The use of combined telemetry and microdialysis techniques to assess 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') effects in rats

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Thesis Abstract

3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') is known to produce hyperthermia and adverse cardiovascular effects in humans following consumption, which can be life threatening. In animals, MDMA also produces similar effects as seen in humans such as increase in core body temperature (Tc) which has been linked to chronic neurotoxicity. Currently, clinical treatment of these adverse effects is inadequate mainly due to limited understanding of the mechanism involved in the acute MDMA-induced adverse effects. Due to ethical reasons, MDMA studies in humans are limited and studies have relied on the use of animal models to investigate MDMA effects. Therefore, it is important to assess MDMA-induced effects using appropriate techniques to relate the findings from animals to humans.

The general aims of this thesis were to investigate effects of different methods used to measure core body temperature and behaviour following MDMA administration and the validity of combined telemetry and microdialysis techniques to assess MDMA and its active metabolite, 3,4-methylenedioxyamphetamine (MDA) effects on body temperature (Tc), behaviour, heart rate (HR), locomotor activity (LMA), and 5-HT extracellular levels in the rat striatum.

The first part of this thesis looked at the influence of methodological approaches used to assess changes in core body temperature and behaviour following MDMA administration. A number of studies used rectal probe measurement which requires handling and restraining of rats which results in confounding effects on the parameters measured including Tc and behaviour. Telemetry has been developed to measure these behavioural parameters without the necessity of handling the rats. The use of rectal probe caused potentiation of 10mg/kg (i.p.) MDMA-induced increase in core body temperature

compared to the use of telemetry to measure Tc during the first 60 minutes following MDMA administration and has also resulted in a lower survival rate. These results demonstrate the importance of using appropriate techniques when measuring these parameters to avoid confounding effects and that telemetry provides a more accurate assessment of MDMA-induced change in core body temperature.

The second part of the thesis looked at the validity of combined telemetry and microdialysis techniques to investigate effects of systemic administration of MDMA and central administration of MDMA and MDA on Tc, HR, LMA and 5-HT extracellular levels in the striatum. Systemic administration of 10mg/kg (i.p.) MDMA produced significant increase in Tc, HR, LMA and 5-HT extracellular levels in the striatum whereas central administration of 100μM MDMA only produced significant increase in 5-HT extracellular levels. Central administration of 5μM MDA produced no significant changes in the parameters measured, which suggests that MDA, at concentration used in this study, does not play a major role in MDMA-induced increase in 5-HT extracellular levels in the striatum and the occurrence of hyperthermia.

In summary, this thesis has demonstrated that a combined telemetry and microdialysis technique provides a better approach to assess MDMA effects in rats, allowing central administration of drugs, and simultaneous measurement of physiological and neurochemical parameters. The combined techniques provided a better tool to investigate the effects of MDMA particularly looking at the relationship between the physiological and neurochemical effects in animal models.

Declaration

I, Intan Sofia Omar certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Intan Omar

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Abbreviations

°C – degree Celcius

4-MTA – 4-methylthioamphetamine

5-HIAA – 5-hydroxyindoleacetic acid

5-HT – serotonin (5-hydroxytryptamine)

aCSF – artificial cerebrospinal fluid

ANOVA – analysis of variance

AUC – area under the curve

CH_3OH – methanol

cm – centimetre

C_{max} – peak concentration

COMT – catechol *O*-methyl transferase

CYP – cytochrome P450

DA – dopamine

DOB – 2,5-dimethoxy-4-bromoamphetamine

DOPAC – 3,4-dihydroxyphenylacetic acid

EDTA – ethylenediaminetetraacetic acid

g – gram

h – hour

HHA – 3,4-dihydroxyamphetamine

HHMA – 3,4-dihydroxymethamphetamine

HMA – 4-hydroxy-3-methoxyamphetamine

HMMA – 4-hydroxy-3-methoxymethamphetamine

HPLC – high performance liquid chromatography

HPLC-ED – high performance liquid chromatography with electrochemical detection

i.m. – intramuscular

i.p. – intraperitoneal

kg – kilogram

M – mol/litre

MAO – monoamine oxidase

MAOI – monoamine oxidase inhibitor

MDA – 3,4-methylenedioxyamphetamine

MDE – 3,4-methylenedioxyethylamphetamine

MDMA – 3,4-methylenedioxymethamphetamine (Ecstasy)

METH – methamphetamine

mg – miligram

min – minute

ml – mililitre

mm – milimitre

NaCl – sodium chloride

NaH_2PO_4 – sodium dihydrogen phosphate

OSA – octanesulphonic acid

PMA – para-methoxyamphetamine

s – second

SD – Sprague-Dawley

SEM – standard error of mean

V – volt

μl – microlitre

Chapter 1 Research Background

1.1 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy')

1.1.1 History of origins

3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') is a psychostimulant drug that is used worldwide in raves and dance clubs and has become increasingly popular in the last three decades. MDMA is a ring-substituted amphetamine derivative. MDMA is also related to mescaline which is a hallucinogenic compound (Hardman et al., 1973; Jonathan et al., 1986; Capela et al., 2009) through its chemical structure and is closely related to methamphetamine, a psychomotor stimulant (Jonathan et al., 1986). As shown in Figure 1, these compounds contain structural modifications such as the addition of the methoxy group at various positions on the benzene ring and variations in the length and branching of the side chain which affect their pharmacological activity (Hardman et al., 1973).

MDMA was primarily synthesized and patented in Germany in 1912 by Merck pharmaceutical as a precursor agent for therapeutically active compounds and it was not intentionally developed for its psychostimulant effects (Green et al., 2003; Freudenmann et al., 2006). The toxicological and behavioral effects of MDMA were first examined in the 1950s, at the University of Michigan by the USA military (Hardman et al., 1973). The study assessed mean lethal doses of MDMA and other mescaline analogues in five animal models, namely rats, mice, dogs, guinea pigs and monkeys, and found that changes in mescaline structure can alter the pharmacological activity of the analogues. The findings were not published until 1973 (Hardman et al., 1973; Parrott, 2001). However, it was not until 1978 that Alexander Shulgin, known as the 'father' of MDMA (Benzenhofer and Passie, 2010) published the findings about the psychoactive properties of MDMA in humans described as an "easily controlled altered state of consciousness with emotional and sensual overtones" (Shulgin and Nichols, 1978). From this point, there has been

research on the potential of this drug in psychotherapy (Greer, 1985) as it was believed to facilitate communication and increase patient self-esteem. During the therapy, MDMA was administered orally at 75-150mg as an adjunct to psychotherapy (Greer and Strassman, 1985).

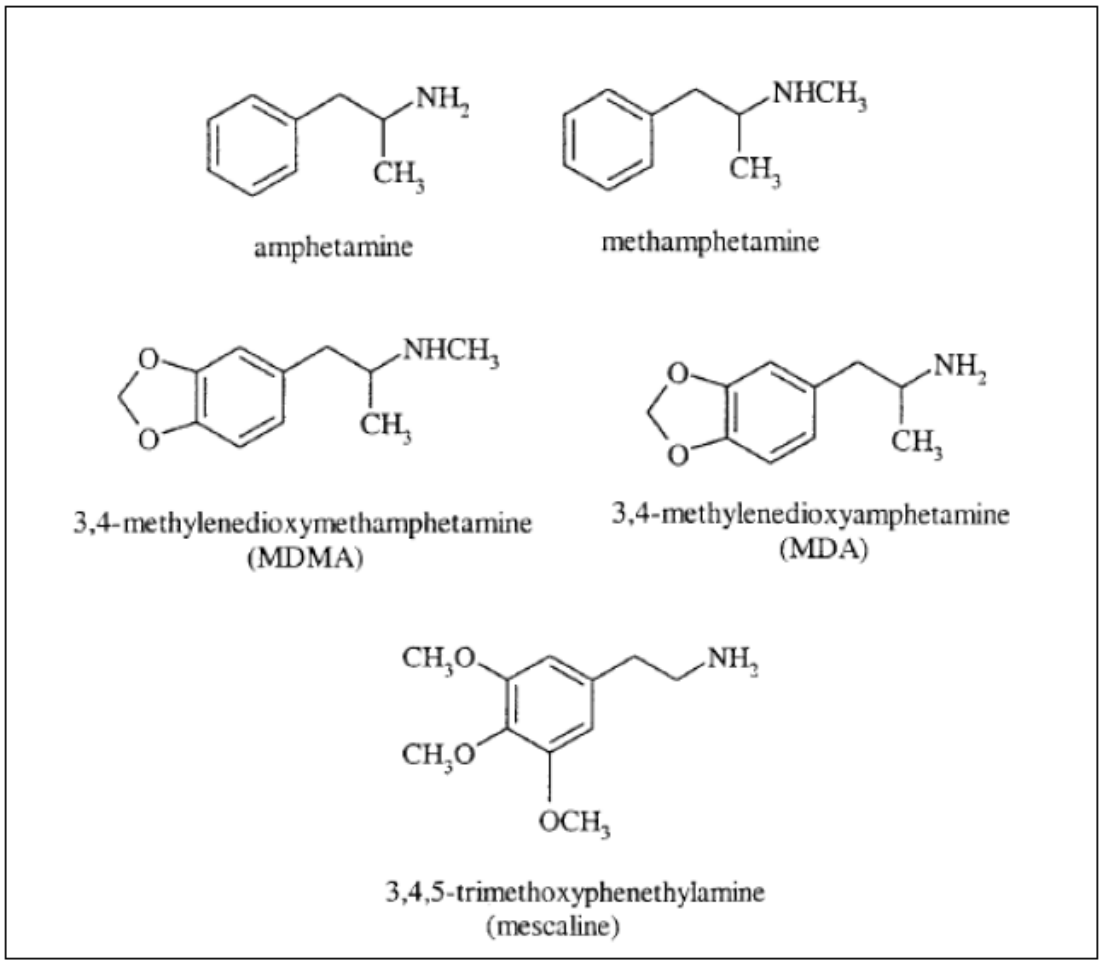


Figure 1. 1: Chemical structures of MDMA and related amphetamine derivatives.

MDMA started to be used recreationally in the 1970's and grew more popular in the 1980's. At that stage, the drug was popularly known as 'Adam' (Capela et al., 2009). Due to its abuse potential and negative effects, in 1977 MDMA was declared illegal and since then has been controlled as a Class A drug by the Misuse of Drugs Act (1971) in the UK. In 1985, the United States Drug Enforcement Administration (DEA) also classified MDMA as a Schedule 1 drug due to findings on its neurotoxic potential (Ricaurte et al., 1985; Schmidt et al., 1987; Peroutka et al., 1988; Parrott, 2001). Meanwhile in Australia,

MDMA was listed in Schedule 9 in 1986 (National Drugs and Poisons Schedule Committee) (2009a). Nevertheless, since then, it has become popular among young adults and is used especially in raves and dance clubs (Green et al., 2003). In recent years, MDMA has also been known by other street names such XTC, beans and 'ecstasy'.

3,4-methylenedioxyamphetamine (MDA), a major MDMA metabolite, was first synthesized in 1910 by two German chemists. The pharmacological properties of MDA was assessed in 1939 in mice as part of amines related study looking at the possibility of exploiting the central effects of MDA therapeutically (Gunn et al., 1939). The first human trial of MDA was done in 1941 as a possible therapy for Parkinson disease (Loman et al., 1941). Following World War II, MDA was studied as part of the USA military programme. MDA was patented as a cough suppressant in 1958, as an ataractic in 1960, and as an appetite suppressant in 1961 (Shulgin and Shulgin, 1990). Following that, MDA was explored more and recommended as an adjunct in psychotherapy (Naranjo et al., 1967). Since then, MDA effects such as increased self-awareness and empathy led to a widespread abuse of the drug. The DEA classified MDA as a Schedule 1 drug in the Controlled Substances Act in 1970 which was earlier than MDMA (Pentney, 2001).

Table 1. 1: History of MDMA (adapted from Freudenmann et al. 2006).

Year	Event
1912	First synthesis of MDMA by Kollisch at Merck (Darmstadt, Germany), secured by German patent 274350
1927	First pharmacological tests with MDMA by Oberlin at Merck
1952	Basic toxicological tests with MDMA by van Schoor at Merck
1953/4	First formal animal study in five species using MDMA and seven other psycotropic drugs (University of Michigan): secret, US army-sponsored study, unpublished until 1973
1959	Re-synthesis of MDMA by Fruhstorfer at Merck
1960	First regular scientific paper on MDMA (in Polish) describing an MDMA synthesis
1970	First detection of MDMA in tablets seized in the streets of Chicago
1978	First MDMA studies in humans by Shulgin and coworkers reporting on chemistry, dosage, kinetics and psychotropic effects
1984	MDMA's street name 'ecstasy' was coined in California
1985-8	MDMA became a Schedule I controlled substance in the United States and banned in most others soon thereafter

1.1.2 Epidemiological studies

According to the United Nations World Drug Report 2010, it is estimated that between 155 and 250 million people (3.5 to 5.7% of the population aged 15-64) used illicit substances at least once in 2008, in which amphetamine-like rank as the second most commonly used drugs after cannabis, followed by cocaine and opiates (2010). In 2010, it was estimated that between 13.7 and 52.9 million people aged 15 to 64 had used amphetamine-like substances, including 10.5 to 25.8 million ecstasy users (0.2% to 0.6% of the population). Oceania, East and South-East Asia, North America, and West and Central Europe have the highest prevalence of amphetamine-like substances. According to the United Nations World Drug Report 2009, Australia has the highest prevalence for ecstasy (4.2%) in the population aged 15-64 years in the world (e.g. Scotland 3.2; New Zealand 2.6; Northern Island 1.8; England and Wales 1.5; Canada 1.3; Republic of Island 1.2; USA 1.1%) (2009b). The percentage of ecstasy users increased from 1.2% in 1993 to 3.5% in 2007, in the Australian population aged 14 years and older (United Nations, 2008b) with the highest prevalence among people aged 20-29 years. In this group, 11.2% (0.3 million) were recent

users of ecstasy, which was the highest rate compared to other groups. The average initiation age of ecstasy users is 22.6 years (Australian Institute of Health and Welfare 2008a) .

1.2 Mechanisms of action of MDMA

1.2.1 Neuropharmacology

MDMA affects the monoaminergic system by acting on the serotonin (5-HT), dopamine (DA) and noradrenaline (NA) systems. MDMA interacts with these systems in various ways resulting in a rapid increase in extracellular concentrations of neurotransmitter in the synaptic cleft and consequent activation of receptors at postsynaptic neurons. The serotonergic system is the main system affected by MDMA in rats, non-human primates, and humans (Ricaurte et al., 1985; Croft et al., 2001; Baumann et al., 2007) whereas the dopaminergic system is the main system affected in mice (Logan et al., 1988; Colado et al., 2004). Therefore, mice may not be a suitable animal model to investigate the effects of MDMA and relating them to effects seen in humans (Logan et al., 1988; Colado et al., 2004).

1.2.1.1 Animal studies

MDMA enters the neuronal system via monoamine reuptake transporters such as the serotonin transporter (5-HTT) and subsequently binds to the vesicular monoamine transporters (VMAT) and causes increased extracellular levels of the neurotransmitters. MDMA administration mainly induces acute increase in extracellular levels of serotonin (5-HT) (Gough et al., 1991; Yamamoto et al., 1995; Gudelsky and Nash, 1996; Callaghan et al., 2005) from the vesicles in presynaptic neurons in many brain regions. Previous studies have shown the release of 5-HT *in vitro* in rat brain slices (Johnson et al., 1986; Schmidt, 1987b; Schmidt, 1987a), rat synaptosomes (Nichols et al., 1982; Berger et al., 1992; O'Loinsigh et al., 2001), and *in vivo* microdialysis studies looking at brain

extracellular 5-HT (Gough et al., 1991; Yamamoto et al., 1995; Gudelsky and Nash, 1996; Nixdorf et al., 2001; Freezer et al., 2005; Stanley et al., 2007). MDMA binds to the 5-HT, DA and NA transporters and induces increase in extracellular levels of the neurotransmitters to the synaptic cleft and prevents the reuptake of the neurotransmitters into the presynaptic neurons (Schmidt et al., 1987; Daws et al., 2000; Rothman et al., 2001; Callaghan et al., 2005).

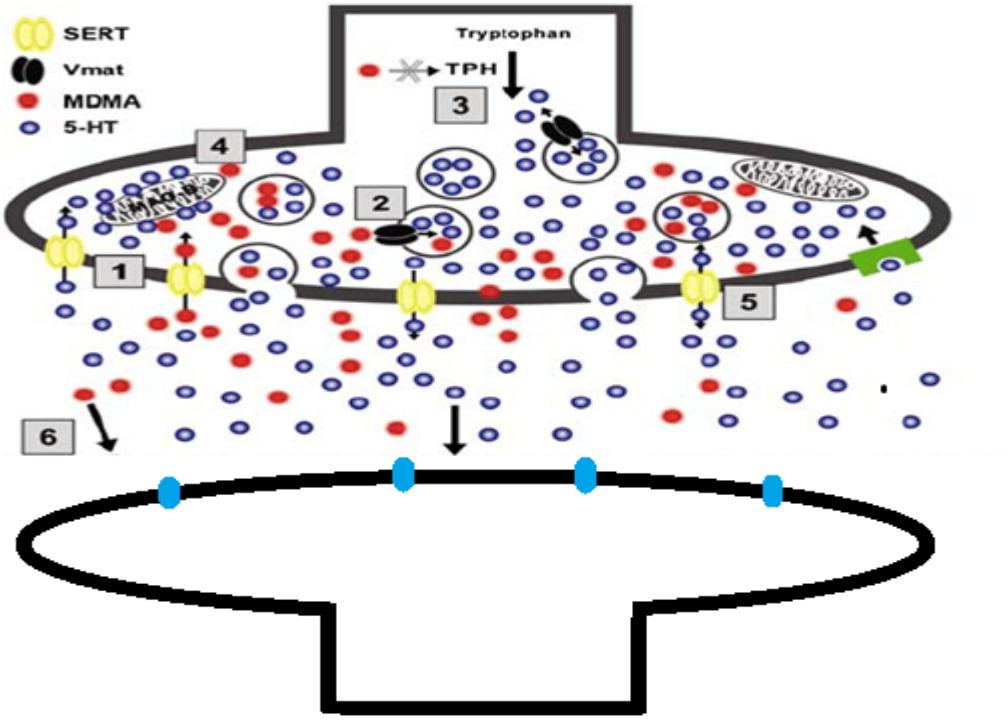


Figure 1. 2: MDMA pharmacological mechanism of action at the neuronal serotonergic terminal and synapse.

1) MDMA, like 5-HT, is a substrate for the 5-HTT and uses the transporters to enter the neuronal terminal, although at high concentration, it may also enter by diffusion. 2) Once inside, MDMA produces an acute and rapid release of 5-HT from storage vesicles, possibly by entering the vesicles via the VMAT and depleting vesicular neurotransmitter stores via a carrier-mediated exchange mechanism. 3) MDMA also inhibits TPH, the rate-limiting enzyme for 5-HT synthesis. 4) MAO-B, located in the outer membrane of the mitochondria of serotonergic neurons, is the enzyme responsible for 5-HT degradation and its activity is partially inhibited by MDMA. 5) Due to an increase in the free cytoplasmic pool of 5-HT, MDMA promotes a rapid release of intracellular 5-HT to the neuronal synapse via reversal of the 5-HTT activity. 6) MDMA hallucinogenic properties are partially a result of agonist activity at the 5-HT_{2A} receptor (Capela et al., 2009).

It has been shown in previous studies that pre-treatment with 5-HT transporter inhibitors such as fluoxetine blocks MDMA-induced 5-HT release (Gudelsky and Nash, 1996; Rothman et al., 2001; Mechan et al., 2002), which suggests MDMA-induced 5-HT release involves a carrier-mediated mechanism. MDMA does not have the same affinity for the different types of monoamine transporters. MDMA, administered intraperitoneally, at doses of 10 and 15 mg/kg, resulted in cerebral extracellular concentration of 11 and 20 μM, respectively. It has the highest potency of inhibiting the 5-HT transporters (Table 1.2) followed by NA transporters and DA transporters which indicate the MDMA physiological effects are probably mediated mainly through these transporters.

Table 1. 2: Affinity of MDMA for major recognition sites in the rat brain. Derived from Battaglia et al. (1988a).

Brain recognition site	**Affinity Ki (μM)**
Serotonin Uptake Transporter	0.61 ± 0.05
Noradrenalin Uptake Transporter	15.7 ± 1.7
Dopamine Uptake Transporter	24.4 ± 1.9
α_1 Adrenoreceptor	18.4 ± 1.2
α_2 Adrenoreceptor	3.6 ± 0.8
β Adrenoreceptor	19.2 ± 2.1
D1 Dopamine Receptor	148 ± 14
D2 dopamine Receptor	95 ± 15
$5HT_1$ Serotonin Receptor	23 ± 1.5
$5HT_2$ Serotonin Receptor	5.1 ± 0.3
M1 Muscarinic Receptor	5.8 ± 0.3
M2 Muscarinic Receptor	15.1 ± 0.1
H1 Histamine Receptor	5.7 ± 2.4

MDMA also interacts with two key enzymes that are responsible for 5-HT biosynthesis and metabolism. Firstly, MDMA inhibits the activity of tryptophan hydroxylase (TPH) which is responsible for synthesizing tryptophan to 5-hydroxytryptophan, precursor of 5-HT hence leads to a decrease in 5-HT formation (Schmidt and Taylor, 1987; Stone et al., 1987; Schmidt and Taylor, 1988). This action causes depletion of 5-HT stores following rapid release of 5-HT into the synaptic cleft. A study by Stone et al., (1987) has shown that administration of a single dose of 10mg/kg MDMA produces significant decrease of the

TPH enzyme activity in the frontal cortex, neostriatum, hippocampus and hypothalamus in rats. This action is followed and closely parallels a decrease in 5-HT levels in the respective brain regions.

Secondly, MDMA inhibits the activity of monoamine oxidase A (MAO-A), which is the enzyme responsible for metabolism of 5-HT to its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) within the nerve terminal (Colzi et al., 1993; Leonardi and Azmitia, 1994; Hewton et al., 2007). The potency of MDMA at MAO-A is approximately 10 times higher (IC_{50} = 44μM) than at monoamine oxidase B (MAO-B) in a rat brain preparation (Leonardi and Azmitia, 1994). However, MAO-A is expressed predominantly in catecholaminergic neurons, whereas MAO-B is expressed in serotonergic neurons, astrocytes, and glia (Nagatsu, 2004). These events also lead to an increase in 5-HT concentration in the synaptic cleft and more 5-HT binding at the postsynaptic receptors. A study by Freezer et al., (2005) has shown that MDMA produces additional increase in striatal 5-HT, 5-HT-related behaviour and hyperthermia when co-administered with moclobemide, a MAO-A inhibitor.

MDMA also interacts with the dopaminergic system. It increases the extracellular levels of DA from several brain regions such as the striatum, cortex, and nucleus accumbens as shown by *in vivo* microdialysis studies (Yamamoto and Spanos, 1988; Gough et al., 1991; Nash and Brodkin, 1991; Gudelsky and Nash, 1996) and *in vitro* studies using tissue slices (Johnson et al., 1986; Schmidt, 1987a). A study by Colado et al., (1999) showed that administration of 15mg/kg MDMA i.p to male Dark Agouti rats produces significant increase in extracellular DA concentrations in the striatum.

As mentioned previously, MDMA-induced 5-HT release results from the interaction of MDMA with the reuptake transporters, since this effect is blocked by fluoxetine, a 5-HT transporter inhibitor (Gudelsky and Nash, 1996). However, the case may be different with DA release, as the involvement of DA uptake transporter in MDMA-induced DA release is unclear. It has been shown via *in vivo* microdialysis that a DA reuptake inhibitor (GBR 12909) blocks the increased DA extracellular levels in the brain (Nash and Brodkin, 1991), and *in vitro* studies using brain slice preparation (Koch and Galloway, 1997).

Several studies have demonstrated that MDMA-induced DA release is associated with the stimulation of 5-HT_2 receptors (Gudelsky et al., 1994). This receptor is mainly involved in mediating the hallucinogenic effects of MDMA (Nichols, 2004). In this study, it was reported that MDMA-induced DA release in the striatum was significantly increased by pretreatment with either 5-HT_2 receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI), or the non-selective 5-HT agonist, 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT). Further support for pretreatment can be found in the fact that central administration of the 5-$HT_{2A/2C}$ receptor antagonists, ritanserin, completely blocks and attenuates MDMA-induced DA release in the substantia nigra and striatum, respectively (Schmidt et al., 1991; Yamamoto et al., 1995) which suggests the involvement of the 5-HT receptors in DA release by MDMA.

MDMA has also been shown to affect the noradrenergic system which is important in cardiovascular and psychiatry effects in humans (Fitzgerald and Reid, 1990; McCann et al., 1996) as well as thermoregulation in animals (Bexis and Docherty, 2005). Following administration of α_2-adrenoreceptor antagonist BRL 44408, MDMA-induced hyperthermia in wild type mice became a biphasic response with an initial hypothermia followed by hyperthermia (Bexis and Docherty, 2005). MDMA causes an increase in NA extracellular

levels *in vitro* from brain tissue and synaptosomal preparation (Fitzgerald and Reid, 1990; Rothman et al., 2001). Research has demonstrated that MDMA has agonist actions at both α_1- and α_2-adrenoreceptors in rats *in vivo* and *in vitro* (McDaid and Docherty, 2001).

1.2.2 Brain regions

Several laboratories have studied specific brain regions following systemic and central administration of MDMA, namely the striatum, nucleus accumbens (NA), hippocampus, and the preoptic area/anterior hypothalamus (PO/AH) (Esteban et al., 2001; Nixdorf et al., 2001; Ishiwata et al., 2002; Freezer et al., 2005; O'Shea et al., 2005; Stanley et al., 2007; Baumann et al., 2008). MDMA action on selected brain regions is consistent with the location of receptors that MDMA has high affinity for in the respective regions, and this is related to the pharmacodynamic effects of MDMA in *in vivo* (Callaghan et al., 2005) and *in vitro* studies (Battaglia et al., 1988a). An increased expression of Fos, a marker for neuronal activation has been shown in the areas of the brain with high levels of DA and 5-HT neurons such as ventral tegmental area and median and dorsal raphe. This expression was also shown in the medial prefrontal cortex, caudate-putamen, nucleus accumbens, striatum, and paraventricular hypothalamus which are important in thermoregulation, locomotion, and psychological or emotional responses (Stephenson et al., 1999; Hargreaves et al., 2007; Colussi-Mas and Schenk, 2008). It has also been shown that an increase in DA extracellular levels in the striatum and nucleus accumbens is important in causing effects on locomotor activity and reinforcing effects (Melega et al., 1995; Jones et al., 1996; Jones et al., 1999). Increase of 5-HT and DA extracellular levels in the nucleus accumbens following MDMA consumption is also important in mediating euphoria, as shown by other stimulants, opiates and alcohol (White et al., 1996). Microdialysis studies have mainly focused on the striatum as this brain region is important for locomotion and cognition function, has a large size and is easily accessible by dialysis probe (Li et al., 2006).

It has been demonstrated that MDMA-induced hyperthermia is mediated by interaction of MDMA on the PO/AH (Benamar et al., 2008). The PO/AH is important in the regulation of body temperature by autonomic and behavioural responses. It has many warm-sensitive neurons sending excitatory and inhibitory signals for heat loss and heat production mechanisms, respectively (Boulant and Dean, 1986; Hasegawa et al., 2005). A microdialysis study by Benamar et al., (2008) administered 20mg/kg MDMA (i.p.) to male Sprague-Dawley rats and measured DA extracellular levels in the PO/AH and found that pretreatment of D_1 DA receptor antagonist SCH 23390 significantly reduced both the hyperthermia and increase DA extracellular levels. Several other studies have also demonstrated the role of PO/AH in thermoregulation (Ishiwata et al., 2002; Ishiwata et al., 2005). It has been shown that perfusion of tetrodotoxin (TTX), a sodium-channel blocker used to block neurotransmission, into the PO/AH using microdialysis produced increase in body temperature under normal and high ambient temperature (23°C and 35°C) in freely moving rats (Ishiwata et al., 2002). These findings indicate the role of the PO/AH in thermoregulation and further studies are needed to understand the mechanism of action of MDMA in the PO/AH in detail.

Table 1. 3: MDMA effects – Roles of different brain regions.

Brain regions	**Roles**	**References**
Striatum	Locomotion and reinforcing effects, cognition	Baumann et al., 2008; Li et al., 2006
Nucleus accumbens	Locomotion and reinforcing effects, mediating euphoria	Baumann et al., 2008; O'Shea et al., 2005; White et al., 1996
PO/AH	Thermoregulation	Boulant and Dean, 1986; Hasegawa et al., 2005

1.3 Effects of MDMA in humans

1.3.1 Psychological effects

MDMA is taken due to its desirable effects such as the feeling of euphoria, increase in self-esteem and a feeling of closeness to others (Parrott, 2001; Green et al., 2003). A study by Davison and Parrott (1997) of 20 recreational drug users aged 18 to 31 years, who have used MDMA at least once, found that the subjects reported feelings of happiness, calmness, relaxation, increased energy and heightened perception of sound, colour, and touch while "on MDMA", which were reported in other studies as well (Peroutka et al., 1988; Sumnall et al., 2006). Due to these desirable effects and lack of prominent acute side effects, MDMA is known as a 'safe drug' in comparison to so-called 'hard' drugs like cocaine and heroin, (Solowij et al., 1992) and because it is taken as tablets and does not involve unpleasant intravenous administration. It has been reported that recreational poly-drug users experienced higher feelings of elation, agreeability and emotional composure when under the influence of MDMA in comparison to amphetamine or LSD (Parrott and Stuart, 1997). However, there have been emerging findings on the hepatotoxicity (Jones and Simpson, 1999), neurotoxicity, psychopathology and abuse potential of designer drugs, which includes MDMA (Hegadoren et al., 1999).

Repeated ecstasy consumption can lead to a decrease in ecstasy effects. MDMA users experienced feelings of lethargy, moodiness, irritability, sleep disturbances, depression, headaches and paranoia several days after consumption of MDMA (Peroutka et al., 1988; Davison and Parrott, 1997; Parrott and Lasky, 1998; Green et al., 2003). It has also been reported that recreational ecstasy users experienced 'low mood' few days after acute MDMA consumption (Curran and Travill, 1997; Parrott and Lasky, 1998). Since MDMA causes acute 5-HT release and inhibits the activity of TPH (to synthesize 5-HT), this causes depletion of the 5-HT stores, which may underlie the moodiness, sleep disturbances

and depression effects. Previous studies have investigated the effects of several 5-HT and DA antagonists and uptake inhibitors (ketanserin, haloperidol, and citalopram) on psychological effects of MDMA (Liechti et al., 2000a; Liechti and Vollenweider, 2000a). Pretreatment with citalopram blocked most of the psychological effects of MDMA which indicates the involvement of 5-HT uptake sites in this action (Liechti et al., 2000a). It was also suggested that MDMA reinforcing properties are associated with increase of DA, as 5-HT_2 receptor antagonist, ketanserin, produces significant decrease in MDMA-induced perceptual changes and emotional excitation but only slightly affects positive mood responses (Liechti et al., 2000b). Dopamine is generally thought to be involved in the euphoria and arousing effects of stimulants. Hence, the stimulant-like effects of MDMA may be caused partially by 5-HT_2-mediated increase of dopamine activity (Liechti et al., 2000b). These results suggested the role of both DA and 5-HT in mediating MDMA-induced psychological effects (Liechti and Vollenweider, 2001).

1.3.2 Physiological effects

There are also physiological effects associated with MDMA use by humans which vary from minor symptoms to those that are rare but potentially life-threatening. MDMA symptoms include muscle aches and tension, jaw clenching and bruxism, elevated blood pressure and heart rate, sweating, tremor, insomnia, nausea, chills, and hyperthermia (McCann et al., 1996; Green et al., 2003; Lyles and Cadet, 2003). Although the incidence of the adverse effects are low, these effects are unpredictable and can lead to morbidity and death (Gowing et al., 2002). The effects of citalopram, a serotonin reuptake inhibitor on MDMA-induced physiological responses are marked (Liechti and Vollenweider, 2000b). Oral administration of 1.5mg/kg MDMA produces a significant increase in both systolic and diastolic blood pressure and heart rate and these effects were blocked by citalopram pretreatment, which indicates that the physiological effects of MDMA are mediated via the

5-HT uptake sites that increase 5-HT extracellular levels through the transporter (Liechti and Vollenweider, 2000b).

Hyperthermia is one of the major symptoms following acute MDMA administration and this leads to other adverse effects and multi-organ failure including rhabdomyolysis, acute renal failure, cardiac arrhythmias, and disseminated intravascular coagulation (Green et al., 2003; Lyles and Cadet, 2003; Hall and Henry, 2006). MDMA-induced hyperthermia is affected by the increase in ambient temperature (Malberg and Seiden, 1998). As ecstasy is usually consumed in raves and clubs, in an over-crowding environment, high ambient temperature settings, and increase physical activity, these conditions potentiate the degree of hyperthermia experienced by ecstasy users (Green et al., 2003). Due to a reaction against feeling too hot and excessive sweating, ecstasy users may drink large amounts of fluids. This causes dilution of sodium and potassium electrolyte levels in the blood, which potentially leads to hyponatraemia with cerebral oedema, a related medical condition following hyperthermia (Green et al., 2003). There have also been sudden death cases associated with MDMA users due to excessive sympathomimetic effects of the drug which mainly affect individuals with undiagnosed cardiomyopathy, hypertension or congenital heart disease (Hall and Henry, 2006).

MDMA users also experience symptoms similar to 'serotonin syndrome'. Serotonin syndrome occurs due to excessive extracellular levels of 5-HT to the synapse which stimulates the serotonergic system (Gillman, 1999). This syndrome occurs rapidly and ecstasy users experience some of these effects that include hyperthermia, tachycardia, shivering, hyperreflexia, tremor, hyperactivity, diarrhoea, mental confusion and others (Gillman, 1999; Parrott, 2002). Users who experiences these effects were usually required to rest in a cool ambient temperature setting but users who are severely affected require

hospitalisation and in some cases, treatment with 5-HT antagonists such as cyproheptadine or chlorpromazine, intubation and rapid cooling (Gillman, 1999).

1.3.3 Long-term effects

Neuroimaging and electrophysiological studies have demonstrated attenuation of serotonergic function and reduction of serotonin transporter density in the brain following repeated exposure to MDMA (McCann et al., 1998; Reneman et al., 2002a; Reneman et al., 2002b; McCann et al., 2005). Several studies have used positron emission tomography (PET) with the 5-HT transporter ligand [^{11}C]McN-5652 to examine 5-HT transporter binding in recreational users of MDMA (McCann et al., 1998; Ricaurte et al., 2000) and it was demonstrated that MDMA users have lower density of brain 5-HT transporter sites. A study by Reneman et al., (2000) which used single photon emission computed tomography (SPECT) with the 5-HT_{2A} receptor ligand [^{123}I]R91150 found significantly lower 5-HT_{2A} receptor binding ratios in current MDMA users compared to abstinent users, which suggests down regulation of 5-HT_{2A} receptors.

It was also postulated that long-term MDMA use may be associated with several psychological effects such as visual hallucinations and paranoid delusions, together with anxiety, depression and panic attacks, and development of memory and cognitive impairment (McCann and Ricaurte, 1991a; Schifano, 1991; McCann and Ricaurte, 1992; McCann et al., 1994; Bolla et al., 1998; Parrott and Lasky, 1998; McCann et al., 1999; Parrott et al., 2000), and the problems continue in the drug-free condition (Bolla et al., 1998).

There are findings that linked recreational MDMA users and the development of cognitive and memory impairment (Parrott and Lasky, 1998; Chummun et al., 2010; Hanson and Luciana, 2010). Bolla et al., (1998) have shown that MDMA users who had used MDMA

on at least 25 occasions (and had abstained from use for more than 2 weeks), exhibited impairment of verbal memory and delayed visual memory than MDMA-naïve subjects. A study by Parrott et al., (2002) has also demonstrated severity of MDMA effects directly correlate with the extent of MDMA use. This study was done as a web questionnaire in novice ecstasy users (1-9 occasions), moderate users (10-99 occasions), and heavy users (more than 100 occasions). It was shown that heavier MDMA users reported more mood fluctuation, anxiety and cognitive problems, and poorer performance in memory task (73% of heavy users, compared to 19% of novice users and 52% of heavy users).

As ecstasy is commonly consumed along with alcohol and other drugs, several studies have also explored at the association of cognitive problems with ecstasy use, especially in heavy users in poly-drug use subjects (Morgan, 1999; Quednow et al., 2006). It was shown that MDMA users have significantly worse immediate and delayed recall compared to other poly-drug users (alcohol, cigarettes, cannabis, amphetamine, LSD, and cocaine) and control subjects (Morgan, 1999). This suggests that past exposure to ecstasy in recreational ecstasy users affected their memory performance, in comparison to other poly-drug use or no drug use (control subjects).

Heavy MDMA users demonstrate marked memory deficits in comparison to cannabis users and drug-naive control subjects in their memory performance (Quednow et al., 2006), and higher impulsivity and lower decision-making performance in comparison to cannabis and non-drug users (Quednow et al., 2007). This suggests that heavy use of MDMA causes these cognitive problems which is possibly mediated by impairment of the 5-HT system. However, precaution is needed in drawing conclusions regarding MDMA effects on cognitive impairment in poly-drug users (Croft et al., 2001). Some MDMA users consume cannabis to lighten the negative experience of MDMA when MDMA-induced euphoria

diminishes. However, cannabis has been shown to interact with the dopaminergic system (Malone and Taylor, 1999; Nava et al., 2000) and DA has been shown to affect 5-HT related impairment in humans (Liechti and Vollenweider, 2001) and animal models (Stone et al., 1988). This indicates that cannabis is a confounding factor in MDMA-induced cognitive impairment and certain MDMA users may have experienced the cognitive problems caused by cannabis use. Apart from cannabis, ecstasy is normally consumed with alcohol (Cassel et al., 2005; Izco et al., 2007). Previous studies have reported that MDMA and alcohol consumption prolonged the duration of euphoria and well being, in comparison to MDMA or alcohol alone (Hernandez-Lopez et al., 2002). MDMA also reverses alcohol sedative effect but did not remove the alcohol-induced psychomotor performance. These results are important in terms of poly-drug users, as they might think that they are feeling better but actually having impairment of psychomotor abilities, which may impact road safety (Hernandez-Lopez et al., 2002). A study by Dumont et al. (2008) investigating the effects of co-administration of 100mg MDMA (orally) and alcohol also found that co-administration of MDMA and alcohol produced significant memory impairment in healthy volunteers.

1.4 Effects of MDMA in animals

1.4.1 Disruption of thermoregulation

In animal studies, systemic doses of 10 and 15mg/kg of MDMA are used as these doses produced reliable changes in body temperature, locomotor activity and biochemical effects such as increase in extracellular levels of 5-HT and DA without causing fatalities (Daws et al., 2000; Jaehne et al., 2005; Stanley et al., 2007). These doses in animals are comparable to those in humans (Green et al., 2003).

Hyperthermia is the major acute effect of MDMA. Under normal ambient temperature (20-22°C), MDMA administration to rats has been reported to produce both hyperthermia

(Colado et al., 1993) and hypothermia (Malberg and Seiden, 1998) responses. Previous findings have demonstrated hyperthermia response (1-2°C) with a peak at about 40 to 60 min following systemic MDMA administration in rats (Che et al., 1995; Malberg et al., 1996; Mechan et al., 2002). Similar to humans, it has also been shown in animal studies that ambient temperature affects MDMA-induced hyperthermia (Malberg and Seiden, 1998). This study found hypothermic response following administration of 20 and 40mg/kg MDMA at ambient temperature of 20-22°C, and hyperthermic response at ambient temperature of 28-30°C in male Holtzman rats. It has also been suggested that increase in core temperature of MDMA-treated animals increase neurotoxicity. A study by Dafters (1994) has demonstrated dose-dependent hypothermic response in rats housed under ambient temperature of 11°C, while a dose-dependent hyperthermic response was observed when rats were housed under ambient temperature of 24°C.

Thermoregulation is important in mammals to maintain normal body temperature. Body temperature is physiologically maintained by a balance between heat production and dissipation (Rusyniak and Sprague, 2005) via regulation of the sympathetic nervous system (Lowell and Spiegelman, 2000). Nevertheless, the mechanism of action that underlies MDMA-induced hyperthermia response remains unclear as the heat production mechanism and impairment in thermoregulation following MDMA administration is not clearly defined (Capela et al., 2009). There have been suggestions regarding the major role of 5-HT (Rothwell, 1994), DA (Cox and Lee, 1980) and NA (Mallick et al., 2002) in the regulation of hypothalamic control of the core body temperature. Stimulant drugs which are known to affect the dynamics of these neurotransmitters level in the brain could potentially disrupt normal thermoregulation. Although MDMA-induced hyperthermia involves 5-HT release (Freezer et al., 2005; Stanley et al., 2007), methamphetamine-induced hyperthermia has been shown to involve DA release (Bronstein and Hong, 1995),

suggesting a role for DA as well in hyperthermic responses. Other studies have also reported the role of DA in MDMA-induced hyperthermia. The D_1 receptor antagonist SCH 23390 (0.3-2 mg/kg) has been shown to attenuate MDMA-induced hyperthermia (Mechan et al., 2002), whereas administration of selective 5-$HT_{2A/2C}$ antagonists, methysergide, and ritanserin, failed to inhibit MDMA-induced hyperthermia (Mechan et al., 2002). Previous microdialysis studies have also shown that increase extracellular 5-HT release has no association with MDMA-induced hyperthermia (Freezer et al., 2005; Stanley et al., 2007), and administration of the selective 5-HT uptake inhibitor fluoxetine blocked the increase in extracellular 5-HT but had no effect on hyperthermia (Schmidt et al., 1990; Berger et al., 1992; Malberg et al., 1996). These results suggest the involvement of both 5-HT and DA in MDMA-induced hyperthermia.

1.4.2 Behavioural effects

Apart from hyperthermia, MDMA also produces acute 'serotonin syndrome' in animals and this effect is potentially caused by significant increase in extracellular 5-HT levels in several brain regions. The 5-HT syndrome include enhanced locomotor activity, head-weaving, forepaw treading, piloerection, penile erection, proptosis, ejaculation, salivation, and defecation (Spanos and Yamamoto, 1989). It was shown that administration of an MAO inhibitor, L-tryptophan (Grahame-Smith, 1971b), non-selective 5-HT agonists (Grahame-Smith, 1971a), and 5-HT1A agonist 8-OH-DPAT (Goodwin and Green, 1985) could produced this serotonin syndrome, suggesting the importance increased in 5-HT activity.

MDMA also produces acute, dose-dependent hyperlocomotor response accompanied by major behavioural features of the serotonin syndrome (Spanos and Yamamoto, 1989; Colado et al., 1993). The enhanced locomotor effects following MDMA administration could be due to increased monoamine extracellular levels and subsequent activation of

monoamine receptors in the brain. Kehne et al., (1996) showed dose-dependent increased in locomotion following administration of 1, 2, 4, 10, and 20 mg/kg MDMA, which was blocked by pretreatment of 1mg/kg 5-HT_{2A} receptor antagonist MDL 100907. It was also shown that MDMA-induced locomotor activity was blocked following pretreatment with fluoxetine (Callaway et al., 1990), suggesting the involvement of 5-HT release and 5-HT_{2A} receptor in MDMA-induced locomotor activity.

1.4.3 Cardiovascular effects

Cardiovascular response to ambient temperature is closely related to core temperature and mechanism for heat production in rats (Chambers et al., 2000). Increased heart rate may provide indication of heat production and impairment of thermoregulation (Gordon, 1990; Gordon et al., 1991; Jaehne et al., 2011). It was reported that MDMA users have increased plasma catecholamine levels, which may be due to noradrenergic hyperactivity and this could linked to cardiovascular effects (Stuerenburg et al., 2002). It has been demonstrated that MDMA administration in rats increase heart rate, as well as locomotor activity and body temperature (Green et al., 2003; Bexis and Docherty, 2006; Jaehne et al., 2008). MDMA affects cardiovascular function in rats as it has cardiac stimulant effects which can lead to tachycardia and arrhythmia (Gordon et al., 1991). This might be due to the action of MDMA displacing NA from adrenergic nerve terminals (Fitzgerald and Reid, 1993) and effects on adrenoreceptors. As mentioned before, MDMA has agonist effects on α_1- and α_2-adrenoreceptors both *in vivo* and *in vitro* (McDaid and Docherty, 2001). These receptors are involved in jaw clenching effect reported by MDMA users, panic attacks (McCann et al., 1996), blood pressure effects in rat (McDaid and Docherty, 2001) and hyperthermia in mice (Bexis and Docherty, 2005). A study by O'Cain et al., (2000) has also shown that MDMA (0.01-3 mg/kg i.v.) produced significant bradycardia and a dose-dependent increase in mean arterial pressure, in which the arterial pressure recorded were comparable to that showed in human study following MDMA consumption (Vollenweider et al., 1998).

1.4.4 Long-term effects

There have been many findings that link MDMA consumption and long-term depletion of 5-HT and 5-HIAA (Schmidt et al., 1987; Battaglia et al., 1988b; McKenna and Peroutka, 1990; Colado et al., 1993). It has been shown in animal studies that administration of MDMA causes damage to axons and serotonin axon terminals. Chronic administration of MDMA to rats leads to problems in serotonergic components in the brain such as a decrease in tryptophan hydroxylase (Stone et al., 1988), 5-HT and its metabolite 5-HIAA in neostriata, cerebral cortex, and hippocampus (Schmidt et al., 1986), loss of 5-HT uptake sites (Battaglia et al., 1988b), 5-HT terminal degeneration (O'Hearn et al., 1988), reduction of 5-HT transporter (5-HTT) binding in cortex (Green et al., 2003), and impairment of central 5-HT function (Hatzidimitriou et al., 1999). Several anatomical observations have also supported these findings using techniques such as immunohistochemistry (O'Hearn et al., 1988), silver impregnation methods (Commins et al., 1987), and Fluoro-Jade B staining for neuronal degeneration (Schmued, 2003; Fornai et al., 2004). A study by Hatzidimitriou et al., (1999) using neuroimaging technique has shown that MDMA-treated monkeys still have diminished 5-HT immunoreactivity seven years after treatment, which indicates the long-term effect of MDMA in causing neurotoxicity.

A study by Callaghan et al., (2006) has shown that repeated MDMA administration produces reductions in cortical 5-HT transporter binding and 5-HT content in rats. Treatment with MDMA once daily for 4 days (10 or 20 mg/kg MDMA) resulted in significant decrease (20 mg/kg; 23% of vehicle treatment) in SERT density one week after final drug administration. Meanwhile, rats treated twice daily for 4 days (10 or 20 mg/kg MDMA) showed significant reductions in cortical 5-HT content (20 mg/kg; 39% of vehicle treatment). In another study, O'Shea et al., (1998) investigated MDMA-induced neurotoxicity in Dark Agouti rats following administration of single doses of MDMA (4,

10, and 15 mg/kg i.p.), repeated low doses (4 mg/kg) once or twice daily for four consecutive days, and repeated low doses (4 mg/kg) twice weekly for eight consecutive weeks. Serotonergic depletion was observed in the hippocampus, cortex, and striatum one week post-treatment following single doses of MDMA (10 and 15 mg/kg). Repeated low doses of MDMA (4 mg/kg) daily for four days had no significant effect on 5-HT and 5-HIAA brain concentrations, whereas 4mg/kg twice daily caused depletion of 5-HT in all brain regions. Interestingly, repeated MDMA doses weekly also had no significant effect on 5-HT and 5-HIAA ceoncentrations. These results suggest that high and frequent doses of MDMA are required to cause neurotoxic effects, which may be critical to recreational MDMA users. However, this is not the case in female DA rats, an animal model of the CYP2D6 poor metabolizer. It was demonstrated that female DA rats produced lower concentrations of neurotoxic metabolites of MDMA, thus were less susceptible to long-term neurotoxic loss of 5-HT in the brain (Colado et al., 1995).

There has been suggestion that hyperthermia influences the severity of MDMA-induced serotonergic neurotoxicity and this has been linked to dopamine receptor activity (Malberg et al., 1996; Malberg and Seiden, 1998; Yuan et al., 2001; Yuan et al., 2002). It has been shown than inducing hypothermia protects agains neurotoxicity and increasing body temperature causes neurotoxicity (Malberg et al., 1996). However, pretreatment with compounds such as fluoxetine and mazindol protected against MDMA-induced neurotoxicity but did not block the MDMA-induced increase in body temperature (Shankaran et al., 1999; Falk et al., 2002), suggesting a mechanism unrelated to the occurrence of hyperthermia. It is important to consider the effects of hyperthermia in interpreting the results on MDMA-induced neurotoxicity.

1.5 Pharmacokinetics of MDMA

1.5.1 Humans

MDMA is usually taken orally and sold in the form of tablets in various designs, logos and colours and may also be available in capsule form. Other routes of administration include intranasal and intravenous. MDMA is usually produced in racemic mixture, in which the S(+) isomer of MDMA (effective dose is 80-120mg) is more potent in causing euphoria and other desired effects than its R(-) isomer (effective dose is about 300mg), which predominantly has mescaline-like effects (Fantegrossi and Godlewski, 2003; de la Torre et al., 2004; Parrott, 2004; Schifano, 2004). However, the doses, purity or the content of 'ecstasy' tablets containing MDMA may vary between batches, within and also between countries (Capela et al., 2009).

According to Green et al. (2003), one tablet of ecstasy contains on average 80 to 150 mg of MDMA. Although common amount of tablets consumed are 1 to 2 tablets, the number may vary until 10 tablets in some users (Parrott, 2001). However, a study by Morefield et al. (2011) looking at the pattern of ecstasy used has shown that the dose of MDMA per pill can range from 0 to 245mg and users consumed from one half to five tablets. The term 'ecstasy' usually refers to MDMA but the tablets may contain other substances such as other amphetamine derivatives (methamphetamine (METH), para-methamphetamine (PMA), 3,4-methylenedioxyamphetamine (MDA), methylenedioxyethylamphetamine (MDE), 2,5-dimethoxy-4-bromoamphetamine (DOB), 4-methylthioamphetamine (4-MTA)), and other substances such as ketamine, caffeine, ephedrine or even not at all (placebo) (Cole et al., 2002; Parrott, 2004; Morefield et al., 2011).

Following oral consumption, MDMA is absorbed from the gastrointestinal tract by the bloodstream. It takes about 20 to 60 min for the onset of MDMA effects to occur. The peak

occurring 60 to 90 minutes after consumption, and the acute effects last for 3 to 5 hours (Green et al., 2003). Following oral ingestion of MDMA by humans (50-125mg), maximum concentration (C_{max}) appears at 2 hours with half-life of 8 hours (de la Torre et al., 2004). After a single dose of MDMA, it takes about 40 hours to eliminate 95% of MDMA from humans. The elimination half-life of 100 mg MDMA is about 8-9 hours, which is lower than methamphetamine (10-12 hours) and amphetamine (12-15 hours) at similar doses with MDMA (de la Torre et al., 2004). According to de la Torre et al. (2000), MDMA has a non-linear pharmacokinetics, in which the increase in plasma and brain concentration of MDMA is not proportional to administration of increasing doses of MDMA. This can be explained by a saturation of MDMA metabolism and interaction of MDMA metabolites with the enzymes involved in its own metabolism pathways. Hence, changes in metabolism can occur if MDMA is taken repeatedly (de la Torre et al., 2000; de la Torre et al., 2004).

1.5.2 Animals

In order to improve our interpretation of animal based studies on MDMA, extensive pharmacodynamics and pharmacokinetics studies have been undertaken in animals. These included mice, rats, and primates. A study by Baumann et al., (2009) looked at the effects of 2mg/kg and 10mg/kg of MDMA and route of administration on pharmacokinetics of MDMA. The study found that at low-dose, oral administration of MDMA produces low levels of MDMA, whereas at high dose, intraperitoneal administration produces high levels of MDMA. The half-life of MDMA was 45 min in rats given 2mg/kg which is shorter than half-life of MDMA in humans which is about 7 to 9 hours (de la Torre et al., 2000). The half-life of MDMA in rats given 10mg/kg MDMA was longer than 1 hour which suggests that longer time for drug elimination at high-dose. The study also found that a low-dose of MDMA (2mg/kg) produces Cmax values (~200ng/ml) comparable to humans given recreational doses of the drug (1.3 to 1.7mg/kg) under controlled conditions (de la Torre et

al., 2000). They also found evidence of non-linear pharmacokinetics of MDMA metabolism in rats which have previously been shown in monkeys and humans (Chu et al., 1996; de la Torre et al., 2000; Mueller et al., 2008).

1.5.3 MDMA metabolites

There are differences between MDMA metabolism in humans and rats. In humans, MDMA *O*-demethylenation to HHMA is the major metabolic pathway whereas in rats *N*-demethylenation to MDA is dominant. This causes differences in the degree of acute effects of MDMA as MDA is an active metabolite (de la Torre and Farre, 2004). However, a study by Baumann et al. (2009) found a lower concentration of MDA in comparison to MDMA and HHMA, suggesting N-demethylation is not the major pathway of MDMA metabolism in rats. However, this does not neglect the fact that MDA is an important active metabolite of MDMA in rats and humans (Maurer et al., 2000; de la Torre et al., 2004). Although N-demethylation to MDA is a minor pathway in humans (~10%), α-MeDA is a metabolite for both MDMA and MDA (Erives et al., 2008), which can undergo this metabolic pathways and producing potential neurotoxic compounds.

Metabolism pathways are another factor that causes difficulty in scaling MDMA data across species. MDMA metabolism in humans and animals occur in the liver (de la Torre et al., 2000; de la Torre and Farre, 2004; Baumann et al., 2009). MDMA has a complex metabolism profile as it involves several biotransformation pathways.

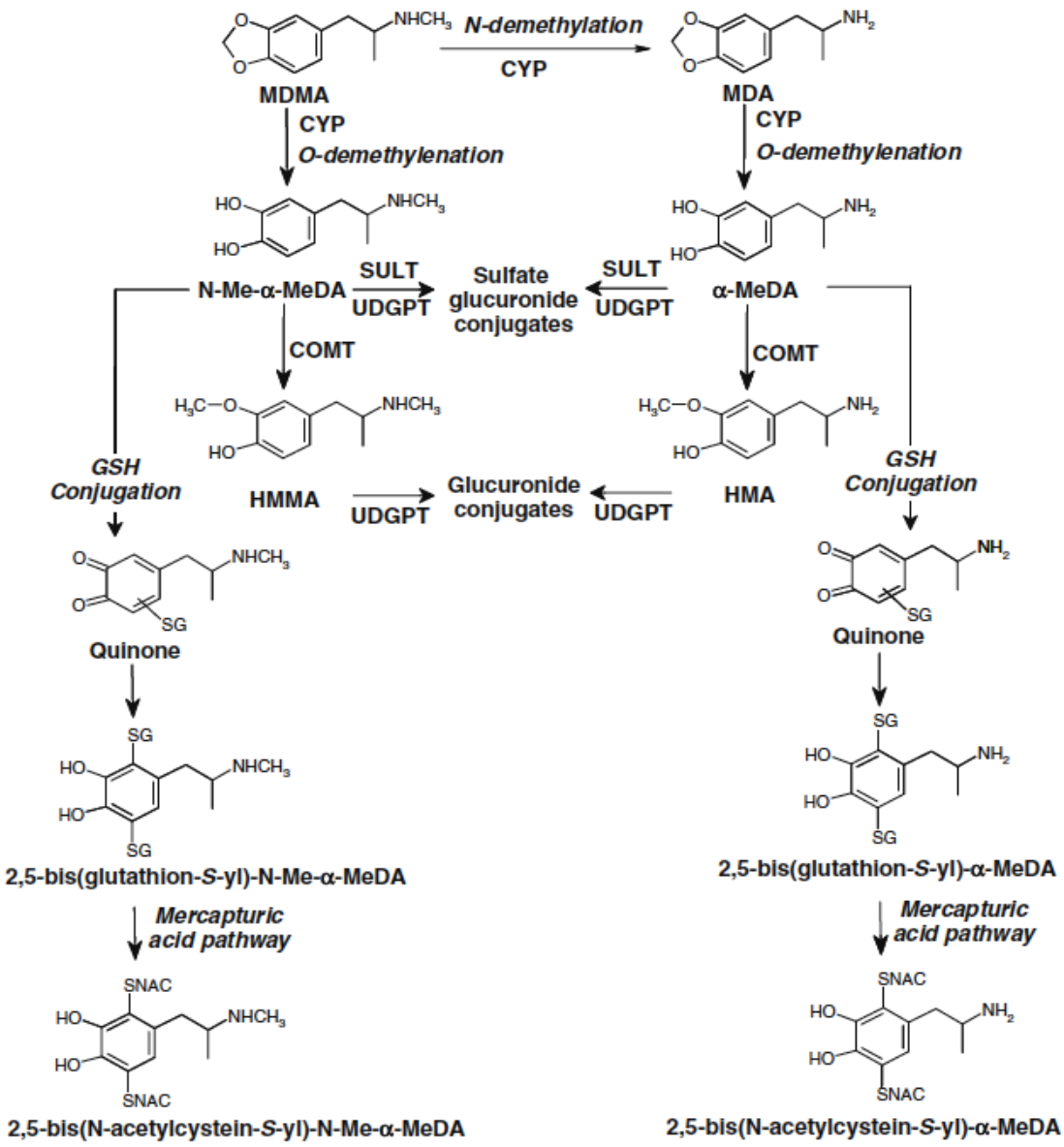


Figure 1. 3: Pathways of MDMA metabolism. Adapted from Capela et al (2009).

There are two major pathways of MDMA metabolism (Figure 1.3). It is *O*-demethylenated to 3,4-dihydroxymethamphetamine (HHMA/N-Me-α-MeDA), a product with neurotoxic potential (Segura et al., 2001) and *N*-demethylenated to 3,4-methylenedioxyamphetamine (MDA) (Goni-Allo et al., 2008a). MDA is further metabolized to 3,4-dihydroxyamphetamine (HHA/α-MeDA) via *O*-demethylenation. HHMA and HHA then undergo *O*-methylation catalysed by catechol-*O*-methyltransferase (COMT) to 4-hydroxy-3-methoxymeth-amphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA), respectively. The half-life for MDA and HMMA following MDMA (10mg/kg i.p) administration is ~2.18 h and 2.73 h, respectively (Baumann et al., 2009). HHMA and

HHA can rapidly undergo oxidaton to the corresponding ortho-quinones, which are highly electrophilic, as they can react with the cysteinyl sulfhydryl group in glutathion (GSH) to form GSH conjugates, which can lead to formation of neurotoxic products (Hiramatsu et al., 1990; Green et al., 2003; de la Torre and Farre, 2004; Erives et al., 2008). Some of MDMA metabolites are pharmacologically active and claimed to be responsible for the hepatotoxic, hyperthermic, and neurotoxic effects (Hiramatsu et al., 1990) which results in difficulty to determine and to assess which metabolites are actually causing the acute and long-term effects of MDMA.

(−)MDA was reported to be more active in humans and has hallucinogenic effect, while (−)MDMA was less active than (+)MDMA and had few or no hallucinogenic effects (Anderson et al., 1978). However, racemic and (+)MDMA produced only amphetamine-liked effects. In contrast, racemic MDA and (−)MDA produced 4-methyl-2,5-dimethoxyphenylisopropylamine (DOM)-liked effects whereas racemic and (+)MDA produced amphetamine-liked effects (Anderson et al., 1978). Since (−)isomers of MDMA and MDA have higher affinities for the radiolabelled 5-HT_2 receptors (Table 1.4), (+)MDMA in humans results in weak hallucinogenic effects, whereas MDA will results in stronger hallucinogenic effects (Teitler et al., 1990).

Table 1. 4: Relative potencies of amphetamine derivatives at selected receptors in the brain, with respect to MDMA. Adapted from Battaglia et al. (1988).

Drug	5HT Uptake	$5HT_2$ Receptor	α_2 Adrenoreceptor	M1 Receptor
MDMA	1	1	1	1
MDA	1.8	0.5	0.5	1.4
MDE	0.4	3.5	3.3	1.8
Amphetamine	4.8	2.6	0.09	4.8
Methamphetamine	3.4	2.4	0.61	3.6

MDMA and MDA metabolism produce α-MeDA (Table 1.5), a reactive compound that can undergo oxidation and conjugation with glutathione (GSH). It has been shown that metabolite of α-MeDA, 5-(Glutathion-S-yl)-α-methyldopamine [5-(GSyl)-α-MeDA] (720nmol, i.c.v.) was rapidly cleared from all brain regions examined (Miller et al., 1995). This finding was in parallel with formation of 5-(cystein-S-yl)-α-methyldopamine [5-(CYS)-α-MeDA, which correlated with regional differences in the distribution of γ-GT. They also indicated the ability of the brain to synthesize mercapturic acids as evidenced by formation of 5-(N-acetyl-ι-cystein-S-yl)-α-MeDA [5-(NAC)-α-MeDA] in all brain regions (hypothalamus > midbrain/diencephalon/telencephalon > pons/medulla > hippocampus > cortex > striatum). Moreover, direct injection of [5-(GSyl)-α-MeDA] and [5-(NAC)-α-MeDA] (Bai et al., 1999), and i.c.v. administration of 2,5-bis-(glutathion-S-yl)-N-Me-α-MeDA (Miller et al., 1997) have been shown to produce prolonged depletion in 5-HT in the striatum, cortex and hippocampus and neurobehavioural changes similar to systemic MDMA and MDA administration.

Table 1. 5: Neurotoxicity of MDMA metabolites. Adapted from Capela et al (2009).

MDMA Metabolite	**Neurotoxicity *in vivo***
HHA	ICV administration was not neurotoxic; s.c. administration produced long term 5-HT neurotoxicity in rats
HHMA	i.p. administration was not neurotoxic; ICV administration produced DA neurotoxicity in mice
HMA	s.c. administration produced long term 5-HT neurotoxicity in rats
5-(GSH)-α-MeDA	ICV administration did not induce long term neurotoxicity; direct intrastriatal or intracortical administration caused long term 5-HT neurotoxicity in rats
5-(NAC)-α-MeDA	ICV administration did not induce long term neurotoxicity; direct intrastriatal or intracortical administration caused long term 5-HT neurotoxicity in rats
5-(NAC)-N-Me-α-MeDA	Direct intrastriatal administration caused long term 5-HT neurotoxicity in rats
2,5-(GSH)-α-MeDA	ICV or direct intrastriatal or intracortical administration caused long term 5-HT neurotoxicity in rats

Despite the findings described above, it may be possible that direct administration of MDMA into the hippocampus and striatum did not produce a hyperthermia response as these brain regions are not involved in the control of body temperature. Hence, it is important to look at the effects of central MDMA and MDA in the preoptic area/anterior hypothalamus (PO/AH) which plays a role in central thermoregulation (Ishiwata et al., 2002; Nagashima, 2006). Apart from that, as mention previously, distribution of [^{3}H]MDA binding to brain membranes is not similar (Zaczek et al., 1989), which suggests uneven effects of MDA on different brain regions.

1.6 Appraisal of methodological approaches used to assess MDMA effects in animal models

Various methods have been used to determine MDMA effects in animal models and the parameters measured include thermoregulatory, behavioural, physiological and neurochemical as summarized in Table 1.6. Although one of the primary aims of these studies were to assess MDMA-induced hyperthermia and subsequent changes in brain neurotransmitter concentrations and resulting neuroroxicity, the methods used to measure these effects have been shown to produce confounding effects following MDMA administration and difficult to relate effects observed in animal models to humans.

References and dose of MDMA	Rats strain	Ambient temperature	Parameters measured		
			Core body temperature	Physiological	Neurochemical
Bexis and Docherty (2006) 20mg/kg s.c.	Male Wistar	22°C	↓2°C (telemetry)	20mmHg MAP (telemetry)	n/a
Clemens et al. (2007) 8mg/kg i.p.	Female Albino Wistar	28°C	↑0.6°C (ear thermometer)	LMA 4000 counts/min (Infrared detector)	↑5-HT 124% in n.acc (microdialysis)
Colado et al. (1995) 10mg/kg i.p.	Male Dark Agouti	21°C	↑1°C (rectal probe)	n/a	↓5-HT 40% long term
Daws et al. (2000) 10mg/kg i.p.	Male Sprague-Dawley	30°C	↑0.5°C (telemetry)	LMA to 250 counts (telemetry)	n/a
Freezer et al. (2005) 10mg/kg i.p.	Male Sprague-Dawley	22°C	↓0.4 then ↑1.8°C (rectal probe)	Behavioural response Score 3-3.5	↑5-HT 600% of baseline (microdialysis)
Gordon et al. (1991) 30mg/kg s.c.	Male Long-Evans	30°C	↑3°C (rectal probe)	LMA 350 units/60 mins (Doppler system)	n/a
Jaehne et al. (2005) 10mg/kg i.p.	Male Sprague-Dawley	30°C	↑3.5°C (telemetry)	HR 450 bpm LMA 1000 counts/min (telemetry)	n/a
McGregor et al. (2003) 4 X 5mg/kg i.p.	Male Wistar	28°C	↑2.8°C (ear thermometer)	LMA 17000/4h counts (Infrared detector)	↓5-HT 60ng/g long term
Nixdorf et al. (2001) 100µM	Male Sprague-Dawley	22°C	No significant change (rectal probe)	n/a	↑DA 80pg/20µl ↑5-HT 8pg/20µl (microdialysis)
Stanley et al. (2007) 10mg/kg i.p.	Male Sprague-Dawley	30°C	↑1.5°C (rectal probe)	Behavioural response Score 3-4	↑5-HT 375% (microdialysis)

Table 1. 6: Summary of a number of MDMA studies.

Some studies used rectal temperature measurement to record core body temperature following MDMA administration (Gallaher et al., 1985; Colado et al., 1995; Marston et al., 1999; Freezer et al., 2005; Stanley et al., 2007). From Table 1.6, at normal ambient temperature, it was shown that Tc measured using rectal probe showed an initial decrease in Tc followed by subsequent increase in Tc (Freezer et al., 2005). Whe Tc was measured using telemetry, the rats showed hypothermia response (Bexis and Docherty, 2006) which is consistent with other studies which reported hypothermia following MDMA administration at normal ambient temperature (Marston et al., 1999). Interestingly, another study which administered MDMA at normal ambient temperature showed no significant change in Tc (Nixdorf et al., 2001). However, this result is consistent with other studies which found no significant change in Tc following central administration of MDMA (Esteban et al., 2001; Goni-Allo et al., 2008a).

This rectal measurement requires handling of rats and repeated insertion of rectal probe into the rectum thus producing stress-induced increase in body temperature (Gordon, 1990) and can also affect their heart rate, blood pressure and also locomotor activity. In Table 1.6, it can be seen that locomotor activity following MDMA administration varies in normal and high ambient temperatures, which makes it difficult to interpret. However, in a study by Clement et al. (1989), it was demonstrated that when mice were handled and pick up for 15 sec, the body temperature and activity increase. In the same study, mice were also handled repeatedly at 5 min intervals for 30 min which resulted in increase in body temperature and activity (Clement et al., 1989). For neurochemical analysis, rats which Tc was measured using rectal probe showed a higher extracellular levels of 5-HT ranging from 375 to 600% from baseline (Freezer et al., 2005; Stanley et al., 2007), in comparison to rats which Tc was measured with less invasive technique such as ear thermometer which showed an increase of 124% from baseline (Clemens et al., 2007).

A method, known as the telemetry, has been developed capable of monitoring core body temperature, blood pressure, heart rate and locomotor activity which does not involve restraining and repeated insertion of rectal probe thus eliminating stress-induced increase in body temperature (Clement et al., 1989; Dilsaver et al., 1990; Irvine et al., 1997) and eliminating confounding effects on heart rate, blood pressure, and locomotor activity (Wright et al., 1989; Guiol et al., 1992; van den Buuse, 1994; Gegout-Pottie et al., 1999). Previous study has also shown that measurement of temperature in rats using rectal probe and thermosensor telemetry produces qualitatively similar results but quantitatively different (Dilsaver et al., 1990). Researchers have used telemetry to measure the effects of MDMA on these parameters in animal models (Bexis and Docherty, 2005; Jaehne et al., 2005; Bexis and Docherty, 2006). In the present study, we will be using telemetry which is used to continuously monitor core body temperature, heart rate and locomotor activity of the rats.

1.6.1 Telemetry

1.6.1.1 History

Telemetry was first used in 1959 for ecology study in which the transmitters were designed for implantation in the woodchucks (Le Munyan et al., 1959). Since then, more approaches have been made to produce miniaturize transmitters that are smaller, more durable and have longer transmitting life expectancies (Houseknecht, 1970). During this period, telemetry was used for simple application and its use was limited for measurement of one physiological parameter, which is body temperature. The technique has evolved to more complex system over the last thirty years capable of measuring several physiological paramaters including cardiovascular (Murray et al., 1968; Fryer et al., 1975; Rubenson et al., 1984), locomotor activity (Clement et al., 1989), heart rate (Gordon et al., 1991; Irvine et al., 2006) variables in large animals. In the 1990s, telemetry has been developed to evaluate continuous spontaneous locomotor activity and body temperature in rodents, in

experimental models of inflammation (Gegout-Pottie et al., 1999), and simultaneous measurement of diastolic and systolic blood pressures and heart rate (Wright et al., 1989; Guiol et al., 1992).

1.6.1.2 Design methodology of telemetric system

The telemetric system has been used to collect physiological data such as core body temperature, heart rate, and locomotor activity (Table 1.7) in conscious, freely moving animals. It consists of an implantable transmitter, receiver cosolidation matrix and computer with acquisition software (Guiol et al., 1992; Rodsiri et al., 2011) as shown in Figure 1.4. The transmitters and receivers are available in variable size and models to allow optimum usage for different animal models ranging from mice and rats to dogs and primate. The transmitter devices are surgically implanted and can continuously transmit data via radio frequency signals to a nearby receiver, which is then collected by the data acquisition system.

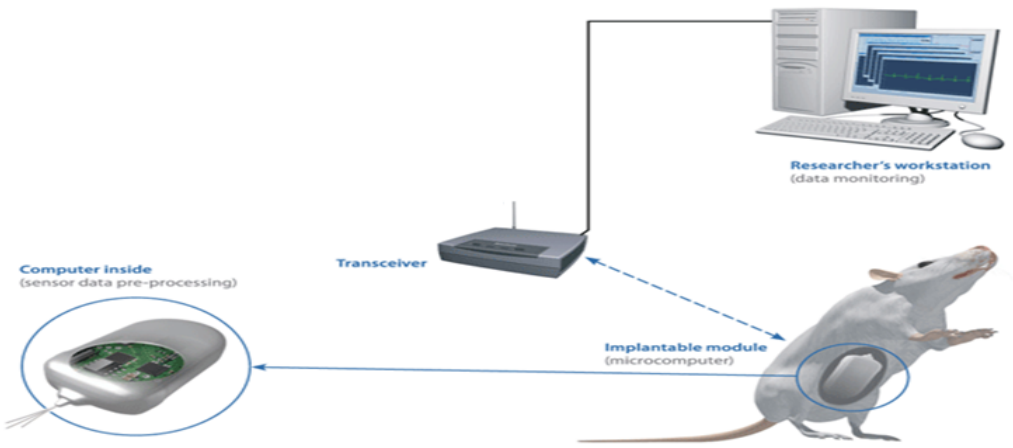


Figure 1. 4: Diagram of a telemetric technique setup.

Table 1. 7: Studies using telemetry to assess the effects of MDMA in animal models.

References	Subject	Parameters measured
Bexis et al. (2005)	Mice	Tc
Bexis and Docherty (2006)	Wistar rats	Tc, HR, LMA
Daws et al. (2000)	SD rats	Tc and LMA
Jaehne et al. (2005, 2007, 2010)	SD rats	Tc, HR, LMA

1.6.2 Microdialysis

1.6.2.1 History

Before the development of microdialysis, previous studies which measured transmitter extracellular levels in anaesthetized and freely moving animals used the push-pull technique and i.c.v perfusion to collect substances such as acetylcholine and 5-HT (Myers, 1970). The i.c.v perfusion technique can only be done in relatively large areas of brain tissue. The push-pull cannula technique allows collection of endogenous neurochemical compounds in the brain (Myers, 1970; Lavenhar and Palanker, 1976; West et al., 1992). The cannula is connected to inlet and outlet tubing. Perfusion is carried out by attaching the inner cannula and the reservoir outflow tube with polyethylene tubing to glass syringes, driven by reciprocating pump (Yaksh and Yamamura, 1974). However, this technique cause lesion in the brain due to the large size of the implanted cannula, and direct contact between the dialysate and the tissue, the flow rate used during perfusion (Redgrave, 1977; Dluzen and Ramirez, 1986; Zhang et al., 1992; Bourne, 2003). In 1966, Bito et al. (1966) introduced the concept of collecting samples from small interstitial tissue using a dialysis bag. This technique is refined by using dialytrode to perfuse solution through the dialysis bag and accessible for collection. Ungerstedt and Pycock (1974) improved the design by increasing the surface area of the dialysis membrane and the efficiency of the probe to collect substances, in which contributed significantly to the use of microdialysis technique nowadays.

1.6.2.2 Design methodology of microdialysis system

Microdialysis is a technique used to measure chemical subtances in a tissue of interest continuously in freely moving animals. The microdialysis system consists of a probe and guide cannula, microinjection pump, inlet and outlet tubing, and collection tube. The probe consists of a small semipermeable hollow fibre membrane, connected to an inlet and outlet tubing. Following the animal recovery, the dialysis tube is perfused with solution of interest by the microinjection pump at certain flow rates through the inlet tubing. Molecules pass the semipermeable membrane will diffuse down their concentration gradient into or out of perfusate (de Lange et al., 2000). This solution (dialysate) will then leave the probe through the outlet tubing and collected using a tube. The concentration of the drug in the dialystes reflect the concentration of the drug in the extracellular fluid around the semipermeable membrane (de Lange et al., 2000). The dialysates can be analyzed using various analytical technique such as High Performance Liquid Chromatography (HPLC) with electrochemical detection and liquid chromatography with mass spectrometry (LC-MS).

The no-net-flux microdialysis method can also be used to determine *in vivo* recovery and involves the perfusion of different concentrations of the neurotransmitter of interest through the dialysis probe under steady-state condition. If a linear relationship and true steady-state conditions exist, the slope of the line gives the dialysis recovery of the neurotransmitter. However, this method of calibration involves lengthy experimental procedure and it is not suitable for the type of experiments conducted in our study (de Lange et al., 1997).

The probe and cannula are stereotaxically placed in the brain of an animal under anaesthesia. They come in various membrane lengths such as 2mm and 4mm to

accommodate different tissue size and region such as in the brain, liver, and kidney (Chefer et al., 2009). Every type of dialysis probe produces certain tissue damage when implanted into the brain (Santiago and Westerink, 1990). It has been demonstrated that neuronal tissue lesion is directly related to the size of the cannulas (Westerink and De Vries, 1988). This study shows that U-shaped cannula causes small ruptures and signs of bleeding in comparison to transtriatal probe which causes no ruptures and occasional signs of bleeding.

The microdialysis technique has several advantages. The advantage of microdialysis in comparison to other *in vivo* perfusionis is due to the probe size (Chefer et al., 2009). The probe is smaller than the devices used in push-pull technique, thus access smaller area of tissue. Second, the membrane provides a physical barrier between the perfusate and tissue, thus protecting the tissue. This technique also allow lower flow rate in comparison to other perfusion technique, thus reducing analyte depletion. Other than that, it allows monitoring of endogenous substances, allows continous and uninterrupted flow overtime without constant visual monitoring, less degradation of compounds (oxidative enzymes do not pass through the membrane) (Myers et al., 1998). Microdialysis also reflects free concentrations in tissues and plasma, which allows access to information on drug transport equilibration across membranes (de Lange et al., 2000) and collection of microdialysates at certain time interval to measure neurotransmitter extracellular levels and drugs concentration in the brain. Microdialysis also allows local perfusion of a drug into a discrete brain region. This is important especially in studies which are interested in metabolism of certain drugs and direct effects of the drugs in a brain region. Since tissue trauma can occur following probe implantation (Drijfhout et al., 1995), it is important to determine the optimal times after the implantation for the substances of interest. It is optimal to collect data 1 and 2 days after implantation, which allow recovery from tissue reactions and before the long-term reactions occur (de Lange et al., 2000). It is also important to be aware of environmental

stress, as this has been shown to linked with an increase in DA and other neurotransmitters, which can result in incorrect measurement of basal levels of the neurotransmitters. However, this limitation can be reduced by placing the animals in the habituation cage and allowing habituation times before data collection (Bourne, 2003). Examples of tissues and compounds analysed by microdialysis are shown in tables 1.8 and 1.9.

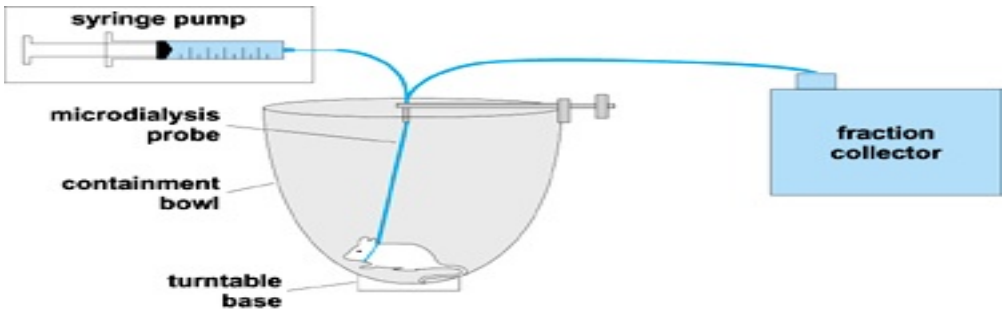


Figure 1. 5: Diagram of a microdialysis technique setup (www.accessscience.com)

Table 1. 8: Examples of tissue analysed by microdialysis. Adapted from Chefer et al. (2009).

Tissues Analyzed by Microdialysis

Organ system	Reference
Adipose tissue	Hallstrom et al., 1989
Adrenal glands	Kuzmin et al., 1990
Blood	Sjoberg et al., 1992
Bone	Thorsen et al., 1996
Brain	Hallstrom et al., 1989
Heart	Kuzmin et al., 1992
Ileum	Fukui et al., 1993
Kidney	Siragy, 1992
Liver	Okuda et al., 1992b
Lung	Larsson, 1991
Luteal tissue	Sauerwein et al., 1992
Muscle	Hallstrom et al., 1989
Pancreas	Jonsson et al., 1992
Retina	Louzada-Junior et al., 1992
Skin	Petersen et al., 1992b
Spinal cord	Linderoth et al., 1992
Spleen	Shimizu et al., 1994
Subcutaneous tissue	Deleu et al., 1993
Uterus	Nordenvall et al., 1989

Table 1. 9: Examples of compounds analysed by microdialysis. Adapted from Chefer et al. (2009).

Compounds Analyzed by Microdialysis

Substance[a]	Reference
N-acetylaspartate	Taylor et al., 1994
N-[^{3}H]acetylaspartylglutamate	Tsai et al., 1988
Acetylcholine	Consolo et al., 1987; Damsma et al., 1987
Adenosine	Chen et al., 1992; Lonnroth et al., 1989
Ascorbate	Hallstrom et al., 1989
Aspartate, D-[^{3}H]aspartate	Kendrick et al., 1988; Nielsen et al., 1989
Bradykinin	Siragy et al., 1993
Carbamazepine, carbamazepine epoxide	Scheyer et al., 1994a
Cortisol and corticotropin-releasing hormone	Cook, 2001
Cyclic AMP	Stone and John, 1992
5-*S*-cysteinyldopa	Blomquist et al., 1991
3,4-dihydroxyphenylethyleneglycol	Itoh et al., 1990
Dopamine and metabolites	Imperato and Di Chiara, 1984; Zetterstrom et a
Eicosanoid	Callaghhan et al., 1994
Endogenous opioid peptides	Maidment et al., 1989
Epinephrine	Dev et al., 1992
Ethanol levels	Ferraro et al., 1990
Follicle stimulating hormone	Lincoln, 1992
GABA	Bourdelais and Kalivas, 1992
Glucose	de Boer et al., 1992
Glutamate	Dietze and Kushinsky, 1992
Glutathione and other thiols	Dizdar et al., 1991
Histamine	Petersen et al., 1992a,[b]
5-hydroxyindoleacetic acid	Guadalupe et al., 1992
5-hydroxytryptophol	Yoshimoto et al., 1992
Hypoxanthine	Hagberg et al., 1987
Imipramine and metabolites	Sato et al., 1994
Inorganic phosphate	Scheller et al., 1992
Inosine	Hagberg et al., 1987
Kynurenic acid	Russi et al., 1992
Lactate	Hallstrom et al., 1989; Okuda et al., 1992a,[b]
Levodopa, DA, and their metabolites	Deleu et al., 1993
LHRH	Steele et al., 1992
Melatonin	Hasegawa and Ebihara, 1992
Methotrexate	Matsuyama et al., 1994
3-*O*-methyldopa	Deleu et al., 1993
MHPG	Kubota et al., 1993
Neurokinin A	Lindefors et al., 1989a
Neuropeptide Y	Lambert et al., 1994

Previously, there have been separate studies looking at the physiological changes following MDMA administration and the extracellular levels of neurotransmitters in the brain (Table 1.10). These studies tried to relate physiological changes following acute MDMA administration with long-term 5-HT and DA concentrations in the rat brain. Hence, these studies are unable to relate acute physiological changes with simultaneous 5-HT and DA extracellular levels in the brain. In addition, as microdialysis causes no net fluid loss, samples can be collected continuously from a single freely moving animals for hours or days (Li et al., 2006). Other studies which sacrifices rats at different time points and measure the exogenous and endogenous substances in the tissue of interest would measure the total concentrations of bound and unbound drugs hence unable to determine extracellular concentrations of the drugs. However, microdialysis allows measurement of the free drug concentrations that bind to the receptors. This will provide pharmacokinetic information for drugs which interact with receptors in the extracellular space (de Lange et al., 2000). Since MDMA is a very lipid soluble substance, it is possible for MDMA to diffuse from the probe site to other brain areas, and removal from the brain via plasma clearance might produce a lower concentration in the brain than that is required to produce pharmacodynamic effects. A study by Esteban et al. (2001) placed a second probe near the first probe (1mm) to recover MDMA from the perfusion of the first probe. Following perfusion of 400μM MDMA for 6 h, the estimated actual concentration of MDMA is between 10.4 and 19.5μM, which is in the range of concentrations found following systemic administration of 10mg/kg MDMA.

Table 1. 10: Previous microdialysis studies looking at the effects of MDMA.

References	Subject	Brain regions	Functions
Freezer et al. (2005)	SD rats	Striatum	5-HT and 5-HIAA collection
Stanley et al. (2007)	SD rats	Striatum	5-HT and 5-HIAA collection
Mechan et al. (2002)	DA rats	Hippocampus	5-HT and DA collection
Koch and Galloway (1997)	SD rats	Striatum	5-HT and DA collection
Goni-Allo et al. (2008)	Wistar rats	Striatum	Central MDMA administration
Nixdorf et al. (2001)	SD rats	Striatum	Central MDMA administration and 5-HT and DA collection
Esteban et al. (2001)	DA rats	Hippocampus	Central MDMA administration and 5-HT, DA and MDMA collection

1.6.2.3 Ethical implications of experimental design

Some studies used methods which involved postmortem analysis at several single time points after drug administration, in which each animal provides data for only one time point. This method is usually performed when analysing drug, blood, plasma, or neurotransmitters concentrations in the brain at certain time points. Since microdialysis is a real-time analysis, it allows continuous sampling during the entire course of drugs administration for each subject. Apart from continuous sampling, the animals can be used again after the drug washout period. These results in the ability to reduce the number of subjects needed in comparison to other methodological approach.

1.6.3 Combined telemetry and microdialysis

It has been demonstrated previously that both telemetry and microdialysis are good methodological approaches to assess MDMA effects in animal models, especially the effect on core body temperature. However, they provide a better methodological approach if they are use simultaneously. Although telemetry and microdialysis have been used previously for decades, the combined telemetry and microdialysis have only been used over the past 5 years (Benamar et al., 2008; Rodsiri et al., 2011). Interestingly, both of these studies used telemetry only to record Tc. Benamar et al. (2008) used different type of telemetry, known as biotelemetric-thermosensor which emits Hertzian waves to a receiver, at a rate directly proportional to temperature (Dilsaver et al., 1990) to measure Tc whereas Rodsiri et al. (2011) used telemetry with radio-transmitter to measure Tc and LMA.

Table 1. 11: Studies of MDMA using combined telemetry and microdialysis techniques.

References and dose	Telemetry	Brain region	Parameters measured		
			Core body temperature	Physiological	Neurochemical
Benamar et al. (2008) 10, 20 and 30mg/kg	Thermosensor	PO/AH	↑1.3, 2.0, and 2.3°C (respectively)	n/a	DA ↑1000%
Rodsiri et al. (2011) 3 and 6mg/kg	Radiotransmitter	Hippocampus	↓1.5, and ↓0.7-↑1.2°C (respectively)	LMA 20 counts/min	5-HT ↑134%, and 555% (respectively)

Benamar et al. (2008) used combined telemtry and microdialysis techniques to evaluate the relationship between MDMA-induced hyperthermia and the extracellular level of DA in the PO/AH. Following MDMA administration, it is shown that 20mg/kg MDMA produces significant hyperthermia accompanied by increase in extracellular DA in the PO/AH. Both of these effects decreased following pretreatment of D1 receptor antagonist, SCH 23390. On the other hand, Rodsiri et al. (2011) used combined telemetry and microdialysis techniques to evaluate the effects of MDMA on Tc, LMA and their relationship with hippocampal 5-HT extracellular levels. It was shown in this study that MDMA administration produces significant changes in Tc (both hypothermia and hyperthermia) and acute hyperactivity. However, it was demonstrated that changes in LMA and Tc are not related to the magnitude of 5-HT extracellular levels in the hippocampus. From both of the studies mention above, it can be seen that combined telemetry and microdialysis techniques provide a better approach to measure physiological and behavioural changes following MDMA administration and to relate the changes to neuropharmacological changes in the brain, in particular changes in extracelullar monoamine concentrations.

Although both of the studies measured Tc following MDMA administration, they did not measure heart rate, a parameter also affected by MDMA. It has been established that MDMA disrupts cardiovascular function and toxicity such as cardiac arrhythmias and myocarditis (Badon et al., 2002). Since MDMA affects cardiovascular system, it is also important to measure heart rate in addition to Tc and LMA. In addition, it has been shown that MDMA effect on Tc is related to its effect on HR. Previously in MDMA studies, mean arterial pressure (MAP) was measured to elucidate the effects of MDMA on cardiovascular effect and HR was derived from MAP reading using a software (O'Cain et al., 2000). Previous studies started measuring HR using telemetry following MDMA administration since the early 1990s. Gordon et al. (1991) was the first to show preliminary data using telemetry on the effects of MDMA in unstressed and unrestrained animal. However, these studies did not measure and compare systemic administration of MDMA and central perfusion of MDMA and MDA at bioequivalent doses within the brain including correlatation of pharmacodynamics responses with changes in striatal extracellular 5-HT concentrations.

Previously, the measurements of body temperature and extracellular neurotransmitters levels have been done in separate groups of animals which resulted in diffculties to relate thermoregulatory effects with neurochemical effects. This combined technique allows continuous simultaneous measurement of core body temperature, heart rate, locomotor activity and changes in extracellular neurotransmitters level. This method is important to elucidate the association between acute physiological changes following MDMA administration and extracellular neurotransmitters levels in a specific brain region. Figure 1.7 shows the advantages of the telemetry and microdialysis techniques. The combined techniques provides several advantages over the previous methodological approach. It allows the researcher to measure Tc, HR, and LMA without the necessity of handling the

rats, thus eliminating stress and other confounding factors on Tc, HR and LMA. This technique allows continuous monitoring and when combined with microdialysis, can be used to relate the physiological and behavioural changes to changes in monoamine levels in the brain. It is also a simple method with real-time analysis using a smaller number of animals which can be used throughout the experiment instead of sacrificing animals at each time points for analysis. The dialysates collected by microdialysis can be used to measure endogenous and exogenous substances in the brain and also free drug concentrations. Apart from that, this technique also allows central administration of drug into the brain continuously. Overall, the combined telemetry and microdialysis techniques provide an essential tool to measure MDMA effects on physiological, behavioural and neuropharmacological changes.

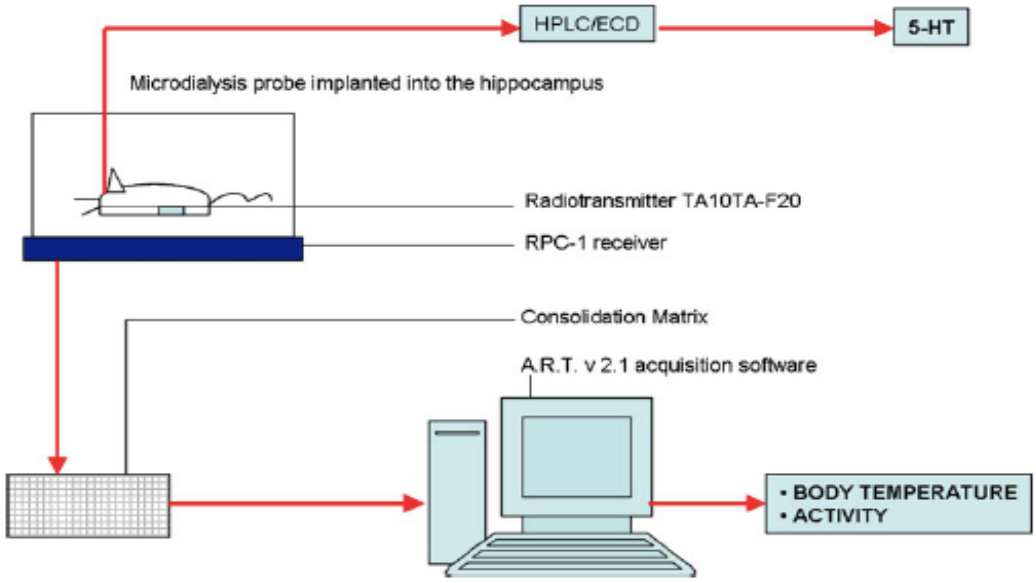


Figure 1. 6: The diagram of combined telemetry and microdialysis techniques setup. Adapted from Rodsiri et al. (2011).

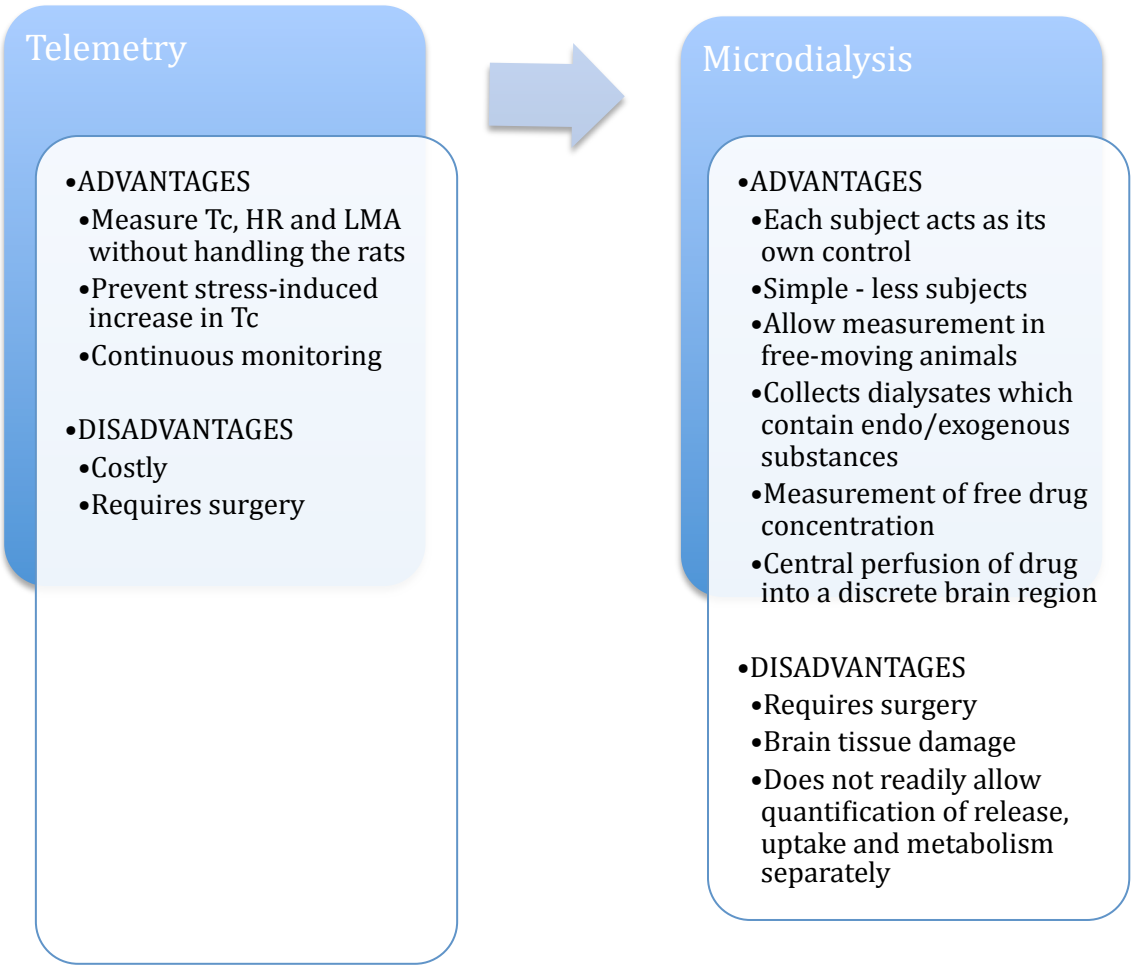


Figure 1. 7: The advantages and disadvantages of telemetry and microdialysis techniques.

1.7 Aims and hypotheses

This study aims to:

1) Investigate effects of different methods (rectal probe and telemetry) used to measure core body temperature and behaviour following MDMA administration which are the telemetry and rectal probe; and
2) Validate the use of combined telemetry and microdialysis techniques to assess MDMA and MDA effects on core body temperature, heart rate, locomotor activity and 5-HT extracellular levels following systemic and central administration.

The hypotheses for this study are:

1) MDMA-induced hyperthermia and behavioural response will be significantly potentiated when Tc is measured using rectal probe in comparison to MDMA-induced hyperthermia and behavioural response when Tc is measured using telemetry.
2) Use of combined telemetry and microdialysis techniques will provide better assessment of measuring and correlating physiological changes with neurochemical changes following systemic administration of MDMA and central administration of MDMA and MDA.
3) Central administration of MDA will produce potentiated increase on physiological and neurochemical changes compared to central administration of MDMA.

Chapter 2 MDMA-induced hyperthermia: The influence of methodological approaches used to measure core body temperature

2.1 INTRODUCTION

The most commonly used method to assess MDMA effects on core body temperature (Tc) in conscious animal model is the rectal probe technique. Rectal temperature measurement is usually done via insertion of a rectal probe 5 – 9 cm into the rectum. A study by Lomax (1966) has demonstrated the importance of distance for probe insertion. It is shown that a distance of 6 to 8 cm was optimal as body temperature measurements are erroneously low and variable if the distances were less than 5 cm (Lomax, 1966). Although this technique is considered as simple, less invasive and does not involve surgery it has been shown to produce stress-induced increase in body temperature (Martin et al., 1977; Poole and Stephenson, 1977; Gordon, 1990).

This stress-induced increase in body temperature occurs due to the need of restraining by hand or restraining device when measuring body temperature, and intermittent or chronic insertion of the probe into the rectum. It has been shown that a single insertion of rectal probe for 1 min caused a sustained increase in body temperature of 1°C for about 70 min after removal of the probe at ambient temperature of 23°C (Poole and Stephenson, 1977). A study by Gallaher et al. (1985) has also demonstrated that rectal temperature measurement causes approximately 1°C increase in body temperature in thermosensor-implanted rats that did not fully recover for over 3 hours.

Restraining can also affect cardiovascular and behavioural parameters aside from body temperature. It was shown that when rats are restrained, it causes significant effects on their heart rate and blood pressure (Irvine et al., 1997). In this study, Irvine et al. (1997)

measured heart rate and blood pressure using telemetry in rats restrained in a plastic tube (100mm in diameter). After a brief restraining, blood pressure and heart rate returned to normal after 20 and 10 min, respectively. This type of restraining is more stressful than restraining during rectal probe measurement. A study by Clement et al. (1989) has demonstrated that handling of mice caused an increase in body temperature and activity in transmitter-implanted mice. In the study, mice were removed from the cage and held for 15 sec as they would be if they were to be injected with a drug. In this study, the increase in core body temperature coincided with an increase in activity (Clement et al., 1989). Although the results showed no significant difference in core body temperature between the two methods used (rectal probe and telemetry), it demonstrated that temperature increase, probably the result of handling the mice. However, most studies using animals have habituated and handled the animals prior to the start of experiment (48 hours earlier) to reduce stress and eliminate confounding factor (Freezer et al., 2005; Stanley et al., 2007; Benamar et al., 2008).

MDMA administration causes significant change in body temperature and it is important to be aware of any confounding factors that could possibly disrupt the measurement of actual MDMA effects during the experiment. It is very important to get accurate reading on core body temperature following MDMA administration as this parameter has an impact on other parameters such as the magnitude of neurotoxicity. A study by Malberg and Seiden (1998) which used temperature-sensitive transmitter to measure Tc have shown that small changes in core body temperature produces significant changes in long-term neurotoxicity. In this study, rats were allowed 1 hour habituation period to prevent hyperthermia induced by exploratory locomotion from interfering with the effects of the drug. Then, they were treated with 20 or 40 mg/kg MDMA at different ambient temperatures from 20 to 30°C and core body temperature and regional 5-HT levels (frontal cortex, somatosensory cortex,

hippocampus, and striatum) were analyzed as a measure of neurotoxicity. It was shown that at ambient temperature of 26 to 30°C, a decrease level of 5-HT was observed in long-term and correlated with core temperature in all brain regions examined (frontal cortex, hippocampus, striatum, and somatosensory cortex). As mentioned in the previous chapter, MDMA administration produces increase in extracellular 5-HT levels. However, stress can also cause neurotransmitters release in the brain. It has been shown that restraint stress significantly increases 5-HT extracellular levels in the central nucleus of the amygdala (Mo et al., 2008). In this study, rats were placed in a restraining tube (6 cm id, 27 cm long) which prevents rats movement for 40 min while dialysates were collected for extracellular 5-HT measurement (Mo et al., 2008). As restraint stress could contribute to increase in extracellular levels of 5-HT in the brain, this effect could potentially lead to stress-induced neurotoxicity. It has been demonstrated that administration of 5 mg/kg MDMA i.p. at ambient temperature of 24°C produced a significant depletion of 5-HT and DA in the striatum 5 days post-treatment, which was potentiated by pre-exposure to chronic unpredictable stress (Johnson and Yamamoto, 2010). Exposure to chronic restraint stress also potentiates metamphetamine induced neurotoxicity in rats (Quinton and Yamamoto, 2007).

Although change of acute extracellular levels of 5-HT in the brain is not used as a marker of MDMA-induced neurotoxicity, it is important to have a better understanding of factors that influence MDMA-induced acute hyperthermia and this includes changes in 5-HT extracellular levels.

Environmental conditions including low or high ambient temperatures influence the magnitude of changes in body temperature in response to MDMA. These changes are relevant to conditions of clubs and raves where the drug is most commonly taken (Green et

al., 2003; Parrott et al., 2008). In the present study, a high ambient temperature of 29±1°C was chosen as it can clearly show the hyperthermic effect following MDMA. Although a number of studies have shown that using rectal probe to measure Tc could result in stress-induced increase in body temperature, no evidence has been presented that compares rectal probe measurement and telemetry in rats, especially their application to assess MDMA effect on core body temperature.

The aim of this study is to compare two methodological approaches to assess MDMA effect on Tc in rats at high ambient temperature (29 ± 1°C). We have evaluated the effects of MDMA on core body temperature using the rectal probe and telemetry. It is hypothesized that MDMA-induced hyperthermia will be significantly potentiated when measured using rectal probe in comparison to telemetry. It is also hypothesized that rats which Tc is measured using rectal probe will have a higher behavioural score in comparison Tc measured using telemetry following MDMA administration.

2.2 MATERIALS AND METHODS

2.2.1 Animals

Male Sprague-Dawley (SD) rats (250-300 g) were supplied by the Laboratory Animal Services, The University of Adelaide (SA, Australia). The rats were housed in groups of 2-4 and were kept under a 12 hour light/dark cycle and constant temperature (22ºC) with free access to food and water. All procedures of the experiment have been approved by the University of Adelaide Animal Ethics Committee and carried out in accordance with National Health and Medical Council of Australia Guidelines for the Care and Use of Laboratory Animals.

2.2.2 Rectal temperature measurement

Temperature measurement was performed using a rectal thermometer with digital readout (Thermalert TH-8 monitor; Physitemp Instruments, Inc., Clinton, NJ), and a lubricated rectal temperature probe with petroleum jelly. Rat was lightly restrained by hand while the probe was inserted 5cm into its rectum for 10 sec until a steady reading was obtained. The behavioural testing was conducted before body temperature measurement every 30 minutes to minimise confounding factors due to the need of restraining the rat by hand.

2.2.3 Radiotelemetry

Rats were anesthetized with sodium pentobarbital (60mg/kg i.p) and placed on water-heated pad (37ºC) to maintain body temperature. An incision was made below the ribcage and the implants were placed into the rats' abdominal cavity. The implants were stitched to the muscle to keep the implants in place. One lead was placed near the chest and held with some stitches. Another lead was placed in the back near the shoulder blade and held with stitches. The abdominal wall was then stitched and covered with topical antibiotic powder. The incision was then closed with suture clips and rats were allowed one-week recovery period before further treatments. After each surgery, the rats' conditions were recorded in a

clinical record sheet (Appendices). The clips were removed 1 or 2 days later. The system consists of telemetry devices (TA11CTA-F40, Data Sciences International, USA), which measure core body temperature (Tc), heart rate (HR) and locomotor activity (LA), as reported previously (Jaehne et al., 2005; Bexis and Docherty, 2006; Benamar et al., 2008; Huetteman and Bogie, 2009). The telemetric system also consists of a radio-receiver (RLA 1020), placed on the bottom of the Perspex bowl during data collection. Radio receivers, received information from the implants and transferred it to a computer which recorded the data using Dataquest LabPro software (Data Sciences International, USA). Data were recorded every two minutes over the experimental sessions and presented as mean of temperature data 1-6 minutes.

2.2.4 Behavioural score

Behavioural responses were scored using a scale used by (Molloy and Waddington, 1988). This scale has been used in previous studies to measure the effects of stimulant drugs on the behavioural responses of male Sprague-Dawley rats, which is similar to the 'serotonin syndrome' observed in rats. For each observation, the rat's behaviour was rated and scored. The scores for the behavioural responses are as the following: 0 = asleep or inactive; 1 = episodes of normal activity; 2 = discontinuous activity with bursts of prominent sniffing or rearing; 3 = continuous stereotyped behaviour along a fixed path; 4 = continuous stereotyped behaviour fixated in one location; 5 = stereotyped behaviour with bursts of licking or gnawing; 6 = continuous licking or gnawing.

2.2.5 Drug treatments

MDMA was given as the hydrochloride salt. Rats were treated with either saline or 10mg/kg MDMA i.p. and the Tc was measured using either rectal probe or telemetry. The rats were placed in the microdialysis bowl in a room where the ambient temperature was maintained at 29ºC, that resulted in MDMA-induced hyperthermia in rats without causing fatalities (Green et al., 2003; Jaehne et al., 2005; Stanley et al., 2007). The rats were

allowed 2 hours pretreatment time. For rats which Tc was measured using rectal probe, rectal temperature measurement was performed every 30min for 240min. The rats were administered with 0.9% saline (1ml/kg) or 10mg/kg MDMA. This systemic dose of 10mg/kg MDMA has been shown to produce reliable changes in body temperature and locomotor activity in rats (Daws et al., 2000; Jaehne et al., 2005; Stanley et al., 2007), and comparable to 'ecstasy' used and blood concentration found in humans (Green et al., 2003; Irvine et al., 2006; Jaehne et al., 2011) as shown in Table 1.6.

2.2.6 Chemicals and reagents

(±)MDMA was obtained from the Australian Government Analytical Laboratories (Sydney, Australia). Chloral hydrate and sodium chloride were purchased from BHD Laboratory Supplies Pty. Limited (Victoria, Australia).

2.2.7 Data analysis

Tc and area under curve (AUC) were analysed with unpaired t-test and repeated measures one-way ANOVA with Tukey post-hoc test as shown in previous study (Jaehne et al., 2008). Due to some missing data as a result of a number of rats not surviving the whole duration of the experiment, two-way repeated measures ANOVA was not used. All relevant data within this study employed two-way repeated measures ANOVA as indicated in chapter three. Behavioural response was analysed with unpaired t-test and Kruskal-Wallis non-parametric test with Dunn's post-test. All results are presented as mean ± SEM and considered statistically significant when $P<0.05$. All calculations and analysis were done using Graph Pad Prism software.

2.3 RESULTS

2.3.1 Core body temperature

In both control groups (saline-treated rats), Tc did not showed significant increase or decrease when measured with rectal probe or telemetry. Systemic administration of 10mg/kg MDMA produced significant increase in Tc in comparison to control. When Tc was measured with rectal probe ($F = 70.94$), MDMA caused an increase in Tc from 30 min (2.408 ± 0.506°C, $P<0.01$, $n = 6$) to 210 min with peak at 60 min post-treatment (3.575 ± 0.339°C, $P<0.001$, $n = 6$), and then a decrease throughout the experiment to 1°C (Figure 2.1). When Tc was measured with telemetry ($F = 39.8$), MDMA caused a slight decrease in Tc at 30 min (-0.234 ± 0.263°C), followed by a significant increase at 90 min to 210 min with peak at 150 min post-treatment (2.321 ± 0.885°C, $P<0.001$, $n = 4$), and then a decrease throughout the experiment to 1.5°C (Figure 2.2). For comparison purposes, the data for telemetry groups taken every 30 minutes were used for Figure 2.3. In Figure 2.3, Tc is significantly higher at 30 and 60 min ($P<0.05$, $n = 4$-6) when measured with rectal probe (differences of 2.2°C and 2.3°C, respectively) in comparison to Tc measured with telemetry. Figure 2.3 (B) shows that Tc in MDMA groups were significantly higher than their respective saline groups (0 – 240 min). Although AUC for the entire 240 minute time course shows no difference between rectal or telemetric recording approaches, the case was different if we are looking at temporal effects and onset of MDMA effects between these two methods of measurements. Figure 2.3 (C) shows that MDMA caused a higher increase in Tc when Tc was measured with rectal probe in comparison to telemetry (0 – 60 min).

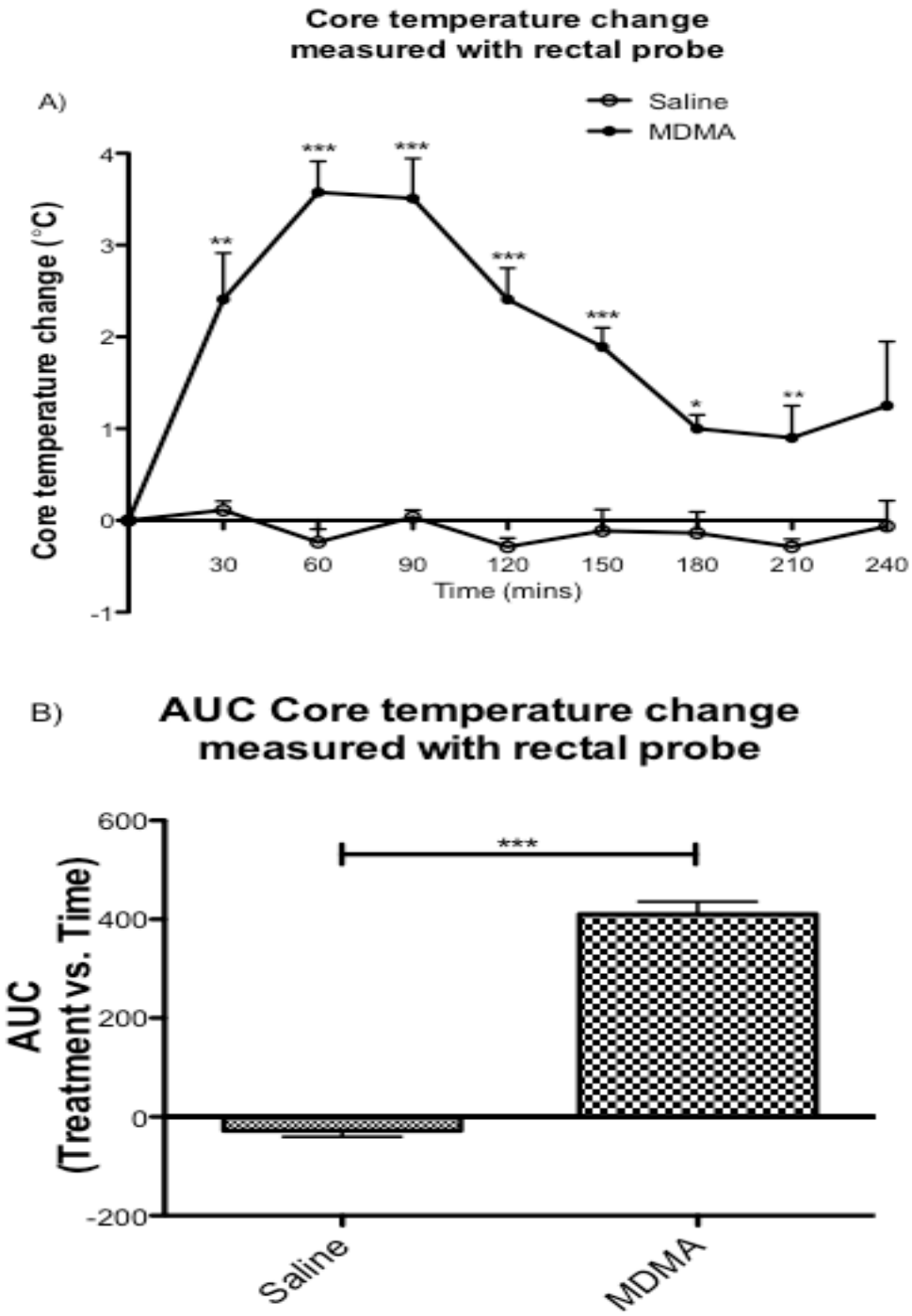


Figure 2. 1: Mean core temperature change measured using rectal probe following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-6). Data was measured from 0 to 240 min after treatment. *P<0.05, **P<0.01, ***P<0.001 compared with unpaired t-test to saline at the respective time points. Column graphs (bottom) represent the AUC of the corresponding line graphs (top).

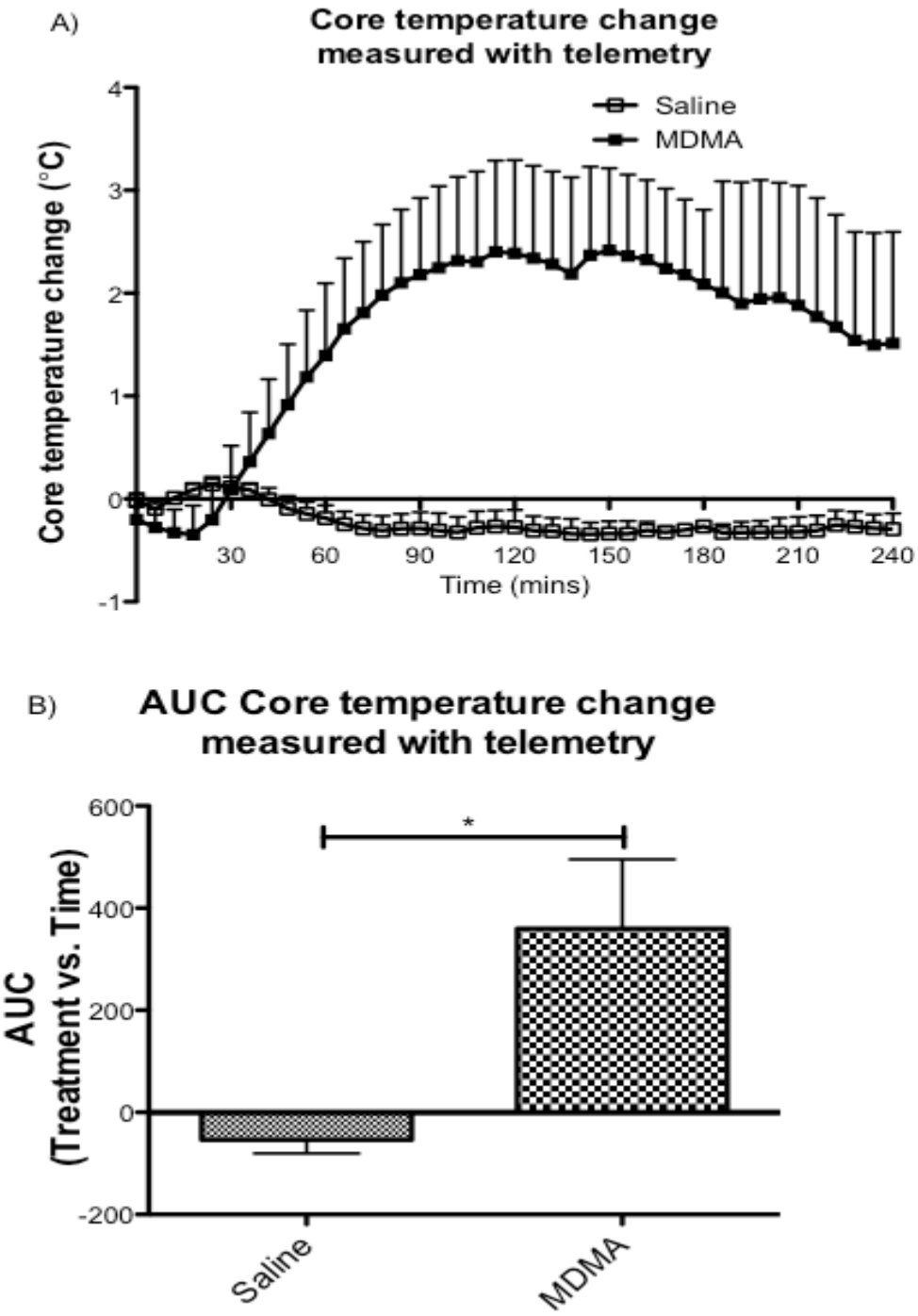
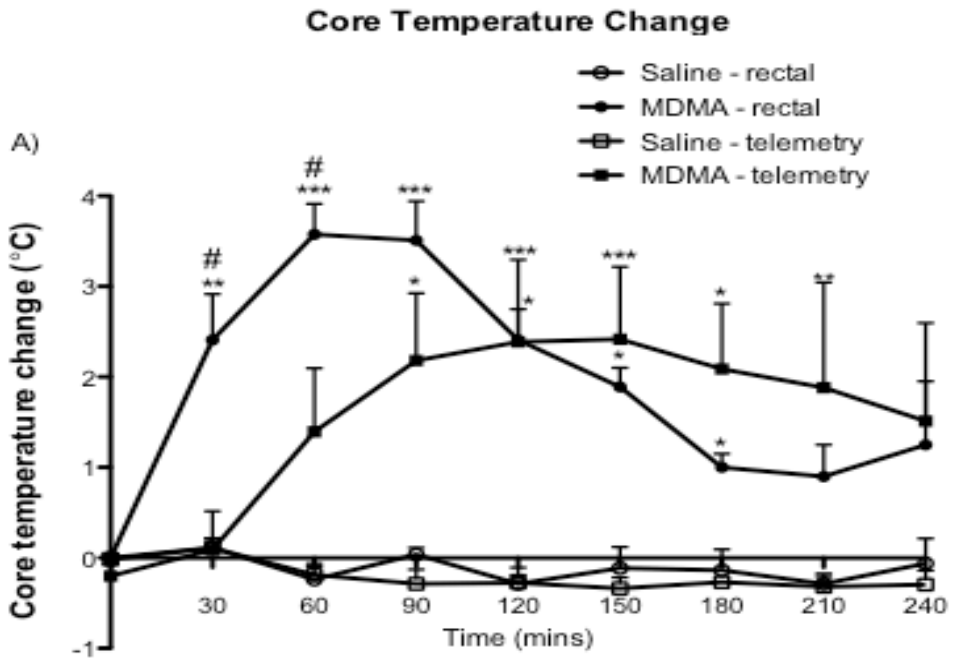
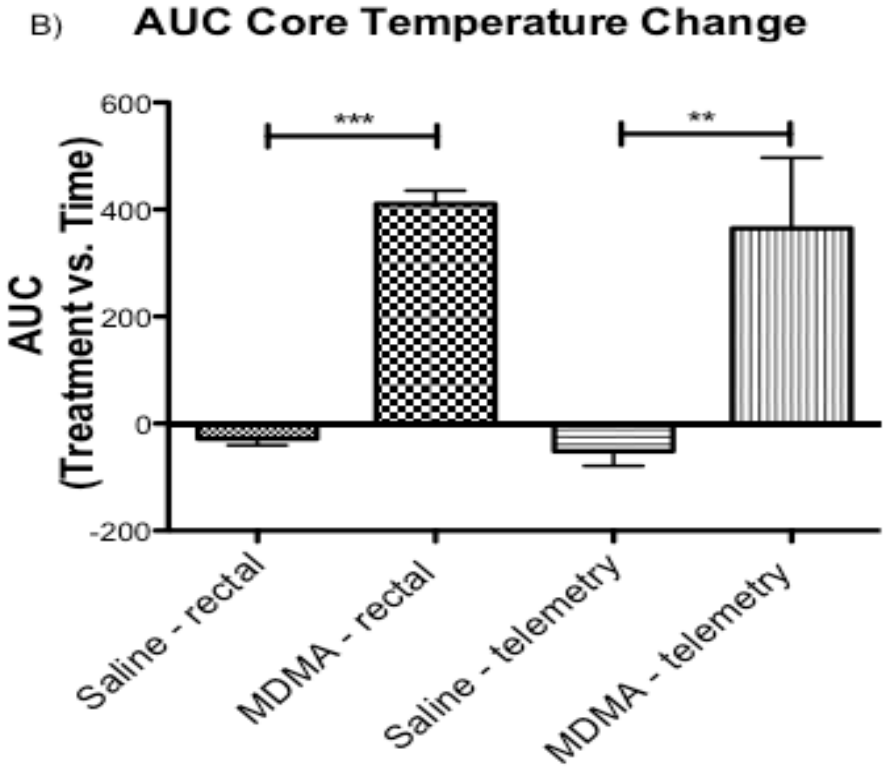


Figure 2. 2: Mean core temperature change measured using telemetry following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 3-4). Data was measured every 2 min but time points are shown for every 6 min from 0 to 240 min after treatment. *P<0.05 compared with unpaired t-test. Column graphs (bottom) represent the AUC of the corresponding line graphs (top).


Core Temperature Change
Saline - rectal
MDMA - rectal
Saline - telemetry
MDMA - telemetry
A)
Core temperature change (°C)
Time (mins)
30
60
90
120
150
180
210
240


B)
AUC Core Temperature Change
AUC
(Treatment vs. Time)
Saline - rectal
MDMA - rectal
Saline - telemetry
MDMA - telemetry

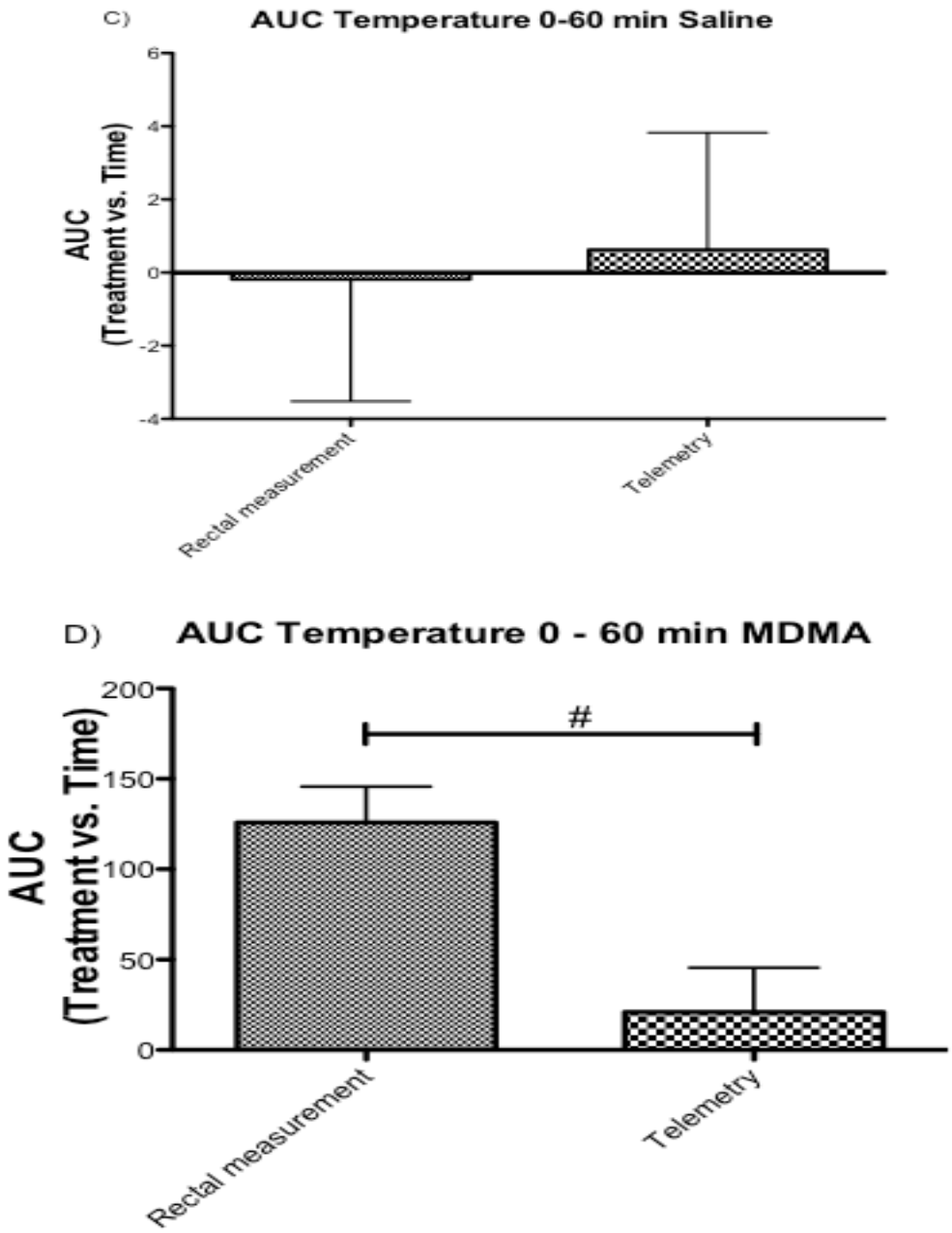


Figure 2. 3: Mean core temperature change measured using rectal probe and telemetry following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-6). Data was measured from 0 to 240 min after treatment. *P<0.05, **P<0.01, ***P<0.001 compared with unpaired t-test to saline at the respective time points. Column graphs (B) (bottom) represent the AUC of the corresponding line graphs (top). **P<0.01, ***P<0.001 compared with one-way ANOVA with Tukey post-hoc test. Column graphs (C) and (D) represent AUC 0 to 60 min for saline and MDMA groups. #P<0.05 compared with unpaired t-test.

2.3.2 Behavioural response

In control groups, the behavioural score did not showed significant change when measured with rectal probe or telemetry. Systemic administration of 10mg/kg MDMA produced significant increase in behavioural score in comparison to control. Behavioural score in rats when Tc was measured with rectal probe showed an increase from 30 min (2.833 ± 0.167, $P<0.001$, n = 6) to 120 min with peak at 60 min post-treatment (3.167 ± 0.307, $P<0.001$, n = 6), and then a decrease throughout the experiment to less than 1 (Figure 2.4). When Tc was measured with telemetry, behavioural score in rats showed an increase at 30 min (2.75 ± 0.25, $P<0.001$, n =4) to 180 min (2.25 ± 0.75, $P<0.05$, n =4) with a plateau from 30 to 120 min and then a decrease throughout the experiment around 2 (Figure 2.5). In Figure 2.6, when Tc was measured with rectal probe, rats shows a higher score followed by a steep decrease throughout the experiment, in comparison to behavioural score when rats was measured with telemetry. In the telemetry group, the score plateau until 120min followed by a gradual decrease throughout the experiment. At 120 min, rats which Tc was measured with telemetry showed significantly higher score ($P<0.01$, n = 4-6) than rats which Tc was measured with rectal probe (Figure 2.6). The latter group showed no significant difference in behavioural score in comparison to control from 150 min. Figure 2.6 (B) shows that behavioural score in MDMA groups were significantly higher than their respective saline groups (0 – 240 min). Since there was no significant change in behavioural score when Tc was measured using rectal probe in control group in comparison to MDMA group, this result suggests that the increased behavioural score was due to MDMA instead of handling the rats.

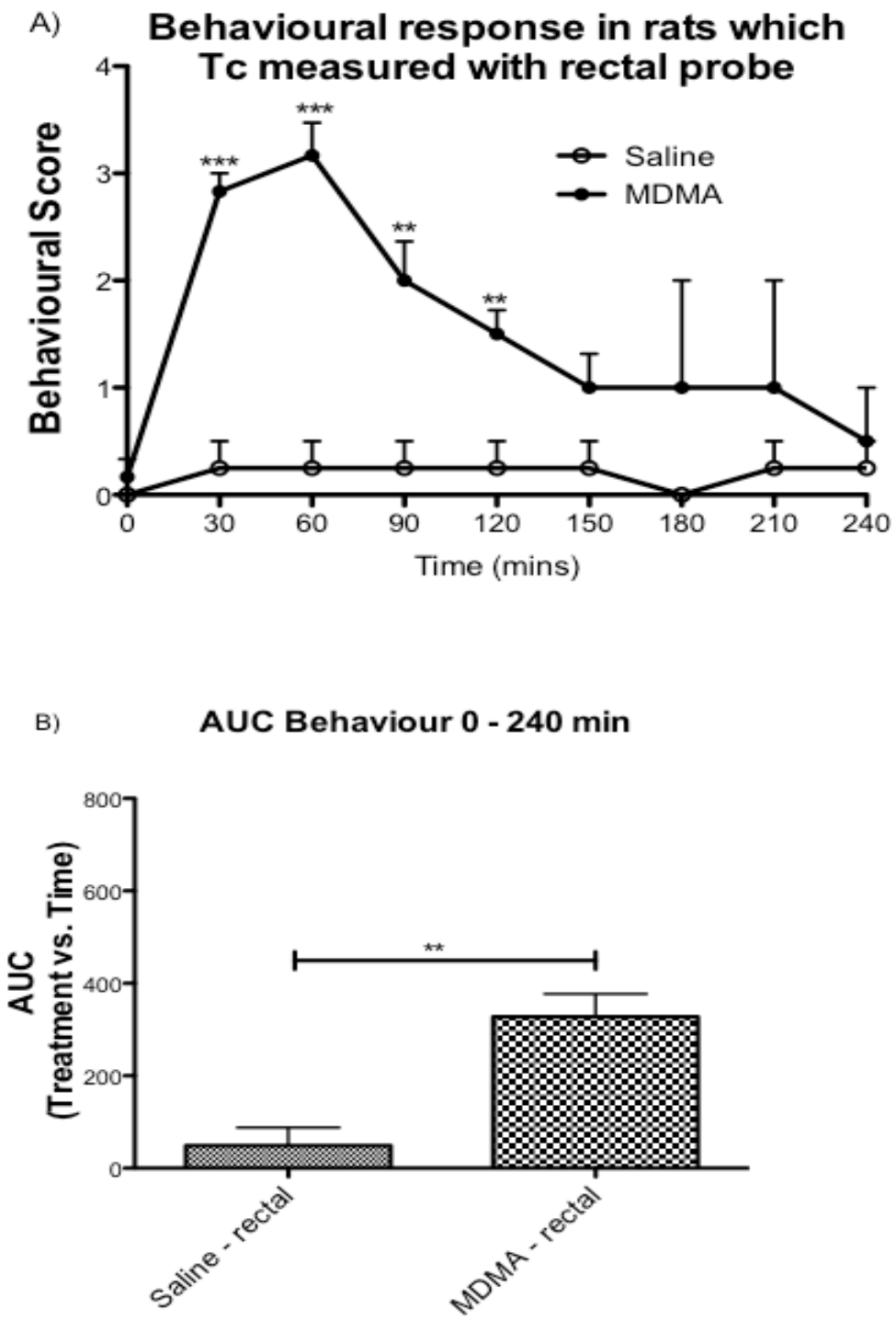


Figure 2. 4: Behavioural response in rats measured using rectal probe following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-6). Data was measured from 0 to 240 min after treatment. **P<0.01, ***P<0.001 compared with unpaired t-test. Column graphs (bottom) represent the AUC of the corresponding line graphs (top).

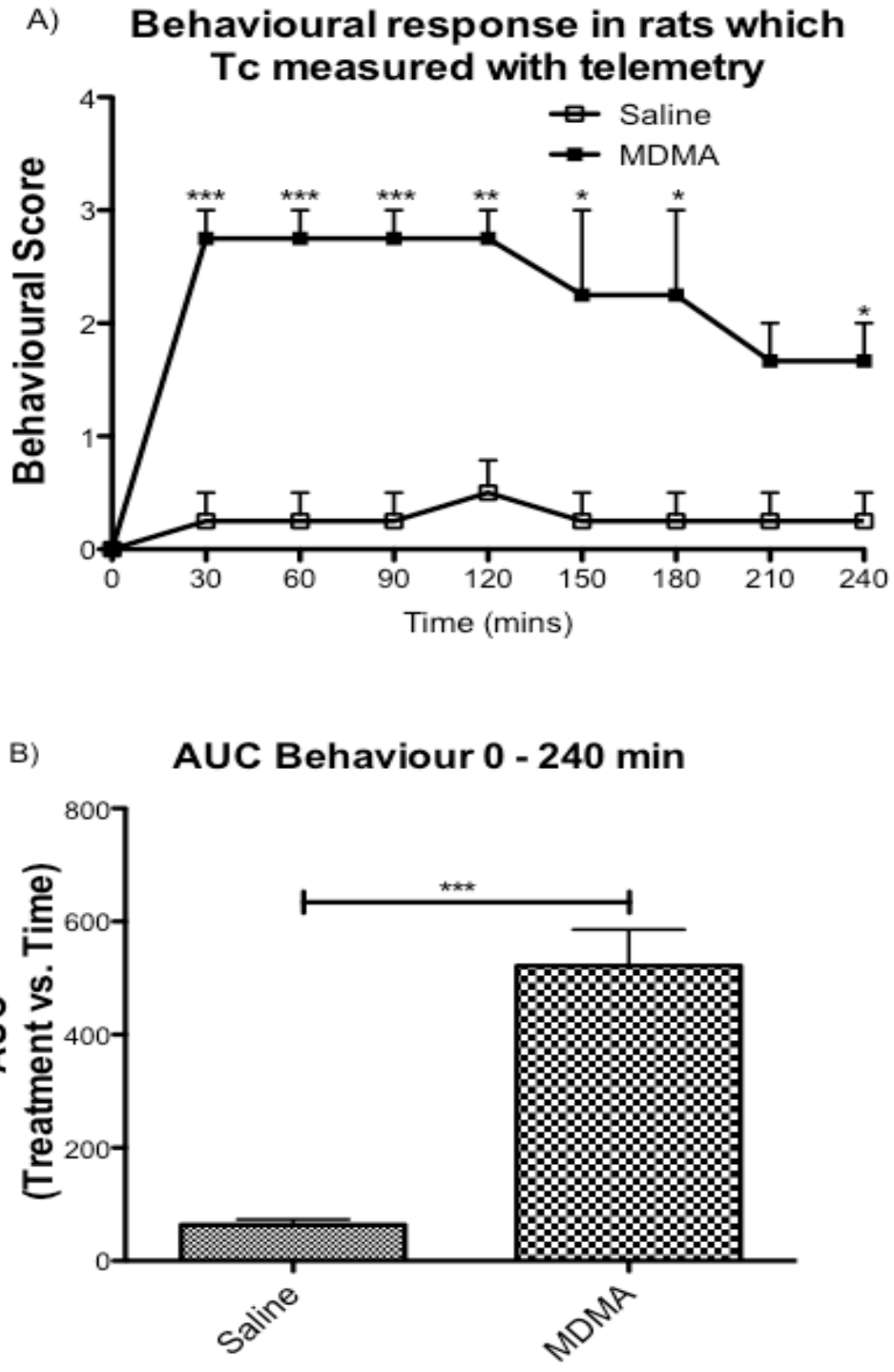


Figure 2. 5: Behavioural response in rats measured using telemetry following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 3-4). Data was measured from 0 to 240 min after treatment. *P<0.05, **P<0.01, ***P<0.001 compared with unpaired t-test. Column graphs (bottom) represent the AUC of the corresponding line graphs (top).

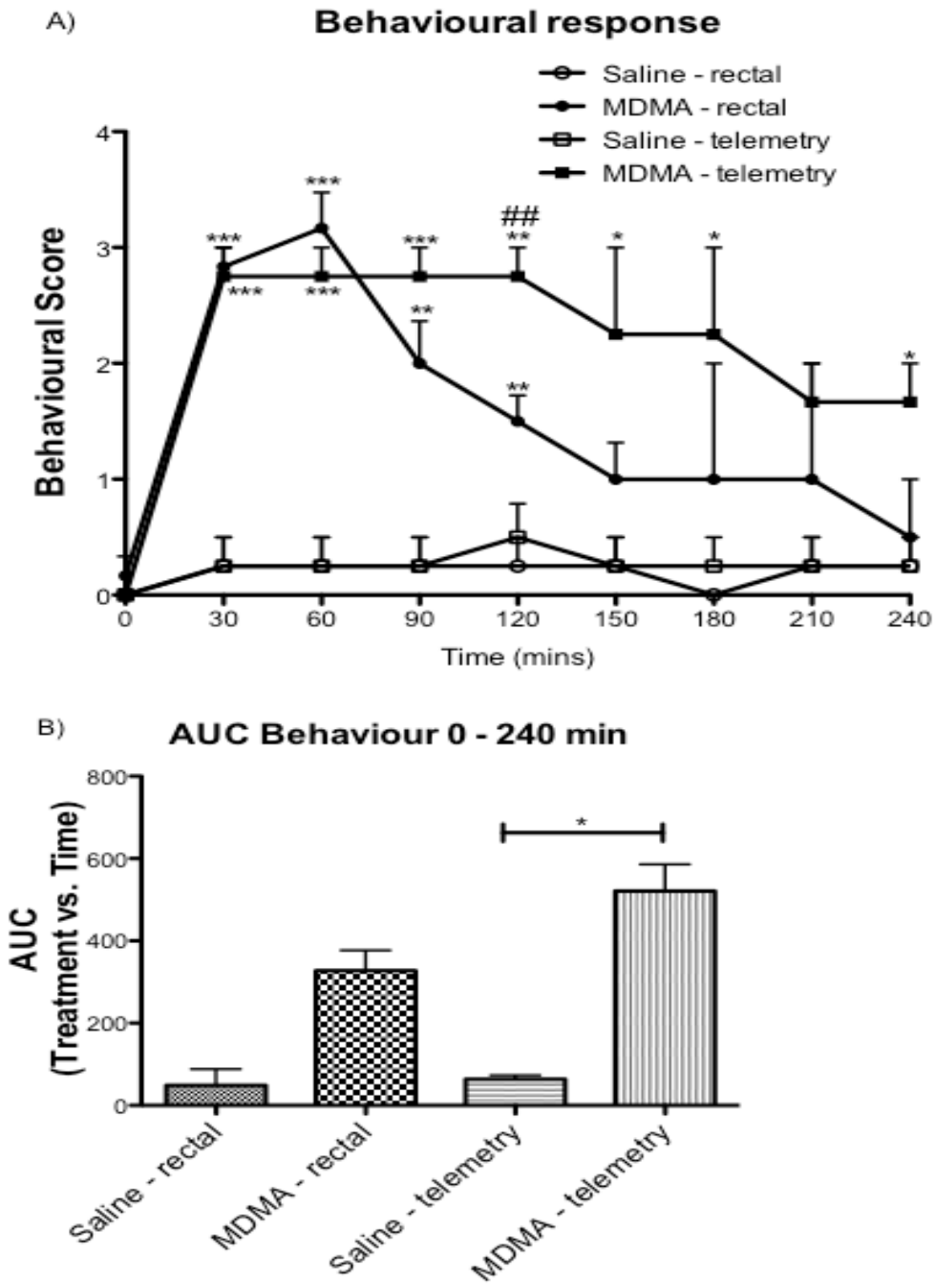


Figure 2. 6: Behavioural response in rats measured using rectal probe and telemetry following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-6). Data was measured from 0 to 240 min after treatment. ##P<0.01 compared with unpaired t-test between MDMA groups at the respective time points. *P<0.05, **P<0.01, ***P<0.001 compared with unpaired t-test between MDMA and their respective control (saline) groups. Column graphs (bottom) represent the AUC of the corresponding line graphs (top). *P<0.05 compared with Kruskal-Wallis non-parametric test with Dunn's post-test.

2.3.3 Survival rate

Table 1 shows the survival rate for each group at each time points following treatment. Rats in the saline groups showed a 100% survival rate. For MDMA-telemetry group, the survival rate at 0 to 180 min was 100%, whereas for MDMA-rectal group the survival rate was 100% only from 0 to 120 min. For MDMA-telemetry group, the rate decreases to 75% at 210 to 240 min. For MDMA-rectal group, the rate decreases to 83.33% at 150 min to 33.33% from 180 to 240 min. Due to ethical reason, the number of rats in MDMA-rectal group was not increased.

Table 2. 1**: **Survival rate (%) at each time points.

		Time points (min)							
Group	0	30	60	90	120	150	180	210	240
Saline-rectal	100	100	100	100	100	100	100	100	100
Saline-telemetry	100	100	100	100	100	100	100	100	100
MDMA-rectal	100	100	100	100	100	83.3	33.3	33.3	33.3
MDMA-telemetry	100	100	100	100	100	100	100	75	75

2.4 DISCUSSION

In the current study, it has been demonstrated that MDMA produced significant increase in Tc and behavioural score in comparison to control. Tc in rats measured using rectal probe showed a significant increase at 30 and 60 min post-treatment in comparison to Tc in rats measured using telemetry. Behavioural score in rats when Tc is measured with telemetry showed a higher score at 120 min post-treatment in comparison to rats which Tc is measured with rectal probe. When Tc was measured with rectal probe, the survival rate was lower than rats which Tc was measured with telemetry in MDMA-treated rats.

This study has demonstrated that the timing of the onset and profile of MDMA-induced hyperthermia were different when measured with rectal probe and telemetry (Figure 2.3). Although the maximum core temperature change was similar regardless of the temperature recording technique, use of rectal probe resulted in a faster onset of temperature increase following MDMA administration (at 30 min) and peak increase (at 60 min) in comparison to rats which when Tc was measured using telemetry, 90 min and 150 min, respectively. This is possibly due continuous handling when measuring Tc throughout the experiment and enhanced effects of MDMA on body temperature in restrained rats. The result of this study is consistent with other reports demonstrating the importance of identifying factors that might influence measurement of body temperature including stress (Clement et al., 1989; Gordon, 1990; van den Buuse, 1994).

A previous study has also demonstrated enhanced effects of drugs in restrained rats in comparison to free-moving rats. A study by Martin et al. (1977) showed restrain alters the effects of morphine and heroin on Tc in rats. In the study, rats were treated with 5, 15 and 30 mg/kg morphine, or 0.1, 1 and 5 mg/kg heroin, in which the administration of 5 mg/kg of heroin or 30 mg/kg of morphine caused hypothermia in restrained rats and hyperthermia

in unrestrained rats (Martin et al., 1977). These results suggest that handling rats can alter the animal's Tc response to the drugs. However, the type of restraint used by Martin et al., (1977) was different than that reported in the current study. In our study, the rats were slightly restrained by hands for a few seconds to measure rectal temperature. Our results have demonstrated that slightly restrained rats in the control group did not display significant change in body temperature, but with the administration of MDMA the changes in Tc were potentitated in rats which were slightly restrained. The Tc in rats measured with telemetry initially showed hypothermia although not significant following MDMA administration, followed by a gradual increase in Tc. It is important to get an accurate reading of Tc, especially during the first hour as MDMA administration has been reported to produce marked hyperthermia with a peak at 40 to 60 min post-treatment (Colado et al., 1993; Dafters, 1994; Malberg et al., 1996; Malberg and Seiden, 1998; O'Shea et al., 1998; Stanley et al., 2007). In a study by O'Shea et al. (1998) which used a thermocouple rectal probe to measure Tc, administration of 10mg/kg MDMA (i.p.) produced a significant increase in Tc of 1.5°C, peak at 60 min post-treatment. Thus, the use of an appropriate method to measure Tc can prevent confounding effects of restraining on Tc following MDMA administration, especially at the onset of effects.

It has also been demonstrated in this study that use of rectal probe resulted a lower survival rate compared to Tc was measured with telemetry. This finding suggests that rats exposed to stress were more susceptible to death than stress-free rats when challenged with a drug known to increase body temperature. These results are consistent with Gallaher et al. (1985) who demonstrated that animals with 1°C rise in body temperature did not recover fully when measured with rectal probe.

This study has also demonstrated that the temperature recording technique influenced the profile of MDMA-induced increase in behavioural activity. Although Tc within the rectal group was measured after behavioural recording, subsequent behavioural testing was still influenced by the previous Tc measurement which involved restraining the rat by hand. When Tc was measured with telemetry, animals showed significantly greater increases in behavioural activity and stereotypical 5-HT – related behaviours in comparison to rats when Tc was measured with rectal probe at 120 min post-treatment. The elavation of extracellular 5-HT is associated with a condition known as 'serotonin syndrome' which has been reported both in humans (Gillman, 1999) and animals (Grahame-Smith, 1971a), caused by monoamine oxidase inhibitors, SSRI, drug interactions, and drugs that prevent clearance and of 5-HT and its metabolism (Stanley et al., 2007). When Tc was measured with telemetry following MDMA administration, the behavioural activity increased and maintained at a higher score (30 min to 240 min), than rats which Tc was measured with rectal probe (immediate peak at 60 min followed by a steep decrease to 150 min). This pattern with the rectal probe group was similar to that reported by Stanley et al. (2007) which demonstrated that MDMA–treated rats began to show signs of heat stress and a significant decrease in their activity until 150 min which they were completely inactive. The steep decrease in behavioural score in the rectal probe group was also probably due to depletion of energy during the first 2 hours of the treatment to accommodate increase in locomotion and heat dissipation (Gordon, 1990; Green et al., 2003; Cadet et al., 2007; Gordon, 2007; Kiyatkin, 2007).

Results of this study demonstrated the importance of obtaining more accurate readings of core body temperature following MDMA administration. It can be observed in this study that when MDMA-treated rats were handled for rectal temperature measurement, it resulted in a high behavioural score and could be attributed to increased extracellular levels

in 5-HT in specific brain regions (Spanos and Yamamoto, 1989; Greene et al., 2008). A study by Baumann et al. (2008) has shown that stereotypy was strongly correlated with levels of dialysate 5-HT in a number of brain regions including the striatum, and with dialysate DA in nucleus accumbens and striatum. In this study, it was also shown that in the rectal probe group, the behavioural response showed a steep decrease throughout the experiment when rats became inactive. The findings in the current study indicates that potentiated MDMA effects on Tc when measuring Tc using rectal probe causes a higher behavioural response which possibly correlate with increase in extracellular levels of 5-HT in the brain (Spanos and Yamamoto, 1989).

Telemetry has some limitation including the requirement for surgery and it is costly compared to rectal probe technique. Even though telemetry is costly in comparison to rectal thermometer, the advantages outweigh the disadvantages. A number of studies have started using telemetry for the past few decades, especially to measure physiological parameters. As a technique that eliminates restraining stress, telemetry provides an alternative that can be used to support studies which used conventional method. The results of this study provide the practical application of telemetry in measuring Tc, over conventional method using rectal probe. The use of telemetry has several advantages, primarily the ability to record Tc without producing confounding effects which allow more quality and quantity of data collection. It provides a better representation of MDMA effects on Tc without producing stress when handling the animals or repeated insertion of rectal probe during a long duration of experiment, more time efficient in data collection and analysis, and provides a more thorough time profile of MDMA effects in animals. Our results demonstrate that telemetry is a valid technique and provides a better approach to record core body temperature, and also other parameters in small animals than the conventional method.

Chapter 3 The effects of systemic administration of MDMA, and central perfusion of MDMA and MDA into the striatum, on core body temperature, heart rate, locomotor activity and striatal serotonin concentration

3.1 INTRODUCTION

It has been suggested that MDMA metabolism plays a major contribution to some of the adverse effects of MDMA (Esteban et al., 2001; Nixdorf et al., 2001; Goni-Allo et al., 2008a). This is mainly due to involvement of several pathways of MDMA metabolism with a number of metabolites forming toxic conjugates (Hiramatsu et al., 1990; Green et al., 2003; de la Torre and Farre, 2004; Erives et al., 2008). MDA is a major metabolite of MDMA and plasma levels in rats rise rapidly in a parallel manner and plateau between 1 and 3 h after MDMA administration (Colado et al., 1995; Chu et al., 1996). MDA increases locomotor activity (Yeh and Hsu, 1991), more potent at producing 5-HT-mediated behaviour (Hiramatsu et al., 1989), induces hyperthermia in rats and mice (Colado et al., 1995; Bexis and Docherty, 2006) and induces 5-HT and dopamine release (Nash and Brodkin, 1991; O'Loinsigh et al., 2001). A study by Fonsart et al. (2008) found a higher hyperthermia response in male rats following systemic administration of 10mg/kg MDA (~4.3°C) than 20mg/kg MDMA (~3.2°C) 1h after drugs administration. Colado et al. (1995) also observed a higher MDA-induced hyperthermia (~2.7°C) than MDMA-induced hyperthermia (~1.0°C) 1h after systemic administration of 10mg/kg MDA and MDMA, respectively. A study by Goni-Allo et al. (2008b) has shown that the period of temperature elevation following systemic MDMA administration parallels the increased in plasma concentrations of MDA, which suggests a role for MDA in MDMA-induced hyperthermia. A study by Bexis and Docherty (2006) has shown that MDA increases heart rate more than MDMA at identical doses (20mg/kg, i.p.). This is probably due to the agonist action of

MDA at 5-HT_{2B} receptors (Setola et al., 2003), which potentiate the cardiac effects of MDA (Droogmans et al., 2007).

Although MDMA-induced hyperthermia and neurotoxicity have been previously demonstrated following systemic MDMA administration, perfusion of MDMA in rats into hippocampus (Molliver et al., 1986; Esteban et al., 2001) including MDA (Molliver et al., 1986) and striatum (Goni-Allo et al., 2008b) failed to cause hyperthermia and neurotoxicity. Direct injection of MDMA into the brain failed to reproduce the adverse effects demonstrated following systemic administration of MDMA (Molliver et al., 1986; Paris and Cunningham, 1990; Esteban et al., 2001; Nixdorf et al., 2001; Goni-Allo et al., 2008a). Previous studies that administer MDMA centrally into the brain used a concentration of 100µM MDMA which is comparable to the concentration found in the brain following systemic administration of 10mg/kg MDMA (i.p.) (Molliver et al., 1986; Paris and Cunningham, 1990; Esteban et al., 2001; Nixdorf et al., 2001; Goni-Allo et al., 2008a). In a study by Goni-Allo et al. (2008), perfusion of 100µM of MDMA did not alter core body temperature nor cause depletion of 5-HT neurons. Yet, combined perfusion of 100µM MDMA directly into the brain and 3 X 5mg/kg MDMA (i.p.) produced acute hyperthermia and neuronal 5-HT loss in the striatum. These findings suggest that peripheral generation of active metabolites contributes to MDMA-induced hyperthermia and the importance of metabolism to produce MDMA-induced hyperthermia and long-term damage of serotonergic neurons.

Additionally, although there were many studies looking at the effects of central administration of MDA and its metabolites on the development of neurotoxicity, the findings on acute effects of MDA such as hyperthermia is sparse. Moreover, systemic MDA administration produces significantly higher hyperthermia (Fonsart et al., 2008),

heart rate (HR) and locomotor activity (LMA) than MDMA at similar doses in rats (Colado et al., 1995; Bexis and Docherty, 2006). MDA is also available in the drug market either as itself or as 'ecstasy' (Pentney, 2001) and it is postulated that MDA users will experience worse adverse effects compared to MDMA users based on the findings on animal studies (Fonsart et al., 2008).

Previous microdialysis studies were conducted in the dorsal striatum as this region is important for locomotion and cognition function, and has a large size and easily accessible by dialysis probe (Li et al., 2006). The behavioural effects of MDMA are also partially mediated by 5-HT release in the striatum (Dafters and Lynch, 1998; Shankaran and Gudelsky, 1999; Mechan et al., 2002). Striatum has also been implicated in MDMA-induced hyperthermia and changes in 5-HT extracellular levels (Malberg et al., 1996; Malberg and Seiden, 1998). It was assumed that increase in 5-HT extracellular levels in one brain region reflects extracellular levels in other brain regions (Rodsiri et al., 2011), hence no previous studies have compared 5-HT extracellular levels using microdialysis following systemic administration of MDMA in several brain regions and correlated them to physiological effects of MDMA. Striatum is unique compared to hippocampus due to the interplay between dopaminergic and serotonergic neurochemistry especially in the presence of MDMA. It is important to measure the dynamics of extracellular 5-HT changes between MDMA and MDA after central administration, especially when locomotor activity and heart rate are measured in the present study. It is also important to perfuse MDMA and MDA centrally to the striatum to eliminate peripheral metabolism confounding the interpretation of effects. This approach is also important to elucidate the brain regions responsible for MDMA and MDA-induced adverse effects especially hyperthermia, and/or the possible role of the active metabolite, MDA in mediating these effects. The present study was done using awake animals rather than anaesthetized animals

which is a more accurate representation of 5-HT extracellular levels in the brain. Since the concentration of 5-HT increase immediately after probe implantation, the rats were allowed 48 hours recovery period before initiation of experiment.

In the current study, we used combined telemetry and microdialysis techniques to measure the effects of MDMA on core body temperature, heart rate, locomotor activity, and striatal 5-HT extracellular levels. The telemetry system prevents from the confounding effects of handling on Tc, HR and LMA, whereas the microdialysis technique allows continuous central perfusion of MDMA and MDA and collection dialysates to measure changes in brain neurotransmitters concentrations using High Performance Liquid Chromatography (HPLC) with electrochemical detection (ED) (Myers et al., 1998). The purpose of this study is to compare and contrast the concurrent pharmacodynamics responses (temperature, heart rate, and locomotor activity) between centrally administered MDMA and MDA and peripheral administration of MDMA, at bioequivalent doses in awake animals at high ambient temperature. It is hypothesised that central perfusion of MDMA into the striatum will not cause significant increase in Tc and HR, as systemic administration of MDMA, and central administration of MDA will produce significant increase in Tc, HR, LMA and striatal 5-HT in comparison to central administration of MDMA.

3.2 MATERIALS AND METHODS

3.2.1 Animals

Supply of animals and methods were as described in Chapter 2.

3.2.2 Radiotelemetry

Supply of animals and methods were as described in Chapter 2.

3.2.3 Brain surgery for probe implantation

After one-week recovery period following the telemetry implant surgery, rats underwent probe implantation surgery. Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p) and placed on water heated pad (37ºC) to maintain body temperature. Once the rat was fully anesthetized, the rat's head was secured in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) and an intercerebral guide cannula (BAS MD-2251, Bioanalytical System Inc., West Lafayette, IN, USA) was implanted into the striatum and held with dental cement (Vertex, Dentimex BV HJ Zeist, Netherlands). The coordinates of the striatum are AP: +1.2 mm and L: +2.2 mm from bregma and D: -5.5 mm from dura, which was referred from a rat brain atlas (Paxinos and Watson, 1986). Rats were allowed to recover on the 37ºC heated pad until they regain full consciousness and were placed individually in a clear Perspex observation bowl (BAS "Bee-keeper" rodent assistance). A further 48 hours recovery was allowed before the telemetry and microdialysis sampling commenced to ensure that the neurotransmitter levels are stable and to minimize tissue damage from the surgery (Westerink, 2000; Esteban et al., 2001; O'Shea et al., 2005). Animals were given *ad libitum* access to food and water.

3.2.4 Experimental protocol

Following the 48 hours recovery period, rats were lightly restrained by hand and the microdialysis probe (2mm membrane, BAS MD-2200, Bioanalytical Systems Inc., Indiana, USA) was inserted through the guide cannula into the striatum. The animals were

then placed in a room where the T_a was maintained at high (29ºC) T_a. Experiments were conducted at ambient temperature of 29°C in order to mimic the hot conditions that MDMA users may be exposed to at raves or nightclubs. Artificial cerebrospinal fluid (aCSF) (in mM: NaCl 125, KCl 2.5, $MgCl_2.6H_2O$ 1.18, Na_2HPO_4 2.0 and $CaCl_2.2H_2O$ 1.2 adjusted to pH 7.4) was perfused for 2 h at a flow rate of 1.5 µl/min. The first two samples following probe insertion were discarded and the next two 30 minutes samples were used as baseline samples. The probe was then perfused with either aCSF (control), MDMA (100µM) or MDA (5µM) for 300 minutes. For systemic (i.p) administration, 0.9% saline (dose volume 1mg/ml) or 10mg/kg MDMA was administered. Dialysates were collected through the same guide cannula every 30 minutes into Eppendorf tube containing 10 µl of 2% acetic acid. Radio receivers, placed on the bottom of the microdialysis bowl, received information from the implants and transferred it to a computer which recorded the data using Dataquest LabPro software (Data Sciences International). Temperature change (Tc), heart rate (HR) and locomotor activity (LA) were recorded via telemetry every two minutes over the experimental period.

3.2.5 High Performance Liquid Chromatography (HPLC) with electrochemical detection (ED)

The system consists of a controller (Decade II; Shimadzu, Kyoto, Japan), VP auto injector (Shimadzu SIL-10AD), VP liquid chromatograph (LC-10AD), degasser (Shimadzu DGU-14A) and BAS LC-4B (Bioanalytical Systems). The mobile phase for MDMA and MDA was composed of sodium acetate 0.1M (pH 4.25) and 12.5% methanol (adjusted according to (Michel et al., 1993). It was filtered, degassed and delivered at a flow rate of 1.18 ml/min. Alltima HP C18 3µ 100×2.1mm column (Alltech Associates, Inc., NSW, Australia) is used to separate MDMA and MDA. The mobile phase for 5-HT and 5-HIAA was composed of (in mM) NaH_2PO_4 102.9, octanesulfonic acid 1.0, ethylenediamine tetraacetic acid (EDTA) 0.1 (adjusted to pH 3.8) and 12.5% methanol and injected at a

flow rate of 0.7 ml/min with working electrode potential set at 0.7V with a range of 100pA (Callaghan et al., 2006). An Ultrasphere ODS 250×4.6mm column (Beckman Coulter, USA) was used for 5-HT and 5-HIAA separation. Data were recorded using the LC Solution programme (Shimadzu).

3.2.6 Reverse dialysis recovery

Reverse dialysis were done to estimate the extracellular concentration of MDMA and MDA in the striatum following drugs administration by assessing the ability of the probe to recover MDMA and MDA in vitro from solutions containing selected concentrations of these drugs. The probe was perfused with 100μM MDMA or 5μM MDA. The drugs were perfused using an automated syringe ('Bee Stinger', Bioanalytical Systems) at 1μl/min. Dialysates were collected every 30 min into an Eppendorf tube for 300 min. Samples were analysed with HPLC-ED to determine MDMA and MDA concentration. The percentage of recovery was calculated from the original solution of the drugs in the syringe. MDMA and MDA peak area was compared to a previously acquired standard curve to obtain an apparent concentration.

3.2.7 Drugs preparation and administration

MDMA and MDA were given as the hydrochloride salt and were dissolved in aCSF to give concentrations of 100μM MDMA and 5μM MDA, respectively. MDMA concentration in the perfusate was chosen based on previous studies which shows this concentration resulted in the same extracellular concentration of MDMA in the brain (~11μM) following systemic dose of 10 mg/kg MDMA (Esteban *et al*., 2001). This systemic dose also produces reliable changes in body temperature and locomotor activity in rats without causing fatalities (Daws et al., 2000; Jaehne et al., 2005; Stanley et al., 2007). The dose is comparable to 'ecstasy' used in humans which is about 100 to 150mg (Green et al., 2003) and produces blood concentration in the rat similar to those found in humans (Irvine et al., 2006; Jaehne et al., 2011). In human, the plasma concentration of

MDA following 1mg/kg (43 to 106mg) and 1.6mg/kg (69 to 150mg) MDMA is 7.5ng/ml and 12.5ng/ml, respectively (Kolbrich et al., 2008). Previous studies which measured peak MDA concentration in the plasma following systemic MDMA administration found ~200 ng/ml (0.9μM) MDA in Flinders Sensitive Line (FSL) rats (Jaehne et al., 2011) and ~350ng/ml MDA in male Wistar rats (Goni-Allo et al., 2008b) following 7.5mg/kg MDMA (i.p.), and ~200ng/ml MDA in male Sprague-Dawley rats (Baumann et al., 2009) following 10mg/kg MDMA (i.p.). Following 7.5mg/kg MDMA (i.p.), the brain Cmax of MDA was ~4000ng/g (Jaehne et al., 2011), whereas 10mg/kg MDMA (oral) produces 3μg/ml (0.0139nM) MDA in the brain (Upreti and Eddington, 2008). We also did an *in vitro* recovery of central MDMA and MDA and found about 10% of the drugs were delivered by the probe (Figure 3.1). From these findings, we chose a concentration of 5μM MDA to be administered centrally to the brain. The present study is the first study which uses microdialysis for continuous perfusion of MDA into a specific region in the brain.

3.2.8 Chemicals and reagents

(±)MDMA and (±)MDA were obtained from the Australian Government Analytical Laboratories (Sydney, Australia). 5-HT, 5-HIAA, sodium salt of octanesulfonic acid and disodium salt of EDTA were purchased from Sigma-Aldrich Co. Ltd (St. Louis, USA). Chloral hydrate, sodium chloride, potassium chloride, magnesium chloride, disodium hydrogen orthophosphate, sodium dihydrogen orthophosphate, acetic acid and methanol were purchased from BHD Laboratory Supplies Pty. Limited (Victoria, Australia).

3.2.9 Data analysis

Core body temperature, heart rate, locomotor activity and changes in 5-HT and 5-HIAA levels were analysed with repeated measures two-way ANOVA with treatment and time as main factors, with Bonferroni post hoc test. The mean 5-HT level from the two samples obtained before treatment was used to determine the baseline value and calculate any subsequent change for each rate as a percentage of this baseline value. Area under the

curve (AUC) for each treatment group was calculated from -30 to 300 min and compared with one-way ANOVA with Tukey post-hoc test. Due to its non-parametric nature, behavioural data were analyzed using a Kruskal-Wallis test followed by Dunn's post-test. All results are presented as mean ± SEM and considered statistically significant when $P<0.05$. All calculations and analysis were done using Graph Pad Prism software.

3.3 RESULTS

3.3.1 HPLC

Validity of the HPLC assay was tested by analysis of intra-day and inter-day variances (Table 3.1). Accuracy = (Estimated value – nominal value)/Nominal value X 100. Precision = (SD/mean) X 100. Nominal value is the known concentration. Coefficient values (r^2) for MDMA and MDA, determined from the calibration curve were 0.9999 and 0.9968, respectively. Coefficient values for 5-HT and 5-HIAA were 0.9854 and 1.000, respectively (n = 4).

3.3.2 Reverse dialysis recovery for 100µM MDMA and 5µM MDA

In vitro recovery (Figure 3.1) for MDMA was 10.41 ± 0.37% whereas for MDA it was 11.22 ± 0.51%.

3.3.3 Core body temperature

Figure 3.2 (a) shows the effects of treatment on Tc. A two-way ANOVA showed a significant interaction effect ($P<0.0001$, $F = 14.23$) and significant effects of treatment ($P<0.0001$, $F = 22.83$) and time ($P< 0.0001$, $F = 10.61$). Only systemic administration of 10mg/kg MDMA produces significant increase in Tc in comparison to control ($P<0.001$). MDMA caused an increase in Tc from 30 min (1.094 ± 0.304 °C) onwards with peak at 78 min post-treatment (3.376 ± 0.306 °C), resulting in a temperature change of +3.467 °C from 0 min and then decreases throughout the experiment. Both central perfusion of

MDMA and MDA did not induced significant changes in Tc in comparison to the control group. Figure 3.2 (b) shows that AUC of Tc in rats treated with systemic administration of MDMA significantly increased compared to control (-30–300 min).

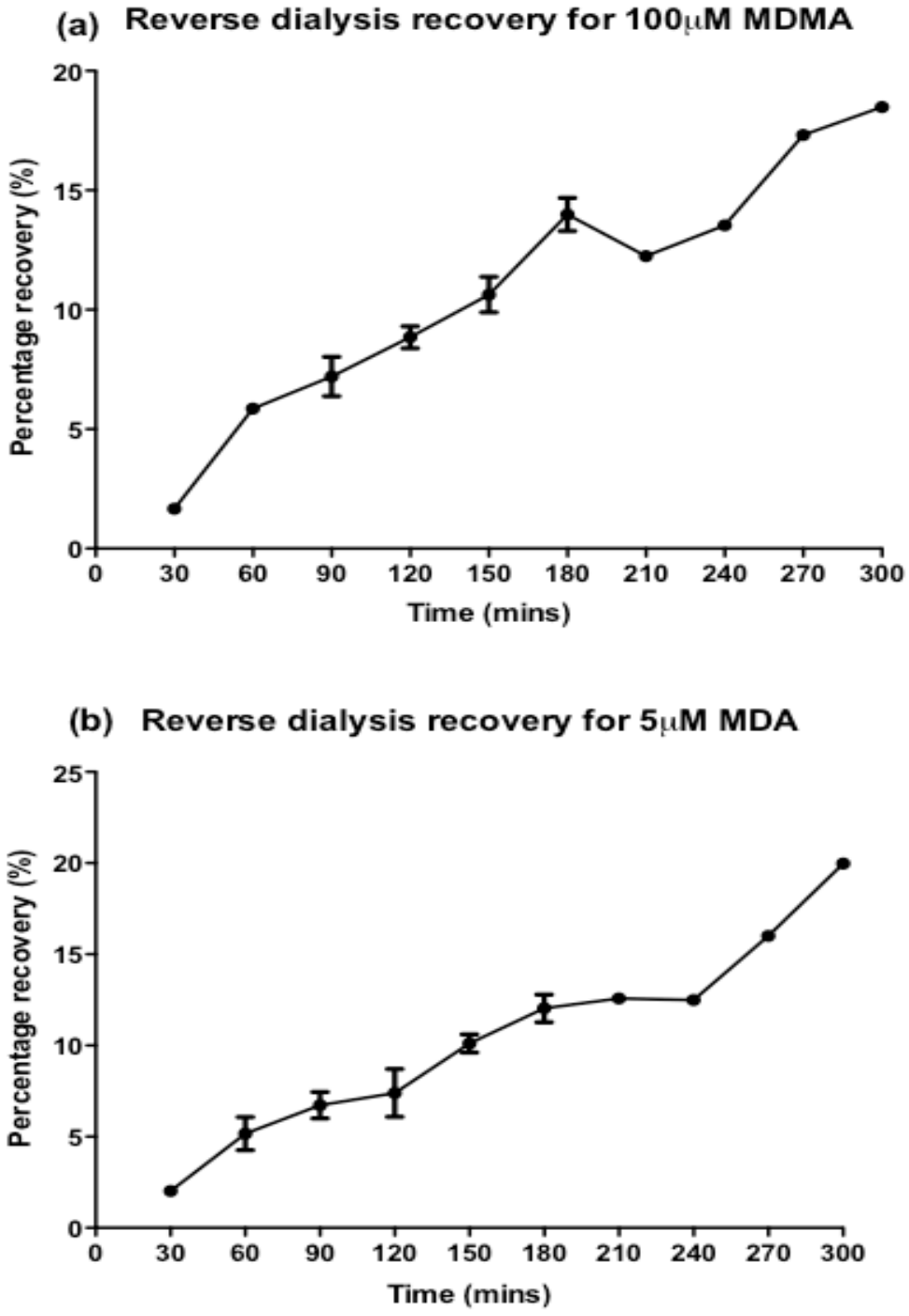


Figure 3. 1: Reverse dialysis recovery for (a)100μM MDMA and (b)5μM MDA.

All data represent mean ± SEM (n = 1-3). Data was measured every 30 min for 300 min.

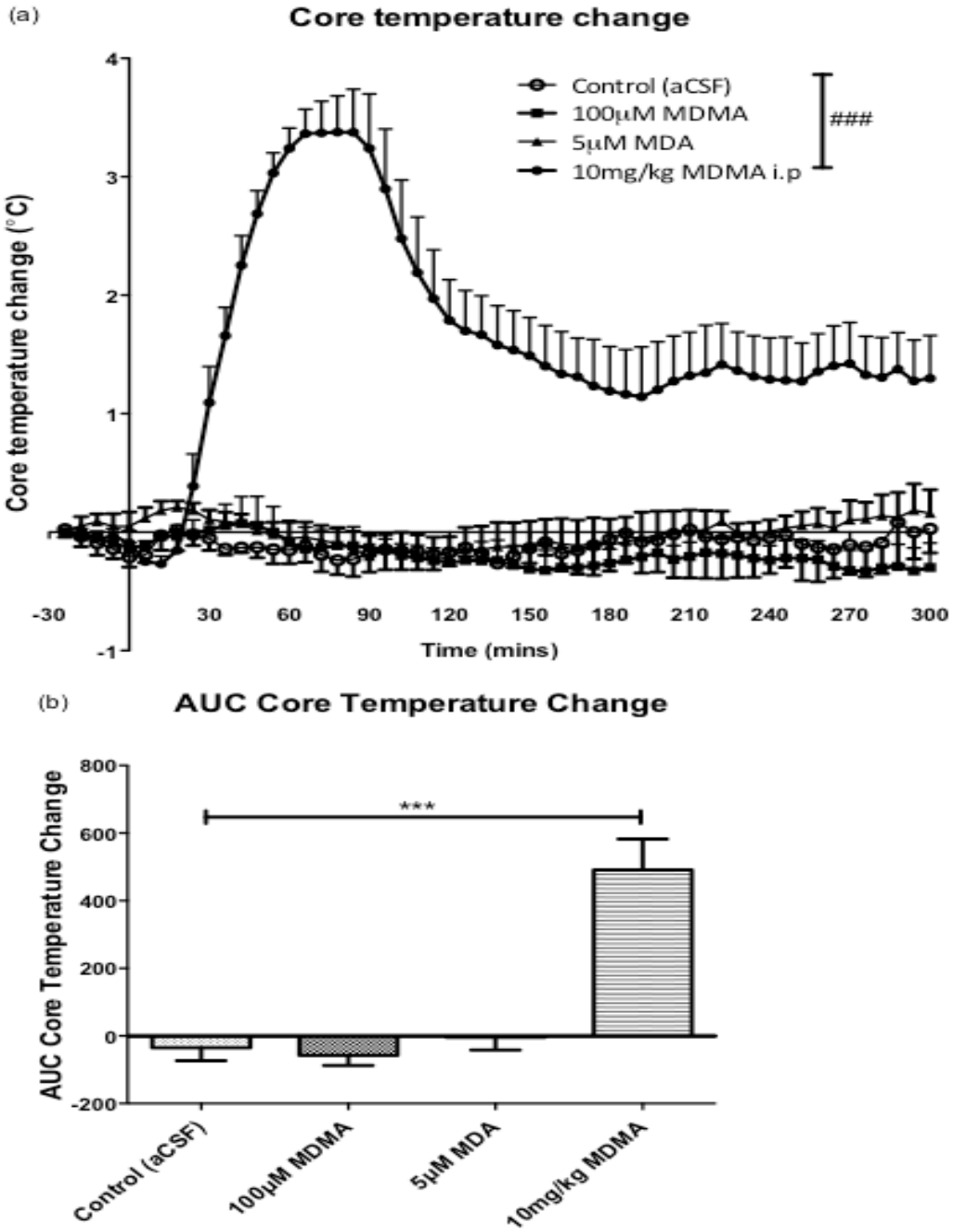


Figure 3. 2: Core temperature response following administration of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-5). Data was measured every 2 min but time points are shown for every 6 min from -30 min before treatment to 300 min after treatment. ###P<0.001 compared with repeated measures two-way ANOVA and Tukey post hoc test. Column graphs (bottom) represent the AUC of the corresponding line graphs (top). ***P<0.001 compared with one-way ANOVA and Tukey post hoc test.

3.3.4 Heart rate

Figure 3.3 shows the effects of treatment on HR. A two-way ANOVA showed a significant interaction effect ($P<0.0001$, $F = 4.38$) and significant effects of treatment ($P<0.0001$, $F = 17.47$) and time ($P< 0.0001$, $F = 2.81$). Only systemic administration of 10mg/kg MDMA produces significant increase in HR in comparison to control ($P<0.001$). MDMA caused an increase in HR from 18 min (415.489 ± 10.787 bpm) onwards with peak at 78 min post-treatment (527.873 ± 24.979 bpm) and then decreases throughout the experiment. Both central perfusion of MDMA and MDA did not induced significant changes in HR in comparison to the control group. Figure 3.3 (b) shows that AUC of HR in rats treated with systemic administration of MDMA significantly increased compared to control (-30–300 min).

3.3.5 Locomotor activity

Figure 3.4 shows the effects of treatment on LMA. A two-way ANOVA showed a significant interaction effect ($P<0.0001$, $F = 21.46$) and significant effects of treatment ($P<0.0001$, $F = 1149.65$) and time ($P< 0.0001$, $F = 22.30$). Only systemic administration of 10mg/kg MDMA produces significant increase in LMA in comparison to control ($P<0.001$). MDMA caused an increase in LMA from 12 min (13.625 ± 8.518 cpm) to 150 min with peak at 60 min post-treatment (51.875 ± 7.704 cpm) and then decreases throughout the experiment. Both central perfusion of MDMA and MDA did not induced significant changes in LMA in comparison to the control group. Figure 3.4 (b) shows that AUC of LMA in rats treated with systemic administration of MDMA significantly increased compared to control (-30–300 min).

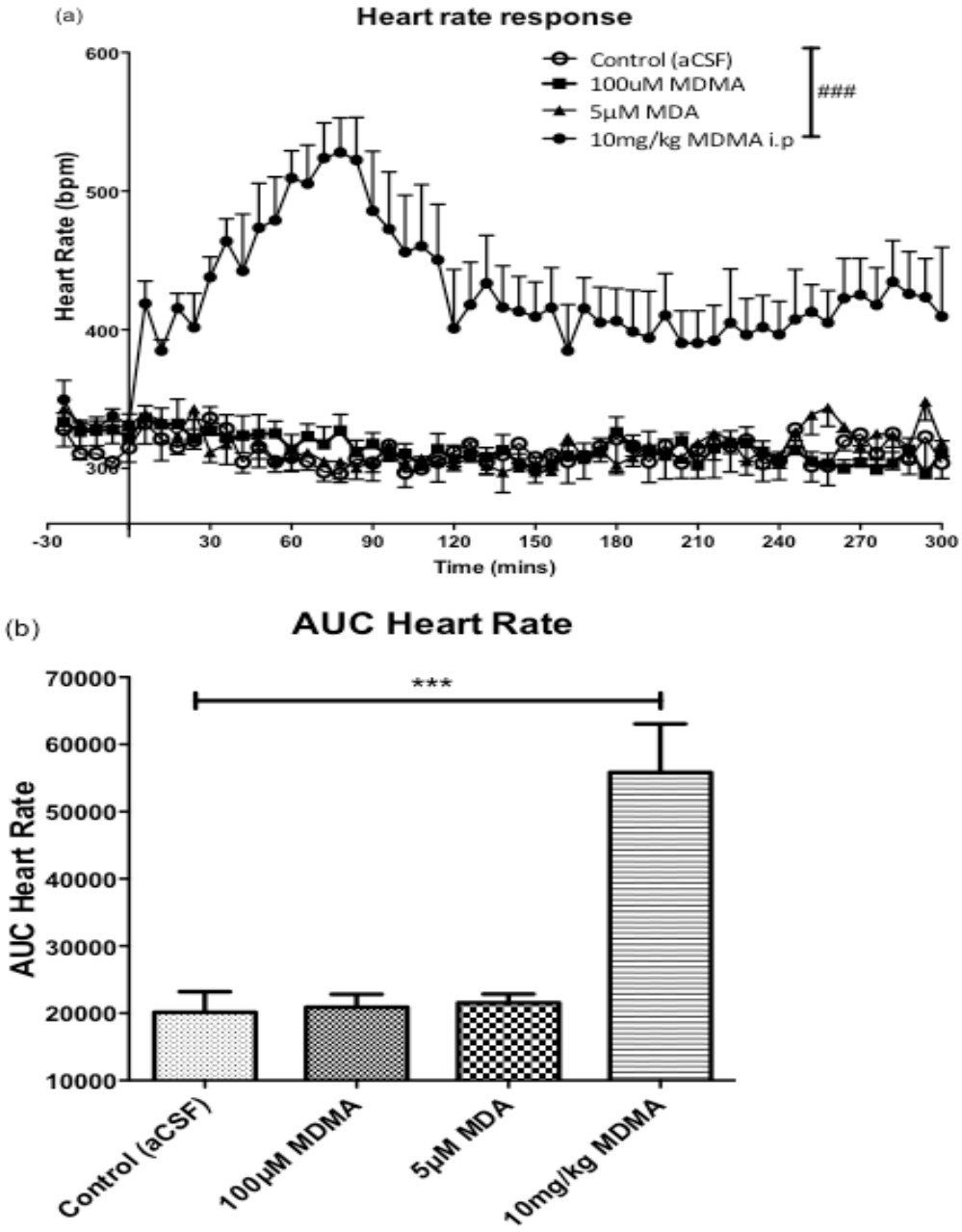


Figure 3. 3: Heart rate response following administration of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-5). Data was measured every 2 min but time points are shown for every 6 min from -30min before treatment to 300 min after treatment. ###P<0.001 compared with repeated measures two-way ANOVA and Tukey post hoc test. Column graphs (bottom) represent the AUC of the corresponding line graphs (top). ***P<0.001 compared with one-way ANOVA and Tukey post hoc test.

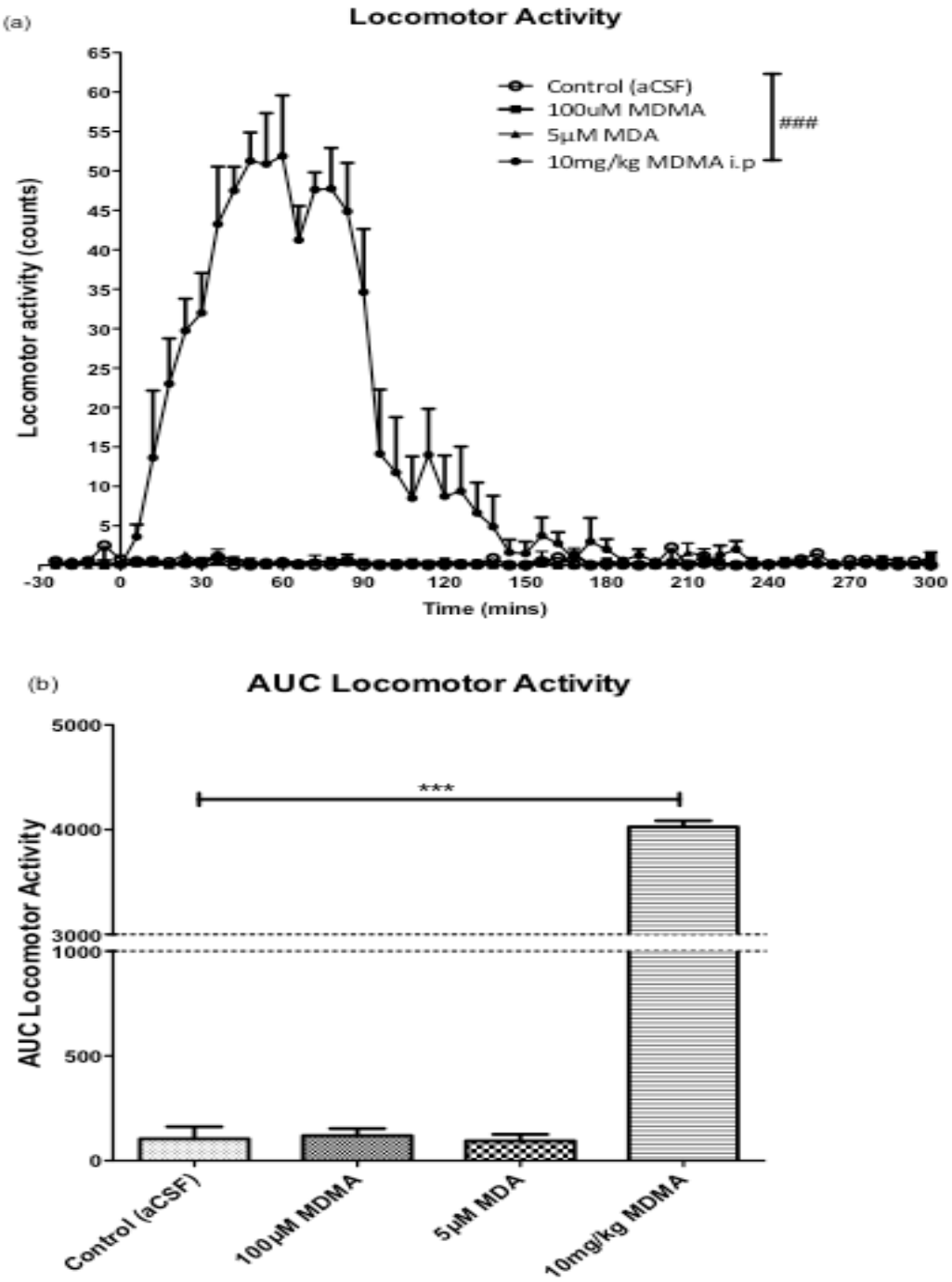


Figure 3. 4: Locomotor activity following administration of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p. at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-5). Data was measured every 2 min but time points are shown for every 6 min from -30min before treatment to 300 min after treatment. ###P<0.001 compared with repeated measures two-way ANOVA and Tukey post hoc test. Column graphs (bottom) represent the AUC of the corresponding line graphs (top). ***P<0.001 compared with one-way ANOVA and Tukey post hoc test.

3.3.6 Standards validation

Table 3. 1: Accuracy and precision data for assay validation, n=4. Validity required accuracy and precision to be within ±15%.

	Intra-day Validation					
	5-HT (ng/ml)			5-HIAA (ng/ml)		
Nominal Conc	5	7.5	10.0	5	7.5	10
Mean Measured	4.981	7.716	10.121	5.012	7.197	9.920
SD	0.053	0.270	0.264	0.070	0.191	0.190
Precision %	1.058	3.495	2.613	1.397	2.652	1.911
Accuracy %	-0.386	2.881	1.214	0.242	-4.037	-0.804

	Inter-day Validation					
	5-HT (ng/ml)			5-HIAA (ng/ml)		
Nominal Conc	5	7.5	10.0	5	7.5	10
Mean Measured	5.239	7.532	10.010	4.927	7.357	10.095
SD	0.397	0.134	0.145	0.178	0.352	0.281
Precision %	7.585	1.780	1.453	3.610	4.785	2.783
Accuracy %	4.782	0.424	0.099	-1.460	-1.905	0.947

3.3.7 Extracellular 5-HT and 5-HIAA concentrations

A mean basal level of 5-HT in the striatum dialysates was 1.265 ± 0.087 pg/µl (n = 4-5). Figure 3.5 (a) shows the effects of 10mg/kg MDMA, 100µM MDMA and 5µM MDA on 5-HT extracellular levels in the striatum. A two-way ANOVA showed a significant interaction effect ($P<0.01$, $F = 2.15$) and no significant effect of treatment ($P>0.05$, $F = 2.52$) and significant effect of time ($P< 0.05$, $F = 1.99$). Data are expressed as dialysate 5-HT (% of baseline) ± standard error of mean (S.E.M.) and as AUC 0 – 300 min following treatment. Systemic MDMA administration produced a significant increase in 5-HT extracellular levels (378.465 ± 133.169 peak 60 min, $P<0.01$, $n = 4$) in comparison to control. Central perfusion of MDMA also produced a significant increase in 5-HT extracellular levels (320.435 ± 71.050 peak 150 min, $P<0.05$, $n = 5$). Meanwhile, central perfusion of MDA did not produce significant change in 5-HT extracellular levels. The AUC of 5-HT response (bottom) shows significant difference between MDMA and the

control group at high Ta ($P<0.05$). For 5-HIAA, a mean basal level in the striatum dialysates was 14.004 ± 3.52 pg/µl (n = 4-5). Figure 3.6 (a) shows the effects of 10mg/kg MDMA, 100µM MDMA and 5µM MDA on 5-HIAA extracellular levels in the striatum. A two-way ANOVA showed a significant interaction effect ($P<0.05$, $F = 1.55$) and no significant effect of treatment ($P>0.05$, $F = 0.59$) and significant effect of time ($P< 0.0001$, $F = 4.39$). Data are expressed as dialysate 5-HIAA (% of baseline) ± standard error of mean (S.E.M.) and as AUC 0 – 300 min following treatment. Only central perfusion of MDA produces a significant increase in 5-HIAA extracellular levels (124.856 ± 24.884 peak 210 min, n = 5).

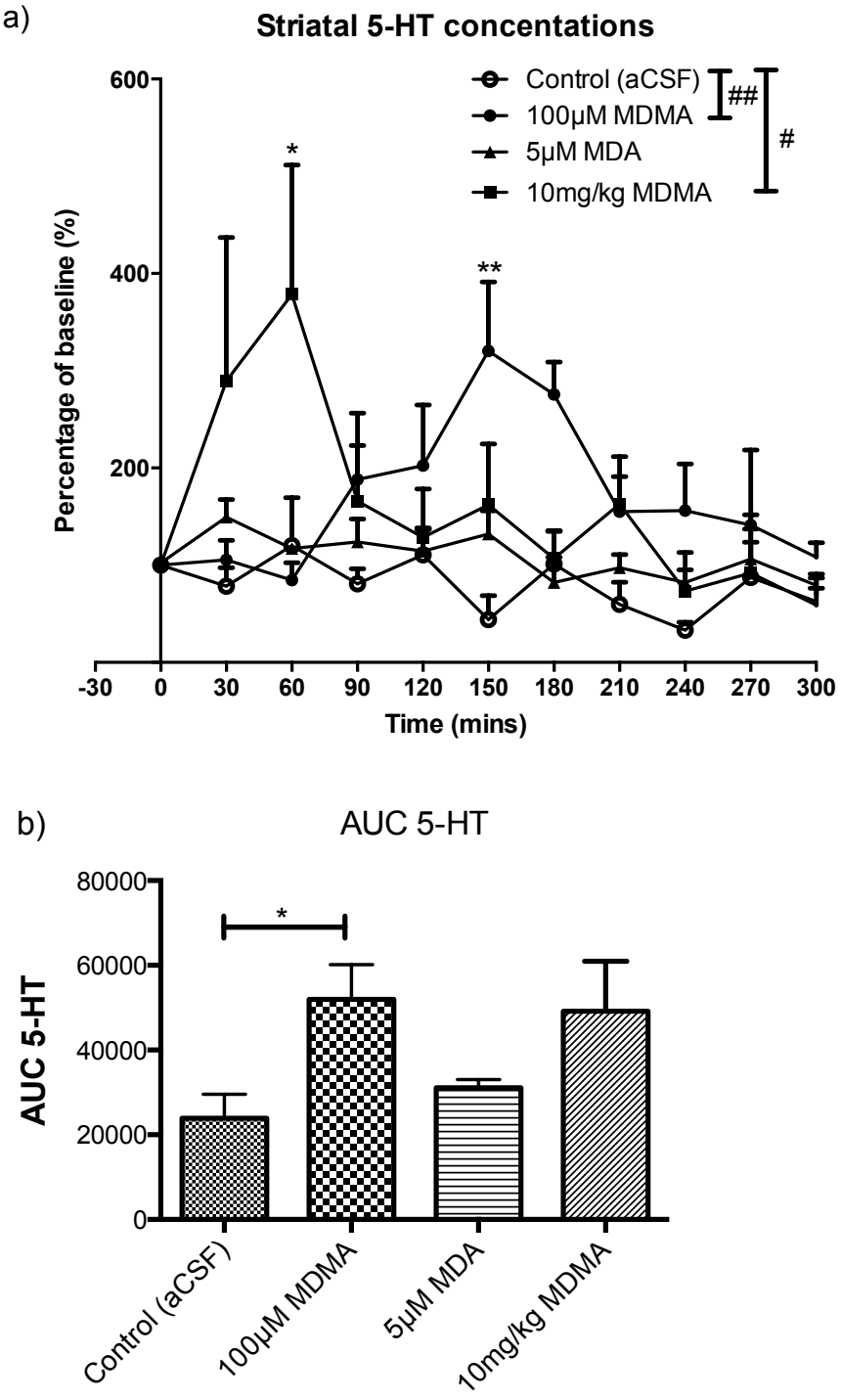


Figure 3. 5: Effect of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p. on striatal 5-HT at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-5). Responses are expressed as percentage of pre-treatment baseline values. #P<0.05, ##P<0.01 compared with repeated measures two-way ANOVA and Tukey post hoc test. *P<0.05, P<0.01 compared with two-way ANOVA and Tukey post hoc test. Column graphs (bottom) represent the AUC of the corresponding line graphs (top). *P<0.05 compared with one-way ANOVA and Tukey post hoc test.

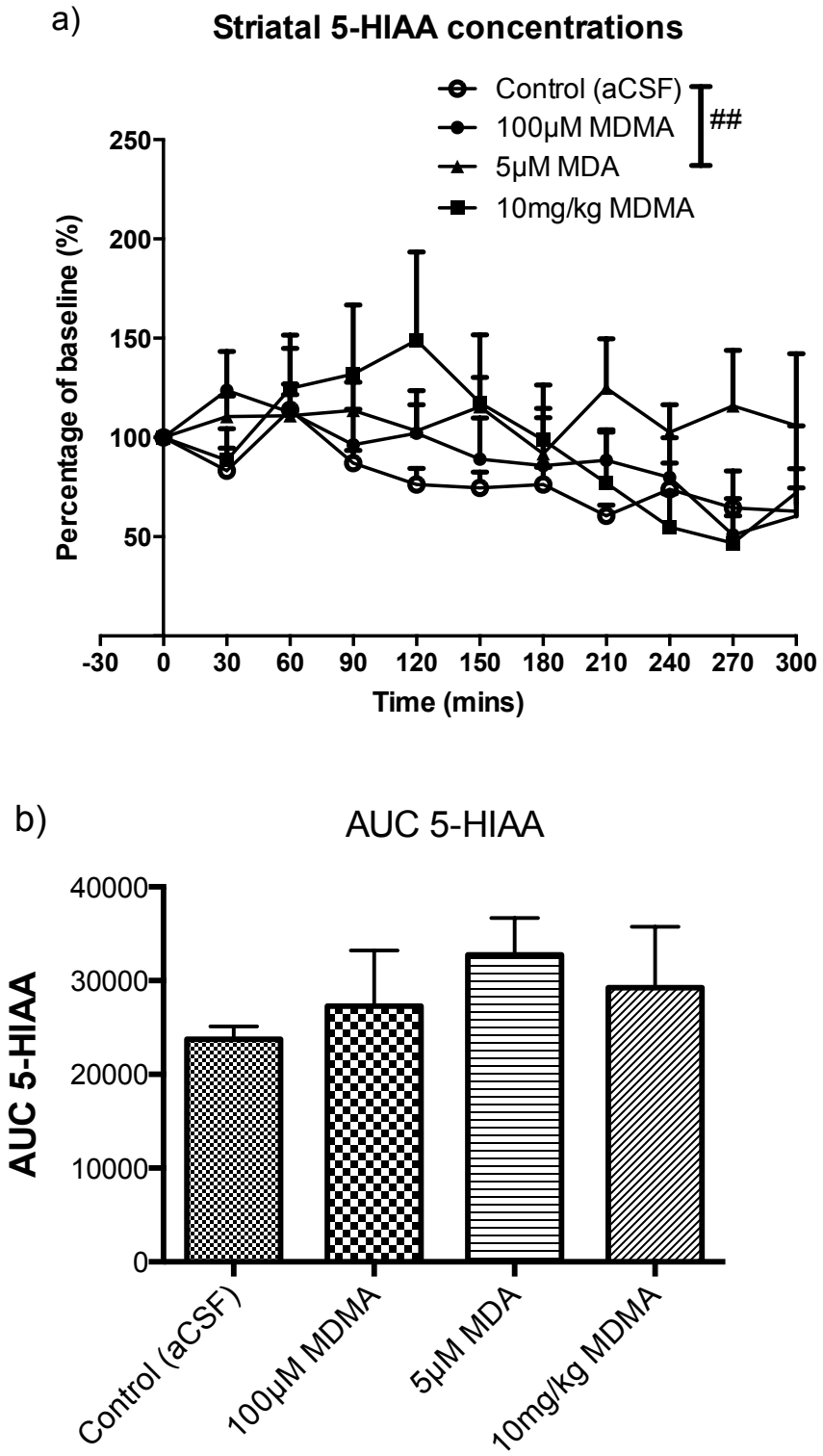


Figure 3. 6: Effect of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p. on striatal 5-HIAA at high (29 ± 1°C) Ta.

All data represent mean ± SEM (n = 4-5). Responses are expressed as percentage of pre-treatment baseline values. ##P<0.01 compared with repeated measures two-way ANOVA and Tukey post hoc test. Column graphs (bottom) represent the AUC of the corresponding line graphs (top).

3.4 DISCUSSION

This study used combined telemetry and microdialysis techniques at high ambient temperature to compare the effects of systemic administration and central perfusion of MDMA and to investigate the effects of central perfusion of MDMA and MDA into the striatum, and on Tc, HR, and LMA, and related the changes to striatal 5-HT extracellular levels. Our study has demonstrated that systemic administration of 10mg/kg (i.p.) MDMA cause a significant increase in Tc, HR, and LMA in comparison to control whereas central perfusion of 100μM MDMA do not produce any significant changes to Tc, HR and LMA. It was also shown that MDMA administered systemically (10mg/kg, i.p.) and centrally (100μM) produce significant striatal 5-HT extracellular levels. Central perfusion of 5μM MDA into the striatum did not produce significant change in the parameters measured but causes an increase in 5-HIAA extracellular levels. These MDMA results suggested that there was no direct association between the physiological parameters and extracellular 5-HT levels in the striatum.

Systemic MDMA administration caused significant increase in Tc, and this result was comparable to previous studies which found hyperthermia following systemic MDMA (10mg/kg i.p.) administration (Green et al., 2003; Stanley et al., 2007). The peak increase in Tc at 60 min was consistent with peak MDMA concentration in the brain measured by Esteban et al. (2001) following systemic administration of 10mg/kg MDMA (i.p.), although the experiment was undertaken at normal ambient temperature. Since MDMA blocks the rat tail vasodilatory heat loss mechanism (Blessing et al., 2003) and affects other physiological mechanisms associated with thermoregulation (Gordon et al., 1991), it is expected that rats demonstrate hyperthermic responses in a hot ambient temperature environment. MDMA disrupts thermoregulation which can produce hypothermia and hyperthermia effects at normal and high ambient temperature conditions (Malberg and

Seiden, 1998; Daws et al., 2000; Bexis et al., 2004). MDMA-induced hyperthermia in rats is thought to be mediated through its actions as 5-HT_{1A} receptor agonist and a 5-HT_{2A} receptor antagonist (Blessing et al., 2003; Blessing, 2005), which indicate the involvement of the serotonergic system in mediating hyperthermia. Activation of 5-HT_{2A} receptors increased Tc and substantially reduced tail temperature and blood flow. Meanwhile, 5-HT_{1A} receptors controls sympathetic outflow to the cutaneous vascular bed (Blessing, 2005).

Central perfusion of 100µM MDMA did not produce significant change in Tc. The concentration of MDMA used in the present study has been shown to produce extracellular concentration of MDMA similar to those found following systemic administration of 10mg/kg i.p. MDMA (Esteban et al., 2001; Nixdorf et al., 2001; Goni-Allo et al., 2008a) and produced an increase in striatal extracellular DA similar to that found after administration of 10mg/kg MDMA i.p. (Nixdorf et al., 2001). Our result was consistent with previous study by Nixdorf et al. (2001) and Goni-Allo et al. (2008a) which found no significant change in body temperature following central perfusion of 100µM MDMA into the striatum. The lack of significant hyperthermia in the present study following central perfusion of MDMA as compared to what is usually observed following systemic MDMA adminstration suggests that peripheral metabolism of MDMA and the formation of metabolites are important factors that result in the acute hyperthermic response. A study by Goni-Allo et al. (2008a) found no significant change in body temperature in male Wistar rats following 5h intrastriatal perfusion of 100µM MDMA at ambient temperature of 21.5°C but an increase in body temperature following intrastriatal perfusion of 100µM MDMA and systemic administration of 3×5mg/kg (i.p) MDMA at the same time. In this study we also perfused MDA centrally into the striatum which resulted in no significant change in Tc. This result was different from those found following systemic MDA

administration of 10 mg/kg i.p. and 20 mg/kg i.p. (Colado et al., 1995; Bexis and Docherty, 2006). MDA shares similar properties with MDMA in terms of causing acute hyperthermia at the same dose with MDMA (Colado et al., 1995; Bexis and Docherty, 2006). Systemic administration of 20mg/kg MDA (s.c) at room temperature to male Wistar rats produced initial hypothermia followed by significant increase in core body temperature, peak at 180 min after administration (Bexis and Docherty, 2006).

In the present study, systemic MDMA administration increased heart rate in conscious rats in comparison to control. However, central perfusion of MDMA and MDA did not produce significant change in HR. Cardiovascular physiology is important to mammals especially when adaptation to ambient temperature is involved (Jaehne et al., 2008). Chambers et al. (2000) demonstrated that cardiovascular responses to ambient temperature are closely related to the mechanism for heat production in rats. As seen in the present study, MDMA, which has cardiac stimulant effects can lead to tachycardia and arrhythmia (Gordon et al., 1991). It has been shown that MDMA acts as an agonist at both α_1- and α_2-adrenoreceptors, as well as 5-HT_2 receptors to increase blood pressure in anaesthetized rats (McDaid and Docherty, 2001).

Systemic MDMA administration causes an increase in LMA which corresponds to increase in Tc and HR. MDMA produces acute, dose-dependant hyperlocomotor response accompanied by the major behavioural features of the serotonin syndrome (Spanos and Yamamoto, 1989; Colado et al., 1993), although serotonin syndrome was not quantified in the present study. The 5-HT syndrome included enhanced locomotor activity, head-weaving, forepaw treading, piloerection, penile erection, proptosis, ejaculation, salivation, and defecation (Spanos and Yamamoto, 1989). The enhanced locomotor effects following MDMA administration was believed to be associated with MDMA-induced 5-HT

extracellular levels in the brain (Baumann et al., 2008). However, a study by Rodsiri et al. (2011) has shown that the binge dosing of 6mg/kg MDMA produced an increase in LMA without significant concomitant increase in extracellular 5-HT. This result could be due to the brain region in which 5-HT was measured, as the study measured 5-HT extracellular levels in the hippocampus which may play a minor role in mediating locomotion hyperactivity effects in comparison to the striatum. However, it has also been shown by previous studies that increase in LMA was not related to 5-HT extracellular levels (Dafters, 1994; O'Shea et al., 2005). In the present study, central perfusion of MDMA and MDA did not produce significant change in LMA as seen following systemic MDMA administration even though it was administered into the striatum, a brain region which is responsible for modulating locomotion. It is postulated that MDMA-induced increase in LMA involves combined effects of MDMA within several brain regions instead of MDMA action on the striatum alone. Other brain region such as the nucleus accumbens also plays an important role in mediating locomotion effects (Melega et al., 1995; Jones et al., 1996; Jones et al., 1999). There is also evidence suggesting activation of multiple 5-HT receptors by an interaction of DA and 5-HT (Bankson and Cunningham, 2002; Green et al., 2003). The present study did not include measurement of striatal extracellular levels of dopamine. Although we realize the importance of measuring striatal extracellular levels of dopamine in relation to MDMA-induced increase in LMA, the HPLC assay method used in this study was primarily optimised for 5-HT and 5-HIAA analysis. Due to low volume of dialysates, it was not possible to conduct further HPLC assay specifically optimised for dopamine and DOPAC.

In the present study, central perfusion of 5μM MDA results in a significant increase in 5-HIAA extracellular levels with no significant change in extracellular 5-HT levels. It has been described previously that MDA has a higher potency at several brain regions than

MDMA to induce 5-HT release, hence resulted in decrease in 5-HT and 5-HIAA concentrations. The present study is the first to observe a significant increase of 5-HIAA extracellular levels in the striatum following central perfusion of MDA. This could be explained due to less potent effect of MDA on MAO, which resulted in more 5-HT being metabolized to 5-HIAA, although this has not been reported in previous literatures.

The lack of hyperthermia following central perfusion of MDMA and MDA shown in the present study could be due to several reasons. The first reason is that MDMA metabolites other than MDA could potentially cause the hyperthermic response seen following systemic MDMA administration. As mentioned previously, MDMA is metabolized via N-demethylenation to MDA and via *O*-demethylenation to HHA, *O*-demethylenation to HHMA and subsequently to HMMA. Although there was limited finding on the effects of MDMA metabolites in MDMA-induced hyperthermia, these metabolites have been shown to produce MDMA-induced neurotoxicity. A study by Goni-Allo et al. (2008a) has demonstrated that *O*-demethylenation of HHMA and MDA to HMMA and HMA, respectively were inhibited by entacapone pretreatment, but plasma concentrations of HHMA and HHA were unchanged suggesting they were not solely metabolized via O-demethylenation pathway. HHMA and HHA are unstable catechols that can form conjugates with GSH that have been related to neurotoxicity (Hiramatsu et al., 1990; Miller et al., 1997; Bai et al., 1999; Bai et al., 2001). Since central perfusion of MDMA and MDA failed to produce hyperthermia at high ambient temperature, there is the possibility that hyperthermia seen following systemic MDMA administration was due to the role of other MDMA metabolites further down the metabolism pathways.

Although systemic administration of MDMA is known to cause acute hyperthermia (Colado et al., 1995; Malberg and Seiden, 1998; Fonsart et al., 2008) the mechanism

involved is still unclear. One of the suggested mechanisms is disruption of thermoregulation due to increase in 5-HT levels (Gordon et al., 1991; Blessing et al., 2003). However, administration of 100μM MDMA centrally into the rat brain using microdialysis resulted in an increase in hippocampal (Esteban et al., 2001) and striatal (Nixdorf et al., 2001) 5-HT levels 1-3h following MDMA administration with no significant increase in body temperature. This result is in accordance with the present study as we found significant increase in 5-HT levels without significant increase in Tc following central perfusion of MDMA. In the present study, we also demonstrated significant change in Tc from 30 min onwards but only significant increase in 5-HT extracellular levels at 60 min following systemic administration of MDMA. These results suggest no direct functional association between these parameters in regard to extracellular 5-HT in striatum. A study by Mechan et al. (2002) has also shown that fluoxetine blocks 5-HT extracellular levels in hippocampus but not hyperthermia, suggesting MDMA-induced hyperthermia in rat was not related to extracellular levels in 5-HT in the brain.

There has been suggestion on the role of DA in mediating hyperthermic effects in rats (Green et al., 2003; Docherty and Green, 2010). MDMA administration *in vivo* has been shown to produce acute increase in striatal DA concentration. Dopamine D_1 receptor antagonist SCH 23390 inhibited MDMA-induced hyperthermia in a dose-dependent manner which suggest that increase in DA extracellular levels, which acts in this receptor leads to hyperthermia (Mechan et al., 2002).

The other reason for the absence of hyperthermia following central MDMA and MDA perfusion into the striatum could be due to lack of direct involvement of striatum in thermoregulation. As explained in the introduction chapter, there were a number of valid reasons for selecting the striatum in our study. However, it is also important to repeat these

experiments in a region with evidence for its role in thermoregulation such as the preoptic area/anterior hypothalamus (PO/AH) (Ishiwata et al., 2002; Nagashima, 2006; Benamar et al., 2008).

In this study, reverse dialysis was done *in vitro* to determine the extracellular concentrations of MDMA and MDA following central administration of 100µM MDMA and 5µM MDA. A previous study which administered 10mg/kg and 15mg/kg MDMA i.p to rats found concentrations of 11µM and 20µM MDMA, respectively in the hippocampus (Esteban et al., 2001). In the present study, 10% *in vitro* recovery was achieved following perfusion of 100µM MDMA and 5µM MDA at 120 and 150 min, respectively and the value of 10µM obtained for MDMA is similar to that reported by Esteban et al., (2001). A study in our lab showed that administration of 7.5mg/kg MDMA produces concentration of ~3200ng/ml (14µM) of MDMA in blood (Jaehne et al., 2010) and this concentration is comparable to previous study which administered 10 and 15mg/kg MDMA and found between 11µM and 20µM of MDMA in the hippocampus (Esteban et al., 2001). A previous study that looked at the effects of MDMA and MDA on neurotoxicity administered 20µg/µl MDA into the cerebral cortex using microinjection found no serotonin neurotoxicity following MDA administration (Molliver et al., 1986). This is among the earlier study to administer MDA directly into the brain. Another study which measured brain MDA concentration found peak concentration of MDA in the brain of 165µM, 45 min following administration of 20mg/kg MDA (s.c.) (Zaczek et al., 1989) Since MDMA and MDA have non-linear pharmacokinetics, it is expected that MDA concentration in the brain following systemic administration is not parallel with increase in dose and can be quite high.

There are a number of limitations to the work that has been conducted in the present study. It is possible that the lack of pharmacodynamic effects following central perfusion of MDMA and MDA was due to the diffusion of these drugs away from the striatum. However, this problem was addressed by Esteban et al. (2001) which placed a double microdialysis probe, constructed so that shafts and tips were parallel and separated by a distance of 1mm. The concentration of MDMA recovered by the second probe reflect an estimated extracellular concentration of MDMA between 10.4 and 19.5 μM which were in the range of that found after peripheral administration of 10 and 15mg/kg MDMA, respectively. Accurate probe placement was confirmed by comparing with samples that already been established in previous work in the lab. The present study did not determine the concentrations of MDMA and MDA in the brain throughout the experiment. This parameter would help to understand the correlation of MDMA and MDA concentrations in the brain at each time points with thermal and physiological changes, and the metabolism of the drug.

The present study has demonstrated that systemic administration of MDMA produced significant increase in Tc, HR, LMA, and striatal 5-HT extracellular levels as shown by previous studies. However, central perfusion of MDMA only produces significant elevation of striatal 5-HT, whereas central perfusion of MDA has no significant effects on Tc, HR, LMA and 5-HT extracellular levels in the striatum. It has been shown that acute physiological changes following MDMA administration has no direct correlation with striatal 5-HT extracellular levels. These findings suggest that striatum plays a minor role in mediating hyperthermia and different mechanisms are involved in mediating MDMA-induced adverse effects on Tc, HR and LMA. It is also suggested that MDA does not play a major role in MDMA-induced hyperthermia and 5-HT extracellular levels in the striatum. Future work could explore the effects of these drugs on the PO/AH and nucleus

accumbens, which play important roles in thermoregulation and locomotion, respectively, and also the role of DA in mediating these effects.

Chapter 4 General Discussion

MDMA has been known to cause unpredictable and life-threatning acute toxic effects and long-term neurotoxic effects. Research on MDMA has been continuously undertaken over the last few decades in an attempt to understand the mechanism of action of this drug. Although progress has been made we are still not in a position to predict when adverse effects in humans will occur. This lack of understanding is a major hurdle to the development and introduction of evidence based preventative and treatment programs.

When MDMA is consumed, it can cause acute increase in Tc, especially when taken at high ambient temperature. This increase in core body temperature can lead to symptoms such as acute renal failure, cardiac arrhythmias, rhabdomyolisis and disseminated intravascular coagulation that can cause death (Gowing et al., 2002; Kaye et al., 2009). MDMA can also cause an increase in HR and blood pressure resulting in clinical adverse effects (O'Cain et al., 2000; Badon et al., 2002). In animal studies, MDMA has been shown to cause long term neurochemical changes such as decreases in intracellular 5-HT concentrations, 5-HT transporters and loss of 5-HT neurons. These neuronal effects are exacerbated by increased in Tc and ambient temperature (Malberg and Seiden, 1998). High ambient temperature can also enhance the rewarding effects of MDMA in rats and human (Parrott, 2002; Cornish et al., 2003; Parrott et al., 2006), which can affect MDMA consumption and usage. It is difficult to investigate the effects of MDMA alone following systemic administration because it is metabolized to several active metabolites, including MDA. Some of the conjugated metabolites have been shown to be neurotoxic (Bai et al., 1999; Goni-Allo et al., 2008a; Baumann et al., 2009). The metabolism of MDMA is also influenced by Tc and high ambient temperature (Malberg and Seiden, 1998), which can lead to different magnitude of neurotoxicity. In pre-clinical studies, MDA has been shown to cause more severe degeneration of serotonin neurons than MDMA. MDMA users are

exposed to MDA in the drug market, where MDA is sold as MDMA or 'ecstasy'. They are also exposed to MDA as it is a major product of the peripheral metabolism of MDMA. These situations are concerning and highlight the need to further understand the potential role of MDA, particularly in the occurrence of hyperthermia following MDMA administration. Determining the specific brain regions involved in mediating MDMA-induced hyperthermia and other adverse effects will provide basis to develop and improve treatment strategies for humans.

A number of methods have been used to assess MDMA effects on thermoregulation in animals including rectal measurement, ear temperature measurement, and tail temperature measurement (Clement et al., 1989; Gordon et al., 1991; Colado et al., 1995; McGregor et al., 2003; Clemens et al., 2007). Of these methods rectal temperature is considered to be most reliable as it is not influenced by operator error or changes in regional blood flows. However, rectal measurement has been shown to produce stress due to handling of animals and repeated insertion of probe into the rectum (Frankel, 1959; Poole and Stephenson, 1977; Clement et al., 1989; Dilsaver et al., 1990), which can possibly influence the actual effects of MDMA on body temperature. Recent development of telemetry methods in small animals may provide a better option to assess MDMA effects on Tc as this method allows continuous measurement of Tc, and other parameters including HR, blood pressure and LMA (Gallaher et al., 1985; Clement et al., 1989; Dilsaver et al., 1990; Brockway et al., 1991; Guiol et al., 1992; van den Buuse, 1994; Huetteman and Bogie, 2009). Previously, there has been no comparison of rectal measurement and telemetry to assess MDMA effects. In the first study described in this thesis, the usage of rectal probe and telemetry to assess MDMA effects at high ambient temperature on Tc were compared. Our results have indicated that telemetry provides a better tool to assess MDMA effects on Tc as it prevented potentiation of MDMA effects on Tc, which occurred with rectal

measurements. The second study was undertaken to determine the validity of combined telemetry and microdialysis use to assess the effects of systemic MDMA administration, and central MDMA and MDA perfusion into the striatum. Previously, animals were sacrificed at different time points to assess acute neurochemical changes following drug administration to relate the changes in physiological and thermoregulatory parameters to neurochemical changes in the brain. At the time of starting work for this thesis, only two studies had used the combined techniques to relate the physiological and neurochemical changes (Benamar et al., 2008; Rodsiri et al., 2011), in which one study used thermosensor telemetry. Benamar et al. (2008) measured the effects of MDMA on Tc and the extracellular levels of dopamine in the PO/AH, whereas Rodsiri et al. (2011) measured the concomitant effects of MDMA binge dosing on hippocampal extracellular 5-HT, locomotion and Tc. The latter demonstrated that acute physiological changes following MDMA administration have no direct correlation with striatal 5-HT extracellular levels. However both of these studies did not look at the effects of MDMA on heart rate or the effects of MDA. Furthermore, the experiments were conducted at normal ambient temperature which did not mimic the raves and clubs condition where 'Ecstasy' is usually consumed. The main aim of the second study was to assess MDMA physiological and neurochemical effects, and the role of MDA in MDMA-induced hyperthermia at high ambient temperature using combined telemetry and microdialysis techniques.

This research comprised two parts. The first part investigated the influence of methodological approaches used to measure MDMA-induced increase in body temperature. The use of rectal probe caused potentiation of MDMA effects on body temperature compared to the use of telemetry to measure Tc and has also resulted in a lower survival rate. The use of telemetry resulted in a higher behavioural score in comparison to the use of rectal

probe has a potential to influence body temperature measurements and ths could be attributed to stress (Matthew, 1997; Clark et al., 2003; Veening et al., 2004).

The second part of this thesis looked at the use of combined telemetry and microdialysis techniques to investigate the effects of systemic MDMA administration and central MDMA and MDA perfusion into the striatum, on the acute pharmacodynamic effects and striatal 5-HT extracellular levels. Systemic MDMA administration produced significant increase in Tc, HR, LMA, and striatal 5-HT extracellular levels and these results were comparable to previous studies which looked at the effects of systemic administration of MDMA in rats (Dafters, 1994; Green et al., 2003; Bexis and Docherty, 2006). We have demonstrated that 10mg/kg MDMA i.p. produces an increase in Tc of around 3°C, when given to SD rats at high ambient temperature of 29°C. This is comparable to the findings demonstrated in previous studies in which MDMA was given at similar doses and ambient temperatures (Malberg and Seiden, 1998; Mechan et al., 2002). Following systemic MDMA administration, MDMA-induced 5-HT release was significantly higher than control at 60 min post-treatment but MDMA-induced hyperthermia was significantly higher than control at earlier time points preceding the increase in brain 5-HT. These results suggested that there was no direct association between MDMA-induced hyperthermia and release of 5-HT in the striatum. We have also shown that systemic administration of MDMA produces an increase in HR at high Ta. Changes in HR after MDMA administration may be a result of direct sympathomimetic effects on the control of the cardiovascular system either centrally or peripherally or by an indirect effect in response to the disruption of the central mechanisms of thermoregulation (Bexis and Docherty, 2005; Bexis and Docherty, 2006).

Central perfusion of MDMA and MDA did not produce any change in Tc, HR and LMA but central perfusion of MDMA produces significant increase in striatal 5-HT extracellular levels. The results obtained for MDMA are consistent with previous studies which administered MDMA centrally to the brain and found no significant change in Tc despite a significant increase in 5-HT extracellular levels in the striatum and the hippocampus (Esteban et al., 2001; Nixdorf et al., 2001). However, studies on central MDA effects are scarce (Molliver et al., 1986; McCann and Ricaurte, 1991b; Elayan et al., 1992) and no previous studies have looked at the acute effects following central MDA perfusion. Central MDMA perfusion produced significant 5-HT extracellular levels at a later stage (150 min) in comparison to systemic MDMA administration (60 min). This is proabably due to the time it takes for central MDMA perfusion to reach the concentration of ~10μM, which is similar to the concentration found in rat brain following systemic 10 mg/kg MDMA administration. This result is supported by the microdialysis probe *in vitro* recovery data indicating that it takes up to 150 min to achieve ~10 μM concentration (Figure 3.1).

Striatal administration of both MDMA and MDA did not produce significant change in Tc, HR, and LMA. The lack of effect on LMA is suprising considering the striatum's important role in locomotion. Our results suggest that MDMA distribution to several brain regions is essential to induce significant increase in locomotion hyperactivity instead of confined perfusion within the striatum. Previous studies have also suggested the role of DA in mediating MDMA-induced locomotion effects (Green et al., 2003; Baumann et al., 2008).

There were some limitations to the work that has been conducted in this research. In the second study, it was shown that MDMA-induced striatal 5-HT extracellular levels has no direct association with MDMA-induced increase in body temperature. However, in this

study the concentration of DA was not analyzed. Previous work has demonstrated that the D_1 receptor antagonist SCH 23390 can significantly attenuate MDMA-induced hyperthermia suggesting that DA extracellular levels is associated with changes in body temperature (Mechan et al., 2002). It was also shown that MDMA-induced ambulation and stereotypy are correlated with increases in dialysate DA concentrations in nucleus accumbens and striatum (Baumann et al., 2008). Since central perfusion of MDMA and MDA into the striatum failed to reproduce hyperthermia that has been demonstrated following systemic administration, it is important to investigate central perfusion of both drugs into other brain regions, especially the PO/AH which has been shown to play a major role in thermoregulation (Boulant and Dean, 1986; Hasegawa et al., 2005; Benamar et al., 2008). In addition, although the doses used in the present study were comparable to those consumed by humans, there were differences in pharmacokinetics between rats and humans which limits direct extrapolation of the results from animal studies into humans. Future studies should also look at long-term effects in relation to thermoregulation following MDMA and MDA perfusion into different brain regions. In conclusion, this research has highlighted some important technical issues in the methodologies applied to investigate MDMA-induced physiological and neurochemical effects in animal models. It also has highlighted problems in assuming drug effects on neurotransmitter extracellular levels is homogeneous throughout the brain and reinforces the need for regional studies within the brain.

The complex pharmacology of MDMA is still setting challenges to researchers. The issues identified in this thesis will contribute to our understanding of MDMA behavioural and neurochemical effects and potentially leading to improved risk reduction and treatment of MDMA adverse effects in humans.

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