chills and excessive sweating (Vollenweider, Gamma et al. 1998). Other side effects that may occur within the first few hours after drug consumption are somatic in nature, namely, nausea, jaw clenching and bruxism (teeth grinding), numbness; psychological immediate side effects inculde anorexia, concentration difficulty, anxiety, irritability, restlessness, panic attacks, transient paranoid psychosis, hallucinations (Series, Boeles et al. 1994; Williamson, Gossop et al. 1997; Baylen and Rosenberg 2006). Prospective memory impairment, when under the influence of MDMA, is accompanied by a decrease in deactivation in the inferior parietal lobules (Ramaekers, Kuypers et al. 2009). Acute adverse effects were reported both by current ecstasy users and ecstasy-naïve subjects (Vollenweider, Gamma et al. 1998). A few independent cases described spontaneous pneumothorax and pneumomediastinatum related to ecstasy ingestion (Mazur and Hitchcock 2001; Mutlu, Silit et al. 2005).

Hyperthermia is one of the major physiological effects of MDMA. The mechanisms of the MDMA-induced hyperthermia have been thoroughly researched in laboratory animals (Dafters and Lynch 1998; Jaehne, Salem et al. 2005; Hargreaves, Hunt et al. 2007; Goni-Allo, B et al. 2008; Jaehne, Salem et al. 2008; Sharma and Ali 2008). Ecstasy is commonly consumed at parties and clubs, i.e. where crowded conditions and increased ambient temperature prevail. Such settings lead to a predisposition towards

the development of MDMA-induced hyperthermia due to disruption in thermoregulation. MDMA-induced disruption in thermoregulation can occur due to the effects of the drug on the 5-HT and DA levels. These neurotransmitters are involved in thermoregulation, and pretreatment with 5-HT and DA antagonists has been able to prevent MDMA-induced hyperthermia in preclinical studies (Shioda, Nisijima et al. 2008). Excessive sweating due to an increase in body temperature in a setting with high ambient temperature leads to the intake of large amounts of fluids. Along with excessive fluid intake, it may cause water-electrolyte imbalance and, consequently, brain swelling. MDMA-induced disruption in thermoregulation may also result in multi-organ failure (Hall and Henry 2006). A decrease in plasma sodium levels can occur as a consequence of hyperthermia (Hartung, Schofield et al. 2002) and inappropriate secretion of vasopressin (antidiuretic hormone) (Satchell and Connaughton 1994; Holden and Jackson 1996). Fatalities associated with hyperthermia due to ecstasy ingestion include rhabdomyolysis, acute renal failure and disseminated intravascular coagulation (Chadwick, Curry et al. 1991; Screaton, Singer et al. 1992; Sprague, Brutcher et al. 2004). Clinically, there is a history of intake of large amounts of fluids following ecstasy consumption, and consequent disturbed behaviour, disorientation, non-responsiveness, drowsiness and sometimes seizures due to cerebral oedema.

Cases of sudden death due to ecstasy use may be caused by excessive sympathetic stimulation in subjects with undiagnosed cardiomyopathy or congenital heart disease (Hall and Henry 2006). Overstimulation of the serotonergic system by MDMA may, in some cases, lead to serotonin syndrome that occurs due to a rapid increase in extracellular 5-HT level (Gillman 1999). Clinically, the serotonin syndrome has a rapid

onset and presents with agitation, mental confusion, hyperpyrexia, tachycardia, hypertension, shivering, diarrhoea, tremor, myoclonus and hyperreflexia (Gillman 1999; Parrott 2002; Hall and Henry 2006). Milder cases should be treated with rest in a place with cool ambient temperature and usually do not require inpatient observation. More severely affected subjects require hospitalisation and, in some cases, prompt aggressive treatment with 5-HT antagonists, including cyproheptadine or chlorpromazine, intubation and ventilation and active cooling (Gillman 1999).

In conclusion, although MDMA can have a range of positive effects on mood and self-esteem that may be beneficial in psychotherapy, one must appreciate that ingestion of the drug can lead to a series of adverse reactions due to the 'neuropharmacological messiness' of MDMA's action (Parrott 2001). This, in turn, although infrequent, still can result in significant morbidity. The following sections discuss the delayed effects and possible long-term consequences of the repeated consumption of ecstasy.

## 1.4. The subacute (rebound) effects of MDMA

The studies on the subacute effects of ecstasy are less numerous than investigations focussing on the acute and long-term consequences of drug consumption. Nevertheless, the psychological effects that develop several days after ecstasy ingestion play an important role in the investigation into the mechanisms and significance of the drug effects on serotonergic system functioning. As discussed previously, one of the mechanisms of MDMA's action in the serotonergic nerve terminal is inhibition of tryptophan hydroxylase (TPH) activity, which results in restriction of synthesis aimed at restoring neurotransmitter levels. The mood,

cognitive and sleep disturbances that occur in the days following ecstasy consumption may be attributable to a decline in 5-HT levels.

The subacute or rebound effects of MDMA in users develop within 24-48 hours after MDMA use and last for several days. Colloquially, such effects are called 'mid-week blues'. Sometimes 1-3 days after ecstasy consumption users can experience mood disturbance, concentration difficulties, sleep disturbance and drowsiness, muscle aches, irritability, tiredness and headaches (Peroutka, Newman et al. 1988; Verheyden, Henry et al. 2003; Hoshi, Pratt et al. 2006; Huxster, Pirona et al. 2006). It has been reported that ecstasy-naïve volunteers experience similar effects 24 hours following drug consumption in a clinical setting (Vollenweider, Gamma et al. 1998). Rapid boost in the intrasynaptic levels of 5-HT shortly after MDMA ingestion is followed by the depletion in the neurotransmitter levels, which can underlie the 'mid-week blues' symptoms.

Differently designed studies have investigated the post-acute effects of ecstasy in recreational users. It has been shown that, 4 days after attending a social gathering and consuming a pill, subjects reported an increase in depressive symptoms and mood disturbance compared with their initial assessment (Verheyden, Hadfield et al. 2002). A more substantial increase in mood disturbance was observed in female ecstasy users. In a study with a similar approach, a gradual increase in depressive symptoms was observed in ecstasy users in the 5 days following drug consumption (Curran and Travill 1997). In both studies, the maximal depression scores reach mild to moderate clinical cut-off levels. However, neither of the studies had a thorough pre-testing assessment for determination of any underlying psychopathology; moreover, the

initial assessment was done at the moment of social gathering, when ecstasy and alcohol consumption may have interfered with the responses.

The findings of another study showed that, in comparison to MDMA-naïve controls, ecstasy users performed poorly in memory tasks shortly after drug self-administration and also 2 and 7 days after it. 48 hours after ecstasy consumption, users also noticed increased intensity of feeling sad, unpleasant and unsociable, whereas ecstasy-naïve controls didn't report any changes in their mood scores over time (Parrott and Lasky 1998).

Aggression is attenuated by ecstasy consumption, however, 4 days following drug ingestion, users reported higher aggression scores (Curran, Rees et al. 2004; Hoshi, Pratt et al. 2006).

Huxster et al (2006) showed that ecstasy users displayed an increase in negative mood scores, which consisted of depressive and anxiety symptoms, rumination and irritability, 24 hours after drug use. The severity of negative mood then gradually decreased and remained on a plateau for three days following the testing period. Disrupted sleep and cognitive disturbance developed approximately 24 hours after MDMA use and persisted for one day, then returning to levels comparable to the control group, which consisted of ecstasy users who attended a social gathering but chose not to consume ecstasy during the testing period. The analysis of the results in the study, controlling for concurrent drug use and pre-existing psychopathological symptoms, didn't reveal changes as drastic as in previous studies of Verheyden et al (2002) and Curran et al (1997). Nevertheless, the presence of impairment in a few psychological spheres as a subacute consequence of ecstasy consumption may play an

important role in the psychopathology associated with the drug use. Although the rebound effects tend to subside a few days after consumption, the disruption in the 5-HT system may lead to more long-term pharmacological and psychological consequences, some which are discussed in the next section.

## 1.5. Long-term effects of MDMA

Repeated consumption of ecstasy may lead to a decline in the magnitude of desired effects (Parrott 2005) and to the development of impairment in various psychological spheres. Long-term use of MDMA has been linked with the development of cognitive and memory impairment and depressive symptoms in continuing and former ecstasy users (Parrott, Lees et al. 1998; MacInnes, Handley et al. 2001; McCardle, Luebbers et al. 2004; Reneman, Schilt et al. 2006; Thomasius, Zapletalova et al. 2006).

The chronic administration of MDMA to rats leads to a decrease in levels of 5-HT and 5-HIAA in cortex, hippocampus and caudate nucleus, reduced synthesis of 5-HT, reduction of 5-HTT binding in cortex and an overall decrease in 5-HT function in brain (Ricaurte, Bryan et al. 1985; Miller, Lau et al. 1997; Green, Mechan et al. 2003; Wang, Baumann et al. 2004; O'Shea, Orio et al. 2006). Decreased axonal density in dorsal neocortex and hypothalamus caused by MDMA long-term administration has also been reported (Fischer, Hatzidimitriou et al. 1995). The neurotoxic potential of MDMA is partially attributable to its metabolites, primarily HHMA (Segura, Ortuno et al. 2001; Jones, Duvauchelle et al. 2005), as it has been established that when administered centrally, MDMA does not produce depletions in the 5-HT uptake sites and other dysfunction in the serotonergic function (Ricaurte, Bryan et al. 1985; Miller, Lau et al. 1997). Moreover, DA may contribute to MDMA neurotoxicity (Stone, Johnson et al.

1988). It has been shown that MDMA administration induces production of reactive oxygen species (ROS) that are released after DA degradation in the 5-HT nerve terminal (Colado, O'Shea et al. 2004).

The findings of the group of McCann show several alterations in 5-HT system functioning in brains of ecstasy users (McCann, Szabo et al. 1998). These changes include lower density of brain 5-HTT and decreases in 5-HIAA levels in CSF (Ricaurte, Finnegan et al. 1990; McCann, Ridenour et al. 1994; Bolla, McCann et al. 1998). Other imaging findings include impaired regulation of 5-$HT_{2A}$ receptors in abstinent ecstasy users, which is possibly linked with a higher risk of cerebrovascular accidents (Reneman, Habraken et al. 2000). In novice and incidental users, no changes in 5-HTT densities have been observed, however, small alterations that may represent axonal damage have been found (de Win, Jager et al. 2008). Ecstasy is commonly consumed along with alcohol and other illicit drugs, therefore, the above-mentioned alterations may occur due to the interaction of several factors. The contribution of potential neurotoxic effects of other substances should be considered.

Notwithstanding this, the neurotoxic potential of MDMA can be considered as one of the underlying mechanisms of the symptoms of depression, memory, and cognitive function impairment that are present in long-term ecstasy users, particularly in those with patterns of heavy use. Depression attributed to the repeated MDMA use in some cases was regarded as very severe and more resistant to conventional antidepressant treatment (Freudenmann, Schonfeldt-Lecuona et al. 2006). This may be due to repeated disruption of the serotonergic system caused by MDMA. Depressive symptoms (MacInnes, Handley et al. 2001; de Win, Reneman et al. 2004), along with

memory deficit and cognitive disturbance attributable to drug use are reported by some of the former ecstasy users (Zakzanis and Young 2001; Thomasius, Zapletalova et al. 2006).

Numerous studies have assessed the rates of depressive symptoms in ecstasy users (MacInnes, Handley et al. 2001; Guillot 2007; Falck, Jichuan et al. 2008; Fisk, Montgomery et al. 2009). The results are usually compared with either polydrug ecstasy-naïve users or subjects who reported no previous exposure to any illicit drugs. A majority of the studies have used validated self-report questionnaires, such as Beck Depression Inventory (BDI) (Beck 1996), Hamilton Rating Scale (Hamilton 1960) or Symptom Checklist 90 (SCL-90) (Derogatis, Lipman et al. 1973). While some studies have considered the role of MDMA use with the development of depressive symptoms as likely (Williamson, Gossop et al. 1997), and have related even sporadic consumption of the drug to persistent anxiety and depressive symptoms (Series, Boeles et al. 1994), more recent studies have found less evidence for the contribution of ecstasy to psychopathological effects (Sumnall and Cole 2005; Medina and Shear 2007; Schilt, Koeter et al. 2009). A number of studies have failed to observe substantially higher depressive scores in ecstasy users in comparison to controls (Guillot and Greenway 2006; Durdle, Lundahl et al. 2007), whereas other have reported higher depressive scores in current and former ecstasy users when compared to drug-naïve subjects (McCardle, Luebbers et al. 2004), but not to polydrug ecstasy-naïve users (Thomasius, Petersen et al. 2003). In a prospective study of depressive symptoms in current and abstinent ecstasy users, an overall decline in BDI scores was observed at a 2-year follow-up in comparison to baseline levels; however heavier ecstasy use was associated with higher depressive scores (Falck, Jichuan et al. 2008).

Ecstasy use has been associated with impairment in cognitive performance (Curran and Travill 1997; Parrott and Lasky 1998; Parrott, Lees et al. 1998; Dafters, Hoshi et al. 2004; McCardle, Luebbers et al. 2004; Lamers, Bechara et al. 2006; Thomasius, Zapletalova et al. 2006; Golding, Groome et al. 2007; Hoshi, Mullins et al. 2007; Schilt, de Win et al. 2007; Fisk, Montgomery et al. 2009). However, when taking into account the confounding factors, little difference between ecstasy users and ecstasy-naïve polydrug controls is observed (Croft, Mackay et al. 2001; Dafters, Hoshi et al. 2004; Groth-Marnat, Howchar et al. 2007; Hoshi, Mullins et al. 2007; Medina and Shear 2007). According to some authors, polydrug use by current ecstasy users, rather than solely ecstasy consumption, may predispose users to cognitive and memory impairment, anxiety and depressive symptoms.

Controversial findings of reports of the long-term consequences of ecstasy use may be due to some limitations in design of the studies. Some of the earlier studies that reported connections between repeated ecstasy use and depression and cognitive impairment did not control for the use of other drugs, with cannabis and alcohol being the substances most frequently used concurrently with MDMA (Gouzoulis-Mayfrank and Daumann 2006). However, the majority of people who take ecstasy are polydrug users. Moreover, the self-reporting of symptoms relating to recreational ecstasy use is the most frequently used approach in studies, and a lot of them use a cross-sectional design. This is, however, associated with significant limitation of accuracy of reported results. Some of the methodological limitations of cross-sectional studies, such as not including pre-existing psychopathology in the analysis, or not matching groups for use of other drugs and lifestyle differences can also contribute to the controversy surrounding the findings. Nonetheless, even though the accuracy of answers may not

be sufficient in some cases, self-reporting is the most feasible form of assessment of subjects, especially in large-scale studies.

Notably, even though numerous studies have linked mood and cognitive impairment with polydrug use rather than ecstasy use alone, neuroimaging and electrophysiological findings indicate that repeated exposure to ecstasy may result in attenuation of 5-HT system functioning and thus underlie depressive symptoms and cognitive impairment (McCann, Szabo et al. 1998; Croft, Klugman et al. 2001; Reneman, de Win et al. 2006). Moreover, evidence from preclinical studies supports the neurotoxic potential of MDMA, and heavy ecstasy users may be at greater risk of negative long-term effects of the drug.

Different grades of exposure to ecstasy can play a role in the severity of side effects. Parrott and colleagues compared the self-reports of problems attributable to ecstasy use between novice users, who reported using ecstasy on up to 9 occasions, moderate users (10-99 occasions), and heavy users, with more than 100 occasions (Parrott, Buchanan et al. 2002). The majority of users reported consuming 1-2 tablets per occasion on average. This study was done as a web-based questionnaire, in which mood, cognitive, physiological and medical problems were included. Subjects with heavier drug use reported more mood, anxiety and cognitive problems, which they attributed to ecstasy use, when they were drug-free. Heavier ecstasy use has been associated with poorer performance in memory tasks by heavy and former ecstasy users (Reneman, Schilt et al. 2006). However, Schilt and colleagues reported impairment in verbal memory in novice ecstasy users with a cumulative lifetime use of 3 tablets in comparison with ecstasy-naïve controls (Schilt, de Win et al. 2007)

Among other problems related to prolonged exposure to MDMA, some current and abstinent users report sleep disturbance, including poor quality of sleep and increased night time awakenings (Carhart-Harris, Nutt et al. 2009). The potential neurotoxic effects of MDMA on the 5-HT system may underlie the disruption of sleeping patterns, as 5-HT is involved in the regulation of sleep (Ursin 2002).

Chronic heavy MDMA use can precipitate underlying psychiatric conditions: there have been cases of development of chronic paranoid psychosis (McGuire and Fahy 1991). Unusual neuropsychiatric complications of MDMA use may develop not only after repeated exposure to ecstasy, but also after a single dose (Vecellio, Schopper et al. 2003).

To conclude, depression is a one of the major side effects of MDMA use: low mood manifests as a rebound and a possible long-term effect of the drug, and depressive symptoms persist in former ecstasy users. Therefore, the following part of the introduction will focus on this pathology more closely.

## 1.6. Depression: an overview

### 1.6.1. Definition

The World Health Organization defines depression as “a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration” (World Health Organisation). This mood disturbance was firstly described by Hippocrates as melancholia in his theory of humoralism (Gelder, López Ibor et al. 2000).

The American Psychiatric Association has developed criteria for diagnosing the major depressive episode and major depressive disorder (MDD). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) developed by the American Psychiatric Association, major depressive episode can be diagnosed when one of two core components – depressed mood and anhedonia (loss of interest or ability to experience pleasure from all or nearly all activities) – are present for at least two weeks along with five of the secondary symptoms. The secondary symptoms include:

1. Significant weight loss or weight gain without changes in diet, or decreased or increased appetite;
2. Sleep disturbance (insomnia or hypersomnia);
3. Psychomotor agitation or retardation;
4. Fatigue or loss of energy;
5. Feelings of low self-worth or inappropriate or excessive guilt;
6. Concentration difficulties or indecisiveness; and
7. Recurrent thoughts of death or suicidal ideation with or without a specific plan, or a suicide attempt.

The above-mentioned symptoms should be new or more severe than those immediately prior to the onset of the major depressive episode; they should be present nearly all day and nearly every day, and they should not be caused by any substance or medical drug use or be a result of normal bereavement (American Psychiatric Association 2000). A history of one or more major depressive episodes can

point to MDD. In children and adolescents, irritable mood can be more characteristic than depressed mood.

Depression is a common mental condition that is spread worldwide and has a high disability potential (World Health Organisation). One may consider depression as a heterogeneous complex of disorders with different etiological factors, development patterns and outcomes. There are a number of treatments that effectively modulate the severity of symptoms and decrease the frequency of relapses, however, to date there is no consensus on the pathogenesis of depression.

### 1.6.2. Mechanisms of development of the depressive symptoms

There are several theories which describe changes in various brain systems that may underlie depressive symptoms. The central role in the theoretical basis of research into depression belongs to the monoamine hypothesis which suggests that dysregulation in the serotonergic, noradrenergic and dopaminergic neurotransmitter systems is implicated in the development of depressive symptoms (Schildkraut 1965). Moreover, impairment in the functioning of the cholinergic system, hypothalamo-pituitary-adrenal (HPA) axis dysregulation, cytokine involvement, altered functioning of gamma-aminobutyric acid (GABA) and neuropeptide Y (NPY) systems may also underlie the development of depressive symptoms. The following part of the review briefly outlines the main aspects of these hypotheses. Apart from the alterations in neurotransmitter systems, several other changes in the brain have been linked with depression. Neuroimaging examination of subjects with a family history of MDD has found that cerebral blood flow (CBF) and glucose metabolism were increased in the medial thalamus, the orbital cortex and the amygdala and decreased in the subgenual

prefrontal cortex (Manji, Drevets et al. 2001). Moreover, structural imaging results and examination of post mortem samples associated reduction in grey matter volumes in the orbital and medial cortex, hippocampal and ventral striatal regions, as well as decreased glial cell counts in the prefrontal cortex and the amygdala (Ongur, Drevets et al. 1998; Rajkowska, Miguel-Hidalgo et al. 1999; Rajkowska 2000).

#### *1.6.2.1. Monoaminergic hypothesis of depression*

##### 1.6.2.1.1. Serotonergic system in depression

Changes in the serotonin system are assumed to play a major role in the formation of depressive symptoms. The serotonergic system in brain comprises of a network spread over many areas. The 5-HT neurons mostly originate from raphe nuclei in brain stem and innervate the amygdala, cortex, hippocampus and basal ganglia. Several alterations in the functioning of serotonergic system are linked with mood disorders. Firstly, decreases in the levels of 5-HT and its main metabolite, 5-hydroxyindoleacetic acid (5-HIAA) have been found in cortico-spinal fluid (CSF), plasma (Sarrias, Artigas et al. 1987) and urine and post mortem brain tissue samples of patients with an MDD (Birkmayer and Riederer 1975; Davis, Koslow et al. 1988). Furthermore, changes in 5-HT neurotransmission, such as decreased 5-HT uptake rate (Modai, Zemishlany et al. 1984; Butler and Leonard 1990) and 5-HTT binding in brain and platelets were detected in patients with depression (Malison, Price et al. 1998; Jayanthi and Ramamoorthy 2005; Uebelhack, Franke et al. 2006).

The involvement of 5-HT receptors can be illustrated with a more rapid onset of an antidepressant action of SSRIs when the $5\text{-HT}_{1A}$ autoreceptors are blocked (Tome, Cloninger et al. 1997; Whale, Terao et al. 2008). This may potentially be explained as

the inhibition of serotonergic autoregulation: increased activity of presynaptic 5-$HT_{1A}$ receptors may lead to the inhibition of 5-HT release and thus may lead to a predisposition to the development of depressive symptoms (Blier and de Montigny 1994). Overall, decreased intrasynaptic levels of 5-HT are considered to be of importance in the development of depressive symptoms by modulating $5HT_{2A}$ receptor density. Increased density of these postsynaptic receptors in the frontal cortex is associated with depression. Furthermore, the efficacy of nefazodone, a combined 5-HT reuptake inhibitor and 5-$HT_{2A}$ receptor antagonist, as an antidepressant, suggests the involvement of receptors in the pathogenesis of depression (Kent 2000). However, neuroimaging studies of patients treated for depression show controversial results: in some, decreased binding was observed (Attar-Levy, Martinot et al. 1999; Yatham, Liddle et al. 1999), whereas others have noted increases (Zanardi, Artigas et al. 2001) or no change (Moses-Kolko, Price et al. 2007) in post-treatment binding to receptors.

Indirectly, the involvement of the 5-HT system in depression pathogenesis is evident from the efficacy of agents that increase intrasynaptic levels of 5-HT, such as clinically prescribed antidepressants, selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) (Aberg-Wistedt 1989). Additionally, the depressogenic effect of depletion of the 5-HT precursor tryptophan (Trp), which leads to decreased 5-HT synthesis, has been previously established in humans and laboratory animals (Blokland, Lieben et al. 2002; Neumeister 2003; van der Stelt, Broersen et al. 2004).

Finally, one of the more easily accessible screening methods for the examination of depressed patients is the assessment of platelet function (Da Prada, Cesura et al. 1988). 5-HT function in platelets may reflect changes that occur in brain, as platelets

possess active 5-HT transport, 5-HT receptors and MAO in mitochondria (Biegon et al. 1987; Wägner et al. 1990), and 5-HT transport kinetics in human platelets and synaptosomes are correlated to some extent (Rausch, Johnson et al. 2005; Uebelhack, Franke et al. 2006). One gene encodes the serotonergic transporters in human brain and platelets (Lesch, Wolozin et al. 1993). Changes in serotonergic system functioning in patients suffering from depression have been found in CNS and peripherally (Ortiz, Artigas et al. 1988). It has been shown that decreased density of 5-HTT binding sites along with reduced 5-HT uptake is present in depressed patients (Tuomisto, Tukiainen et al. 1979; Modai, Zemishlany et al. 1984; Sheline, Bardgett et al. 1995). The chronic administration of SSRIs and other antidepressants to depressed patients, similarly to their central action, has the potential to normalise the 5-HT levels, as well as 5-HT uptake and imipramine binding in platelets (Sarrias, Artigas et al. 1987; Wägner, Montero et al. 1990; Perez et al. 1998; Axelson et al. 2005; Alati et al. 2008).

#### 1.6.2.1.2. Noradrenergic system in depression

The noradrenergic hypothesis of depression is based on the findings of impaired NA system functioning in the CNS and peripherally. The locus coeruleus is the main noradrenergic centre in brain, and noradrenergic projections are widely spread, reaching the hippocampus and the prefrontal cortex among other areas (Anand and Charney 2000). The role of the locus coeruleus in the regulation of the response to stressful environmental stimuli correlates with the involvement of the noradrenergic system in the pathogenesis of depressive and anxiety disorders (Kostowski 1985; Weiss and Simson 1985).

Depressive symptoms may be associated with reduced levels of the principal metabolite of NA, 3-methoxy-4-hydroxyphenylglycol (MHPG), in CSF, plasma and urine (Pickar, Sweeney et al. 1978; Charney, Heninger et al. 1981), and decreased plasma levels of NA (Anand and Charney 2000). However, the variability of NA and MHPG levels is considerable, and, according to some authors, decreased levels may be more representative of a bipolar disorder than unipolar depression (Schildkraut 1965; Koslow, Maas et al. 1983), whereas an increased excretion of catecholamines was detected in subjects with unipolar depression in another study (Schatzberg, Samson et al. 1989). Moreover, changes in NA and its metabolite levels vary depending on the duration of treatment (Tang, Helmeste et al. 1978). A challenge with a TPH inhibitor, alpha-methylparatyrosine (AMPT), leading to a rapid depletion in catecholamine levels in brain, has been shown to induce depressive symptoms in healthy subjects (Engelman, Jequier et al. 1968) and to induce a relapse in depressed patients (Berman, Narasimhan et al. 1999).

Similarly to changes in 5-HT receptor function, an increased sensitivity of presynaptic $\alpha_2$-adrenoreceptors, which are present in serotonergic neurons, has been associated with depression. This action has been demonstrated in studies with yohimbine, an $\alpha_2$-receptor antagonist (Anand and Charney 2000). Indirectly, the noradrenergic system dysfunction in depression is supported by the efficacy of antidepressant agents that increase NA levels, such as imipramine (TCA) (Melia, Nestler et al. 1992) and phenelzine (MAOI) (Blier, De Montigny et al. 1986).

#### 1.6.2.1.3. Dopaminergic system in depression

The dopaminergic system is involved in the regulation of sensitivity to reward, and motivation (Lippa, Antelman et al. 1973). Alterations in the DA system are likely to underlie symptoms of depression, such as loss of motivation, anhedonia and psychomotor retardation. Dopaminergic dysfunction associated with depression includes changes similar to other neurotransmitter systems, namely: reduced DA transporter binding, reduced levels of DA and homovanillic acid (HVA), the major metabolite of DA in CSF, plasma and urine (Hamner and Diamond 1996; Engstrom, Alling et al. 1999) and increased DA receptor binding in striatum and basal ganglia (D'Haenen and Bossuyt 1994).

Indirect evidence of DA system involvement in the development of depression includes blunted neuroendocrine and temperature responses to DA agonists in an animal model of depression (Overstreet, Friedman et al. 2005), and antidepressant efficacy of agents that increase DA brain levels, such as MAOIs (Bortolato, Chen et al. 2008). Finally, the association of depressive symptoms with Parkinson's disease, in which reduced activity of DA system predominates, may provide further indirect evidence of the involvement of the dopaminergic system in depression (Manji, Drevets et al. 2001).

### *1.6.2.2. Cholinergic system in depression*

The cholinergic-adrenergic hypothesis of depression was proposed in the 1970s (Janowsky, el-Yousef et al. 1972). According to the hypothesis, in a normally functioning brain, a balance between cholinergic and adrenergic (or serotonergic) systems exists; in depression, the activity of the cholinergic system prevails. In support of this hypothesis, cholinomimetic agents have been shown to reverse the stimulant

effects of amphetamine derivatives (Janowsky, el-Yousef et al. 1972). Furthermore, TCAs have anticholinergic properties (Blackwell, Lipkin et al. 1972). Acetylcholine modulates neuroendocrine and behavioural responses to stress. Depressed patients exhibit an exaggerated sensitivity to cholinergic agents (Janowsky, Overstreet et al. 1994). Scopolamine, a muscarinic cholinergic receptor antagonist, has been shown to improve the state of patients with a partial response to treatment with monoaminergic antidepressants (Furey and Drevets 2006). In addition, the Flinders Sensitive Line rat model of depression, when initially developed, started to be considered as a model of a human condition primarily due to increased sensitivity to cholinomimetic agents. Subsequently, impairment in other systems that play a role in depression has been observed in this model.

#### *1.6.2.3. Role of the hypothalamo-pituitary adrenal axis and cytokines in depression*

The interaction of the CNS with endocrine and immune systems is one aspect of the regulation of affective behaviour (Simmons and Broderick 2005). Impairment in the hypothalamo-pituitary-adrenal (HPA) axis may also be a cause of depression development. The HPA axis is involved in the regulation of responses to stress (Nemeroff, Widerlov et al. 1984). Reactions to stressful stimuli are initiated by increased release of corticotropin-releasing factor (CRF) by the hypothalamus, which subsequently stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland, which, in turn, induces the release of cortisol from the adrenal glands (Leonard 2005). Chronic stress may induce an exaggerated secretion of cortisol, which can lead to blunted negative feedback to CNS centres and thus, to an overall

imbalance in the HPA axis. In mood disorders, disruption in the HPA axis functioning due to chronic uncontrollable stress can result in increased brain and CSF levels of CRF and higher serum ACTH and cortisol levels, along with adrenal and pituitary hypertrophy (Checkley 1996). Indirectly, the role of the HPA axis in depression has been shown by the presence of depressogenic effects of CRF agonists in preclinical models and normalisation of hypercortisolemia by treatment with antidepressants (Pacifici, Zuccaro et al. 2000; Leonard 2005; Braw, Malkesman et al. 2006).

The serotonergic system takes part in HPA regulation: activation of postsynaptic 5-$HT_{1A}$ receptors in the paraventricular nuclei of the hypothalamus leads to increased release of ACTH (Calogero, Bagdy et al. 1990; Pan and Gilbert 1992). Chronic treatment with SSRIs has been shown to normalise the excessive responsiveness of the HPA axis (Jensen, Jessop et al. 1999) (Holsboer and Barden 1996). The underlying mechanism of SSRIs likely includes a reduction of CRF neuronal activity via a decrease in CRF mRNA expression in the hippocampus and the amygdala (Nemeroff and Owens 2004).

Cytokines play a regulatory role in immune responses and are produced by macrophages and lymphocytes. There are two groups of cytokines: pro-inflammatory, such as interkeukin-1 (IL-1), IL-2, IL-6, and tumour necrosis factor alpha (TNFα), and anti-inflammatory, such as IL-4 and IL-10. Corticosteroids are implicated in the regulation of cytokine production. A number of neurotransmitters, including 5-HT, DA and NA, have the potential to regulate the HPA axis (Calogero, Gallucci et al. 1988; Calogero, Bagdy et al. 1990). Sequentially, the HPA axis controls the levels of corticosteroids, and thus, both HPA axis and neurotransmitter systems are indirectly involved in the regulation of immune system functioning (Schiepers, Wichers et al.

2005). In turn, it has been reported that cytokines are able to affect neurotransmitter system functioning, including 5-HT and other monoamines (Linthorst, Flachskamm et al. 1995; Lacosta, Merali et al. 2000; Song 2000). IL-1 administration has been shown to cause induction of 5-HT and monoaminergic activity in limbic regions and the hypothalamus, whereas IL-6 can stimulate the 5-HT system in the prefrontal cortex, the hippocampus and the nucleus accumbens (Dunn, Wang et al. 1999).

Cytokine involvement in depression has been demonstrated by peripheral changes in blood, such as increased lymphocyte count, along with an increased release of proinflammatory cytokines by macrophages and T-cells (Maes 1995). Moreover, cytokines used as therapeutic compounds, such as interferon-alpha (IFN-α) in treatment of chronic hepatitis C or IL-2 therapy for malignancies, has a depressogenic potential (Lotrich, Ferrell et al. 2009). Severity of depressive symptoms has been correlated with the plasma levels of IL-1 and IL-6 (Maes 1995).

#### *1.6.2.4. Role of GABA and neuropeptides in depression*

A disturbance in the GABAergic and NPYergic systems, such as reduced CSF and plasma GABA levels and decreased NPY levels in post-mortem brain, may accompany manifestations of depression (Manji, Drevets et al. 2001). Indirect evidence of GABAergic system involvement in the development of depressive symptoms includes the weak antidepressant-like activity of benzodiazepines (Morishita 2009).

NPY is widely distributed in the CNS and has anticonvulsant properties, regulates food intake, modulates neuronal activity in the hippocampus, affects the release of glutamate and monoamines, plays a role in circadian rhythm regulation, affects HPA axis activity and is involved in the regulation of cognition (Redrobe, Dumont et al.

2002). Its role in depression may be indirectly supported by evidence of an increase in NPY levels following repeated electroconvulsive shock administration (Nikisch and Mathe 2008). Moreover, pharmacotherapy with antidepressants has also increased the levels of NPY and its receptor mRNA levels (Bellmann and Sperk 1993) and stimulated immunoreactivity in the frontal cortex (Heilig, Wahlestedt et al. 1988). NPY immunoreactivity is significantly lower in the Flinders Sensitive Line animal model of depression (Caberlotto, Jimenez et al. 1999).

Impaired regulation of neurosteroids may play a role in the pathogenesis of many psychiatric conditions, including depression, PTSD, Alzheimer's and Parkinson's diseases, anxiety disorders and schizophrenia (Longone, Rupprecht et al. 2008). The levels of 3α,5α-tetrahydroprogesterone (3α,5α-THP), which is a selective positive modulator of $GABA_A$ receptors, are decreased in plasma and CSF of patients with MDD (Romeo 1998, (Romeo, Strohle et al. 1998; Uzunova, Sheline et al. 1998). Fluoxetine administration has been associated with an increase in the 3α,5α-THP levels (Pinna, Costa et al. 2006).

#### *1.6.2.5. Neurotrophic hypothesis of depression*

Other hypotheses for the development of depressive symptoms include the involvement of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF). Related changes in some intracellular transduction pathways, and the modulation of synaptic plasticity (Spedding, Neau et al. 2003), have also been proposed. The neurotrophic hypothesis of depression discusses the role of decreased levels of BDNF and other factors in hippocampal atrophy, which can be reversed by antidepressant treatment (Duman and Monteggia 2006).

### 1.6.3. Mechanisms of action of clinically prescribed antidepressants

The monoamine hypothesis of depression suggests that a deficiency in the functioning of the serotonergic and other monoaminergic systems may underlie the development of depressive symptoms (Schildkraut 1965). The mechanisms of action of the main classes of clinically prescribed antidepressants aim to restore the neurotransmitter levels in the synaptic cleft.

The first class of antidepressants with proven efficacy in the treatment of mood disorders was MAO inhibitors. They were used to treat depression for over three decades until the appearance of selective serotonin reuptake inhibitors in the 1980s. The mechanism of action of MAO inhibitors consists of irreversible (phenelzine) and reversible, such as moclobemide (MAO type A inhibitor), blocking of the breakdown of neurotransmitters within the nerve terminal (Bortolato, Chen et al. 2008). MAOIs have many prominent adverse effects, such as hypertensive crisis, orthostatic hypotension, fatigue, blurred vision, nausea, sleep disruption, dry mouth and constipation (Thase and Denko 2008).

Tricyclic antidepressants (TCAs), such as imipramine and amitryptiline, were introduced about 50 years ago. The antidepressant activity of TCAs is reached via 5 different pathways: these compounds have serotonergic and noradrenergic reuptake inhibitory properties, and also possess anticholinergic-antimuscarinic, antiadrenergic and antihistamine activities (Lopez-Munoz and Alamo 2009). Due to their lack of selectivity, the spectrum of side effects of TCAs is similar to that of MAOIs, and is greater than for SSRIs, therefore, with the introduction of fluoxetine and related drugs, the prescription rates of TCAs diminished considerably.

Fluoxetine, citalopram, paroxetine and sertraline belong to the SSRI group, which are the most widely prescribed class of antidepressants at present (Mant, Rendle et al. 2004; Smith, Sketris et al. 2008; Moore, Yuen et al. 2009). They comprise of compounds which act by blocking the 5-HT reuptake pump, thus increasing the extracellular levels of 5-HT. Apart from mood disorders, SSRIs are used in treatment of a number of anxiety disorders, such as social phobia, panic disorder, PTSD, bulimia nervosa and some other conditions (Vaswani, Linda et al. 2003). The benefits of SSRIs include less severe side effects than classical antidepressants and the convenience of dose adjustment in treatment. Usually 2-3 weeks of continuous treatment with SSRIs are required for a clinical effect to appear (Thompson 2002). This delay may be due to the sequence of action of these antidepressants. The inhibition of the reuptake of neurotransmitters firstly occurs in the somatodendritic region of the serotonergic neuron; an enhanced release of 5-HT in this area then stimulates the down-regulation of presynaptic 5-$HT_{1A}$ receptors, which, in turn, activates the release of 5-HT from the axon of the neuron (Stahl 1998). SSRIs also inhibit the activity of $VMAT_2$ *in vitro* (Yasumoto, Tamura et al. 2009) thus increasing the available pool of 5-HT and contributing to its increased release in the intrasynaptic cleft.

Other classes of antidepressants include 5-HT-NA reuptake inhibitors (SNRIs), such as venlafaxine and milnacipran; tetracyclic noradrenergic and specific serotonergic antidepressants, such as mirtazapine and mianserin; bupropion, a DA-NA reuptake inhibitor; and 5-$HT_2$ receptor antagonists (nefazodone) (Kent 2000; Sen and Sanacora 2008). SSRIs may sometimes be effective when used along with atypical antipsychotics in managing treatment-resistant depression (DeBattista and Hawkins 2009).

### 1.6.4. Genetics of depression

There is a growing body of evidence of the role of genetic factors in susceptibility to depression. Extensive research in mice provides insight into the genes that are likely to underlie the development of depressive symptoms.

According to the findings of numerous twin and family-based studies, the risk of developing a major depressive episode with first-degree relatives of subjects suffering from major depression is almost three times higher than in the general population (Lesch 2004). Parental depression also predicts earlier onset of depression in offspring (Lieb, Isensee et al. 2002). Pre-determined lower threshold to depression development may be further lowered by environmental factors, such as stress and life threatening events.

There are a number of genes that are associated with a susceptibility to depressive conditions. Genomic studies of families with recurrent major depressive disorder linked genes coding proteins involved in cellular signalling pathways, such as cyclic adenosine monophosphate (cAMP) response element binding protein 1 (CREB), with the vulnerability to depression (Zubenko, Maher et al. 2003; Blendy 2006). Subsequently, potential target regions on different chromosomes have been identified using the same genomic scanning approach (Abkevich, Camp et al. 2003; Levinson, Evgrafov et al. 2007).

The involvement of the serotonin system in the development of depressive symptoms is of major interest in research into the pathogenesis of depression (Ueno 2003; Lesch 2004). Carrying certain allelic variants of the gene coding the serotonergic transporter predisposes subjects to an increased risk of depression and other affective disorders.

The solute carrier family 6, member 4 (SLC6A4) gene coding 5-HTT is located on chromosome 17q11.1-17q12 (Lesch, Wolozin et al. 1993). There are two types of 5-HT transporter gene polymorphism, which are implicated in an increased risk of depression.

The second intron in the 5-HTT coding gene contains a polymorphic region with a variable-number-tandem-repeat (VNTR) element (Lesch, Balling et al. 1994; Ogilvie, Battersby et al. 1996). Polymorphism produces 9, 10 and 12 repeats of VNTR. 12 repeats are most frequently present in the general population (Fan and Sklar 2005). It has been shown that carrying 9 VNTR copies is associated with a risk of unipolar depression (Battersby, Ogilvie et al. 1996; Ogilvie, Battersby et al. 1996), and individuals who are homozygous for the 12 copies of repeat allele tend to have less 5-HTT availability (Bah, Lindstrom et al. 2008).

A functional polymorphism of the promoter region of the 5-HTT coding gene has been associated with altered emotional processing and increased susceptibility to depression. The 5-HTT linked polymorphic region (5-HTTLPR) polymorphism produces two alleles, a short variant with 14 repeats and a long allele with 16 repeats, and thus modulates lower and higher 5-HTT expression, respectively (Mandelli, Serretti et al. 2007). Subjects who are homozygous for the short allele have increased susceptibility to depression, especially when exposed to life-threatening situations (Cervilla, Rivera et al. 2006; Cervilla, Molina et al. 2007). Moreover, the short 5-HTTLPR variant predisposes ecstasy users to negative emotional traits and a higher risk of mood disorders (Roiser, Cook et al. 2005; Martin-Santos, Torrens et al. 2009), but does not affect memory function (Reneman, Schilt et al. 2006). Interestingly, the association of

the 5-HTTLPR polymorphism with depression as a side effect of treatment has been found for interferon-alpha (Lotrich, Ferrell et al. 2009). The short allele is also more frequently found in people with anxiety disorders (Schinka, Busch et al. 2004), as is a 12-copy VNTR repeat (Ohara, Suzuki et al. 1999).

Other candidate genes that potentially increase the susceptibility to depression are genes that code enzymes and proteins involved in neurotransmitter system functioning (Garriock, Delgado et al. 2006). These genes code for the 5-$HT_{1A}$ receptor (Lemonde, Turecki et al. 2003), MAO type A (Sabol, Hu et al. 1998), COMT (Mandelli, Serretti et al. 2007; Wray, James et al. 2008), TPH (Walther and Bader 2003), DAT (Vandenbergh, Persico et al. 1992) and the D4 DA receptor (Lichter, Barr et al. 1993).

In short, several genetic targets have been found to be associated with predisposition to depression and other affective disorders. Further research into the genetic basis of depression may provide valuable insight into the pathogenesis of and new treatment options for this condition.

Additionally, CYP2D6 metabolic profile has been shown to play an important role in response to antidepressant treatment (Binder and Holsboer 2006). Poor metabolisers, who lack the enzyme, are at increased risk of toxic effects of the drugs, whereas extensive metabolisers are likely to have suboptimal drug plasma concentrations. Thus, genetic screening prior to the start of pharmacotherapy of MDD or other depressive disorders may be beneficial to predict clinical efficacy of antidepressants on individual level.

### 1.6.5. Depression as a comorbidity or a side effect of drugs

Depressive symptoms can occur separately, as in a major depressive episode or disorder. Additionally, depression can be a feature of bipolar disorder (American Psychiatric Association 2000) and as an associate state in psychiatric and medical conditions. Depressive symptoms sometimes accompany treatment with certain medications.

Depression has been linked with various severe diseases, such as cancer, chronic infections, neurodegenerative diseases (Alzheimer's and Parkinson's diseases), schizophrenia and diabetes mellitus (Goodnick and Hernandez 2000; Giunta, Somboonwit et al. 2007; Rabkin 2008; Reich 2008; Buckley, Miller et al. 2009) . It can also develop in patients who have suffered from coronary event or stroke (Fang and Cheng 2009; Marano, Harnic et al. 2009). In these cases, depression can be regarded as a psychological reaction to the burden of the condition. Postpartum depression can occur in some women shortly after delivery (Almond 2009). The development of postpartum depression and depressive symptoms in peri- and postmenopausal women is likely to be associated with fluctuations of hormones (Payne, Palmer et al. 2009).

Moreover, depressive symptoms are sometimes reported by patients who receive treatment with corticosteroids, IFN-α, β-adrenoblockers, and some antipsychotics (Patten and Love 1993; Patten and Barbui 2004). Finally, depression is associated with drugs of abuse: alcohol, cocaine and substituted amphetamines, opiates and nicotine. The comorbidity of depression with substance use is not an uncommon finding (Buckner and Mandell 1990; Currie, Patten et al. 2005; Davis, Uezato et al. 2008). The association between depression and substance use can be ambiguous: the presence of

depressive symptoms has been associated with a higher risk of subsequent substance use, and drug users report higher rates of depression than the general population (Jane-Llopis and Matytsina 2006). The connection between depressive symptoms and MDMA use is discussed in Section 1.8 of this Chapter. Moreover, it has been proposed that depression and drug dependence are different phenotypes of one underlying pathogenetic mechanism (Markou, Kosten et al. 1998).

To conclude, a combination of various genetic, environmental and neurochemical factors and the simultaneous interaction between several systems may trigger the development of depressive symptoms. However, the precise pathways of the interrelation of different neurotransmitter and other systems are yet to be discovered. Preclinical studies in animals play an important role in the investigation of causal traits, pathogenesis of and treatment options for depression and related disorders. Further sections of this Chapter will overview preclinical models of depression that are used at present.

## 1.7. Preclinical studies of depression

### 1.7.1. Overview of the animal models of depression

The investigation of the links between mood disturbance and MDMA use requires a fundamental approach on both preclinical and clinical levels. A good animal model is essential in order to accumulate mechanistic data, using methods not applicable in clinical research.

Animal models have been developed for many medical and psychiatric conditions, including depression. A model is aimed to represent various aspects of a condition. The mechanisms of the development of symptoms or investigation of certain physiological,

psychological and biochemical changes can be studied using a model, and hence provide a knowledge basis for the pathogenesis and possible treatment options of the mimicked condition.

There are certain limitations in the translation of some symptoms of the depression in laboratory animals. Indeed, one of the core criteria for the diagnosis of major depressive disorder is the presence of low mood. This cannot be readily assessed in a laboratory animal. However, a number of approaches have been introduced to detect a so-called depressive-like state in animals. Anhedonia, or an inability to experience pleasure from events that are usually enjoyable, is another core symptom of depression. Several tests used in preclinical settings have been developed to measure the state of anhedonia in laboratory animals. As for the secondary symptoms of a MDD, some of them, like psychomotor agitation or retardation or changes in weight, can be readily reproduced in an animal model of depression, whereas others, like suicidal ideation and feelings of guilt and low self-esteem, do not have a direct analogue in animals. Nevertheless, depression is a complex of pathologies with different underlying mechanisms, triggers and development patterns; therefore, development of an animal model that would address all aspects of the disorder would, at this stage, be unlikely. Moreover, as has been illustrated above, the exact mechanisms of the development of the depression are still unclear. Accordingly, the animal models of depression that have been previously established have neurochemical and pharmacological similarities with the human condition, which may underlie the resemblance of animal behaviour to symptoms observed in humans (McArthur and Borsini 2006).

Research into the effects of MDMA has been extensively conducted in different laboratory species and in human subjects. The limitations of extrapolating preclinical findings to a clinical level have various explanations. The pharmacokinetic profiles of MDMA differ between species (see Section 1.2.1 of this Chapter). Moreover, depending on the aims of the research, the doses used in rats and other animals differ from those commonly taken by humans recreationally. The acute physiological effects of the drug are similar in humans and rats. MDMA-induced disrupted thermoregulation leads to hyperthermia, which is one of the central immediate adverse effects of the drug (Green, Mechan et al. 2003). The consequences of hyperthermia and associated neurotoxicity have been extensively studied in laboratory animals (Dafters and Lynch 1998; Jaehne, Salem et al. 2005; Hargreaves, Hunt et al. 2007; Goni-Allo, B et al. 2008; Jaehne, Salem et al. 2008; Sharma and Ali 2008). Other physiological effects present in humans, such as increased heart rate and hypertension, have also been recorded in rats (Jaehne, Salem et al. 2008). The prosocial effects of MDMA, which are frequently reported by ecstasy users, are present in rats following acute drug administration (Morley and McGregor 2000; Cornish, Shahnawaz et al. 2003; Thompson, Callaghan et al. 2007). Increased locomotor activity, Repeated MDMA administration, akin to chronic ecstasy use by humans, impairs performance in memory tasks in animals (Morley, Gallate et al. 2001).

At present there are a substantial number of animal models of depression. Some of them are briefly introduced later in this chapter. Well-developed animal models of psychiatric disorders should resemble the condition based on several criteria. The etiological validity, or how similar the causes of a condition in humans and in a model setting are, is not an central criterion for a model of depression, as the causes of

depressive symptomatology are heterogenous and complex, and the exact interaction of causal factors leading to the development of the disorder are still unknown. The face validity of a model shows how closely the model corresponds to the representation of depressive symptoms. The construct validity of a model is present when the model has similar pathophysiological mechanisms to the condition it resembles. Finally, the predictive, or pharmacological, validity of a model shows that the efficacy of drugs and the time course of treatment is similar to that observed in patients (Yadid, Nakash et al. 2000; Frazer and Morilak 2005).

The studies on the depressive-like state that are described also include neurochemical models, behavioural models, such as chronic mild stress (Willner 1997; Grippo, Francis et al. 2005) or maternal deprivation (McArthur and Borsini 2006; Wortwein, Husum et al. 2006), olfactory bulbectomy, in which the depressive-like state is induced by a surgical manipulation (Song and Leonard 2005), and rat strains which possess similarities to depressed patients. The first two groups of models are based on induction of depression and depend on long-term stress, whereas in genetic rodent models, animals are selectively bred for different aspects of the depressive-like state. There are three rat strains that are putative animal models of depression, namely Fawn-Hooded rats (FH), Wistar Kyoto rats (WKY) and Flinders Sensitive Line rats (FSL). Unlike other animal models, where the depressive-like state is induced by environmental or social manipulations, these three rat strains have genetically pre-determined depressive-like characteristics. FH rats have a hereditary 5-HT storage defect, and are used as a model of depression and alcohol abuse (Overstreet and Rezvani 1996; Overstreet, Rezvani et al. 1999; Hall, Huang et al. 2000; Kantor, Anheuer et al. 2000; Chen and Lawrence 2003; Broderick and Hope 2006; Overstreet, Rezvani et

al. 2007), whereas WKY rats are regarded as a model of comorbid anxiety and depression (Lahmame, del Arco et al. 1997; De La Garza and Mahoney 2004; Braw, Malkesman et al. 2006). The FSL strain is a putative model of depression and a predisposition to depression, as some depressive-like characteristics in these animals develop after application of external stressful stimuli (Overstreet 1986; Overstreet, Janowsky et al. 1986; Overstreet 1993; Yadid, Nakash et al. 2000). Progress in gene technology allowed the development of various mouse models to investigate the functions of certain proteins, receptors or transporters, and their role in the development of depressive symptoms (Urani, Chourbaji et al. 2005; Cryan and Slattery 2007).

### 1.7.2. Neurochemical models of depression

Numerous models of depression have been developed in the past few decades (Yadid, Nakash et al. 2000; McArthur and Borsini 2006). The first models appeared in the mid-1960s, when a monoamine hypothesis of affective disorders was formulated (Schildkraut 1965). Based on this hypothesis, a few neurochemical models of depression were proposed. These models included the administration of drugs, such as reserpine, that caused depletion in catecholamine levels in the brain. Tricyclic antidepressants and MAO inhibitors were able to reverse the behavioural and neurochemical effects of reserpine (Askew 1963; Bruhwyler, Liegeois et al. 1998). However, the reserpine antagonism test lacked efficacy in detecting antidepressants that do not primarily affect the reuptake of monoamines (Bourin 1990). Other neurochemical models supported the monoamine theory of depression by showing potentiation of the effects of monoamine receptor agonists by administration of

antidepressants (Lapin 1980; Silvestrini 1982), and by demonstrating the involvement of serotonergic, adrenergic and dopaminergic receptors functioning in the pathogenesis of depression (Kalia 2005).

### 1.7.3. Environmental and social stress-induced models of depression

Environmental factors are important in the development of depressive symptoms. Environmental stress-induced models include learned helplessness and chronic mild stress (Seligman 1972; Willner 1997). These paradigms are putative models of depression, as, to a certain extent, they have behavioural and pharmacological similarities to the human condition. Social stress and maternal separation paradigms explore induction of the depressive-like behaviour by modulating the interaction with other rodents.

#### *1.7.3.1. Learned Helplessness*

In the Learned Helplessness model animals are trained to develop escape avoidance. Specifically, the animals are placed in a chamber and foot shock or other aversive stimuli are administered in an unpredictable and uncontrollable manner. Initially, the attempts to escape are ruled by the expectation that they would be successful, but subsequent exposure to the aversive stimuli reduces this incentive, resulting in passivity and cessation of any attempts to avoid the stimuli (Seligman, Maier et al. 1968; Wagner, Hall et al. 1977). The face and predictive validity of this model have been previously established (Seligman and Beagley 1975; Vollmayr and Henn 2001) . The animals exhibit changes in weight, psychomotor retardation, sleep alterations and anhedonia, akin to depressed patients (Seligman and Beagley 1975; 2000). Moreover, it has been shown that the administration of antidepressants improves behaviour in

animals previously placed in a stressful, uncontrollable setting (Willner, Towell et al. 1987; Takamori, Yoshida et al. 2001). Alterations in the serotonergic system functioning were noted in the Learned Helplessness paradigm. In particular, amelioration of the Learned Helplessness state was observed after administration of a presynaptic 5-$HT_{1A}$ receptor agonist (Zazpe, Artaiz et al. 2007). Moreover, the density of 5-HT receptors was reduced in some area in rats with helplessness (Wu, Kramer et al. 1999); a decreased release of 5-HT, when corrected with antidepressants, also improved Learned Helplessness (Petty, Kramer et al. 1992).

The genetic component of Learned Helplessness has been investigated in mice and rats. A congenitally learned helpless rat strain was developed (Lachman, Papolos et al. 1992). These animals exhibit diminished tolerance to stressors, decreased cognitive functioning after exposure to stress, and have impaired regulation of 5-HT receptors (Shumake, Barrett et al. 2005).

To conclude, the Learned Helplessness paradigm is considered as one of the models of stress-induced depression with potential in assessing the efficacy of various classes of antidepressants and the effects of psychostimulants that interact with the monoaminergic systems in brain.

#### *1.7.3.2. Chronic Mild Stress*

Chronic Mild Stress, like other putative models of depression, has an established face, construct and predictive validity and, in contrast with animal tests of depression, the observed changes in behaviour persist up to 3 months (Yadid, Nakash et al. 2000). The administration of a series of mild unpredictable stressors for extended periods of time induces a state of anhedonia in rats (Willner, Towell et al. 1987; Willner 1997).

Assessment of anhedonia in laboratory animals is done by the sucrose or saccharin preference test (Muscat, Kyprianou et al. 1991; Muscat, Papp et al. 1992; Rygula, Abumaria et al. 2005). The Chronic Mild Stress model is analogous to the model proposed by Katz and colleagues, where stressors with higher intensity were used (Katz, Roth et al. 1981). Mild stressors used in this model include short periods of food and water deprivation, cage tilt, housing in cages with wet bedding, continuous illumination, change of cage-mate, and periods of exposure to cold ambient temperature (Willner, Towell et al. 1987). Animals are repeatedly exposed to stressors for weeks to months. The development of the anhedonic state in animals is subsequently assessed using sucrose or saccharin preference tests (See Section 1.7.5.3). Apart from anhedonia, animals exposed to Chronic Mild Stress exhibit other characteristic depressive-like behavioural aspects, such as impaired sleep and decreased locomotor activity as a marker of psychomotor retardation and decreased sexual and investigative behaviours (D'Aquila, Brain et al. 1994; Cheeta, Ruigt et al. 1997). HPA axis functioning is also affected, resulting in increased secretion of corticosterone (Ayensu, Pucilowski et al. 1995). As a result, the Chronic Mild Stress model possesses evident face validity with melancholic states.

Exposure to a repeated series of mild stressors leads to neurochemical changes that are consistent with dysregulation of neurotransmitter systems in depression. Increased dopaminergic activity and decreased serotonergic activity in the prefrontal cortex, an increase in 5-HT functioning in the hippocampus and a decrease in dopaminergic activity in the striatum were detected after application of Chronic Mild Stress in rats (Bekris, Antoniou et al. 2005). The changes in neurotransmitter systems were normalised following treatment with imipramine.

The effects of all of the main classes of antidepressants have been studied in this model. Chronic administration of antidepressants, usually over 3-4 weeks, improves behaviour in animals previously exposed to stressors. Similarly to the use of antidepressants in humans, the drugs have little impact on the behaviour of animals not exposed to stressors (Willner 1997). TCAs, the MAO inhibitor moclobemide, the SSRI fluoxetine, the atypical antidepressant mianserin and the specific NA reuptake inhibitor maprotiline were effective in correcting the state of anhedonia induced by chronic mild stress (Willner, Towell et al. 1987; Muscat, Papp et al. 1992; Moreau, Jenck et al. 1993; Moreau, Bourson et al. 1994). Selective 5-HT releasers (Marona-Lewicka and Nichols 1997), as well as 5-$HT_2$ (Moreau, Bos et al. 1996) and 5-$HT_{1A}$ receptor agonists (Przegalinski, Moryl et al. 1995) have also been shown to effectively reverse the state of anhedonia. Apart from clinically prescribed antidepressants, some psychotropic agents, such as amphetamine (Papp, Moryl et al. 1996), haloperidol, and risperidone (Moreau 1997), were also administered to stressed rats, however, they caused no change in behaviour.

The reversal of the anhedonic state in animals exposed to chronic mild stressors by the main classes of antidepressants that are used in clinical practice at present and similar time courses of treatment provide support for the predictive validity of this model of depression.

The effects of MDMA in a Chronic Mild Stress paradigm have been previously studied (Cunningham, Raudensky et al. 2009; Leon, Landeira-Fernandez et al. 2009). In one of the studies, rats were treated with MDMA and subsequently exposed to a series of chronic mild unpredictable stressors. The chosen regime of treatment and stress

application resulted in reduced spatial learning capability in rats, but did not affect serotonergic system functioning (Cunningham, Raudensky et al. 2009). Another study showed that MDMA facilitates the performance of rats exposed to chronic mild stress in a stressor avoidance task (Leon, Landeira-Fernandez et al. 2009). This finding provides information about the possible anxiolytic-like effect of MDMA in the Chronic Mild Stress setting.

Chronic Mild Stress is a valid model of induction of depressive-like symptoms and, to a certain extent, mimics several signs and symptoms of a human condition, including anhedonia, the core component of the MDD.

#### *1.7.3.3. Social stress and maternal separation models*

Certain social settings have an ability to induce stress that leads to alterations in behaviour. In preclinical models, several techniques are used to study the effects of social stress on behaviour, namely, social isolation, maternal separation and social interaction. The latter is linked to social hierarchy and also includes a so-called resident-intruder paradigm.

Extended periods of social isolation have been shown to induce aggressive behaviour in rodents (Wongwitdecha and Marsden 1996; Toth, Halasz et al. 2008). In rats it provokes muricidal behaviour, i.e., the killing of mice (Yoshimura and Ueki 1977). This model of depression is more closely related to features of the disorder in adolescents, when irritability and aggression prevail (Leussis and Andersen 2008). The aggressive behaviour in rodents following social stress responds to antidepressants (Kostowski, Valzelli et al. 1984).

Another social stress model that was proposed over two decades ago is based on social hierarchy and subordination (Malatynska and Kostowski 1984). In this paradigm, animals are housed in pairs and food is provided only during a restricted period of time. This results in the differentiation between the dominant and the submissive animal in a cage. The rat that dominates is thus able to consume a sufficient amount of food. When antidepressants are administered to an unassertive rodent, its behaviour changes and it spends a longer time in competing for access to the food (Pinhasov, Crooke et al. 2005). This model was first established in rats, and consequently was adapted for studies in mice and primates (Raleigh, McGuire et al. 1991; Malatynska, Rapp et al. 2005).

A resident-intruder paradigm involves two rats, one of them is isolated prior to the test, and another is placed in a cage with the pre-isolated animal. Social interaction of these animals is then observed. Residents usually show increased social exploration and aggression towards the intruder (Raab, Dantzer et al. 1986). The main classes of antidepressants, TCAs, MAOIs and SSRIs, after a shorter period of administration, reduce the aggression of the resident animal, however, chronic treatment subsequently exacerbates the aggressive behaviour towards the intruder animal (Mitchell and Redfern 2005). This model was also used in mice (Strekalova, Spanagel et al. 2004). A resident-intruder paradigm can be carried out in another setting, when an intruder is placed in a home cage of a heavier and more aggressive rat (Meerlo, Overkamp et al. 1996). The setting of such social defeat is repeated. The housing of the submissive animal close to the cage of the aggressor can also enhance social stress (McArthur and Borsini 2006). Consequently, the subordinate animal develops behavioural and neurochemical depressive-like changes, namely anhedonia and loss of

appetite, and impaired function of 5-$HT_{1A}$ receptors (Korte, Buwalda et al. 1995; Von Frijtag, Reijmers et al. 2000). Social defeat-induced behavioural deficits can be reversed with antidepressant treatment (Rygula, Abumaria et al. 2006).

The maternal separation paradigm studies the role of early life experiences in the development of depressive symptoms, increased susceptibility to stress and higher risks of substance use (Beatson and Taryan 2003; Reijneveld, Crone et al. 2003). The effect of maternal separation has been extensively studied in humans and laboratory animals, including primates and rats. When pups are separated from their mother in early infancy, they initially develop behaviour that resembles despair (Matthews, Wilkinson et al. 1996). The separation induces changes in HPA axis functioning, including the increased release of corticotropin-releasing factor and corticosterone (Aisa, Tordera et al. 2008). Antidepressants can correct the behavioural changes in previously separated rats (MacQueen, Ramakrishnan et al. 2003). Alterations in 5-HT system functioning have also been found (Arborelius and Eklund 2007; Lee, Kim et al. 2007; Lambas-Senas, Mnie-Filali et al. 2009). Furthermore, exposure to such stressors as maternal separation in early life consequently affects social interaction with offspring and maternal behaviour in previously separated animals (Meaney 2001).

It would be interesting to address the issues of predisposition to depression and MDMA use later in life using the maternal separation paradigm. To date, only one study used MDMA to assess serotonergic modulation of the ultrasonic call of rat pups (Winslow and Insel 1991). However, the effects of MDMA on the depressive-like state in animals, which have previously experienced maternal separation, are yet to be investigated.

### 1.7.4. Olfactory bulbectomy

In the olfactory bulbectomy model of depression, after surgical removal of olfactory bulbs animals exhibit similar behaviour to depressed patients (Song and Leonard 2005). The detection of pheromones plays an important role in psychosocial interaction in rodents (Shepherd 2006). Ablation of olfactory bulbs interferes with functioning of the limbic system and consequently results in an increased sensitivity to stress, psychomotor agitation, disturbed sleep patterns and weight loss (Song and Leonard 2005). The behavioural deficits in mice and rats are corrected after chronic treatment with antidepressants (Broekkamp, Garrigou et al. 1980; Jarosik, Legutko et al. 2007; Roche, Harkin et al. 2007). To date, the olfactory bulbectomy model has not yet been used to study the effects of MDMA on behaviour, but it has been shown to lower the reward-enhancing effects of amphetamine. The induction of locomotor activity was greater following amphetamine administration in rats with olfactory bulbectomy (Romeas, Morissette et al. 2009). Features of psychomotor agitation following olfactory bulbectomy are represented by an increase in locomotor activity. Behaviour of bulbectomised animals resembles that induced by psychostimulants. Therefore, it may be interesting to study the effects of MDMA in this model.

### 1.7.5. Animal tests of depression

The animal tests of depression described below are used to assess the depressive-like state in laboratory animals, as well as the efficacy of antidepressants. These tests may not be considered as models of depression per se, as they are done to assess different aspects of the depressive-like state, as well as the effects of treatment with antidepressants and other drugs. The sucrose preference test is aimed to measure the

state of anhedonia in laboratory animals, and also to assess the hedonic and anhedonic effects of treatment with antidepressants or other psychotropic drugs.

#### *1.7.5.1. Tail Suspension Test*

The Tail Suspension Test is usually used in mice (Steru, Chermat et al. 1985). The principle of this test is similar to the Forced Swimming Test, when an animal is placed in an inescapable situation, and usually initial attempts to escape are followed by prolonged periods of immobility. The duration of the test is 6 minutes, during which an animal is suspended by its tail. Treatment with different types of antidepressants, including TCAs and SSRIs, has been shown to reduce the immobility of mice in this test (Steru, Chermat et al. 1985; Perrault, Morel et al. 1992). The Tail Suspension Test has been previously used to investigate the effects of chronic administration of MDMA on serotonergic neurotransmission in 5-HTT knockout and wild-type mice (Renoir, Paizanis et al. 2008).

#### *1.7.5.2. Forced Swimming Test*

One of the main tests used in preclinical studies of the depressive-like state is the Forced Swimming Test (FST). It is also the most widely used test for screening the efficacy of antidepressants (Porsolt, Le Pichon et al. 1977). During the testing session an animal is placed in a restricted space, usually in a transparent cylinder 18 cm in diameter and 40 cm in height. The tank is filled with water at 25-27$^{o}$C, to the level sufficient that the rat cannot reach the bottom with its hind paws or escape from the top. Initial active attempts to escape subsequently decrease in length and intensity, and immobility periods, when the animal starts to float, performing minimal movements in order not to drown, predominate. Cessation of attempts to escape and

immobility are considered to reflect the state of 'despair' in animals (Porsolt, Anton et al. 1978). The original protocol included a 15-minute pre-test, 24 hours prior to the actual 5-minute test. The aim of the preliminary trial is to induce a constant and reproducible level of immobility in the subsequent test (Porsolt, Anton et al. 1978). Sometimes the pre-test is omitted due to the fact that behaviour observed during the subsequent immersion 24 hours after an initial test. According to some authors, behaviour in the subsequent test may not be a reflection of 'despair', but an adaptation to a stressful situation, since the animal learned in the pre-test that there is no escape from the tank (Hawkins, Hicks et al. 1978). Justification for not using a pre-test has been based on the lack of difference in total immobility time during the actual test between animals who did and did not have opportunity to escape during a pre-test session (O'Neill and Valentino 1982).

Moreover, the modifications of the original FST paradigm include a more structured assessment of observed active behaviour, and a distinction between swimming and climbing (Lucki 1997). SSRIs like fluoxetine, and other compounds mainly affecting the serotonergic system function, affect swimming to a greater extent, whereas active climbing is mainly attributable to the effects of TCAs and other compounds affecting monoaminergic transmission in general (Detke, Rickels et al. 1995; Cryan, Page et al. 2005).

Since its introduction, the FST has been extensively used to screen compounds with potential and established antidepressant activity (Borsini and Meli 1988). Chronic treatment with TCAs (Kitada, Miyauchi et al. 1981; Hedou, Pryce et al. 2001; Kubera, Basta-Kaim et al. 2006), SSRIs (Bianchi, Moser et al. 2002; Sanchez, Bergqvist et al.

2003; Dulawa, Holick et al. 2004), MAOIs (Shimazu, Minami et al. 2005), 5-HT and NA reuptake inhibitors (Mochizuki, Tsujita et al. 2002; Reneric, Bouvard et al. 2002) and the tetracyclic antidepressant mianserin (Kitada, Miyauchi et al. 1981) decreased the total immobility time in the FST. It is believed that increased immobility time may reflect a depressive-like state in laboratory animals. Similarly to clinical settings, when antidepressants start to take effect after 2-3 weeks of continuous administration, changes in immobility in the FST are more evident after 14 days of treatment in comparison to a series of 3 injections, which was proposed in the original FST protocol (Porsolt, Anton et al. 1978; Zangen, Overstreet et al. 1997; Cryan, Page et al. 2005)

The efficacy of antidepressants and related substances has been analysed in the FST paradigm in mice (Shimazu, Minami et al. 2005) and guinea pigs (Wicke, Rex et al. 2007), where similar behaviour to rats has been observed.

The effects of psychostimulants were assessed in the FST paradigm. Amphetamine decreased immobility time, but this finding was attributed to a general induction in locomotor activity that was also observed in the open field test (Porsolt, Anton et al. 1978). Amphetamine thus has been traditionally considered as a false positive in the FST. The effects of methamphetamine are similar to amphetamine (Kitada, Miyauchi et al. 1981; Wicke, Rex et al. 2007), and the use of these compounds in FST as false positives is based on their lesser potential as antidepressants in humans than SSRIs and other therapeutic compounds used in clinical practice at present.

MDMA action in the FST was studied in light of the effects of antidepressants on MDMA-induced 5-HT neuronal loss (Thompson, Li et al. 2004; Durkin, Prendergast et al. 2008). The long-term consequences of MDMA, methamphetamine and their

combination were also assessed using the FST paradigm (Clemens, Cornish et al. 2007). Finally, no effects of the prenatal exposure to MDMA on the behaviour in FST in rats were observed in a recent study (Adori, Zelena et al. 2009).

To conclude, the FST paradigm is well validated and is frequently used to assess depressive-like behaviour in laboratory animals and the efficacy for novel antidepressants. Since there is a lot of evidence of the efficacy of serotonergic compounds to alter behaviour in the FST, the potential antidepressant-like activity of MDMA may be apparent in this test.

#### *1.7.5.3. Sucrose preference test*

The sucrose preference test is used to assess the presence of the state of anhedonia, one of the central depressive symptoms, in laboratory animals. This test has been frequently used in conjunction with Chronic Mild Stress (Willner, Towell et al. 1987; Muscat, Papp et al. 1992), in assessment of the depressive-like state in other animal models of depression (Overstreet 1993; Malkesman, Braw et al. 2005) and as effects of treatment (Sammut, Goodall et al. 2001; Sammut, Bethus et al. 2002). A preference for palatable sucrose and saccharin solutions is reduced in animals with the development of an anhedonic state and reflects their blunted sensitivity to reward (Papp, Willner et al. 1991). The test is usually administered after a period of food and water deprivation for 4-24 hours (Prendergast, Yells et al. 2002; Sammut, Bethus et al. 2002). During the tests, bottles of either sucrose or saccharine solutions are placed in cages and rats are allowed access for an hour. There are several variants of the sucrose preference test. In some studies, preference for either saccharin or a weak sucrose solution (0.8-1%) is compared with water consumption (Grippo, Francis et al. 2005; Rygula, Abumaria et al.

2005; Tonissaar, Herm et al. 2006; Romeas, Morissette et al. 2009). A single bottle test, using only saccharin or sucrose, has also been used in some studies (Muscat, Papp et al. 1992; Malkesman, Braw et al. 2005). Other test modifications use three different sucrose solutions, 1%, 8% and 32% (Sammut, Bethus et al. 2002) or 0.7%, 7% and 34% (Muscat, Kyprianou et al. 1991; Phillips, Willner et al. 1991). In this test, increased preference for the strongest solution has been associated with changes in behaviour due to the development of anhedonia, which is sensitive to repeated antidepressant administration (Sammut, Bethus et al. 2002). Reciprocal changes in the preference for the weaker solutions, namely decreased intake, also represent a state of anhedonia (Phillips, Willner et al. 1991; Sammut, Bethus et al. 2002).

Sensitivity to reward is primarily controlled by the dopaminergic neuronal circuitry (Lippa, Antelman et al. 1973). Decreased sucrose consumption in comparison to water, or increased preference for strong solutions and decreased preference for weaker, more palatable solutions, are likely to represent blunted sensitivity to reward. Indeed, such changes in sucrose preference were induced by administration of DA antagonists (Muscat, Kyprianou et al. 1991; Phillips, Willner et al. 1991).

The sucrose preference test in MDMA studies has been done to assess the involvement of prenatal MDMA exposure on behavioural and neurochemical functioning in rats (Galineau, Belzung et al. 2005; Adori, Zelena et al. 2009).

In summary, the animal tests described above aim to assess the behavioural changes which are present in genetic models of depression, or induced by stress or treatment. Similarities in the behaviour of animals in these tests with depressed patients and the ability of antidepressants to reverse the equivalent of ‘despair’ or anhedonia

contribute to face and predictive validity of animal models of depression. Characteristics of three of the selectively bred rat strains, which are putative models of depression, are described below.

### 1.7.6. Genetic rodent models of depression

#### *1.7.6.1. Fawn-Hooded rats*

Fawn-Hooded rats (FH) are an animal model of depression, anxiety and alcohol abuse (Rezvani, Overstreet et al. 1990; Kantor, Anheuer et al. 2000). These animals exhibit an exaggerated immobility in the FST and voluntarily consume increased volumes of ethanol in comparison with other rat strains (Overstreet, Rezvani et al. 1992). Moreover, the FH rats are substantially more anxious in the social interaction test than Wistar and Sprague-Dawley (SD) rats (Kantor, Anheuer et al. 2000). The administration of SSRIs and other antidepressants to FH rats reduced their depressive-like state and exaggerated consumption of ethanol. In addition, subchronic treatment with 5 mg/kg of MDMA significantly decreased ethanol consumption in FH rats (Rezvani, Garges et al. 1992). This finding further supports the presence of a 5-HT deficiency in FH rats, as MDMA is a very potent 5-HT release inducer.

The FH animal model was originally developed to investigate bleeding disorders as they have a hereditary platelet storage pool deficiency (Tschopp and Zucker 1972; Raymond and Dodds 1975). The utility of this strain in the research of bleeding disorders and depression is due to their genetic predisposition to 5-HT system dysfunction both in the CNS and periphery. The FH rat strain was also regarded as an animal model of spontaneous hypertension (Magro, Rudofsky et al. 1986).

The impaired functioning of the serotonergic system in FH rats is due to 5-HT storage abnormalities, as well as impaired 5-HTT and 5-HT receptor expression (Gudelsky, Koenig et al. 1985; Morecroft, Loughlin et al. 2005). The hereditary serotonergic dysfunction contributes to their utility as an animal model of depression, whereas face and construct validity of the FH strain as a model of alcoholism is associated more with changes in dopaminergic mesolimbic reward system functioning, as the serotonergic system interacts with it (Chen and Lawrence 2003). FH rats have increased reuptake of 5-HT in synaptopsomes in the limbic regions in comparison to Sprague-Dawley rats; this leads to depletion of extracellular 5-HT levels and thus may underlie the depressive-like behaviour on one hand, and increased voluntary ethanol intake as a 'self-medication' remedy against exhaustion of serotonin levels on the other (Chen and Lawrence 2000). The exaggerated serotonergic uptake may be due to increased density of 5-HTT sites in some brain regions of FH rats (Chen and Lawrence 2003). Increased expression of $5\text{-HT}_{1A}$ receptors further contributes to the serotonergic dysfunction in FH rats (Chen and Lawrence 2000).

Changes in HPA activity has been also observed in some studies of this animal model of depression. Increased corticosterone levels, susceptible to antidepressant treatment, were documented for this model of depression (Aulakh, Wozniak et al. 1988; Aulakh, Hill et al. 1993), however, these findings were not replicated in other studies (Lahmame, Gomez et al. 1996; Gomez, Grauges et al. 1999). Gómez and colleagues (1999) did not observe elevation in basal corticosterone and ACTH levels, however, decreased serum corticosterone binding capacity was noted. In contrast, no changes in HPA axis functioning or any depressive-like behaviour was reported in another study (Lahmame, Gomez et al. 1996).

In addition, the FH rat strain is heterogeneous, with marked behavioural differences between substrains that originate from different colonies (Overstreet, Rezvani et al. 2007). Voluntary ethanol intake is mostly increased in the FH/Wjd substrain, whereas FH/Har rats are markedly less immobile in the FST than rats of other FH strain subtypes (Overstreet, Rezvani et al. 1999).

The effects of psychostimulants, including cocaine (Broderick and Hope 2006) and MDMA (Rezvani, Garges et al. 1992; Schechter 1997; Schechter 1998), have been previously studied in the FH rat strain. In one of the studies, responses to cocaine of FH and SD rats were compared. The stimulant caused increases in 5-HT and DA release in SD, whereas in FH rats, the release of neurotransmitters was decreased. Moreover, the behaviour of FH rats, when administered with cocaine, differed from SD rats, and their locomotor activity was less pronounced than in a 'normal' strain. Observed differences between strains were likely due to the 5-HT deficiency in the CNS of FH rats.

Studies with MDMA were focussed on the drug's potential to rapidly increase 5-HT levels in brain, thus improving the state in animals and decreasing their ethanol intake (Rezvani, Garges et al. 1992).

Due to genetically predisposed 5-HT system dysfunction, the FH strain can be considered as a model of co-existing disorders, such as depression, substance abuse (including alcoholism), and anxiety. Platelet pathology and hypertension are also modelled using FH rats. Due to mirroring of such diverse disorders, the FH rat strain may not an ideal animal model to assess depressive symptoms solely. However, as ecstasy is frequently consumed along with alcohol, the role of the FH rat strain in investigation of the effects of concomitant use of MDMA in subjects with depressive and anxiety disorders is worth considering. Moreover, existing 5-HT depletion may

predispose to 'self-medication' techniques, and MDMA, being an efficient inducer of monoamine release, may be ingested in order to improve the state. It would be interesting to investigate MDMA's potential in a self-administration setting in the FH rat model of depression.

#### *1.7.6.2. Wistar Kyoto rats*

The Wistar Kyoto rat strain (WKY) is another preclinical model of depression. Originally the WKY rats were bred for use as a control strain for Spontaneously Hypertensive rats, however, examination of the behavioural, neurochemical and neuruendocrine characteristics of WKY rats led to their use as a model of depression. The WKY rats exhibit depressive-like and anxiety-like behaviour and are also considered to be a model of childhood depression (Malkesman, Braw et al. 2005; Braw, Malkesman et al. 2006; Malkesman, Braw et al. 2006). Their depressive-like state develops after exposure to stress (Pare 1989; Lahmame, del Arco et al. 1997; De La Garza and Mahoney 2004).

These rats are more immobile in the FST than SD and Spontaneously Hypertensive rats (Pare 1989; Lahmame and Armario 1996). They have decreased responses to serotonergic agonists, including a selective 5-$HT_{1A}$ receptor agonist 8-OH-DPAT (Lahmame and Armario 1996; Lopez-Rubalcava and Lucki 2000), decreased density of 5-HT reuptake sites in hippocampus (Pollier, Sarre et al. 2000), increased 5-HT turnover in prefrontal cortex, striatum and nucleus accumbens and decreased tissue 5-HIAA levels in prefrontal cortex, amygdala and nucleus accumbens after exposure to stress (De La Garza and Mahoney 2004). Impairment in functioning in DA and NA systems have also been reported. WKY rats have decreased tissue levels of DA and its

metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), suggesting lower DA output and lower DAT binding (Jiao, Pare et al. 2003; De La Garza and Mahoney 2004). The number of NAT binding sites is decreased in WKY rats when they are exposed to acute stress (Tejani-Butt, Pare et al. 1994).

The chronic administration of imipramine improved performance of WKY rats in the FST, however the effect was less pronounced than in 'non-depressed' strains (Lahmame, del Arco et al. 1997).

One of the substrains of the WKY animal model shows exaggerated responsiveness to antidepressants (Will, Aird et al. 2003). Animals with polar behaviour in the FST were compared. In the 'most immobile' substrain administration of TCA and MAOI significantly reduced the immobility of rats in the FST, whereas in its control, the 'WKY least immobile' substrain, the effect was much less pronounced.

Comorbidity of anxiety and depressive disorders is fairly common (Middeldorp, Cath et al. 2005), and ecstasy is known to have some anxiolytic properties as one of its subjective effects. Moreover, the antidepressant-like potential of MDMA, which is examined in the present study, may provide the basis for investigating the drug's effects on the co-existing anxiety-like and depressive-like states in this animal model.

#### *1.7.6.3. Flinders Sensitive Line rats*

Overstreet and colleagues at the Flinders University in Adelaide developed the Flinders Sensitive Line (FSL) rat strain in the 1980s. Originally FSL rats and their genetic counterparts, Flinders Resistant Line rats (FRL), were selectively bred from Sprague-Dawley (SD) rats based on their differences in sensitivity to cholinergic agents (Overstreet 1986). FSL rats have a hypothermic response to a cholinesterase inhibitor,

diisopropyl fluorophosphate (DFP), whereas FRL rats are as resistant to DFP as 'normal' rats (Overstreet, Russell et al. 1979). Subsequently, the use of this rat strain to assess cholinergic mechanisms similar to those of depressed patients, who are more sensitive to cholinergic activity, was proposed (Janowsky, Overstreet et al. 1994), and further research with FSL rats revealed more behavioural and neurochemical similarities with patients suffering from mood disorders (Overstreet 1986).

Indeed, the FSL rat strain is considered to be a suitable model of depression, as it has a considerable degree of face, construct and predictive validity. These animals have reduced body weight and decreased appetite compared with SD rats, and features of psychomotor retardation, as FSL rats are less active in an open field test and have slower response in operant responding tests in comparison with SD rats (Overstreet 1993; Bushnell, Levin et al. 1995). Moreover, it has been established that FSL rats have altered sleep patterns, namely, increased amounts and reduced latency of REM sleep in comparison to the SD strain, from which they originated (Shiromani, Armstrong et al. 1988). The depressive-like state of FSL rats is reproduced as increased immobility in the FST compared with FRL and SD rats (Overstreet, Janowsky et al. 1986; Pucilowski and Overstreet 1993; Overstreet and Griebel 2004; Overstreet, Hlavka et al. 2004). However, anhedonia is not present in a non-stressed FSL rat. Nevertheless, after application of chronic mild stress, the sucrose preference of FSL rats is decreased when compared with SD rats (Overstreet 1993). This allows consideration of the FSL rat strain not only as a putative model of depression, but also as a genetic model of predisposition to depression.

Cytokine activity has been associated with depression (Schiepers, Wichers et al. 2005). Impairment in immunological system function, such as increased levels of circulating cytokines and decreased natural killer cytotoxicity and lymphocyte proliferative responses (Friedman, Berman et al. 2006) similar to observations in depressed patients (Herbert and Cohen 1993), have been found in FSL rats, which provides further evidence of the utility of this strain as an animal model of depression.

The construct validity of the FSL model is presented by the changes in neurotransmitter systems similar to the dysfunction observed in depressed patients. It has been found that FSL rats have prominent alterations in the 5-HT system, including: exaggerated sensitivity to the $5\text{-HT}_{1A}$ receptor agonists, such as 8-OH-DPAT (Overstreet, Rezvani et al. 1994), increased tissue 5-HT and 5-HIAA levels in limbic regions (Zangen, Overstreet et al. 1997), and decreased levels of vesicular transporter ($VMAT_2$) expression in limbic regions (Schwartz, Yadid et al. 2003), decreased 5-HT synthesis in several limbic and cortical regions, compared both to FRL and SD rats, probably as a compensatory mechanism for other changes in the neurotransmitter system (Hasegawa, Nishi et al. 2006). Increased tissue levels of serotonin and its main metabolite are normalised following repeated administration of antidepressants (Zangen, Overstreet et al. 1997). Furthermore, chronic administration of citalopram and buspirone has been shown to increase 5-HT synthesis to levels similar to SD rats (Kanemaru, Nishi et al. 2009; Nishi, Kanemaru et al. 2009).

Abnormal functioning of the monoaminergic neurotransmitter systems in FSL rats further supports the construct validity of this model of depression. It has been reported that FSL rats have increased activity of tyrosine hydroxylase (TH) activity in

the ventral tegmental area (VTA), from which neurons of the mesocorticolimbic pathway originate (Serova, Sabban et al. 1998), thus resulting in higher levels in DA and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in striatum, the hypothalamus, the hippocampus and nucleus accumbens in comparison to SD rats (Zangen, Overstreet et al. 1999). Moreover, similar observations were reported for noradrenergic system functioning in FSL rats: when compared with SD rats, the levels of NA in FSL rats were found to be significantly higher in the prefrontal cortex, nucleus accumbens, the hippocampus and median raphe; and the levels of homovanillic acid (HVA), a catecholamine metabolite, were markedly higher in the striatum, hypothalamus and nucleus accumbens (Zangen, Overstreet et al. 1999). Increased tissue levels of 5-HT, DA and NA may reflect decreased release of the neurotransmitters in the intrasynaptic space.

Investigation of the parameters related to HPA axis functioning in FSL rats not exposed to any stressors revealed lower CRF levels in locus coeruleus and diminished ACTH levels in plasma in comparison to FRL rats, with no differences in corticosterone concentrations (Owens, Overstreet et al. 1991). In contrast, an elevation of corticosterone serum levels was observed in response to the administration of a cholinergic agonist (Overstreet, Booth et al. 1986). Additionally, Malkesman et al found increased basal levels of corticosterone, but not ACTH, in FSL rats compared with SD rats, which is a broader control strain for FSL rats; a social isolation paradigm did not affect the levels in SD, but produced a marked decrease in corticosterone concentration in FSL rats (Malkesman, Maayan et al. 2006).

The predictive validity of the FSL model of depression has been extensively investigated using a wide spectrum of antidepressant drugs, including TCAs (desipramine), SSRIs (paroxetine) and SNRIs (nefazodone) (Zangen, Overstreet et al. 1999; Yadid, Nakash et al. 2000). Novel compounds with antidepressant potential have also been studied in this model (Overstreet and Griebel 2004; Overstreet, Hlavka et al. 2004; Overstreet, Keeney et al. 2004). Thus, given that chronic administration of antidepressants significantly improved performance of FSL rats in the FST, and normalise the neurotransmitter levels (Zangen, Overstreet et al. 1997; Zangen, Overstreet et al. 1999), this rat model also has a substantial predictive validity.

Interestingly, stimulant drugs, such as amphetamine, had no effect on FST performance in FSL rats after chronic administration (Overstreet, Pucilowski et al. 1995). This may reflect the fact that amphetamine primarily influences the dopaminergic system and, to a much lesser extent, the serotonergic system. However, amphetamine and other psychostimulants have been regarded as false positives in the FST due to an induction in activity shortly after administration (Borsini and Meli 1988). Nevertheless, MDMA, having a substantial influence firstly on the serotonergic system, may be effective in improvement of the depressive-like state in animals. To date, the effects of MDMA have not yet been studied in the FSL model of depression. As the FSL rat model has a considerable face, construct and predictive validity, this model may be more suitable to investigate the antidepressant-like potential of MDMA than other models that are used in preclinical studies at present.

## 1.8. MDMA and depression: what are the links?

The growing body of research into the psychological environment of MDMA use suggests that the link between ecstasy use and depressive symptoms is likely to be complex. This part of the review will explore some of the possible explanations of this connection.

According to recent publications, there is a global trend of increases in the rates of diagnosis of depression in young people. The 12-month prevalence of MDD in people aged 18-29 was 6.39% in the USA (Hasin, Goodwin et al. 2005). According to a recent mental health survey, the 12-month prevalence of affective disorders was approximately 7% in people aged 15-34 years in Australia (Australian Bureau of Statistics 2007). The trend in the diagnosis of depression may be evident from the increase in the prescription of antidepressants, which has grown substantially since the 1990s, when SSRIs were indroduced (Mant, Rendle et al. 2004; Katz, Kozyrskyj et al. 2008; Moore, Yuen et al. 2009). In Australia, the prescription of antidepressants in the 15-34 years age group, represented as defined daily dose/1000 people/day, grew from approximately 2 in 1990 to 20 in 2001 for males and from 6 to 44 for females (Hall, Mant et al. 2003). However, such an increase may also reflect greater willingness of medical practitioners to prescribe antidepressants (Kendrick, King et al. 2005).

In comparison with the general population, rates of mood disorders are increased in drug users, and at the same time substance abuse is more common in people with depression (Swadi and Bobier 2003; Currie, Patten et al. 2005). The existence of a mood disorder with concurrent drug use may be explained through different hypotheses. Repeated consumption of illicit substances may lead to disruption in the

functioning of various neurotransmitter systems in brain. This, in turn, can underlie the development of the depressive symptoms. As has been discussed previously, MDMA causes a rapid increase in extracellular 5-HT levels, followed by depletion in stores in the following days. This may underlie depressive and other psychological symptoms as a manifestation of the so-called 'mid-week blues' that users note a few days after ecstasy consumption. MDMA may have the potential to cause long-term depletions of 5-HT stores following regular use, which may lead to depressive symptoms in heavy and former ecstasy users (Parrott, Sisk et al. 2000; MacInnes, Handley et al. 2001; Parrott, Buchanan et al. 2002). The neurotoxic effects of MDMA have been demonstrated in a large number of animal studies (Ricaurte, Bryan et al. 1985; Colado, Williams et al. 1995; Dafters and Lynch 1998; Morley, Gallate et al. 2001; Gurtman, Morley et al. 2002; Wang, Baumann et al. 2004; O'Shea, Orio et al. 2006; Baumann, Wang et al. 2007; Jaehne, Salem et al. 2008). However, inconsistent findings regarding the delayed effects of MDMA on mental health in humans, which are likely to be due to polydrug use, and not exclusively to ecstasy, warrants further research into this aspect of MDMA's actions, with longitudinal design being more appropriate. This improved approach will resolve the confounding factor of polydrug use and will help identify psychiatric effects of MDMA.

Another possible relationship between depression and substance use is that these disorders may be different manifestations of one underlying pathogenetic mechanism (Markou, Kosten et al. 1998). This has been discussed in light of drug dependence, but may relate to substance use as well. However, it is not clear whether MDMA use can be explained in such a way. One of the possible explanations may be that the mean

age of initiation of ecstasy use is close to the age when the onset of psychiatric illness can occur (Soar, Turner et al. 2001).

Finally, one of the explanations of the occurrence of depressive symptoms in illicit substance users is the so-called self-medication hypothesis (SMH). This hypothesis was first suggested over 30 years ago by Khantzian and was mainly focussed on drug dependence (Khantzian 1974). However, the principle of the self-medication hypothesis may be applicable to the connection between a predisposition to depression and ecstasy consumption by users who do not meet the criteria for dependence. According to this hypothesis, the use of a particular drug by people may be explained by their desire to control their emotional state in order to be able to cope with negative aspects of a pre-existing psychiatric condition (Khantzian 1977). The SMH may explain why drug users, despite the negative effects of the drugs, continue to consume them in order to alleviate their psychological distress caused by co-existing depressive or other affective disorders that they suffer from (Khantzian 1997). Due to an inability to tolerate negative affect, people may choose to take drugs to alter their emotional state so that a so-called 'affect deficit' diminishes (Hall and Queener 2007).

A few studies focussed on predicting substance use based on susceptibility to mood and anxiety disorders in childhood and adolescence found that certain parental anxiety and depression disorders (Lieb, Isensee et al. 2002), parental use of alcohol and nicotine (Alati, Najman et al. 2005) and delinquent behaviour in childhood predispose individuals to substance use and abuse later in life (King, Iacono et al. 2004). Some studies were focussed on early predictors of nicotine use and dependence (Fergusson, Goodwin et al. 2003; King, Iacono et al. 2004), alcohol use (Wu, Bird et al. 2006) and

polysubstance use (Wu, Hoven et al. 2008). Experiencing a life-threatening event or disaster in adolescence have been associated with subsequent depressive and anxiety symptoms, as well as excessive alcohol use (Reijneveld, Crone et al. 2003). One of the recent studies found a positive association between the high intensity of depressive symptoms in children and the earlier onset of illicit drug use in general (Wu, Hoven et al. 2008). However, research into depression in early life as a predictor of MDMA use is still scant. To date, three large-scale longitudinal studies have been undertaken to approach this area of the relationship between depressive symptoms and MDMA use (Lieb, Schuetz et al. 2002; Huizink, Ferdinand et al. 2006; Alati, Kinner et al. 2008).

Lieb et al (2002) were the first to address the cause-effect relationship of ecstasy use and depression. In a prospective longitudinal study, almost 70% of respondents aged 14-24 years who reported having used ecstasy in their lifetime also reported at least one mental disorder. An increased incidence of affective disorders was observed in ecstasy users compared with ecstasy-naïve controls, and in almost 90% of cases the onset of mental disorder was reported prior to the initiation of ecstasy use. Phobic disorders were the most frequently reported mental problems that preceded ecstasy use. Interestingly, depression was one of the most frequently reported mental disorders that developed after the start of ecstasy use (Lieb, Schuetz et al. 2002).

A large-scale longitudinal population-based study in the Netherlands Huizink and colleagues (2006) explored the relationship between early depression and anxiety symptoms with MDMA use. In this study, children who reported increased depression and anxiety scores at baseline had a significantly increased risk of initiation of MDMA use at a 14-year follow-up (Huizink, Ferdinand et al. 2006). In a multi-centre,

community-based study, analysis of economic and psychological factors influencing ecstasy use has shown that depression may be considered as a predictor of increased drug consumption, likely through potentiation of motivation to take ecstasy (Abdallah, Scheier et al. 2007). Correspondingly, Falck et al argued that for those subjects who experienced depression prior to initiation of ecstasy use, the drug would be more appealing due to its empathic and mood enhancing properties (Falck, Wang et al. 2006; Falck, Jichuan et al. 2008). This is in concordance with the self-medication potential of ecstasy in depressed individuals, who may experience more prominent positive effects of the drug than subjects with normal 5-HT functioning, and this may lead to more frequent use of ecstasy by individuals with pre-existing 5-HT impairment (Croft, Klugman et al. 2001).

An Australian study used a similar approach to the Dutch study (Alati, Kinner et al. 2008). When assessed at the age of 21 years, participants who have had aggressive and delinquent behaviour, or who consumed alcohol and nicotine at 14 years, were at increased risk of ecstasy use disorder. However, no association between anxiety and mood disorders in childhood and increased risk of ecstasy use later in life was observed. Similarly, no association between mental health problems and subsequent greater ecstasy use was found in another smaller-scale study (de Win, Schilt et al. 2006; de Win, Reneman et al. 2007).

Preliminary psychiatric disturbance may underlie an increased intensity of psychological problems that develop due to ecstasy consumption. Impairment in the functioning of the serotonergic system is likely to cause the development of depression, but additional stimulation of the 5-HT system by MDMA may lead to more

prominent disruption in system functioning and thus more negative consequences. A pre-existing susceptibility to mental problems has been discussed in a study in which users who experienced psychological problems related to ecstasy use, also had higher scores for depression and anxiety and higher levels of somatisation in comparison with users who were not considered to have any ecstasy-related problems (Soar, Turner et al. 2006). Authors suggested that preliminary psychiatric condition may be the cause of a more prominent vulnerability to the toxic effects of ecstasy. This may contribute to users' perception of adverse effects of ecstasy.

Furthermore, increased sensitivity to MDMA-induced disturbance of the 5-HT system may be genetically predefined. The 5-HTT gene polymorphism, as described earlier in this review, is a major contributor to genetic predisposition to mood disturbance. Carrying the short allele produced by a functional polymorphism of the 5-HTT gene has been associated with a more significant emotional disturbance in ecstasy users in comparison to ecstasy-naïve controls that carry the same allele (Roiser, Cook et al. 2005). Additionally, the 5-HTTPLR functional gene polymorphism is associated with an increased incidence of mood disorders in ecstasy users (Martin-Santos, Torrens et al. 2009).

In sum, depressive symptoms may precede, co-exist and develop due to ecstasy use. Increased susceptibility to depressive and other mental disorders has been associated with a higher risk of ecstasy initiation, and, possibly, with more psychopathological problems associated with repeated MDMA use.

## 1.9. Summary, aims and hypotheses

A number of research studies on the effects of MDMA and the consequences of drug use have emerged in recent years. MDMA, commonly sold as ecstasy tablets, is a psychostimulant illicit amphetamine derivative with unique properties. Ecstasy culture is a widespread phenomenon, and the drug is especially popular among young people who attend raves and dance parties. The drug produces empathic and prosocial effects shortly after consumption. The pharmacological action of MDMA includes stimulation of the serotonergic system in the brain leading to a rapid increase in the 5-HT levels in the intrasynaptic space, which underlies the immediate effects of the drug. Due to some similarities in its mechanisms of action with clinically prescribed antidepressants, MDMA may potentially have some antidepressant-like properties. However, differences between MDMA and clinically prescribed antidepressants in some of their pharmacological actions may underlie the differences in the onset of action: subjective effects of MDMA develop within an hour after consumption, whereas clinical improvement in subjects suffering from depression becomes apparent after several weeks of continuous treatment with SSRIs or other prescribed antidepressants.

Apart from the potential beneficial effects on mood and self-esteem, which might find application in psychotherapy, MDMA-induced disruption in the serotonergic and monoaminergic systems functioning in brain may underlie a spectrum of adverse effects, including hyperthermia, which in some cases can have negative physical and mental health consequences of various intensity. Moreover, the neurotoxic potential of MDMA has been extensively investigated in laboratory animals and reproduced in some observations in ecstasy users.

MDMA effects on the serotonergic system and to a lesser extent noradrenergic and dopaminergic neurotransmitter system may underlie the development of depressive symptoms associated with exposure to the drug. Ecstasy users sometimes experience low mood, or a so-called 'mid-week blues', presumed to be due to depletion in 5-HT stores in the days following consumption. Depressive symptoms in long-term and former users have also been associated with repeated ecstasy consumption.

On the other hand, individuals with a predisposition to psychiatric disorders, including depression, have a higher risk of using illicit drugs in an attempt to 'normalise' their state. Subjects with pre-existing alterations in the 5-HT system or increased susceptibility to depression due to environmental or genetic factors may experience a more prominent positive immediate effect of MDMA than users with no mood disturbance. In this regard, ecstasy may be more appealing to subjects with a predisposition to depression. Such positive 'self-medicating' effects of MDMA may contribute to more frequent use of ecstasy by individuals susceptible to mood disorders. Exploration of the antidepressant-like potential of MDMA may thus provide better understanding of the patterns of drug use.

The main focus of this study is to explore the likelihood of MDMA use as a self-medication tool by subjects with a pre-existing vulnerability to depression. The development of depressive symptoms is heterogeneous, and, to date, the exact pathogenesis is still not fully understood. The changes in CNS functioning that are associated with depression affect serotonergic, monoaminergic, cholinergic and some neuropeptide neurotransmitter systems, as well as the neuroendocrine and immune systems. There are a significant number of animal models of depression, which, to a

certain extent, correlate with the symptoms and neurochemical changes observed in patients suffering from depression. It is important to address this relationship between MDMA and depression both on preclinical and clinical levels. The Flinders Sensitive Line rat strain represents a putative model of depression and a predisposition to depression. The validity of this model has been extensively evaluated and it would likely to be suitable for investigation of the relationship between MDMA effects and predisposition to depression. However, to date, the possible antidepressant-like action of MDMA has not been investigated in preclinical settings.

The overall aim of the present study was to investigate the antidepressant-like effects of MDMA. An animal study and a clinical study were carried out.

The animal study addressed the following aims:

1. To determine whether MDMA has a dose-dependent antidepressant-like effect in an animal model of depression;
    a. To determine whether this effect occurs after acute or repeated drug administration; and
2. To confirm that presumed antidepressant-like activity of MDMA is not a consequence of a general stimulant effect of the drug.

The following aims were formulated for the clinical study:

1. To estimate the severity of depressive symptoms in ecstasy users;

2. To compare rates of depression in those with a pre-existing mood disturbance and those without a predisposition to depression;
3. To determine whether depressive symptoms are alleviated following ecstasy consumption and if it occurs in subjects with and those without a predisposition to depression; and
4. To determine the effects of ecstasy use on mood in individuals with a varying previous exposure to the drug.

The preliminary hypotheses regarding the possible links between the MDMA use and depression include:

1. The acute administration of MDMA to rats in an animal model of depression will reduce the depressive-like state, which will be represented by changes in the performance in the FST paradigm. This will result in similar behaviour between FSL and SD rats.
2. Repeated administration of MDMA to FSL rats may result in a regression to the depressive-like features that are observed at the baseline (i.e. prior to MDMA administration) in FSL rats; consequently, the depressive-like state in FSL rats due to repeated MDMA administration will be more prominent than the one observed in their counterparts;
3. Ecstasy users with a predisposition to depression will report more prominent mood disturbance and depressive symptoms than users without such predisposition, when they are not under the influence of the drug;

4. The antidepressant-like effect of ecstasy will be more evident in subjects with a predisposition to depression following pill consumption;
5. Predisposition to depression will be associated with more frequent ecstasy use; and
6. Subjects with a greater previous exposure to ecstasy will have more prominent changes in mood and depressive symptoms when they are under the influence of the drug compared to those with a lower total amount of ecstasy pills consumed in their lifetime.

The following chapters describe the results of the animal study and the clinical study and discuss the possible implications of the findings.

# Chapter 2. Effects of MDMA on behaviour in an animal model of depression

## 2.1. Introduction

The next three chapters describe the results of the preclinical part of the project. These studies were designed to address the question of the role of a predisposition to depression in the effects of MDMA using approaches that are not applicable in clinical research. Antidepressant-like activity of MDMA may exist due to similarities between some mechanisms of action of the drug in the brain to those of antidepressants used in clinical practice at present, as described above (see Section 1.2 of Chapter 1). However, the antidepressant-like activity of MDMA has not been yet investigated on a preclinical level. A good animal model is essential to better understand the mechanisms that may underlie such action of MDMA. The accumulation of mechanistic data on the antidepressant-like activity of MDMA may consequently add knowledge to the area of ecstasy use and mood disorders, and allow a better understanding of the patterns and sequelae of drug use.

Modelling the mechanisms of mental disorders on a preclinical level can sometime be challenging due to certain limitations. Indeed, some characteristic symptoms of depression, e.g. suicidal ideation, cannot be reproduced in laboratory animals. Nevertheless, for most depressive symptoms, including the two core ones, depressed mood and anhedonia, behavioural equivalents have been found in laboratory animals. In preclinical research, the equivalents of depressive behaviour are widely assessed by validated tests. As mentioned previously, the Forced Swimming Test may be now

considered as a 'gold standard' in the assessment of the depressive-like state in animals.

In order to investigate the effects of MDMA on behaviour, the FST was used at two different time points: shortly after a single drug injection and after a series of injections over 3 weeks. This protocol was aimed to look not only at the onset of the presumed antidepressant-like action of MDMA, but also to compare it with clinically prescribed antidepressants. It has been previously established that the effects of the main classes of antidepressants become evident after continuous administration over 2-3 weeks (Blier and de Montigny 1994; Stahl 1998; Thompson 2002). Moreover, subacute treatment with antidepressants does not have as prominent an effect on animal behaviour as chronic administration over 14 days (Cryan, Page et al. 2005).

The FSL rat strain is considered not only an animal model of depression, but also predisposition to depression (Overstreet, Janowsky et al. 1986; Overstreet 1993). The depressive-like state of FSL rats can be induced by the application of Chronic Mild Stress (Ayensu, Pucilowski et al. 1995). The effects of MDMA have not been previously investigated in this animal model. The FSL rat model of depression was originally developed with an outbred control strain, the Flinders Resistant Line (FRL). When investigating all aspects of this animal model of depression, the FRL rats were commonly used as controls (Overstreet 1986; Overstreet, Pucilowski et al. 1995; Overstreet, Friedman et al. 2005). In some studies, however, SD rats were used as counterparts for the FSL rats (Overstreet, Janowsky et al. 1986; Zangen, Overstreet et al. 1997; Schwartz, Yadid et al. 2003; Hasegawa, Nishi et al. 2006; Malkesman, Maayan et al. 2006; Kanemaru and Diksic 2009). This project compared the behavioural and

biochemical parameters of FSL rats with SD rats, as there is a very extensive body of research into the MDMA action in SD rats. FSL rats were originally outbred from SD rats and it has been shown that FRL rats do not differ from SD in the parameters that were the focus of this project (Hasegawa, Nishi et al. 2006).

The hypothesis addressed in this chapter is as follows: MDMA will cause a dose-dependent reversal of the depressive-like state in the FSL model of depression and this will occur as an acute effect. This effect will be more pronounced in FSL than in SD rats. The antidepressant-like effect of MDMA may not be evident after continuous drug administration, in contrast with therapeutic antidepressants, such as SSRIs and TCAs, possibly due to difference in underlying mechanisms of action. The subjective effects of MDMA in humans develop shortly after drug ingestion (Baylen and Rosenberg 2006). Accordingly, in rats, MDMA produces changes in behaviour similar to humans 30-60 minutes after administration: the drug stimulates social interaction, decreases aggressive behaviour and increases locomotor activity (Morley and McGregor 2000).

In sum, the preclinical part of this project was designed to investigate effects of MDMA in an animal model of depression and compare changes in behaviour of these animals with 'normal' rats. It was hypothesised that a greater positive effect of MDMA will be observed in an animal model of depression, namely the depressive-like state will be reversed following drug administration in the acute setting. Repeated drug administration may, however, exacerbate the depressive-like behaviour in FSL animals, due to pre-existing dysregulation of the 5-HT system. This may be consistent with the self-medication hypothesis, as subjects with pre-existing mood disturbance are more likely to have greater reinforcement due to initial MDMA action, followed by a greater

risk of more profound negative effects on mood and behaviour due to repeated exposure to MDMA.

Finally, the protocol of this study included methamphetamine (METH) as another tested drug. METH and MDMA are both amphetamine derivatives with psychostimulant properties. The stimulant effects of both drugs are evident after acute administration in humans and laboratory animals (Dumont and Verkes 2006; Clemens, McGregor et al. 2007; Hart, Gunderson et al. 2008). In the earlier studies of the depressive-like state of FSL rats, amphetamine was used as a positive control, when the rats underwent the FST following acute drug administration. It has been argued that the significant reduction in immobility time in the FST following drug administration could be simply due to a general induction of activity (Overstreet, Pucilowski et al. 1995). To distinguish the general stimulant and antidepressant-like effects of MDMA, additional behavioural experiments were included in the protocol, where MDMA was compared with METH (see Materials and Methods in this chapter).

## 2.2. Materials and methods

### 2.2.1. Animals

32 male Sprague-Dawley (SD) and 32 Flinders Sensitive Line (FSL) male rats were used in the main experimental protocol. Additionally, 4 rats of each strain were used in each of the 3 treatment groups that underwent the locomotor activity assessment. All animals in the main experimental group were 7 weeks of age at the start of the experiments. Rats used for the locomotor activity test were 11 weeks of age. Animals were matched by age as it has been shown that FSL rats have significantly lower body weight than their counterparts; this is another feature of this model of depression that

gives it face validity (Overstreet 1986). Mean weight was 279.9±4.43g for SD rats and 185.4±5.89g for FSL rats at the start of experiments (mean±SEM). SD rats were supplied from the Waite Campus Animal Facility of the University of Adelaide; FSL rats were supplied from the colony of the Sydney University of Technology, which subsequently was transferred to the Veterinary Services of the Institute of Medical and Veterinary Science in Adelaide and then to the University of Adelaide. Animals were housed in groups of four in acrylic cages at the Animal Facility of the Medical School of the University of Adelaide under general conditions (12h:12h light/dark cycle, lights on at 07.00 am, room temperature 20-22$^0$C). Food and water were provided *ad libitum*. Animals were allowed to adapt themselves to the new environment for one week prior to the start of the behavioural tests. All animal procedures were approved by the University of Adelaide Animal Ethics Committee.

### 2.2.2. Drugs

3,4-(±)-methylenedyoxy-N-methylamphetamine (MDMA) and (±)-methylamphetamine hydrochloride (METH) were supplied from the National Measurement Institute, Sydney, Australia. Normal saline (0.9% NaCl solution) was prepared prior to the testing day and stored at room temperature. Pentobarbitone (60 mg/kg) was used as an anaesthetic.

### 2.2.3. Experimental protocol

#### *2.2.3.1. Weighing of animals*

The animals' weight was measured on each experimental day prior to any manipulations or tests.

#### *2.2.3.2. Preparation and administration of drugs*

MDMA and METH were dissolved in 0.9% sodium chloride (normal saline) to reach final concentrations of 5 and 10 mg/ml for MDMA and 2 mg/ml for METH. MDMA and METH solutions were prepared just before each injection session. Vehicle (normal saline) and drugs were administered intraperitoneally (i.p.) in volumes of 1 ml/kg.

#### *2.2.3.3. Injections schedule*

Animals were divided into 4 groups according to the drug injected, with 8 rats of each strain (SD and FSL) in each group. Control animals received i.p. injections of normal saline (saline group); rats in the MDMA5, MDMA10 and METH treatment groups received MDMA in the doses of 5 mg/kg and 10 mg/kg and METH 2 mg/kg, respectively. MDMA doses were chosen as those that produce moderate to high stimulant effects in laboratory animals (Paulus and Geyer 1991). Moreover, using an interspecies dose scaling conversion (Baumann, Wang et al. 2007), 10 mg/kg and 5 mg/kg doses may be regarded as an approximate equivalent of 2 mg/kg and 1 mg/kg doses in humans, respectively. However, due to marked differences in the pharmacokinetic profiles between rodents and humans, the extrapolation of doses used in rats to human research should be exercised with caution (Green, Gabrielsson et al. 2009). METH at a dose of 2 mg/kg was included in the protocol as a positive control to MDMA 10 mg/kg, as these doses were previously shown to produce similar effects on induction of locomotor activity in rats (Paulus and Geyer 1992).

On the first day of the experimental period, the rats were injected with either vehicle, MDMA or METH. Starting from the second day of the experiments, rats were injected daily with either vehicle or drugs of interest for 3 consecutive days in a week for 3

weeks in total. There were 4-day washout periods between each of the 3 injection sessions. The chosen regime of injections was aimed to imitate a common schedule in humans, as they usually consume pills during social gatherings and parties, which usually occur on weekends.

#### *2.2.3.4. Forced Swimming Test*

During both sessions a single 5-minutes FST test was carried out without the traditional 15-minutes pre-test the previous day, as the pre-test was originally aimed to induce exaggerated immobility in the actual test; the exaggerated immobility of the FSL rats was well established, hence a modified FST was used (Porsolt, Le Pichon et al. 1977; Overstreet 1986; Pucilowski and Overstreet 1993) (Please see Section 1.8.6.3 for more details). A transparent cylinder 18 cm in diameter and 40 cm in height was used. The tank was filled with tap water at $25^0$C to the level at which the animal could not reach the bottom with its hind paws. All behavioural tests were videotaped and the total immobility time was calculated. A researcher, who was blind to the rats' strain and treatment, assessed eight randomly selected videotaped FST sessions. The assessment results were not significantly different from the original observations. The total immobility time was calculated when all videotaped tests were analysed in random order. When analysing the recorded tests the morphological differences between SD and FSL rats of the same age were not highly evident, thus preserving 'blindness' to rats' strain. All tests were carried out in the morning between 9 and 11 AM in a laboratory with daylight as the source of illumination. After the tests, all animals were dried and kept near the heater for 30 minutes before returning to the home cage. Animals who underwent the test were kept separately from the rest until

completion of the FST by all rats. The initial FST session was carried out 30 min after the first injection of either vehicle or drug. The second FST session was carried out 48 h after the last injection. As previously reported, repeated FST sessions carried out three weeks apart are unlikely to affect performance due to memory or adaptation mechanisms (Kusmider, Faron-Gorecka et al. 2006).

#### *2.2.3.5. Sucrose preference test*

Prior to the first week of injections, the first modified sucrose preference test was carried out. 3 subsequent test sessions were carried out 24h after the last injection at the end of each series of injections. The animals fasted from food and water for 4 hours prior to the test; this period of food and water deprivation has been previously used in the sucrose preference test (Willner, Towell et al. 1987). Bottles with 32% sucrose solutions were then introduced to each cage, and rats were allowed access to them for 1 hour. After the test, food and water were returned to the cages. Mean volumes of each sucrose solution were calculated for each cage. As described previously, a sucrose test is used to assess response to reward (Muscat, Kyprianou et al. 1991). Decreased response to reward may be regarded as representing anhedonia in humans, one of the key symptoms of depression (American Psychiatric Association 2000). A 32% sucrose solution is used in a combination with 1% and 8% solutions in a so-called 'three-bottle' test, which was shown to work well in the assessment of drug-induced depressive-like states in laboratory animals (Muscat, Kyprianou et al. 1991; Sammut, Goodall et al. 2001; Sammut, Bethus et al. 2002). The strongest 32% solution is usually most preferred by animals, contributing to up to 90% of total consumption (Sammut, Goodall et al. 2001), therefore, in this study, assessment of the 32% sucrose

solution was evaluated. Increases in the consumption of the stronger sucrose solutions have been associated with decreased sensitivity to reward, modelling anhedonia in laboratory animals (Sammut, Bethus et al. 2002).

#### *2.2.3.6. Locomotor activity assessment*

##### 2.2.3.6.1. Equipment

Locomotor activity was analysed using an apparatus custom-made at Monash University (Melbourne, Australia). In brief, 4 square bases 25x25 cm and 17 cm in height equipped with infrared beam activity sensors, which are distributed along the inner sides of the borders 2.5 cm apart, were connected to a computer. Each base has a central area where a plastic transparent square compartment was placed. Each compartment has a detachable netting lid to prevent animals from escaping from the compartment during the test. Locomotor activity counts were detected from each base and the total number of counts was calculated using software which was developed specifically for the apparatus.

##### 2.2.3.6.2. Experimental protocol

Rats were taken from the their home cages and placed in individual clear plastic compartments and were allowed to habituate for 30 min prior to injections. Animals were then administered with vehicle (normal saline), MDMA 10 mg/kg or METH 2 mg/kg (i.p.) and subsequently observed for 2 hours. Total activity counts were recorded at the end of each 30-minute period, i.e. at 30, 60, 90 and 120 min post-dose. After the experiment animals were returned to their home cages.

### 2.2.4. Statistical analysis

The mean weight between strains in each treatment group were analysed using a two-way Repeated Measures (RM) analysis of variance (ANOVA) with Bonferroni post-hoc tests, and experimental day and strain as between-subjects factors. Total immobility time in the FST at each session was analysed using a 2-way ANOVA with Bonferroni post-hoc tests. Rats' strain and received treatment were used as between-subjects factors. Cohen's d was used to determine the effect size (r) of MDMA administration after acute and repeated administration. Results of the sucrose preference test were analysed by Mr Thomas Sullivan (Public Health, The University of Adelaide) using a between-within design Linear Mixed Model (LMM) with the effect of treatment, strain and test session on consumption of the 32% solution, allowing for interactions between the predictors, correlation in results over time and a heterogeneous variance structure. Total peak areas of locomotor activity time-response curves were compared using ordinary two-way ANOVA with Bonferroni post-hoc tests between strains and treatment groups.

A difference with p values less than 0.05 was considered statistically significant. All data analysis was carried out using Microsoft Excel, GraphPad Prism and SAS software.

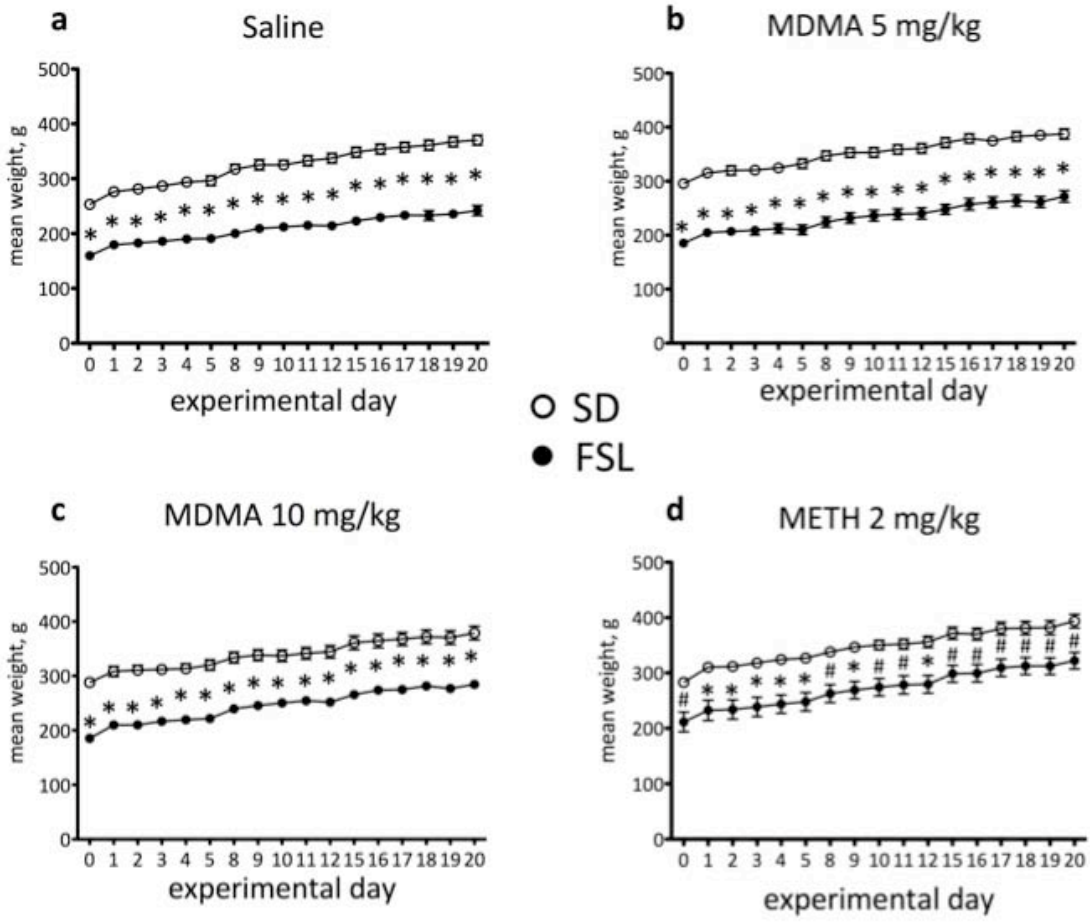
## 2.3. Results

### 2.3.1. Weight

FSL rats in all treatment groups weighed significantly less than SD rats throughout the experimental period (Fig. 2.1). The rate of change in mean weight, however, was similar between the strains from the start of the experiment (Fig. 2.2). A 2-way ANOVA showed a significant interaction effect of strain and experimental day on the weight of

animals in the control and MDMA treatment groups; the difference between the mean weight of SD and FSL rats within each treatment group was statistically significant over the experimental period (saline: $F(16,224)=20.41$, $p<0.0001$; MDMA5: $F(16,224)=4.96$, $p<0.0001$; MDMA10: $F(16,224)=2.38$, $p=0.0026$). In the METH-treated group, significant main effects of time and strain were observed; throughout the whole experimental period, FSL rats were lighter than SD rats (effect of strain $F(1,224)=16.68$, $p=0.0011$).

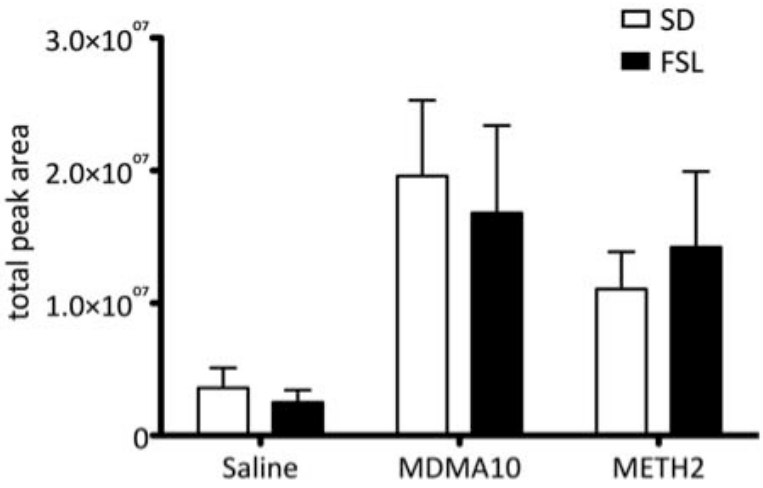
***Figure 2.1. Mean weight of SD and FSL rats in treatment groups throughout the experimental period***




Mean weight (g) of SD and FSL rats in the saline (a), MDMA5 (b), MDMA10 (c) and METH (d) treatment groups throughout the experimental period. All data represent mean±SEM (n=8 of each strain per group). Mean weight in each treatment group was compared using a two-way Repeated Measures ANOVA with Bonferroni post-hoc tests (* $p<0.001$, # $p<0.01$).

### 2.3.2. Locomotor activity

***Figure 2.2. Mean total peak areas for the locomotor activity time-response curves of SD and FSL rats in the saline, MDMA10 and METH2 treatment groups after drug administration***




Mean total peak areas for the time-response curves in SD and FSL rats in the saline, MDMA10 (MDMA 10 mg/kg) and METH2 (METH 2 mg/kg) treatment groups. All data represent mean±SEM (n=4 of each strain in each treatment group). Data were analysed using a two-way ANOVA with Bonferroni post-hoc tests. Locomotor activity count was recorded 30, 60, 90 and 120 min after drug administration.

The assessment of locomotor activity didn't reveal any difference between the strains in each treatment group ($F(1,18)=0.004$, $p=0.95$), nor there was an interaction effect of strain and treatment on the results ($F(2,18)=0.24$, $p=0.79$). 2 mg/kg dose of METH was equivalent to 10 mg/kg MDMA in stimulating locomotor activity in both strains. Areas under the curve (AUC) were used to compare locomotor activity counts after injection with either vehicle (normal saline), or MDMA 10 mg/kg or METH 2 mg/kg. MDMA 10 mg/kg and METH 2 mg/kg caused a similar induction of locomotor activity in both strains in comparison with controls ($F(2,18)=5.87$, $p=0.01$) (Fig. 2.3). The mean total peak areas were as follows: in the saline group SD 3625000±1512000 and FSL

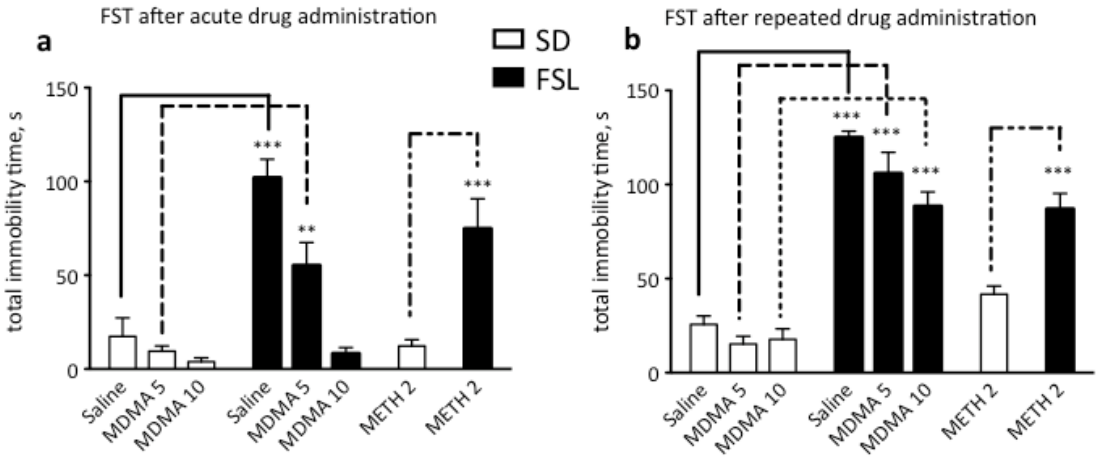
2546000±914100; MDMA 10 mg/kg SD 19590000±5702000 and FSL 16810000±6585000; METH 2 mg/kg SD 11070000±2801000 and FSL 14230000±5703000. Moreover, MDMA 10 mg/kg and METH 2 mg/kg caused similar induction in locomotor activity (difference 5551875±4460499, p=0.688).

### 2.3.3. Forced Swimming Test

The results of the FST after initial drug administration are presented in Fig 2.4a. A 2-way ANOVA analysis showed that there was a significant interaction effect of strain and treatment on performance in the FST after acute drug administration: $F(3,56)=7.26$, $p=0.0003$. In the saline-treated group, an expected difference between SD and FSL strains was observed: the FSL rats were almost 5-fold more immobile than their counterparts (101.9±9.92s for FSL and 17.38±9.81s for SD, $p<0.001$). There was a dose-dependent decrease in immobility following MDMA administration. This antidepressant-like effect was more evident in the animal model of depression. The FSL rats treated with MDMA 5 mg/kg were still more immobile than SD rats which received the same treatment (FSL 55.13±12.29s and SD 9.5±2.80s, $p<0.01$), but MDMA 10 mg/kg had a more prominent effect, and no difference between the strains was observed (FSL 8.125±3.27s and SD 3.88±2.03s, $p=0.29$). Acute METH administration didn't cause an effect similar to MDMA 10 mg/kg: animals treated with METH 2 mg/kg were not different from saline-treated animals of the same strain (SD METH2 vs. saline $p=0.87$, FSL METH2 vs. saline $p=0.06$). Moreover, the difference between strains in the METH treatment group was statistically significant, and FSL rats were more immobile than SD counterparts (FSL 74.75±15.98s and SD 12.25±3.35s, $p<0.001$). Furthermore, the behaviour of animals treated with METH was similar to that observed in the

control group, whereas MDMA-treated animals, especially after the 10 mg/kg dose, exhibited different behaviour with increased periods of swimming.

***Figure 2.3. Total immobility time in the FST after acute and repeated drug administration***




Mean total immobility time of SD and FSL rats in the saline, MDMA5 (MDMA 5 mg/kg), MDMA10 (MDMA 10 mg/kg) and METH2 (METH 2 mg/kg) treatment groups after acute (a) and repeated (b) drug administration. All data represent mean±SEM (n=8 of each strain in each treatment group). Data were analysed using a two-way ANOVA with Bonferroni post-hoc tests for each test session.
* Indicates significant difference with SD rats in the same treatment group (** p<0.01, *** p<0.001).

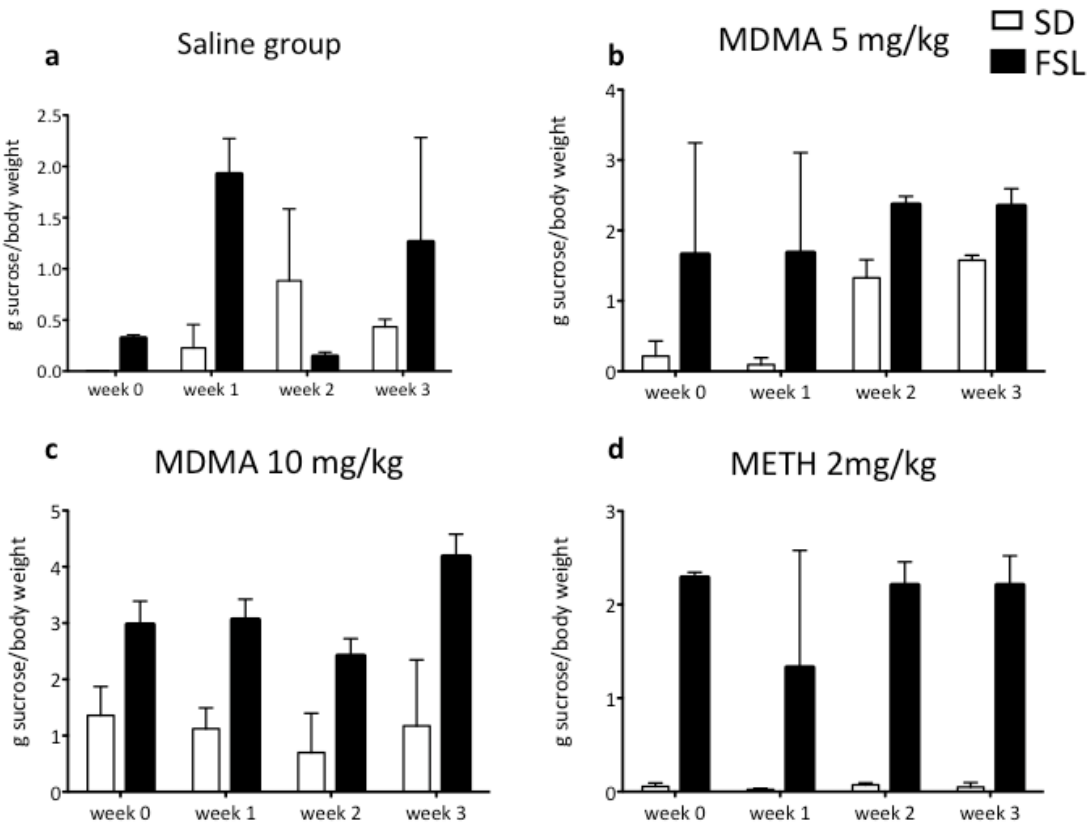
After three weeks of treatment, a significant interaction effect between strain and received treatment was also observed ($F(3,54)=6.23$, $p=0.001$). The saline-treated animals performed similarly to the first FST session, and the difference between strains was significant (FSL 125.0±3.34s and SD 25.67±4.46s, $p<0.001$). FSL rats that received 5 mg/kg of MDMA and 2 mg/kg of METH were significantly more immobile than their SD counterparts (MDMA5: FSL 105.9±11.28s and SD 15.25±4.14s, $p<0.001$; METH: FSL 87.0±8.2s and SD 41.75±4.33s, $p<0.001$) (Fig. 2.4b). There was still some

antidepressant-like effect of MDMA 10 mg/kg observed in the FSL rats (125.0±3.34s controls and 88.38±7.70s MDMA10), but it was of much less magnitude than after acute drug administration (MDMA10 FSL: acute FST 8.125±3.27s and chronic FST 88.38±7.70s; Cohen's d = -.4.87, r= -0.93 (large size effect)). Total immobility time in the MDMA5 FSL cohort returned to the higher levels comparable with FSL controls, and the FSL rats were significantly more immobile than SD rats (FSL 105.9±11.23s and SD 15.25±4.14s, $p<0.001$). METH treated FSL rats also were more immobile than SD counterparts treated with the same drug (FSL 87.00±8.21s and SD 41.75±4.33s, $p<0.001$).

### 2.3.4. Sucrose preference test

No three-way or two-way interaction effects between the strain, treatment group and time on consumption of the 32% sucrose solution were observed. There was no significant effect of time on consumption over the testing period ($F(2,30)=1.39$, $p=0.26$), but received treatment and strain significantly affected sucrose consumption (strain: $F(1,11)=31.07$, $p=0.0002$; treatment: $F(3,11)=5.12$, $p=0.019$). Independently of time of assessment and treatment, FSL rats consumed the sucrose solution in greater proportions than their SD counterparts (adjusted means: FSL 2.1±0.19g sucrose/body weight and SD 0.63±0.19g sucrose/body weight, $p<0.0001$). An increase in consumption of the 32% sucrose solution was most evident in the FSL group treated with MDMA 10 mg/kg (Bonferroni post-hoc tests: difference = 3.009, $p<0.05$) (Fig. 2.5d).

***Figure 2.4. Consumption of the 32% sucrose solution by SD and FSL rats in the saline, MDMA5, MDMA10 and METH2 treatment groups***




Mean consumption of the 32% sucrose solution by SD and FSL rats in the saline (a), MDMA5 (MDMA 5 mg/kg) (b), MDMA10 (MDMA 10 mg/kg) (c) and METH2 (METH 2 mg/kg) (d) treatment groups before (week 0) and after each week of injections (weeks 1-3). All data represent mean±SEM (n=2 cages for each strain in each treatment group). Data were analysed using the Linear Mixed Model analysis.

Moreover, independently of the time of the test and strain, the rats that received MDMA, especially the dose of 10 mg/kg, consumed more sucrose solution than animals treated with METH and saline (adjusted means: saline 0.82±0.26g sucrose/body weight, METH 0.97±0.26g sucrose/body weight, MDMA5 1.56±0.26g sucrose/body weight, MDMA10 2.12±0.26g sucrose/body weight).

## 2.4. Discussion

This project was the first to demonstrate the immediate antidepressant-like MDMA effects, which are more evident in 'depressed' than in 'non-depressed' rats.

The face validity of an animal model of a disorder or condition is represented by behavioural resemblance to symptoms. A significant change in weight is one of the symptoms of MDD. The lower weight of FSL rats in comparison with FRL and SD rats remained stable throughout time, therefore not fully mimicking the symptom observed in humans (Overstreet 1993). The difference in weight between FSL and SD rats remained similar over the whole experimental period of the current study. However, the changes in mean weight from day to day were similar in both strains. This is consistent with previous data on the lighter weight of FSL rats (Overstreet 1993).

The depressive-like state in laboratory animals can be observed in behavioural tests, with FST being the main test to assess it in a preclinical setting. Anhedonia, another core feature of the depression, can be assessed with the sucrose preference test. In this study, the most remarkable findings were obtained in the FST. The expected differences between strains were observed in the saline-treated group, where rats from the animal model of depression were significantly more immobile than 'non-depressed' SD counterparts (Fig. 2.4). 30 minutes after the initial drug administration, a dose-dependent decrease in immobility was observed in both strains, but it was more pronounced in FSL rats. Moreover, the depressive-like behaviour of FSL rats disappeared following administration of a higher MDMA dose, and total immobility time didn't differ from SD rats. Immobility time in the saline-treated FSL rats was

comparable to previously published results (Overstreet 1986; Pucilowski and Overstreet 1993; Zangen, Overstreet et al. 1997; Overstreet, Hlavka et al. 2004). The original FST protocol includes a 15-minute pre-test to make immobility more evident in the actual 5-minute test (Porsolt, Le Pichon et al. 1977). However, many previous studies used a modified single 5-minute session, as immobility of FSL rats in FST is well established (Pucilowski and Overstreet 1993). Moreover, exclusion of the pre-test from the protocol precluded the development of adaptation to the test situation as a possible confounder (see Section 1.7.5.2 of Chapter 1).

Methamphetamine shortly after injection produced a significant decrease in immobility, and the observed behaviour was similar to that of saline-treated rats: periods of activity consisted of swimming and climbing. This finding is not consistent with previously published results, where behaviour induced by 1 mg/kg of D-methamphetamine was regarded as a general stimulant effect of the drug, as observed behaviour differed from presumed active attempts to escape: the animals were actively swimming, but not trying to escape (Kitada, Miyauchi et al. 1981). However, no locomotor activity assessment was done in parallel with FST in the study of Kitada et al.

The behaviour of animals treated with MDMA, especially evident at a dose of 10 mg/kg, was similar to that induced by other serotonergic agents (Detke, Rickels et al. 1995; Cryan, Page et al. 2005). However, the changes in behaviour induced by MDMA developed shortly after drug administration, whereas it has been noted that the chronic administration of fluoxetine and other SSRIs over 14 days reduces immobility and increases swimming time. Accordingly, this was regarded as an antidepressant

effect of the drugs. In this study the behaviour of animals treated with MDMA consisted of decreased immobility periods and an increased number of swimming periods in comparison with control animals. Such an effect, which was more pronounced in FSL rats, can be considered a representation of the antidepressant-like activity of MDMA.

After repeated MDMA administration a weak antidepressant-like effect was observed only in the FSL rats treated with MDMA 10 mg/kg, however, the difference between saline and MDMA-treated animals was not as marked as in the acute setting (Fig. 2.4). A similar tendency was observed in the SD animals; however, the changes and differences were not as evident in comparison to FSL rats. The reduction in effect on the depressive-like state after repeated administration may be due to the development of tolerance to the antidepressant-like action of MDMA. This is consistent with reports that some users observe a decrease in the magnitude of the positive effects of ecstasy on mood following repeated consumption of the drug (Peroutka, Newman et al. 1988; Parrott 2005). In some test settings, observed immobility after repeated exposure of animals to the FST paradigm may be regarded as adaptation, when animals may have learned the sequences in the test procedure. It has been previously shown that, if administered repeatedly, 2 weeks between the sessions does not affect performance in the FST (Kusmider, Faron-Gorecka et al. 2006). The 3-week period between the sessions was used to eliminate the possible learning confounder, therefore, the observed behaviour was unlikely to represent adaptation.

A marked contrast between the effects of MDMA in this study and previous data on the effects of clinically prescribed antidepressants can be clearly observed. Typically,

SSRIs, e.g. fluoxetine, and other antidepressants, like desipramine, were shown to reverse the increased immobility of the FSL rats only after 2-3 weeks of continuous treatment (Pucilowski and Overstreet 1993; Zangen, Overstreet et al. 1997; Overstreet and Griebel 2004; Overstreet, Hlavka et al. 2004).

The findings of the present study add evidence regarding the hypothesised difference between the onset of action between MDMA and clinically prescribed antidepressants. The antidepressant-like action of MDMA was apparent 30 min after drug administration, whereas previous data have shown that the reversal of the depressive-like state in FSL rats usually occurred after 14 or more days of continuous treatment with antidepressants. In contrast, MDMA, although administered 3 times per week, but not daily, over 3 weeks had a reduced effect on immobility in the FSL animal model of depression. This may be due to the development of tolerance to the antidepressant-like activity of MDMA. Moreover, these findings support the utility of the FSL animal model of depression in research into the antidepressant effects of various drugs: the effects of prescribed antidepressants are delayed, akin to the mood improvement reported by humans after 2-3 weeks of treatment, while ecstasy users report the onset of positive effects of the drug on mood and self-esteem 30-60 min after pill consumption (Peroutka, Newman et al. 1988; Thompson 2002; Baylen and Rosenberg 2006).

MDMA is a stimulant drug, as are other amphetamine derivatives. One of the features of the action of this class of drugs is increased locomotor activity. Although the FST has been extensively used to assess the depressive-like state in laboratory animals, one may assume that observed changes in behaviour following the administration of

MDMA, especially to animals with the depressive-like state, may not be a true representation of the antidepressant-like activity of the drug, but just an increase in locomotor activity. Indeed, previous studies focussed on characteristics of the FSL strain as an animal model of depression have used amphetamine as a positive control in the FST (Overstreet, Pucilowski et al. 1995). Therefore, the protocol of this study included another treatment group that received METH at a dose of 2 mg/kg. The assessment of the effects of MDMA and METH on the locomotor activity of rats didn't show any significantly different patterns between strains. This may be counted as a reproduction of the results of the study that the choice of the doses used in this study was based upon (Paulus and Geyer 1992). In contrast, the behaviour of SD and FSL rats in the FST after administration of either MDMA 10 mg/kg or METH 2 mg/kg was quite different. It was observed, especially in the acute setting, that METH-treated animals behaved similarly to saline-treated animals, whereas a marked decrease in immobility time was observed following MDMA injection (Fig. 2.4a). After repeated drug administration, a weak antidepressant-like effect was observed in an animal model of depression after treatment with MDMA 10 mg/kg, whereas METH-treated animals again behaved similarly to saline controls (Fig. 2.4b). Additionally, the sucrose consumption test showed that the consumption of the 32% solution was not affected by METH treatment of animals of either strain (Fig. 2.5d). Therefore, one may conclude that METH doesn't have a marked antidepressant-like activity in comparison with MDMA. This may be explained by the difference in pharmacological action of these two substituted amphetamines, namely: MDMA primarily affects the serotonergic system, whereas METH mainly causes changes in the levels of DA (Clemens, McGregor et al. 2007).

The results of the modified sucrose preference test showed an overall greater consumption of the sucrose solution of high concentration by the rats in the animal model of depression. It was most evident in the MDMA-treated groups (Fig. 2.5). A Linear Mixed Model analysis didn't show any apparent changes in the levels of consumption over time; however, a trend towards an increase in consumption was noted in SD rats treated with MDMA 5 mg/kg (Fig. 2.5b) and FSL rats treated with MDMA 10 mg/kg (Fig. 2.5c). Greater consumption of the 32% sucrose solution has been linked with a drug-induced anhedonic state in rats, which was reversed by chronic treatment with fluoxetine and desipramine (Sammut, Bethus et al. 2002). It has been suggested that increased consumption of a strong sucrose solution, which is less palatable than a weaker solution, may be due to impaired sensitivity to reward. This, in turn, may occur due to changes in functioning of the dopaminergic mesocorticolimbic pathway, however, the activity of the dopaminergic neurotransmitter system was not assessed in the present study. The observed results further confirmed differences between the antidepressant-like activity of MDMA and that of clinically prescribed drugs. The latter have been shown to reduce consumption of the 32% solution after 15 days of continuous administration (Sammut, Bethus et al. 2002). MDMA, however, didn't affect the difference in sucrose preference between strains after repeated administration. Although MDMA affects thermoregulation following acute administration (Dafters and Lynch 1998), in the present study sucrose test was administered 24-96 h after drug administration, therefore the observed findings may not be regarded as direct effect of MDMA on water balance and thirst.

To conclude, observed changes in the behaviour of rats in the FST, especially apparent in the FSL strain, could be characterised as the antidepressant-like activity of MDMA.

As hypothesised, this effect is more evident in an animal model of depression, perhaps due to the pre-existing depressive-like state. Observed changes in swimming and immobility time in the FST are not likely due to a general stimulant effect of the drug, as doses of MDMA and METH that caused similar induction of locomotor activity had different effects in the FST (Fig. 2.3). Finally, the antidepressant-like activity of MDMA is different from the effects of commonly prescribed antidepressants, primarily in the onset of action. The following two chapters describe experiments which were designed to further research the possible basis for the differences in the effects of MDMA between 'normal' rats and an animal model of depression.

# Chapter 3. Pharmacokinetics of MDMA in an animal model of depression

## 3.1. Introduction

MDMA pharmacokinetics have been previously studied in different rat strains, including SD, Dark Agouti and Wistar rats, but not in FSL rats (Chu, Kumagai et al. 1996; Tomita, Nakashima et al. 2007; Fonsart, Menet et al. 2008; Upreti, Eddington et al. 2009). If present, differences in the pharmacokinetics between the strains may explain certain variations in behaviour after drug administration. To date, there are several studies which analysed contribution of strain and gender to the pharmacokinetic and metabolic profiles of MDMA and consequently to potential differences in MDMA's effects on behaviour. It has been previously reported that SD and Dark Agouti rats have different metabolism rates following (+)-MDMA administration, namely SD female rats have lower MDA concentrations than SD males (Fonsart, Menet et al. 2008) and Dark Agouti female rats (Chu, Kumagai et al. 1996). Moreover, the MDMA concentrations in brain and plasma were significantly lower in SD rats than in Dark Agouti rats (Chu, Kumagai et al. 1996). The Dark Agouti rat strain is an established model of the poor metabolisers, as they are deficient in the CYP2D6 enzyme which plays a central role in MDMA metabolism in humans (Tucker, Lennard et al. 1994; Colado, Williams et al. 1995). Deficiency in CYP2D6 occurs in the general population (Sistonen, Sajantila et al. 2007), and as this isoform is involved in the metabolism of many compounds, including MDMA, poor metabolisers are likely to have increased concentration of MDMA and MDA after drug ingestion, and thus be at a greater risk to

develop negative effects of the drug. MDA is the main metabolite of MDMA in rats, and it has been implicated in the neurotoxic potential of the drug, thus, higher resulting concentrations may contribute to the prominence of negative effects of MDMA (Monks, Jones et al. 2004). While FSL rats were outbred from SD rat strain, which does not have a poor metaboliser phenotype, it is unlikely that differences in metabolism between the two strains may underlie the differences in behaviour and neurochemical parameters. Nevertheless, an experiment looking at the pharmacokinetics and metabolism of MDMA in SD and FSL rats was conducted in collaboration with Ms Emily Jaehne (Jaehne, Majumder et al. 2010) in order to determine whether there are any differences in MDMA metabolism between SD and FSL rats that may contribute to the differences in observed behaviour. For that, MDMA and MDA concentrations at different time points after drug administration were determined in this experiment.

## 3.2. Animals and methods

The results of this experiment comprised a part of the manuscript that has been recently accepted for publication to Addiction Biology (Jaehne, Majumder et al. 2010). The methodology is briefly described below.

### 3.2.1. Animals

18 SD and 18 FSL male rats at 11 weeks of age were used in the experiment. Animals were housed individually before drug administration with free access to food and water.

### 3.2.2. Drug preparation and administration

3,4-(±)-methylenedyoxy-N-methylamphetamine (MDMA) was supplied from the National Measurement Institute, Sydney, Australia. MDMA was dissolved in normal saline prior to injections to reach final concentration of 7.5 mg/ml. Rats received intraperitoneal injections of MDMA in volumes of 1ml/kg. Pentobarbitone (60 mg/kg) was used as an anaesthetic.

### 3.2.3. Experimental protocol

MDMA and MDA concentrations were determined in whole blood and cortex samples from SD and FSL rats at different time points. After a single MDMA injection, rats were subsequently anesthetised at 30, 60, 120 and 240 min after the injection, and whole blood samples were obtained via cardiac puncture (n=4-5 of each strain per time point). Animals were then decapitated, and whole brain samples were collected and stored at -70$^0$C until further analysis.

### 3.2.4. Determination of MDMA and MDA concentrations in cortex and blood

Blood samples were prepared immediately after collection for the high performance liquid chromatography (HPLC) analysis using a method described previously (Michel, Rege et al. 1993). Cortex samples were thawed and prepared using the same method as for 5-HT and 5-HIAA concentrations (see Materials and Methods in Chapter 4) (Callaghan, Farrand et al. 2006). The Alltima HP C18 3µ 100x2.1mm column (Alltech Associates, Inc, NSW, Australia) was used to separate MDMA and MDA in cortex and whole blood samples. The HPLC parameters for determination of MDMA and MDA concentrations were as follows: potential was set at 1.2 mV, range 20 nA, and the detector was housed at 25$^0$C. The mobile phase was prepared from 12.5% methanol

($CH_3OH$) and 0.1M sodium acetate ($CH_3COONa$) with final pH 4.25. The rate of the mobile phase delivery was set at 0.120 ml/min.

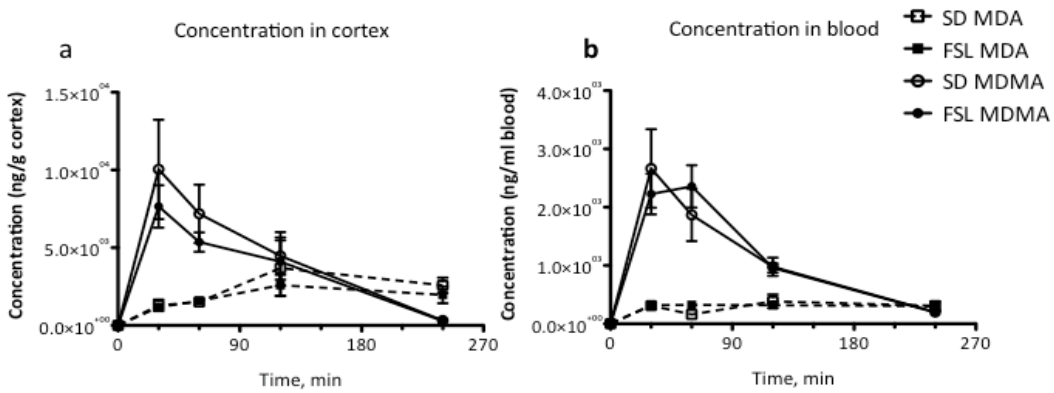
### 3.2.5. Statistical analysis

Concentrations of MDMA and MDA in cortex and whole blood were compared between strains using unpaired, two-tailed Student's t-tests for the AUC of the concentration-time curves, half-lives ($t_{1/2}$) and peak concentrations ($C_{max}$). Differences with p values less than 0.05 were considered statistically significant. All data are represented as mean±SEM.

## 3.3. Results

No difference in MDMA and MDA concentrations in cortex and blood was observed between strains at any time points (Fig. 3.1). Table 3.1 shows the pharmacokinetic and metabolic profile for SD and FSL rats.

***Figure 3.1. MDMA and MDA concentrations in cortex and whole blood samples in SD and FSL rats***



MDMA and MDA concentrations in cortex (a) and whole blood (b) samples of SD and FSL rats after a single injection of 7.5 mg/kg MDMA. All data represent mean±SEM (n=4-5 of each strain per time point). Data were analysed using unpaired two-tailed Student's t-tests for the AUC of the concentration-time curves.

***Table 3.1. Pharmacokinetic and metabolic characteristics of MDMA and MDA in SD and FSL rats***

| | **SD (n=4-5 per time point)** | **FSL (n=4-5 per time point)** | **P value** |
|---|---|---|---|
| **$AUC_{MDMA}$ (cortex)** | $1.06\times10^6 \pm 3.80\times10^5$ | $8.46\times10^5 \pm 2.12\times10^5$ | 0.638 |
| **$AUC_{MDMA}$ (blood)** | 6209 ± 2006 | 5622 ± 1327 | 0.816 |
| **$t_{1/2}$ MDMA (cortex)** | 28.03 ± 7.99 min | 39.76 ± 1.99 min | 0.204 |
| **$t_{1/2}$ MDMA (blood)** | 48.53 ± 1.04 min | 43.58 ± 8.74 min | 0.723 |
| **$C_{max}$ MDMA (cortex)** | 1004 ± 320 ng/g tissue | 765 ± 136 ng/g tissue | 0.512 |
| **$C_{max}$ MDMA (blood)** | 3239 ± 933 ng/ml | 2226 ± 498 ng/ml | 0.376 |
| **$AUC_{MDA}$ (cortex)** | $59\times10^5 \pm 2.11\times10^5$ | $4.55\times10^5 \pm 1.08\times10^5$ | 0.578 |
| **$AUC_{MDA}$ (blood)** | 1020 ± 364 | 1086 ± 265 | 0.888 |

Data represent mean±SEM. All data were analysed using unpaired two-tailed Student's t-tests (n=4-5 of each strain time point).

## 3.4. Discussion

The experiment to determine the pharmacokinetic and metabolic profiles in SD and FSL rats was carried out to investigate whether there is evidence for differences in drug disposition, and, if present, whether such differences may explain the observed variance in behaviour. The pharmacokinetics of MDMA have not been previously assessed in the FSL model of depression. The used dose was chosen as intermediate

between a non-neurotoxic (5 mg/kg) and a moderate neurotoxic dose (10 mg/kg) (Baumann, Wang et al. 2007), and it has been previously used in research on MDMA effects in SD rats in the laboratory (Jaehne, Salem et al. 2005; Callaghan, Farrand et al. 2006). MDMA at a dose of 7.5 mg/kg produced similar pharmacokinetic profiles in SD and FSL rats. Moreover, the metabolic ratios, represented as AUC for MDA, were not statistically different. As shown in Fig 3.1, MDMA and MDA concentrations in cortex and whole blood were similar at all time points in SD and FSL rats. Accordingly, no difference in MDMA and MDA levels in whole blood and cortex suggests that there is no difference in drug bioavailability, distribution or access to CNS of MDMA and its active metabolite between strains. Administration of similar MDMA doses to rats in previous studies produced blood and cortical concentrations comparable to those observed in the present experiment (Baumann, Zolkowska et al. 2009; Hamida, Tracqui et al. 2009). However, no previous studies have included the FSL rat strain. Accordingly, the differences in FST performance in response to MDMA administration, which are reported in Chapter 2, are not likely to be explained by the differences in pharmacokinetics and metabolism of MDMA between strains. Observed variations in behaviour are more likely to be due to different functioning of the serotonergic system in the two strains of rats, therefore, a more thorough investigation into the effects of MDMA on neurotransmitter systems functioning in FSL rats is warranted to compare the behavioural outcomes with SD rats that have been extensively used to study most aspects of MDMA.

# Chapter 4. Effects of repeated administration of MDMA on the cortical levels of 5-HT and 5-HIAA in an animal model of depression

## 4.1. Introduction

Differences in the functioning of neurotransmitter systems, primarily the serotonergic system, have been linked with the development of depressive symptoms in humans (Lopez-Ibor 1988; Meltzer 1989; Vaidya and Duman 2001; Middlemiss, Price et al. 2002; Stockmeier 2003; Kalia 2005). Clinically prescribed antidepressants that are used at present act via various ways, with the resulting effect of increasing the levels of 5-HT in the synaptic cleft. SSRIs are the most widely prescribed class of antidepressants for depression at present (Smith, Sketris et al. 2008). Usually the onset of the beneficial effect of antidepressants is delayed. In contrast, positive effects on mood develop within an hour after MDMA consumption. As has been previously described, MDMA affects the serotonergic system in various ways, resulting in a rapid and substantial boost in 5-HT levels in the synaptic cleft. Similarities in the mechanisms of action of MDMA and prescription antidepressants suggest that the former may have antidepressant-like activity.

However, as discussed in Chapter 2 of this study, there is a marked contrast between ecstasy and antidepressants in the onset of action. The improvement of mood after 2-3 weeks of continuous treatment reported by patients has been reproduced as a reversal of the depressive-like state in the FSL animal model of depression following

repeated administration of the main classes of antidepressants (Overstreet, Pucilowski et al. 1995; Zangen, Overstreet et al. 1997; Zangen, Overstreet et al. 1999; Overstreet, Hlavka et al. 2004; Overstreet, Keeney et al. 2004). FSL rats have increased tissue levels of 5-HT and its main metabolite, 5-HIAA, in the prefrontal cortex, nucleus accumbens and other brain regions; these levels were reduced to normal after chronic treatment with desipramine (Zangen, Overstreet et al. 1997). The increased tissue levels of 5-HT levels in FSL rats in comparison with FRL (Zangen, Overstreet et al. 1997) and SD rats (Yadid, Nakash et al. 2000) may reflect either increased synthesis as a compensation of decreased 5-HT neurotransmission, or increased elimination of 5-HT and its metabolite. Hasegawa and colleagues reported decreased 5-HT synthesis in limbic regions of FSL rats in comparison with SD and FRL rats (Hasegawa, Nishi et al. 2006). Moreover, decreased $VMAT_2$ expression in limbic regions of FSL rats (Schwartz, Yadid et al. 2003) and, thus, decreased density of these transporters, may contribute to decreased serotonergic neurotransmission, which, in turn, can result in increased tissue levels of 5-HT. Normalisation of exaggerated tissue levels of 5-HT and other monoamines by antidepressants that restore the serotonergic and monoaminergic neurotransmission may provide further evidence of compensatory nature of increased levels of 5-HT and 5-HIAA due to decreased 5-HT transporter functioning (Zangen, Overstreet et al. 1997; Zangen, Overstreet et al. 1999).

The present analysis was carried out to investigate how repeated MDMA treatment affects the levels of 5-HT and 5-HIAA in cortex in 'normal' rats and in an animal model of depression.

## 4.2. Animals and methods

### 4.2.1. Animals, drugs and experimental protocol

The animal characteristics and treatment protocol were described previously (please see Section 2.2 of Chapter 2). In brief, 24 SD and 24 FSL rats were divided into 3 treatment groups (n=8 of each strain per group): control animals received i.p. injections of normal saline; MDMA5 and MDMA10 groups received MDMA doses of 5 mg/kg and 10 mg/kg, respectively. Overall, 10 injections were administered to each animal over the period of 3 weeks: an initial injection followed by the first session of FST, and 3 series of daily injections for 3 consecutive days, with a 4-day washout period. 48 hours after the last injection, the animals underwent the second FST test, after which the samples were collected for analysis of the neurotransmitter levels.

### 4.2.2. Measurement of 5-HT and 5-HIAA levels in cortex

Approximately one hour after the second FST, animals were anaesthetised, and after decapitation, whole brains were collected and stored in a freezer at -80$^{o}$C for further analysis. The brain samples were then thawed and the cortex was dissected from one half of the brain. Samples for the analysis were prepared using a technique used previously in the laboratory (Callaghan, Farrand et al. 2006; Jaehne, Salem et al. 2008). In short, each cortex sample was added to 0.1M perchloric acid (PCA) at 400 µl/100 g cortex, and 40 µl of 1 µg/ml of N-methyl-5-HT (NMe5-HT, the internal standard for 5-HT) solution was added. The mixture was then homogenised using an Ultra-Turrax TP 18-10 homogeniser, transferred to eppendorf tubes and centrifuged at 4$^{0}$C 9000g for 10 min (Sigma 4K15 centrifuge). The supernatant was collected into an eppendorf tube and centrifuged again under the same settings. All samples were then stored at -70$^{o}$C

for further analysis. The extraction efficiency was 67.8%. The levels of 5-HT and 5-HIAA in cortex were analysed using high performance liquid chromatography (HPLC) with electro-chemical detection (ECD). The system used for the analysis had a CBM-20A HPLC system controller (Shimadzu, Kyoto, Japan) with an electrochemical detector (Antec Leyden Decade II) housed at $25^0$C. The electrode potential was at 0.7 V and a range of 10 nA. The mobile phase components were as follows: sodium dihydrogen orthophosphate, anhydrous ($NaH_2PO_4$) 102.9 mM; octanesulphonic acid (OSA) 0.5 mM; ethylenediaminetetraacetic acid (EDTA) 0.1 mM and 10% methanol ($CH_3OH$), pH 3.8. The mobile phase was delivered at 1 ml/min. An Alltima HP C18 3µ 100x2.1 mm column (Alltech Associates, Inc, NSW, Australia) was used in the analysis. Results were recorded with a Shimadzu LCSolution program.

### 4.2.3. Statistical analysis

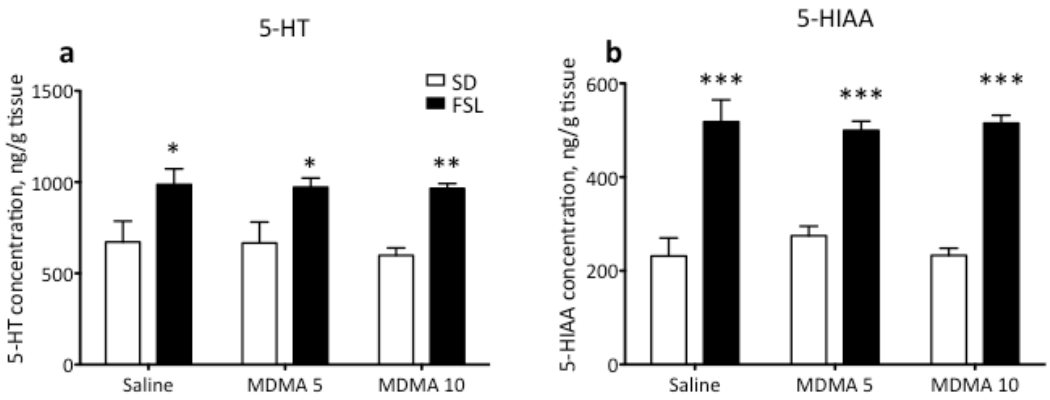
Cortical levels of 5-HT and 5-HIAA measured by HPLC were compared with a two-way ANOVA with Bonferroni post-hoc tests, with strain and treatment group as between-subjects factors. All data are represented as mean±SEM. A p value less than 0.05 was considered statistically significant.

## 4.3. Results

There was a significant main effect of strain on the levels of both 5-HT and its main metabolite 5-HIAA in cortex after 3 weeks of repeated administration (5-HT: $F(1,42)=25.42$, $p<0.0001$; 5-HIAA: $F(1,42)=126.10$, $p<0.0001$). As anticipated, the cortical levels of 5-HT and 5-HIAA were significantly higher in FSL than in SD rats in the control group (SD 671.9±114.3 ng/g tissue and FSL 987.6±85.05 ng/g tissue, $p<0.05$).

The chosen schedule of treatment with the two doses of MDMA didn't affect the levels of 5-HT and its metabolite in either strain (Fig. 4.1).

***Figure 4.1. 5-HT and 5-HIAA levels in the cortex of SD and FSL rats after three weeks of treatment***




Cortical 5-HT (a) and 5-HIAA (b) concentrations in SD and FSL rats in the saline, MDMA5 (MDMA 5 mg/kg) and MDMA10 (MDMA 10 mg/kg) treatment groups after three weeks of treatment. All data represent mean±SEM (n=8 of each strain per group). Data were analysed using a two-way ANOVA with Bonferroni post-hoc tests.
* Indicates significant difference with SD rats in the same treatment group (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

## 4.4. Discussion

The levels of 5-HT and 5-HIAA in the cortex of saline-treated animals from both strains are consistent with previously published studies (Zangen, Overstreet et al. 1997). Repeated MDMA administration at either dose did not cause any changes in the concentrations of either compound in either strain (Fig 4.1). This is in contrast with previous findings regarding the main classes of prescribed antidepressants that have been shown to normalise the serotonergic levels by either decreasing tissue concentrations or by increasing 5-HT synthesis (Zangen, Overstreet et al. 1997; Kanemaru, Nishi et al. 2009). The results suggest that the chosen regime of treatment

does not cause a significant change in serotonergic system functioning in brain. However, given that cortical samples were collected 48 hours after the last injection, the observed findings may be interpreted not only as tolerance to drug's effects, but also as long-term improvement of serotonergic function. Complex nature of MDMA's effects over time the makes the issue of long-term effects of the drug ambiguous. Comparison of other neuropharmacological parameters, including 5-HTT and 5-HT receptor binding, is needed to broaden our knowledge of MDMA's effects in this animal model of depression. As has been discussed previously, tissue levels of 5-HT and 5-HIAA may reflect decreased neurotransmission, increased synthesis or increased elimination of the compounds. To further understand the impact of the chosen dosing regime of MDMA in an animal model of depression, studies of the 5-HTT binding, activity of MAO and assessment of 5-HT synthesis may provide further insight in the changes the serotonergic system functioning due to MDMA administration. Furthermore, the chosen dosing regime included 10 injections overall, with 4 days of washout periods between the series of administration. In contrast, studies with SSRIs and other antidepressants in FSL rats were done using a chronic regime over 14-18 days, after which normalisation of serotonergic system functioning was observed (Zangen, Overstreet et al. 1997; Kanemaru, Nishi et al. 2009). In accordance with the neurochemical findings, normalisation of the behaviour in the animal model of depression using the FST was also observed, and they were not significantly more immobile than 'non-depressed' rats (Overstreet and Griebel 2004; Overstreet, Hlavka et al. 2004; Overstreet, Keeney et al. 2004; Matrisciano, Caruso et al. 2008).

Previous studies on the effects of MDMA in rats, where 5-HT and 5-HIAA depletions following repeated injections of MDMA were observed, used different protocols. Callaghan and colleagues (2006) detected depletions of 5-HT content in SD rats two weeks after twice-daily doses of MDMA 10 mg/kg and 20 mg/kg for four days. In another study, decrease in 5-HT content was observed after treatment of Wistar rats with weekly doses of 8 mg/kg MDMA for 16 weeks (Clemens, Cornish et al. 2007). Another study reported marked and persistent depletions of 5-HT contents in various brain regions following administration of three 'binge' doses of MDMA at 7.5 mg/kg 2 hours apart (Baumann, Clark et al. 2008). Difference in dosing regimes may thus underlie lack of effect of repeated administration of MDMA on neurotransmitter levels in SD and FSL rats.

Another limitation of the present protocol was the lack of assessment of neurotransmitter levels following acute MDMA administration, when the drug had its most prominent effects on behaviour and causes a rapid increase in the intrasynaptic levels of 5-HT. It may be appropriate to include a separate group treated with either saline or MDMA followed by a FST session, and measurement of neurotransmitter levels in cortex. This, however, could only be feasible if the animals were sacrificed straight after the FST.

In sum, the animal studies in this project provided further evidence of the utility of the FSL rat strain as an animal model of depression. Furthermore, an antidepressant-like effect of MDMA was observed as an acute effect of drug administration, and it was more prominent in animals with a pre-existing disturbance in behaviour and serotonergic system functioning. Such an effect was not due to the general stimulant

activity of MDMA, as the behaviour of MDMA-treated rats was different from that of METH-treated animals. The antidepressant-like effect of MDMA was diminished following a series of injections, possibly due to the development of tolerance, however, the chosen dosing regime did not affect the 5-HT and 5-HIAA levels in cortex. Finally, the observed contrast in behaviour between strains was not due to differences in the pharmacokinetic and metabolic profiles of SD and FSL rats. The findings of this study also provide evidence of the unique properties of MDMA's action on the depressive-like state in laboratory animals, with marked differences to the antidepressants that are used in clinical practice, especially in the onset of action.

Overall, the FSL model of depression may be useful in preclinical studies of the role of a predisposition to depression in MDMA use, and further research into the effects of the drug on the neurotransmitter systems functioning in this model of depression may add to understanding of the pathogenesis of depression and concurrent drug use.

# Chapter 5. Effects of MDMA on mood in subjects with and without a predisposition to depression

## 5.1. Introduction

Depressive symptoms with MDMA use are generally considered to be a side effect of the drug. Ecstasy ingestion causes a rapid increase in the 5-HT levels in the intrasynaptic space followed by depletion of neuronal stores of 5-HT in the following days. Repeated excessive activation of the serotonergic system may lead to its overall impairment. However, there is no consensus on the role of long-term ecstasy use in the development of psychiatric side effects: while some authors reported depressive symptoms and cognitive impairment in long-term ecstasy users (Parrott, Lees et al. 1998; MacInnes, Handley et al. 2001; Lamers, Bechara et al. 2006; Reneman, Schilt et al. 2006), others suggest that observed psychiatric effects are more likely to be due to polydrug use, and not solely ecstasy (Roiser and Sahakian 2004; Sumnall, Wagstaff et al. 2004; Schilt, Koeter et al. 2009). Notwithstanding that, evidence from recent neuroimaging studies in ecstasy users shows impairment in functioning of the serotonergic system (McCann, Szabo et al. 1998; Reneman, de Win et al. 2006; McCann, Szabo et al. 2008), which can underlie the development of depressive symptoms.

In recent years, several theories around the links between mood disorders and drug use and dependence have emerged. Many illicit drugs are used and abused due to certain positive effects that outweigh the mental and physical health hazards that drug

use poses to users. Ecstasy, in particular, belongs to a group of substances that produce multiple immediate positive effects on mood and other emotional spheres in people. This, as proposed previously, may be a representation of the antidepressant-like activity of MDMA. As a result, people with an established mood disorder, or with a predisposition to depression, may be at a greater risk of starting to take ecstasy, and, consequently, follow more regular patterns of use, compared with people with no underlying mood condition. In people who are more prone to developing depression, the immediate beneficial effects of MDMA on mood may be more pronounced than in others (Croft, Klugman et al. 2001). This, in turn, may increase risk of more regular use and abuse. Consequently, higher frequency of MDMA consumption and a greater amount of ecstasy pills consumed may induce a greater risk of suffering from the negative psychological and physiological effects of MDMA, especially in the long-term. Blunted response to ecstasy after repeated administration may be due to the development of tolerance (Parrott 2005). This may occur at an earlier stage in more regular users, among which there may be many subjects with a predisposition to mood disorders. Another potential risk in this group is the use of techniques to enhance the positive effects of ecstasy. It can be a conscious process, i.e., using MAOIs before, along with and/or after ecstasy consumption in order to 'boost' positive effects on mood and to prevent negative effects due to subsequent depletion of serotonin stores. On the other hand, people with diagnosed depression may be treated for the disorder and taking ecstasy recreationally along with treatment, whether realising or not the potential enhanced joint action of MDMA and antidepressants on the

serotonergic system (Kelly 2009). This puts them at the risk of developing serotonin syndrome, a potentially lethal condition (Gillman 1999).

A better understanding of the patterns of drug use in people with a predisposition to affective disorders is important. Recent trends suggest that there is an increasing number of young people with diagnosed mood disorders, and substance use is most common in this age group. The accumulation of knowledge on the potential links between pre-existing mood disturbance and subsequent drug use may help to provide information on the effects and risks associated with ecstasy and raise public health awareness on certain patterns of drug use. It may also contribute to the search for therapeutic options in the management of depression and drug-induced depression.

The following study was designed to investigate the immediate effects of MDMA on mood and the intensity of the depressive symptoms in users with and without a predisposition to depression.

## 5.2. Subjects and methods

### 5.2.1. Subjects

40 healthy volunteers, 21 male and 19 females, completed the study. All subjects reported previous use of ecstasy, including on at least 3 occasions in the 6 months prior to the testing. Two participants in the control group reported consuming ecstasy on at least 3 occasions 6.5 months prior to the testing. All study procedures were approved by the Royal Adelaide Hospital Research Ethics Committee.

58 subjects who were eligible to participate signed an informed consent form, 43 of them completed study assessments, two were not eligible to proceed with the testing

at the time of the screening interview, and three were excluded from the study after completion. This gave an overall response rate of 77.5%. Two participants consumed ecstasy pills that didn't contain any MDMA or other amphetamine derivatives (confirmed by testing urine samples using a liquid chromatography-mass spectrometry (LC-MS)); one participant consumed an ecstasy pill at the same time as LSD (acid), and at the time of assessment the effects of LSD were predominant over the effects of MDMA. As no other participants had similar circumstances during the assessment at the social gathering (MDMA was detected in all other urine samples of participants who chose to consume a pill, and no-one else consumed other drugs at the time of pill ingestion), the above-mentioned subjects were excluded. Most participants who didn't complete the study didn't attend the initial session and subsequently couldn't be contacted.

### 5.2.2. Recruitment

Participants were to be recruited from a number of sources, which were approved by the Ethics Committee, namely:

- Contacts in the ' ecstasy scene';
- 'Word of mouth' ('snowballing');
- Participants in other departmental studies, if suitable;
- Advertising through the following media:
    - Street press ("Rip it Up" magazine);
    - Ecstasy-related websites (www.bluelight.ru);

- Youth health services (University Health Practice, University Councelling Services);
- University notice boards;
- Clubs/Bars/Raves/Dance parties.

This recruitment approach was used in previous studies at the department and has proven to be effective. For this study, recruitment via 'snowballing' (35%), university notice boards (30%), street press (27.5%), and from another department study (7.5%) were the most effective.

#### *5.2.2.1. Inclusion criteria*

Participants were included in the study if they met the following criteria:

- Aged 18 years or older;
- Agreeable to and capable of providing written, informed consent prior to study participation;
- Able to communicate in English;
- Willing and able to complete the study requirements at screening and testing intervals;
- Physically well enough to participate in the testing procedures;
- Willing to provide a urine sample at the testing day;
- Willing to provide a blood sample at the testing day;
- Previous use of ecstasy on at least 3 occasions in the past 6 months (6.5 months for 2 participants in a control group);

- Indicate intention to attend a social gathering ('rave', nightclub, bar or private party);
- Reasonable venous access for blood sampling.

#### *5.2.2.2. Exclusion criteria*

Potential participants were excluded from the study if any of the following criteria were found:

- Unable, unwilling or unlikely to comply with study protocol;
- A serious current medical condition requiring medication;
- A pre-existing psychiatric disorder requiring medication, severe behaviour disturbance or psychotic symptoms;
- Current treatment with antidepressants, and treatment with antidepressants within previous three months;
- Currently pregnant or lactating;
- Opioid, amphetamine or alcohol dependence.

Inclusion and exclusion criteria were assessed at the time of the initial phone interview prior to screening by the investigator. If the subject met the inclusion criteria and no exclusion criteria were present, a screening interview was consequently scheduled. At the screening interview, all participants were provided with the written information sheet. If they expressed a willingness to participate, they were asked to sign an informed consent form. Demographic data, a brief assessment of their history of mood disorders and a history of concurrent drug use was then collected and further testing sessions were scheduled.

### 5.2.3. Study testing schedule

A mixed between and within factor design was used in the study. Assessment was carried out in two steps – a baseline session and a party session. All subjects were randomised prior to the start of the testing and assignment to either control or ecstasy groups – with either baseline session before the party session (n=21) or party before baseline (n=19).

#### *5.2.3.1. Baseline session*

The baseline session was carried out when participants hadn't used any illicit drugs for at least 7 days prior. The assessments were scheduled between 8 and 10 AM, and the participants were asked to fast overnight. During this session, participants had to provide a urine sample that was used to screen for the presence of common drugs of abuse, namely: benzodiazepines, cocaine, opiates, methamphetamine, cannabis, amphetamine and MDMA. If the screening urine test was negative for all drugs, with the exception of cannabis, the baseline assessment was then continued. Participants were asked to fill out three self-assessment questionnaires: the Brief Symptom Inventory (BSI), the Beck Depression Inventory (BDI) and Profile of Mood States (POMS). Finally, a blood sample was obtained via venepuncture. Platelet-rich and platelet-poor plasma samples were prepared for the 5-HT uptake and measurement of 5-HT and 5-HIAA concentrations using HPLC. A 4ml whole blood sample was sent to the IMVS Haematology laboratory for platelet count; another 4ml whole blood sample was stored at $-70^0$C for further genotyping analysis. 5-HT uptake was performed using freshly prepared plasma samples. The remainder of the plasma samples were stored at $-70^0$C until further analysis of 5-HT and 5-HIAA quantification.

#### *5.2.3.2. Party session*

The party session was scheduled when participants attended a social gathering. They were assigned to either the ecstasy or control group according to whether they planned to consume a pill during the social gathering. Under no circumstances were they encouraged to consume a pill for the purposes of the study. Prior to the party session, participants were provided with a sterile plastic 5 ml tube, which they had to take with them to the social gathering. Subjects in the ecstasy group notified the investigator when they consumed a pill via text message, and 60 minutes later, the investigator contacted them by telephone and administered the BDI and POMS, i.e. participants were to verbally fill these two questionnaires out, based on how they were feeling at the moment of assessment. For the time of assessment all participants were asked to relocate in a quiet area to minimise distractions and facilitate administration of psychological questionnaires. At the same point in time, all participants were asked to provide a saliva sample in a plastic tube. The day after the social gathering, participants met the investigator for the follow-up, at which they returned the tube with the saliva sample, filled out a form about the drug and alcohol use during the party period and provided a urine sample that was used to detect common drugs of abuse. If the result of the screening urine test was doubtful and didn't match the information provided by subjects, it was sent to the IMVS Toxicology laboratory for confirmatory LC-MS analysis of the presence of MDMA and related substances.

Subjects in the control group chose not to take a pill during the social gathering. All of the assessments were the same as for the ecstasy group. The time of assessment was

matched with the time of the ecstasy group (within 1 hour). Participants in the control group had to abstain from consuming a pill during the entire social gathering. The follow-up protocol was the same for both groups.

### 5.2.4. Demographic data

At the time of the screening interview, the following information was collected from each subject: age, sex, ethnicity, highest level of education completed, current occupation, past and family history of depression, past history of PTSD, and past treatment with antidepressants. If a participant indicated a past diagnosis of depression or PTSD, or previous treatment with antidepressants for any reason other than MDD, time of diagnosis, prescribed treatment, duration and side effects of treatment were recorded. If a family history of depression was mentioned, the relative was recorded. The duration and side effects of treatment with antidepressants for other reasons than MDD, if indicated, were also recorded along with the diagnosis that required the treatment. Moreover, a history of lifetime and current use of the following licit and illicit drugs was collected: ecstasy, methamphetamine (speed, crystal meth, ice), pharmacological stimulants (Dexamphetamine, Ritalin), lysergic acid diethylamide (LSD, acid), cocaine, MDA, amyl nitrate (rush), cannabis, magic mushrooms, nitrous oxide (bulbs), gamma-hydroxybutyric acid (GHB, fantasy), tobacco, heroin, methadone, buprenorphine and any other opioids, antidepressants and benzodiazepines used recreationally, and other illicit drugs that participants had used previously (e.g. dimethyltryptamine (DMT)). For each drug, the following information was obtained: age of first use, duration and frequency of use, total amount of the drug consumed over their lifetime and in the past month prior to

assessment, average amount of the drug consumed per occasion over their lifetime and in the month prior to assessment.

### 5.2.5. Psychological assessment

#### *5.2.5.1. Brief Symptom Inventory (BSI)*

The BSI is a 53-item self-report screening tool that assesses distress in various psychological spheres in people (Derogatis and Melisaratos 1983). It includes nine dimensions of symptoms and three indices that help estimate overall psychological distress. The primary symptom dimensions included in the BSI assess main psychological spheres, namely: Somatisation (SOM), Obsessive-Compulsive (O-C), Interpersonal Sensitivity (I-S), Depression (DEP), Anxiety (ANX), Hostility (HOS), Phobic Anxiety (PHOB), Paranoid Ideation (PAR), and Psychoticism (PSY). Additionally, the BSI allows a calculation of three Global Distress Indices: Global Severity Index (GSI), Positive Symptom Total (PST) and Positive Symptom Distress Index (PSDI) (Derogatis 1993). The Inventory uses a Likert-type scale with five options: 'not at all', 'a little', 'moderately', 'quite a bit' and 'extremely'. For each item, the participant was to indicate how the described problem (e.g. 'feeling no interest in things' or 'trouble concentrating') had distressed him or her over the past 5 years.

The BSI evolved from the Symptom Checklist 90 (Derogatis, Lipman et al. 1973), which has been previously used in some studies on the psychological effects of MDMA (Parrott, Sisk et al. 2000; Thomasius, Zapletalova et al. 2006). The original instructions for the BSI included a period of two weeks prior to testing. In this study, a modified time frame was used: participants were asked to fill out the form indicating distress in

each of the problems for the overall period of 5 preceding years. This was done to cover a broader time frame in order to assess a predisposition to depression.

T-scores for each of the dimensions in the BSI, which are calculated from raw scores, have been established for different populations, including healthy individuals and psychiatric outpatients (Derogatis and Melisaratos 1983). Conversion of raw scores into T-scores depends on the sex and the psychiatric background of the respondent, e.g. in the Depression Dimension for adult non-patients, raw scores of 0.50 for males and 0.66 for females yield a T-score of 60; raw scores of 1.99 for healthy males yield a T-score of 80, whereas the same raw score for male psychiatric outpatients is an equivalent of a T-score of 53 (Derogatis 1993). This study used an adult non-patient scale for the analysis of results. It has been previously established that a T-score higher than or equal to 63 is of clinical significance (Endermann 2005; Lang, Norman et al. 2009). This cut-off point for the Depression Dimension was used in the present study to assign participants to groups with and without a predisposition to depression.

#### *5.2.5.2. Beck Depression Inventory (BDI)*

The BDI is a widely used and validated instrument which assesses the presence and severity of depressive symptoms, based on the DSM-IV criteria for mood disorders (Beck 1996; American Psychiatric Association 2000). Participants were asked to fill out a modified BDI-II form, in which they had to choose which statement best described how they were feeling at the moment of assessment. Due to the choice of the 'right now' assessment, an item referring to sleep was excluded from the inventory. The 'right now' setting was chosen in order to compare mood scores in participants during the social gathering with the baseline results. Use of the modified version of the BDI

was aimed to primarily assess not the severity of depressive symptoms, but to trace changes in mood perception by subjects with and without a predisposition to depression when they were under the influence of ecstasy or at a social gathering, and when they were drug-free. The wording of the questions in the original version of the Inventory was constructed to cover a time frame of two preceding weeks, and it was not modifiable for the assessment using a 'right now' setting. This was taken into account in this study, and the original questions were administered in such a way that participants were to choose the most appropriate answers based how they were feeling at the time of the administration of the test.

#### *5.2.5.3. Profile of Mood States (POMS)*

The Profile of Mood States questionnaire was introduced in 1971 (McNair, Lorr et al. 1971). The original form of the instrument consisted of 65 items that allow assessment of six different mood factors, namely Tension/Anxiety, Depression/Dejection, Anger/Hostility, Vigour/Activity, Fatigue/Inertia, and Confusion/Bewilderment. A cumulative Total Mood Disturbance (TMD) can be calculated based on total scores of the mood factors. A brief, 30-item, version of POMS was used in this study (McNair 1992). This form included the same POMS factors as the original form. Participants were asked to complete the POMS forms at the baseline and party sessions, based on how they were feeling at the moment (the 'right now' option).

### 5.2.6. MDMA and nicotine exposure

To indirectly assess the contents of the pill consumed by the participants, MDMA, MDA, methamphetamine and amphetamine were quantified in 17 oral fluid samples

from the ecstasy group using LC-MS. Two participants did not return the saliva samples after the party session, and the volume of one sample was insufficient. The analysis was carried out by Mr Peter Felgate at Forensic Science South Australia (Adelaide). The levels of nicotine and its metabolite, cotinine, were also assessed as another parameter possibly associated with a predisposition to depression. The cut-off detection point was 5 ng/ml for amphetamine and its derivatives, and 10 ng/ml for nicotine and cotinine.

### 5.2.7. Statistical analysis

Ecstasy consumption patterns, namely, the total number of pills consumed, the average number of pills consumed per session, the mean duration of ecstasy use and the frequency of use, as well as nicotine exposure parameters, were compared between the subjects with and without a predisposition to depression using unpaired two-tailed Student's t-tests. BSI scores for each dimension and index were also compared between the groups predisposed to depression and not predisposed to depression using unpaired two-tailed Student's t-tests.

Demographic parameters represented as frequencies, such as sex distribution, education, occupation, current and lifetime use of drugs were compared with the chi-square test.

Alcohol consumption at the social gathering was compared between groups using a two-way ANOVA with Bonferroni post-hoc tests, with group and a predisposition to depression as factors.

BDI and TMD POMS scores were analysed using a between-within design Linear Mixed Model with the effect of drug group (ecstasy vs. control), predisposition to depression

(yes/no) and time point of assessment (baseline vs. party) on POMS TMD and BDI total scores, as well as scores for six separate POMS sales (Tension/Anxiety, Depression/Dejection, Anger/Hostility, Vigour/Acitivity, Fatigue/Inertia, Confusion/Bewilderment), allowing for interactions between the predictors, correlation in responses over time and a heterogeneous variance structure. The Linear Mixed Model analysis was done by Mr Thomas Sullivan, statistician (Department of Public Health, The University of Adelaide, Australia).

Additionally, a correlational analysis of MDMA exposure, past and family history of depression, age, POMS, BSI and BDI scores was carried out in the groups with and without a predisposition to depression using Pearson's correlation coefficient. A partial correlation controlling for the choice of pill consumption at the party session was done to explore correlations between the changes in POMS TMD and BDI scores with other parameters. Zero-order correlations were done for the demographic parameters.

If the difference between the groups did not reach a statistically significant level, but a trend towards such a difference was detected, Cohen's d was used to calculate the effect size.

All data were analysed using Microsoft Excel, GraphPad Prism, SPSS and SAS Statistics software. All data are represented as mean±SEM or percentage values. Differences with p values of less than 0.05 were considered statistically significant.

## 5.3. Results

### 5.3.1. Demographic data

Collected demographic data, brief psychiatric history and concurrent drug use are presented in Tables 5.1, 5.2, 5.3 and 5.4.

***Table 5.1. Demographic data of subjects with and without a predisposition to depression***

| | | No Predisposition (n=20) | With a Predisposition (n=20) |
|---|---|---|---|
| **Age (years)** | | 22.1±0.9 | 22.4±1.2 |
| **Sex** | Males | 35% | 70%* |
| | Females | 65% | 30%* |
| **Ethnicity** | Caucasian | 100% | 90% |
| | Other | 0% | 10% (5% Middle Eastern, 5% Sri Lankan) |
| **Education** | Year 10 | 5% | 0% |
| | Year 11 | 5% | 10% |
| | Year 12 | 70% | 75% |
| | TAFE | 10% | 15% |
| | University | 10% | 0% |
| **Employment/ Occupation** | Student | 55% | 55% |
| | Casual | 5% | 10% |
| | Part-time | 10% | 25% |
| | Full-time | 15% | 0% |
| | Unemployed | 15% | 10% |
| **History of mood disorders** | Past history of depression | 0% | 10% |
| | Family history of depression | 25% | 5% |
| | Past history of PTSD | 0% (5%)[1] | 0% |
| | Past treatment with antidepressants | 5% (social phobia) | 0% |

Age is represented as mean±SEM (n=20 in each group). Data were compared using an unpaired two-tailed Student's t-test and the chi-square test.
* Indicates significant difference with the No Predisposition group, $p<0.05$.

[1] One subject self-reported an episode of PTSD, however, it was not diagnosed by a medical practitioner and no treatment was prescribed

***Table 5.2. Ecstasy use by subjects with and without a predisposition to depression***

| | No Predisposition (n=20) | With a Predisposition (n=20) | P value |
|---|---|---|---|
| Age of first use (years) | 18.6±0.6 | 18.5±0.5 | 0.95 |
| Duration of use (years) | 3.3±0.5 | 3.9±1.3 | 0.65 |
| Total number of pills consumed | 106.6±34.0 | 168.7±72.6 | 0.44 |
| Frequency of use (occasions/year) | 14.4±2.7 | 15.9±2.8 | 0.70 |
| Average number of pills per session | 2.1±1.5 | 2.1±1.3 | 0.91 |

Data represent mean±SEM (n=20 in each group). Data were analysed using an unpaired two-tailed Student's t-test.

***Table 5.3. Minimum and maximum values for ecstasy use parameters***

| | No Predisposition (n=20) | | With a Predisposition (n=20) | |
|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum |
| Age of first use (years) | 16 | 26 | 15 | 23 |
| Duration of use (years) | 0.6 | 8 | 0.5 | 24 |
| Total number of pills consumed | 5 | 500 | 4 | 1248 |
| Frequency of use (occasions/year) | 0.7 | 40 | 0.3 | 48.9 |
| Average number of pills per session | 1 | 7.5 | 1 | 5 |

***Table 5.4. Lifetime and current use of other illicit drugs by subjects with and without a predisposition to depression***

| | No Predisposition (n=20) | | With a Predisposition (n=20) | |
|---|---|---|---|---|
| | Lifetime | Current (last 12 months) | Lifetime | Current (last 12 months) |
| Alcohol | 100% | 100% | 100% | 100% |
| Cannabis | 95% | 65% | 100% | 85% |
| Tobacco | 85% | 65% | 85% | 55% |
| Methamphetamine | 55% | 5% | 65% | 20% |
| Magic mushrooms | 50% | 35%* | 35% | 5% |
| LSD | 45% | 20% | 75% | 40% |
| Nitrous oxide (bulbs) | 40% | 20% | 45% | 30% |
| Cocaine | 35% | 15% | 30% | 20% |
| Pharmacological stimulants | 25% | 15% | 40% | 5% |
| Amyl nitrate (rush) | 25% | 0% | 15% | 5% |
| Other | 20% | 10% | 5% | 0% |
| GHB | 15% | 5% | 10% | 0% |
| Benzodiazepines | 10% | 0% | 25% | 0% |
| Heroin | 5% | 0% | 0% | 0% |
| Other opiates | 5% | 0% | 15% | 0% |
| Buprenorphine | 0% | 0% | 0% | 0% |
| Methadone | 0% | 0% | 0% | 0% |
| Antidepressants (recreational use) | 0% | 0% | 0% | 0% |

Data were analysed using the chi-square test.

* Indicates significant difference with the No predisposition group, $p<0.05$.

Subjects from the two groups were of the same age. The majority of participants were Caucasian. There was an unequal distribution according to sex in groups: approximately 2/3rds of the group without a predisposition to depression were females, whereas in the group with a predisposition to depression the male to female ratio was 2:1 ($\chi^2(1)=4.912$, $p=0.027$). The rest of the demographic factors presented in Table 5.1 were similar between the groups.

The majority of participants had completed secondary education. 55% of subjects in each group were currently enrolled as students at a tertiary institution. Two subjects in the group with a predisposition to depression had been previously diagnosed with depression. A quarter of the subjects with lower BSI scores had at least one first-degree relative who was diagnosed with depression, whereas only one participant in the group with a predisposition to depression reported a family history of depression. None of the participants had been previously diagnosed with PTSD, however, one subject had self-diagnosed PTSD. One subject in the group with no predisposition to depression reported previous treatment with antidepressants for social phobia.

No significant difference between different aspects of ecstasy use was observed between groups. This may be due to the large variance in responses. The mean age of first use of MDMA was approximately 18.5 years in both groups. The participants with a predisposition to depression reported having consumed a higher number of pills overall, and a slightly larger duration and frequency of use, compared with the group with no predisposition to depression. However, this difference didn't reach a statistically significant level due to high variability (Tables 5.2 and 5.3). Participants from both groups consumed approximately 2.1 pills per occasion.

All participants used other substances as well as ecstasy, with alcohol, tobacco and cannabis being most frequently reported as used over their lifetime and recently (in the last 12 months). Subjects from both groups also used methamphetamine, LSD, magic mushrooms, dexamphetamine, methylphenidate, and cocaine, as well as nitrous oxide more frequently than other drugs (Table 5.4). Subjects without a predisposition

to depression were more likely to report current use of magic mushrooms than counterparts predisposed to depression ($\chi^2(1)=5.63$, p=0.018).

### 5.3.2. MDMA and nicotine exposure

All analysed oral fluid samples contained MDMA. The mean (±SEM) concentration of MDMA was 896±132.1 ng/ml. MDA was detected in 36.84% of the samples with a mean (±SEM) concentration of 81±36.85 ng/ml. Methamphetamine and amphetamine were not detected in any of the samples. Nicotine was detected in 47.1% of samples. The mean concentration of nicotine was 2426±1258.74 ng/ml. Cotinine was detected in 35.3% of samples with a mean concentration of 148±96.83 ng/ml.

Nicotine exposure was analysed separately in subjects who reported current use. 65% (n=13) of subjects with no predisposition to depression and 55% (n=11) of subjects predisposed to depression reported current use of nicotine. No difference between the groups was observed in several parameters. The average age of starting to smoke was 16.46±0.37y for the No Predisposition group and 16.82±0.55y for With a Predisposition group, p=0.59. No difference was observed in the years of use (No Predisposition 4.05±0.52y and With a Predisposition 4.52±2.01y, p=0.81) and average number of cigarettes smoked per day or occasion (No predisposition 9.35±3.02 cigarettes and With a Predisposition 9.86±2.33, p=0.90). 46.15% of subjects with no predisposition to depression and 45.45% of subjects with a predisposition to depression were daily smokers. Other participants reported smoking socially or occasionally (i.e. 3-4 times a week). At the social gathering, 69% of current smokers without a predisposition to depression reported use of nicotine, with 7.15±2.56 cigarettes smoked on average, whereas in the group with a predisposition to

depression 81.8% of current smokers reported having 4.0±1.51 cigarettes on average (p=0.32). There was no difference between groups in the frequency of social and daily smokers ($\chi^2(3)=3.16$, p=0.368). In this study no association between current smoking and BSI Depression Dimension scores was observed (r=0.29, p=0.86).

### 5.3.3. Use of ecstasy and other substances at the party session

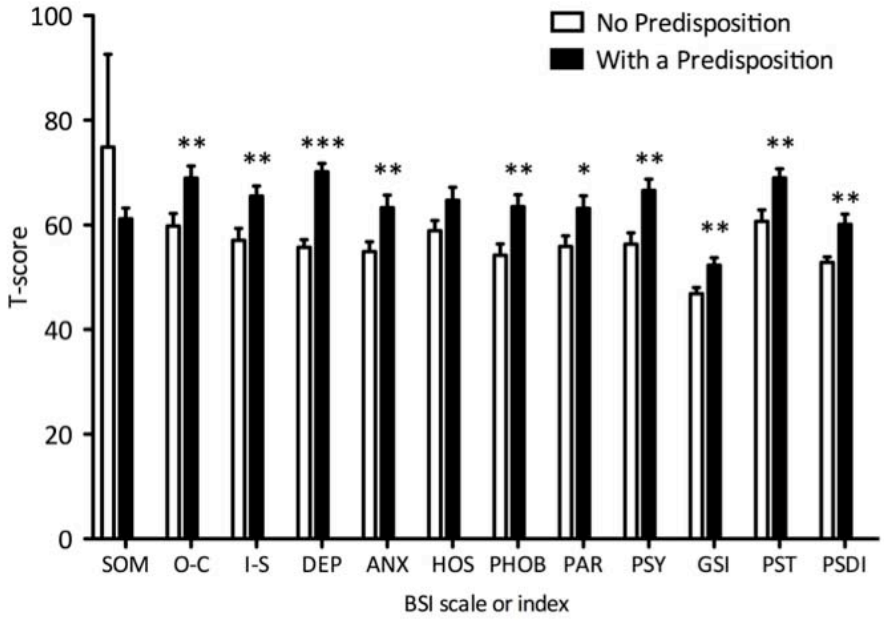
During the social gathering subjects in the No Predisposition group reported consuming 1.7±0.33 pills, and subjects in the With a Predisposition group 1.4±0.22 pills on average (p=0.45). The median time of pill consumption was 21:47 for the No Predisposition group and 20:40 for the With a Predisposition group. Alcohol was the most frequent compound consumed during the party session: only 1 participant in the ecstasy group and 3 control participants didn't report consuming alcohol at the social gathering. There was no effect of predisposition to depression (F(1,35)=1.43, p=0.24) or choice of pill consumption (F(1,35)=0.31, p=0.58) on the number of standard drinks consumed at the social gathering (No Predisposition: control group 5.4±1.22 standard drinks, ecstasy group 7.8±1.77 standard drinks; With a Predisposition: control group 9.4±4.05 standard drinks, ecstasy group 9.78±1.79 standard drinks). Cannabis and tobacco were two other popular compounds, and no difference in their use was observed between the groups. 30% of participants in the ecstasy and control groups with no predisposition to depression, as well as 30% of the control subjects with a predisposition to depression and 40% of those in the ecstasy With a Predisposition group reported smoking cannabis at some time at the social gathering. However, none of the participants in either group reported consuming cannabis at the same time as ecstasy. Thirty percent of the control participants and 50% in the ecstasy group with

no predisposition to depression, and 20% of the controls and 30% of the ecstasy group with a predisposition to depression reported smoking tobacco at the social gathering. Moreover, three participants with a predisposition to depression, one in the ecstasy group and two controls, reported using LSD; however, the time of consumption was either 7 hours before or 8 hours after the assessment. Finally, one control participant with a predisposition to depression reported consuming ketamine at the social gathering 2 hours after the assessment.

### 5.3.4. Psychological tests

#### *5.3.4.1. Brief Symptom Inventory*

***Figure 5.1. Brief Symptom Inventory results***




All data represent mean±SEM (n=20 in each group).
SOM - Somatisation, O-C - Obsessive Compulsive, I-S - Interpersonal Sensitivity, DEP - Depression, ANX - Anxiety, HOS - Hostility, PHOB - Phobic Anxiety, PAR - Paranoid Ideation, PSY - Psychoticism, GSI - Global Severity Index, PST - Positive Symptom Total, PSDI - Positive Symptom Distress Index.
Data were compared between groups using unpaired two-tailed Student's t-tests for each dimension and index, * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with the group with no predisposition to depression.

The results of the BSI are presented in Table 5.5. Subjects with a predisposition to depression; that is, with a Depression dimension T-score of 63 or higher, reported significantly higher distress in all primary symptom dimensions, except for Somatisation and Hostility, compared with subjects without such predisposition (Fig. 5.1). The Global Severity Index, Positive Symptom Total and Positive Symptom Distress Index were also significantly higher in the group with a predisposition to depression. The difference between the groups was most evident in the Depression dimension ($p<0.0001$).

***Table 5.5. Brief Symptom Inventory T-scores***

| Symptom dimensions and indices | No Predisposition (n=20) | With a Predisposition (n=20) | P value |
|---|---|---|---|
| Somatisation (SOM) | 74.9 ± 17.7 | 61.2 ± 2.1 | 0.45 |
| Obsessive-Compulsive (O-C) | 59.8 ± 2.4 | 69.0 ± 2.3 | **0.0082** |
| Interpersonal Sensitivity (I-S) | 57.1 ± 2.2 | 65.6 ± 1.9 | **0.0064** |
| Depression (DEP) | 55.8 ± 1.5 | 70.2 ± 1.6 | **< 0.0001** |
| Anxiety (ANX) | 55.0 ± 1.8 | 63.3 ± 2.4 | **0.0085** |
| Hostility (HOS) | 58.9 ± 2.0 | 64.7 ± 2.5 | 0.0076 |
| Phobic Anxiety (PHOB) | 54.3 ± 2.1 | 63.6 ± 2.3 | **0.005** |
| Paranoid Ideation (PAR) | 55.9 ± 2.0 | 63.2 ± 2.4 | **0.0254** |
| Psychoticism (PSY) | 56.4 ± 2.1 | 66.6 ± 2.1 | **0.0016** |
| Global Severity Index (GSI) | 47.0 ± 1.2 | 52.4 ± 1.4 | **0.0052** |
| Positive Symptom Total (PST) | 60.7 ± 2.2 | 69.0 ± 1.7 | **0.0051** |
| Positive Symptom Distress Index (PSDI) | 52.9 ± 1.1 | 60.2 ± 1.9 | **0.0022** |

Data represent mean±SEM (n=20 in each group). Data were compared using unpaired two-tailed Student's t-tests for each dimension and index.

#### *5.3.4.2. Profile of Mood States*

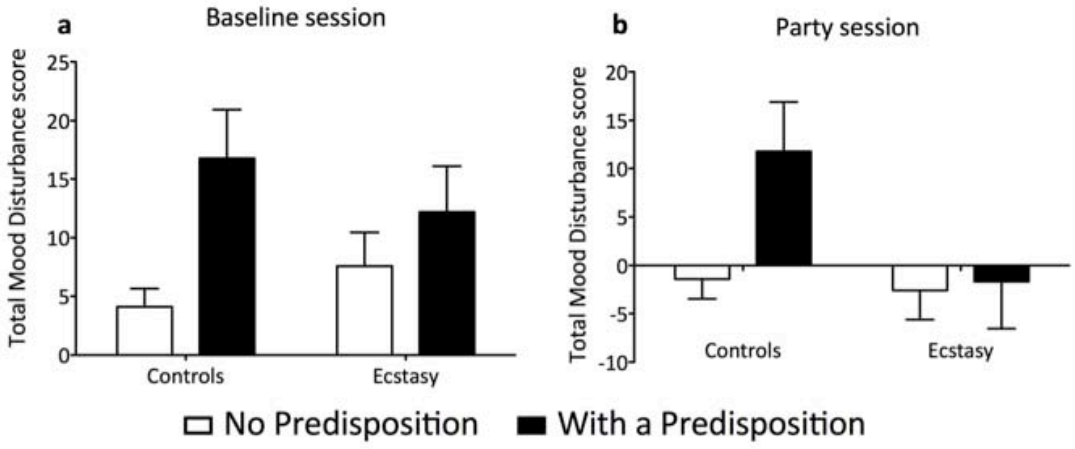
##### 5.3.4.2.1. Total Mood Disturbance score

No evidence for a three-way interaction effect between the drug group (controls/ecstasy), predisposition to depression (yes/no) and time of assessment was observed ($F(1,72)=0.17$, $p=0.684$). There was a significant two-way interaction effect between drug group and predisposition to depression ($F(1,75)=4$, $p=0.049$). Independently of the time of assessment, the participants with a predisposition to depression in the control group had significantly higher POMS TMD values than the other three groups (adjusted means: control group - With a Predisposition 14.30±3.22, No Predisposition 1.35±1.30; ecstasy group - With a Predisposition 5.25±3.12, No Predisposition 2.50±2.06) (Fig. 5.2). Moreover, the time of assessment had a significant main effect on the TMD scores ($F(1,75)=13.98$). Independently of drug group and predisposition to depression, the TMD scores were significantly decreased in all groups during the party session when compared with the baseline session (adjusted means: baseline 9.53±1.61 vs. party 2.17±1.61, $p = 0.0004$). Notably, the mean TMD scores at the party session in both control and ecstasy groups with no predisposition to depression were negative (-1.4±2.1 and -2.6±3.0, respectively), whereas in the group with a predisposition to depression the mean TMD scores remained positive in the control group (11.8±5.1), whereas in the ecstasy group they decreased to a negative level (-1.7±4.8) (Fig. 5.2).

Separate analyses of each of the POMS scales didn't reveal any three-way interaction between the drug group (control/ecstasy), predisposition to depression (yes/no) and

the time of assessment (baseline/party). Results of the scores for all POMS scales are presented in Fig. 5.3 and Fig. 5.4.

***Figure 5.2. Profile of Mood States Total Mood Disturbance scores at the baseline and party sessions***

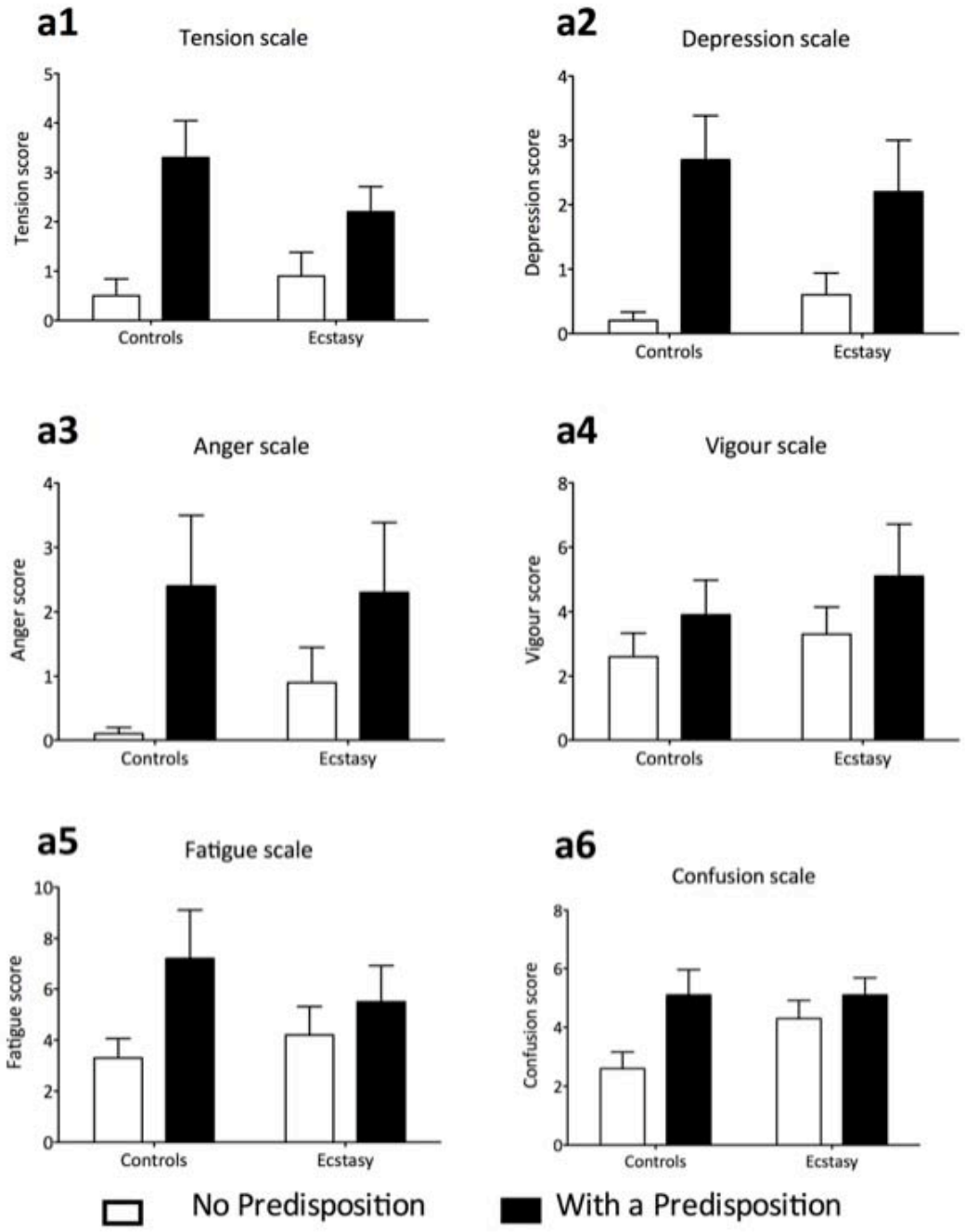



Profile of Mood States Total Mood Disturbance scores at the baseline (a) and party (b) sessions.
Data represent mean±SEM (n=10 in each group).
Data were compared between using a Linear Mixed Model analysis.

5.3.4.2.2. Tension/Anxiety scale

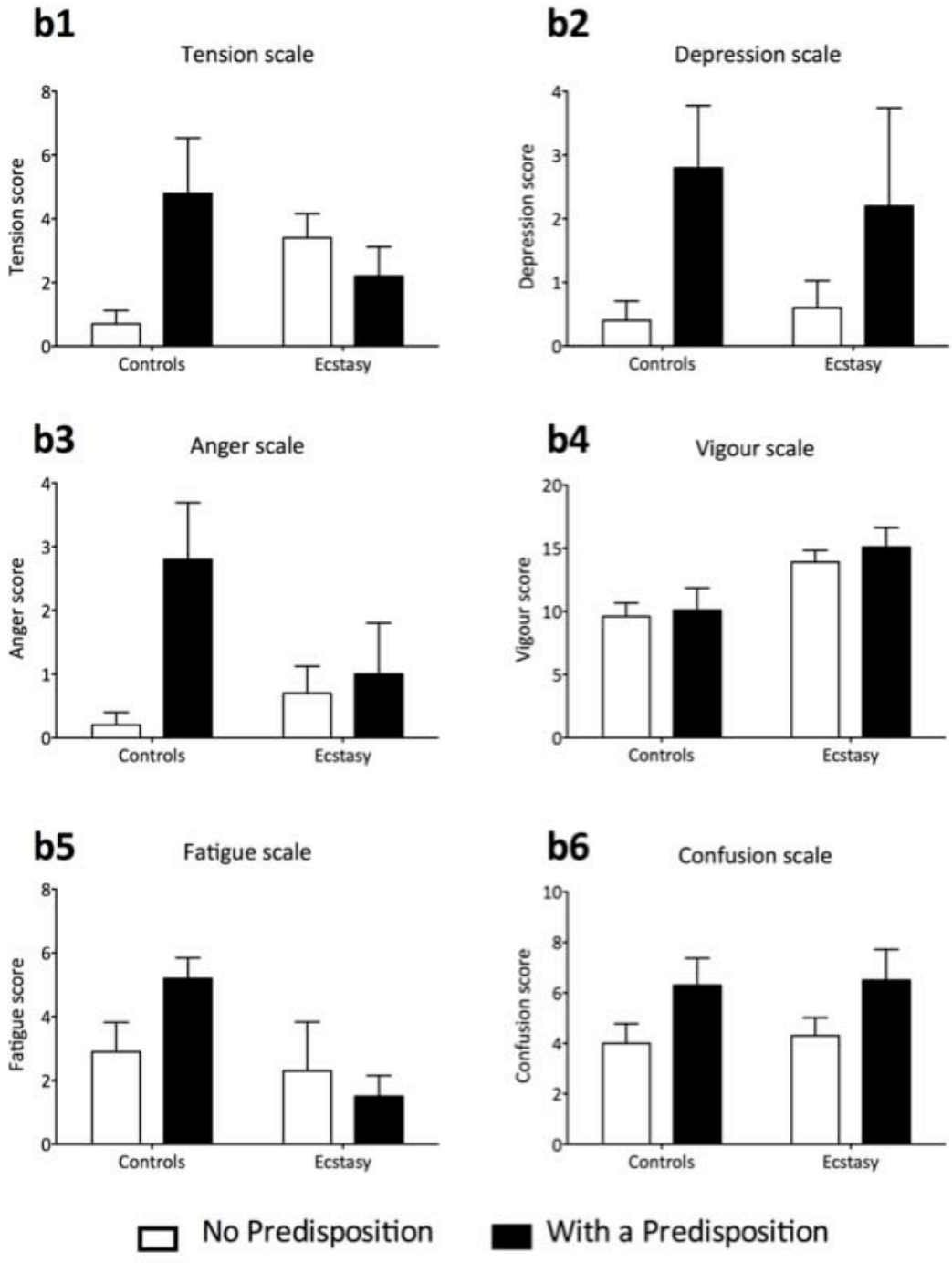
A significant interaction effect between drug group and predisposition to depression was observed for the Tension total scores in groups ($F(1,75)=8.02$, $p=0.0059$). Independently of the time of assessment, subjects in the control group without a predisposition to depression reported lower Tension scores than subjects in other groups (control group: No Predisposition 0.60±0.27 and With a Predisposition 4.05±0.93; ecstasy group: No Predisposition 2.20±0.52 and With a Predisposition 2.15±0.49, $p<0.05$).

***Figure 5.3. Profile of Mood State scales scores at the baseline session***




Profile of Mood State scores of Tension (a1), Depression (a2), Anger (a3), Vigour (a4), Fatigue (a5) and Confusion (a6) scales at the baseline session.
Data represent mean±SEM (n=10 in each group). Data were compared between groups using a Linear Mixed Model analysis.

***Figure 5.4. Profile of Mood State scales scores at the party session***




Profile of Mood State scores of Tension (b1), Depression (b2), Anger (b3), Vigour (b4), Fatigue (b5) and Confusion (b6) scales at the party session.
Data represent mean±SEM (n=10 in each group). Data were compared between groups using a Linear Mixed Model analysis.

5.3.4.2.3. Depression/Dejection scale

No significant interaction effect of drug group and predisposition to depression was observed for the Depression scale scores ($F(1,72)<1$, $p=0.44$). However, a significant main effect of predisposition to depression was found ($F(1,76)=19.22$, $p<0.0001$). Independently of whether subjects chose to take a pill during the party session and the time of assessment, participants with a predisposition to depression reported higher Depression scores than their counterparts (2.61±0.48 and 0.43±0.16, respectively).

5.3.4.2.4. Anger/Hostility scale

Similar to the results of the Depression scale, participants with a predisposition to depression reported higher Anger scores independently of the time of assessment and drug group than subjects not predisposed to depression (2.11±0.49 and 0.40±0.17, $F(1,76)=10.79$, $p=0.0015$).

5.3.4.2.5. Vigour/Activity scale

A significant interaction effect of time of assessment and drug group was observed when analysing total Vigour scores ($F(1,75)=5.77$, $p=0.02$). Irrespective of their predisposition to depression, subjects in the control and ecstasy groups didn't differ in Vigour scores at the baseline ($p=0.45$), but, during the party assessment, participants in the ecstasy group reported significantly higher Vigour scores than control subjects (14.50±0.80 and 9.99±0.79, respectively). Vigour scores were significantly increased in both the control and ecstasy groups during the party session in comparison with baseline scores (control group: baseline 3.23±0.79 vs. party session 9.99±0.79; ecstasy group: baseline 4.05±0.80 vs. party 14.50±0.80).

5.3.4.2.6. Fatigue/Inertia scale

There were significant main effects of predisposition to depression and time of assessment on the reported Fatigue scores (F(1,76)=4.21, p=0437 for the predisposition to depression, and F(1,76)=5.02, p=0.028 for the time of assessment). Fatigue scores were higher in subjects with a predisposition to depression than in those without it (4.68±0.64 and 2.93±0.54, respectively), and being at a party alleviated fatigue irrespective of predisposition to depression and choice of pill consumption (2.91±0.57 party vs. 4.70±0.57 at baseline).

5.3.4.2.7. Confusion/Bewilderment scale

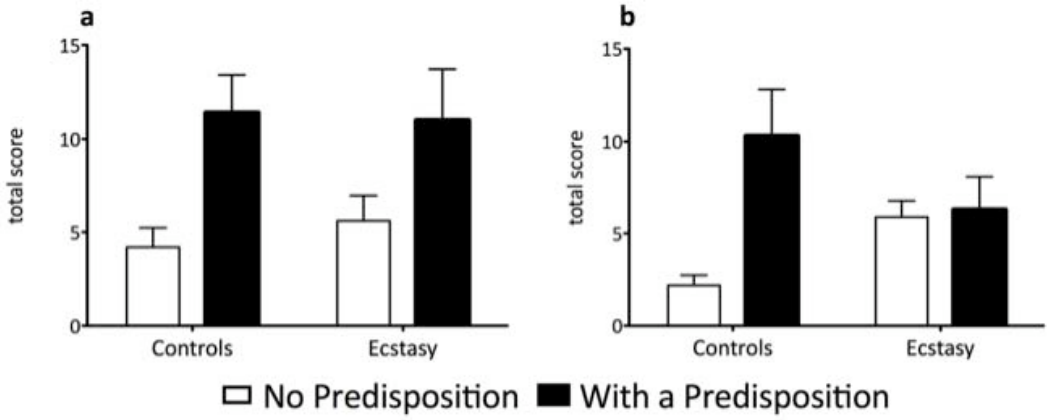
Predisposition to depression had a significant main effect on total Confusion scores. Subjects with a predisposition to depression, independently of the drug group and assessment time, reported higher Confusion scores than users without a predisposition to depression (With a Predisposition 5.75±0.47, No Predisposition 3.80±0.33, F(1,76)=11.31, p=0.0012).

### *5.3.4.3. Beck Depression Inventory*

There was no evidence for a three-way or any two-way interactions between drug group, predisposition to depression and time of assessment. However, a significant main effect of predisposition to depression was found: independently of the time of assessment and drug group, subjects with a predisposition to depression scored significantly higher on the BDI than participants not predisposed to depression (14.07±3.35 vs. 6.97±1.97, p=0.0001). There was no evidence of a change in BDI over time (F(1,75)=2.51, p=0.117) or between control and ecstasy groups (F(1,75)=3.88, p=0.053). However, a trend towards a decrease in the BDI scores was observed at the

party session in the ecstasy group with a predisposition to depression (baseline 11.0±2.72, party 6.30±1.80, Cohen's d=0.65, r=0.3 (medium size effect)). Total BDI scores reported by subjects with a predisposition to depression at baseline (11.0±2.72), and control subjects predisposed to depression during the party assessment (10.30±2.51) were relatively close to the cut-off scores for mild depression (Beck 1996). However, as the subjects were to answer the questions of the Inventory based on how they were feeling at the time of assessment, and not over the preceding 2 weeks, as in the original BDI version, the resulting scores were used only to indicate the level of the depressive symptoms at the time of assessment.

***Figure 5.5. Beck Depression Inventory scores***




Beck Depression Inventory scores at the baseline (a) and party (b) sessions.
Data represent mean±SEM (n=10 in each group).
Data were compared between using a Linear Mixed Model analysis.

### 5.3.5. Correlational analysis

In addition to group analysis described above, a correlational analysis was done to investigate possible effects of demographic and MDMA use parameters on psychological scores. For that, in both No Predisposition and With a Predisposition

groups, zero-order correlations were used to explore relationships between the data collected at the baseline, including demographic parameters, previous MDMA exposure, past and family history of depression, BSI scores, as well as baseline POMS TMD and BDI scores. Moreover, a partial correlational analysis in each of the groups controlling for the choice of pill consumption during the party assessment (control/ecstasy) was done to analyse the relationship between demographic parameters.

Expected relationships between some of the previous MDMA exposure parameters were observed, namely: a greater total amount of pills consumed over the lifetime was associated with longer duration of use (No Predisposition $r=0.56$, $p=0.01$, With a Predisposition $r=0.962$, $p<0.001$) and with a greater average amount of pills consumed per session (No Predisposition $r=0.681$, $p=0.001$).

Interestingly, in the group without a predisposition to depression, the presence of past or family history of depression or past treatment with antidepressants was significantly associated with a greater total amount of pills consumed ($r=0.472$, $p=0.036$). Moreover, subjects in the No predisposition group who started to use ecstasy earlier and who were currently younger than others were more likely to report higher BSI Depression Dimension scores ($r=-0.460$, $p=0.041$ and $r=-0.530$, $p=0.016$, respectively). In addition, older age of subjects without a predisposition to depression significantly correlated with the lower baseline BDI scores ($r=-0.468$, $p=0.043$). No association between past history of mood disorders and BSI scores was found in the group with a predisposition to depression ($r=-0.134$, $p=0.57$), however, a correlation between age and BSI Depression scores, similar to that in the No Predisposition group,

was observed, and older subjects were more likely to report higher BSI Depression Dimension scores (r=0.449, p=0.047).

Analysis of the association between the psychological scores revealed a strong correlation between the baseline BDI and TMD POMS scores in both groups (No Predisposition: r=0.608, p=0.006, With a Predisposition: r=0.566, p=0.009). In the group with no predisposition to depression, higher baseline BDI scores were also associated with higher BSI Depression Dimension scores (r=0.555, p=0.014), whereas a similar correlation in the group with a predisposition to depression did not reach a statistically significant level (r=0.428, p=0.06).

Partial correlational analysis of the changes in psychological scores was done to investigate the efficacy of concurrent use of POMS and BDI in the assessment of mood and depressive symptoms and also as an indirect measure of the effects of ecstasy consumption on the parameters of interest. Changes in the BDI scores significantly correlated only with baseline scores (r=-0.79, p<0.0001), but not party BDI scores (r=0.266, p=0.27) in the No Predisposition group, whereas in the With a Predisposition group changes in the BDI scores were associated with both baseline and party scores (r=-0.64, p=0.003 and r=0.541, p=0.17, respectively). Moreover, in the group with a predisposition to depression, a significant association between the BDI and POMS TMD scores at the party session was observed (r=0.674, p=0.002)

Finally, the following correlations depict the contribution of previous MDMA exposure to the results of psychological assessment at the party session. In the group without a predisposition to depression higher BDI scores at the party session were more likely to be reported by subjects with a lower total amount of pills consumed (r=-0.578, p=0.01)

and a shorter duration of use (r=-0.467, p=0.044). Furthermore, older subjects tended to report lower POMS mood disturbance scores at the party session (r=-0.546, p=0.016). Lower POMS TMD scores at the party session also correlated with a higher average amount of pills consumed per session in the No Predisposition group (r=-0.478, p=0.038). In the group with a predisposition to depression, older age of subjects and longer previous ecstasy use were associated with the lower TMD scores at the party session (r=-0.526, p=0.021 and r=-0.487, p=0.034, respectively).

It must be acknowledged that due to a small sample size (n=20) observed correlations should be interpreted with caution. Notwithstanding that, as the present project was primarily focussed on the analysis of main effects of MDMA and a predisposition to depression on mood in current ecstasy users, correlational analysis was used as an additional measure of possible relationships between the background factors and measured parameters.

## 5.4. Discussion

The present study is the first to assess mood disturbance and depressive symptoms in ecstasy users with and without a predisposition to depression in different settings. The results of the study showed no difference in the patterns of ecstasy use and the majority of demographic parameters and concurrent drug use between the groups. The results of the psychological assessment revealed higher overall distress in individuals with a predisposition to depression, as well as greater intensity of depressive symptoms and mood disturbance, when not under the influence of ecstasy. During the party assessment, no statistically significant effect of ecstasy consumption on mood disturbance was present in either group, whereas being at the social

gathering significantly reduced mood disturbance in all groups. A trend towards a decrease in the intensity of depressive symptoms was observed only in the group with a predisposition to depression following ecstasy ingestion. Finally, significant associations of some of the parameters of previous MDMA exposure with mood disturbance and depressive symptoms, which were observed in the correlational analysis, may explain the patterns of ecstasy use.

The hypothesis of self-medicating use of ecstasy by subjects vulnerable to mood disorders is relatively new, and only a few authors have previously discussed the possibility of this link (Croft, Klugman et al. 2001; Lieb, Schuetz et al. 2002; Huizink, Ferdinand et al. 2006; Sessa and Nutt 2007; Alati, Kinner et al. 2008). However, previous research did not include the assessment of mood changes as an immediate effect of MDMA, rather, the association between different psychological factors with subsequent ecstasy use was investigated.

In this project, the two groups did not differ in demographic parameters and previous and current use of other drugs, including current use of nicotine. Young people, who mainly were university students, represented the cohort of subjects that participated in this study. Due to the diversity of ecstasy exposure, an additional analysis was conducted to investigate whether there are differences in the psychological parameters between users who consumed different amounts of the drug over their lifetime (see Chapter 7).

Cigarette smoking has been linked with depression (Johnson and Breslau 2006). Another hypothesis of the links between nicotine and MDMA use can be that cigarette smoking along with pill consumption can possibly potentiate the effects of MDMA.

Such considerations were expressed by some of the participants. Nicotine has MAO inhibiting properties, therefore may be considered as an antidepressant-like agent (Berlin and Anthenelli 2001). In this study, no statistically significant difference in the patterns of nicotine use was observed between groups, and no association between cigarette smoking and BSI Depression scores was found. However, the impact of nicotine use on predisposition to depression and ecstasy use was not the primary area of interest of this study. A study specifically looking at the possible association between nicotine and ecstasy use and depressive symptomatology may be of interest in the area of patterns of drug use.

The determination of predisposition to depression was done using the BSI, a screening psychological tool, which was previously used to assess psychological distress in ecstasy users (Parrott, Sisk et al. 2000; Singer, Linares et al. 2004; Soar, Turner et al. 2006; Thomasius, Zapletalova et al. 2006). This Inventory has been shown to have high predictive validity as a screening tool for psychopathological states (Derogatis 1993). It was also used as a measure of the risk of the development of depressive symptomatology in young users of psychoactive substances (Buckner and Mandell 1990). One of the recent studies used the BSI Depression Dimension scale in the assessment of the role of affective temperaments and family history of affective disorders in the development of depressive symptoms in a general population (Lazary, Gonda et al. 2009). The BSI is also commonly used in its original and shorter configurations to assess distress in various emotional spheres in the general population (Derogatis and Melisaratos 1983), as well as in specific cohorts of people, including primary care patients (Lang, Norman et al. 2009), adult psychiatric inpatients

(Piersma, Reaume et al. 1994), HIV-positive subjects (Grassi, Righi et al. 1999), cancer patients (Carlson, Angen et al. 2004; Andritsch, Dietmaier et al. 2007; Low, Gessler et al. 2009), haemodialysis patients (Gillanders, Wild et al. 2008), subjects with ischaemic heart disease (Doering, Moser et al. 2009), and drug court participants (Joosen, Garrity et al. 2005).

The BSI configuration was made to assess distress in different psychological spheres over the 5 years preceding the testing. The chosen time frame could be considered as relatively inaccurate for interpretation of psychological distress (Derogatis 1993). There could be multiple periods of increased distress in a certain emotional sphere within the 5 years preceding the testing session, which could potentially confound the results of the test. Moreover, memory distortions could potentially contribute to the inaccuracy of answers. To overcome the potential distortions, certain precautions were taken. When explaining the instructions for the Inventory, participants were asked to answer the questions based on their average perception of each of the problems over the period of 5 years. It was also stressed that the questionnaire had to be filled out based on how the participants were feeling in general, irrespective of use of ecstasy or other drugs. This allowed the assessment of distress in various emotional spheres, with a focus on the Depression dimension. In a study of Soar et al (2006), problematic ecstasy users, i.e., those who reported experiencing side effects of the drug, reported significantly higher Depression Dimension scores compared with non-problematic ecstasy users and drug-naïve controls. Moreover, a greater number of problematic users had past or family history of psychiatric disorders (Soar, Turner et al. 2006). The authors argued that observed increased levels of psychopathology in

problematic ecstasy users may be due to pre-existing susceptibility to psychiatric conditions. However, as the original configuration of the BSI was used, covering a period of precedent 7 days, the assessment of possible pre-existing psychopathology was thus not included.

The BSI in the chosen configuration may be a sensitive instrument to distinguish predisposition to depression: in this study, the difference in the Depression Dimension scores between the groups was almost three-fold; moreover, higher BSI Depression scores were significantly associated with higher baseline BDI scores in the group with no predisposition to depression, and a similar, but not statistically significant trend ($p=0.06$) was observed in subjects with a predisposition to depression. Interestingly, subjects with a predisposition to depression were more likely to indicate distress in most psychological spheres covered in the BSI, however the difference between the groups was of a lesser magnitude than in the Depression dimension. The reference cut-off point in distinguishing distress was 63 for the Depression dimension (Piersma, Reaume et al. 1994; Endermann 2005; Lang, Norman et al. 2009). Other dimensions were not analysed as closely, as they were not the primary area of interest in this project. The average duration of ecstasy use was approximately 3.5 years in both groups. Thus, the BSI results were aimed to cover the period exceeding the period of ecstasy use.

Assessment during the social gathering, when participants were under the influence of ecstasy, was carried out similarly to some previous studies (Curran and Travill 1997; Parrott and Lasky 1998; Huxster, Pirona et al. 2006). The difference was that a telephone interview was used in the present study. Another project conducted at the

department of Pharmacology used an 'in-scene' approach, where all assessments took place during the social gathering (Morefield, Keane et al. 2008; Morefield, Irvine et al. 2009). Curran and colleagues conducted a study in which they were assessing memory, mood and depressive symptoms (using BDI and mood rating VAS), and physical symptoms. The control group included participants who had consumed alcohol during the social event when the assessment took place. However, the control group had diverse previous exposure to ecstasy, and 40% of control participants reported never taking MDMA previously. The psychological assessment was done during the social gathering, and subsequently 1 and 4 days following. However, the exact time relationship between the ecstasy consumption and assessment during the first day is unclear.

Most previously published studies of the acute effects of ecstasy used visual analogue scales (VAS) to measure the subjective effects of MDMA (Cami, Farre et al. 2000; Liechti, Baumann et al. 2000; Dumont and Verkes 2006; Tancer and Johanson 2007; Kolbrich, Goodwin et al. 2008). The protocol of the current study was designed in a way that administration of VAS perhaps would not be the best way of assessment, as it was not a controlled setting, and the assessment was one via a telephone interview. Providing participants with paper forms and instructing them to evaluate their psychological state exactly 1 hour after pill consumption may have posed additional inconvenience for the subjects and decreased the response and compliance rates. Moreover, the chosen psychological instruments were well validated and have been widely used in different clinical and research settings. The structure of the POMS and BDI questionnaires is suitable for administration in the form of a telephone interview,

and, hence, provided a more effective way to carry out the assessment at the necessary time.

Previous studies that have used the BDI in the psychological assessment of ecstasy users commonly administered the questionnaire to measure the level of depressive symptoms in users when they were not under the influence of the drug (de Win, Reneman et al. 2004; Roiser and Sahakian 2004; Falck, Wang et al. 2006; Lamers, Bechara et al. 2006; Falck, Jichuan et al. 2008). Usually this questionnaire was used when the primary aim of the study was to investigate depressive symptoms as a side effect of the use of ecstasy and other drugs. The original configuration of the Inventory covered a period of 2 previous weeks, as it was consistent with the definitions of the Major Depressive Episode required to establish the presence and extent of symptoms (American Psychiatric Association 2000).

In contrast, the primary aim of use of the BDI in this study was to assess the prominence of depressive symptoms at the time of assessment, which was done in different settings, namely, when participants were not under the influence of any drugs, and when they were at a social gathering, during which some chose to consume a pill. For that, a modified version of the BDI was used. Participants were asked to choose one point in each group of statements based on how they were feeling at that moment. In other words, this configuration of the BDI was not aimed to assess the severity of the depressive symptoms in users with and without a predisposition to depression, but to compare how being in a social setting and ecstasy consumption changes their depressive state. The findings of this study suggest that the BDI with a

'right now' option is sensitive in distinguishing participants with and without a predisposition to depression.

The baseline scores in the group with a predisposition to depression were somewhat close to the level of mild depression, but still in the minimal depression range of 0 to 13 points (Beck 1996). However, it must be noted that a modified 20-item inventory was used in the present study, as the item about the quality of sleep was excluded due to the use of the 'right now' option. Inclusion of this item would have been confusing, even if participants were asked to choose the best description of their sleep patterns based on how they were feeling at the moment of assessment, akin to the Epworth Sleepiness Scale. Although not strictly comparable due to a different time frame in the instructions, the baseline BDI scores were in concordance with previous findings: Falck et al reported that the total BDI-II scores of the majority of respondents in their study fell within a minimal depression range (Falck, Wang et al. 2006; Falck, Jichuan et al. 2008). Curran and colleagues administered an earlier version of the BDI at different time points, including at a social gathering, when MDMA users reported total scores of 3 on average, whereas control users who reported consuming only alcohol scored approximately 6 during that assessment (Curran and Travill 1997). Another study reported a mean BDI score of 9.82 in current MDMA and cannabis users (Lamers, Bechara et al. 2006).

Moreover, a trend towards a decrease in the depressive scores was observed in the group with a predisposition to depression if they chose to take a pill, however it didn't reach a statistically significant level ($p=0.053$) (Fig 5.5b). Nevertheless, the magnitude of this effect was sufficient (Cohen's $d=0.65$) to provide evidence for drug's potential

to improve the perception of depressive symptoms more in subjects predisposed to mood disorders than in those with no such predisposition. Tyle II error may underlie the lack of identification of such an effect of ecstasy

The POMS questionnaire has been used in a few studies examining the effects of ecstasy (Cami, Farre et al. 2000; Tancer and Johanson 2001; Tancer and Johanson 2007). In the controlled study conducted by Tancer et al (2001), like the present, participants were included if they had used ecstasy previously on at least 3 occasions. The authors used a full version of POMS with the 'right now' configuration. The questionnaire was administered every 60 minutes after administration of either MDMA or placebo for 6 hours. Peak changes were observed 120 min after drug administration. Higher MDMA doses (110mg/70kg and 145 mg/70kg) caused a significant increase in anxiety and confusion scores, whereas a lower dose (75mg/70kg) caused an increase in the Friendly and Elation scales in comparison with placebo. However, the exact scores were not reported. In a later study from this group, administration of 1.5 mg/kg of MDMA caused a significant increase in POMS scores in the Anxiety, Elation, Vigour, Arousal and Positive Mood scales compared with placebo (Tancer and Johanson 2007). In another controlled study, a full version of POMS was administered at different time points following drug administration (Cami, Farre et al. 2000). Compared with placebo, subjects who received 125 mg of MDMA reported increased Elation, Confusion and Positive Mood scores.

POMS results in the present study did not show a statistically significant effect of MDMA consumption on mood disturbance in subjects with and without a predisposition to depression. While TMD scores were significantly higher at baseline in

control subjects with a predisposition to depression, being at the social gathering significantly improved mood irrespective of predisposition to depression and choice of ecstasy consumption. TMD scores decreased to negative values in both groups without a predisposition to depression, whereas in the control group with a predisposition to depression mean TMD scores at the party session remained in a positive range, while subjects with a predisposition to depression reported negative TMD scores on average. However, as no significant interaction effect of drug group, predisposition to depression and time of assessment was observed, these observations do not represent a direct effect of ecstasy on mood.

Somewhat consistently with the BSI results and the BDI results for the POMS Depression scale, subjects with a predisposition to depression reported a disturbance in some of the psychological spheres analysed in the POMS. Subjects without any predisposition to depression reported feeling less tense and anxious, whereas individuals with a predisposition to depression reported feeling more depressed, confused, tired and angry than their counterparts, irrespective of the time of assessment. Vigour scores did not differ between groups at baseline, however, irrespective of predisposition to depression, being at the party session led to an increase in activity, which was more pronounced in the ecstasy group compared with the controls. Similarly, irrespective of the groups, subjects reported feeling less tired at a social gathering than at baseline.

The findings of the present study are consistent with previous observations. Although neither the brief version of POMS, nor the BDI-II in the chosen configuration have been used in other studies on the effects of MDMA, the psychological profile of ecstasy

users at the social gathering and at baseline are in accordance with the existing body of research into the effects of MDMA on mood. The BDI scores at baseline are similar to findings of other studies that included current ecstasy users (Curran and Travill 1997; Falck, Wang et al. 2006; Lamers, Bechara et al. 2006; Falck, Jichuan et al. 2008). Ecstasy consumption was associated with an increase in activity levels, which was reflected by increased POMS Vigour scores in the present study, and similar observations were reported previously (Tancer and Johanson 2007).

Finally, an additional correlational analysis revealed several significant relationships between demographic and psychological parameters. Of interest is the association between a past or family history of depression with a higher lifetime amount of pills consumed, and a correlation of a younger age and earlier start of ecstasy use with higher BSI Depression dimension scores in the group with no predisposition to depression. No association between the past and family history of depression and previous ecstasy exposure parameters in the With a Predisposition group may be due to the restricted number of subjects who reported previous diagnosis of depression (n=2) or having a first-degree relative with MDD (n=1). Moreover, correlations between the POMS and BDI scores in the groups with and without a predisposition to depression depict concordance in the accuracy of assessment of mood and depressive symptoms, when these two scales are used in the same study. Additionally, the significant correlation between the BDI and TMD scores at the party session in the With a Predisposition group may indirectly describe the presence of the antidepressant-like potential of MDMA: although improvement of mood in subjects occurred at a social gathering irrespective of their choice of ecstasy consumption,

there was a tendency for depressive symptoms to decrease after pill consumption. This may indirectly illustrate the antidepressant-like potential of MDMA in subjects with a predisposition to depression. In contrast, no changes in the BDI scores between the baseline and party assessments were observed in the group without a predisposition to depression.

Furthermore, the association between higher ecstasy exposure with lower mood disturbance and depressive scores during the party assessment may explain certain aspects of patterns of ecstasy use, namely, subjects with more positive immediate effects of the drug are more likely to consume ecstasy at higher rates than those who don't experience such a positive feedback following pill ingestion. The influence of ecstasy exposure on the changes in psychological parameters is discussed in more detail in Chapter 7.

The correlation between baseline POMS and TMD scores in both predisposition groups are used in the next chapter, which describes another approach to the evaluation of a predisposition to depression in ecstasy users.

In sum, this study showed limited evidence of antidepressant-like potential of MDMA, which was observed as a trend in subjects with a predisposition to depression. As hypothesised, individuals with an increased susceptibility to depression reported higher distress in mood and other emotional spheres than subjects with no increased risk of depression development when they were not under the influence of drugs. Finally, although the ecstasy exposure parameters did not differ between groups, ecstasy users with greater exposure had less mood disturbance following ecstasy consumption, which may explain their proneness to more intense drug use. The

findings may suggest some support for the self-medication hypothesis of ecstasy use and a predisposition to depression.

# Chapter 6. Effects of MDMA on serotonergic function in users with and without a predisposition to depression

## 6.1. Introduction

Dysfunction in the serotonergic system has been extensively associated with the development of depressive symptoms (Meltzer 1989; Owens and Nemeroff 1994; Dhaenen 2001; Middlemiss, Price et al. 2002). The serotonergic neurotransmitter system, especially the functioning of the 5-HT transporters, is also a primary target for MDMA action (White, Obradovic et al. 1996; Parrott 2001). As ecstasy use has been associated with disturbance in the functioning of the serotonergic system, it may be that if there is already any pre-existing impairment in this neurotransmitter system, ecstasy use may result in greater negative consequences. On the other hand, the association of depression with depleted 5-HT levels in the intrasynaptic space and reciprocal MDMA action resulting in a significant influx of 5-HT from the nerve terminals may possibly explain the more positive immediate effects of ecstasy in users with a pre-existing serotonergic dysfunction (Croft, Klugman et al. 2001). This part of the study was aimed to investigate whether there are any differences in serotonergic system functioning in ecstasy users with and without a predisposition to depression as another background parameter of susceptibility to mood disorders. If present, the differences may explain some differences in patterns of ecstasy use.

There are certain limitations in the assessment of neurotransmitter functioning in the human brain. Investigation of serotonergic system functioning in clinical research can

be performed in different ways. In preclinical studies, the range of methods is greater than in clinical studies. The methods include measuring 5-HT uptake in various brain regions, receptor and 5-HTT binding, and measurement of compound concentrations in brain samples, as well as microdialysis techniques that allow the measurement of exact levels of 5-HT and 5-HIAA *in vivo*. Due to certain limitations, examination of serotonergic system functioning in humans is less readily available. Platelets are the main 5-HT storage site in the periphery, and have been previously proposed to represent a model of neurons (Da Prada, Cesura et al. 1988). Measurement of the 5-HTT functioning in platelets correlates, to a certain extent, with that in the brain and thus may provide indirect evidence of serotonergic neurotransmission (Rausch, Johnson et al. 2005). Another indirect, but more invasive method is the measurement of 5-HT and 5-HIAA levels in CSF (Engbaek and Voldby 1982). Decreased levels of 5-HIAA were found in long-term ecstasy users (McCann, Ridenour et al. 1994). However, one of the more direct measurements of 5-HT function in the human brain can be done with neuroimaging studies, including 'real-time' functional imaging (McCann, Szabo et al. 1998; Reneman, de Win et al. 2006; Cowan 2007; de Win, Jager et al. 2008; McCann, Szabo et al. 2008). Unfortunately, the inclusion of neuroimaging studies in the protocol has some limitations: the equipment may not readily available, such studies are associated with significant costs, and there are certain technical aspects, such as ligand preparation, that are complex and time-consuming. Finally, the imaging requires a separate series of inclusion criteria that must be met in order to enrol a participant in the study.

There is growing evidence that certain genetic factors may underlie the increased susceptibility to the development of depressive symptoms (Lesch 2004) (also see Section 1.6.4 of Chapter 1). A family history of depression has been linked with a greater risk of developing depression (Roy 1987; Sullivan, Wells et al. 1996; Lieb, Isensee et al. 2002). The 5-HTT activity plays an important role in the pathogenesis of depression, as well as in its treatment. Similarly to SSRIs, MDMA inhibits the activity of the 5-HT reuptake transporter. It was identified that a 5-HTT gene polymorphism may be linked with an increased vulnerability to affective disorders, including depression (Ogilvie, Battersby et al. 1996; Furlong, Ho et al. 1998; Mandelli, Serretti et al. 2007). One of the sites of polymorphism is a variable-number-tandem-repeat (VNTR) in the second intron of the gene coding 5-HTT (Ogilvie, Battersby et al. 1996). The presence of 9 or 12 copies of the repeat is associated with an increased risk of developing depression and with decreased expression of 5-HTT (Battersby, Ogilvie et al. 1996; Ogilvie, Battersby et al. 1996; Bah, Lindstrom et al. 2008). Functional polymorphism of the serotonin transporter linked polymorphic region (5-HTTLPR) in the promoter produces a 'short' (S) and a 'long' (L) allele (Lesch, Bengel et al. 1996). It has been reported that carriers of the short allele are at increased risk of development of mood disorders (Collier, Stober et al. 1996; Gutierrez, Pintor et al. 1998), including in ecstasy users (Martin-Santos, Torrens et al. 2009). It has been previously found that ecstasy users who carry the short allele have impaired emotional processing (Roiser, Cook et al. 2005) and cognitive functioning (Roiser, Rogers et al. 2006).

The present study was aimed to explore functioning of the serotonergic system in ecstasy users with and without a predisposition to depression, using analysis of

peripheral 5-HT uptake rates and 5-HT and 5-HIAA concentrations, as well as genetic predisposition via determination of the 5-HTT gene polymorphism. Additionally, the possible role of a genetic predisposition to mood disorders in the ecstasy use patterns was explored using a correlational analysis.

## 6.2. Subjects and methods

### 6.2.1. Subjects

The subjects' characteristics are described in detail in Section 5.2 of Chapter 5. In brief, 40 current ecstasy users with and without a predisposition to depression were assessed at the baseline session, when they were drug-free for at least 7 days, and at a party session, when participants attended a social gathering. Blood samples for the 5-HT uptake, 5-HT and 5-HIAA levels in platelet-rich plasma and 5-HTT polymorphism were collected from all participants at the baseline section.

### 6.2.2. Measurement of 5-HT and 5-HIAA levels in platelet-rich plasma (PRP)

PRP samples were prepared by centrifuging whole blood samples (collected into 9 ml Vacuette® purple top tubes containing $K_3$EDTA) at 200g for 20 min using an Eppendorf centrifuge 5810 (Crown Scientific). The resulting plasma was transferred into 5 ml plastic tubes and platelet-poor plasma (PPP) was obtained by spinning the samples at 1800g for 10 min. The plasma samples were stored at -70$^0$C until further analysis.

Plasma samples were thawed before the HPLC analysis. Preparation of the plasma samples was based on methods used previously in the analysis of 5-HT and 5-HIAA levels in cortex in laboratory animals (Callaghan, Farrand et al. 2006). In brief, 400 µl of 1M PCA and 40 µl of NMe5-HT were added to 100 µl of plasma, and centrifuged twice

at $4^0$C at 9000g for 10 min each time in a Sigma 4K15 centrifuge. The resulting supernatant was transferred into eppendorf tubes. The extraction efficiency was 89.7%. 40 µl of each sample were injected in the HPLC system. For the analysis, a column Alltima HP C18 3µ 100x2.1 mm (Alltech Associates Inc, NSW, Australia) was used, with a working electrode potential of 0.7V, the flow rate was set for 0.12 ml/min, range 20 nA and temperature $26^0$C. The retention time for 5-HIAA was 9.3 min, 5-HT 11.1 min and NMe5-HT 12.5 min. The mobile phase consisted of $NaH_2PO_4$ 102.9 mM, octanesulphonic acid (OSA) 0.5 mM, EDTA 0.1 mM, methanol 12.5% and pH adjusted to 3.8. This mobile phase was used in analysis of 5-HT and 5-HIAA levels in previous studies in our laboratory (Callaghan, Farrand et al. 2006; Jaehne, Salem et al. 2008; Jaehne, Majumder et al. 2010).

### 6.2.3. Measurement of 5-HT uptake in platelet-rich plasma

5-HT uptake was done on fresh PRP samples, which were prepared as described in Section 6.2.1. The protocol for the 5-HT uptake was based on previous work at the department of Pharmacology (Sluggett 2001). 50 µl of plasma were added to 400 µl of 0.1M phosphate buffered saline (PBS) and then 50 µl of $^{14}$C-5-HT in the concentrations from 10 to 50 µM (hydroxytryptamine binoxalate, 5-[$2^{-14}$C]-(serotonin), stock 250 µCi (9.25 Mbq), 4.82 mCi/mmol, Perkin Elmer, USA). The samples were allowed to sit on a bench for 5 minutes. The uptake was stopped by filtration of the samples. Filtered paper was then transferred into scintillation vials, and 5 ml of CytoScint Scintillation Cocktail was added to each vial. The scintillation count was determined in an LS3801 scintillation counter (Beckman, USA).

### 6.2.4. Determination of the 5-HT transporter gene polymorphism

Whole blood samples for genotyping were collected at the baseline session in 4 ml Vacuette tubes and stored at -70$^0$C until further analysis.

Genomic desoxyribonuceic acid (DNA) was extracted from whole blood samples using Quiagen® QIAmp DNA Mini kits (QIAGEN Pty Ltd, VIC, Australia). DNA concentrations in prepared samples were confirmed using a UV-1601 spectrophotometer (Shimadzu).

Primers for assays for the VNTR and functional (insertion/deletion) polymorphism of the promoter region of the gene coding 5-HTT were supplied from GeneWorks (SA, Australia). Both assays were optimised and validated by Mr Daniel Barratt (Department of Pharmacology, The University of Adelaide). Additionally, 40-50% of samples were analysed independently by Mr Daniel Barratt and subsequently compared with the results of the study to ensure validity of the assay.

#### *6.2.4.1. 5-HTTLPR (Insertion/deletion) assay*

The genotyping protocol used an assay previously described (Roiser, Cook et al. 2005). In brief, the genotypes were detected using a polymerase-chain reaction technique (PCR) (MJ Research PTC-200 Peltier Thermal Cycler). All DNA samples were diluted in autoclaved distilled water to a final concentration of 20 ng/µl. PCR reactions for the insertion/deletion assay were carried out in 0.2ml PCR tubes using the following protocol: 30µl total volumes were prepared from 100 ng of genomic DNA, 10x PCR buffer (NEB, Gene Search, Australia), 2.5 mM dNTP (Finnzymes, Genesearch, Australia), 1mM 7-deaza-dGTP (BD Life Sciences, Australia), STPR 5 and 3 (reverse and forward primers) (GeneWorks, SA, Australia), and TAQ polymerase (NEB, GeneSearch, Australia).

Sequences of the samples with LL, LS and SS genotypes were confirmed at the Institute of Medical and Veterinary Science (IMVS) Sequencing Centre and these samples were used as controls.

The PCR reactions consisted of the following sequence of cycles: 1 cycle at $94^0$C for 5 min, 45 cycles of 30s at $94^0$C, 30s at $63^0$C and 1.5 min at $72^0$C, and 1 cycle for 10 min at $72^0$C, with subsequent soak at $4^0$C. A blank DNA-free water sample and 3 control samples were included in each of the runs.

#### *6.2.4.2. VNTR assay*

In this study a previously described assay for the VNTR genotyping was used (Ogilvie, Battersby et al. 1996). The same protocol was used as for the 5-HTTLPR assay, and the optimised assay conditions were as follows: 10x PCR buffer (NEB, Gene Search, Australia), 2.5 mM dNTP (Finnzymes, Genesearch, Australia), 10µM 8823 and 10µM 8824 primers (reverse and forward primers) (GeneWorks, SA, Australia), Taq DNA polymerase (5U/µl) (NEB, GeneSearch, Australia) and autoclaved water were added to 5 µl of 20ng/µl genomic DNA samples in 0.2 thin-walled PCR tubes.

The PCR cycle program consisted of 1 cycle at $94^0$C for 5 min, 35 cycles of 30s at $94^0$C, 30s at $60^0$C and 1 min at $72^0$C, and 1 cycle for 10 min at $72^0$C, with a subsequent soak at $4^0$C.

PCR products of both assays were visualised using a low melting 2% Omnigel-Sieve (Adelab Scientific, Thebarton, Australia) agarose in 1x TBE buffer containing ethidium bromide, with a 50 base pair marker (NEB, GeneSearch, Australia) used for reference.

### 6.2.5. Statistical analysis

5-HT and 5-HIAA levels in PRP and levels of 5-HT uptake rate at each of the $^{14}$C-5-HT concentrations were compared between groups using unpaired two-tailed Student's t-tests. The distribution of allele and genotype frequencies in groups was determined using Pearson's $\chi^2$-test. Relationship between the genotypes, the results of psychological assessment and demographic parameters was done using Pearson's correlation coefficient. All data are represented as mean±SEM or percentages. Differences with $p<0.05$ were considered statistically significant. All results were analysed using the Microfost Excel, GraphPad Prism and SPSS software.

## 6.3. Results

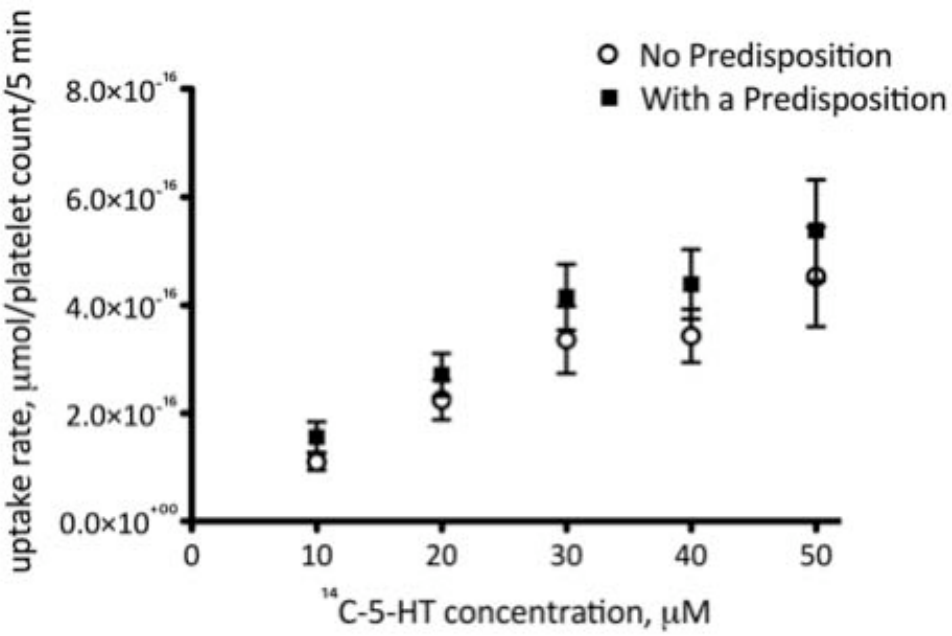
### 6.3.1. 5-HT and 5-HIAA levels in platelet-rich plasma

There were no differences in the levels of 5-HT and 5-HIAA in PRP. 5-HT wasn't detected in PPP. No difference in the levels of 5-HIAA between the No Predisposition and With a Predisposition groups was observed. Mean concentrations of 5-HT in PRP of subjects with and without a predisposition to depression were 802.6±86.03 and 770.5±93.44 ng/ml, respectively ($p=0.80$). Mean concentrations of 5-HIAA in PRP of subjects with and without a predisposition to depression were 156.8±7.72 and 157.4±12.7 ng/ml, respectively ($p=0.97$).

### 6.3.2. 5-HT uptake in platelet-rich plasma

The rate of 5-HT uptake in PRP was similar in samples from subjects with and without a predisposition to depression (Fig. 6.1). No differences in uptake rate were found between the groups at any added $^{14}$C-5-HT concentrations ($p=0.16$-$0.54$).

***Figure 6.1. The 5-HT uptake in platelets in subjects with and without a predisposition to depression***




Data represent mean±SEM (n=20 in each group).
Data were analysed using unpaired two-tailed Student's t-tests at each of the $^{14}$C-5-HT concentrations.

### 6.3.3. Genotyping

#### *6.3.3.1. 5-HTTLPR polymorphism*

The distribution of genotypes in groups with and without a predisposition to depression was as follows: 7 (35%) of the subjects without a predisposition to depression had an LL genotype, 11 (55%) were heterozygous (LS genotype), and 2

(10%) carried both short alleles. In the group with a predisposition to depression, 11 (55%) were heterozygous, 5 (25%) carried two long alleles, and 4 (20%) had an SS genotype. There was no statistically significant difference in the distribution of genotypes ($\chi^2(2)=1$, $p=0.61$) and alleles ($\chi^2(1)=0.82$, $p=0.37$) between groups. The distribution of genotype frequencies in both groups did not differ from the expected frequencies based on Hardy-Weinberg assumptions ($\chi^2(2)=0.21$, $p=0.90$).

#### *6.3.3.2. VNTR polymorphism*

The genotypes were distributed as follows: in the group without a predisposition to depression, 25% of participants had a 10/10 genotype, 40% were heterozygous (12/10 genotype), 50% had a 12/12 genotype. One subject with a predisposition to depression had a 9/12 genotype, 10% of the subjects predisposed to depression had a 10/10 genotype, 40% were heterozygous and 50% were homozygous with 12 repeats. There was no statistically significant difference in the genotype and allele frequencies between the groups (genotypes: $\chi^2(3)=2.51$, $p=0.47$, alleles $\chi^2(2)=1.58$, $p=0.46$). The observed distribution of frequencies didn't violate the Hardy-Weinberg assumption ($\chi^2(3)=0.1$, $p=0.99$).

#### *6.3.3.3. Correlational analysis*

A correlational analysis was done to determine whether there is a possible contribution of genotypes to the effects of ecstasy on mood and depressive symptoms in subjects with and without a predisposition to depression.

In the group with no predisposition to depression, the correlational analysis didn't reveal any effect of 5-HTT gene polymorphisms on any of the demographic or ecstasy

exposure parameters (r=-0.20-0.380, p=0.934-0.099). The association of carrying the short allele with higher BSI Depression Dimension scores didn't reach a statistically significant level (r=0.380, p=0.099). In contrast, in the group with a predisposition to depression, the 5-HTTLPR polymorphism was significantly associated with the BSI Depression Dimension scores, and subjects who carried the short allele reported lower mood distress (r=-.503, p=0.024). Other correlations were non-significant (r=0.14- -0.411, p=0.954-0.072).

The relationship between the genotype variants and changes in BDI and POMS TMD scores was analysed in the ecstasy group using a partial correlation controlling for the baseline scores. Changes in BDI scores significantly correlated with baseline scores in both groups (No Predisposition r=-0.791, p=0.006, With a Predisposition r=-0.866, p=0.001), however, scores at the party session significantly contributed to the changes in BDI scores only in the ecstasy group with a predisposition to depression (No Predisposition r=0.450, p=0.191, With a Predisposition r=0.657, p=0.039).

In the ecstasy group without a predisposition to depression, greater changes in BDI scores at the party assessment were associated with carrying 12 copies of VNTR (r=0.702, p=0.035), but not with the SS 5-HTTLPR genotype (r=0.21, p=0.588). In the group with a predisposition to depression, a strong correlation between the 5-HTTLPR polymorphism and changes in BDI scores was observed (r=0.702, p=0.035), whereas no association between the VNTR polymorphism and BDI scores was detected (r=0.191, p=0.622). The 5-HTTLPR polymorphism was significantly associated with higher BDI scores during the party assessment in the group with a predisposition to depression (r=0.702, p=0.035), whereas the VNTR polymorphism contributed to higher scores at

the party in the group not predisposed to depression (r=0.702, p=0.035). Thus, carrying a short allele or 12 repeats of the VNTR was associated with a greater change in BDI scores and a higher intensity of depressive symptoms even after pill consumption. Neither of the 5-HTT gene polymorphisms had any effect on the changes in POMS TMD scores (r=0.147-0.466, p=0.206-0.706).

## 6.4. Discussion

In the present study, no differences in peripheral serotonergic system functioning parameters were observed between the groups. However, the functioning of the peripheral serotonergic system is not an ideal model for determining changes in the serotonergic system in brain. Functional neuroimaging studies allow direct assessment of the 5-HT system in CNS. However, due to certain limitations, this method was not applicable in the present project.

The levels of 5-HT in PRP and the rate of the 5-HT uptake observed in this study were comparable with the levels in healthy subjects (Vatassery, Sheridan et al. 1981; Modai, Zemishlany et al. 1984; Sarrias, Artigas et al. 1987). No differences between the groups with and without a predisposition to depression were found, perhaps due to the fact that none of the participants had a diagnosed MDD at the time of the study. Indeed, current and recent treatment with antidepressants was one of the exclusion criteria. Moreover, as blood samples were collected at baseline, when participants were drug-free for at least 7 days prior to the assessment, the effect of any substances, including amphetamine-type compounds, was highly unlikely. Finally, all subjects were asked to fast overnight before the baseline assessment, to ensure that no compounds, such as Trp, that are present in foods interfered with the 5-HT measurements.

Certain variations in the gene coding of 5-HTT led to decreased expression of the transporter, which, in turn, can predispose individuals to mood disorders (Battersby, Ogilvie et al. 1996; Ogilvie, Battersby et al. 1996; Bah, Lindstrom et al. 2008). Moreover, as MDMA primarily affects the 5-HT transporter system, decreased availability of 5-HTT may be also linked with increased susceptibility to negative effects of MDMA on mood. In this study, the assessment of two variants of the 5-HTT gene polymorphism was done partly as another measurement of predisposition to depression. No difference between the groups was found in the distribution of the genotypes. As the cohort consisted of only 40 subjects, any differences between the groups were unlikely to reach a statistically significant level. The inclusion of a greater number of participants would probably assist in investigating the distribution of genotypes in cohorts of users with and without a predisposition to depression. The genetic background of ecstasy users was not, however, the principal area of interest in the present study.

A functional polymorphism of the promoter region of the gene coding 5-HTT was linked with altered emotional processing in ecstasy users (Roiser, Cook et al. 2005). In this study all participants were current ecstasy users, and SS and LS genotypes were associated with higher BDI scores. The assessments were completed when the subjects were not under the influence of MDMA. In the present study, it has been observed that carrying the short allele did not significantly affect distress in the Depression Dimension in users, whose cumulative scores were lower than a clinically significant cut-off level (T-score of 63). However, users with a predisposition to depression according to their performance on the BSI were more likely to have lower scores,

which were closer to the cut-off level, if they carried the short allele. This may indicate that the 5-HTTLPR polymorphism is not a principal marker of the intensity of mood disturbance in ecstasy users. Nevertheless, due to a small sample size, the interpretation of results should be done with consideration of the impact of Type II error.

Assessment of the VNTR polymorphism has been previously performed as a measure of susceptibility to affective disorders, including unipolar depression (Ogilvie, Battersby et al. 1996; Furlong, Ho et al. 1998). However, it has not been determined specifically in current ecstasy users. The distribution of genotypes between groups was similar, most likely due to a restricted number of subjects. A single rare genotype with 9 repeats was obtained in the group with a predisposition to depression, and the distribution of alleles and genotypes met the Hardy-Weinberg assumption. No influence of the VNTR polymorphism was noted for any background parameters.

The results of this study showed that the genotype variants, which were previously associated with altered emotional processing and increased risk of the development of depression, were linked with more prominent depressive symptoms in ecstasy users even if they chose to consume a pill at a social gathering. In particular, in the ecstasy group without a predisposition to depression, carriers of 12 copies of the VNTR reported higher BDI scores at the social gathering, whereas carrying the short allele of the 5-HTTLPR polymorphism was associated with higher BDI scores at the party session in the ecstasy group with a predisposition to depression. As these variants of both 5-HTT gene polymorphisms have been previously associated with lower expression of 5-HTT (Bah, Lindstrom et al. 2008), observed findings may indicate that a lower

availability of 5-HTT sites may lead to a less prominent antidepressant-like effect of MDMA following its ingestion. Further research on the density of 5-HTT sites in ecstasy users with different genotypes may provide more evidence of the contribution of genetic background to the intensity of an antidepressant-like activity of MDMA in users with different vulnerability to mood disorders.

The present study did not include an ecstasy-naïve control group. In the study of Roiser et al (2005), the BDI scores reported by ecstasy users were compared with cannabis users and healthy drug-naïve controls. Association of the SS genotype with higher BDI scores was found in the ecstasy group, but not in the comparison group (Roiser, Cook et al. 2005). However, the present study provides further basis for the association between the SS genotype and higher depressive scores in ecstasy users and it was first to demonstrate such a link between genetic background and immediate effects of MDMA on mood. In addition, this study was first to include the VNTR polymorphism as another possible contributor to susceptibility to mood disorders in ecstasy users.

In sum, the assessment of peripheral 5-HT system functioning may not be the most appropriate and sensitive measure of a predisposition to depression in ecstasy users. A larger scale study on the impact of the 5-HTTLPR and VNTR gene polymorphisms in ecstasy users with inclusion of an ecstasy-naïve group may result in a more significant difference between groups of interest, and, thus, provide evidence for a genetic basis of the role of a predisposition to depression in mood changes due to MDMA use.

# Chapter 7. Effects of MDMA on mood in ecstasy users based on their previous exposure to the drug

## 7.1. Introduction

Repeated ecstasy use may lead to long-term psychopathological impairment in various spheres, including mood, memory and cognitive performance. Patterns of MDMA use, in particular, the frequency of use and the average and total number of pills consumed may impact the risks of MDMA-related long-term side effects. Indeed, in some studies, the most prominent long-term changes in various psychological spheres were observed in heavy ecstasy users (Parrott, Buchanan et al. 2002; Fisk, Montgomery et al. 2009; Schilt, Koeter et al. 2009). This may be due to the repeated over-stimulation of the serotonergic system by MDMA, and a larger number of tablets consumed over the person's lifetime, along with a more frequent use may underlie the psychopathological changes in long-term ecstasy users. However, the role of the effects of MDMA alone on long-term impairment in various psychological spheres has been questioned. Most individuals who consume ecstasy also use other drugs, and many authors have attributed long-term psychopathological features to polydrug use, rather than to exclusively to ecstasy use (Gouzoulis-Mayfrank and Daumann 2006; Medina and Shear 2007; Krebs, Johansen et al. 2009).

Differences in the patterns of ecstasy use may be attributable to the intensity of the positive and negative effects of MDMA. Subjects who experience more positive feedback from ecstasy consumption and less subsequent negative effects on mood,

memory, sleep and cognition, are more likely to use the drug more frequently. On the other hand, more frequent ecstasy use has been linked with the development of tolerance to the positive effects on mood and self-esteem (Parrott 2005). To overcome such diminished effects, users are more likely to consume more tablets per occasion, which, in turn, may be associated with increased risk of long-term deficits.

The present study included participants which had a diverse exposure to ecstasy, ranging from 4 to 1268 pills consumed in their lifetime. The following part of the study aimed to investigate one aspect of the contribution of previous ecstasy use to the drug's effects, namely, whether the grade of ecstasy exposure based on the total amount of pills consumed over the person's lifetime leads to different changes in mood and depressive symptoms in current ecstasy users, when they are under the influence of the drug.

## 7.2. Subjects and methods

### 7.2.1. Subjects

The same cohort of participants who participated in the study described in Chapter 5, was included in the present analysis. For this analysis, subjects were divided into two groups, with less and more ecstasy exposure, respectively, according to the median lifetime use of MDMA of 37.5 pills overall.

### 7.2.2. Analysis protocol

All data collected previously, including demographic characteristics, previous and current use of ecstasy and other drugs, were compared between the groups. The protocol of the study, as described in Chapter 5, included assessment at baseline,

when participants were drug-free for at least 7 days prior, and a party session, when participants attended a social gathering. At the baseline session, participants completed a modified BSI form as a measure of distress in various psychological spheres over a period of the previous 5 years. During both sessions, participants were asked to fill out the POMS and a modified BDI-II form based on how they were feeling at the moment of the assessment.

For the analysis of POMS and BDI scores, participants were divided into 2 additional groups in each of the exposure groups, according to their choice to consume a pill at the party session. As a result, 12 control subjects and 8 subjects who chose to take a pill during the party assessment were assigned to a 'less ecstasy exposure' group, whereas in the 'more ecstasy exposure' group there were 8 control subjects and 12 subjects in the ecstasy group. The party assessment occurred 1 hour after pill consumption in the ecstasy group, or at a matched time with the ecstasy group for those individuals who abstained from using ecstasy at the social gatherings.

### 7.2.3. Statistical analysis

Demographic data, past and current drug use, distribution of genotypes, and psychiatric history were compared between the groups using the chi-square test. The differences in BDI and POMS scores between the party and baseline sessions were analysed using a two-way ANOVA with Bonferroni post-hoc tests, and ecstasy exposure and time of assessment as factors. BSI scores and ecstasy exposure parameters were compared between the groups using unpaired two-tailed Student's t-tests. All data are represented as mean±SEM or percentage values. Results were

considered significantly different at $p<0.05$. All data were analysed using Microsoft Excel, GraphPad Prism and SPSS software.

## 7.3. Results

### 7.3.1. Demographic data

The age of participants with more ecstasy exposure was significantly greater, compared with the group with less exposure (23.90±1.27y vs. 20.55±0.54y, $p<0.05$). The rest of the demographic parameters did not differ between the groups (Figure 7.1). Moreover, no differences in the distribution of genotypes between the groups was observed (VNTR $\chi^2(3)=2.32$, $p=0.51$, 5-HTTLPR $\chi^2(3)=2.18$, $p=0.336$). A significantly greater number of participants with more exposure to ecstasy reported using methamphetamine ($\chi^2(1)=10.42$, $p=0.001$), cocaine ($\chi^2(1)=5.58$, $p=0.018$) and ketamine ($\chi^2(1)=4.33$, $p=0.037$) sometime in their lifetime. The groups were similar in lifetime and current use of other drugs.

The age of first ecstasy use was similar in both groups (less exposure 18.75±0.428y and more exposure 18.30±0.576y, $p=0.643$). Apart from the total number of pills consumed, as anticipated, subjects with the greater ecstasy exposure used the drug over a longer period of time (5.20±1.23y vs. 1.84±0.518y, $p=0.024$), and consumed more pills per occasion (2.63±0.37 pills vs. 1.5±0.15 pills, $p=0.01$) and reported more frequent use of ecstasy (21.58±2.87 occasions per year vs. 8.65±1.59 occasions per year, $p=0.003$) than individuals with less exposure. The mean number of tablets consumed in their lifetime was 16.55±2.13 for the group with less exposure and 258.7±70.50 for the group with more exposure ($p<0.001$).

***Table 7.1. Demographic data distribution between groups with different ecstasy exposure***

| | | Less exposure (n=20) | More exposure (n=20) |
|---|---|---|---|
| **Age (years)** | | 20.55±0.54 | 23.90±1.27* |
| **Sex** | Males | 60% | 45% |
| | Females | 40% | 55% |
| **Ethnicity** | Caucasian | 95% | 95% |
| | Other | 5% (Sri Lankan) | 5% (Middle Eastern) |
| **Education** | Year 10 | 0% | 5% |
| | Year 11 | 5% | 10% |
| | Year 12 | 80% | 65% |
| | TAFE | 10% | 15% |
| | University | 5% | 5% |
| **Employment/ Occupation** | Student | 65% | 45% |
| | Casual | 0% | 15% |
| | Part-time | 15% | 20% |
| | Full-time | 0% | 15% |
| | Unemployed | 20% | 5% |
| **History of mood disorders** | Past history of depression | 5% | 5% |
| | Family history of depression | 25% | 5% |
| | Past history of PTSD | 0% (5%)[2] | 0% |
| | Past treatment with antidepressants | 0% | 5% (social phobia) |

Age is represented as mean±SEM (n=20 in each group). Data were analysed using unpaired two-tailed Student's t-tests and the chi-square test.
* Indicates significant difference $p<0.05$.

[2] One subject self-reported an episode of PTSD, however, it was not diagnosed by a medical practitioner and no treatment was prescribed.

During the social gatherings, subjects with less previous exposure consumed 1.3±0.21 tablets on average, whereas participants with more exposure in the ecstasy group reported taking 1.8±0.32 pills (p=0.21).

### 7.3.2. Psychological tests

#### *7.3.2.1. Brief Symptom Inventory*

There was no difference in mean scores for any of the BSI scales, as well as indices, between the two groups at baseline (Table 7.2).

***Table 7.2. BSI T-scores reported by ecstasy users with different exposure to the drug***

| Symptom dimensions and indices | Less exposure (n=20) | More exposure (n=20) | P value |
|---|---|---|---|
| Somatisation (SOM) | 58.94 ± 1.952 | 77.11 ± 17.65 | 0.31 |
| Obsessive-Compulsive (O-C) | 64.66 ± 2.72 | 64.14 ± 2.373 | 0.89 |
| Interpersonal Sensitivity (I-S) | 63.95 ± 1.941 | 58.75 ± 2.446 | 0.10 |
| Depression (DEP) | 64.05 ± 2.16 | 61.90 ± 2.308 | 0.50 |
| Anxiety (ANX) | 60.21 ± 2.134 | 58.09 ± 2.505 | 0.52 |
| Hostility (HOS) | 60.25 ± 2.422 | 63.40 ± 2.224 | 0.34 |
| Phobic Anxiety (PHOB) | 60.56 ± 2.278 | 57.28 ± 2.539 | 0.34 |
| Paranoid Ideation (PAR) | 60.38 ± 2.219 | 58.78 ± 2.499 | 0.63 |
| Psychoticism (PSY) | 62.66 ± 2.631 | 60.35 ± 2.190 | 0.50 |
| Global Severity Index (GSI) | 50.31 ± 1.293 | 49.01 ± 1.550 | 0.52 |
| Positive Symptom Total (PST) | 67.00 ± 1.922 | 62.70 ± 2.326 | 0.16 |
| Positive Symptom Distress Index (PSDI) | 56.40 ± 1.688 | 56.63 ± 1.859 | 0.93 |

Data represent mean±SEM (n=20 in each group). Data were compared using unpaired two-tailed Student's t-tests for each dimension and index.

#### *7.3.2.2. Beck Depression Inventory*

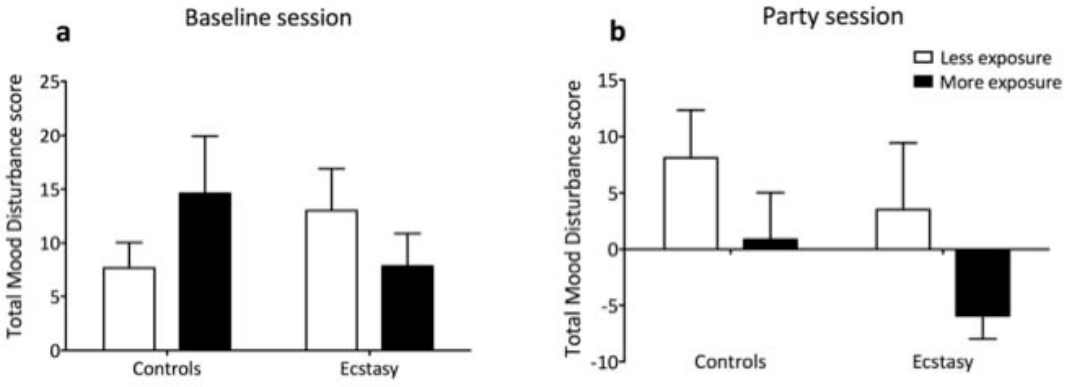
The was no evidence for an interaction effect between ecstasy exposure and drug group ($F(1,36)=0.33$, $p=0.57$), nor a significant main effect of previous ecstasy use ($F(1,36)=0.40$, $p=0.53$) or drug group ($F(1,36)=0.03$, $p=0.86$) on BDI scores. A large variance in responses contributed to the lack of statistical significance. In the control group, the mean BDI scores at baseline were 7.17±1.73 and 8.56±2.10 for users with less and more exposure, respectively; during the party session the scores for these subjects were 6.67±2.31 and 5.22±1.77, respectively. The users who chose to take a pill during the party assessment with a lesser and larger lifetime amount of pills consumed reported mean BDI scores at baseline of 9.5±3.65 and 7.55±1.41, respectively, and 7.38±1.83 and 5.55±1.15 at the party, respectively.

#### *7.3.2.3. Profile of Mood States*

The change in the mean TMD scores between the party and baseline sessions was identical in the ecstasy group: -13.75±3.73 for subjects with less previous exposure and -13.75±3.52 for those with more lifetime exposure. In the control group, the TMD scores changed at a significantly greater rate in users with more ecstasy exposure than in those with less pills consumed over their lifetime (-13.75±5.00 vs. 0.42±3.73, $p<0.05$). There was no significant interaction between ecstasy exposure and drug group on the difference in TMD scores in both groups ($F(1,36)=3.55$, $p=0.0675$), which may be due to a large variance in responses. However, a trend towards a more pronounced difference between baseline and the social gathering was observed in the control and ecstasy groups with more previous exposure, as well as in the ecstasy group with less previous exposure, whereas control participants in the group with less

exposured had a tendency to report similar POMS mood disturbance scores at the party session as at baseline. At baseline, TMD scores were reported as follows: in the control group they were 7.67±2.37 and 14.63±5.33 for users with less and more exposure, respectively; in the ecstasy group the scores were 13±5.33 and 7.83±3.05 for users with less and more exposure to ecstasy, respectively (Fig 7.1). At the party session TMD scores in the control group were 8.08±4.25 and 0.88±4.16 for users with less and more exposure, respectively, and in the ecstasy group, subjects with a lesser number of pills over lifetime had TMD scores of -0.75±4.16 on average, and those with more lifetime exposure -5.92±2.04.

***Figure 7.1. Profile of Mood States Total Mood Disturbance scores in the groups with different ecstasy exposure***



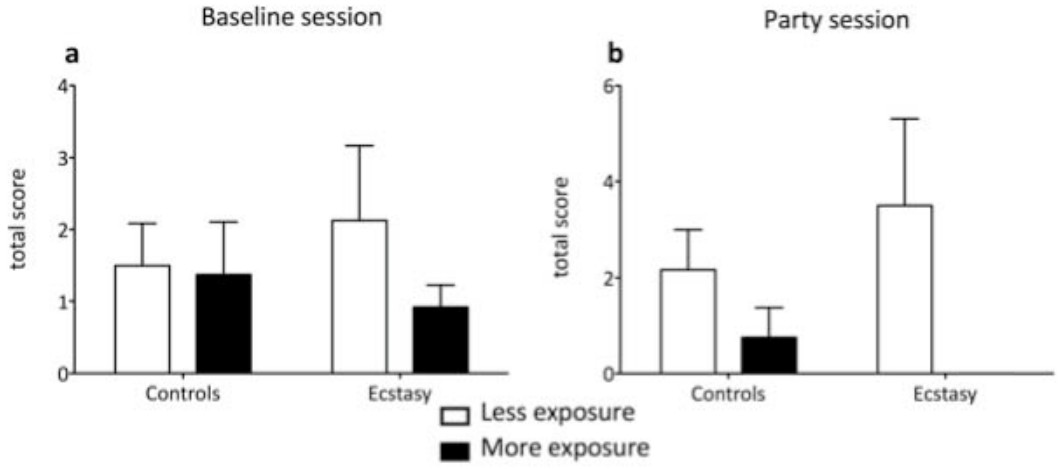

Profile of Mood States Total Mood Disturbance scores at the baseline (a) and party (b) sessions.
Data represent mean±SEM (n=10 in each group). Changes in scores between the baseline and party sessions were analysed using a two-way ANOVA with Bonferroni post-hoc tests.

The results of the POMS test were further analysed for each of the scales. No significant interaction or main effects of ecstasy exposure and choice of pill consumption at the party session between groups were observed in Tension/Anxiety,

Vigour/Activity and Confusion/Bewilderment scales (p=0.33-0.71). However, independent from ecstasy exposure, the changes in Vigour scores in the ecstasy group were somewhat greater than in control group, however, the difference didn't reach statistical significance (F(1,36)=3.55, p=0.068).

No significant two-way interaction effect was observed in the remaining POMS scales (p=0.12-0.47). However, ecstasy exposure had a significant main effect on changes in Depression, Anger and Fatigue scores in both groups (Depression F(1,36)=6.79, p=0.013, Anger F(1,36)=5.74, p=0.02, Fatigue F(1,36)=4.45, p=0.04).

***Figure 7.2. Profile of Mood States Depression/Dejection scores in the groups with different ecstasy exposure***




Profile of Mood States Depression/Dejection scores at the baseline (a) and party (b) sessions.
Data represent mean±SEM (n=10 in each group). Changes in scores between the baseline and party sessions were analysed using a two-way ANOVA with Bonferroni post-hoc tests.

The assessment at the party session revealed that users in the ecstasy group with less exposure reported an increase in Depression scores, whereas a decrease in these

scores was detected in the ecstasy group with more exposure (less exposure 1.38±1,43 vs. more exposure -0.92±0.31, $p<0.05$). In fact, none of the users with more previous exposure who chose to consume a pill at a social gathering reported any feelings of low mood during the party assessment (Fig. 7.2b). The changes in the control group were similar in users with different ecstasy exposure (less exposure 0.67±0.38 vs. more exposure -0.63±0.56, $p>0.05$).

A pattern similar to the one observed for the Depression/Dejection scale was observed for the Anger/Hostility scores: control MDMA users with less exposure reported an increase in angry feelings during the party assessment, whereas control users with more ecstasy exposure reported feeling less angry at the time of social gathering than at baseline (less exposure 1.42±0.65 vs. more exposure -1.5±0.87, $p<0.05$). A similar decrease in Anger scores was observed in the ecstasy group (less exposure -0.38±0.96 vs. more exposure -1.0±0.55, $p>0.05$).

A significant effect of ecstasy exposure was present when assessing changes in the Fatigue scale scores. However, Bonferroni post-hoc tests didn't reveal any statistically significant difference between groups due to large variances (less exposure -0,4±1.0 vs. more exposure -2.95±1.04, $p>0.05$).

## 7.4. Discussion

The present analysis was done to investigate whether previous exposure to ecstasy differently affects mood in users when they are under the influence of the drug. The effects of heavy ecstasy use on mood and cognition have been mainly investigated in a long-term setting, when the contribution of previous ecstasy use to the prominence of

depressive symptoms and cognitive dysfunction in current and abstinent users has been explored (Parrott, Sisk et al. 2000; Fox, Parrott et al. 2001; Parrott, Buchanan et al. 2002; de Win, Reneman et al. 2004; Schilt, Koeter et al. 2009). The present study, however, aimed to explore the immediate effects of the drug on mood in users with different exposure to ecstasy.

Previous studies on the differences in psychological parameters as a consequence of ecstasy use distinguished heavy, moderate and novice or light users based on either a number of occasions of drug consumption (Parrott and Lasky 1998; Parrott, Buchanan et al. 2002; Scholey, Parrott et al. 2004), or cumulative dose of ecstasy (De Win, Jager et al. 2005; de Win, Reneman et al. 2007; Schilt, de Win et al. 2007; Sterk, Theall et al. 2007). As subjects, especially heavy users, are more likely to consume more than one pill per occasion (Parrott and Lasky 1998; Scholey, Parrott et al. 2004; Sterk, Theall et al. 2007), cumulative dose of ecstasy consumed in their lifetime was chosen in this study as a measurement of ecstasy exposure.

The groups were divided according to the median lifetime amount of pills consumed, which resulted in mean cumulative doses of 16.5 pills and 258.7 pills for the groups with less and more exposure, respectively. It has been reported previously that moderate ecstasy use can be considered less than 50 tablets consumed overall, whereas consumption of 50 or more pills is considered to be heavy ecstasy use (de Win, Reneman et al. 2004). In this study the median total number of ecstasy tablets consumed in lifetime was 37.5. As no other parameters of ecstasy use were taken into consideration when comparing the groups in the present study, subjects were

considered to have less and more previous exposure to the drug, rather than heavy or moderate use.

The results of the present analysis didn't reveal any differences in the demographic and genetic background between the groups. However, as one would anticipate, ecstasy users with more exposure were older than their counterparts with less exposure. The mean age of first ecstasy use was similar in both groups, but other parameters of ecstasy use were greater in the group with more exposure. Of note are the higher average doses per occasion reported by users with more exposure and more frequent use of ecstasy. It has been previously reported that heavy users were more likely to have used or tried stimulants and hallucinogenic drugs, including cocaine, amphetamine and magic mushrooms, in comparison to lighter users (Scholey, Parrott et al. 2004). Consistent with these findings, in this study, in comparison to users with less exposure, a greater proportion of the participants with more exposure reported having used or tried cocaine, methamphetamine or ketamine in their lifetime.

Participants with more exposure did not report higher distress in any psychological spheres in comparison to their counterparts with less exposure. This may indicate that an underlying psychopathology, even if present, is not likely to lead to consumption of a greater number of ecstasy tablets.

During the party assessment, participants consumed a similar amount of ecstasy. Analysis of the POMS test showed that subjects who consumed a greater amount of ecstasy pills in their lifetime were more likely to get more prominent positive effects of the drug during the social gathering. The mood disturbance decreased at a similar rate

in users with different exposure to ecstasy, if they chose to take a pill during a social gathering. However, the mean TMD score in the group with less exposure was still positive, indicating some rate of mood disturbance, whereas in the group with more exposure, it was negative, which reflects an improvement of mood. Notwithstanding that, the rate of change in TMD scores was identical in the ecstasy group between participants with less and more previous exposure. Interestingly, an improvement of mood was also observed in the group of subjects who consumed more pills previously, but abstained from ecstasy at the social gathering. Moreover, the consumption of ecstasy had a different effect on the intensity of sad and depressed feelings in users with different previous exposure. Notably, the results of the POMS Depression scale showed that ecstasy consumption during the party assessment caused a complete disappearance of depressive feelings in users who consumed more pills previously, whereas subjects with a lesser cumulative dose reported a slight increase in depressive symptoms compared to their baseline answers. However, BDI results in the chosen configuration did not reveal any differences between the depressive scores between the groups. Parrott and colleagues assessed mood and memory function in regular and novice users and observed a similar decrease in the intensity of feeling sad in both ecstasy groups (Parrott and Lasky 1998). However, in this study VAS were used to assess mood and were administered 2-8 hours after pill consumption. Finally, irrespective of the previous amount of tablets consumed, ecstasy consumption at the social gathering tended to decrease feelings of anger and fatigue and increase vigour.

In sum, in the present study, rate of changes in mood following ecstasy consumption was similar between users with a greater and lesser cumulative dose of the drug, when

they chose to consume a pill at the social gathering. Resulting TMD scores at the party session remained positive in the ecstasy group with less exposure, whereas in the group with more exposure they decreased to a negative level, suggesting mood improvement. The observed differences in mood improvement following ecstasy consumption were not due to an increased number of pills consumed at the social gathering by the individuals with more exposure. Ecstasy users who consumed more pills in their lifetime were more likely to report no depressive feelings following drug consumption, resulting in a decrease in POMS Depression scores compared with individuals with less exposure. The findings may explain the differences in patterns of ecstasy use between users with lower and greater previous exposure to the drug: subjects who experience more intense positive immediate effects of ecstasy on mood and self-esteem may be more likely to use the drug more frequently, resulting in a greater total number of pills consumed.

# Chapter 8. Conclusion

## 8.1. Summary of findings

This project was designed to investigate the antidepressant-like potential of ecstasy (MDMA). MDMA primarily affects the serotonergic neurotransmitter system, causing a rapid and considerable increase in the intrasynaptic levels of 5-HT, which may underlie a spectrum of its immediate positive effects on mood and self-esteem (Green, Mechan et al. 2003). The unique entactogenic properties of MDMA distinguish it from other amphetamine-type stimulants (Nichols 1986). Furthermore, some mechanisms of action of MDMA in the brain are similar to those of clinically prescribed antidepressants. SSRIs and other antidepressants cause an increase in neurotransmitter levels within the synaptic cleft, which may underlie their mood-improving properties (Vaswani, Linda et al. 2003). Thus, based on the pharmacological action of MDMA and the comparability of some neurochemical mechanisms with therapeutic antidepressants, antidepressant-like activity of MDMA was considered as probable.

Such activity of MDMA, akin to the efficacy of antidepressants in depressed patients, but not in healthy individuals, is likely to be more evident in subjects with depression or an increased risk of mood disorders. Indeed, a predisposition to depression may result in different patterns of MDMA use. This has been discussed in light of the so-called 'self-medication' hypothesis (Khantzian 1997), according to which subjects with a pre-existing mood disorder, serotonergic dysfunction or increased susceptibility to depression may find MDMA more appealing, as its consumption may cause more

enhanced positive effects than in users with no pre-existing disturbance (Croft, Klugman et al. 2001). This more prominent positive feedback of ecstasy may lead to more frequent use, which, in turn, may result in an increased risk of the development of long-term negative effects due to the neurotoxic potential of MDMA.

The present study was the first to demonstrate the antidepressant-like potential of MDMA on both preclinical and clinical levels. The results provide further insight into the unique effect of MDMA on mood. In a putative animal model of depression, MDMA had a prominent antidepressant-like effect shortly after administration. In users with a predisposition to depression limited evidence of such an effect of ecstasy was observed. In the animal study it was apparent in the behavioural FST after acute drug administration, and in the clinical study it was reproduced as a trend in the BDI scores ($p=0.053$), where subjects with a predisposition to depression in the ecstasy group reported a relative decrease in the intensity of depressive feelings at the time of assessment. A dose-dependent antidepressant-like effect of MDMA observed in the animal model of depression following acute drug administration was not due to general stimulant effect of the drug, as the behaviour of MDMA-treated animals differed from METH-treated animals in the FST, although both amphetamine derivatives caused a similar induction in locomotor activity in both rat strains. Moreover, the contrast in MDMA's effects on behaviour between a 'non-depressed' strain and an animal model of depression was neither due to the differences in the drug bioavailability, as the pharmacokinetic profile of MDMA was similar in both strains, neither as a result of changes in disposition altering concentration of MDA, the major active metabolite of MDMA in rats. The antidepressant-like effect of MDMA was diminished after repeated MDMA administration, possibly due to the development of

tolerance. The presence of anhedonic features in FSL rats was apparent in a modified sucrose preference test, in which animals in the animal model of depression showed greater preference for the 32% sucrose solution, with no effect of repeated MDMA administration. The chosen dosing regime didn't affect cortical levels of 5-HT and 5-HIAA in SD and FSL rats. All in all, the FSL rat strain can be considered as an appropriate animal model of depression to study the effects of MDMA and related psychostimulants on animal behaviour. Finally, the findings of the animal part of the project illustrated that the antidepressant-like activity of MDMA is markedly different from that of commonly prescribed antidepressants in onset of action. The observed effects of MDMA were most prominent after initial injection, in contrast with the efficacy of antidepressants to decrease the intensity of the depressive-like state after 2-3 weeks of continuous administration (Detke, Rickels et al. 1995; Cryan, Page et al. 2005).

The second part of the study focussed on the immediate effects of MDMA in users with and without a predisposition to depression. To date, this is the first study to address the role of predisposition to depression on the immediate effects of ecstasy on mood and self-esteem. It has been observed that, in comparison to subjects not predisposed to depression, users with a predisposition to depression reported higher depressive symptoms when they were not under the influence of the drug, but the average scores on the depressive scale didn't reach clinically significant levels (BDI-II scores of 13 and higher) in either group. Furthermore, individuals with a predisposition to depression reported higher general psychological distress in various spheres, which may indicate their increased vulnerability to psychopathology in general. Association of family and past history with a greater total number of ecstasy tablets consumed was

present in the group with no predisposition to depression, however, no differences in the patterns of ecstasy use were observed between the groups with and without a predisposition to depression. In this study, the genotype variants, which were previously identified as predisposing to depression, did not contribute to the patterns of drug use. Thus, no indication of more frequent ecstasy use by subjects with a predisposition to depression was observed in this study, which, however, may be due to large variance in the ecstasy use by participants in both groups.

When participants attended a social gathering, they reported improved mood irrespective of presence of their predisposition to depression and whether or not they consumed a pill. However, no changes in the levels of depressive symptoms were noted in the group without a predisposition to depression. In contrast, subjects with a predisposition to depression tended to report a relative decrease in the intensity of the depressive symptoms ($p=0.053$) only if they chose to consume a pill at a social gathering. Additionally, a contribution of a genetic background to the intensity of depressive symptoms in ecstasy users was found: participants who were carriers of allele variants which have been previously associated with increased susceptibility to mood disorders, were more likely to report higher BDI scores at the social gathering, even though they were under the influence of the drug. Therefore, genetically pre-determined vulnerability may contribute to the prominence of the antidepressant-like effect of MDMA and, consequently, may influence patterns of drug use. The observed findings provide further evidence of the role of possible underlying serotonergic dysfunction in MDMA use: a relative, although not quite significant decrease in the intensity of depressive symptoms is present in users with a predisposition to

depression, and this provides limited support for the antidepressant-like potential of the drug.

Finally, evidence of a contribution of more prominent positive effects of MDMA to more frequent ecstasy use was observed. Subjects with a greater number of ecstasy pills consumed in their lifetime were more likely to use the drug more frequently and consume more pills per occasion, as the drug caused a decrease in depression symptoms, when consumed at a social gathering. The rate of change in mood disturbance scores between the baseline and party sessions in the groups with less and more exposure to ecstasy was similar. However, at the social gathering the TMD scores remained in a positive range in the group with less exposure, whereas in the group with more exposure observed decrease reached a negative level. These observations provide some support for the hypothesis that users with a greater total amount of ecstasy tablets consumed in their lifetime use the drug more frequently, as they find the positive effects of it on mood more intense.

In sum, the findings provide some limited evidence for the presence of an antidepressant-like activity of MDMA, which may be more prominent, if an increased susceptibility to depression is present. This may be consistent with the self-medication hypothesis of MDMA use due to a predisposition to depression.

## 8.2. Limitations of the study

There were certain limitations of this research into the antidepressant-like potential of MDMA at both preclinical and clinical levels. In the animal study the levels of neurotransmitters and 5-HTT function were not assessed after the initial drug administration, when the most prominent changes in behaviour were observed. The

most appropriate measure at the time of the initial FST would be assessment of 5HTT functioning, as it is a primary site of MDMA action. The technical difficulties due to the test setting did not allow an *in vivo* measurement of 5-HTT function, which can be done via microdialysis. Furthermore, after the series of injections, only concentrations of 5-HT and 5-HIAA were assessed. Inclusion of the investigation of 5-HTT function in various brain regions following initial MDMA administration and after repeated administration could provide more information on the differences in serotonergic system functioning between SD and FSL rats. However, such analysis after initial administration would be only possible if the animals are sacrificed straight after the FST session.

Furthermore, although the doses that were used in the present study are comparable to those consumed by humans in recreational settings if an interspecies scaling approach is used, the differences in pharmacokinetics between rodents and humans warrant caution in directly extrapolating the results from the animal research into clinical settings. Furthermore, the administration of MDMA at a dose of 10 mg/kg has been associated with neurotoxic potential, whereas for an equivalent dose in humans, based on an interspecies scale extrapolation, there is no consensus on its long-term negative impact. Nevertheless, in this study, a similar antidepressant-like effect of MDMA was observed in an animal model of depression and in human subjects with a predisposition to depression.

The main limitation of the clinical part of this project is its cross-sectional design, which is associated with certain inaccuracies in some of the background data, such as information on use of ecstasy and use of other drugs. Furthermore, all psychological

assessment was done with the use of self-report forms, which may also be associated with inaccurate responses.

Another limitation of the present study is the inability to control the dose of MDMA consumed by participants. Due to ethical and legal restrictions, a controlled study could only be performed in a laboratory. This would eliminate one important aspect of the assessment, being at a social gathering, which, as the findings suggest, plays a major role in the perception of mood and other psychological parameters by users when they are under the influence of ecstasy. In the present study, data were collected from users who voluntarily chose to consume a pill at a social gathering and the results were compared with the subjects who abstained from ecstasy use during the assessment. The contents of consumed pills were indirectly assessed via measurement of amphetamine-type substances in the saliva samples. However, a correlation of the psychological outcomes with plasma concentrations would be a more accurate way of assessing the psychological status of participants following drug consumption. Providing participants with a known dose of MDMA to consume in a controlled setting at a social gathering, with the presence of a research team, including a medical officer, might perhaps be more appropriate for the research purpose, however, this approach would be most likely prohibited due to ethical and legal concerns, and it would be linked with a number of risks, especially with compliance.

Due to the cross-sectional design of the study, a limited number of participants were recruited. Inclusion of a larger number of subjects could have provided more statistical significance to findings and also contribute to the distribution of genotypes between the groups. Nevertheless, the design of the study allowed observation of an

antidepressant-like effect of MDMA in subjects with a predisposition to depression. A prospective longitudinal study with a large number of participants that can represent the community is perhaps a more appropriate design to address the question of whether depressive symptoms reported by ecstasy users are predominantly due to drug consumption, or to an underlying condition, or a combination of both. In such a setting, predisposition to depression may be assessed more accurately, using methods other than self-report questionnaires. However, due to the illicit nature of MDMA, it would not be ethical to carry out a prospective study where MDMA would be administered in controlled setting to a group of interest on several occasions, and mood would be assessed, when subjects are under the influence of ecstasy and when they are not.

## 8.3. Future directions

The FSL rat model of depression proved to be appropriate for investigating the antidepressant-like potential of MDMA. Further research into MDMA effects in this model of depression, including the long-term effects on the functioning of the serotonergic and other neurotransmitter systems, may provide more understanding of the involvement of a pre-existing serotonergic dysfunction in the consequences of MDMA use. Furthermore, a comparison of the effects of MDMA self-administration between FSL and 'non-depressed' rats may contribute to our understanding of the factors associated with MDMA administration in animal models.

A cause-effect relationship between mood disturbance and MDMA and other drug use is usually difficult to assess in cross-sectional studies. An ideal study to address the question of the links between ecstasy use and depressive symptoms would be a large-

scale prospective longitudinal study, where participants are closely observed for a number of years from an age prior to first drug use. Such a study could be designed similar to the one that was a part of the Netherlands Ecstasy Toxicity study (De Win, Jager et al. 2005; Jager, de Win et al. 2007). The target population would consist of adolescents with a potential risk of drug use in the future. Assessment of the risks of ecstasy use initiation, such as peer pressure, could be further related to the start of MDMA use. Mood and risks of the development of depression, family history and assessment of environmental risks could be included in the assessments at the start of the study, prior to the first ecstasy use. Participants could then be observed over a number of years, and those who commenced MDMA use would be included in further investigation of mood disturbance and depressive symptoms, when they are under the influence (several assessment time points) and when they are drug-free. Such a study would provide more information on the patterns of ecstasy use by people with and without a predisposition to depression, and would be able to distinguish a pre-existing mood disturbance from the effects of exposure to MDMA more clearly.

Furthermore, a comparison of MDMA's effects between males and females with and without an increased susceptibility to mood disorders would also be appropriate, as previous findings indicate different rates of depression and different susceptibility to MDMA's effects between sexes.

Depression is a worldwide problem that affects people of all ages. Substance abuse is a common issue in youth. The present study provides a basis for further research into the patterns of drug use by young people and insight into a spectrum of positive and negative effects of the drug in subjects with a different predisposition to depression.

Furthermore, it may assist in research into the mechanisms of the development of depressive symptoms, and, hence, provide a potential basis for the development of new antidepressant medications.

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# Appendix 1. Publications in support of the thesis

## Publications

- Jaehne EJ, Majumder I, Salem A, Irvine RJ. "Increased effects of 3,4-methylenedioxymethamphetamine (ecstasy) in a rat model of depression". Addict Biol, Epub 2010 Feb 26.
- Majumder I, White JM, Irvine RJ. "Antidepressant-like effects of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) in an animal model of depression". 2009. Manuscript in preparation.
- Majumder I, Irvine RJ, White JM. "Antidepressant-like effects of ecstasy in subjects with a predisposition to depression". 2009. Manuscript in preparation.
- Majumder I, White JM, Irvine RJ. "Association between ecstasy (MDMA) use and depression: a review". 2009. Manuscript in preparation.

## Conference papers

- "Reversal of depressive-like state in Flinders Sensitive Line (FSL) rats by acute methylenedioxymethamphetamine (MDMA) administration". Postgraduate Research Expo, Faculty of Health Sciences, University of Adelaide, July 2008.
- "Reversal of depressive-like state in Flinders Sensitive Line (FSL) rats by acute methylenedioxymethamphetamine (MDMA) administration". International conference "Psychobiology of MDMA or ecstasy", Swinburne University of Technology, Melbourne, Australia, September 2008.

- “Antidepressant-like effects of methylenedioxymethamphetamine (MDMA, ecstasy)”. Mental Health Research Day “The Importance of Co-Morbidity in Mental Health”, Adelaide, Australia, October 2008.
- I. Majumder, J.M. White, R.J. Irvine “Antidepressant-like effects of methylenedioxymethamphetamine (MDMA, ecstasy) in an animal model of depression”. 4th Australian Health and Medical Research Congress, November 2008, Brisbane, Australia.
- E.J. Jaehne, I. Majumder, A. Salem, R.J. Irvine “Thermoregulatory and behavioural effects of MDMA in Flinders Sensitive Line rats”. 4th Australian Health and Medical Research Congress, November 2008, Brisbane, Australia.
- I. Majumder, J.M. White, R.J. Irvine “Antidepressant-like effects of MDMA in an animal model of depression”. 71st Meeting of the College on Problems of Drug Dependence, June 2009, Reno, NV, USA.
- I. Majumder, J.M. White, R.J. Irvine. “Antidepressant-like effects of ecstasy (MDMA) in young people”. Postgraduate Research Expo, Faculty of Health Sciences, University of Adelaide, September 2009.